CHAPTER 4

RESULTS

4.1 CHEMICAL ANALYSIS OF GREEN TEA EXTRACT

TLC of methanolic extract of green tea extract showed 8 bands in UV 254 nm. After spraying with 5% methanolic ferric chloride solution and DPPH showed 5 bands.

TLC fingerprint profile of extract with standard solution of gallic acid and catechin showed presence of gallic acid and catechin in the sample solution. It is well resolved in the solvent system of Toluene: Ethyl acetate: Formic acid (6: 6: 1). From the TLC plate derivatized with ferric chloride and DPPH, it can be concluded that phenolic are responsible for the antioxidant activity of the extract (Fig.4.1 and Table 4.1).

Fig.4.1: TLC densitometric chromatogram of methanolic extract of green tea extract with gallic acid standard and catechin standard solution.
E: Extract, 1: gallic acid, 2: catechin standard solution

Fig.4.1

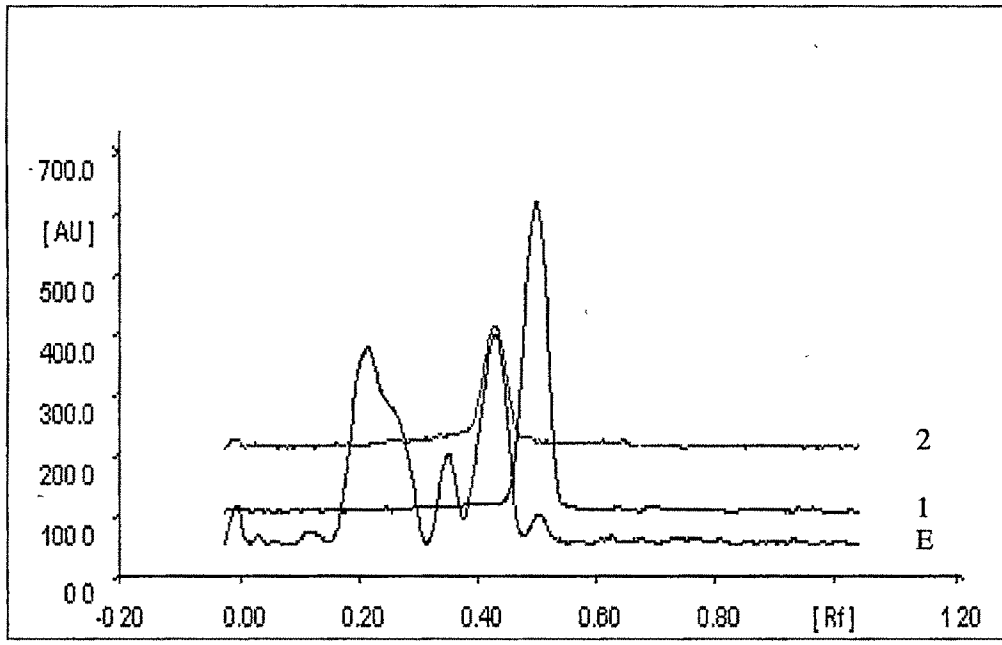

Table 4.1: Details of fingerprint chromatograms of green tea extract after scanning at 254 nm.

Extract	Solvent system	No. of spots
Methanolic extract	Toluene: Ethyl acetate: Formic acid (6: 6: 1).	8
Rf values	0.03, 0.12, 0.22, 0.35, 0.43, 0.50, 0.63, 0.68	
Relative %	3.30, 1.84, 33.03, 15.11, 35.09, 4.99, 1.27,1.05	

4.2 IN VITRO STUDIES

The solutions of green tea extract, melatonin, lovastatin, and resveratrol exhibited different levels of antioxidant activity in the models studied.

4.2.1 DPPH ASSAY

It was observed that green tea extract exhibited significant ($P<0.05$) scavenging activity on DPPH radicals at lower concentration (20 μg/ml) and highly significant ($P<0.001$) activity at higher concentrations (50, 75 and 100 μg/ml). Curcumin also exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.2).

Melatonin also exhibited highly significant ($P<0.001$) scavenging activity on DPPH radicals at higher concentrations (20, 50, 75 and 100 μg/ml). Curcumin also exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.3).

Lovastatin did not exhibit any scavenging activity on DPPH radicals. It formed colour similar to that of control indicating no scavenging activity. However curcumin exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.4).

Resveratrol exhibited highly significant ($P<0.001$) scavenging activity on DPPH radicals at higher concentrations (20, 50, 75 and 100 μg/ml). Curcumin exhibited highly significant ($P< 0.001$) activity at various concentrations. (Fig.4.5).

Fig. 4.2: Effect of green tea extract on DPPH radical formation as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to DPPH solution (% Change 0).

All values are mean ± SEM. The changes in presence of either green tea extract or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig. 4.3: Effect of melatonin on DPPH radical formation as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to DPPH solution (% Change 0).

All values are mean ± SEM. The changes in presence of either melatonin or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig. 4.4: Effect of lovastatin on DPPH radical formation as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to DPPH solution (% Change 0).

All values are mean ± SEM. The changes in presence of either lovastatin or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig. 4.5: Effect of resveratrol on DPPH radical formation as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to DPPH solution (% Change 0).

All values are mean ± SEM. The changes in presence of either resveratrol or standard were compared with control.

(*P<0.05, **P<0.01, *** P<0.001, NS: not significant).

Fig. 4.2

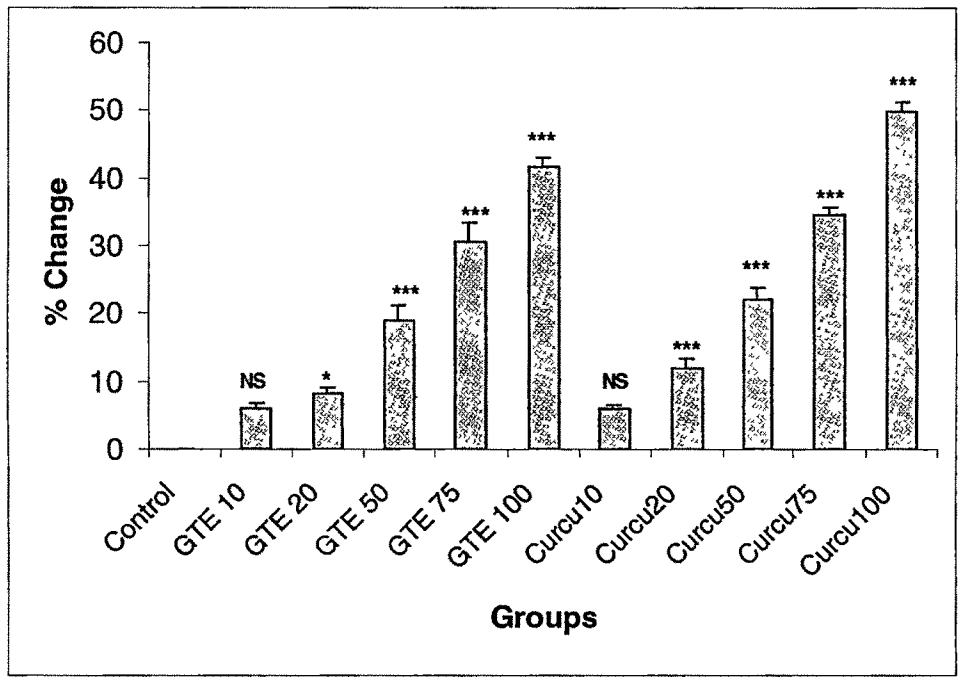
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Fig. 4.3

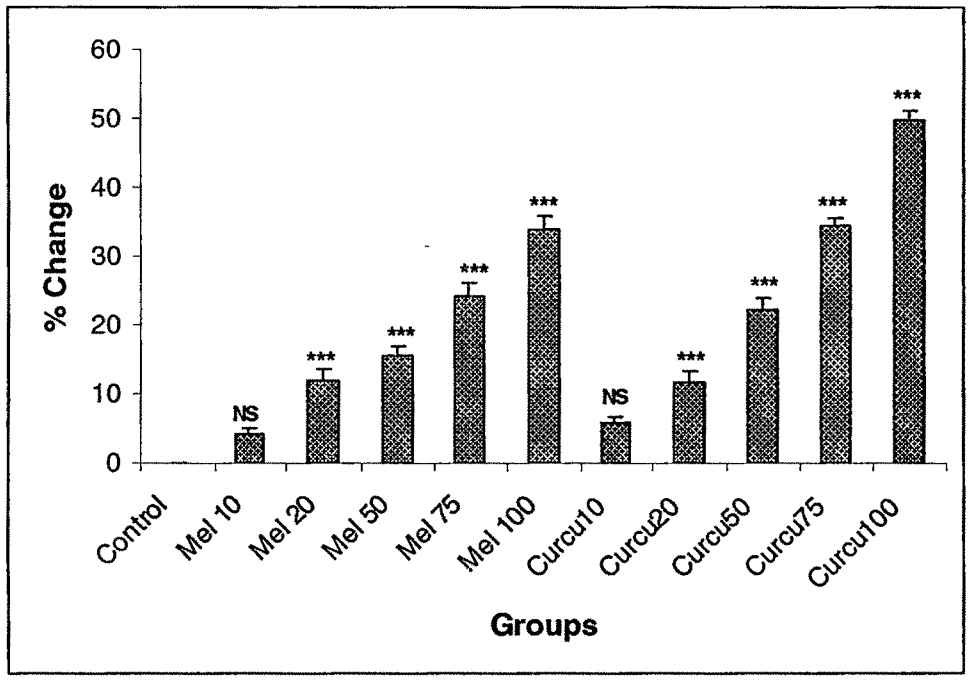
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Fig. 4.4

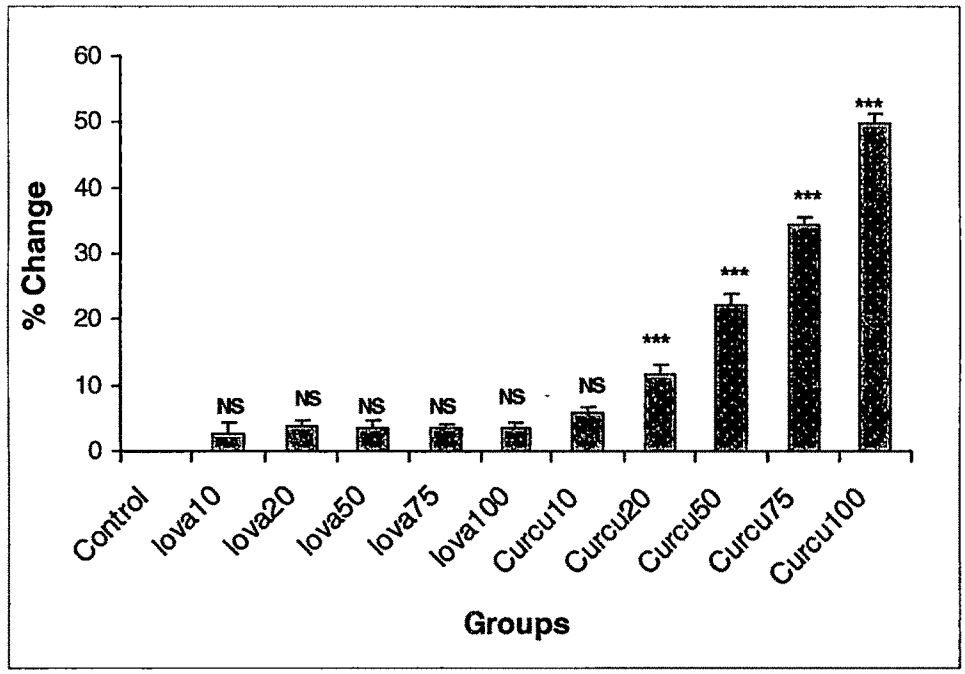
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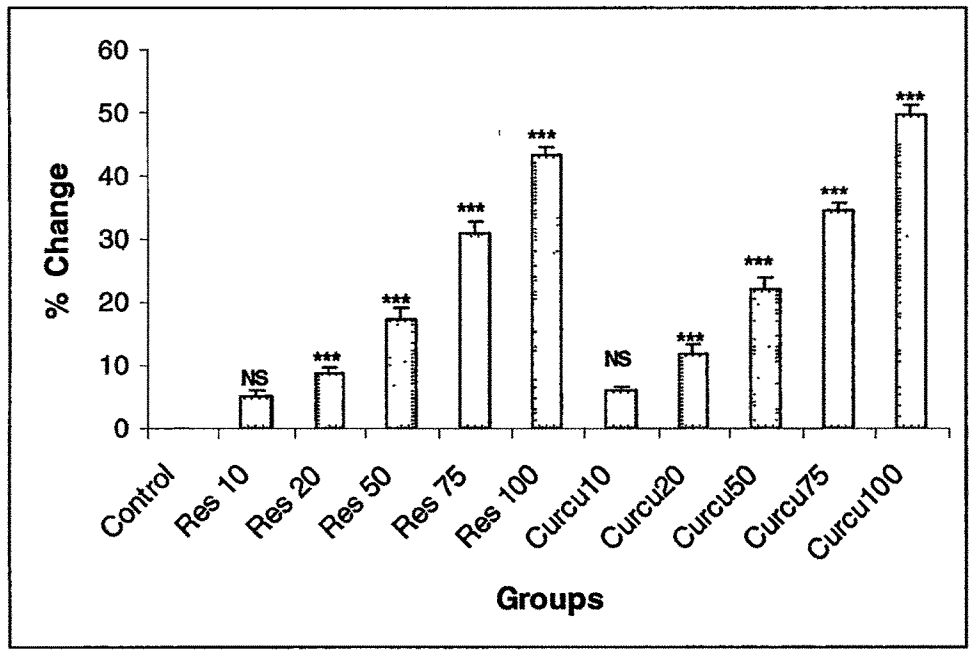
Fig.4.5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4.2.2 ASSAY FOR SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Green tea extract exhibited significant (P<0.001) scavenging activity on superoxide radicals at various concentrations (20, 50, 75 and 100μg/ml) as compared to control. Curcumin exhibited highly significant (P<0.001) activity at all concentrations (Fig.4.6).

Melatonin did not exhibit scavenging activity on superoxide radicals. It formed colour similar to that of control indicating no scavenging activity. However curcumin exhibited highly significant (P< 0.001) activity at all concentrations (Fig.4.7).

Lovastatin did not exhibit scavenging activity on superoxide radicals. It formed colour similar to that of control indicating no scavenging activity. However curcumin exhibited highly significant (P< 0.001) activity at various concentrations (Fig.4.8).

Resveratrol showed significant (P<0.05, P<0.001) scavenging activity on superoxide radicals at higher concentrations (75 and 100 μg/ml) as compared to control and curcumin exhibited highly significant (P<0.001) activity at various concentrations (Fig.4.9).

Fig. 4.6: Effect of green tea extract on NBT-Riboflavin-light induced superoxide release as compared to scavenging activity of curcumin at similar concentrations (10-100 μg/ml). Percentage change as compared to NBT-Riboflavin-light reaction mixture (% Change 0).

All values are mean ± SEM. The changes in presence of either green tea extract or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig. 4.7: Effect of melatonin on NBT-Riboflavin-light induced superoxide release as compared to scavenging activity of curcumin at similar concentrations (10-100 μg/ml). Percentage change as compared to NBT-Riboflavin-light reaction mixture (% Change 0).

All values are mean ± SEM. The changes in presence of either melatonin or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig. 4.8: Effect of lovastatin on NBT-Riboflavin-light induced superoxide release as compared to scavenging activity of curcumin at similar concentrations (10-100 μg/ml). Percentage change as compared to NBT-Riboflavin-light reaction mixture (% Change 0).

All values are mean ± SEM. The changes in presence of either lovastatin or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig. 4.9: Effect of resveratrol on NBT-Riboflavin-light induced superoxide release as compared to scavenging activity of curcumin at similar concentrations (10-100 μg/ml). Percentage change as compared to NBT-Riboflavin-light reaction mixture (% Change 0).

All values are mean ± SEM. The changes in presence of either resveratrol or standard were compared with control.

(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant).

Fig.4.6

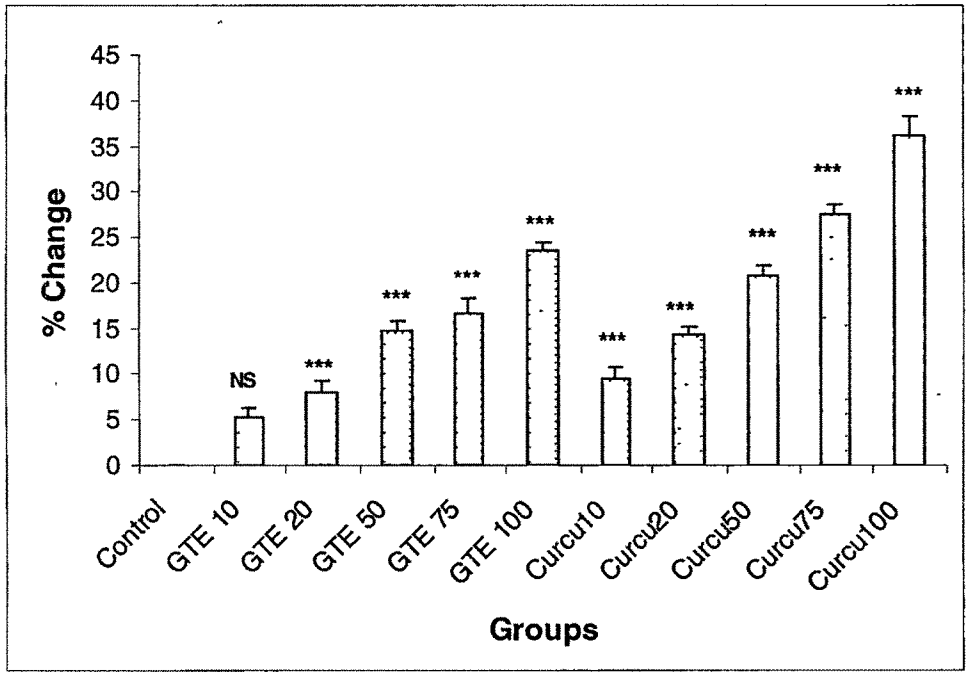
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Fig.4.7

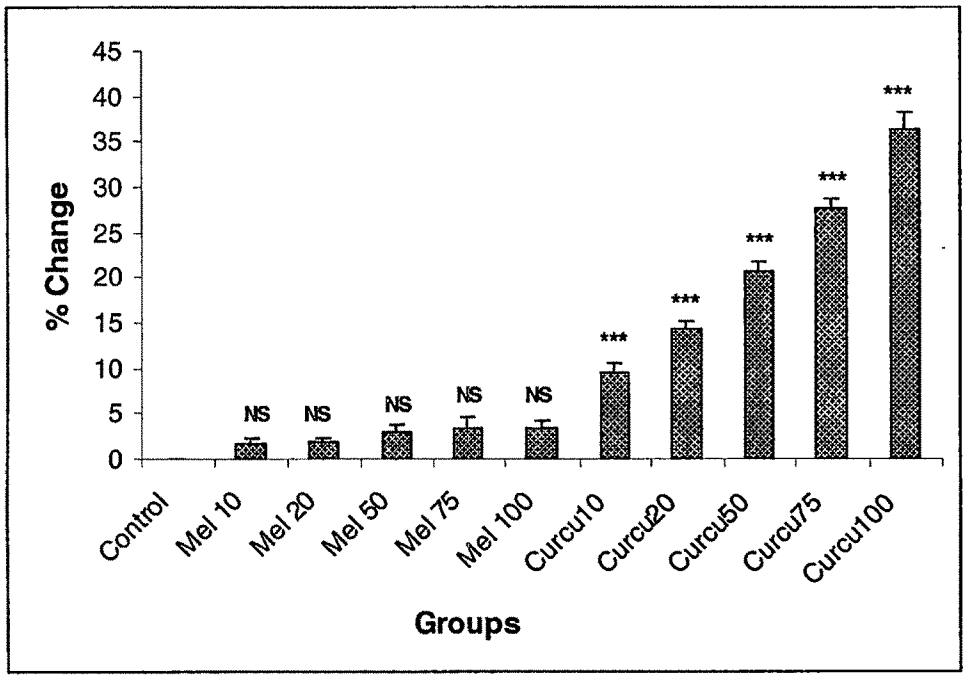
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Fig.4.8

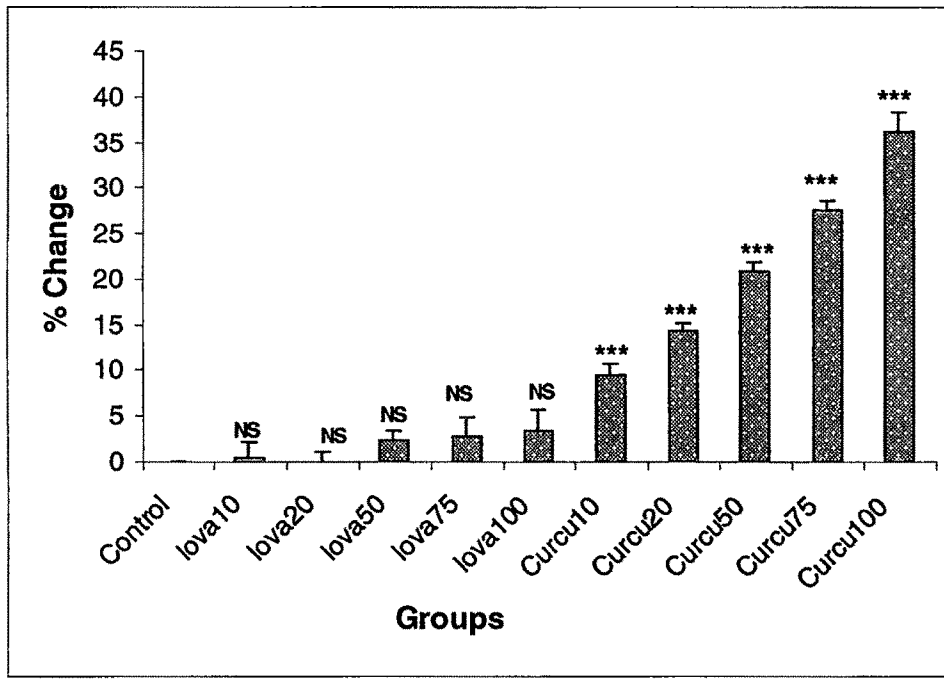
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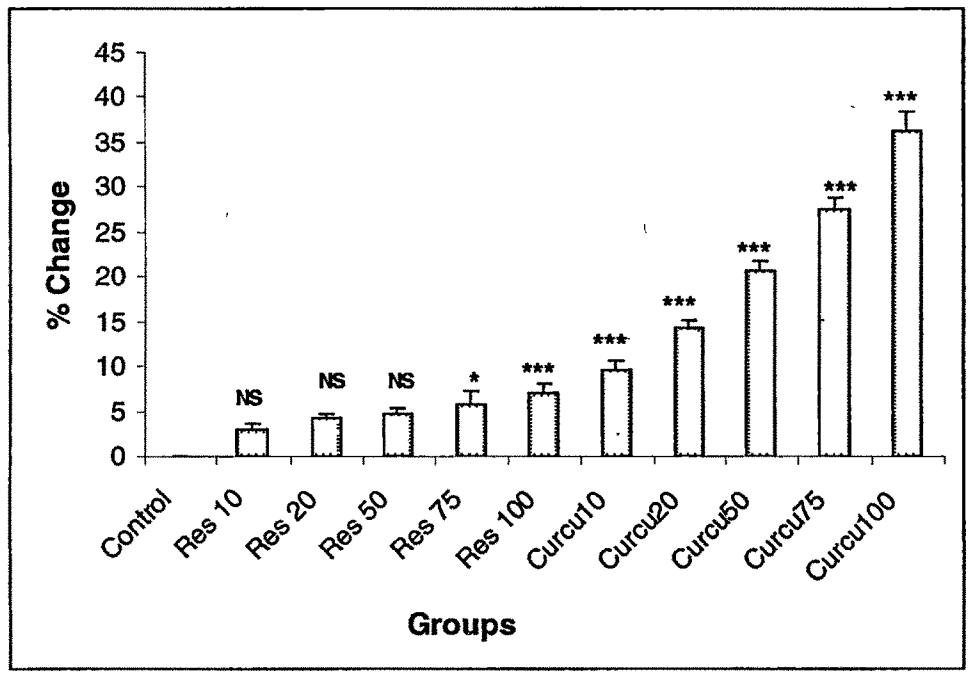
Fig.4.9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4.2.3 ASSAY FOR NITRIC OXIDE SCAVENGING ACTIVITY

There was a significant ($P<0.001$) scavenging activity on nitric oxide radicals by green tea extract at all concentrations (10, 20, 50, 75 and 100μg/ml). Curcumin exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.10).

There was a significant ($P<0.05$, $P<0.01$, $P<0.001$) scavenging activity on nitric oxide radicals by melatonin at higher concentrations (20, 50, 75 and 100μg/ml). Curcumin exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.11).

Lovastatin exhibited significant ($P<0.001$) scavenging activity on nitric oxide radicals at all concentrations (10, 20, 50, 75 and 100μg/ml). Curcumin exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.12).

Resveratrol exhibited significant ($P<0.05$) scavenging activity on nitric oxide radicals only at highest concentration (100μg/ml). However curcumin exhibited highly significant ($P<0.001$) activity at various concentrations (Fig.4.13).

Fig. 4.10: Effect of green tea extract on nitrite formation from released nitric oxide as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to Sodium nitroprusside - Griss reagent reaction mixture (% Change 0).
All values are mean ± SEM. The changes in presence of either green tea extract or standard were compared with control.
(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant).

Fig. 4.11:
Effect of melatonin on nitrite formation from released nitric oxide as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to Sodium nitroprusside - Griss reagent reaction mixture (% Change 0). All values are mean ± SEM. The changes in presence of either melatonin or standard were compared with control.
(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant).

Fig. 4.12: Effect of lovastatin on nitrite formation from released nitric oxide as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to Sodium nitroprusside - Griss reagent reaction mixture (% Change 0).
All values are mean ± SEM. The changes in presence of either lovastatin or standard were compared with control.
(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant).

Fig. 4.13: Effect of resveratrol on nitrite formation from released nitric oxide as compared to scavenging activity of curcumin at similar concentrations (10-100 µg/ml). Percentage change as compared to Sodium nitroprusside - Griss reagent reaction mixture (% Change 0).
All values are mean ± SEM. The changes in presence of either resveratrol or standard were compared with control.
(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant).

Fig. 4.10

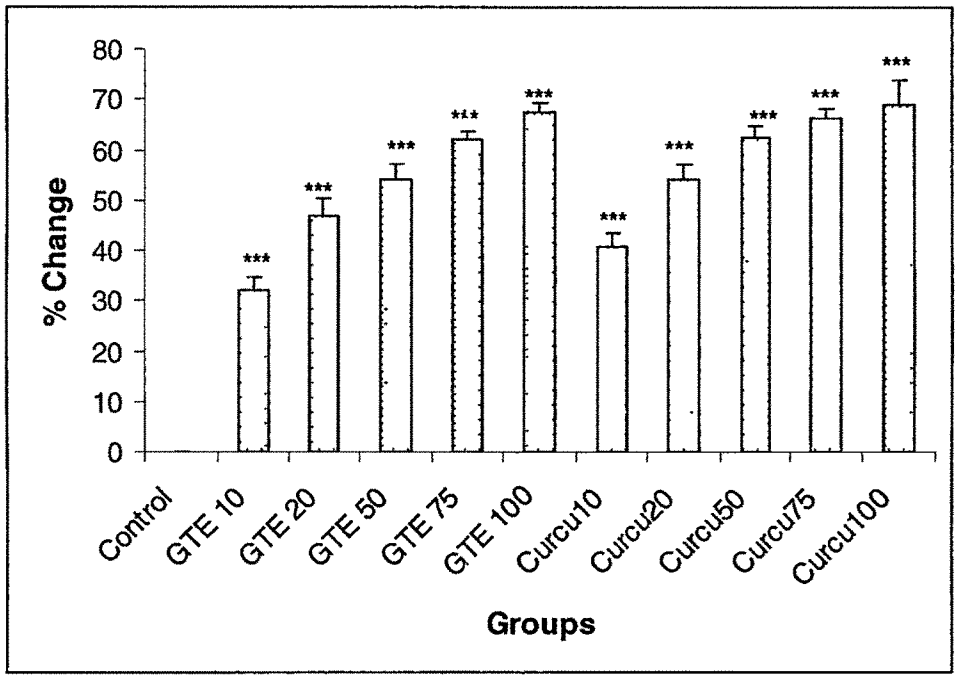
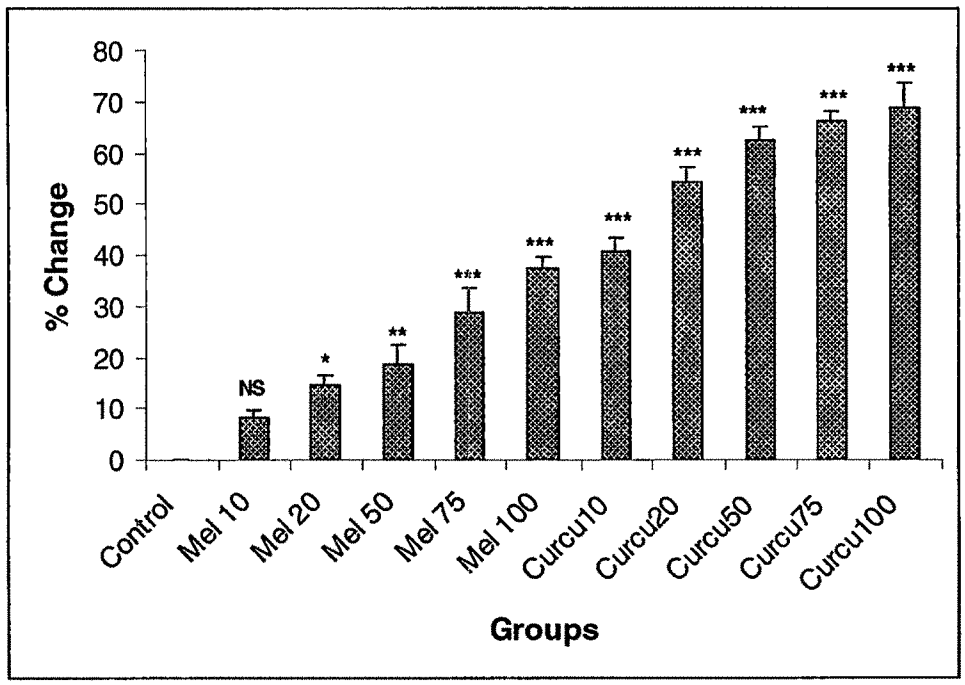


Fig 4.11



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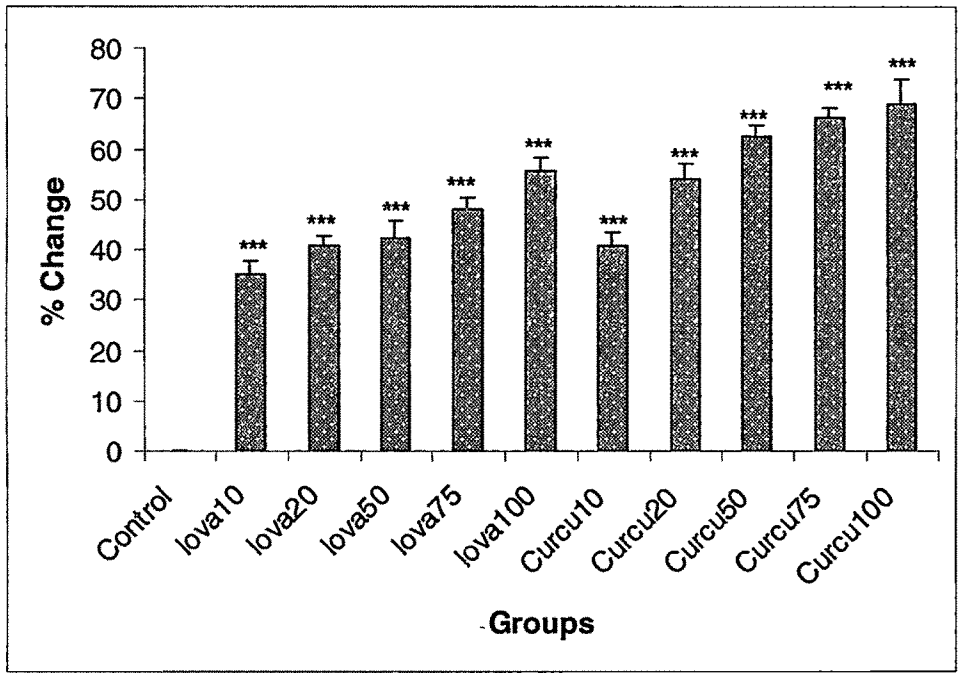

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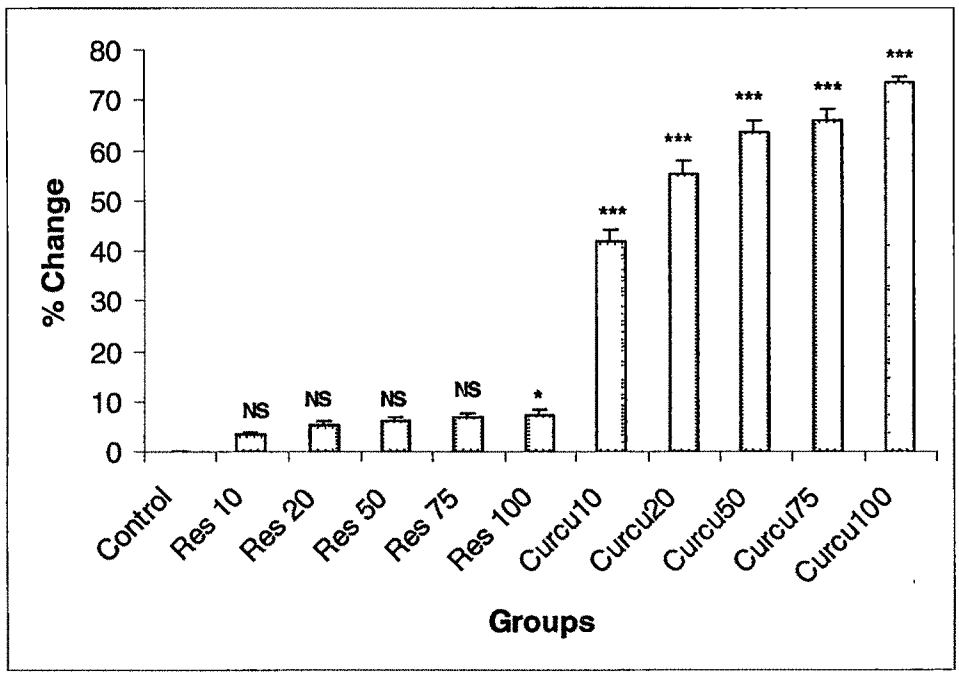


4.2.4 MEASUREMENT OF EFFECT ON LIPID PEROXIDATION IN RAT LIVER HOMOGENATE

There was a significant ($P<0.01$, $P<0.001$) reduction in malondialdehyde formation by green tea extract at all concentrations (10, 20, 50, 75 and 100 μg/ml) (Fig.4.14).

Melatonin produced significant reduction ($P<0.01$, $P<0.001$) in malondialdehyde formation at higher concentrations (20, 50, 75 and 100 μg/ml) (Fig.4.15).

There was a significant ($P<0.01$, $P<0.001$) reduction in malondialdehyde formation with lovastatin at higher concentrations (50, 75 and 100 μg/ml). Curcumin also exhibited highly significant ($P< 0.001$) activity (Fig.4.16).

Resveratrol exhibited significant ($P<0.01$, $P<0.001$) reduction in malondialdehyde formation at higher concentrations (75 and 100 μg/ml). Curcumin also exhibited highly significant ($P<0.001$) activity (Fig.4.17).

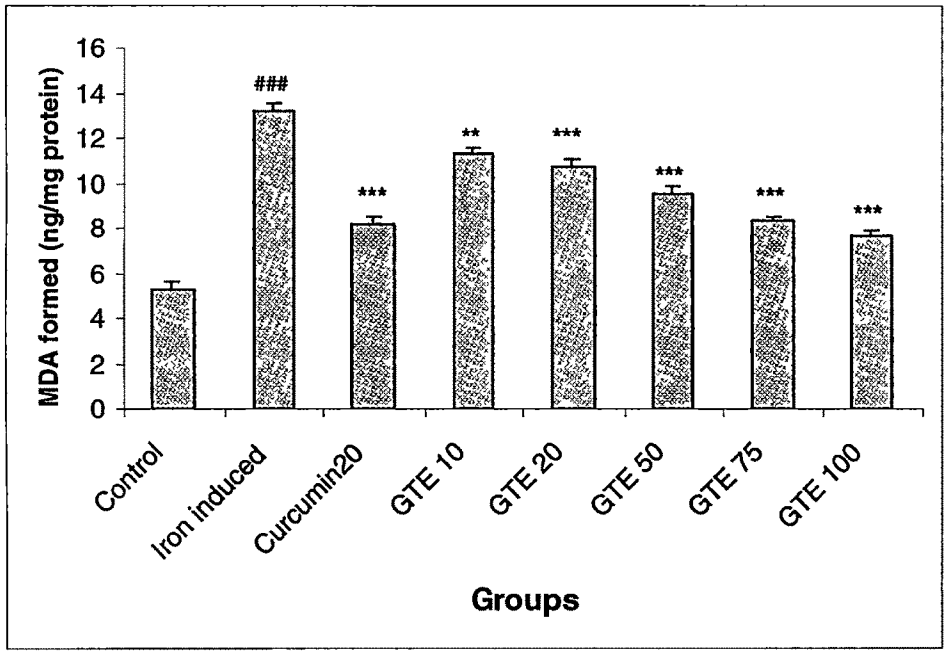
Fig.4.14: Effect of green tea extract at concentrations (10-100 µg/ml) on iron catalyzed lipid peroxidation as compared to inhibitory activity of curcumin at concentration (20µg/ml) on MDA formation (ng/mg protein). All values are mean ± SEM. The group treated with iron was compared with control (### P<0.001) while groups treated with curcumin and green tea extract were compared with the iron treated group. (* P<0.05, ** P<0.01, *** P<0.001, NS: not significant)

Fig.4.15: Effect of melatonin at concentrations (10-100 µg/ml) on iron catalyzed lipid peroxidation as compared to inhibitory activity of curcumin at concentration (20µg/ml) on MDA formation (ng/mg protein). All values are mean ± SEM. The group treated with iron was compared with control (### P<0.001) while groups treated with curcumin and melatonin were compared with the iron treated group.
(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant)

Fig.4.16: Effect of lovastatin at concentrations (10-100 µg/ml) on iron catalyzed lipid peroxidation as compared to inhibitory activity of curcumin at concentration (20µg/ml) on MDA formation (ng/mg protein). All values are mean ± SEM. The group treated with iron was compared with control (### P<0.001) while groups treated with curcumin and lovastatin were compared with the iron treated group. (* P<0.05, ** P<0.01, *** P<0.001, NS: not significant)

Fig.4.17: Effect of resveratrol at concentrations (10-100 µg/ml) on iron catalyzed lipid peroxidation as compared to inhibitory activity of curcumin at concentration (20µg/ml) on MDA formation (ng/mg protein). All values are mean ± SEM. The group treated with iron was compared with control (### P<0.001) while groups treated with curcumin and resveratrol were compared with the iron treated group.
(* P<0.05, ** P<0.01, *** P<0.001, NS: not significant)

Fig.4.14



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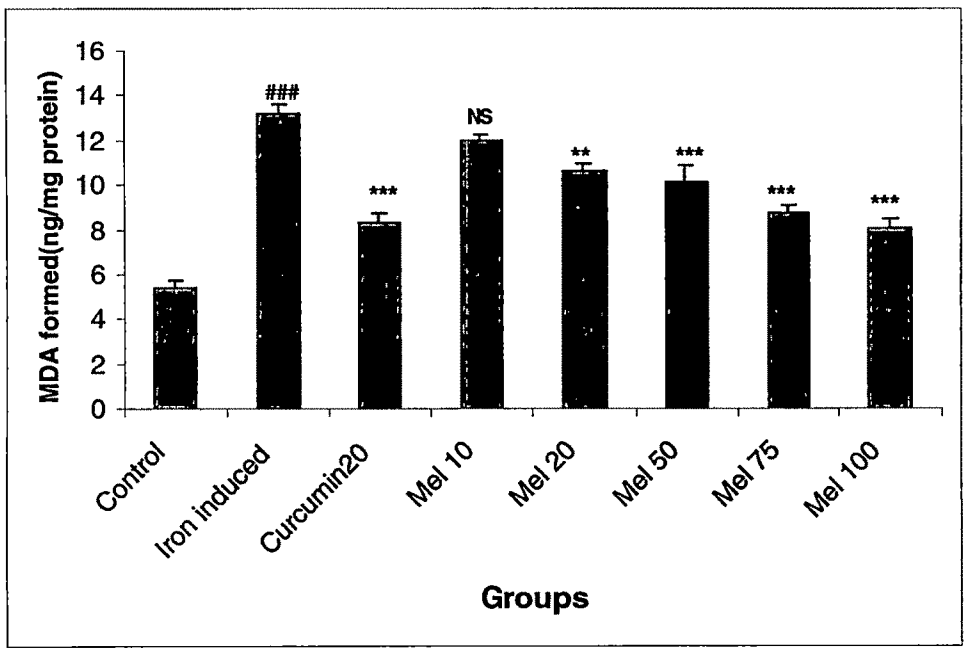

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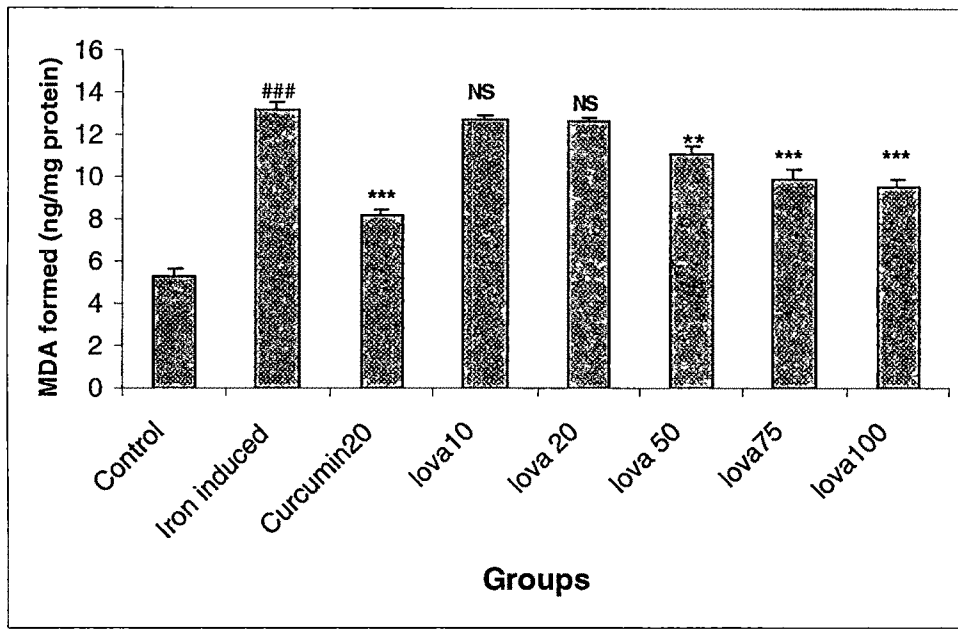

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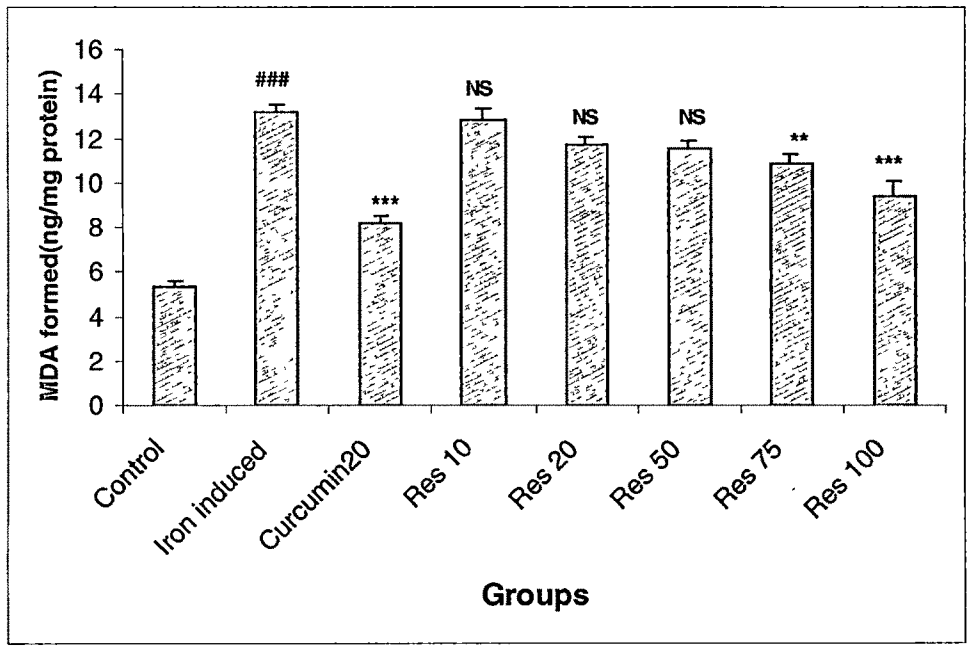


4.3 IN VIVO STUDIES

4.3.1 DOXORUBICIN INDUCED CARDIOTOXICITY

4.3.1.1: ACUTE STUDY IN RATS

4.3.1.1.1 SERUM PARAMETERS

4.3.1.1.1.1 Effect of drugs on Creatine Kinase (CK)

Acute doxorubicin (10 mg/kg i.v.) administration produced a significant ($P<0.001$) increase in the activity of serum CK as compared to control group.

Pretreatment of green tea extract (25 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce any significant reduction in the level of serum CK; but at the higher doses (50 and 100 mg/kg/day p.o. for 30 day), it significantly ($P<0.05$, $P<0.001$) decreased these levels as compared to doxorubicin treated group (Fig.4.18).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce any significant reduction in the level of serum CK; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) decreased these levels as compared to doxorubicin treated group (Fig.4.20).

Pretreatment of lovastatin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce any significant reduction in the level of serum CK; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) decreased these levels as compared to doxorubicin treated group (Fig.4.22).

4.3.1.1.1.2 Effect of drugs on Lactate dehydrogenase (LDH)

Acute doxorubicin (10 mg/kg i.v.) administration produced a significant ($P<0.001$) increase in the activity of LDH in serum of rats as compared to control group.

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day)

administration significantly (P<0.01,P<0.001) decreased the levels of LDH as compared to doxorubicin treated group (Fig.4.18).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.001) reduction in the level of serum LDH as compared to doxorubicin treated group (Fig.4.20).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration significantly (P<0.05, P<0.001) reduced the level of serum LDH as compared to doxorubicin treated group (Fig.4.22).

4.3.1.1.1.3 Effect of drugs on SGOT

Acute doxorubicin (10 mg/kg i.v.) produced a significant (P<0.001) increase in the activity of SGOT in serum of rats as compared to control group.

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration significantly (P<0.001) reduced the level of SGOT as compared to doxorubicin treated group (Fig.4.19).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration significantly (P<0.001) reduced the level of SGOT as compared to doxorubicin treated group (Fig.4.21).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.05, P<0.001) reduction in the level of SGOT as compared to doxorubicin treated group (Fig.4.23).

Fig.4.18: Effect of green tea extract on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.19: Effect of green tea extract on the levels of SGOT in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.20: Effect of melatonin on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.21: Effect of melatonin on the levels of SGOT in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.22: Effect of lovastatin on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.23: Effect of lovastatin on the levels of SGOT in doxorubicin induced cardiotoxicity (acute study) in rats.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control.
Groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
Groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
Groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.18

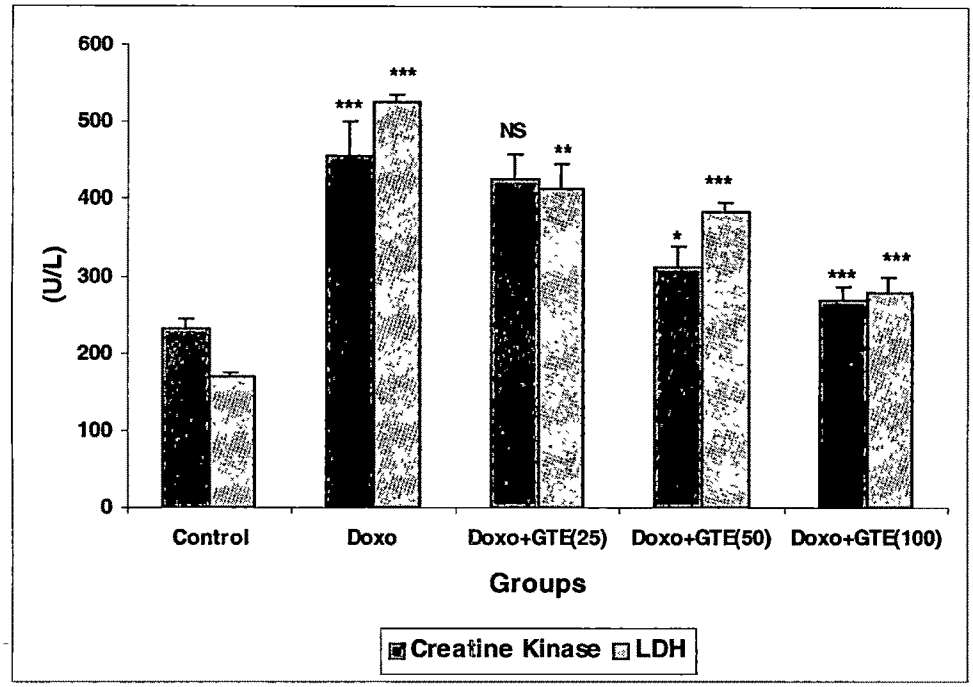
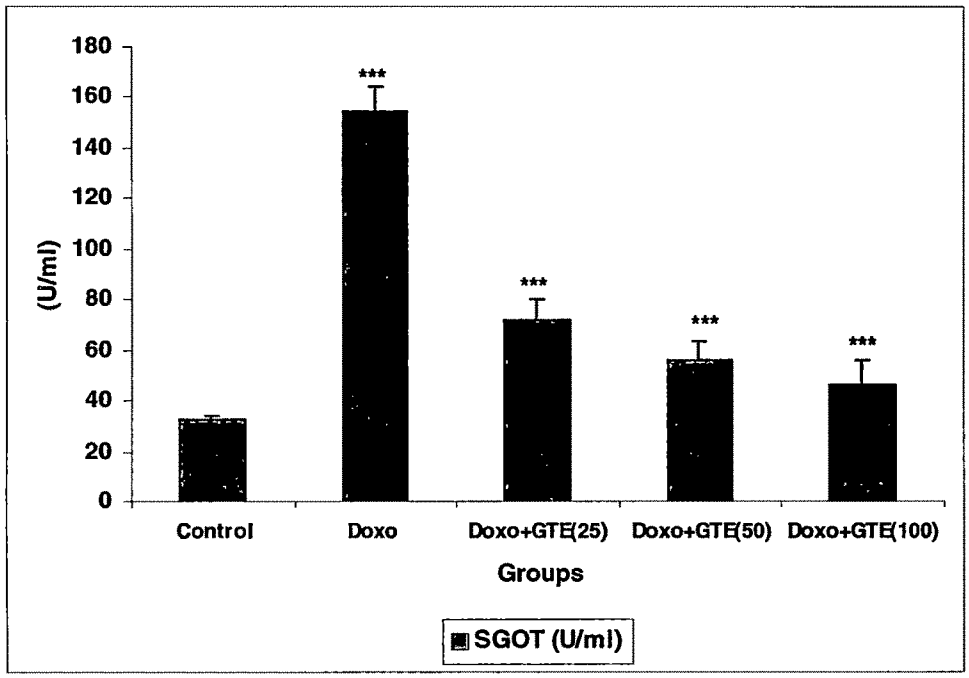


Fig.4.19



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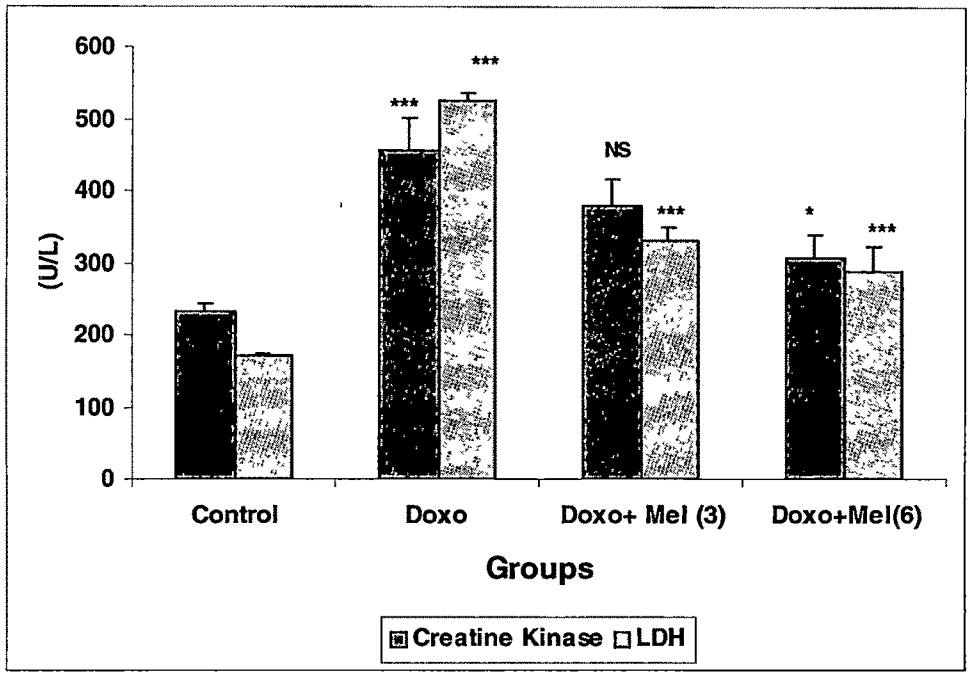

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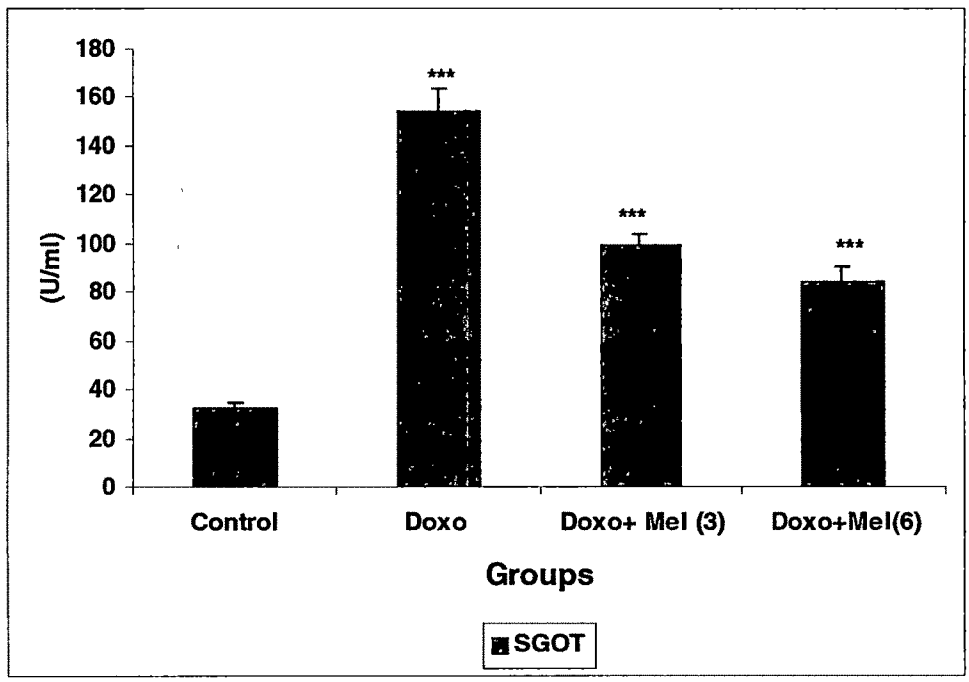

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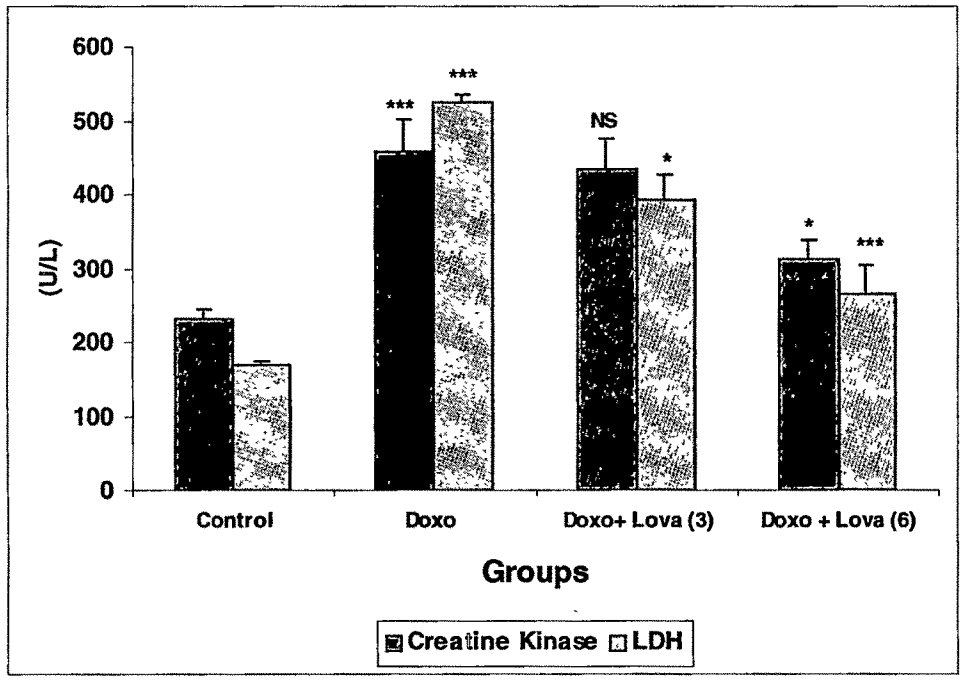
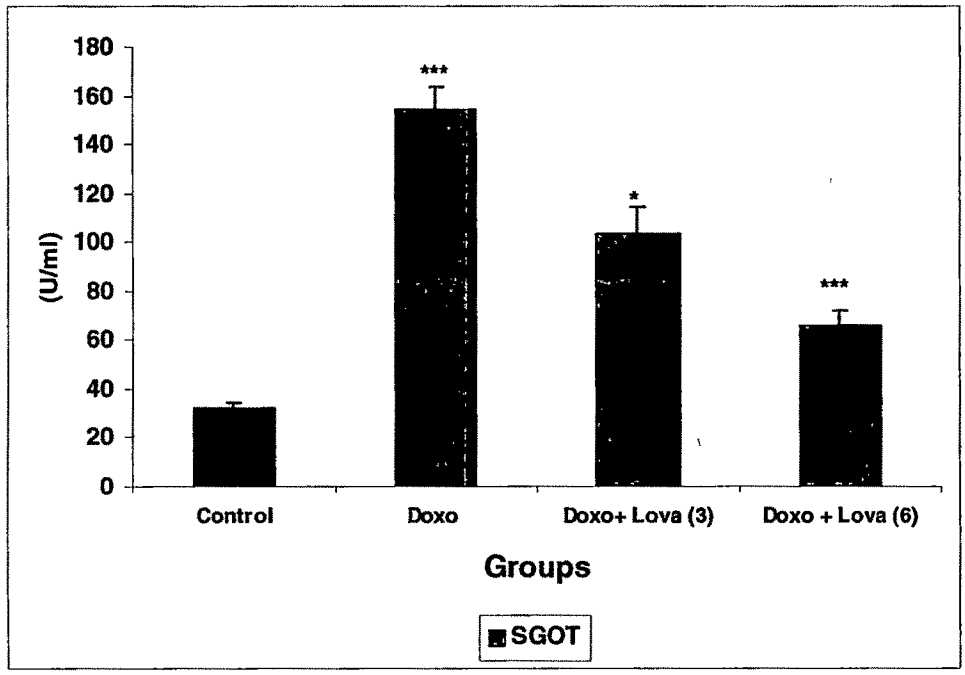


Fig.4.23



4.3.1.1.2 ELECTROCARDIOGRAFIC CHANGES

4.3.1.1.2.1 Effects of drugs on ST interval

Acute doxorubicin (10 mg/kg i.v.) administration induced severe injury to myocardium thereby producing ECG abnormalities as indicated by significant (P<0.001) increase in ST interval as compared to control animals (Fig 4.24 and 4.25).

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.01, P<0.001) decrease in ST interval as compared to doxorubicin treated group (Fig 4.24 and 4.25).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant decrease in ST interval; but higher dose of melatonin (6 mg/kg) significantly (P<0.05) decreased ST interval as compared to doxorubicin treated group (Fig 4.27 and 4.28).

Pretreatment of Lovastatin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant decrease in ST interval; but at higher dose (6 mg/kg), it produced significant (P<0.05) decrease in ST interval as compared to doxorubicin treated group (Fig 4.30 and 4.31).

4.3.1.1.2.2 Effects of drugs on QT interval

Acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.001) increase in QT interval as compared to control animals.

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.01, P<0.001) decrease in QT interval as compared to doxorubicin treated group (Fig 4.24 and 4.25).

Melatonin (3 mg/kg/day p.o. for 30 day) pretreatment followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant decrease in QT interval; but at higher dose (6 mg/kg), it produced a significant (P<0.05) decrease in QT interval as compared to doxorubicin treated group (Fig 4.27 and 4.28).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.05,P<0.01) decrease in QT interval as compared to doxorubicin treated groups (Fig 4.30 and 4.31).

4.3.1.1.2. 3 Effects of drugs on Heart Rate

Acute doxorubicin (10 mg/kg i.v.on 30th day) administration produced a significant (P<0.001) decrease in heart rate as compared to control animals.

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant change in heart rate; but at higher dose (100 mg/kg), it significantly (P<0.05) increased the heart rate as compared to doxorubicin group (Fig 4.26).

Pretreatment of melatonin (3 and 6 mg/kg /day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant change in heart rate as compared to doxorubicin group (Fig 4.29).

Pretreatment of lovastatin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant change in heart rate; but at higher dose (6 mg/kg), it produced a significant (P<0.05) increase in heart rate as compared to doxorubicin treated group (Fig 4.32).

Fig 4.24: Recordings showing changes in Electrocardiographic (ECG) parameters.

4.24 a: ECG recording in control group

4.24 b: ECG recording in doxorubicin alone group

4.24 c: ECG recording in Doxo+ GTE (25) group

4.24 d: ECG recording in Doxo+ GTE (50) group

4.24 e: ECG recording in Doxo+ GTE (100) group

Fig.4.25

Effect of green tea extract on ST interval and QT interval of ECG in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.26

Effect of green tea extract on Heart Rate in doxorubicin induced cardiotoxicity (acute study) in rats.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig 4.24 a

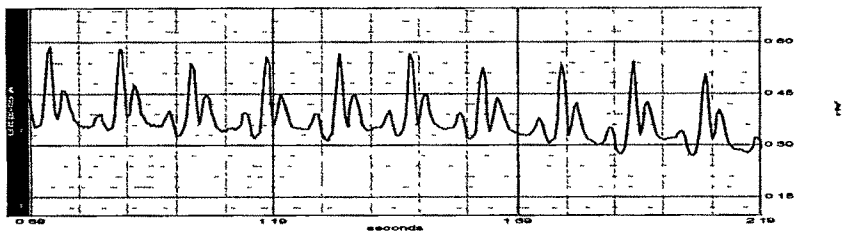


Fig 4.24 b

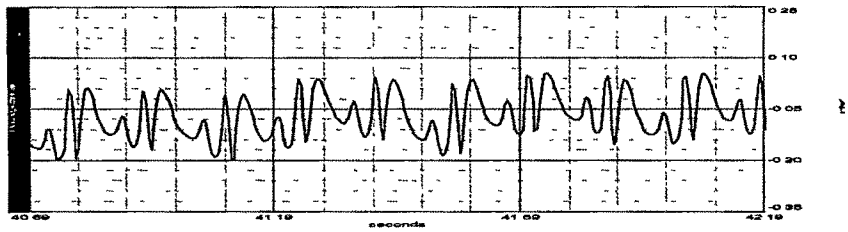
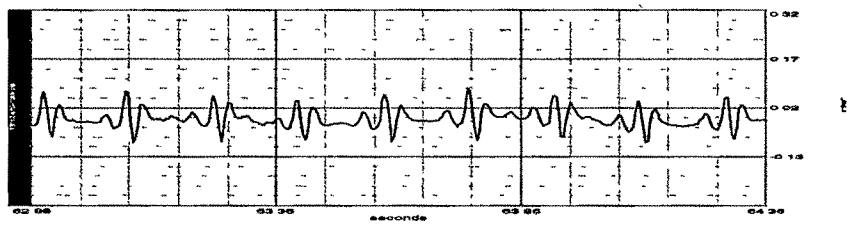


Fig 4.24 c



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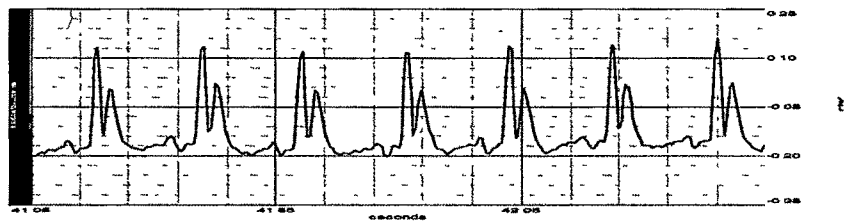


Fig 4.24 e

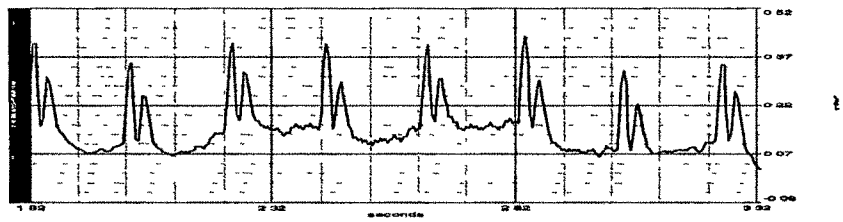


Fig.4.25

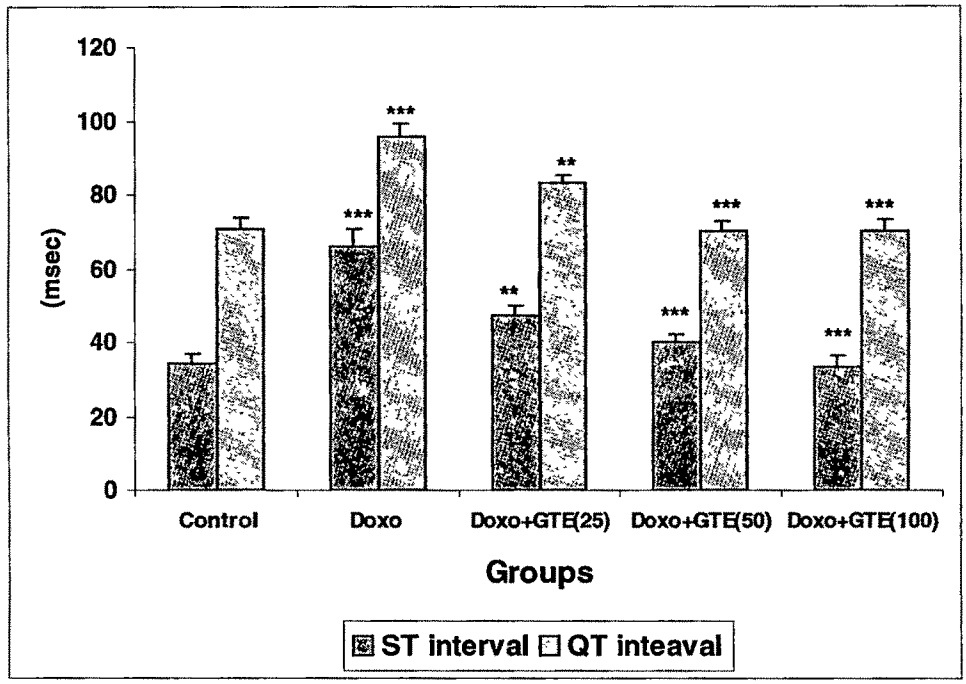


Fig.4.26

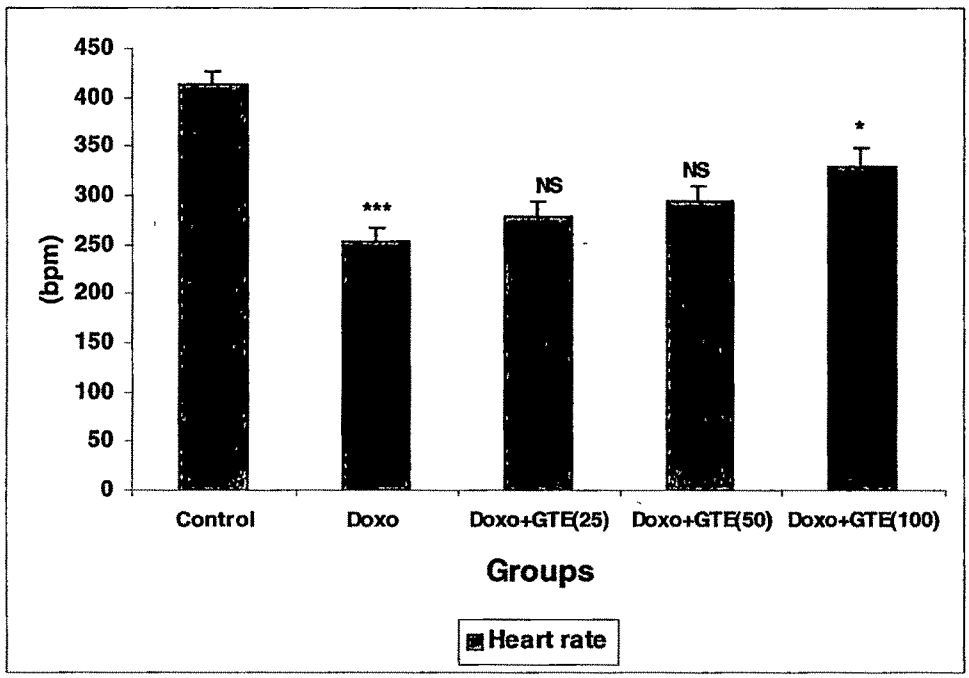


Fig 4.27: Recordings showing changes in Electrocardiographic (ECG) parameters.

4.27a: ECG recording in control group

4.27 b: ECG recording in doxorubicin alone group

4.27 c: ECG recording in Doxo+ Mel (3) group

4.27 d: ECG recording in Doxo+ Mel (6) group

Fig.4.28

Effect of melatonin on ST interval and QT interval of ECG in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.29

Effect of melatonin on Heart Rate in doxorubicin induced cardiotoxicity (acute study) in rats.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Fig 4.27 a

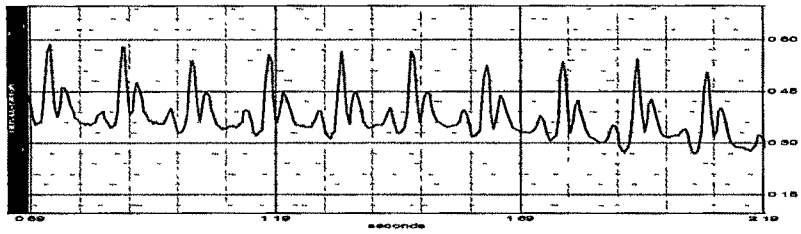


Fig 4.27 b

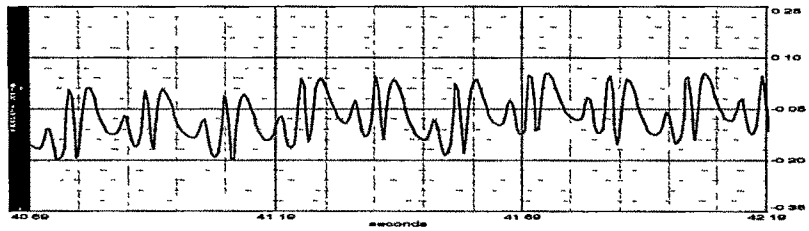


Fig 4.27 c

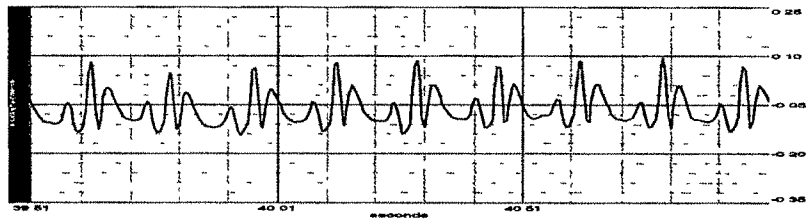


Fig 4.27 d

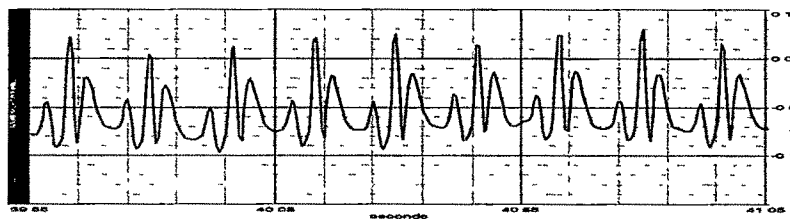
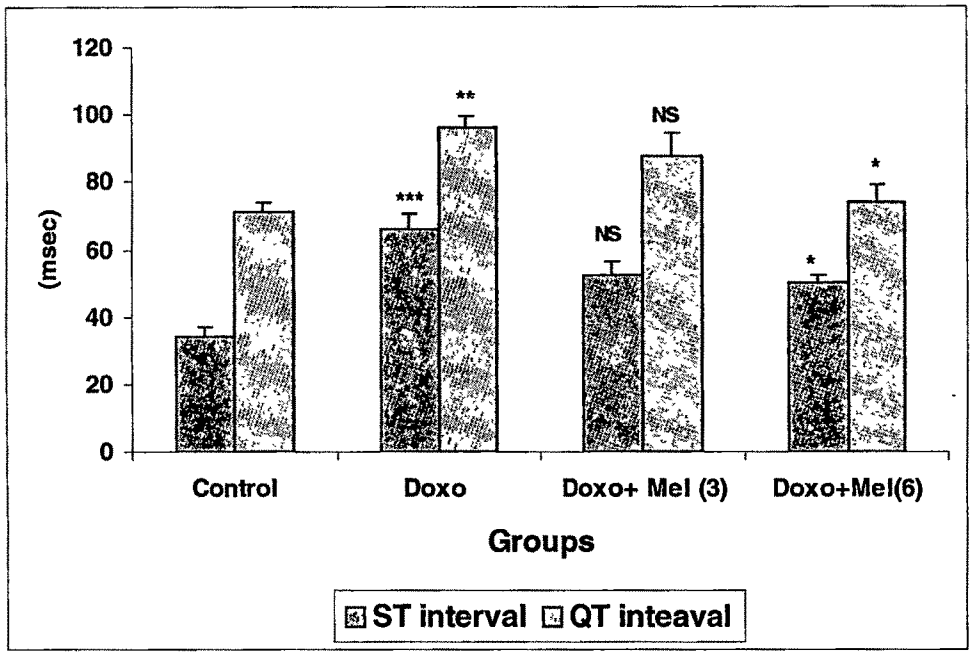


Fig.4.28



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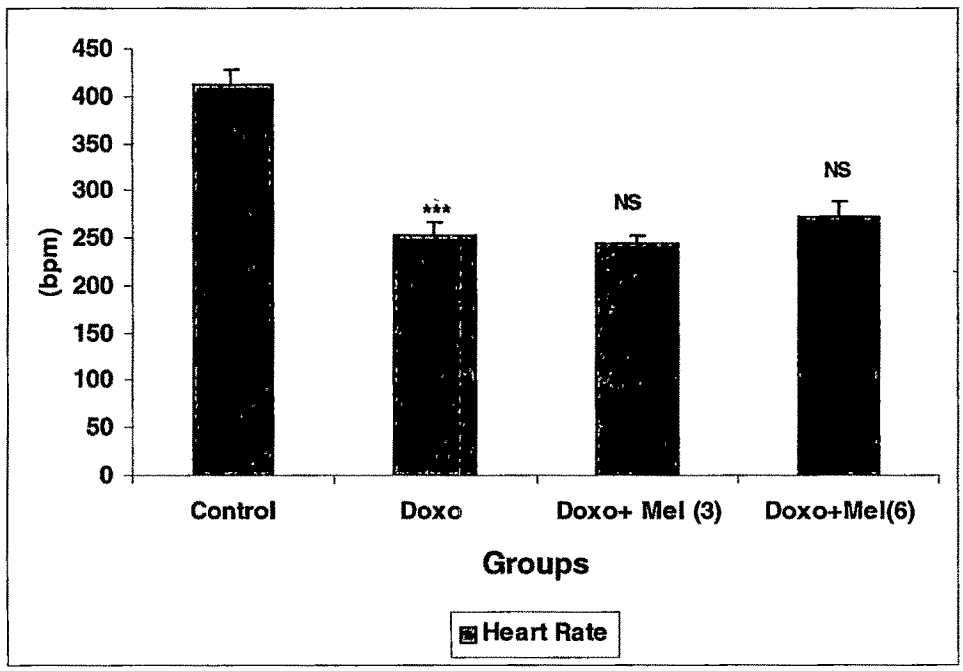


Fig 4.30: Recordings showing changes in Electrocardiographic (ECG) parameters.

4.30 a: ECG recording in control group

4.30 b: ECG recording in doxorubicin alone group

4.30 c: ECG recording in Doxo+ Lova (3) group

4.30 d: ECG recording in Doxo+ Lova (6) group

Fig.4.31

Effect of lovastatin on ST interval and QT interval of ECG in doxorubicin induced cardiotoxicity (acute study) in rats.

Fig.4.32

Effect of lovastatin on Heart Rate in doxorubicin induced cardiotoxicity (acute study) in rats.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Fig. 4.30 a

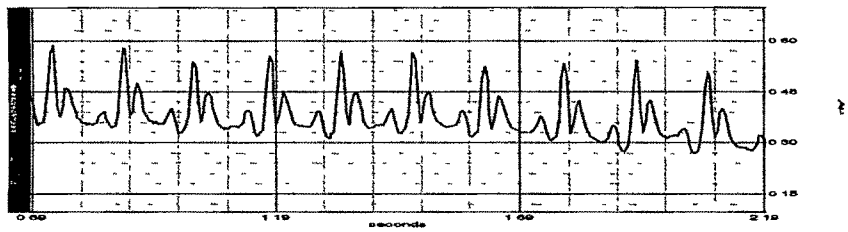


Fig. 4.30 b

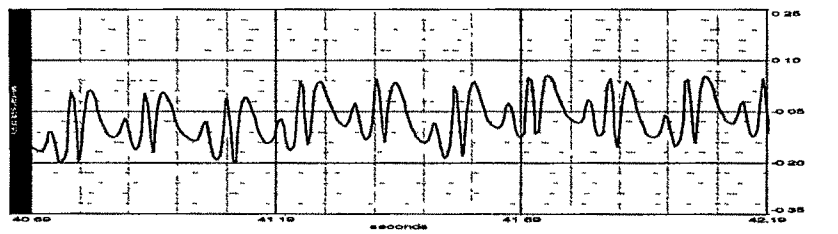


Fig. 4.30 c

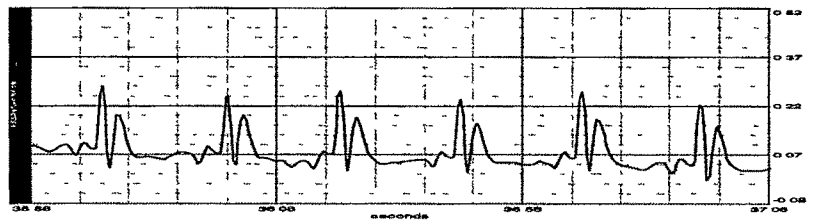


Fig. 4.30 d

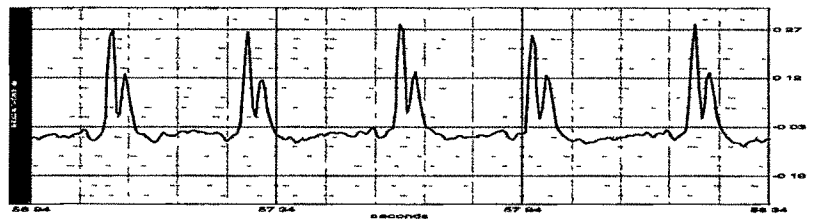


Fig.4.31

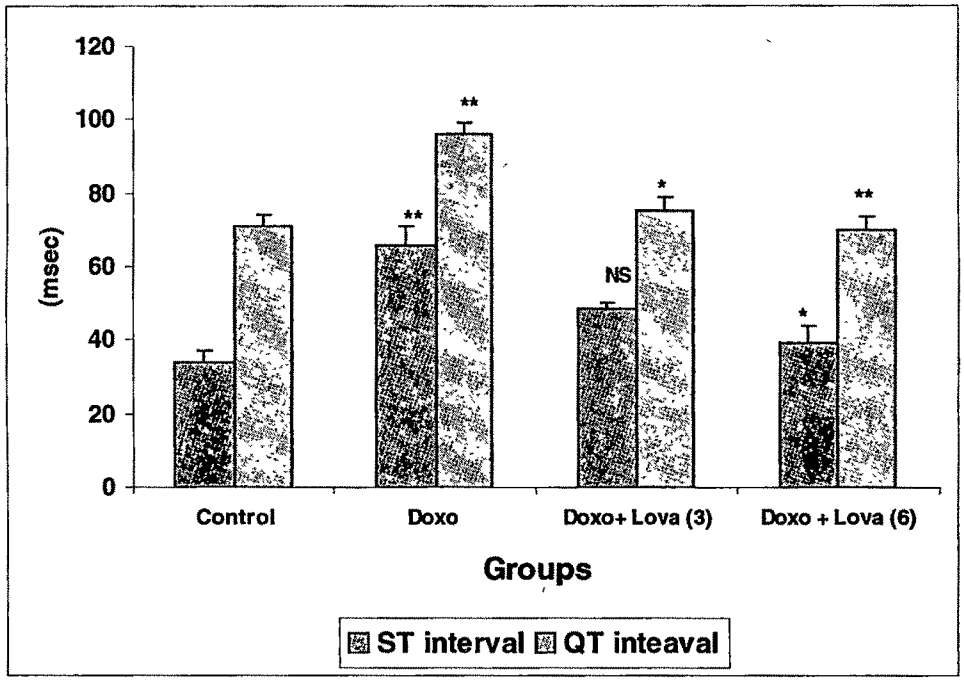
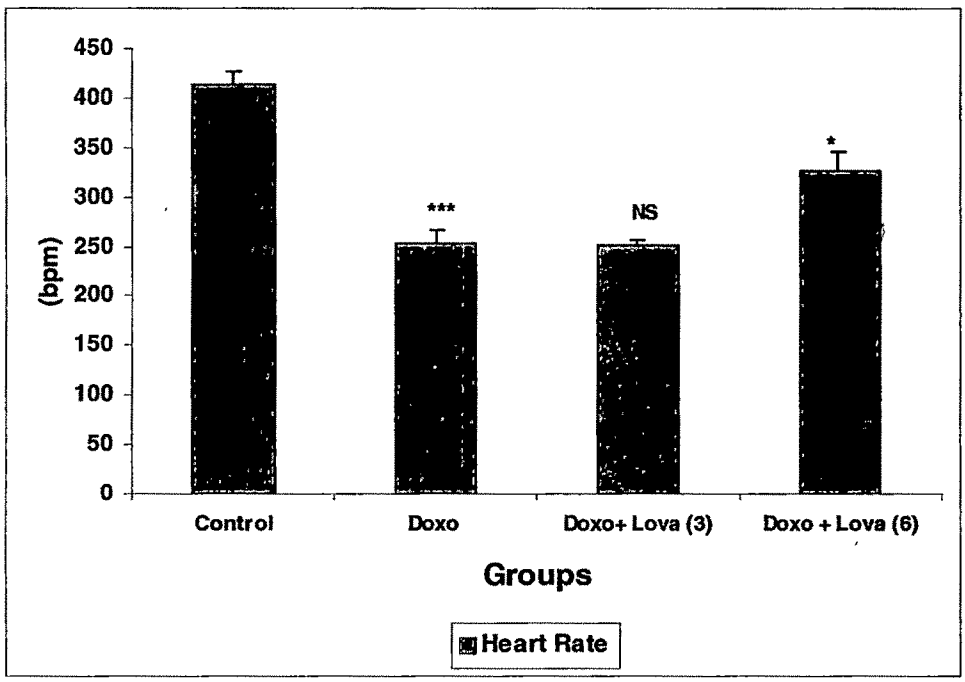


Fig.4.32



4.3.1.1.3 TISSUE PARAMETERS

4.3.1.1.3.1 Effect on lipid peroxidation

Acute doxorubicin (10 mg/kg i.v.) administration (Group 2) to rats led to a significant (P<0.001) increase in lipid peroxidation or MDA content in the heart as compared to the control group (Group 1).

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration significantly (P<0.001) reduced the levels of MDA as compared to doxorubicin treated group (Table 4.2).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration produced a significant (P<0.05, P<0.001) decrease in the MDA content as compared to doxorubicin treated group (Table 4.3).

Pretreatment of lovastatin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant change in the level of lipid peroxidation (MDA content); but the higher dose (6mg/kg) of lovastatin significantly (P<0.01) decreased lipid peroxidation when compared with doxorubicin treated group (Table 4.4).

4.3.1.1.3.2 Effect on endogenous antioxidants

4.3.1.1.3.2.1 Effect on Superoxide dismutase

Acute doxorubicin (10 mg/kg i.v.) administration (Group 2) reduced the SOD activity significantly (P<0.001) in the heart as compared to control (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant changes in the level of SOD; but the higher dose (100 mg/kg) of green tea extract significantly (P<0.001) rise SOD content as compared to doxorubicin treated group (Tables 4.2).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant increase in the SOD content as compared to doxorubicin treated group (Table 4.3).

Pretreatment of lovastatin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant change in the level of SOD; but higher dose (6mg/kg) of lovastatin significantly (P<0.05) increased the level of SOD as compared to doxorubicin treated group (Table 4.4).

4.3.1.1.3.2.2 Effect on Catalase

The catalase activity in doxorubicin (10 mg/kg i.v. on 30th day) treated animals (Group 2) was significantly (P<0.001) reduced as compared to control animals (Group 1).

Pretreatment of green tea extract (25 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant change in the level of catalase enzyme; but at higher doses (50 and 100 mg/kg), it significantly (P<0.01,P<0.001) increased these levels as compared to doxorubicin treated group (Table 4.2).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant change in the level of catalase enzyme; but higher dose (6 mg/kg) of melatonin significantly (P<0.05) increased these levels as compared to doxorubicin treated group (Table 4.3).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration produced a significant (P<0.05 P<0.001) increase in the level of catalase enzyme as compared to doxorubicin treated group (Table 4.4).

4.3.1.1.3.2.3 Effect on Reduced glutathione

A significant (P<0.001) reduction in reduced glutathione concentration was observed in doxorubicin (10 mg/kg i.v. on 30th day) treated rats (Group 2) as compared to control animal (Group 1).

Green tea extract (25 mg/kg/day p.o. for 30 day) pretreatment followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant increase in GSH content; but the higher doses, (50 and 100 mg/kg) of green tea extract significantly (P<0.05,P<0.01) increased the GSH content as compared to doxorubicin treated group (Table 4.2).

Melatonin (3 mg/kg/day p.o.for 30 day) pretreatment followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not produce significant change in the level of GSH; but at the higher dose (6 mg/kg), it significantly ($P<0.01$) increased these levels as compared to doxorubicin treated group (Table 4.3).

Lovastatin (3 mg/kg/day p.o.for 30 day) pretreatment followed by acute doxorubicin (10 mg/kg i.v. on 30th day) administration did not alter the GSH content in heart as compared to the doxorubicin treated group; but at the higher dose (6 mg/kg), the drug led to a significant ($P<0.01$) rise in GSH levels as compared to doxorubicin treated group (Table 4.4).

Table 4.2: Effect of green tea extract on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in doxorubicin induced cardiotoxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	3.06 ±0.16	9.45 ± 1.21	2.33 ± 0.36	4.02 ± 0.3
Group 2	4.61±0.09***	4.17±0.28***	0.53±0.09***	1.82±0.09***
Group 3	3.57 ±0.17***	5.94 ± 0.5NS	1.23±0.1 NS	1.96±0.15 NS
Group 4	3.49±0.05***	6.73± 0.15*	1.38±0.11NS	2.95± 0.11**
Group 5	3.07±0.09***	7.34± 0.17**	2.07±0.33***	4.0 ± 0.17***
F value	**25.82**	**9.95**	**9.26**	**30.54**
P value	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control.

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.3: Effect of melatonin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in doxorubicin induced cardiotoxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA /mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/mg Protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	3.06±0.16	9.45±1.21	2.33±0.36	4.02±0.32
Group 2	4.61±0.09***	4.17±0.28***	0.53±0.09***	1.82±0.09***
Group 3	3.84±0.15*	6.46±0.56^{NS}	1.18±0.088^{NS}	2.815±0.35^{NS}
Group 4	3.41±0.19***	8.66±0.47**	1.31±0.13^{NS}	3.63±0.39*
F value	**18.32**	**10.71**	**13.09**	**9.43**
P value	**P<0.0001**	**P=0.0174**	**P=0.0030**	**P=0.045**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

*P<0.05; ** P<0.01; ***P<0.001; NS = Non Significant

Table 4.4: Effect of lovastatin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in doxorubicin induced cardiotoxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA /mg protein	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/mg Protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein
Group 1	3.06±0.16	9.45±1.21	2.33±0.36	4.02±0.32
Group 2	4.61±0.09**	4.17±0.28***	0.53±0.09***	1.82±0.09***
Group 3	3.41±0.35NS	5.69±0.58NS	0.88±0.10NS	3.58±0.38*
Group 4	3.03±0.17**	8.59±0.71**	1.40±0.19*	4.76±0.31***
F value	**6.63**	**10.42**	**13.77**	**16.21**
P value	**P=0.0009**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.1.1.3.3 Effect on membrane bound enzymes

4.3.1.1.3.3.1 Effect on Sodium Potassium ATPase

In the heart of doxorubicin (10 mg/kg, i.v.on 30th day) treated rats (Group 2) the activity of Na^+K^+ATPase enzyme was significantly ($P<0.01$) reduced as compared to the control animals (Group1).

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not alter the Na^+K^+ATPase levels as compared to doxorubicin treated group (Table 4.5).

Similarly, Pretreatment of melatonin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not produce significant change in Na^+K^+ATPase levels as compared to doxorubicin treated group (Table 4.6).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not alter the Na^+K^+ATPase levels as compared to doxorubicin treated group (Table 4.7).

4.3.1.1.3.3.2 Effect on Calcium ATPase

Administration of doxorubicin (10 mg/kg, i.v.on 30th day) resulted in a significant ($P<0.001$) decrease in the Ca^{2+}ATPase activity as compared to the control (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not alter the Ca^{2+}ATPase levels as compared to doxorubicin treated group; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.001$) increased these levels as compared to doxorubicin treated group (Table 4.5).

Pretreatment of melatonin (3 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not produce significant increase in Ca^{2+}ATPase levels; but at higher dose (6mg/kg), it significantly ($P<0.01$) increased these levels as compared to doxorubicin treated group (Table 4.6).

Pretreatment of lovastatin (3 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not produce significant change in the level of Ca^{2+}ATPase; but the higher dose (6mg/kg) of lovastatin significantly ($P<0.01$) increased the level as compared to doxorubicin treated group (Table 4.7).

4.3.1.1.3.3.3 Effect on Magnesium ATPase

In the heart of doxorubicin (10 mg/kg, i.v.on 30th day) treated rats (Group 2) the activity of Mg^{2+}ATPase enzyme was significantly ($P<0.05$) reduced as compared to the control animals (Group1).

Pretreatment of green tea extract (25 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not alter the Ca^{2+}ATPase levels as compared to doxorubicin treated group; but at the higher doses (50 mg/kg and 100 mg/kg), it significantly ($P<0.05$,$P<0.001$) increased these levels as compared to doxorubicin treated group (Table 4.5).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not produce significant increase in Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.6).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute doxorubicin (10 mg/kg, i.v.on 30th day) administration did not alter the Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.7).

Table 4.6: Effect of melatonin on membrane bound enzymes in the heart of rats in doxorubicin induced cardiotoxicity (acute study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	7.0±0.2	3.86±0.17	3.01±0.17
Group 2	4.04±0.45***	2.09±0.17***	2.04±0.22*
Group 3	4.15±0.46NS	2.11±0.17NS	2.22±0.24NS
Group 4	4.78±0.33NS	2.75±0.21**	2.74±0.28NS
F value	**12.99**	**9.43**	**3.663**
P value	**P<0.0001**	**P=0.0004**	**P=0.0298**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day)

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.7: Effect of lovastatin on membrane bound enzymes in the heart of rats in doxorubicin induced cardiotoxicity (acute study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	7.0±0.2	3.86±0.17	3.01±0.17
Group 2	4.04±0.45**	2.09±0.17***	2.04±0.22^{NS}
Group 3	5.22±0.53^{NS}	3.08±0.36^{NS}	2.43±0.20^{NS}
Group 4	5.80±0.51^{NS}	3.39±0.26**	2.97±0.22^{NS}
F value	**7.13**	**9.83**	**3.5**
P value	**P=0.0006**	**P<0.0001**	**0.021**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day)

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg, i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.1.1.4 HISTOPATHOLOGY

Fig 4.33(A) depicted the normal architecture of heart in rats of control group (Group 1) on histological examination. The figure reflects the organized arrangement, well-defined boundaries and distinct bundles of myocardial fibres.

A massive necrosis of heart muscle fibres along with focal loss and marked fragmentation was observed in acute doxorubicin (10 mg/kg i.v.) treated group (Group 2). Disorganized arrangements with no well-defined boundaries or distinct bundles of myocardial fibres were observed. Nuclei were scattered, some were lost and some were pyknotic in nature [Fig. 4.33 (B)].

Pretreatment of green tea extract exhibited decreased degree of necrosis with less fragmentation of fibres and well-defined boundaries or distinct bundles of myocardial fibres with increasing doses [Fig. 4.33(C)-4.33(E)].

The degree of myocardial damage in melatonin treated group (3 mg/kg) was similar to doxorubicin treated group in regard to morphological changes showing occasional loss and fragmentation of muscle fibres with disorganized arrangement [Fig 4.34(B)]. With increasing dose of melatonin (6 mg/kg) there was lesser loss of myofibre. Bundles of myocardial fibres with more or less distinct boundaries were present [Fig 4.34(C) – Fig. 4.34 (D)].

Necrosis of heart muscle fibres along with focal loss and marked fragmentation similar to doxorubicin administered group was observed in lovastatin treated groups (3 mg/kg). Disorganized arrangement with no well-defined boundaries or distinct bundles of myocardial fibres was observed. Nuclei were scattered and were pyknotic in nature [Fig. 4.35(C)]. The degree of necrosis was reduced at the dose of 6 mg/kg [Fig. 4.35(D)]. Nuclei were not lost and were not pyknotic in nature.

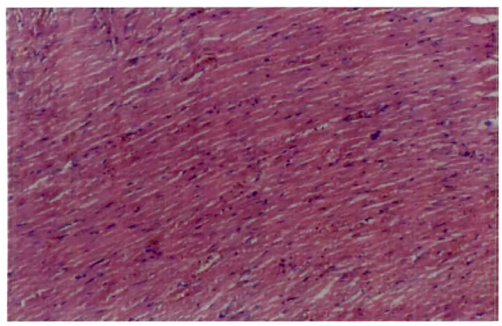
Fig. 4.33: Photomicrographs showing effect of green tea extract on the heart of doxorubicin treated rats (acute study).

(Magnification 10 X)

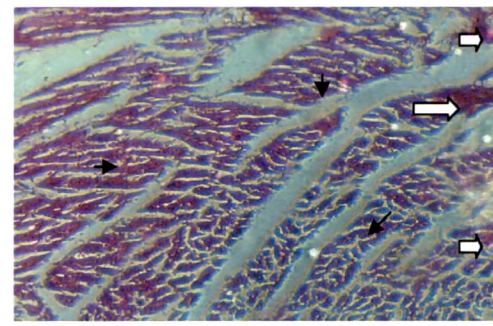
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

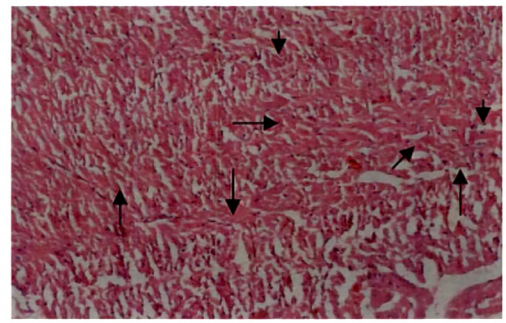
A: Normal control



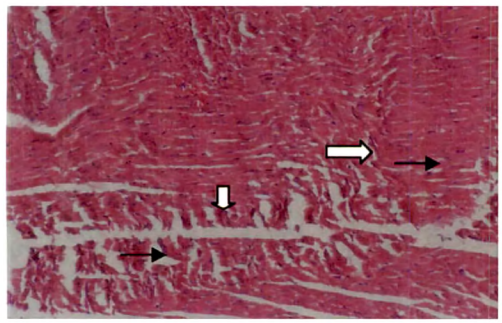
B: Doxorubicin (10 mg/kg i.v.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

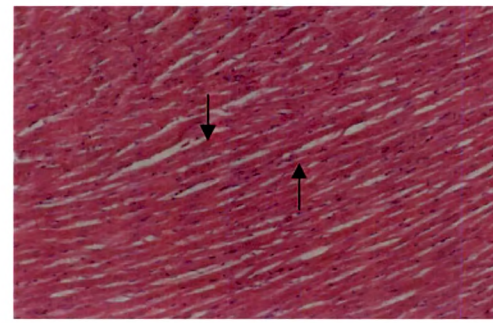


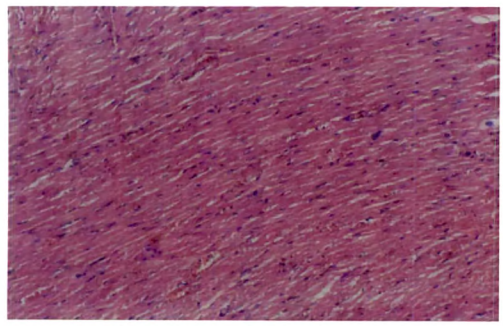
Fig. 4.34: Photomicrographs showing effect of melatonin on the heart of doxorubicin treated rats (acute study).

(Magnification 10 X)

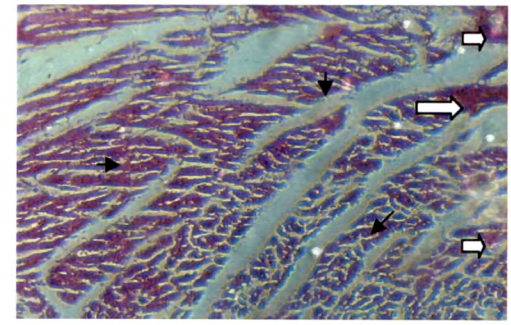
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

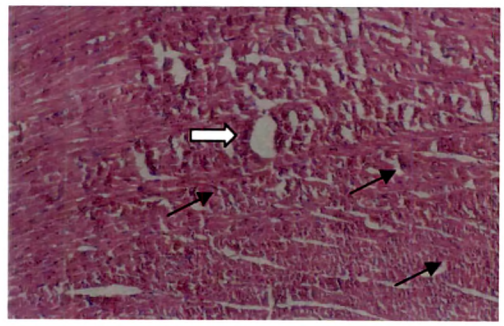
A: Normal control



B: Doxorubicin (10 mg/kg i.v.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

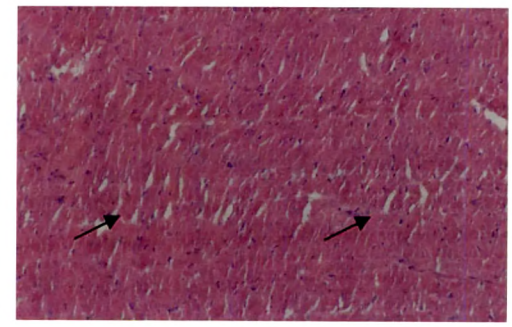


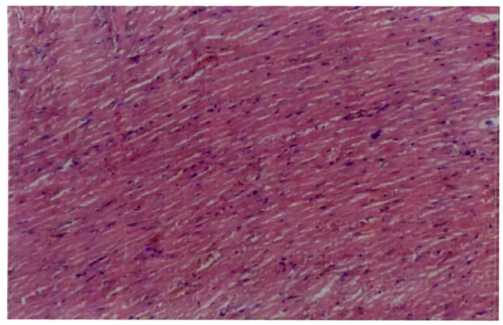
Fig. 4.35: Photomicrographs showing effect of lovastatin on the heart of doxorubicin treated rats (acute study).

(Magnification 10 X)

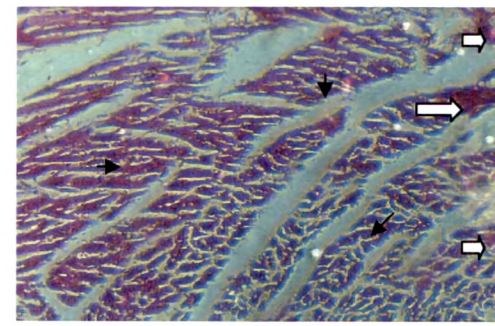
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

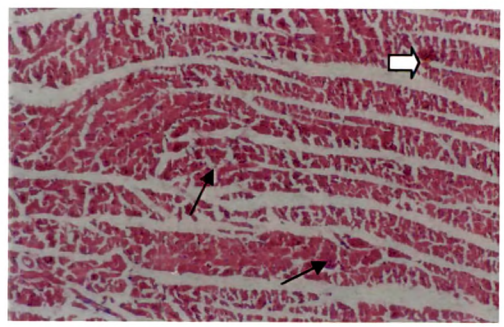
A: Normal control



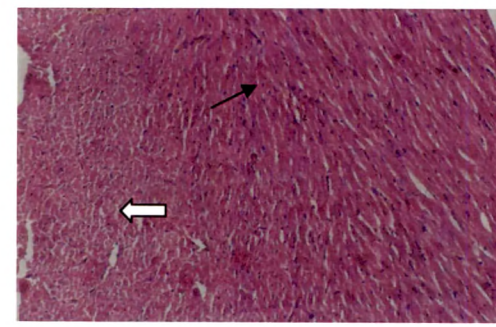
B: Doxorubicin (10 mg/kgi. V.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.1 DOXORUBICIN INDUCED CARDIOTOXICITY

4.3.1.2: CHRONIC STUDY IN RATS

4.3.1.2.1 SERUM PARAMETERS

4.3.1.2.1.1 Effect of drugs on Creatine Kinase (CK)

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) to rats resulted in a significant (P<0.001) increase in the serum concentration of CK as compared to control group.

Treatment of green tea extract (25,50 and 100 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.05, P<0.001) reduction in the level of serum CK when compared with doxorubicin treated group (Fig.4.36).

Melatonin (3 and 6 mg/kg/day p.o. for 30 day) treatment along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.001) decrease in the levels of serum CK as compared to doxorubicin treated group (Fig.4.38).

Treatment of lovastatin (3 mg/kg and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.001) reduction in the level of serum CK as compared to doxorubicin treated group (Fig.4.40).

4.3.1.2.1.2 Effect of drugs on Lactate dehydrogenase (LDH)

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) to rats produced a significant (P<0.001) increase in the activity of serum LDH as compared to control group.

Treatment of green tea extract (25,50 and 100 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.05, P<0.001) reduction in the level of serum LDH when compared with doxorubicin treated group (Fig.4.36).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28)

administration produced a significant (P<0.01,P<0.001) reduction in the level of serum LDH when compared with doxorubicin treated group (Fig.4.38).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration significantly (P< 0.01,P<0.001) reduced the level of serum LDH as compared to doxorubicin treated group (Fig.4.40).

4.3.1.2.1.3 Effect of drugs on SGOT

Chronic treatment with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) to rats resulted in a significant (P<0.001) increase in the activity of SGOT as compared to control group (Group 1).

Treatment of green tea extract (25,50 and 100 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.05, P<0.001) reduction in the level of SGOT as compared to doxorubicin treated group (Fig.4.37).

Treatment of melatonin (3 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant reduction in the level of SGOT; but the higher dose (6 mg/kg) of melatonin significantly (P<0.01) decreased these levels as compared to doxorubicin treated group (Fig.4.39).

Treatment of lovastatin (3 mg/kg and 6 mg/kg day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.01, P<0.001) reduction in the level of SGOT as compared to doxorubicin treated group (Fig.4.41).

Fig.4.36: Effect of green tea extract on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.37: Effect of green tea extract on the levels of SGOT in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.38: Effect of melatonin on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.39: Effect of melatonin on the levels of SGOT in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.40: Effect of lovastatin on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.41: Effect of lovastatin on the levels of SGOT in doxorubicin induced cardiotoxicity (chronic study) in rats.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control.
Groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
Groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
Groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.36

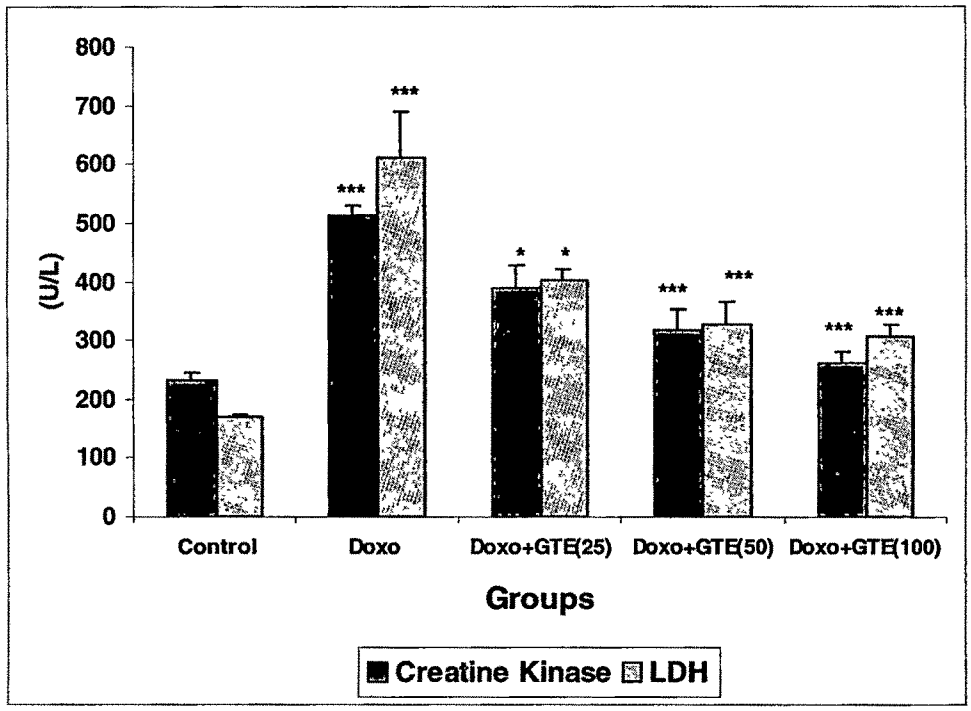


Fig.4.37

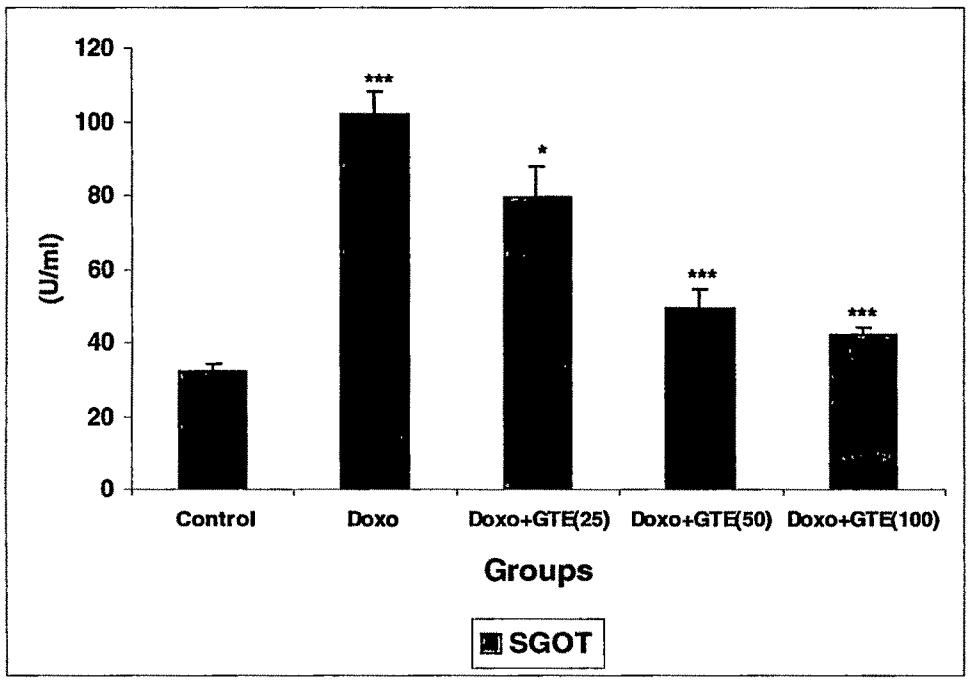


Fig.4.38

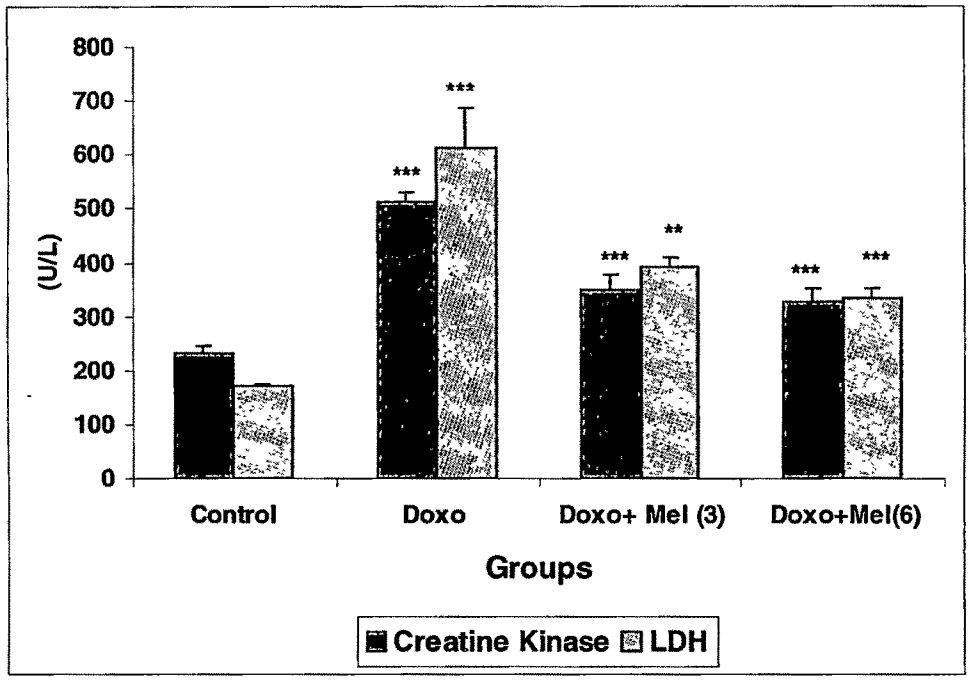


Fig.4.39

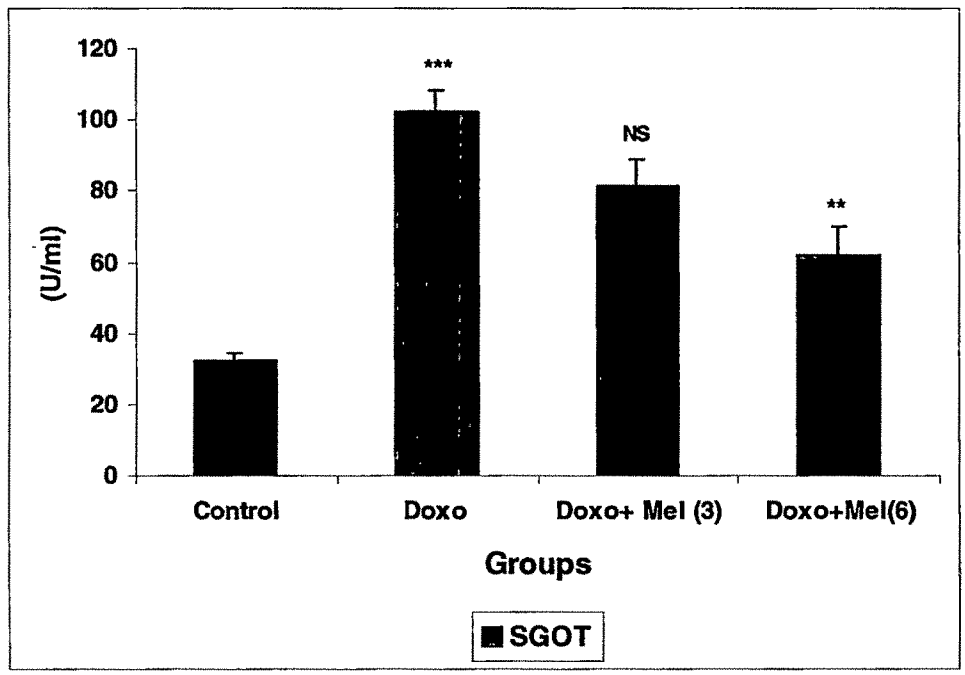


Fig.4.40

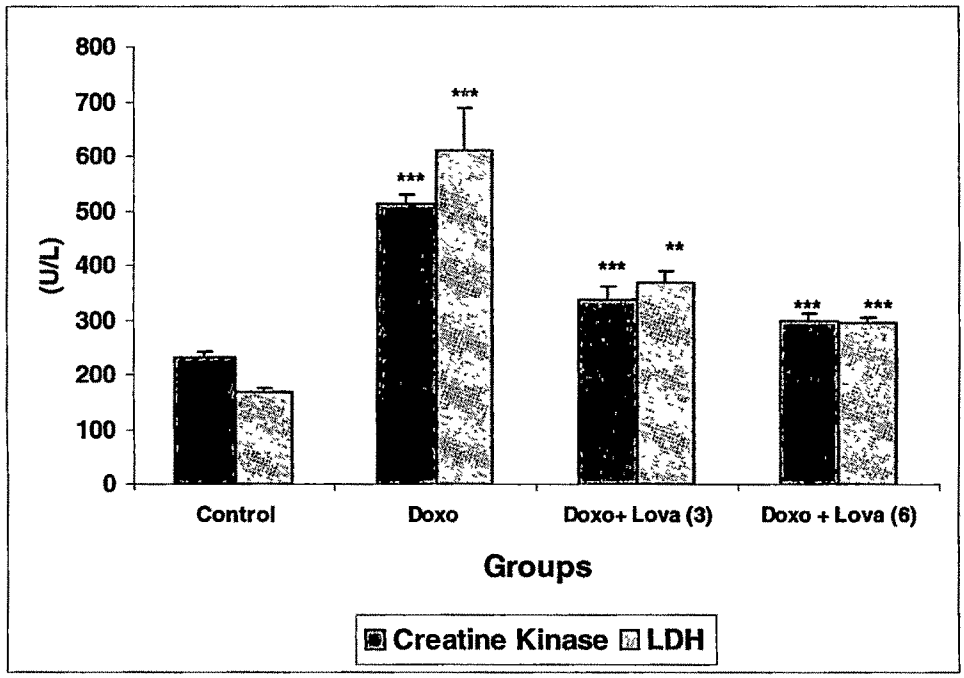
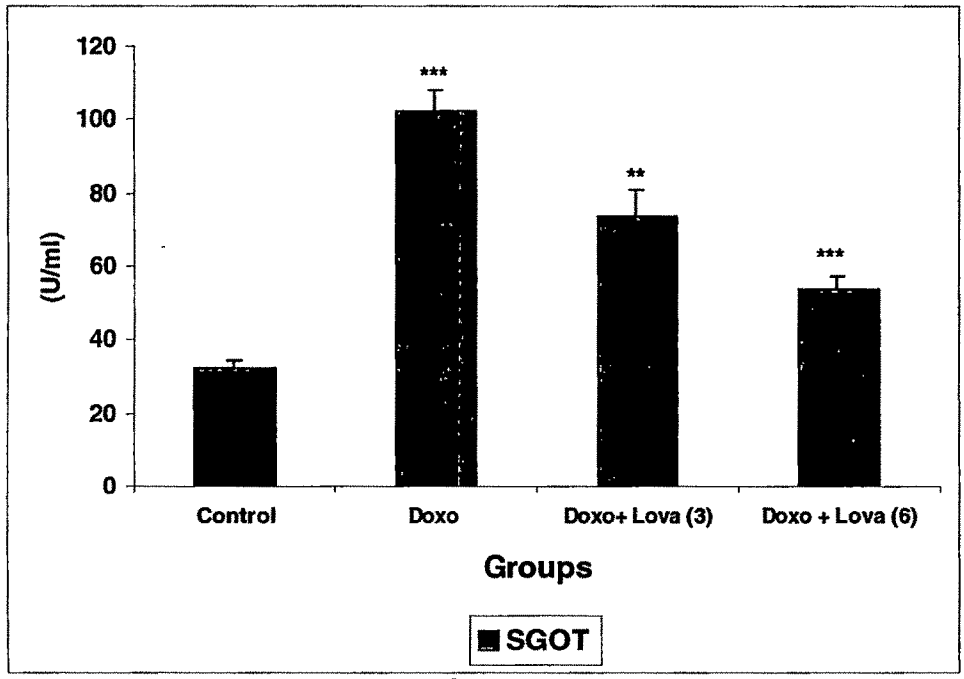
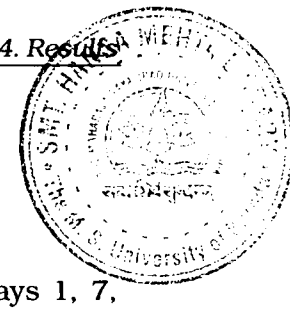


Fig.4.41





4.3.1.2.2 ELECTROCARDIOGRAFIC CHANGES

4.3.1.2.2.1 Effects of drugs on ST interval

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) induced severe injury to myocardium thereby producing ECG abnormalities as indicated by significant ($P<0.001$) increase in ST interval as compared to control animals.

Treatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.05$, $P<0.001$) decrease in ST interval as compared to doxorubicin treated group (Fig 4.42 and 4.43).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.001$) decrease in ST interval as compared to doxorubicin treated group (Fig 4.45 and 4.46).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.001$) reduction in ST interval when compared with doxorubicin treated group (Fig.4.48 and 4.49).

4.3.1.2.2.2 Effects of drugs on QT interval

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) produced a significant ($P<0.001$) increase in QT interval as compared to control animals.

Treatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.05$, $P<0.001$) decrease in QT interval as compared to doxorubicin treated group (Fig 4.42 and 4.43).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.01$,$P<0.001$) decrease in QT interval as compared to doxorubicin treated group (Fig 4.45 and 4.46).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28)

administration produced a significant (P<0.01,P<0.001) reduction in QT interval as compared to doxorubicin treated group (Fig.4.48 and 4.49).

4.3.1.2.2.3 Effects of drugs on Heart Rate

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) produced a significant (P<0.001) decrease in heart rate as compared to control animals.

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in heart rate as compared to doxorubicin group; but higher doses (50 and 100 mg/kg) of green tea extract significantly (P<0.05,P<0.001) increased the heart rate (Fig 4.44).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in heart rate; but at higher dose (6 mg/kg), it significantly (P<0.001) increased the heart rate as compared to doxorubicin group (Fig 4.47).

Treatment of lovastatin (3 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in heart rate; but at higher dose (6 mg/kg), it produced a significant (P<0.05) increase in heart rate as compared to doxorubicin treated group (Fig 4.50).

Fig 4.42: Recordings showing changes in Electrocardiographic (ECG) parameters.

4.42 a: ECG recording in control group

4.42 b: ECG recording in doxorubicin alone group

4.42 c: ECG recording in Doxo+ GTE (25) group

4.42 d: ECG recording in Doxo+ GTE (50) group

4.42 e: ECG recording in Doxo+ GTE (100) group

Fig.4.43

Effect of green tea extract on ST interval and QT interval of ECG in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.44

Effect of green tea extract on Heart Rate in doxorubicin induced cardiotoxicity (chronic study) in rats.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig 4.42 a

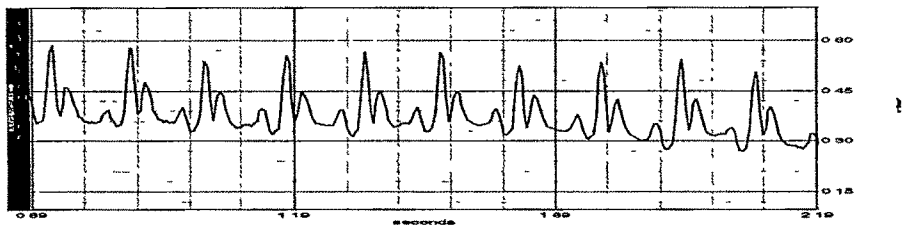


Fig 4.42 b

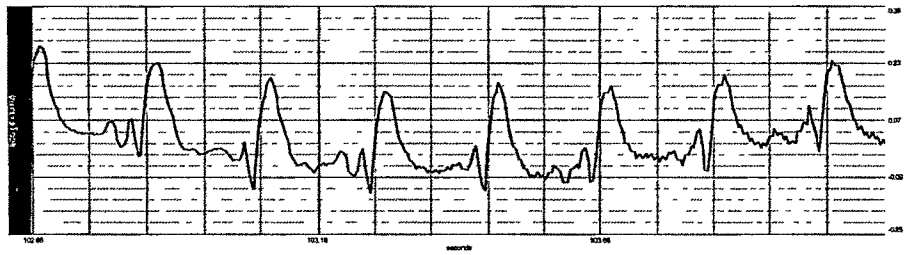


Fig 4.42 c

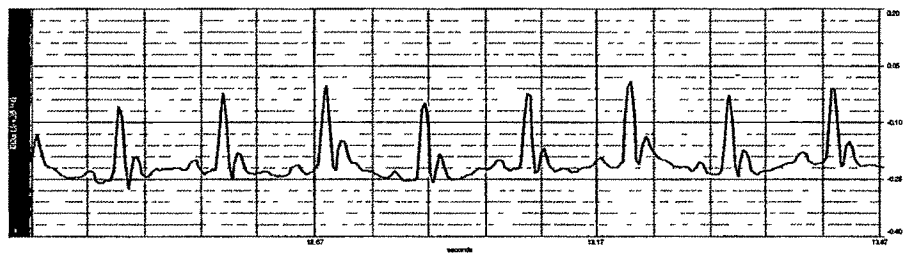
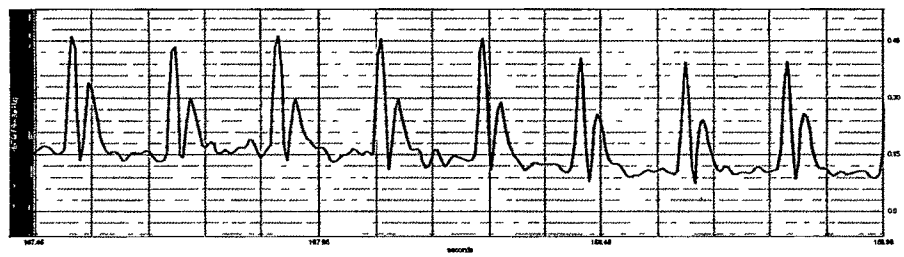


Fig 4.42 d



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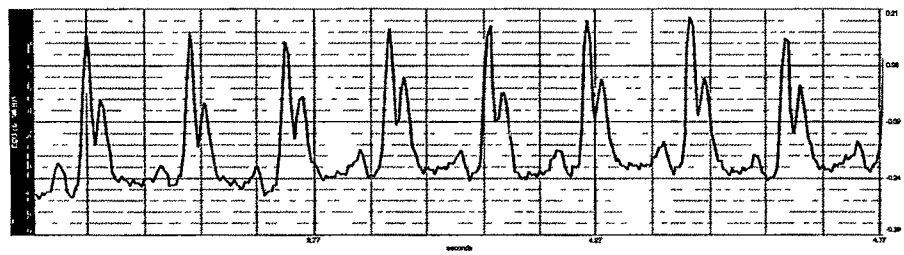


Fig.4.43

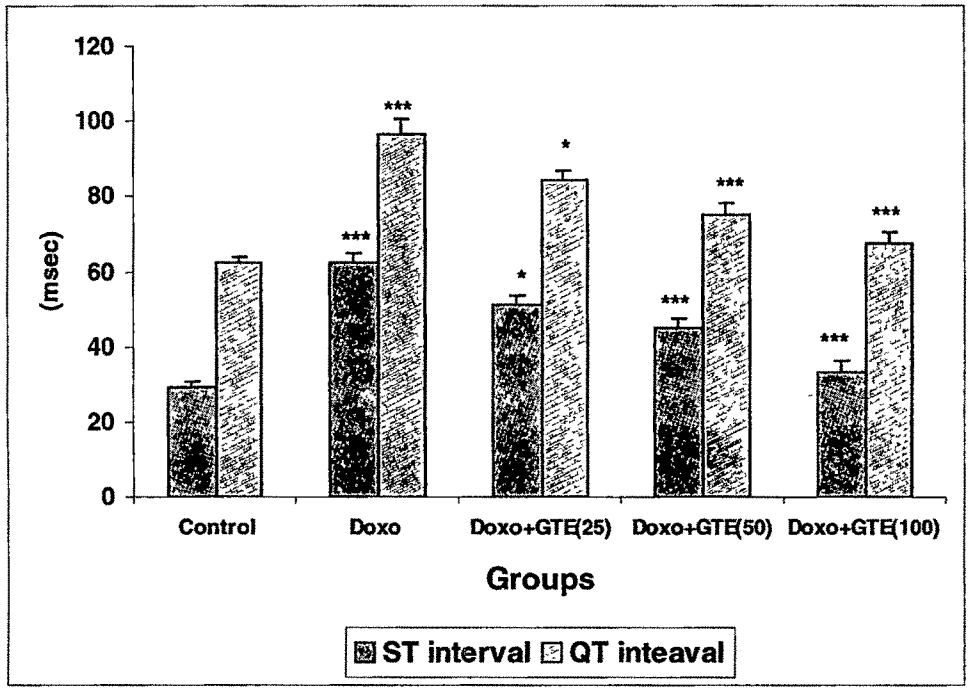


Fig.4.44

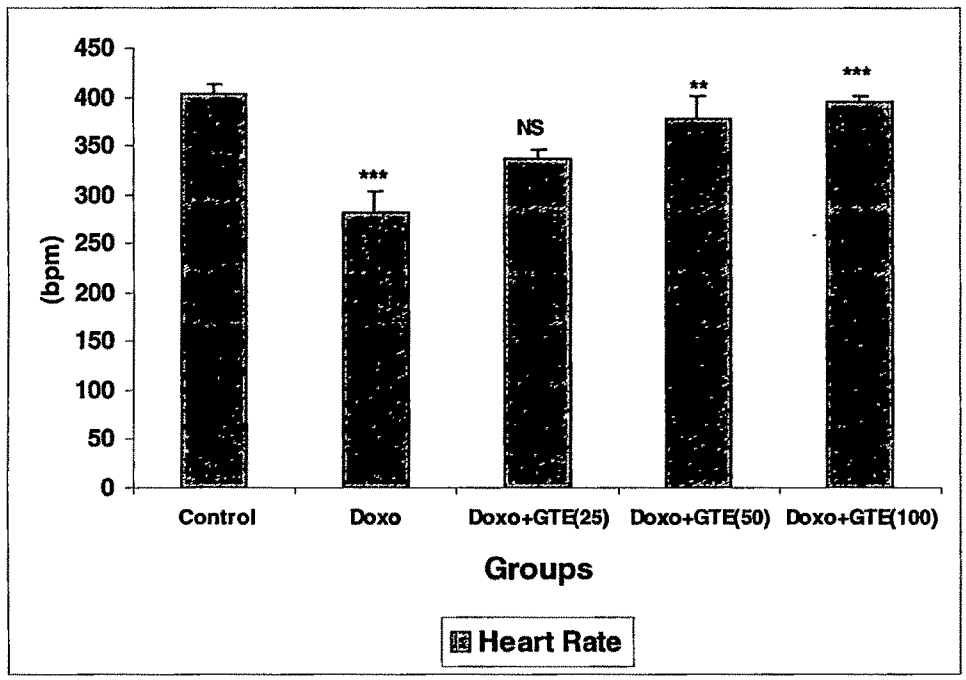


Fig 4.45: Recordings showing changes in Electrocardiographic (ECG) parameters.

4. 45 a: ECG recording in control group

4. 45 b: ECG recording in doxorubicin alone group

4. 45 c: ECG recording in Doxo+ Mel (3) group

4. 45 d: ECG recording in Doxo+ Mel (6) group

Fig.4.46

Effect of melatonin on ST interval and QT interval of ECG in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.47

Effect of melatonin on Heart Rate in doxorubicin induced cardiotoxicity (chronic study) in rats.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Fig 4. 45 a

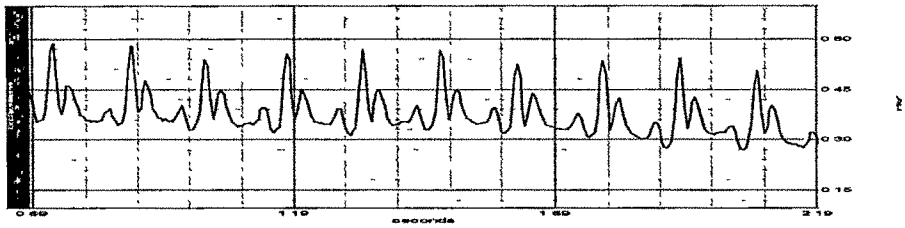


Fig 4. 45 b

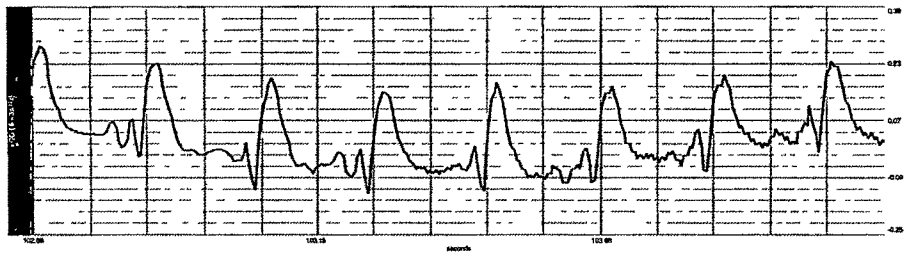


Fig 4. 45 c

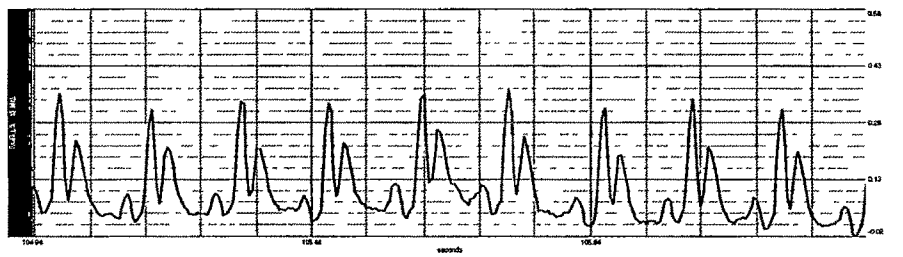


Fig 4. 45 d

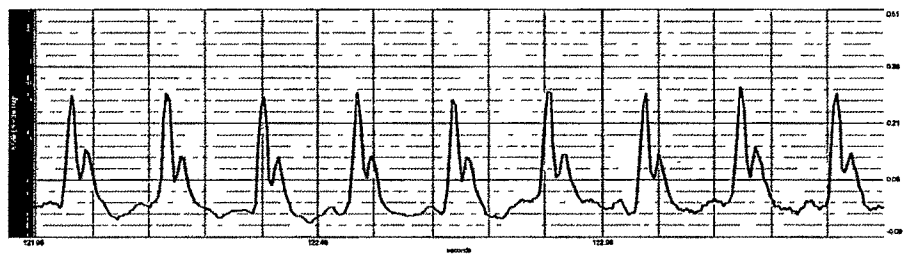


Fig.4.46

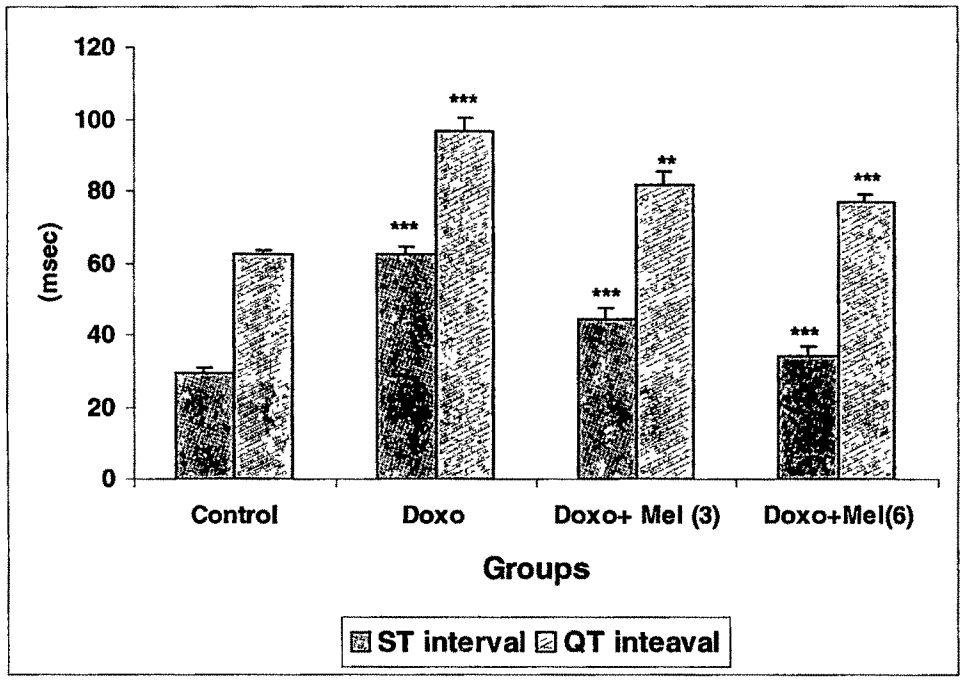


Fig.4.47

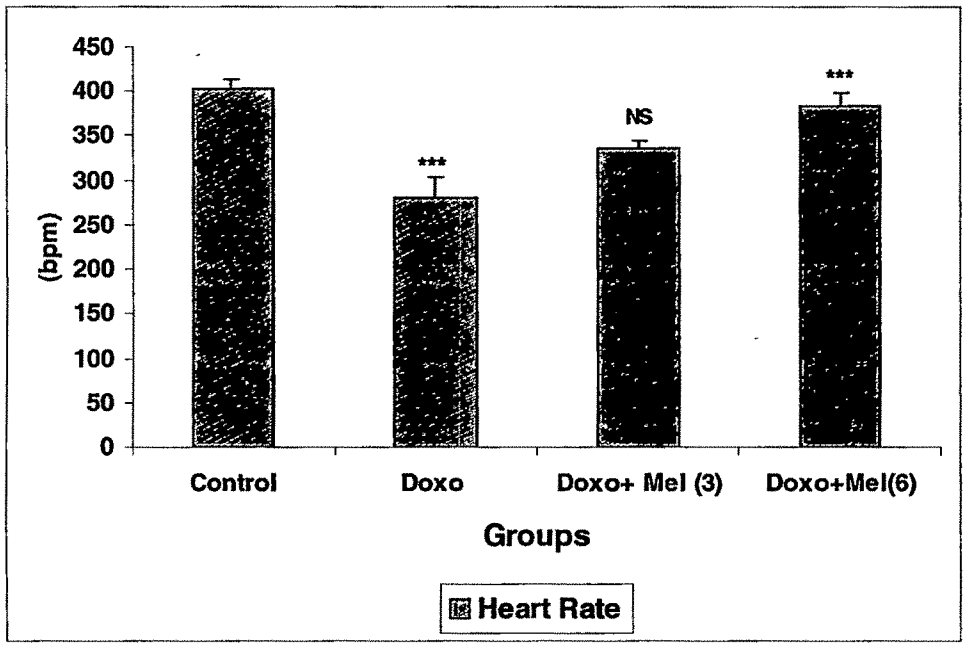


Fig 4.48: Recordings showing changes in Electrocardiographic (ECG) parameters.

4. 48 a: ECG recording in control group

4. 48 b: ECG recording in doxorubicin alone group

4. 48 c: ECG recording in Doxo+ Lova (3) group

4. 48 d: ECG recording in Doxo+ Lova (6) group

Fig.4.49

Effect of lovastatin on ST interval and QT interval of ECG in doxorubicin induced cardiotoxicity (chronic study) in rats.

Fig.4.50

Effect of lovastatin on Heart Rate in doxorubicin induced cardiotoxicity (chronic study) in rats.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig 4.48 a

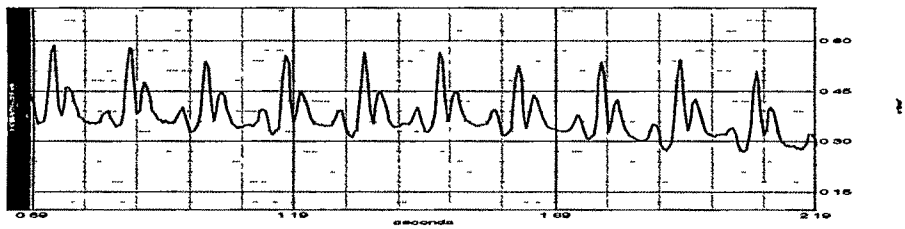


Fig 4.48 b

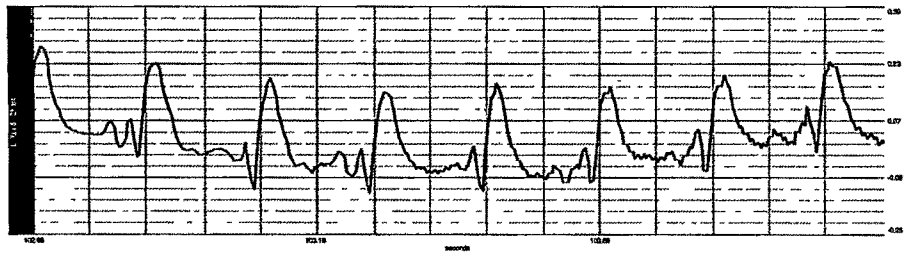


Fig 4.48 c

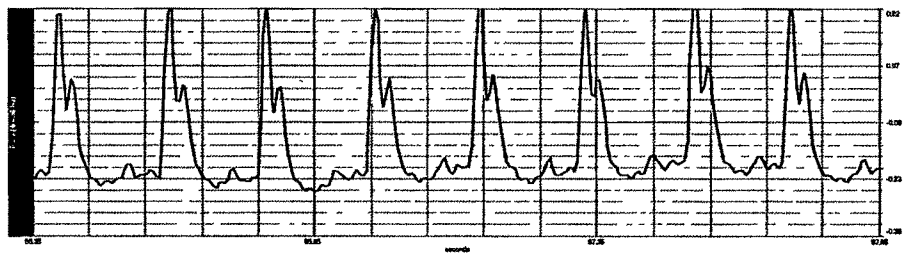


Fig 4.48 d

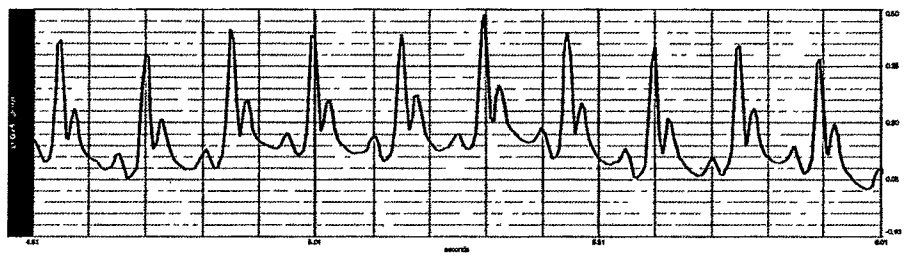


Fig.4.49

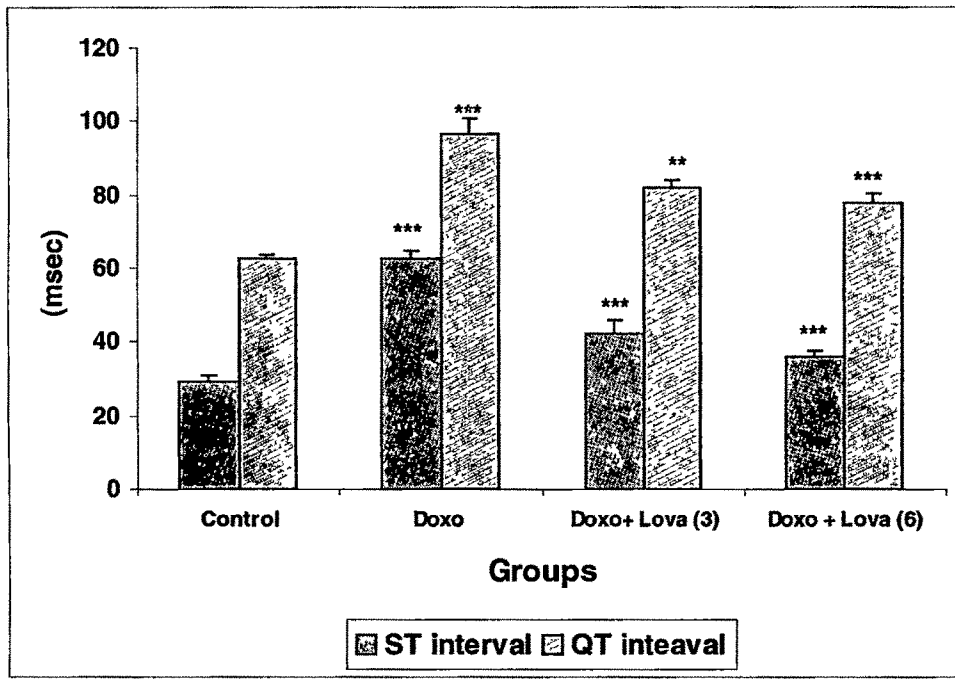
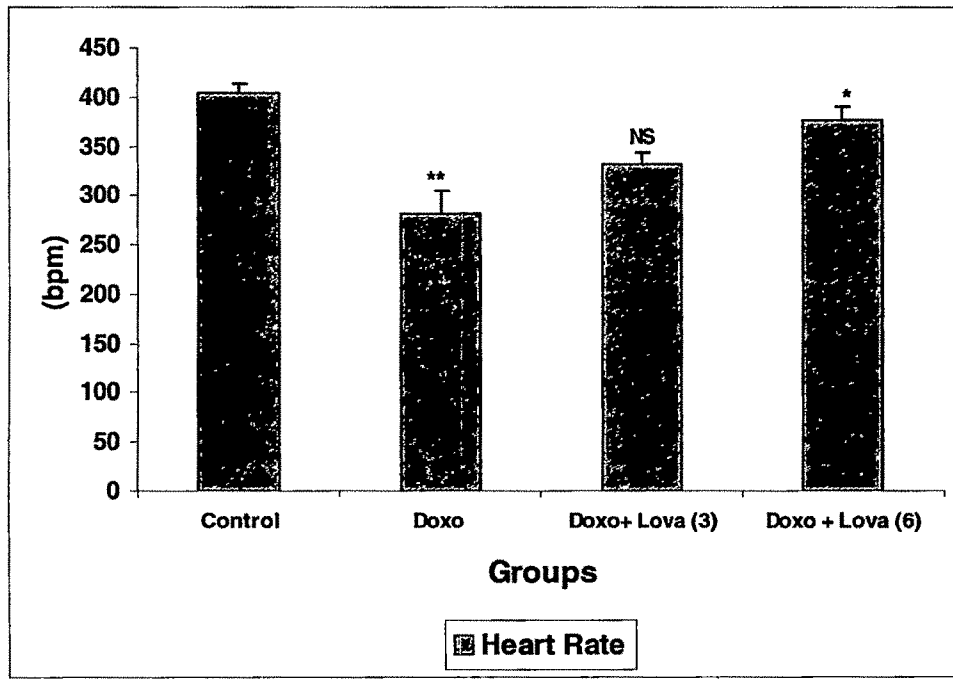


Fig.4.50



4.3.1.2.3 TISSUE PARAMETERS

4.3.1.2.3.1 Effect on lipid peroxidation

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) to rats led to a significant ($P<0.001$) increase in lipid peroxidation or MDA content in heart of rats as compared to the control group.

Treatment of green tea extract (25,50 and 100 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration significantly ($P<0.05$, $P<0.001$) reduced the levels of MDA as compared to doxorubicin treated group (Table 4.8).

Treatment of melatonin (3 and 6 mg/kg /day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.001$) decrease in the MDA content as compared to doxorubicin treated group (Table 4.9).

Lovastatin treatment (3 and 6 mg/kg /day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration significantly ($P<0.001$) reduced the level of lipid peroxidation (MDA) as compared to doxorubicin treated group (Table 4.10).

4.3.1.2.3.2 Effect on endogenous antioxidants

4.3.1.2.3.2.1 Effect on Superoxide dismutase

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) to rats significantly ($P<0.01$) reduced the SOD activity in heart of rats as compared to control (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant changes in the level of SOD; but the higher dose (100 mg/kg) led to a significant ($P<0.01$) rise in SOD content as compared to doxorubicin treated group (Tables 4.8).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin administration produced a significant ($P<0.05$,$P<0.01$) increase in the SOD content as compared to doxorubicin treated group (Table 4.9).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin administration produced a significant (P<0.05,P<0.001) increase in the SOD content as compared to doxorubicin treated group (Table 4.10).

4.3.1.2.3.2.2 Effect on Catalase

The catalase activity in doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treated group (Group 2) was significantly (P<0.001) reduced as compared to control group (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant change in the level of catalase enzyme; but higher doses (50 and 100 mg/kg) of green tea extract significantly (P<0.01,P<0.001) increased these levels as compared to doxorubicin treated group (Table 4.8).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.05,P<0.001) increase in the level of catalase enzyme as compared to doxorubicin treated group (Table 4.9).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant (P<0.05,P<0.001) increase in the level of catalase enzyme as compared to doxorubicin treated group (Table 4.10).

4.3.1.2.3.2.3 Effect on Reduced glutathione

A significant (P<0.001) reduction in reduced glutathione concentration was observed in doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treated rats as compared to control animal.

Green tea extract treatment (25 and 50 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in GSH content; but at the higher dose (100 mg/kg), it significantly (P<0.01) increased the GSH content as compared to doxorubicin treated group (Table 4.8).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28)

administration produced a significant ($P<0.05$,$P<0.01$) increase in the level of GSH as compared to doxorubicin treated group (Table 4.9).

Lovastatin treatment (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.05$,$P<0.01$) increase in the level of GSH as compared to doxorubicin treated group (Table 4.10).

Table 4.8: Effect of green tea extract on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in doxorubicin induced cardiotoxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	3.06 ± 0.16	9.45 ± 1.21	2.33 ± 0.36	4.02±0.32
Group 2	4.75±0.28***	5.14±0.15***	0.6±0.18**	1.85±0.18***
Group 3	3.9±0.22*	6.46±0.36 NS	1.05±0.25 NS	2.03±0.18 NS
Group 4	3.51±0.08***	7.44±0.19 NS	1.56±0.23 NS	3.21±0.15**
Group 5	2.98±0.06***	8.40±0.23**	2.15±0.27**	4.61±0.29***
F value	**15.25**	**8.052**	**7.210**	**25.35**
P value	**P<0.0001**	**P=0.0003**	**P=0.00052**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (3 mg/kg i.p. on days 1,7,14,21and 28).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.9: Effect of melatonin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in doxorubicin induced cardiotoxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	3.06±0.16	9.45±1.21	2.33±0.36	4.02±0.32
Group 2	4.75±0.28***	5.14±0.15***	0.6±0.18***	1.85±0.18***
Group 3	3.15±0.22***	8.24±0.34*	1.58±0.14*	2.81±0.19*
Group 4	2.65±0.21***	8.74±0.50**	2.05±0.22**	3.55±0.14***
F value	**16.69**	**7.66**	**9.5**	**17.46**
P value	**P<0.0001**	**P=0.0013**	**P=0.0004**	**P<0.0001**

Group 1: Normal control

Group2: Doxorubicin treated group (3 mg/kg i.p. on days 1,7,14,21and 28).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.10: Effect of lovastatin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in doxorubicin induced cardiotoxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	3.06±0.16	9.45±1.21	2.33±0.36	4.02±0.32
Group 2	4.75±0.28***	5.14±0.15***	0.6±0.18***	1.85±0.18***
Group 3	2.88±0.24***	7.90±0.35*	1.68±0.96*	2.90±0.18*
Group 4	2.61±0.19***	9.19±0.42**	2.36±0.15***	3.54±0.22***
F value	**16.80**	**10.37**	**13.58**	**17.51**
P value	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (3 mg/kg i.p.on days 1,7,14,21and 28)

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.1.2.3.3 Effect on membrane bound enzymes

4.3.1.2.3.3.1 Effect on Sodium Potassium ATPase

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) resulted in a significant ($P<0.001$) decrease in the Na^+K^+ATPase activity as compared to the control (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce significant increase in Na^+K^+ATPase levels; but the higher doses (50 and 100 mg/kg) of green tea extract significantly ($P<0.01$,$P<0.001$) increased the levels of the enzyme as compared to doxorubicin treated group (Table 4.11).

Melatonin treatment (3mg /kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce significant increase in the levels of Na^+K^+ATPase; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased the level of the enzyme as compared to doxorubicin treated group (Table 4.12).

Treatment of lovastatin (3mg /kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce significant increase in the levels of Na^+K^+ATPase; but the higher dose (6 mg/kg) of lovastatin significantly ($P<0.05$) increased the level of the enzyme as compared to doxorubicin treated group (Table 4.13).

4.3.1.2.3.3.2 Effect on Calcium ATPase

Chronic administration with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) resulted in a significant ($P<0.01$) decrease in the Ca^{2+}ATPase activity as compared to the control (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not alter the Ca^{2+}ATPase levels as compared to doxorubicin treated group; but at the higher dose (100 mg/kg), it significantly ($P<0.01$) increased these levels as compared to doxorubicin treated group (Table 4.11).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce any significant increase in Ca^{2+}ATPase levels as compared to

doxorubicin treated group; but higher dose (6mg/kg) of melatonin significantly (P<0.05) increased these levels as compared to doxorubicin treated group (Table 4.12).

Treatment of lovastatin (3 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce any significant increase in Ca^{2+}ATPase levels as compared to doxorubicin treated group; but at higher dose (6mg/kg), it significantly (P<0.05) increased these levels as compared to doxorubicin treated group (Table 4.13).

4.3.1.2.3.3.3 Effect on Magnesium ATPase

In the heart of chronically doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treated rats (Group 2) the activity of Mg^{2+}ATPase enzyme was not significantly reduced as compared to the control (Group1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not alter the Ca^{2+}ATPase levels as compared to doxorubicin treated group; but at the higher doses (100 mg/kg), it significantly (P<0.05) increased these levels as compared to doxorubicin treated group (Table 4.11).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce any significant increase in Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.12).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1,7,14,21and 28) administration did not produce any significant increase in Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.13).

Table 4.11: Effect of green tea extract on membrane bound enzymes in the heart of rats in doxorubicin induced cardiotoxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	7.0 ± 0.2	3.86 ± 0.17	3.01 ± 0.17
Group 2	4.54±0.16***	2.74±0.24**	2.23±0.37 NS
Group 3	4.83±0.27 NS	2.90±0.075 NS	2.78±0.24 NS
Group 4	6.10±0.35**	3.02±0.11 NS	2.94±0.24 NS
Group 5	7.75±0.18***	3.75±0.21**	3.38±0.15*
F value	**31.30**	**8.36**	**2.76**
P value	**P<0.0001**	**P=0.0002**	**P=0.0499**

Group 1: Normal control

Group2: Doxorubicin treated group (3 mg/kg i.p.on days 1,7,14,21and 28).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.12: Effect of melatonin on membrane bound enzymes in the heart of rats in doxorubicin induced cardiotoxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	7.0±0.2	3.86±0.17	3.01±0.17
Group 2	4.54±0.16***	2.74±0.24*	2.23±0.37 NS
Group 3	5.18±0.22NS	2.81±0.21NS	2.86±0.28NS
Group 4	5.66±0.33*	3.94±0.35*	3.35±0.30NS
F value	**19.18**	**6.48**	**1.25**
P value	**P<0.0001**	**P=0.003**	**P=0.316**

Group 1: Normal control

Group2: Doxorubicin treated group (3 mg/kg i.p.on days 1,7,14,21and 28).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.13: Effect of lovastatin on membrane bound enzymes in the heart of rats in doxorubicin induced cardiotoxicity (chronic study) in rats.

Groups	$Na^+K^+ATPase$ (µmoles of inorganic phosphorus liberated/min/mg protein)	$Ca^{2+}ATPase$ (µmoles of inorganic phosphorus liberated/min/mg protein)	$Mg^{2+}ATPase$ (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	7.0±0.2	3.86±0.17	3.01±0.17
Group 2	4.54±0.16***	2.74±0.24**	2.23±0.37 NS
Group 3	4.98±0.24NS	2.89±0.25NS	2.99±0.30NS
Group 4	5.67±0.29*	3.77±0.17*	3.27±0.24NS
F value	**14.29**	**7.02**	**0.67**
P value	**P<0.0001**	**P=0.0006**	**P=0.61**

Group 1: Normal control

Group 2:Doxorubicin treated group (3 mg/kg i.p.on days 1,7,14,21and 28)

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p.on days 1,7,14,21and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.1.2.4 HEMODYNAMIC MEASUREMENT

4.3.1.2.4.1 Measurement of Blood pressure by Non invasive method (indirect method)

4.3.1.2.4.1.1 Effect on Diastolic BP

Chronic administration of doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) produced a significant (P<0.001) increase in diastolic BP as compared to control animals (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration did not produce significant decrease in diastolic BP; but the higher doses (50 and 100 mg/kg) of green tea extract significantly (P<0.01,P<0.001) decreased the diastolic BP as compared to doxorubicin treated group (Fig.4.51, Fig.4.52).

Melatonin treatment (3 and 6 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration produced a significant (P<0.001) decrease the diastolic BP as compared to doxorubicin treated group (Fig.4.57, Fig.4.58).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration produced a significant (P<0.001) decrease the diastolic BP as compared to doxorubicin treated group (Fig.4.63, Fig.4.64).

4.3.1.2.4.1.2 Effect on Systolic BP

Chronic doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) administration produced a significant (P<0.001) increase in Systolic BP as compared to control animals (Group 1).

Green tea extract treatment (25 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration did not produce any significant decrease in Systolic BP; but the higher doses (50 and 100 mg/kg) of green tea extract significantly (P<0.01,P<0.001) decreased the Systolic BP as compared to doxorubicin treated group (Fig.4.53, Fig.4.54).

Melatonin treatment (3 and 6 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration

produced a significant (P<0.001) decrease the Systolic BP as compared to doxorubicin treated group (Fig.4.59, Fig.4.60).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration produced a significant (P<0.001) decrease the Systolic BP as compared to doxorubicin treated group (Fig.4.65, Fig.4.66).

4.3.1.2.4.1.3 Effect on Mean BP

Chronic administration of doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) produced a significant (P<0.001) increase in Mean BP as compared to control animals (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration did not produce significant decrease in Mean BP; but at the higher doses (50 and 100 mg/kg), it significantly (P<0.01,P<0.001) decreased the Mean BP as compared to doxorubicin treated group (Fig.4.55, Fig.4.56).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration produced a significant (P<0.001) decrease in Mean BP as compared to doxorubicin treated group (Fig.4.61, Fig.4.62).

Lovastatin treatment (3 and 6 mg/kg/day p.o.for 30 days) along with chronic doxorubicin (3mg/kg i.p. on days 1,7,14, 21 and 28) administration produced a significant (P<0.001) decrease in Mean BP as compared to doxorubicin treated group (Fig.4.67, Fig.4.68).

Fig.4.51: Effect of green tea extract on time course of changes in Diastolic BP in animals chronically treated with doxorubicin.

Fig.4.52: Effect of green tea extract on Diastolic BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Fig.4.53: Effect of green tea extract on time course of changes in Systolic BP in animals chronically treated with doxorubicin.

Fig.4.54: Effect of green tea extract on systolic BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Fig.4.55: Effect of green tea extract on time course of changes in Mean BP in animals chronically treated with doxorubicin.

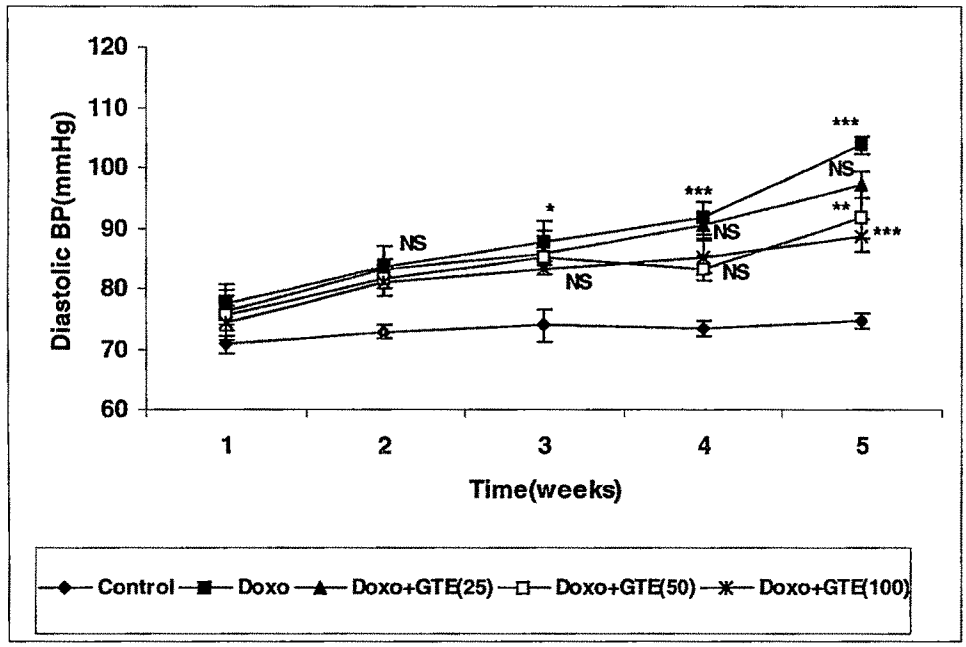
Fig.4.56: Effect of green tea extract on Mean BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.51



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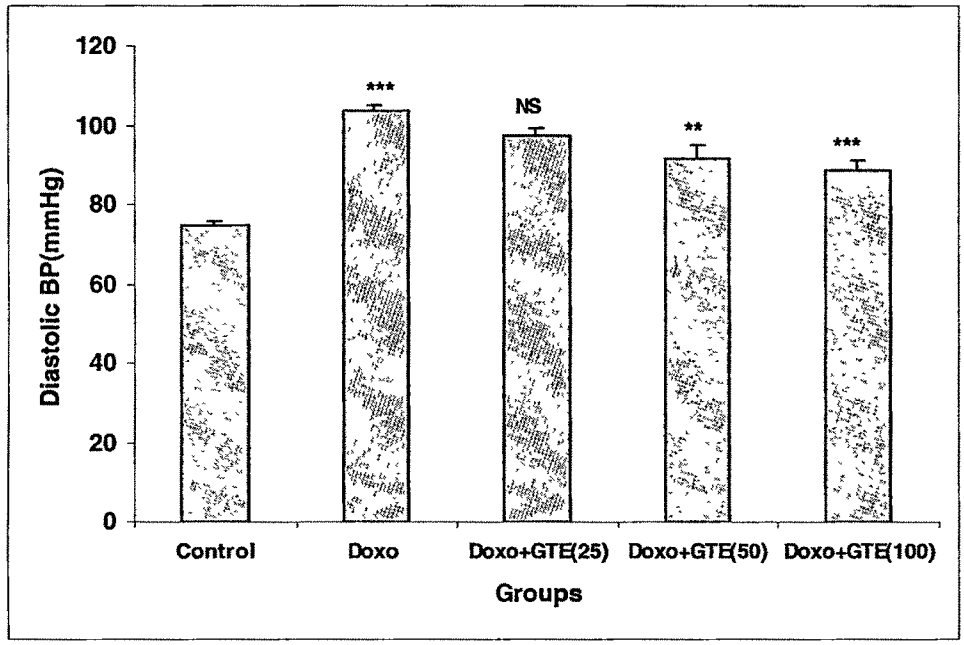


Fig.4.53

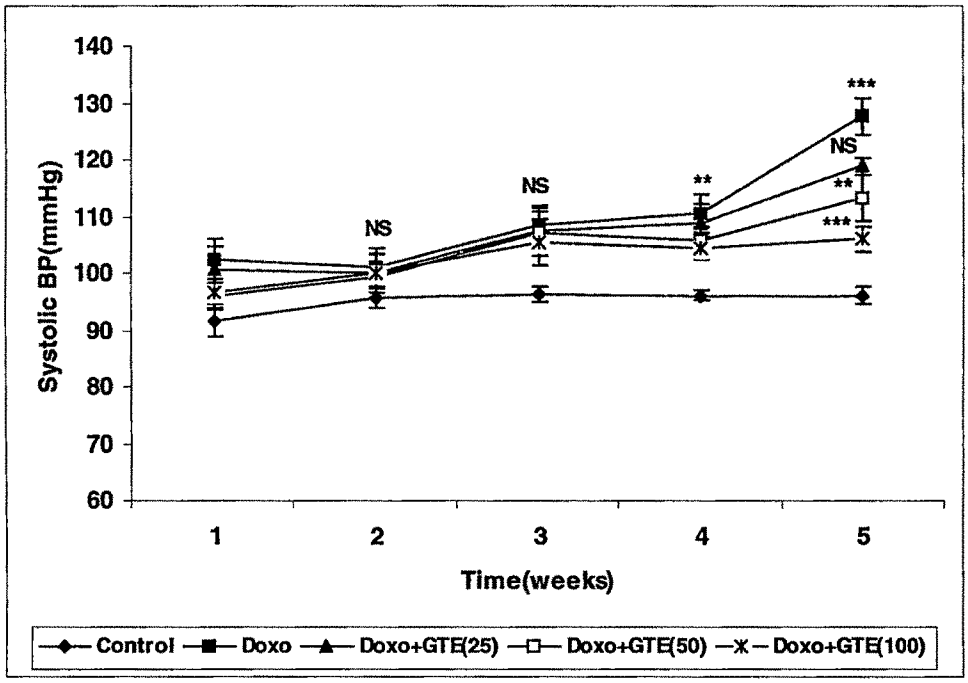


Fig.4.54

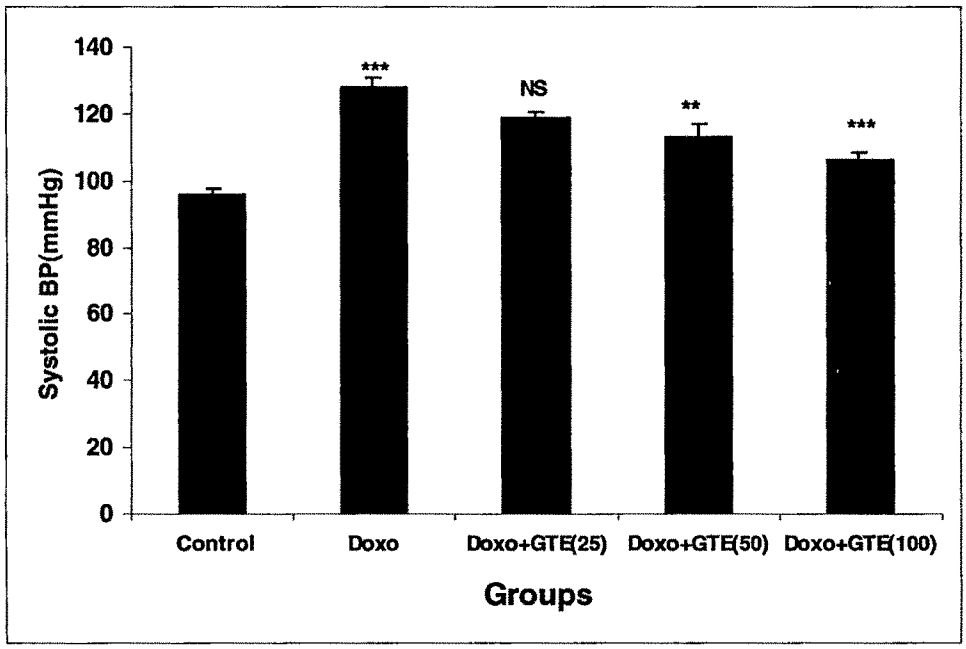


Fig.4.55

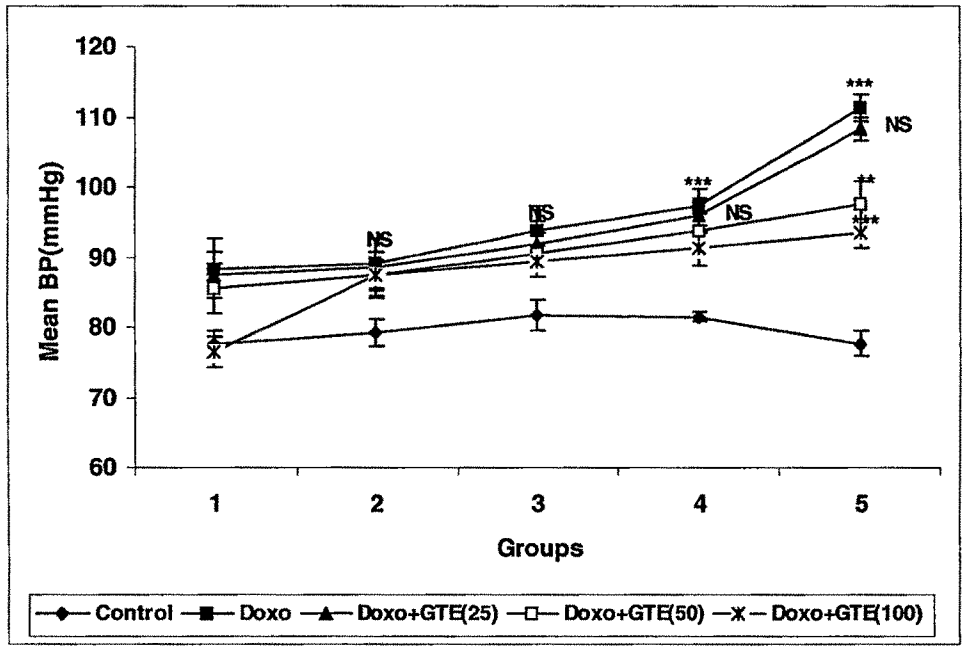


Fig.4.56

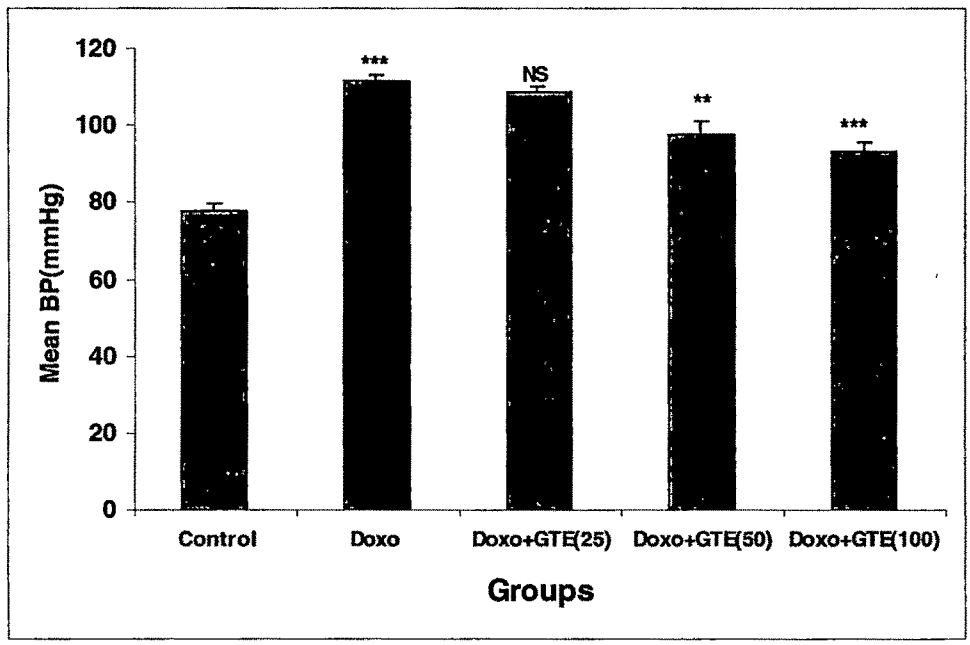


Fig.4.57: Effect of melatonin on time course of changes in Diastolic BP in animals chronically treated with doxorubicin.

Fig.4.58: Effect of melatonin on Diastolic BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Fig.4.59: Effect of melatonin on time course of changes in Systolic BP in animals chronically treated with doxorubicin.

Fig.4.60: Effect of melatonin on Systolic BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Fig.4.61: Effect of melatonin on time course of changes in Mean BP in animals chronically treated with doxorubicin.

Fig.4.62: Effect of melatonin on Mean BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.57

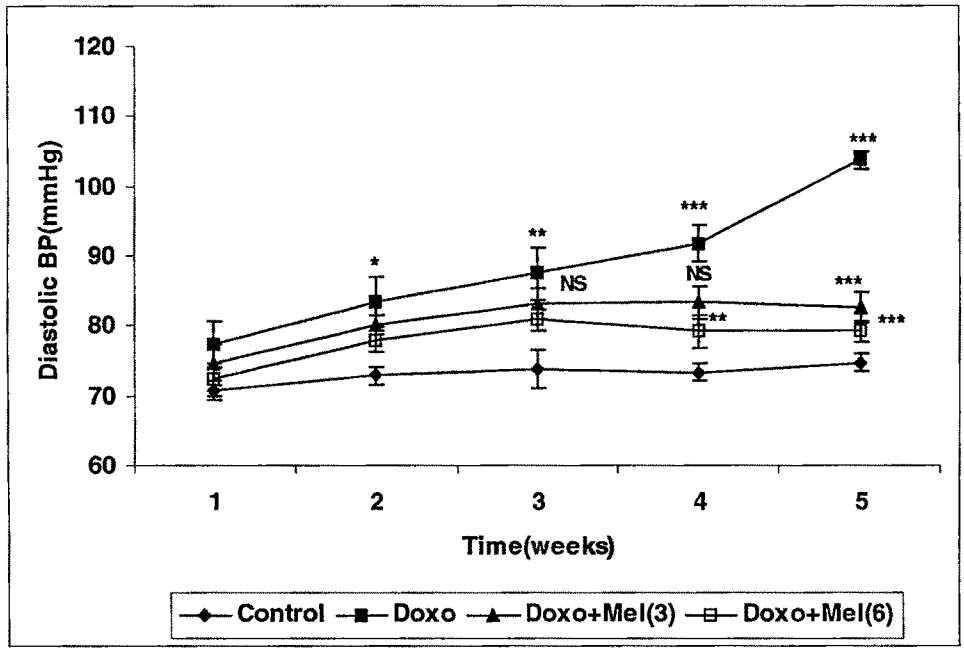


Fig.4.58

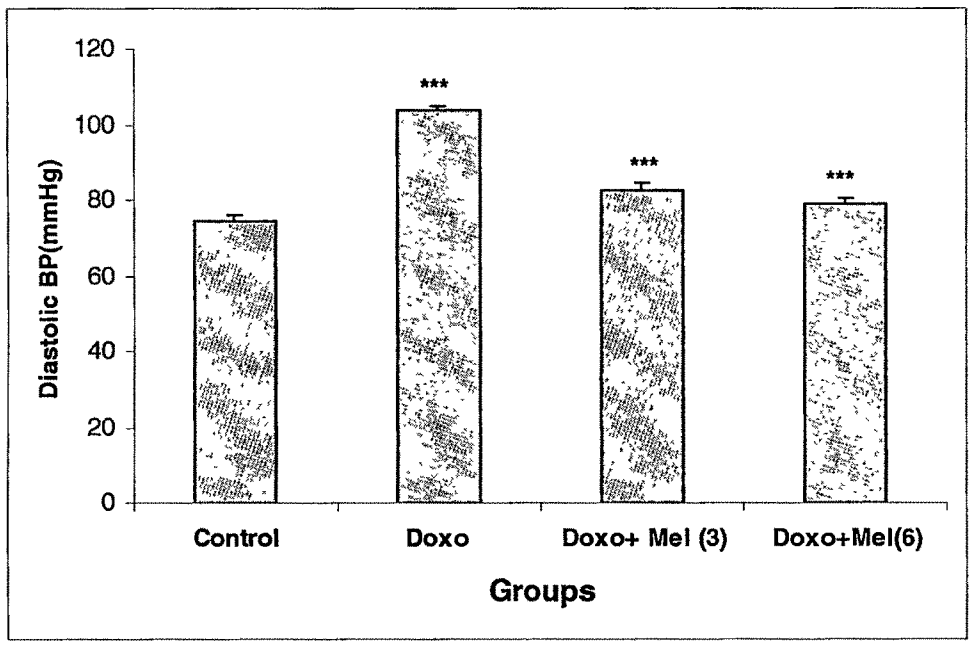


Fig.4.59

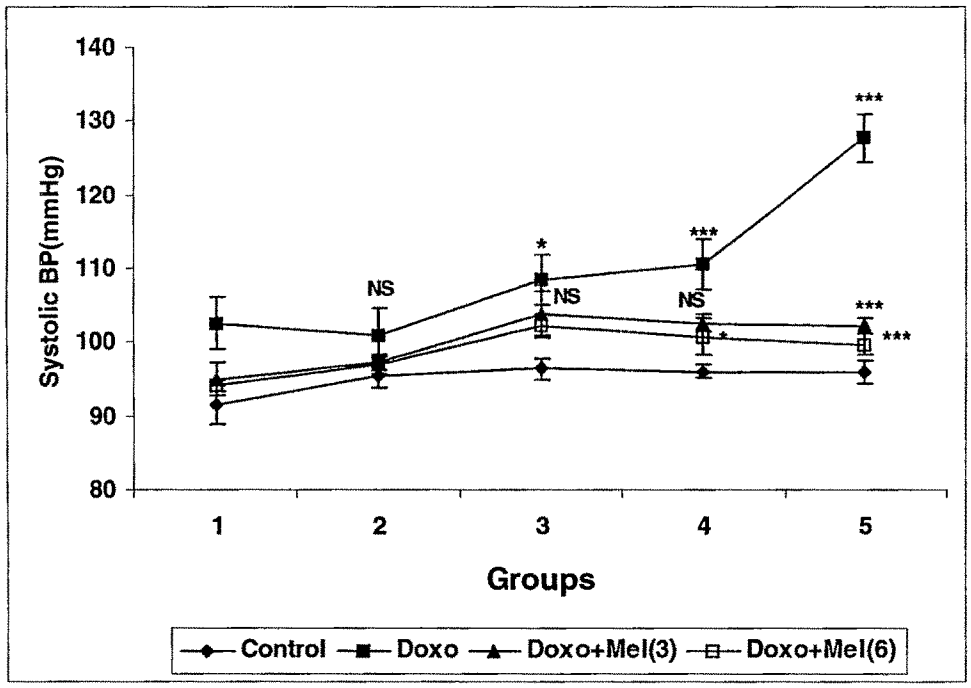


Fig.4.60

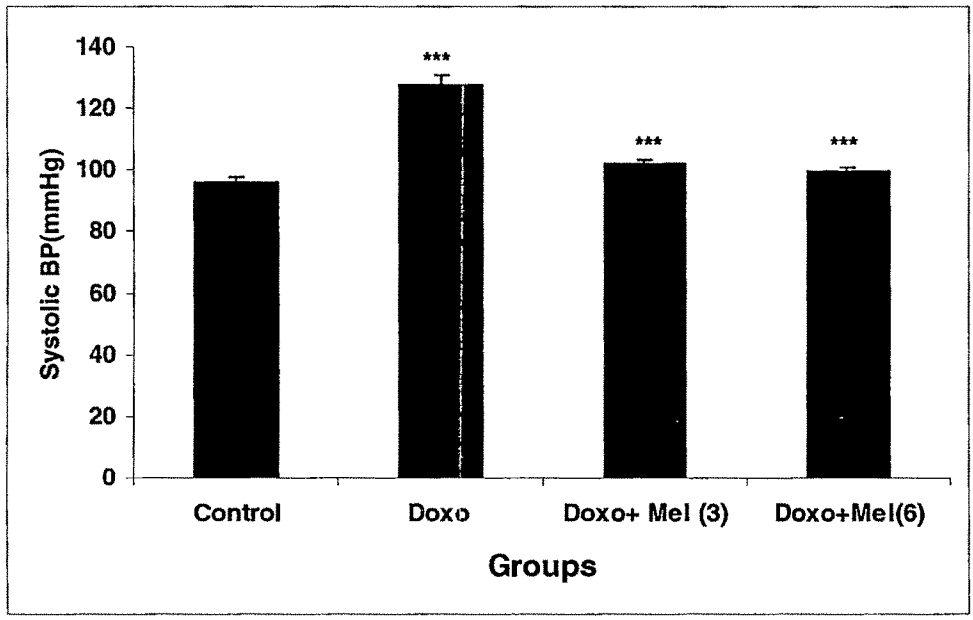


Fig.4.61

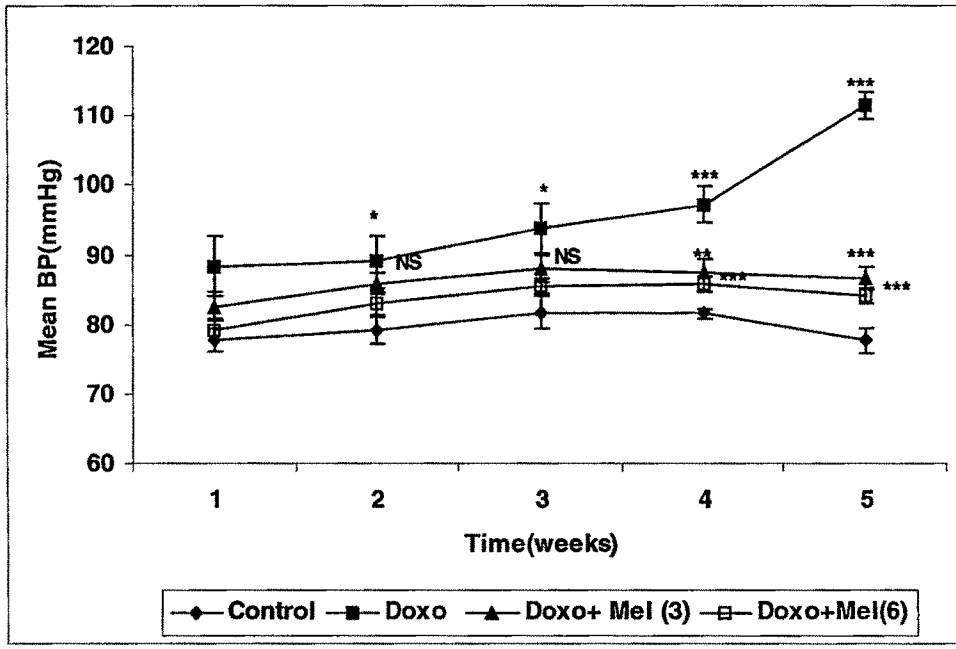


Fig.4.62

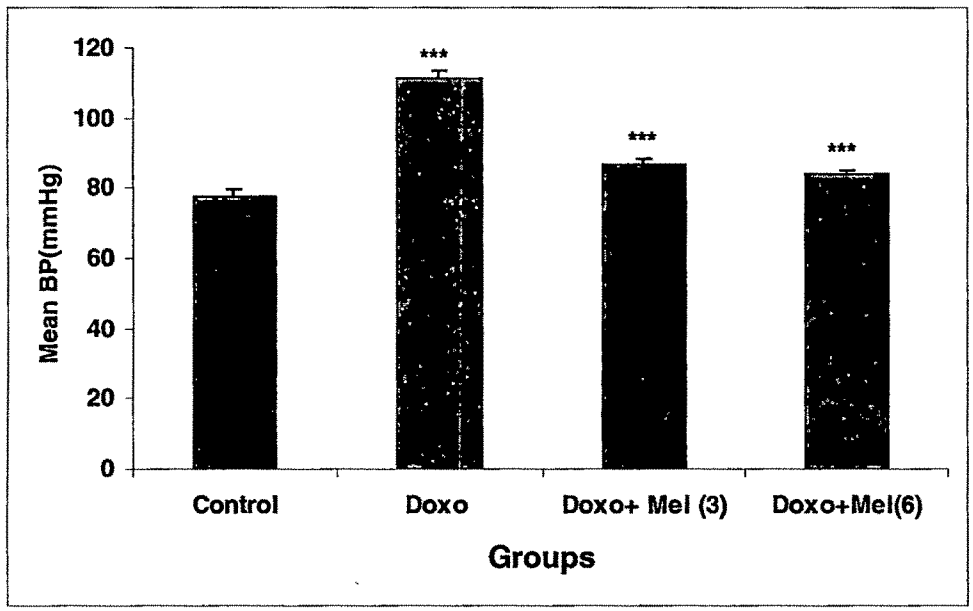


Fig.4.63: Effect of lovastatin on time course of changes in Diastolic BP in animals chronically treated with doxorubicin.

Fig.4.64 Effect of lovastatin on Diastolic BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Fig.4.65: Effect of lovastatin on time course of changes in Systolic BP in animals chronically treated with doxorubicin.

Fig.4.66: Effect of lovastatin on Systolic BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Fig.4.67: Effect of lovastatin on time course of changes in Mean BP in animals chronically treated with doxorubicin.

Fig.4.68: Effect of lovastatin on Mean BP after completion of treatment schedule in animals chronically treated with doxorubicin.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.63

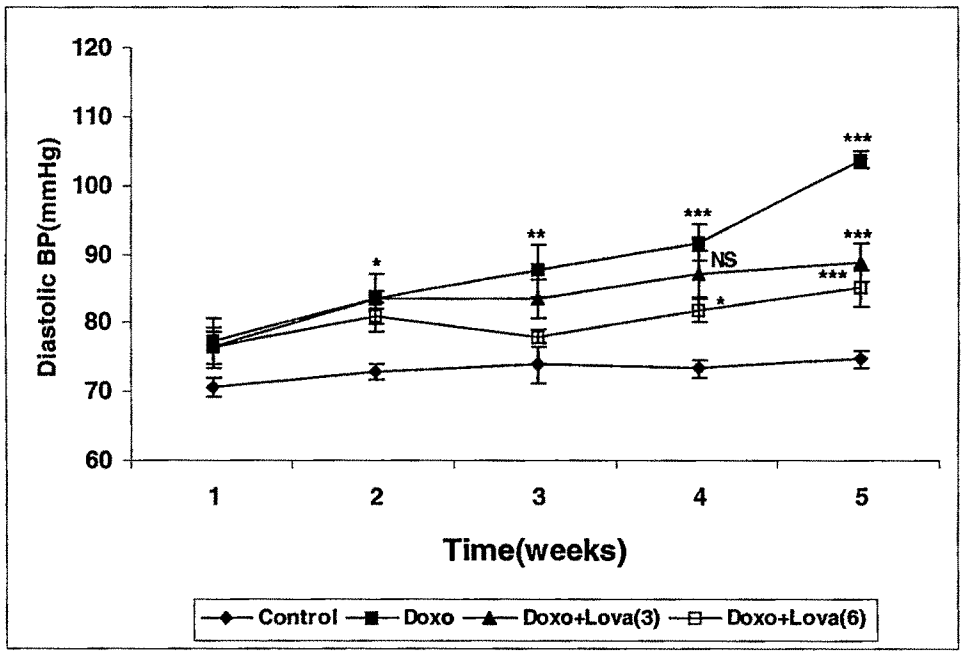


Fig.4.64

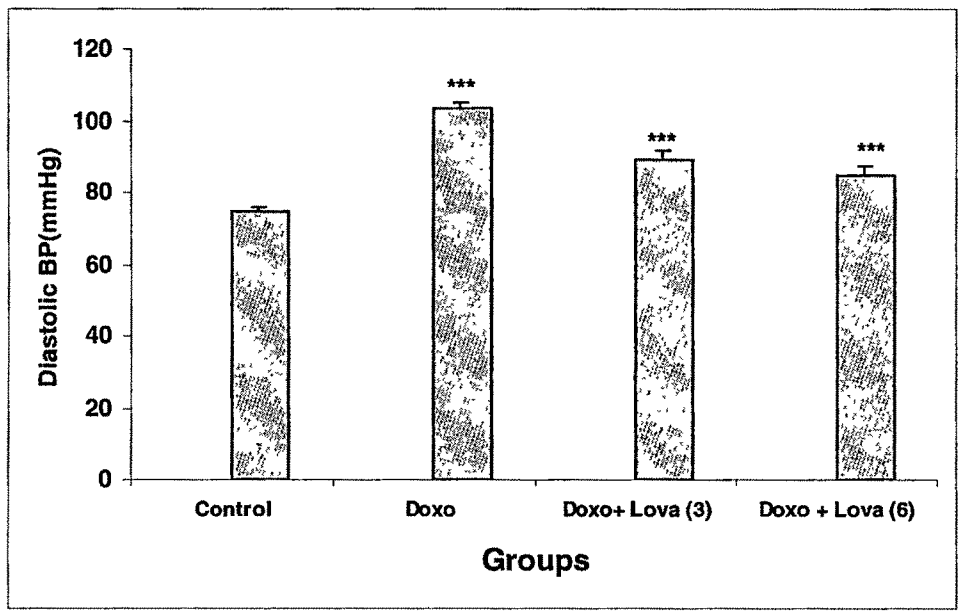


Fig.4.65

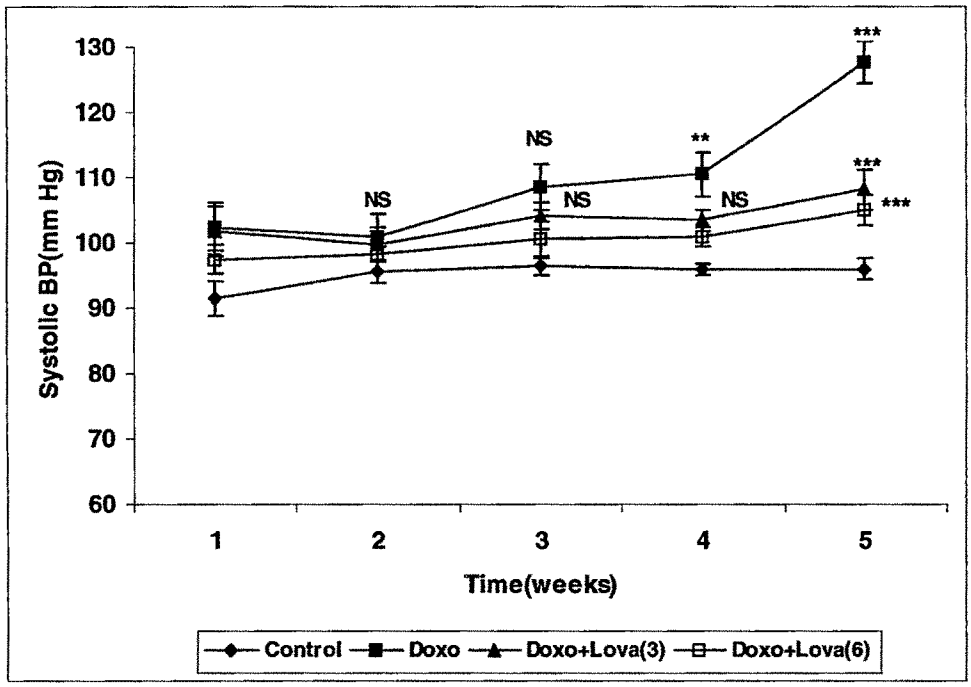


Fig.4.66

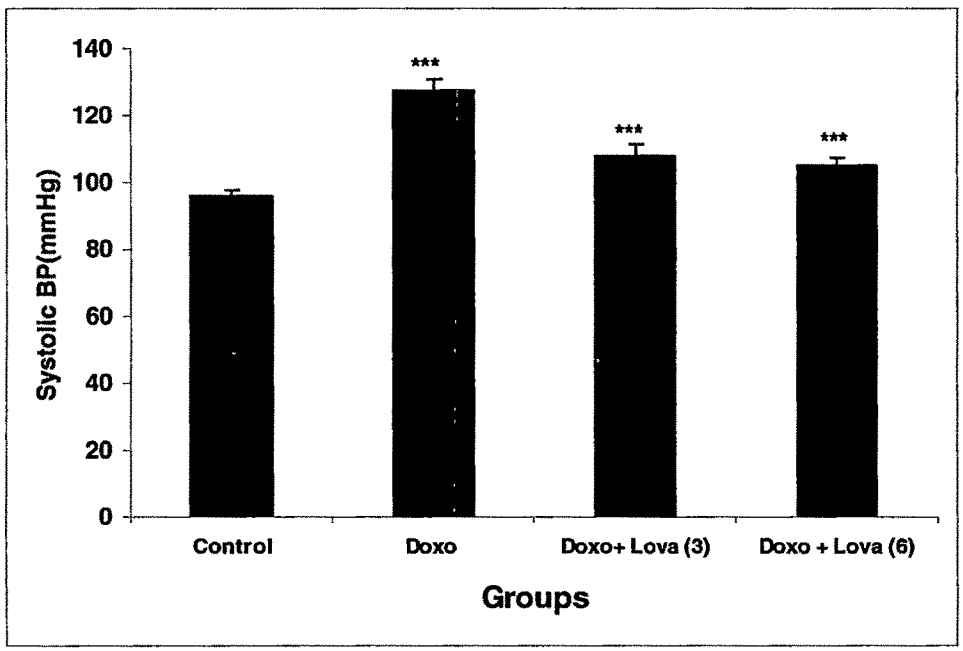


Fig.4.67

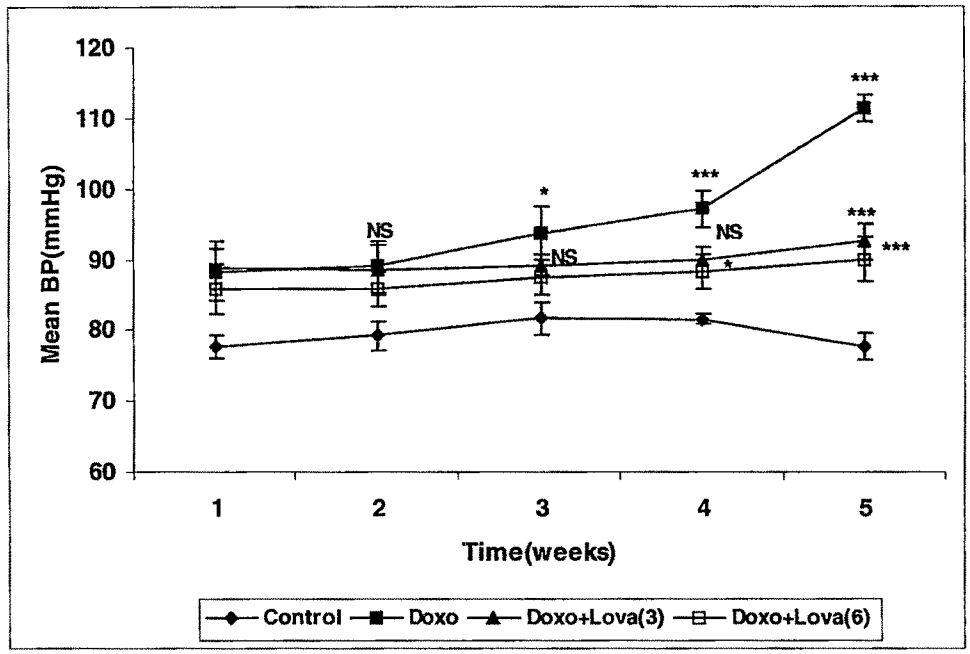
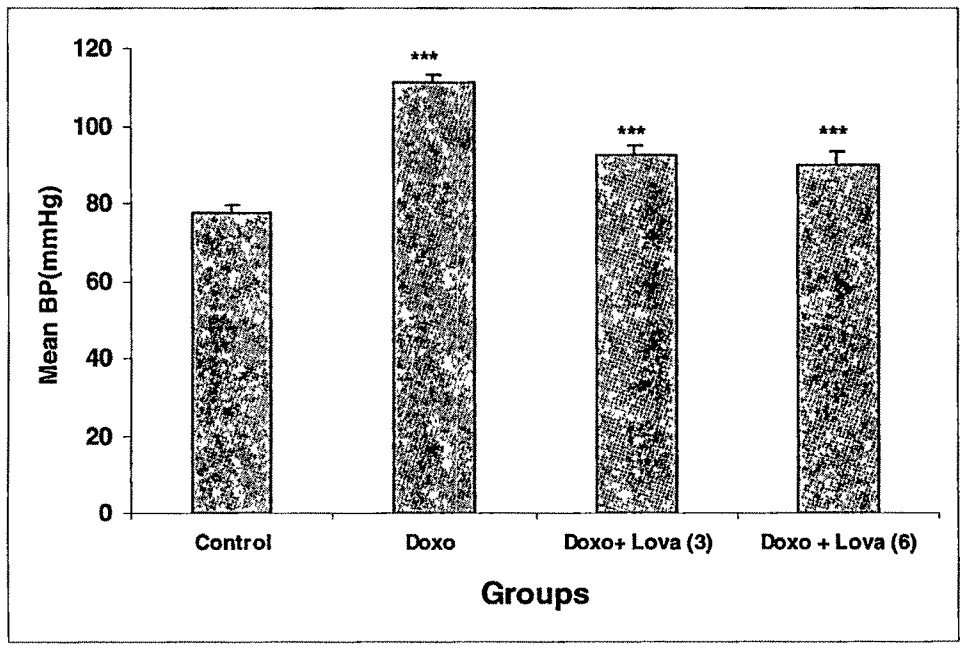


Fig.4.68



4.3.1.2.4.2 Measurement of Blood pressure by Invasive method (direct method)

4.3.1.2.4.2.1 Vascular reactivity to noradrenaline

Pressor responses to noradrenaline (1 and 2 μg/kg i.v.) in chronically doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) treated rats did not alter significantly as compared to control animals.

In animals treated with green tea extract (25,50 and 100 mg/kg), melatonin (3 and 6 mg/kg) and lovastatin (3 and 6 mg/kg), there was no change in pressor response to noradrenaline (1 and 2 μg/kg i.v.) as compared to doxorubicin treated animals (Fig.4.69, Fig.4.75, Fig.81).

4.3.1.2.4.2.2 Vascular reactivity to adrenaline

Pressor responses to adrenaline (1 and 2 μg/kg i.v.) in chronically doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) treated rats did not alter significantly as compared to control animals.

In animals treated with green tea extract (25,50 and 100 mg/kg), melatonin (3 and 6 mg/kg) and lovastatin (3 and 6 mg/kg), there was no change in pressor response to adrenaline (1 and 2 μg/kg i.v.) as compared to doxorubicin treated animals (Fig.4.70, Fig.4.76, Fig.82).

4.3.1.2.4.2.3 Vascular reactivity to isoprenaline

Depressor responses to isoprenaline (1 and 2 μg/kg i.v.) in chronically doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) treated rats altered significantly ($P<0.01$,$P<0.001$) as compared to control animals.

Administration of green tea extract 9 25 and 50 mg/kg) did not alter depressor response but at higher dose (100 mg/kg), depressor responses to isoprenaline (1 and 2 μg/kg i.v.) significantly ($P<0.05$,$P<0.01$) increased as compared to doxorubicin treated animals (Fig.4.71).

Administration of melatonin (3 mg/kg) did not alter depressor response, but at higher dose (6 mg/kg), depressor responses to isoprenaline (1 and 2μg/kg) significantly ($P<0.05$,$P<0.001$) increased as compared to doxorubicin treated animals (Fig.4.77).

Administration of lovastatin (3 and 6 mg/kg) did not alter depressor response to isoprenaline (1 and 2μg/kg) as compared to doxorubicin treated animals (Fig.4.83).

4.3.1.2.4.2.4 Vascular reactivity to phenylephrine

Pressor responses to phenylephrine (1 and 2μg/kg i.v.) in chronically doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) treated rats significantly ($P<0.05$) altered as compared to control animals.

Administration of green tea extract (25,50 and 100 mg/kg), melatonin (3 and 6mg/kg) and lovastatin (3 and 6 mg/kg) did not alter pressor response to phenylephrine as compared to doxorubicin treated animals (Fig.4.72, Fig.4.78, Fig.84).

4.3.1.2.4.2.5 Vascular reactivity to angiotensin

Pressor responses to angiotensin (1 and 2μg/kg i.v.) in chronically doxorubicin (3mg/kg i.p.on days 1,7,14,21and 28) treated rats did not alter significantly as compared to control animals.

In animals treated with green tea extract (25,50 and 100 mg/kg), melatonin (3 and 6 mg/kg) and lovastatin (3 and 6 mg/kg), there was no change in pressor response to angiotensin (1 and 2 μg/kg i.v.) as compared to doxorubicin treated animals (Fig.4.73, Fig.4.79, Fig.85).

Fig.4.69: Effect of green tea extract on vascular reactivity to noradrenaline (1 and 2 μg / kg) in animals chronically treated with doxorubicin.

Fig.4.70: Effect of green tea extract on vascular reactivity to adrenaline (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.71: Effect of green tea extract on vascular reactivity to isoprenaline (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.72: Effect of green tea extract on vascular reactivity to phenylephrine (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.73: Effect of green tea extract on vascular reactivity to angiotensin (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig 4.74: Tracings of arterial BP of anaesthetized rat after administration of noradrenaline, adrenaline, isoprenaline, phenylephrine and angiotensin.
4.74 a: Control group
4.74 b: Doxorubicin alone group
4.74 c: Doxo+ GTE (25) group
4.74 d: Doxo+ GTE (50) group
4.74 e: Doxo+ GTE (100) group

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.69

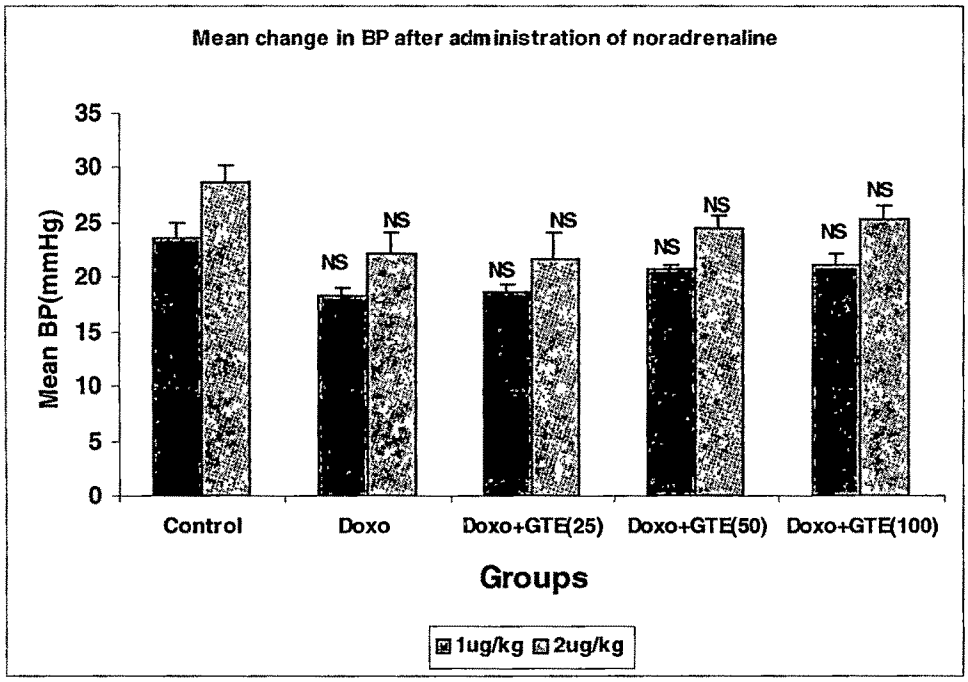


Fig.4.70

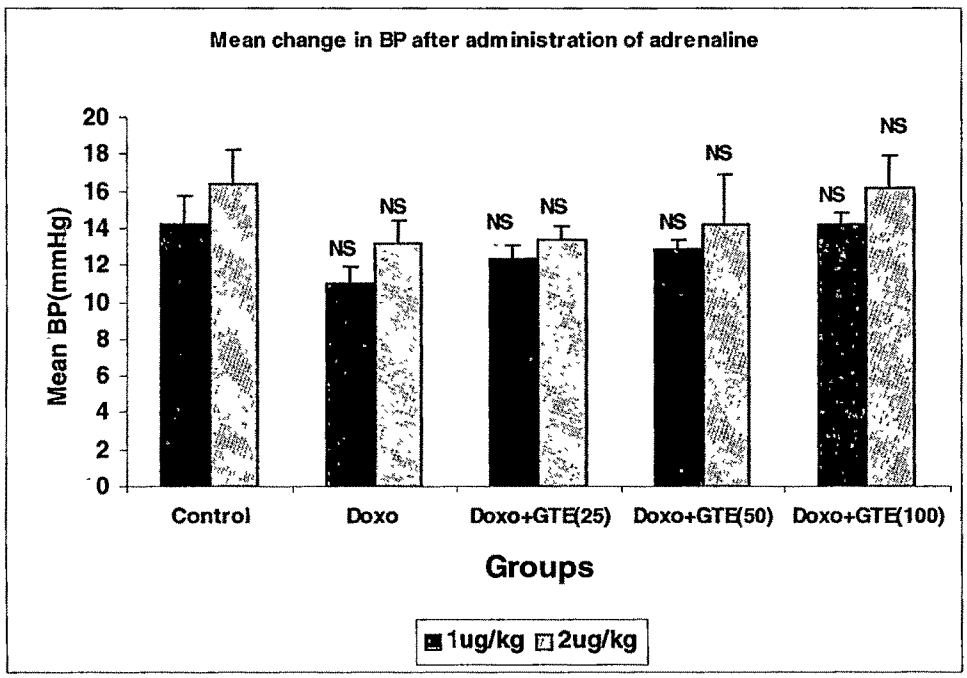


Fig.4.71

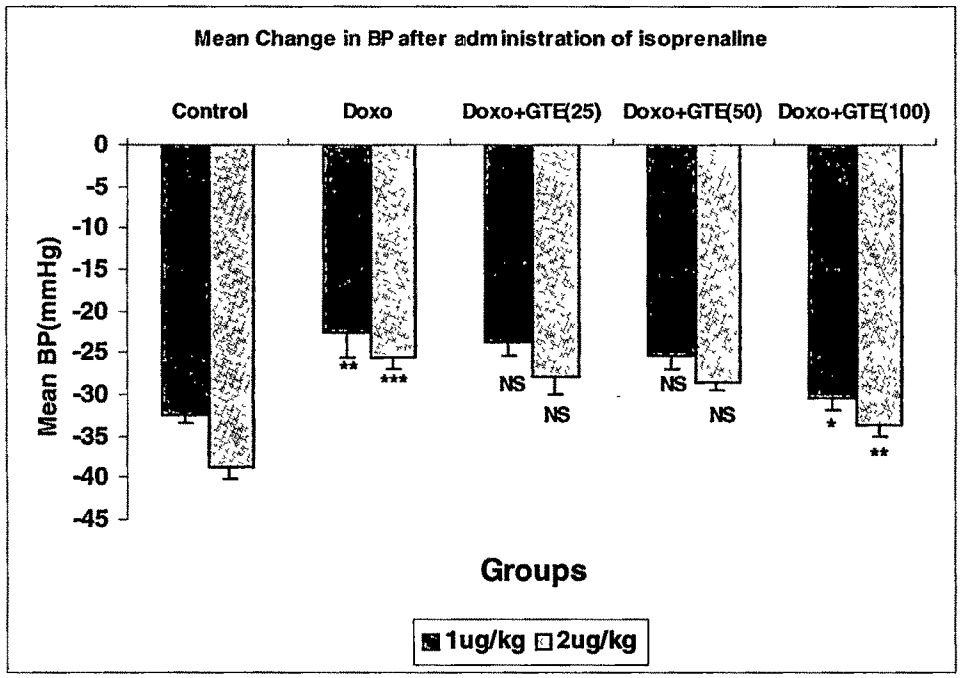


Fig.4.72

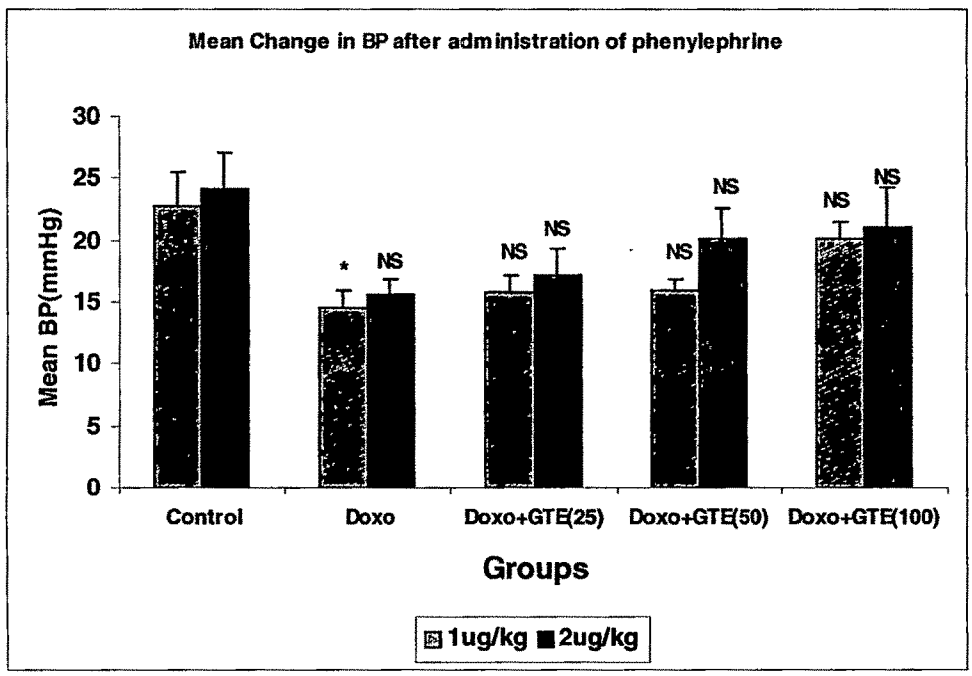


Fig.4.73

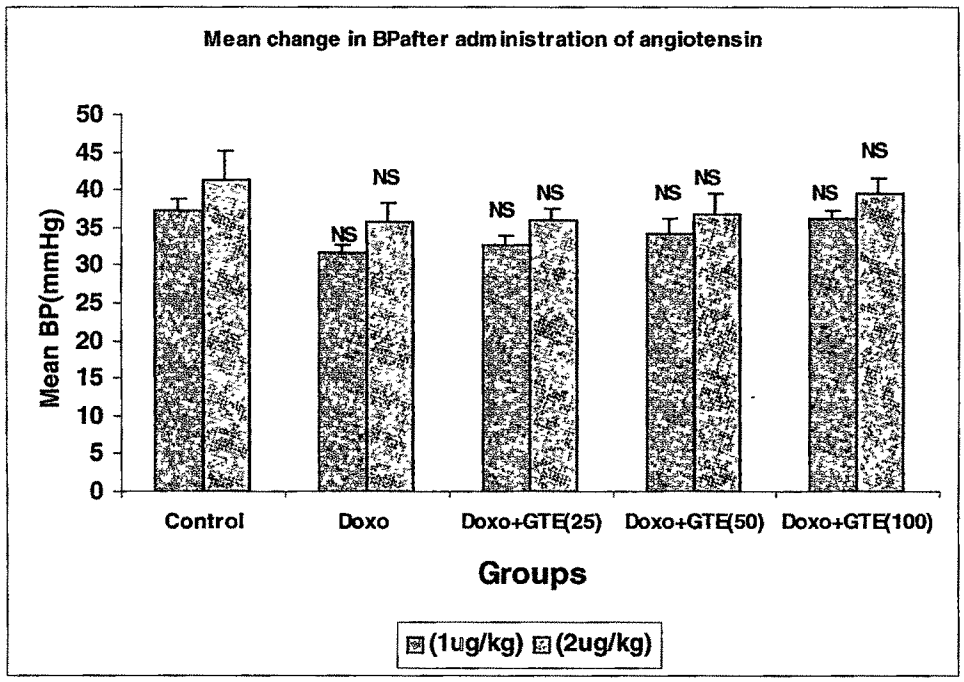

Mean change in BPafter administration of angiotensin
Mean BP(mmHg)
50
45
40
35
30
25
20
15
10
5
0
NS
Control
Doxo
Doxo+GTE(25)
Doxo+GTE(50)
Doxo+GTE(100)
Groups
(1ug/kg)
(2ug/kg)

Fig.4.74 a

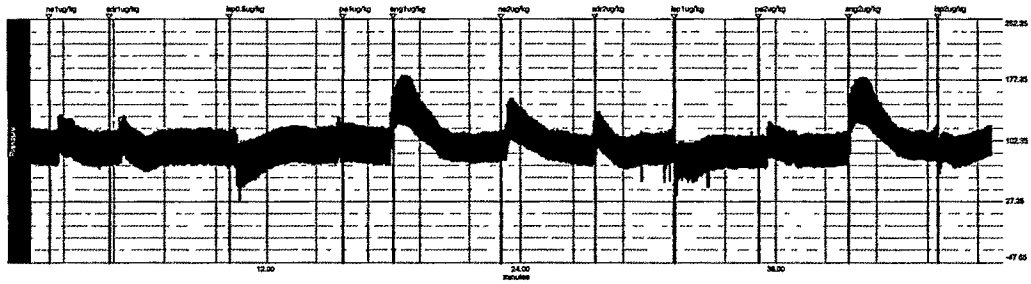


Fig.4.74 b

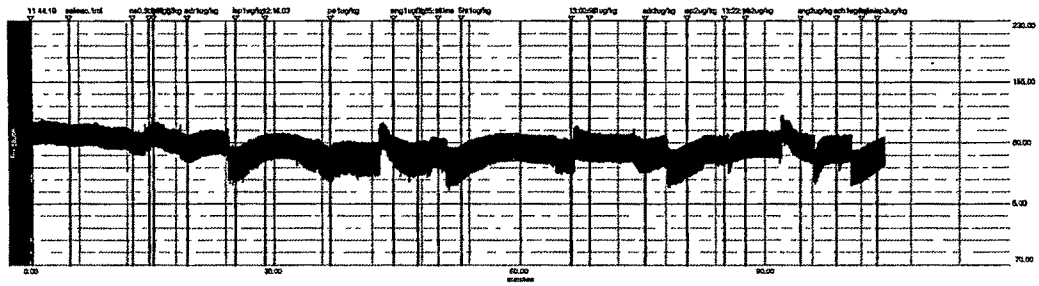


Fig.4.74 c

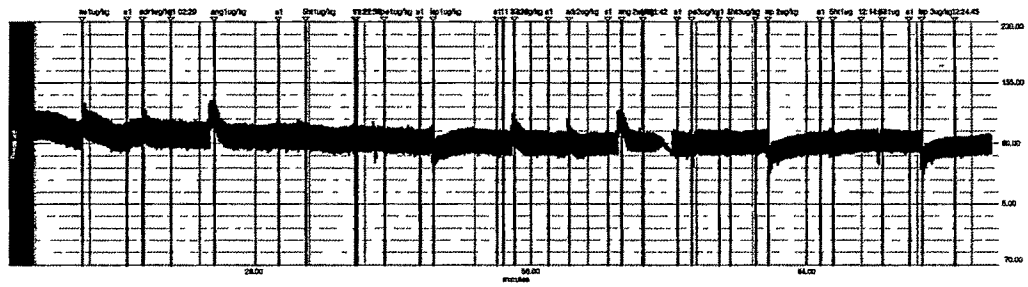


Fig.4.74 d

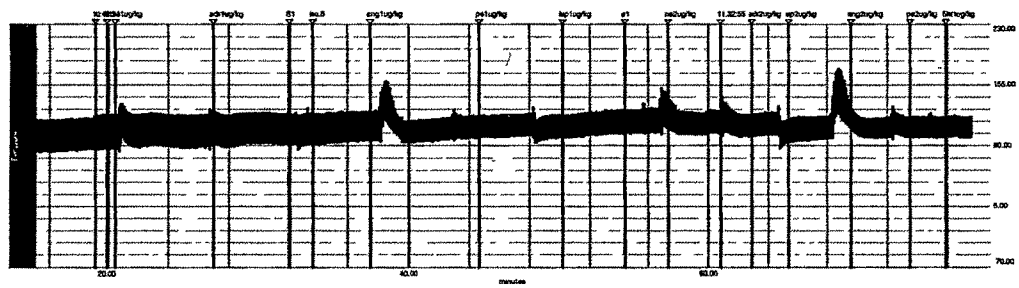


Fig.4.74 e

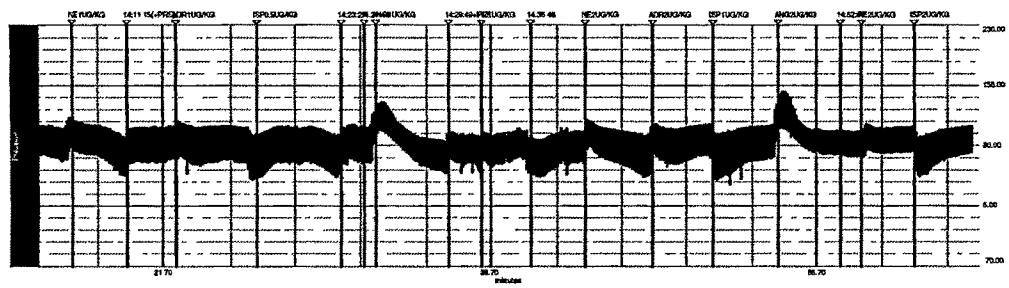


Fig.4.75: Effect of melatonin on vascular reactivity to noradrenaline (1 and 2 μg / kg) in animals chronically treated with doxorubicin.

Fig.4.76: Effect of melatonin on vascular reactivity to adrenaline (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.77: Effect of melatonin on vascular reactivity to isoprenaline (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.78: Effect of melatonin on vascular reactivity to phenylephrine (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.79: Effect of melatonin on vascular reactivity to angiotensin (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig 4.80: Tracings of arterial BP of anaesthetized rat after administration of noradrenaline, adrenaline, isoprenaline, phenylephrine and angiotensin.
4.80 a: Control group
4.80 b: Doxorubicin alone group
4.80 c: Doxo+ Mel (3) group
4.80 d: Doxo+ Mel (6) group

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.75

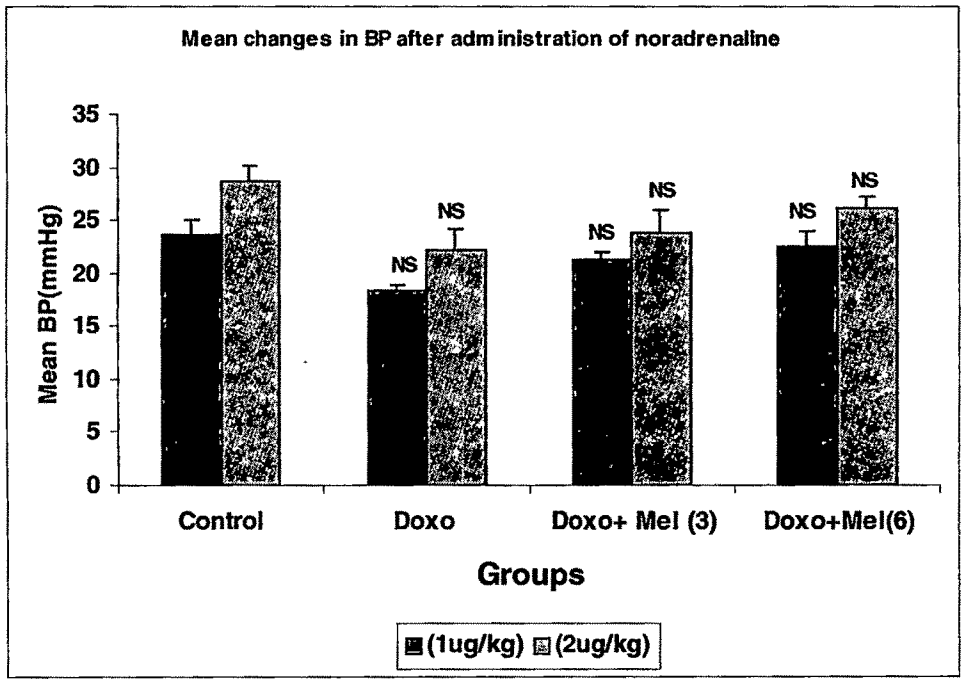


Fig.4.76

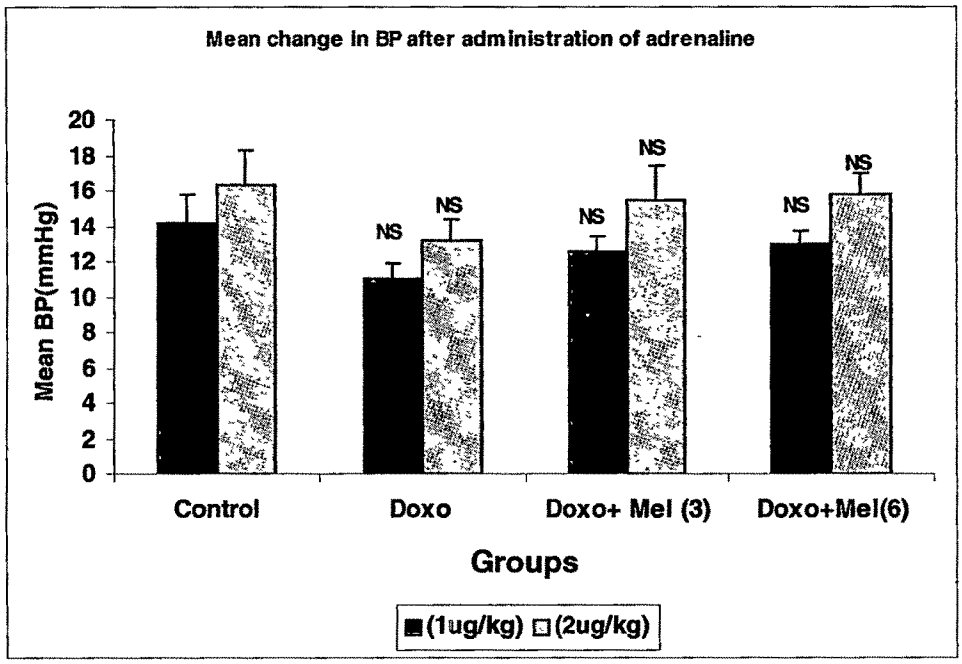


Fig.4.77

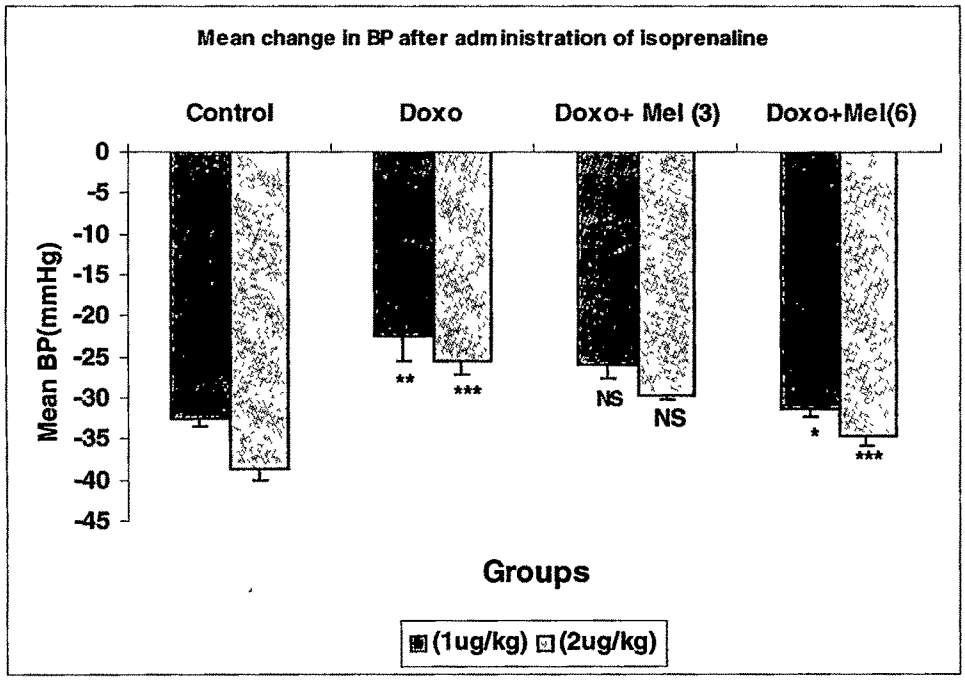


Fig.4.78

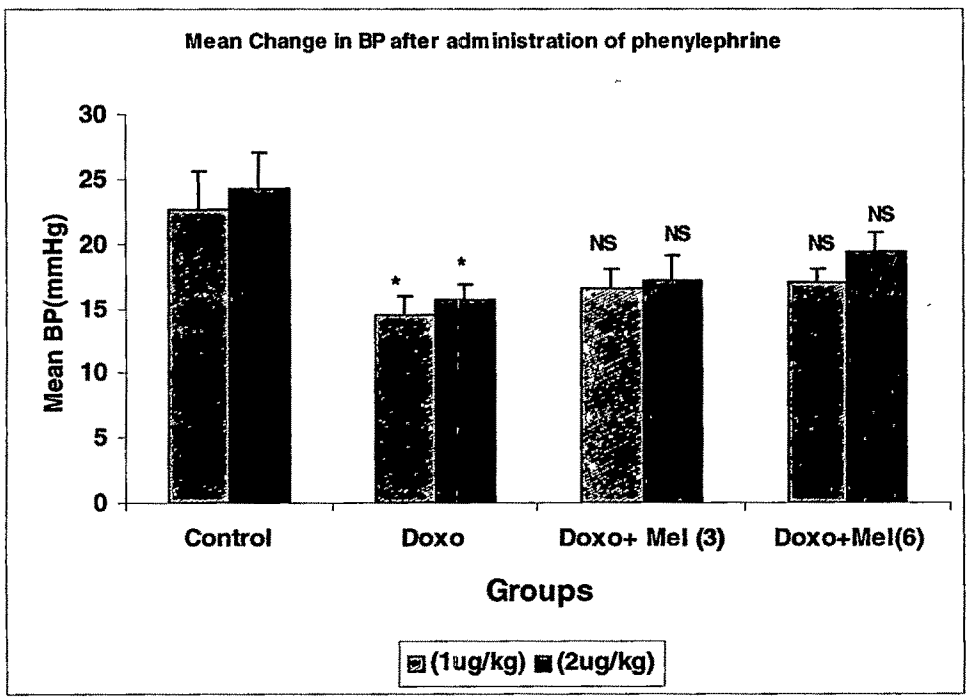


Fig.4.79

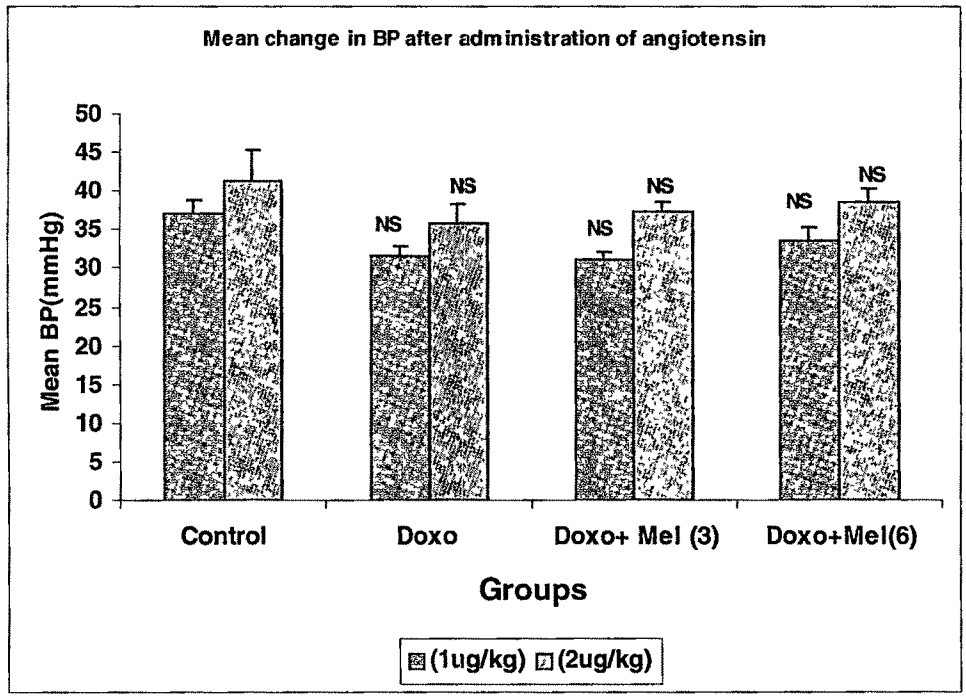

Mean change in BP after administration of angiotensin
Mean BP(mmHg)
50
45
40
35
30
25
20
15
10
5
0
NS
NS
NS
NS
NS
NS
Control
Doxo
Doxo+ Mel (3)
Doxo+Mel(6)
Groups
(1ug/kg)
(2ug/kg)

Fig.4.80 a

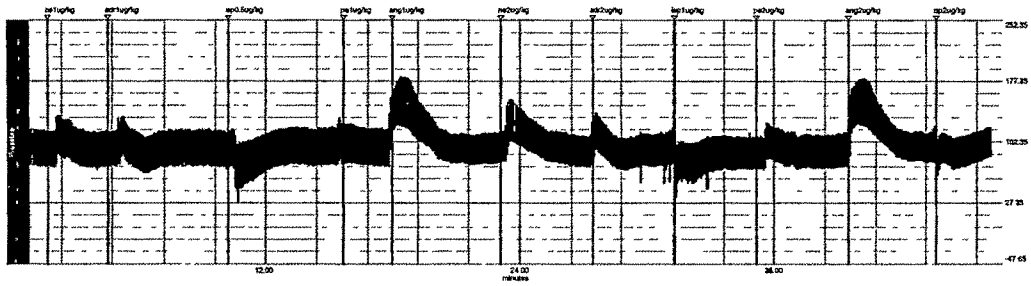


Fig.4.80 b

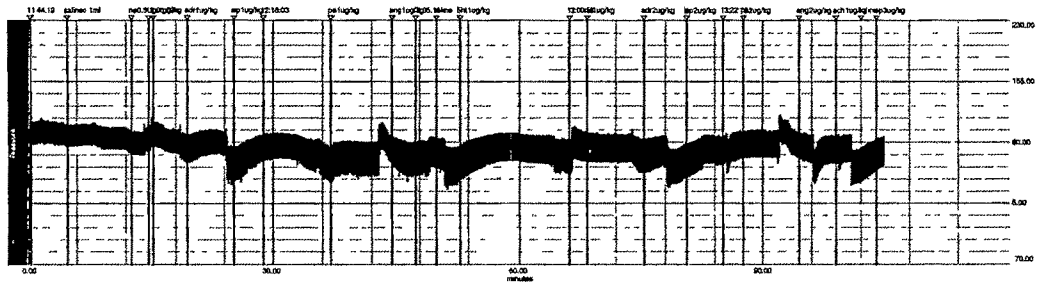


Fig.4.80 c

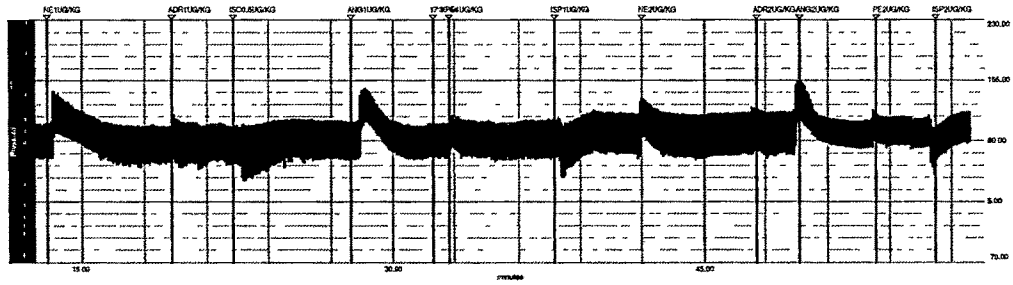


Fig.4.80 d

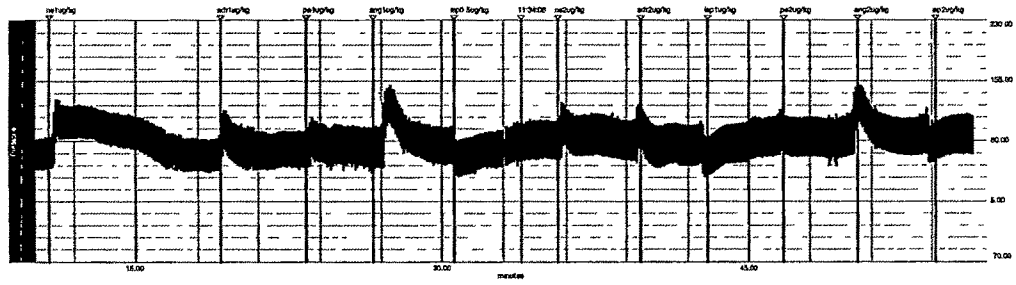


Fig.4.81: Effect of lovastatin on vascular reactivity to noradrenaline (1 and 2 μg / kg) in animals chronically treated with doxorubicin.

Fig.4.82: Effect of lovastatin on vascular reactivity to adrenaline (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.83: Effect of lovastatin on vascular reactivity to isoprenaline (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.84: Effect of lovastatin on vascular reactivity to phenylephrine (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig.4.85: Effect of lovastatin on vascular reactivity to angiotensin (1 and 2 μg/kg) in animals chronically treated with doxorubicin.

Fig 4.86: Tracings of arterial BP of anaesthetized rat after administration of noradrenaline, adrenaline, isoprenaline, phenylephrine and angiotensin.
4.86 a: Control group
4.86 b: Doxorubicin alone group
4.86 c: Doxo+ Lova (3) group
4.86 d: Doxo+ Lova (6) group

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control while groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.81

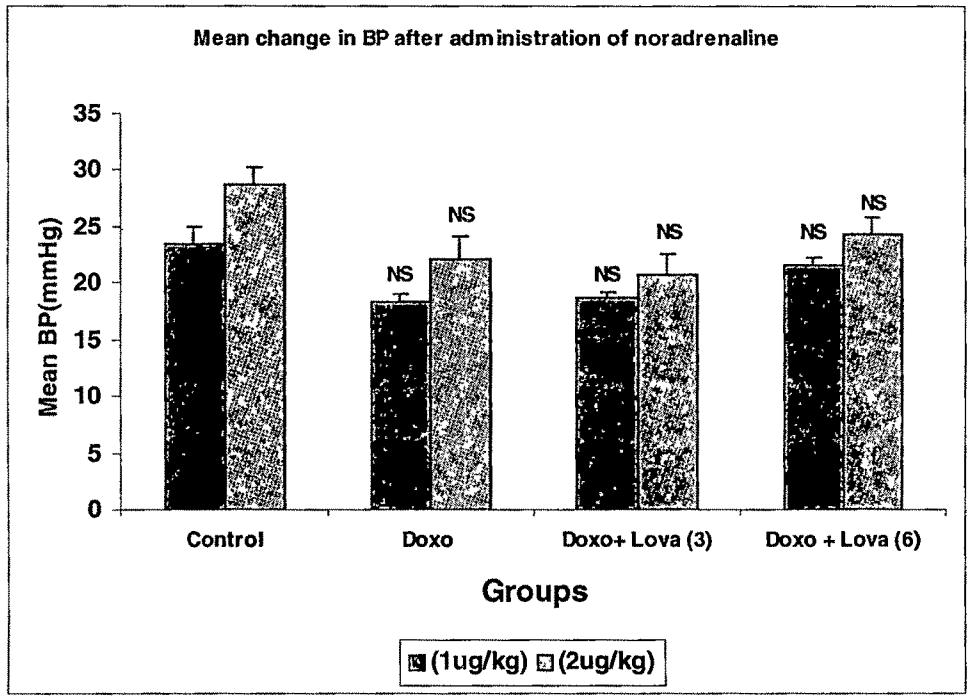


Fig.4.82

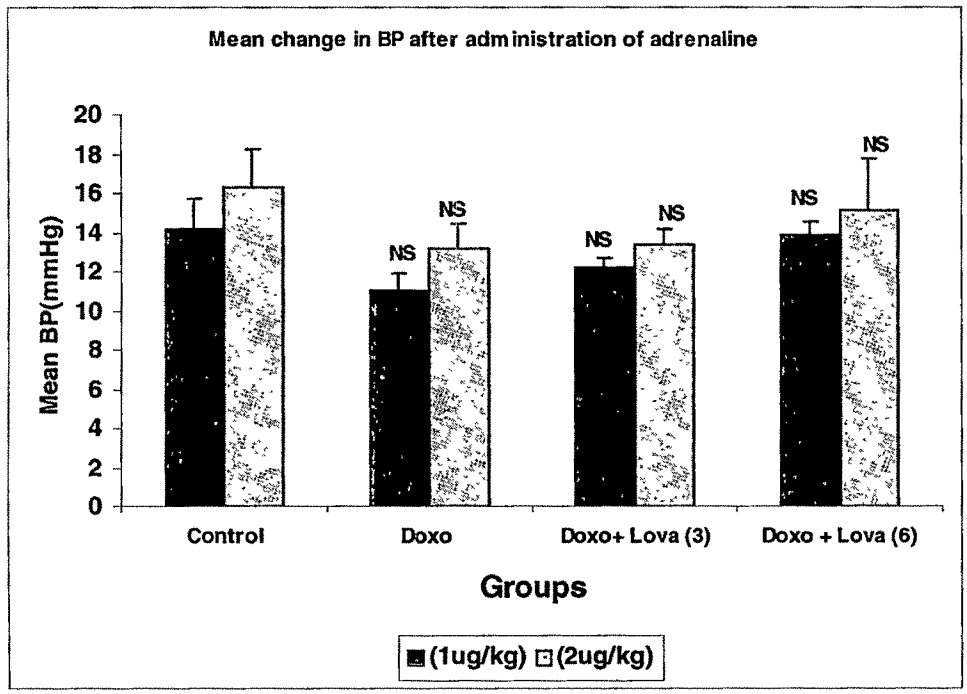
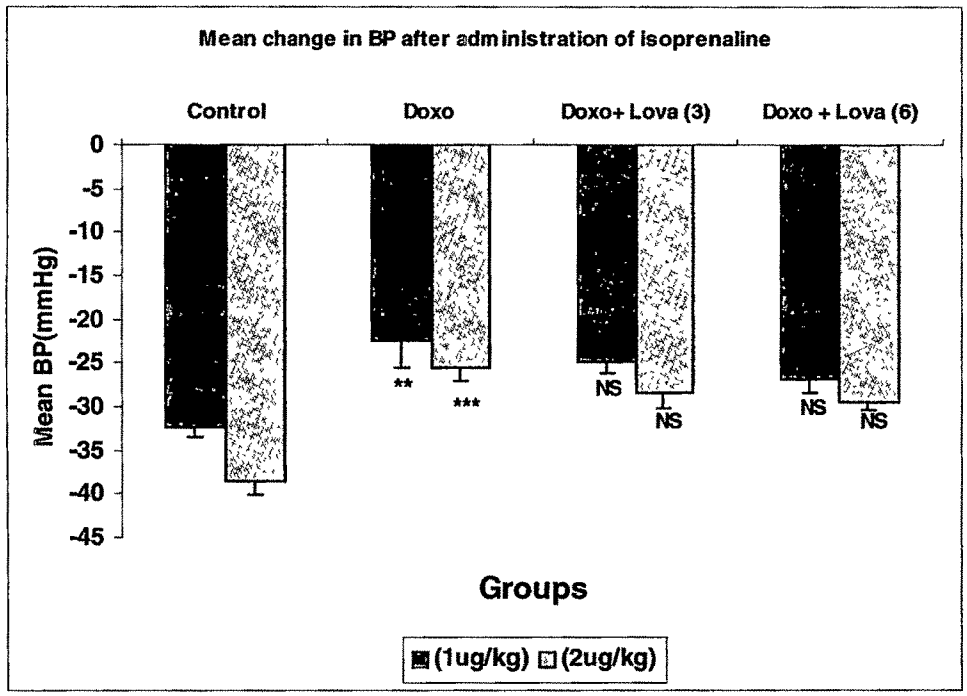


Fig.4.83



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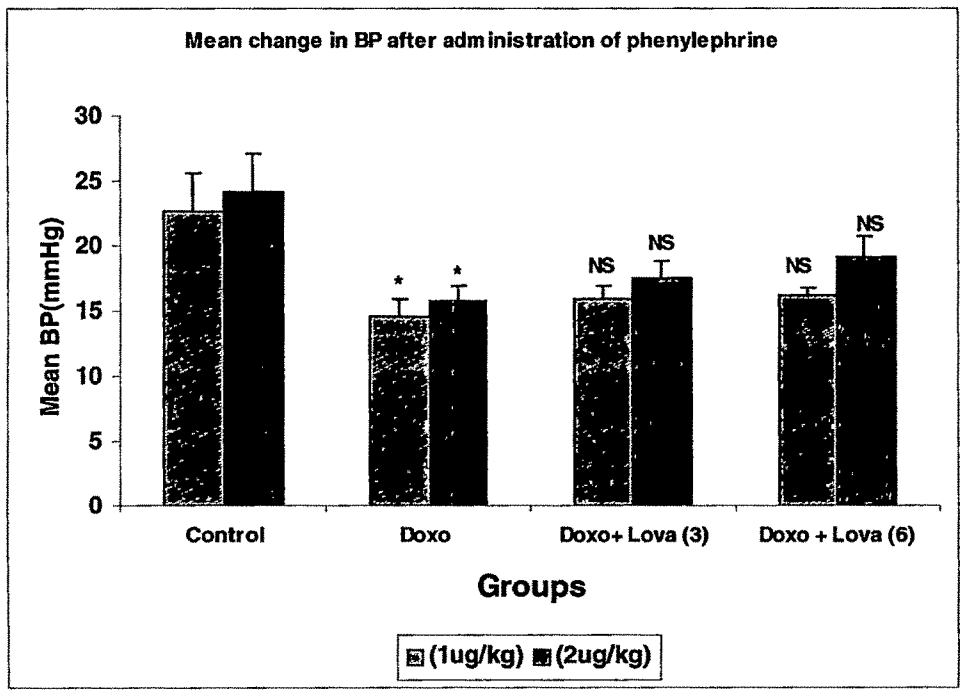


Fig.4.85

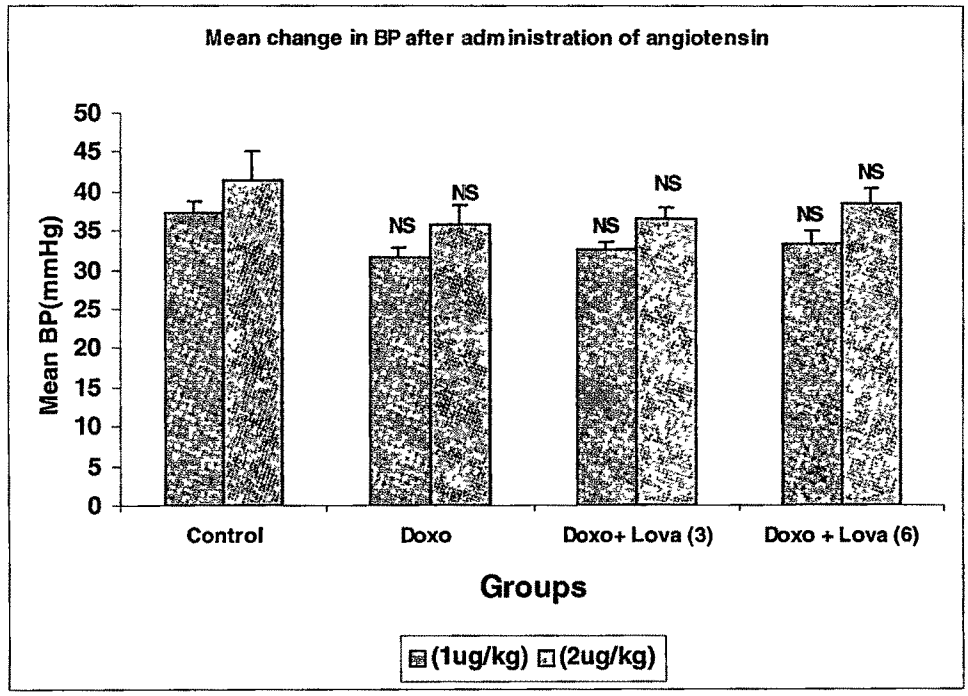

Mean change in BP after administration of angiotensin
Mean BP(mmHg)
50
45
40
35
30
25
20
15
10
5
0
NS
NS
NS
NS
NS
NS
Control
Doxo
Doxo+ Lova (3)
Doxo + Lova (6)
Groups
(1ug/kg)
(2ug/kg)

Fig.4.86 a

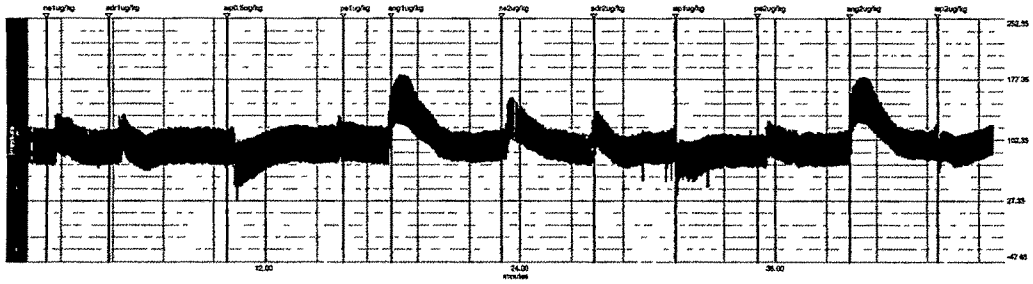


Fig.4.86 b

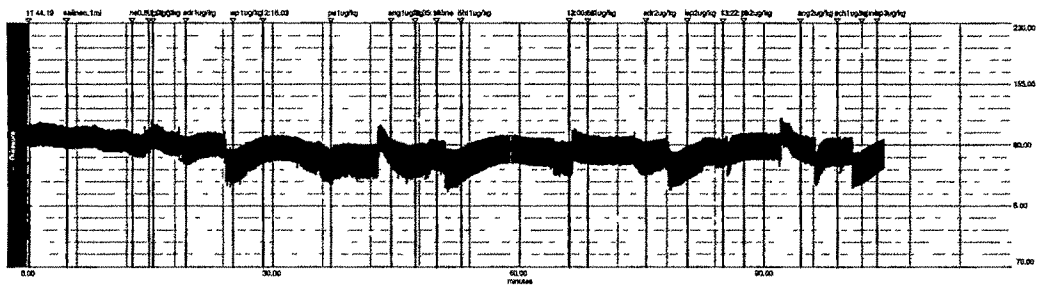


Fig.4.86 c

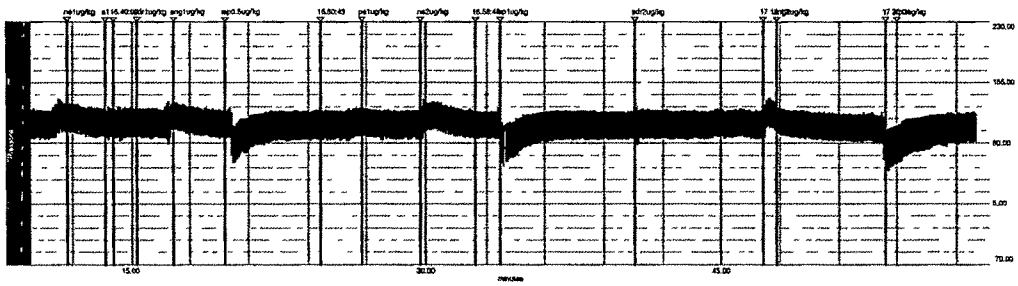
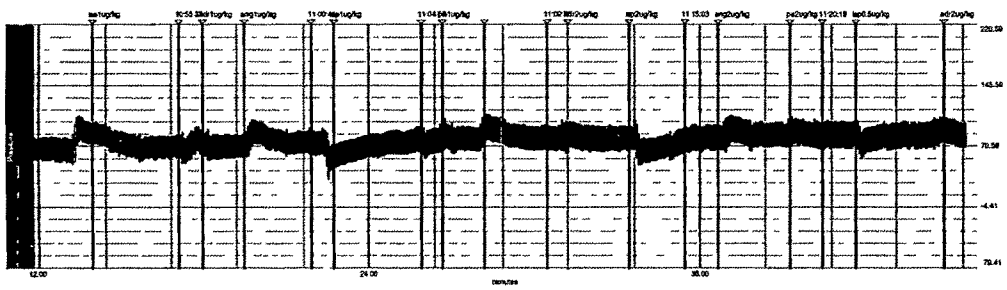


Fig.4.86 d



4.3.1.2.5 HISTOPATHOLOGY

Fig. 4.87(A) depicted the normal architecture of heart in rats of control group (Group 1) on histological examination. The figure reflects the organized arrangement, well-defined boundaries and distinct bundles of myocardial fibres.

A massive degenerative changes indicated by splaying and fragmentation of muscle fibers, edema and obvious infiltrations of inflammatory cells along with observation of cells with either pyknotic nuclei or anucleic cells and necrosis of heart muscle fibres was observed in animals chronically treated with doxorubicin (Group 2). Disorganized arrangements with no well-defined boundaries or distinct bundles of myocardial fibres were observed. [Fig. 4.87(B)].

Treatment of green tea extract along with chronic doxorubicin exhibited decreased degree of necrosis with less fragmentation of fibres and well-defined boundaries or distinct bundles of myocardial fibres with increasing doses [Fig. 4.87(C)-4.87(E)].

The degree of myocardial damage in melatonin treated group (3 mg/kg) was similar to doxorubicin treated group in regard to morphological changes showing occasional loss and fragmentation of muscle fibres with disorganized arrangement [Fig 4.88(B)]. With increasing dose of melatonin (6 mg/kg), there was lesser loss of myofibre. Bundles of myocardial fibres with more or less distinct boundaries were present [Fig 4.88(C) – Fig. 4.88 (D)].

Necrosis of heart muscle fibres along with focal loss and marked fragmentation similar to doxorubicin administered group was observed in lovastatin treated groups (3 mg/kg). Disorganized arrangement with no well-defined boundaries or distinct bundles of myocardial fibres was observed. Nuclei were scattered and were pyknotic in nature [Fig. 4.89(C)]. The degree of necrosis was reduced at the dose of 6 mg/kg [Fig. 4.89(D)]. Nuclei were not lost and were not pyknotic in nature.

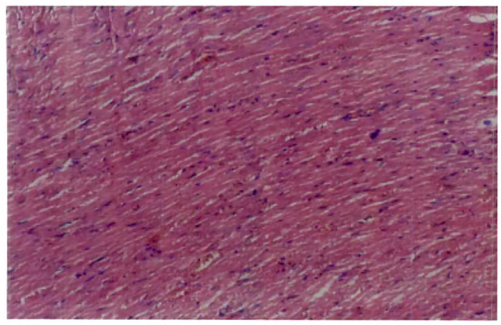
Fig. 4.87. Photomicrographs showing effect of green tea extract on the heart of doxorubicin treated rats (chronic study).

(Magnification 10 X)

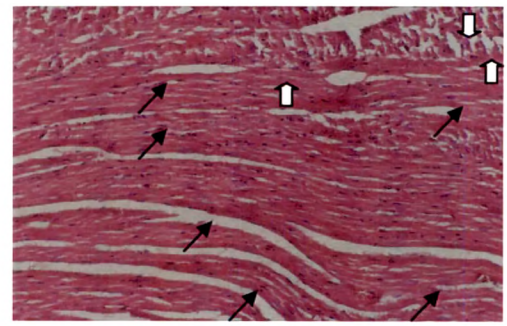
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

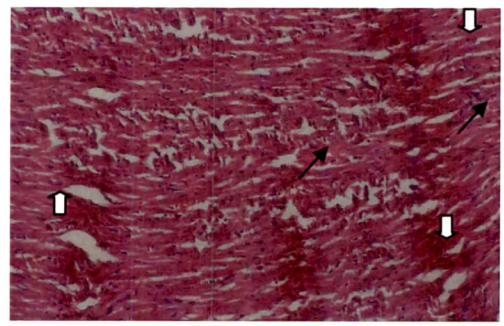
A: Normal control



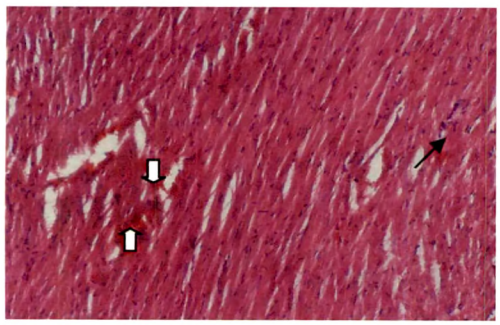
B: Doxorubicin (3mg/kg i.p.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

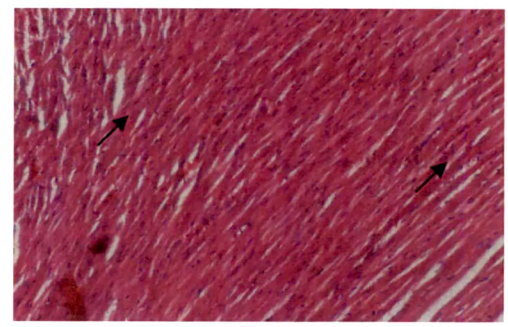


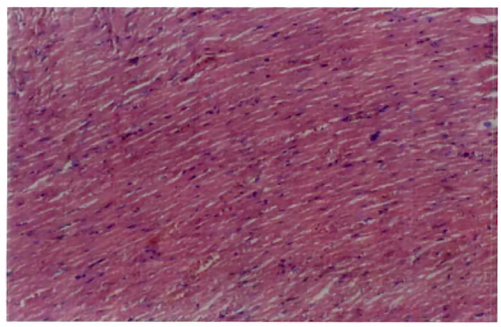
Fig. 4.88. Photomicrographs showing effect of melatonin on the heart of doxorubicin treated rats (chronic study).

(Magnification 10 X)

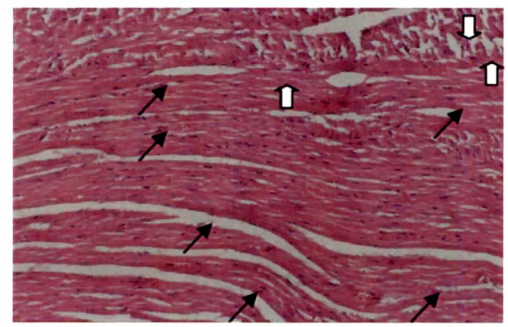
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

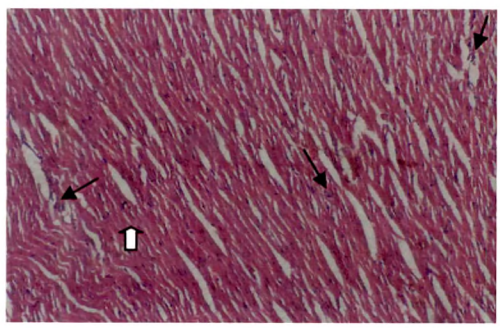
A: Normal control



B: Doxorubicin (3mg/kg i.p.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

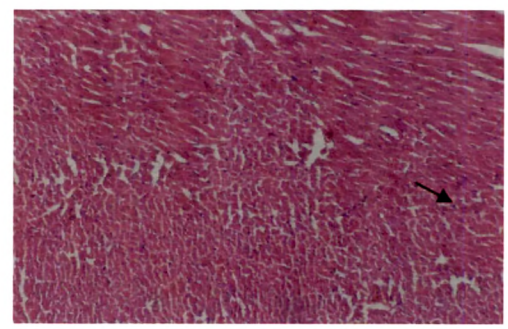


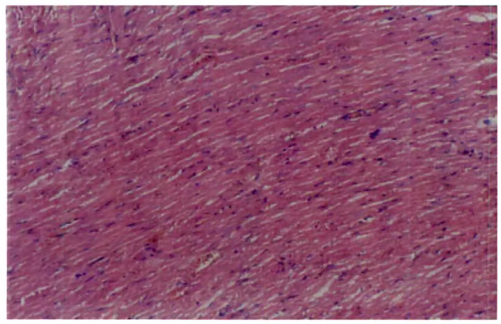
Fig. 4.89. Photomicrographs showing effect of lovastatin on the heart of doxorubicin treated rats (chronic study).

(Magnification 10 X)

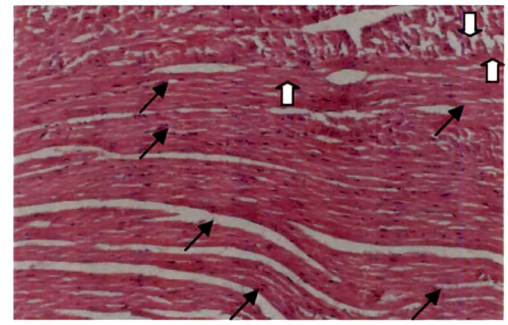
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

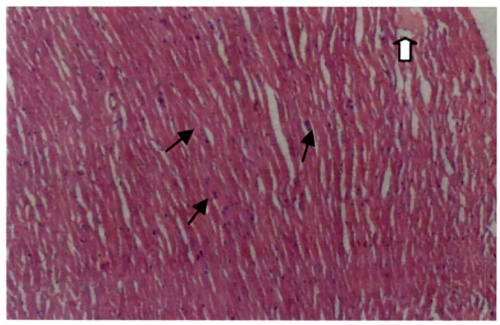
A: Normal control



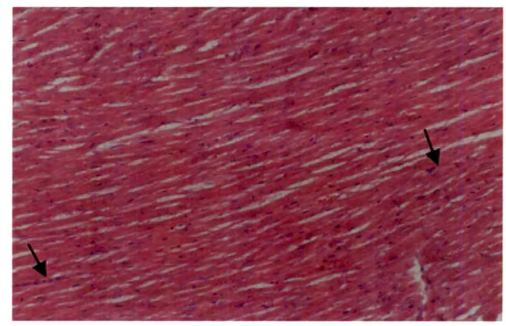
B: Doxorubicin (3mg/kg i.p.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.2 CISPLATIN INDUCED NEPHROTOXICITY

4.3.2.1: ACUTE STUDY IN RATS

4.3.2.1.1 SERUM PARAMETERS

4.3.2.1.1.1 Effect of drugs on Creatinine

Acute administration of cisplatin (5 mg/kg, i.p.) resulted in a significant (P<0.001) increase in the serum concentration of creatinine in rats (Group 2) as compared to control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg, i.p.) administration did not produce significant reduction in the level of serum creatinine; but the higher dose (100 mg/kg) of green tea extract significantly (P<0.01) decreased these levels when compared with cisplatin treated group (Fig.4.90).

Pretreatment of melatonin (3 and 6 mg/kg) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg, i.p.) administration did not produce a significant change in the level of serum creatinine as compared to cisplatin treated group (Fig.4.92, Fig.4.94).

4.3.2.1.1.2 Effect of drugs on uric acid

Acute cisplatin (5 mg/kg i.p.on 30th day) administration resulted in a significant (P<0.001) increase in the serum concentration of uric acid as compared to control group.

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce significant reduction in the level of serum uric acid; but at the higher dose (100 mg/kg), it significantly (P<0.05) decreased these levels when compared with cisplatin treated group (Fig.4.90).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce a significant decrease in the level of serum uric acid as compared to cisplatin treated group (Fig.4.92, Fig.4.94).

4.3.2.1.1.3 Effect of drugs on urea

Acute cisplatin (5 mg/kg i.p.on 30th day) treatment resulted in a significant (P<0.001) increase in the serum concentration of urea as compared to control group (Group 1).

Green tea extract pretreatment (25 and 50mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of serum urea; but at higher dose (100 mg/kg), it significantly (P<0.05) decreased the levels of urea as compared to cisplatin group (Fig.4.91).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce any significant change in the levels of serum urea; but at the higher dose (6 mg/kg), it significantly (P<0.01) decreased the levels of urea as compared to cisplatin group (Fig.4.93).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce a significant decrease in the level of serum urea as compared to cisplatin treated group (Fig.4.95).

4.3.2.1.1.4 Effect of drugs on blood urea nitrogen (BUN)

Acute cisplatin (5 mg/kg i.p.on 30th day) administration resulted in a significant (P<0.001) increase in the serum concentration of BUN as compared to control group (Group 1).

Green tea extract pretreatment (25 and 50mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of serum BUN; but at higher dose (100 mg/kg), it significantly (P<0.05) decreased the levels of BUN as compared to cisplatin group (Fig.4.91).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce any significant change in the levels of serum BUN; but at the higher dose (6 mg/kg), it significantly (P<0.01) decreased the levels of BUN as compared to cisplatin group (Fig.4.93).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce a significant decrease in the level of serum BUN as compared to cisplatin treated group (Fig.4.95).

Fig. 4.90: Effect of green tea extract on the serum levels of creatinine and uric acid in cisplatin induced nephrotoxicity (acute study) in rats.

Fig. 4.91: Effect of green tea extract on the serum levels of urea and BUN in cisplatin induced nephrotoxicity (acute study) in rats.

Fig. 4.92: Effect of melatonin on the serum levels of creatinine and uric acid in cisplatin induced nephrotoxicity (acute study) in rats.

Fig. 4.93: Effect of melatonin on the serum levels of urea and BUN in cisplatin induced nephrotoxicity (acute study) in rats.

Fig. 4.94: Effect of lovastatin on the serum levels of creatinine and uric acid in cisplatin induced nephrotoxicity (acute study) in rats.

Fig. 4.95: Effect of lovastatin on the serum levels of urea and BUN in cisplatin induced nephrotoxicity (acute study) in rats.

Values are expressed as mean ± SEM.
The group treated with cisplatin alone (Cis) was compared with control. Groups treated with green tea extract {Cis + GTE (25), Cis + GTE (50) and Cis + GTE (100)} were compared with Cis alone group.
Groups treated with melatonin {Cis + Mel (3) and Cis + Mel (6)} were compared with Cis alone group.
Groups treated with lovastatin {Cis + Lova (3) and Cis + Lova (6)} were compared with Cis alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig. 4.90

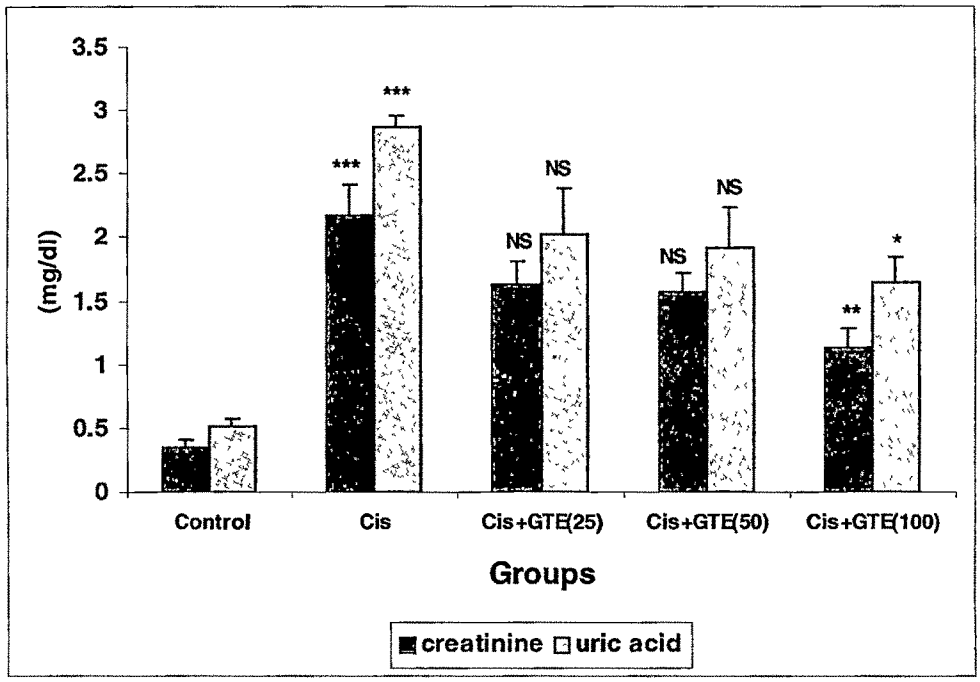


Fig. 4.91

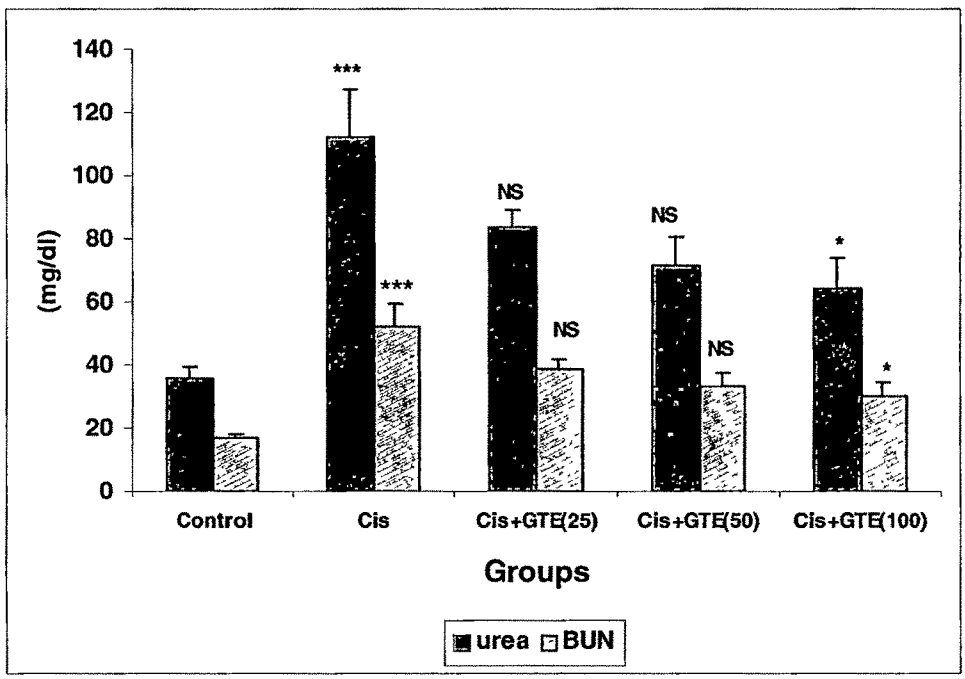


Fig. 4.92

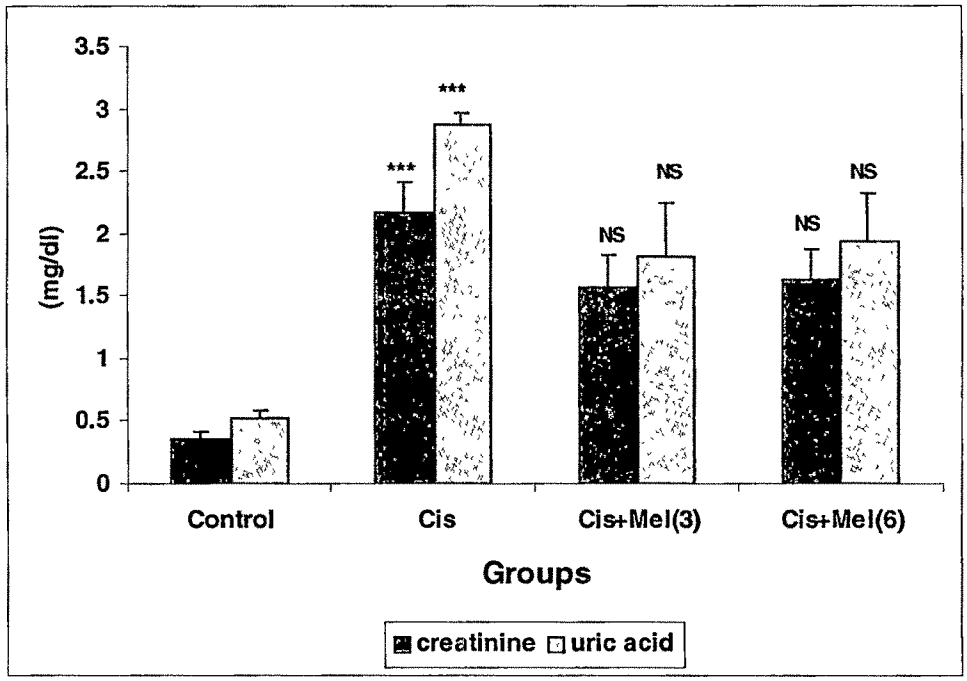


Fig. 4.93

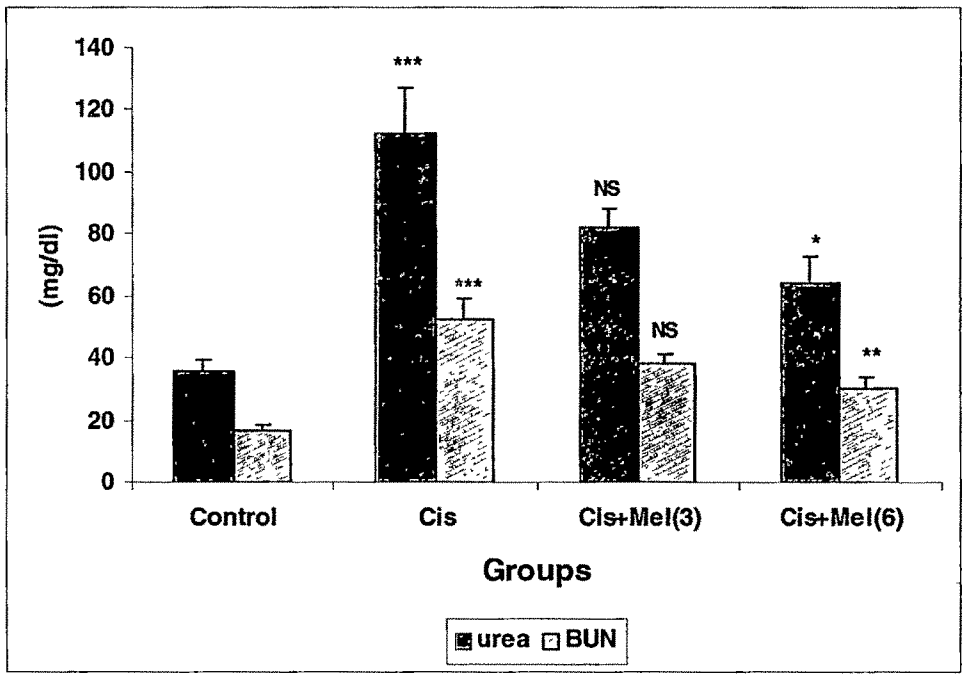


Fig. 4.94

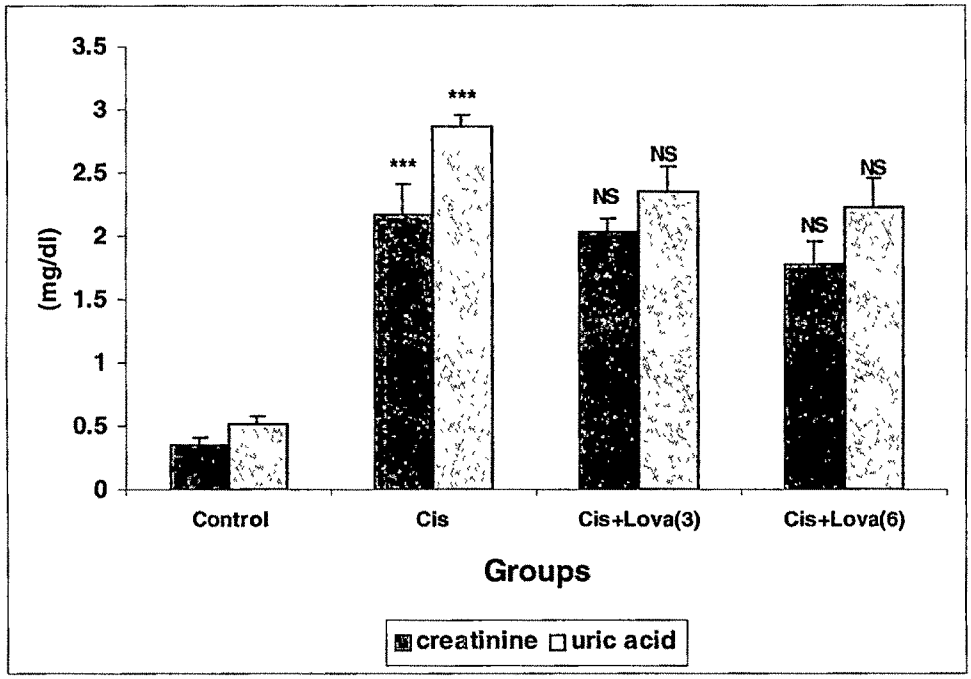
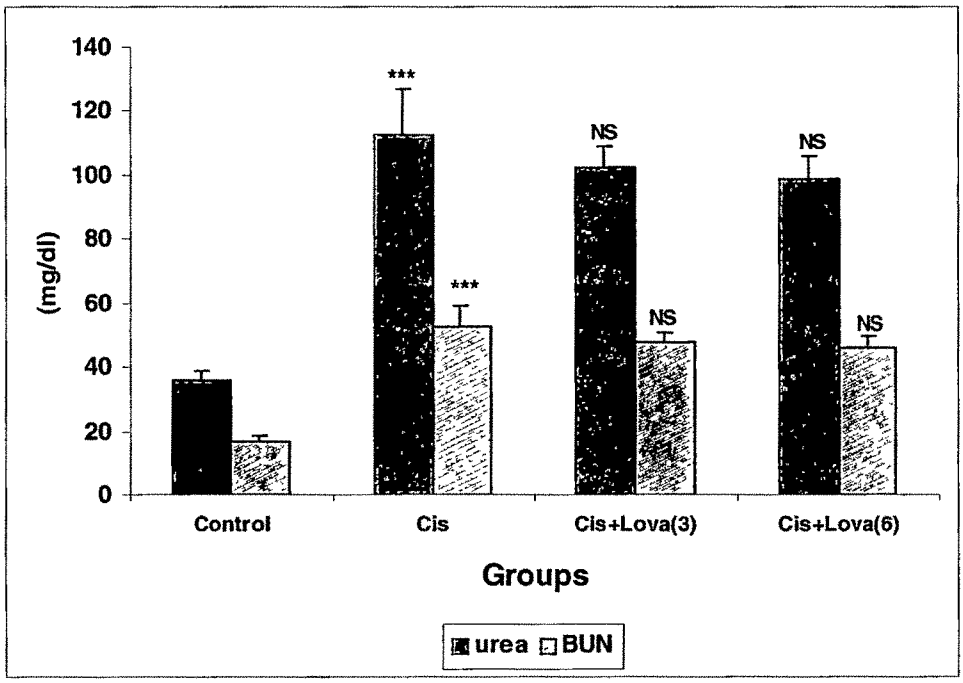


Fig. 4.95



4.3.2.1.2 TISSUE PARAMETERS

4.3.2.1.2.1 Effect on lipid peroxidation

Acute cisplatin (5 mg/kg i.p.) administration to rats led to a significant (P<0.001) increase in lipid peroxidation or MDA content in kidneys as compared to the control group (Group 1).

Pretreatment of green tea extract (25, 50, and 100 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration significantly (P<0.01, P<0.001) reduced the levels of MDA as compared to cisplatin treated group (Table 4.14).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration produced a significant (P<0.01, P<0.001) decrease in the MDA content as compared to cisplatin treated group (Table 4.15).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of as compared to the cisplatin group (Tables 4.16).

4.3.2.1.2.2 Effect on endogenous antioxidants

4.3.2.1.2.2.1 Effect on Superoxide dismutase

Acute cisplatin (5 mg/kg i.p.) administration reduced the SOD activity significantly (P<0.001) in kidneys of rats as compared to control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce any significant changes in the level of SOD. At the higher dose (100 mg/kg), led to a significant (P<0.01) rise in SOD content as compared to cisplatin treated group (Tables 4.14).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of SOD as compared to the cisplatin group (Tables 4.15).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of SOD as compared to the cisplatin group (Tables 4.16).

4.3.2.1.2.2.2 Effect on Catalase

The catalase activity in cisplatin (5 mg/kg i.p.on 30th day) treated group (Group 2) was significantly (P<0.001) reduced as compared to control group (Group 1).

Pretreatment of green tea extract (25 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce significant changes in the level of catalase. At the higher doses (50 and 100 mg/kg/day p.o. for 30 day), led to a significant (P<0.05,P<0.01) rise in catalase level as compared to cisplatin treated group (Tables 4.14).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of catalase as compared to the cisplatin group (Tables 4.15).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute (5 mg/kg i.p.on 30th day) cisplatin administration did not significantly alter the levels of catalase as compared to the cisplatin group (Tables 4.16).

4.3.2.1.2.2.3 Effect on Reduced glutathione

A significant (P<0.001) reduction in reduced glutathione concentration was observed in cisplatin (5 mg/kg i.p.on 30th day) treated rats (Group 2) as compared to the normal control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce any significant changes in the level of GSH. At the higher dose (100 mg/kg), led to a significant (P<0.05) rise in GSH content as compared to cisplatin treated group (Tables 4.14).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not produce any significant changes in the level of GSH. At the higher dose (6 mg/kg), led to a significant (P<0.01) rise in GSH level as compared to cisplatin treated group (Tables 4.15).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) administration did not significantly alter the levels of GSH as compared to the cisplatin group (Tables 4.16).

Table 4.14: Effect of green tea extract on the levels of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the kidney of rats in cisplatin induced nephrotoxicity (acute study).

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	2.0±0.08	4.25±0.54	2.95±0.49	3.82±0.15
Group 2	3.56±0.27***	1.72±0.1***	0.52±0.13***	2.07±0.21***
Group 3	2.54±0.13**	2.26±0.27^{NS}	0.86±0.07^{NS}	2.38±0.13^{NS}
Group 4	2.17±0.15***	2.71±0.23^{NS}	1.53±0.27^{NS}	2.80±0.21*
Group 5	1.75±0.19***	3.33±0.25*	1.99±0.24**	3.05±0.12**
F value	**15.55**	**9.63**	**11.65**	**15.38**
P value	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p. on 30th day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with cisplatin alone group.

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.15: Effect of melatonin on the levels of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the kidney of rats in cisplatin induced nephrotoxicity (acute study).

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	2.00±0.08	4.25±0.54	2.95±0.49	3.82±0.15
Group 2	3.56±0.27***	1.72±0.1***	0.52±0.13***	2.07±0.21***
Group 3	2.46±0.20**	2.64±0.33^{NS}	0.94±0.08^{NS}	2.17±0.12^{NS}
Group 4	2.22±0.20***	3.57±0.23**	1.62±0.24^{NS}	2.71±0.26^{NS}
F value	**11.56**	**10.32**	**13.8**	**16.5**
P value	**P=0.0001**	**P=0.0003**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p.on 30th day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.16: Effect of lovastatin on the levels of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the kidney of rats in cisplatin induced nephrotoxicity (acute study).

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	2.0±0.08	4.25±0.54	2.95±0.49	3.82±0.15
Group 2	3.56±0.27**	1.72±0.1***	0.52±0.13***	2.07±0.21***
Group 3	2.71±0.2 NS	1.80±0.26 NS	0.99±0.17 NS	2.07±0.12NS
Group 4	2.05±0.12 NS	2.50±0.25 NS	1.21±0.18 NS	2.33±0.2NS
F value	**6.11**	**9.87**	**12.98**	**13.91**
P value	**P=0.0014**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p. on 30th day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.2.1.2.3 Effect on membrane bound enzymes

4.3.2.1.2.3.1 Effect on Sodium Potassium ATPase

In the kidneys of cisplatin (5 mg/kg i.p.) treated rats (Group 2) the activity of Na^+K^+ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not produce any significant increase in Na^+K^+ATPase levels; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the levels of the enzyme as compared to cisplatin treated group (Table 4.17).

Pretreatment of melatonin (3 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not produce any significant change in Na^+K^+ATPase levels; but the higher dose (6 mg/kg) of melatonin significantly ($P<0.05$) increased the levels of the enzyme as compared to cisplatin treated group (Table 4.18).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not produce any significant increase in the levels of Na^+K^+ATPase as compared to the cisplatin treated group (Tables 4.19).

4.3.2.1.2.3.2 Effect on Calcium ATPase

Acute administration of cisplatin (5 mg/kg i.p.on 30th day) resulted in a significant ($P<0.001$) decrease in the Ca^{2+}ATPase activity as compared to the control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day), melatonin (3 and 6 mg/kg/day p.o.for 30 days) and lovastatin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not significantly alter the levels of Ca^{2+}ATPase compared to cisplatin treated group (Tables 4.17, 4.18, and 4.19).

4.3.2.1.2.3.3 Effect on Magnesium ATPase

Acute administration of cisplatin (5 mg/kg i.p.on 30th day) resulted in a significant ($P<0.001$) decrease in the Mg^{2+}ATPase activity as compared to the control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not produce any significant increase in Mg^{2+}ATPase levels; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the levels of the enzyme as compared to cisplatin treated group (Table 4.17).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o.for 30 days) and lovastatin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not significantly alter the levels of Mg^{2+}ATPase as compared to cisplatin treated group (Tables 4.18, and 4.19).

Table 4.17: Effect of green tea extract on membrane bound enzymes in the kidney of rats in cisplatin induced nephrotoxicity (acute study).

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	12.67±0.61	6.98±0.24	9.98±0.38
Group 2	8.55±0.62***	4.65±0.37**	4.80±0.37***
Group 3	8.70±0.17 NS	5.20±0.50 NS	5.56±0.28NS
Group 4	9.45±0.38NS	6.24±0.37 NS	6.29±0.46NS
Group 5	10.58±0.48*	6.33±0.53 NS	6.74±0.40*
F value	**12.16**	**5.003**	**26.36**
P value	**P<0.0001**	**P=0.0042**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p. on 30th day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.18: Effect of melatonin on membrane bound enzymes in the kidney of rats in cisplatin induced nephrotoxicity (acute study).

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	12.67±0.61	6.98±0.24	9.98±0.38
Group 2	8.55±0.62***	4.65±0.37***	4.80±0.37***
Group 3	9.34±0.42^{NS}	4.78±0.34^{NS}	5.90±0.46^{NS}
Group 4	10.75±0.3*	5.64±0.13^{NS}	5.98±0.28^{NS}
F value	**12.49**	**13.60**	**35.5**
P value	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p.on 30th day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.19: Effect of lovastatin on membrane bound enzymes in the kidney of rats in cisplatin induced nephrotoxicity (acute study).

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	12.67±0.61	6.98±0.24	9.98±0.38
Group 2	8.55±0.62***	4.65±0.37**	4.80±0.37***
Group 3	8.54±0.32^{NS}	4.73±0.39^{NS}	4.87±0.39^{NS}
Group 4	9.09±0.32^{NS}	5.44±0.42 ^{NS}	4.98±0.52^{NS}
F value	**14.56**	**7.96**	**21.91**
P value	**P<0.0001**	**P=0.0003**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p. on 30th day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg, i.p. on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.2.1.3 HISTOPATHOLOGY

Fig. 4.96(A) showed kidney from control rats with normal morphology of the proximal tubule located in the outer stripe of the outer medulla. The tubules were lined up with cells forming a proper lumen inside.

Acute cisplatin (5 mg/kg i.p.) administration to the rats revealed a remarkable proximal tubular necrosis, with extensive epithelial vacuolization, swelling and tubular dilation as compared to control. Widespread necrosis and sloughing of proximal tubular epithelial cells was noted.

Pretreatment with green tea extract (25 50 and 100 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration (Fig. 4.96(C) to Fig. 4.96(E)] and melatonin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration [Fig. 4.97(C) to Fig. 4.96(D)] decreased the necrosis with increasing doses and the cell structure regained to normal with the increasing doses of the drug.

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 days) followed by acute cisplatin (5 mg/kg, i.p. on 30th day) administration did not protect the kidney from cisplatin induced histopathological changes [Fig. 4.98(C) to Fig. 4.98(D)].

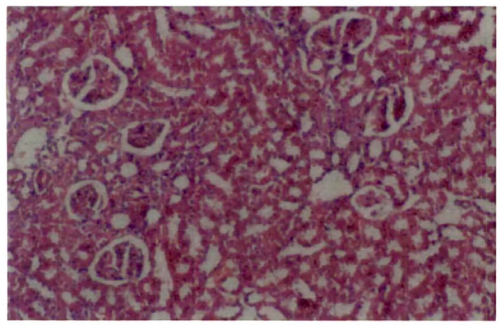
Fig. 4.96: Photomicrographs showing effect of green tea extract on the kidney of cisplatin treated rats (acute study).

(Magnification 10 X)

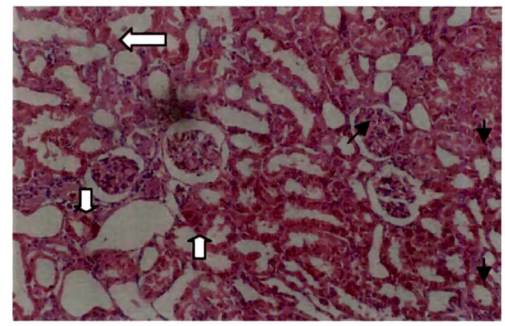
⇒ **Epithelial cell vacuolization**

→ **Proximal tubule dilation**

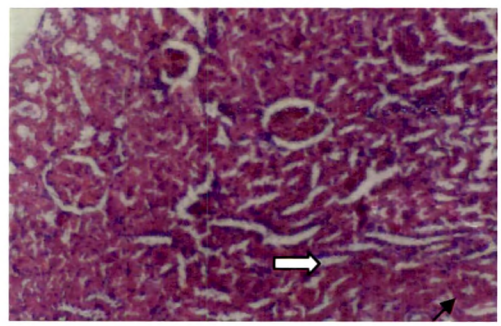
A: Normal Control



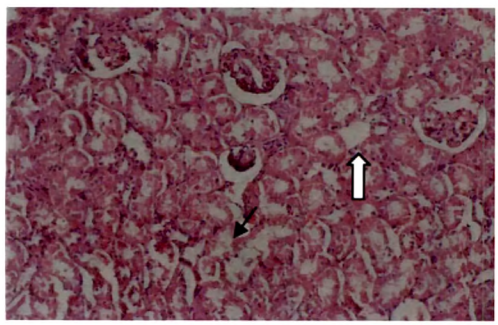
B: Cisplatin (5mg/kg, i.p.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

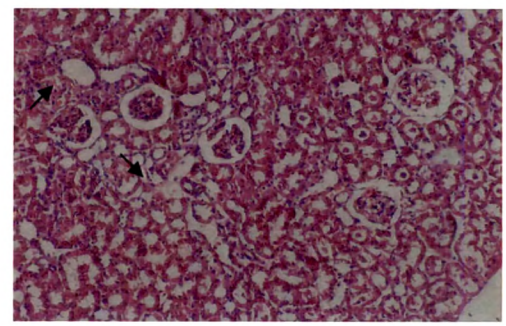


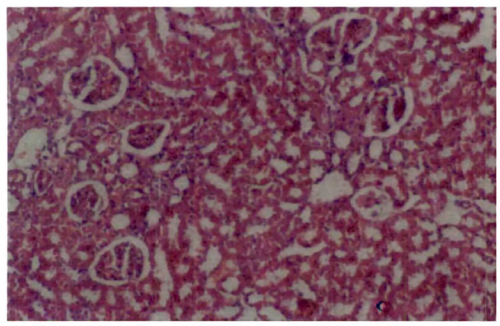
Fig. 4.97: Photomicrographs showing effect of melatonin on the kidney of cisplatin treated rats (acute study).

(Magnification 10 X)

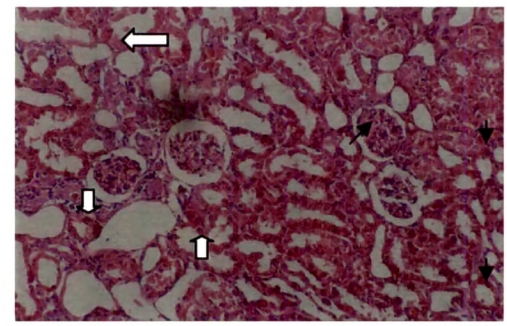
⇨ **Epithelial cell vacuolization**

→ **Proximal tubule dilation**

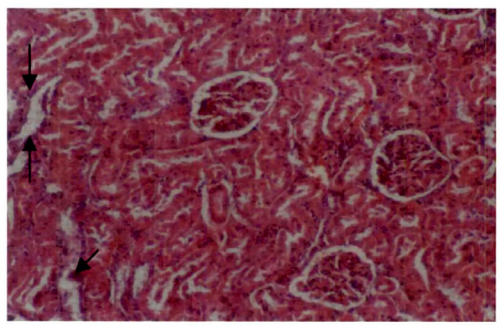
A: Normal Control



B: Cisplatin (5mg/kg, i.p.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

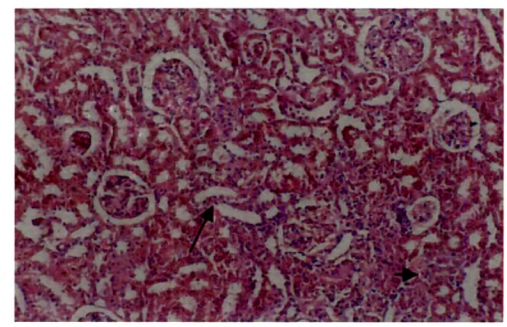


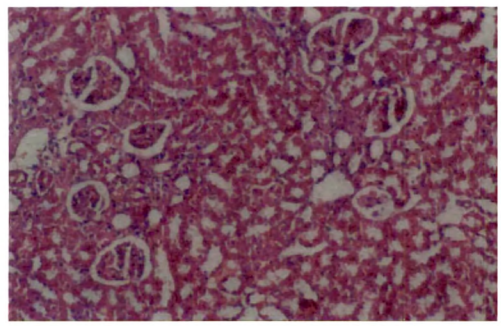
Fig. 4.98: Photomicrographs showing effect of lovastatin on the kidney of cisplatin treated rats (acute study).

(Magnification 10 X)

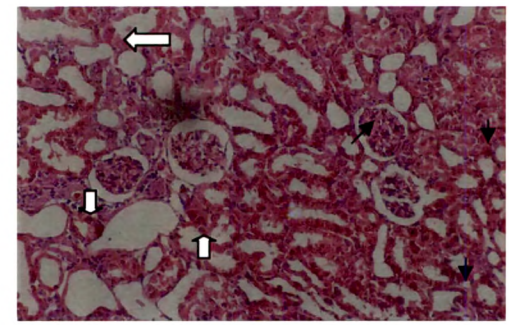
⟹ **Epithelial cell vacuolization**

⟶ **Proximal tubule dilation**

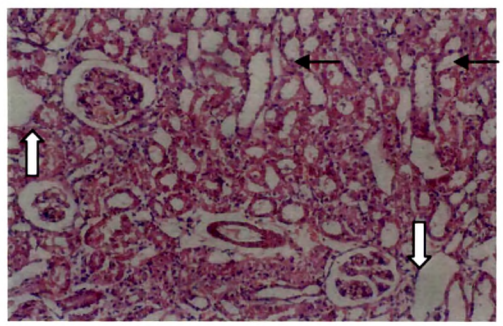
A: Normal Control



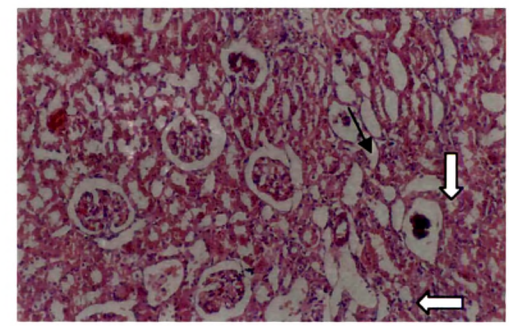
B: Cisplatin (5mg/kg, i.p.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.2 CISPLATIN INDUCED NEPHROTOXICITY

4.3.2.2: CHRONIC STUDY IN RATS

4.3.2.2.1 SERUM PARAMETERS

4.3.2.2.1.1 Effect of drugs on Creatinine

Chronic administration of cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) resulted in a significant (P<0.001) increase in the serum concentration of creatinine in rats (Group 2) as compared to control group (Group 1).

Treatment of green tea extract (25, 50, and 100 mg/kg/day p.o. for 30 day) along with chronic cisplatin (3 mg/kg i.p.on 1, 7,14,21 and 28 day) administration produced a significant (P<0.001) decrease in the levels of serum creatinine as compared to cisplatin treated group (Fig. 4.99).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration significantly (P<0.01,P<0.001) reduced the levels of creatinine as compared to cisplatin control (Fig. 4.101).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce a significant decrease in the level of serum creatinine as compared to cisplatin treated group (Fig.4.103).

4.3.2.2.1.2 Effect of drugs on uric acid

Chronic administration of cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) resulted in a significant (P<0.001) increase in the serum concentration of uric acid as compared to control group.

Treatment of green tea extract (25, 50, and 100 mg/kg/day p.o. for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration produced a significant (P<0.001) decrease in the levels of serum uric acid as compared to the cisplatin treated group (Fig. 4.99).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day)

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant change in the levels of serum BUN; but the higher dose (6 mg/kg) of melatonin significantly ($P<0.01$) decreased the levels of BUN as compared to cisplatin group (Fig.4.102).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin treatment did not produce a significant decrease in the level of serum BUN as compared to cisplatin treated group (Fig.4.104).

Fig. 4.99: Effect of green tea extract on the serum levels of creatinine and uric acid in cisplatin induced nephrotoxicity (chronic study) in rats.

Fig. 4.100: Effect of green tea extract on the serum levels of urea and BUN in cisplatin induced nephrotoxicity (chronic study) in rats.

Fig. 4.101: Effect of melatonin on the serum levels of creatinine and uric acid in cisplatin induced nephrotoxicity (chronic study) in rats.

Fig. 4.102: Effect of melatonin on the serum levels of urea and BUN in cisplatin induced nephrotoxicity (chronic study) in rats.

Fig. 4.103: Effect of lovastatin on the serum levels of creatinine and uric acid in cisplatin induced nephrotoxicity (chronic study) in rats.

Fig. 4.104: Effect of lovastatin on the serum levels of urea and BUN in cisplatin-induced nephrotoxicity (chronic study) in rats.

Values are expressed as mean ± SEM.
The group treated with cisplatin alone (Cis) was compared with control.
Groups treated with green tea extract {Cis + GTE (25), Cis + GTE (50) and Cis + GTE (100)} were compared with Cis alone group.
Groups treated with melatonin {Cis + Mel (3) and Cis + Mel (6)} were compared with Cis alone group.
Groups treated with lovastatin {Cis + Lova (3) and Cis + Lova (6)} were compared with Cis alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig. 4.99

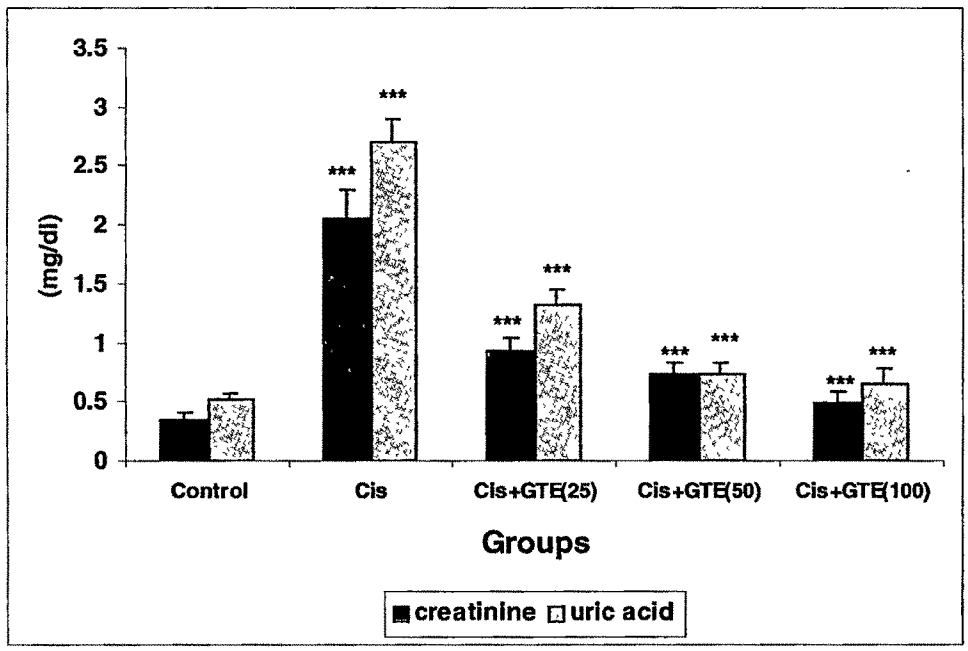


Fig. 4.100

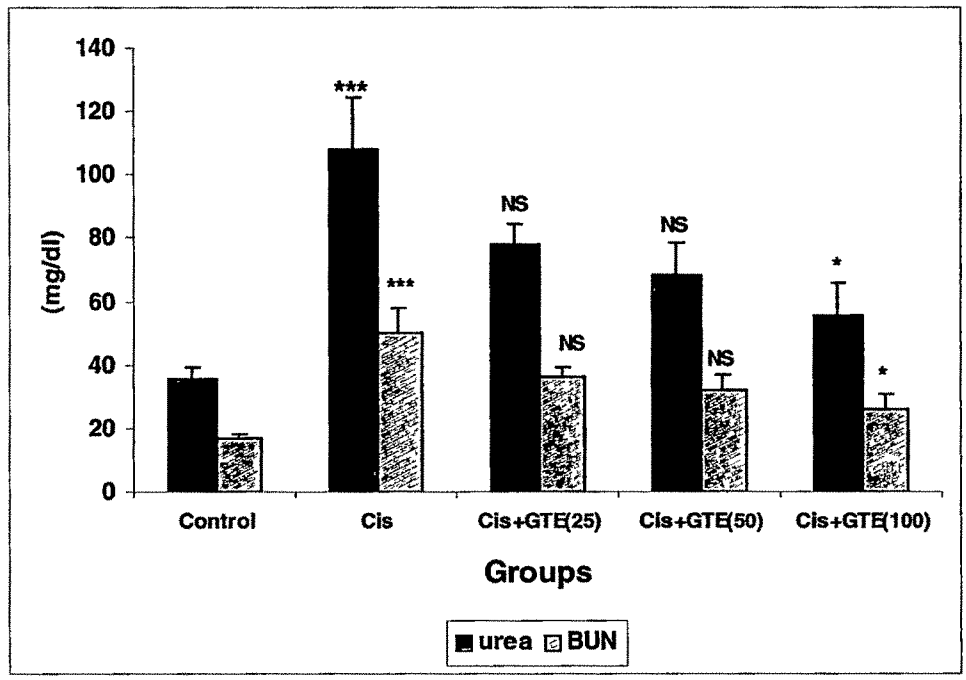


Fig. 4.101

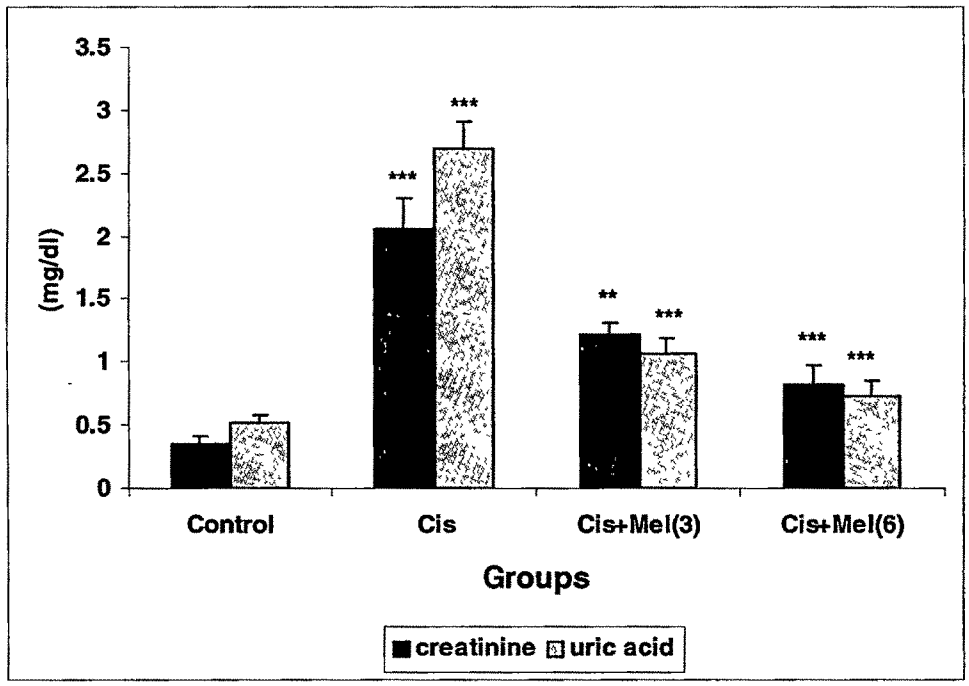


Fig. 4.102

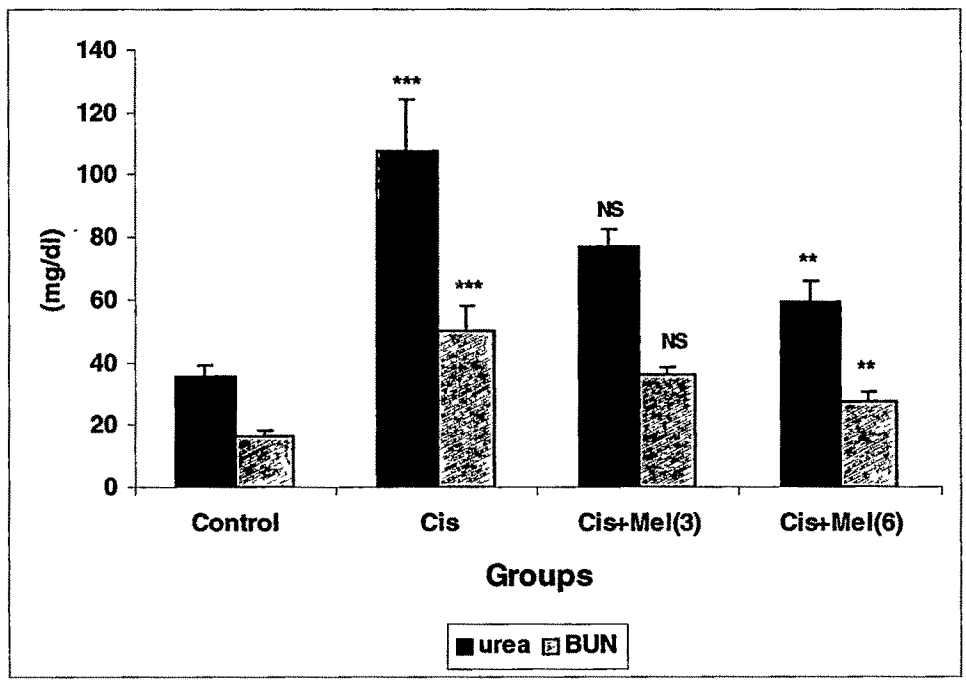


Fig. 4.103

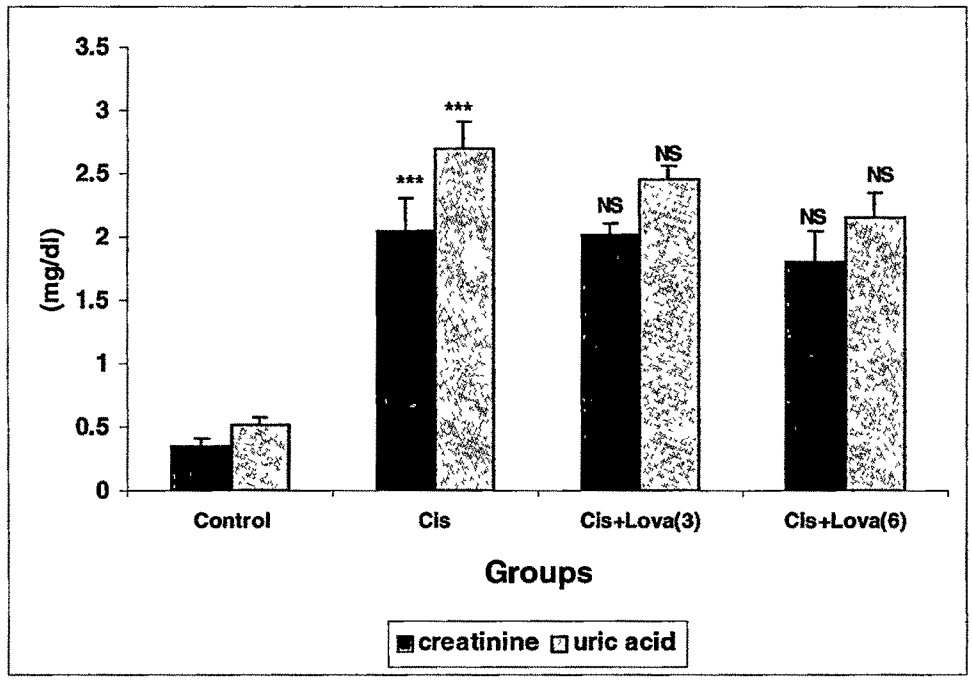
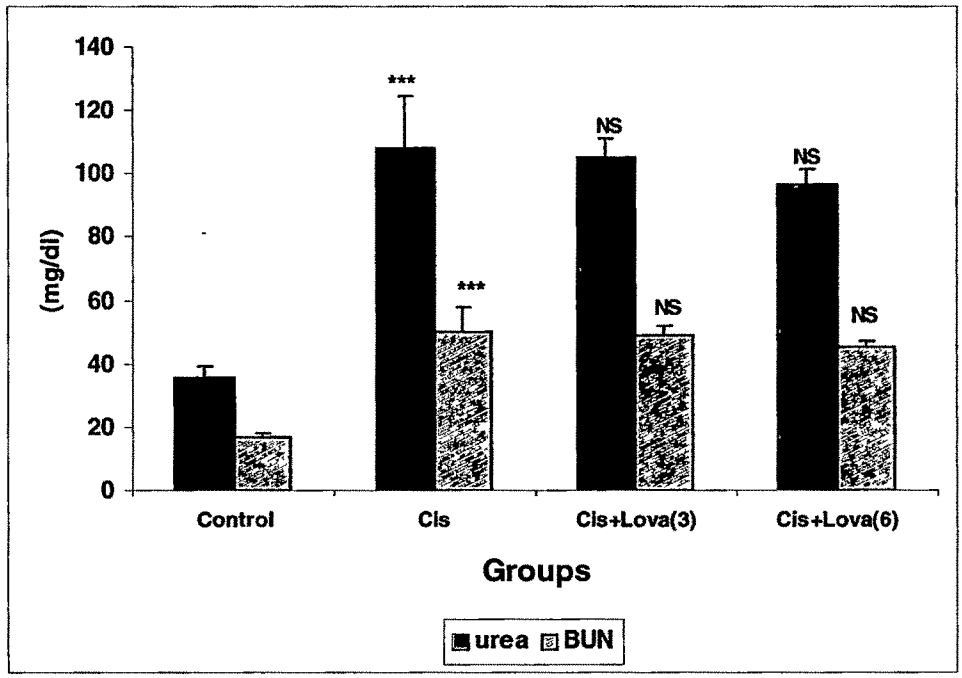


Fig. 4.104



4.3.2.2.2 TISSUE PARAMETERS

4.3.2.2.2.1 Effect on lipid peroxidation

Chronic cisplatin administration (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) to rats led to a significant ($P<0.01$) increase in lipid peroxidation or MDA content in kidneys as compared to the control group (Group 1).

Treatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration significantly ($P<0.01$,$P<0.001$) reduced the levels of MDA as compared to cisplatin treated group (Table 4.20).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration produced a significant ($P<0.01$, $P<0.001$) decrease in the MDA content as compared to cisplatin treated group (Table 4.21).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not significantly alter the levels of MDA as compared to the cisplatin treated group (Tables 4.22).

4.3.2.2.2.2 Effect on endogenous antioxidants

4.3.2.2.2.2.1 Effect on Superoxide dismutase

Chronic cisplatin administration (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) reduced the SOD activity significantly ($P<0.001$) in kidneys of rats as compared to control group.

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant changes in the level of SOD. At the higher dose (50 and 100 mg/kg), led to a significant ($P<0.05$,$P<0.01$) rise in SOD content as compared to cisplatin treated group (Tables 4.20).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not significantly alter the levels of SOD. At the higher dose (6 mg/kg), led to a significant ($P<0.05$) rise in SOD content as compared to the cisplatin treated group (Tables 4.21).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration

did not significantly alter the levels of SOD as compared to the cisplatin treated group (Tables 4.22).

4.3.2.2.2.2.2 Effect on Catalase

The catalase activity in chronically cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treated animals (Group 2) was significantly ($P<0.001$) reduced as compared to control group (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant changes in the level of catalase. At the higher dose (100 mg/kg), led to a significant ($P<0.001$) rise in catalase level as compared to cisplatin treated group (Tables 4.20).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration produced a significant ($P<0.01$, $P<0.001$) increase in catalase level compared to cisplatin treated group (Table 4.21).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not significantly alter the levels of catalase as compared to the cisplatin treated group (Tables 4.22).

4.3.2.2.2.2.3 Effect on Reduced glutathione

A significant ($P<0.001$) reduction in reduced glutathione concentration was observed in chronically cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treated rats (Group 2) as compared to the normal control rats (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant changes in the level of GSH. At the higher doses (50 and 100 mg/kg), led to a significant ($P<0.05$,$P<0.01$) rise in GSH content as compared to cisplatin treated group (Tables 4.20).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration produced a significant ($P<0.01$, $P<0.001$) increase in GSH level as compared to cisplatin treated group (Table 4.21).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin administration did not significantly alter the levels of GSH as compared to the cisplatin treated group (Tables 4.22).

Table 4.20: Effect of green tea extract on the levels of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the kidney of rats in cisplatin induced nephrotoxicity (chronic study).

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	2.0±0.08	4.25±0.54	2.95±0.49	3.82±0.15
Group 2	3.36±0.31**	1.91±0.16***	0.71±0.11***	2.31±0.13***
Group 3	2.45±0.07**	2.92±0.4^{NS}	1.01±0.13^{NS}	2.60±0.08^{NS}
Group 4	2.24±0.11***	3.53±0.27*	2.15±0.31*	2.8±0.07^{NS}
Group 5	1.9±0.08***	4.19±0.12***	2.58±0.46**	3.23±0.13***
F value	**12.87**	**8.1**	**8.1**	**23.45**
P value	**P<0.0001**	**P=0.0002**	**P=0.0002**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day)

Group 3: GTE (25 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.21: Effect of melatonin on the levels of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the kidney of rats in cisplatin induced nephrotoxicity (chronic study).

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	2.0±0.08	4.25±0.54	2.95±0.49	3.82±0.15
Group 2	3.36±0.31**	1.91±0.16***	0.71±0.11***	2.31±0.13***
Group 3	2.03±0.26**	3.16±0.15*	1.81±0.21NS	3.03±0.15**
Group 4	1.86±0.19***	3.44±0.19*	2.06±0.29*	3.35±0.12***
F value	**9.19**	**9.84**	**8.77**	**19.89**
P value	**P=0.0005**	**P=0.0003**	**P=0.0006**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin control (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.22: Effect of lovastatin on the levels of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the kidney of rats in cisplatin induced nephrotoxicity (chronic study).

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	2.0±0.08	4.25±0.54	2.95±0.49	3.82±0.15
Group 2	3.36±0.31**	1.91±0.16***	0.71±0.11***	2.31±0.13***
Group 3	2.88±0.11NS	1.96±0.13NS	1.01±0.04NS	2.06±0.13NS
Group 4	2.24±0.14 NS	2.47±0.11 NS	1.19±0.15NS	2.34±0.19NS
F value	**5.22**	**13.52**	**13.58**	**17.67**
P value	**P=0.0034**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin control (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.2.2.3 Effect on membrane bound enzymes

4.3.2.2.3.1 Effect on Sodium Potassium ATPase

In the kidneys of chronically cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treated rats, the activity of Na^+K^+ATPase enzyme was significantly ($P<0.01$) reduced as compared to the control (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant increase in Na^+K^+ATPase levels; but the higher doses (50 and 100 mg/kg) of green tea extract significantly ($P<0.05$,$P<0.01$) increased the levels of the enzyme as compared to cisplatin group (Table 4.23).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant increase in Na^+K^+ATPase levels; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased the levels of the enzyme as compared to cisplatin control (Table 4.24).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant increase in the levels of Na^+K^+ATPase as compared to the cisplatin treated group (Tables 4.25).

4.3.2.2.3.2 Effect on Calcium ATPase

Chronic treatment with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) resulted in a significant ($P<0.05$) decrease in the Ca^{2+}ATPase activity as compared to the control group (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant increase in Ca^{2+}ATPase levels; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the levels of the enzyme as compared to cisplatin group (Table 4.23).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant increase in Ca^{2+}ATPase levels; but at the

higher dose (6 mg/kg), it significantly (P<0.05) increased the levels of the enzyme as compared to cisplatin control (Table 4.24).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not significantly alter the levels of Ca^{2+}ATPase at any of doses as compared to cisplatin group (Table 4.25).

4.3.2.2.3.3 Effect on Magnesium ATPase

Chronic treatment with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) resulted in a significant (P<0.001) decrease in the Mg^{2+}ATPase activity as compared to the control group (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not produce any significant increase in Mg^{2+}ATPase levels; but the higher dose (50 and 100 mg/kg) of green tea extract significantly (P<0.001) increased the levels of the enzyme as compared to cisplatin group (Table 4.23).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration produce significant (P<0.001) increase in Ca^{2+}ATPase levels as compared to cisplatin group (Table 4.24).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not significantly alter the levels of Mg^{2+}ATPase at any of doses as compared to cisplatin group (Table 4.25).

Table 4.23: Effect of green tea extract on membrane bound enzymes in the kidney of rats in cisplatin induced nephrotoxicity (chronic study).

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	12.67±0.61	6.98±0.24	9.98±0.38
Group 2	8.22±0.08**	5.34±0.54*	4.25±0.24***
Group 3	10.10±0.89NS	6.02±0.26NS	5.58±0.41NS
Group 4	11.44±0.61*	6.84±0.25NS	7.02±0.26***
Group 5	12.12±0.21**	7.07±0.50*	7.32±0.41***
F value	**5.66**	**3.68**	**37.26**
P value	**P=0.0022**	**P=0.0172**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin control (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.24: Effect of melatonin on membrane bound enzymes in the kidney of rats in cisplatin induced nephrotoxicity (chronic study).

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	12.67±0.61	6.98±0.24	9.98±0.38
Group 2	8.22±0.08**	5.34±0.54*	4.25±0.24***
Group 3	10.34±0.24NS	5.77±0.30NS	7.67±0.24***
Group 4	11.5±0.56*	6.79±0.17*	8.32±0.42***
F value	**7.38**	**5.15**	**51.99**
P value	**P=0.0016**	**P=0.0084**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin control (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.25: Effect of lovastatin on membrane bound enzymes in the kidney of rats in cisplatin induced nephrotoxicity (chronic study).

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	12.67±0.61	6.98±0.24	9.98±0.38
Group 2	8.22±0.08**	5.34±0.54*	4.25±0.24***
Group 3	8.80±0.26^{NS}	5.41±0.52^{NS}	4.67±0.25^{NS}
Group 4	9.28±0.29^{NS}	5.83±0.56^{NS}	5.01±0.51^{NS}
F value	**8.6**	**2.36**	**31.86**
P value	**P=0.0002**	**P=0.080**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin control (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.2.2.4 HISTOPATHOLOGY

Fig. 4.105(A) showed kidney from control rats with normal morphology of the proximal tubule located in the outer stripe of the outer medulla. The tubules were lined up with cells forming a proper lumen inside.

Chronic treatment with cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) to the rats revealed a remarkable proximal tubular necrosis, with extensive epithelial vacuolization, swelling and tubular dilation as compared to control. Widespread necrosis and sloughing of proximal tubular epithelial cells was noted.

Treatment with green tea extract (25,50 and100 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration [Fig. 4.105(C) to Fig. 4.105(E)] and melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration [Fig. 4.106(C) to Fig. 4.106(D)] decreased the necrosis with increasing doses and the cell structure regained to normal with the increasing doses of the drug.

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p. on 1, 7, 14, 21 and 28 day) administration did not significantly protect the kidney from cisplatin induced histopathological changes [Fig. 4.107(C) to Fig. 4.107(D)].

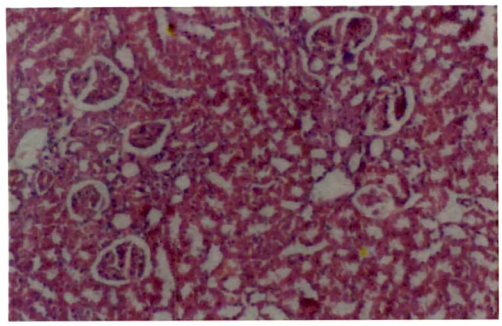
Fig. 4.105: Photomicrographs showing effect of green tea extract on the kidney of cisplatin treated rats (chronic study).

(Magnification 10 X)

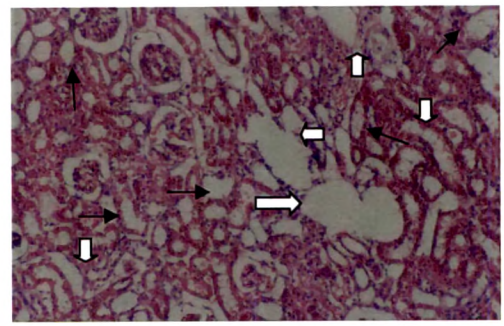
⇨ **Epithelial cell vacuolization**

→ **Proximal tubule dilation**

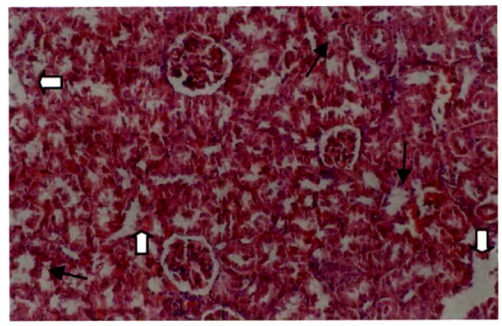
A: Normal Control



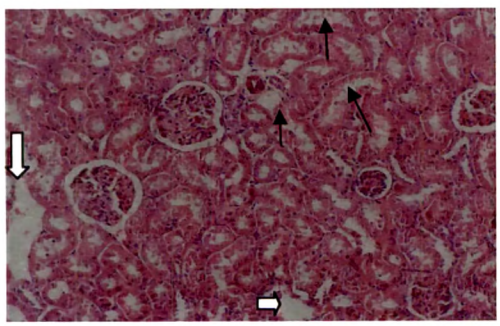
B: Cisplatin (3 mg/kg, i.p.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

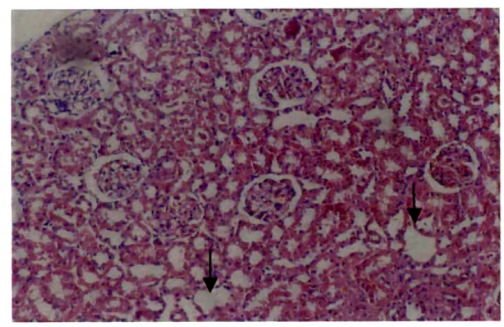


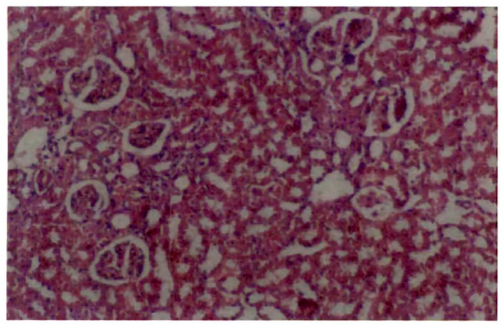
Fig. 4.106: Photomicrographs showing effect of melatonin on the kidney of cisplatin treated rats (chronic study).

(Magnification 10 X)

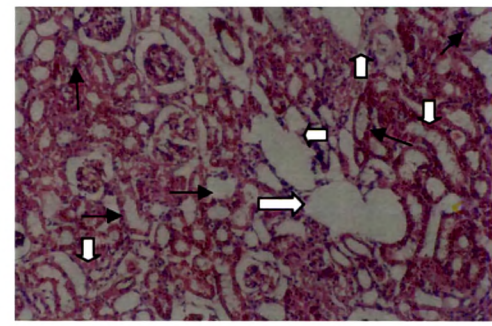
⟹ **Epithelial cell vacuolization**

⟶ **Proximal tubule dilation**

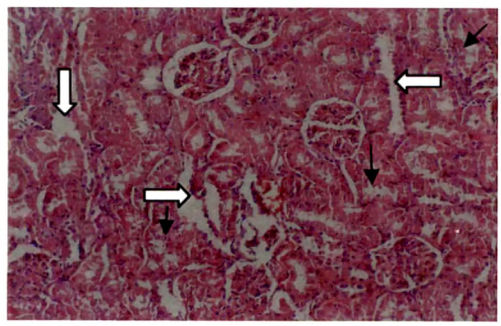
A: Normal Control



B: Cisplatin (3 mg/kg, i.p.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

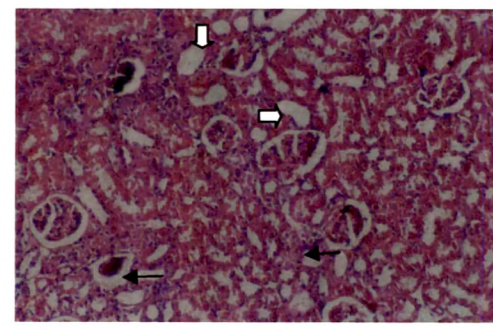


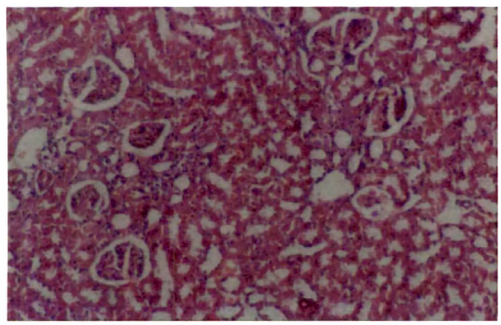
Fig. 4.107: Photomicrographs showing effect of lovastatin on the kidney of cisplatin treated rats (chronic study).

(Magnification 10 X)

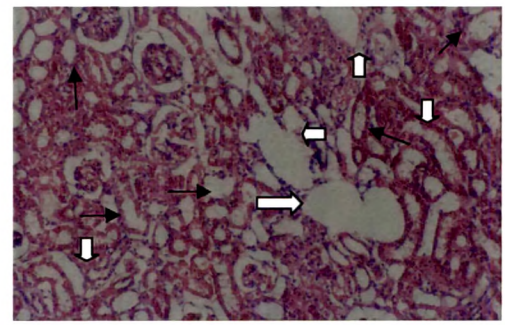
⇒ **Epithelial cell vacuolization**

→ **Proximal tubule dilation**

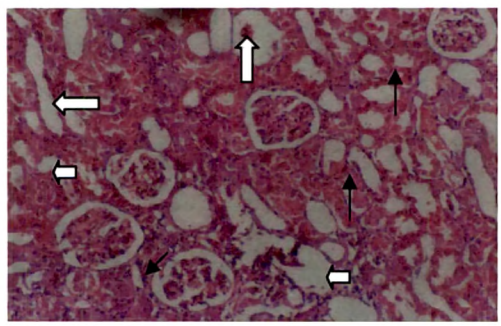
A: Normal Control



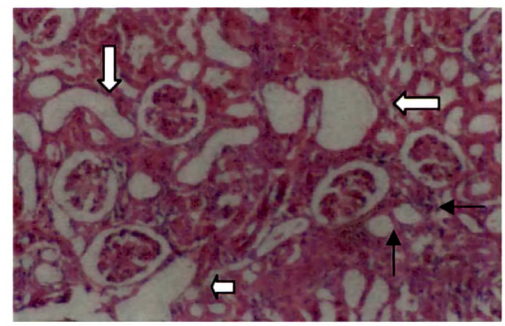
B: Cisplatin (3 mg/kg, i.p.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.3 DOXORUBICIN INDUCED TESTICULAR TOXICITY

4.3.3.1: ACUTE STUDY IN RATS

4.3.3.1.1 Effect of drugs on Testosterone

Acute doxorubicin (10 mg/kg i.v.) administration produced a significant ($P<0.001$) decrease in the activity of testosterone in serum of rats (Group 2) as compared to control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant change in the level of serum testosterone; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.01$) increased the testosterone level as compared to doxorubicin treated group (Fig.4.108).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant change in the level of serum testosterone as compared to doxorubicin treated group (Fig.4.109).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant change in the level of serum testosterone as compared to doxorubicin treated group (Fig.4.110).

Fig.4.108: Effect of green tea extract on the serum testosterone in doxorubicin induced testicular toxicity (acute study) in rats.

Fig.4.109: Effect of melatonin on the serum testosterone in doxorubicin induced testicular toxicity (acute study) in rats.

Fig.4.110: Effect of lovastatin on the serum testosterone in doxorubicin induced testicular toxicity (acute study) in rats.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control.
Groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
Groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
Groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.108

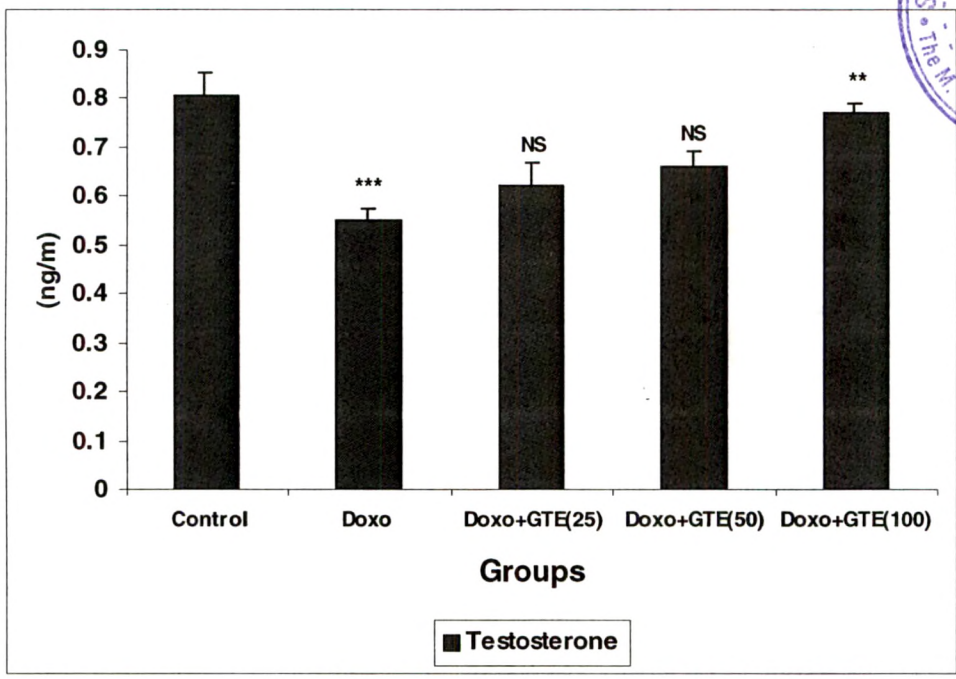


Fig.4.109

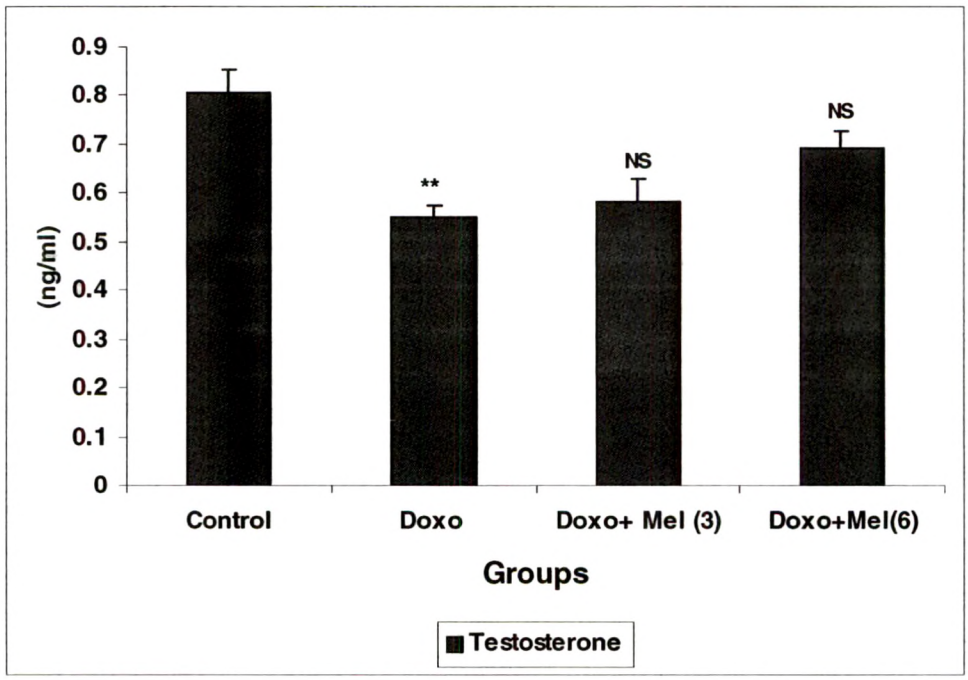
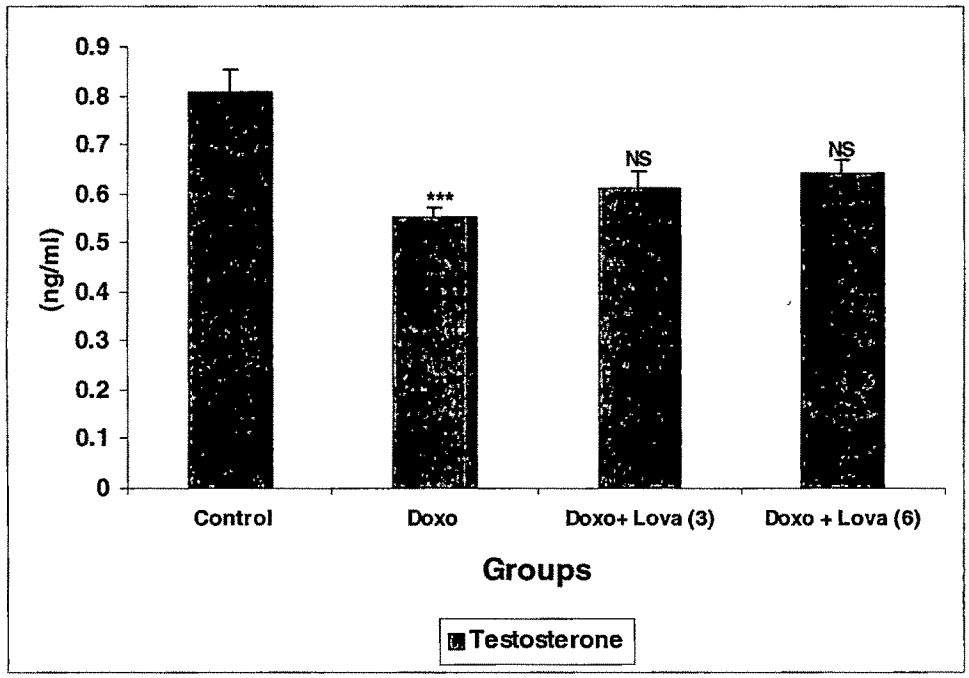


Fig.4.110


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(ng/ml)
***
NS
NS
Control
Doxo
Doxo+ Lova (3)
Doxo + Lova (6)
Groups
Testosterone

4.3.3.1.2 Effect of drugs on Sperm count

Acute doxorubicin (10 mg/kg i.v.) administration produced a significant (P<0.001) decrease in the sperm count of rats (Group 2) as compared to control group (Group 1).

Pretreatment of green tea extract (25 mg/kg and 50 mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant increase in the sperm count; but the higher dose (100 mg/kg) of green tea extract significantly (P<0.05) increased the sperm count as compared to doxorubicin treated group (Fig.4.111).

Pretreatment of melatonin (3 mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration (10mg/kg i.v.on 30th day) did not produce significant increase in the sperm count; but at the higher dose (6 mg/kg), it significantly (P<0.05) increased the sperm count as compared to doxorubicin treated group (Fig.4.112).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration (10mg/kg i.v.on 30th day) did not produce significant increase in the sperm count as compared to doxorubicin treated group (Fig.4.113).

Fig.4.111: Effect of green tea extract on sperm count in doxorubicin induced testicular toxicity (acute study) in rats.

Fig.4.112: Effect of melatonin on sperm count in doxorubicin induced testicular toxicity (acute study) in rats.

Fig.4.113: Effect of lovastatin on sperm count in doxorubicin induced testicular toxicity (acute study) in rats.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control.
Groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
Groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
Groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.111

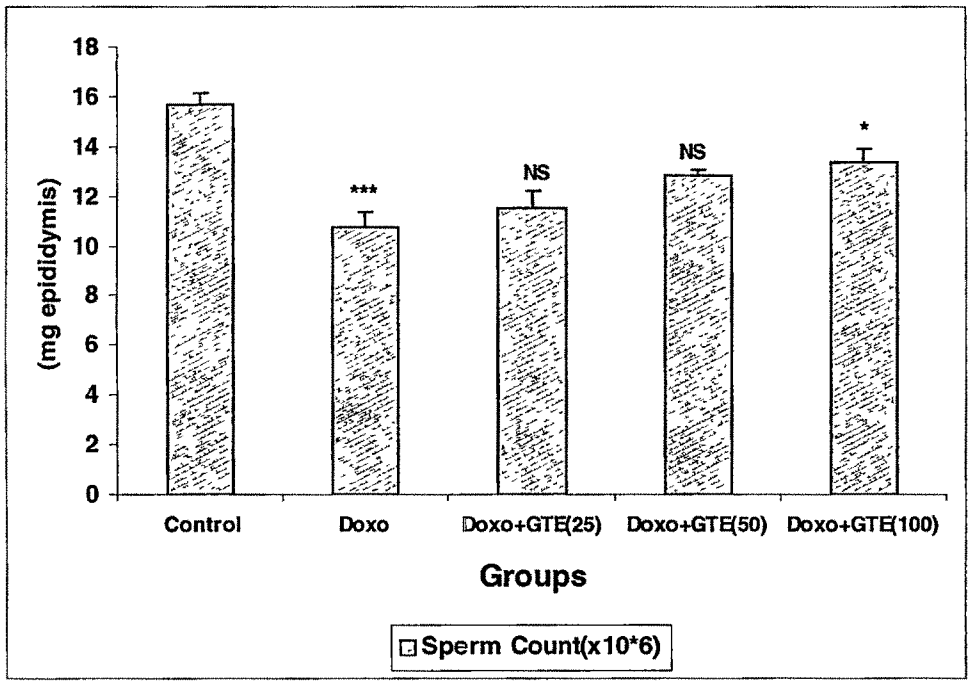


Fig.4.112

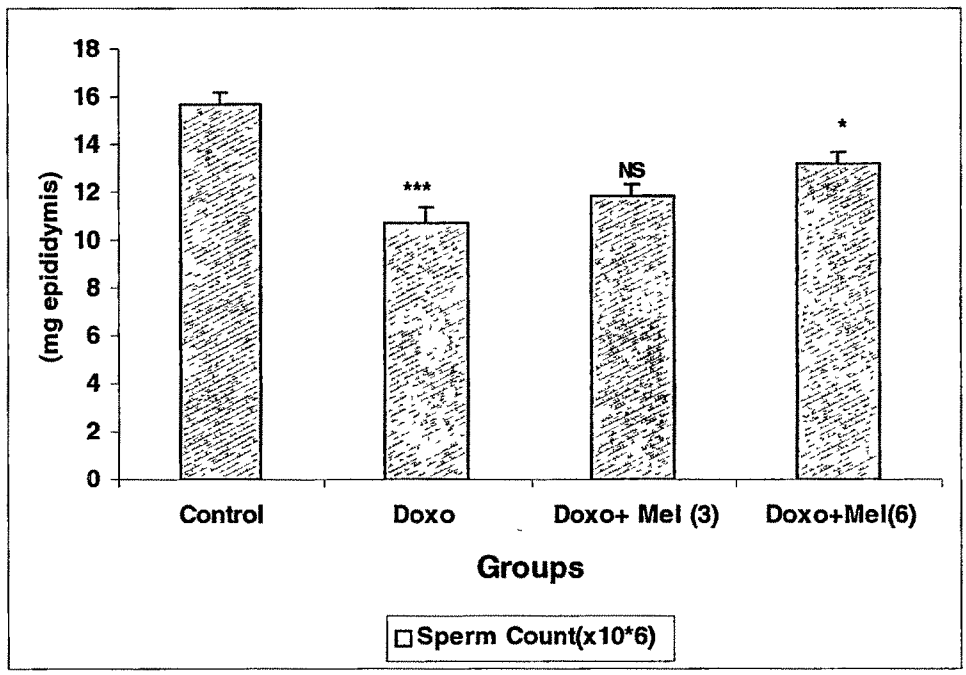
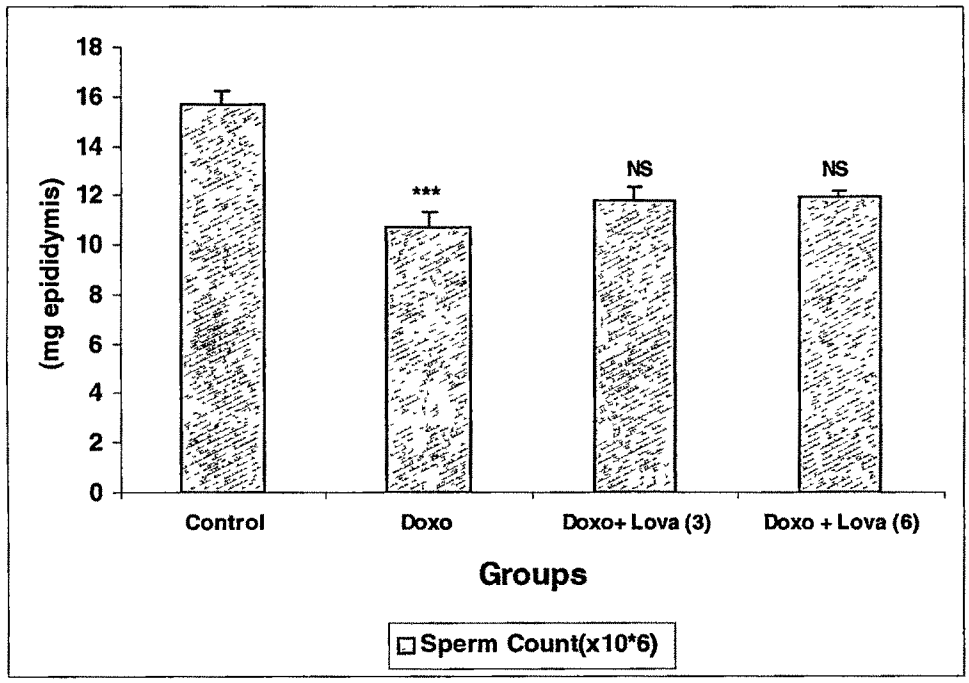


Fig.4.113


18
16
14
12
10
8
6
4
2
0
(mg epididymis)
***
NS
NS
Control
Doxo
Doxo+ Lova (3)
Doxo + Lova (6)
Groups
Sperm Count(x10*6)

4.3.3.1.3 TISSUE PARAMETERS

4.3.3.1.3.1 Effect on lipid peroxidation

Acute doxorubicin (10 mg/kg i.v.) administration to rats led to a significant (P<0.001) increase in lipid peroxidation or MDA content in testes of rats as compared to the control group (Group 1).

Pretreatment of green tea extract (25 and 50mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant change in the level of lipid peroxidation (MDA content); but the higher dose (100 mg/kg) of green tea extract significantly (P<0.05) decreased these levels as compared to doxorubicin treated group (Table 4.26).

Pretreatment of melatonin (3mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant change in the level of lipid peroxidation (MDA content); but at the higher dose (6 mg/kg), it significantly (P<0.01) decreased these levels as compared to doxorubicin treated group (Table 4.27).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration (10 mg/kg i.v.on 30th day) did not produce significant change in the level of lipid peroxidation (MDA content as compared to doxorubicin treated group (Table 4.28).

4.3.3.1.3.2 Effect on endogenous antioxidants

4.3.3.1.3.2.1 Effect on Superoxide dismutase

Acute doxorubicin (10 mg/kg i.v.) administration reduced the SOD activity significantly (P<0.01) in testes of rats as compared to control (Group1).

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce any significant changes in the level of SOD content as compared to doxorubicin treated group (Tables 4.26).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant changes in the level of SOD content as compared to doxorubicin treated group (Tables 4.27).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant changes in the level of SOD content as compared to doxorubicin treated group (Tables 4.28).

4.3.3.1.3.2.2 Effect on Catalase

The catalase activity in doxorubicin (10 mg/kg i.v.) treated group was significantly ($P<0.001$) reduced as compared to control.

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant change in the level of catalase; but at the higher dose (100 mg/kg), it significantly ($P<0.05$) increased these levels when compared with doxorubicin treated group (Table 4.26).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin administration did not produce significant changes in the level of catalase content as compared to doxorubicin treated group (Tables 4.27).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce any significant changes in the level of catalase as compared to doxorubicin treated group (Tables 4.28).

4.3.3.1.3.2.3 Effect on Reduced glutathione

A significant ($P<0.001$) reduction in reduced glutathione concentration was observed in doxorubicin (10 mg/kg i.v.) treated rats as compared to control animal.

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce any significant change in the level of GSH; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.01$) increased these levels when compared with doxorubicin treated group (Table 4.26).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant changes in the level of GSH content as compared to doxorubicin treated group (Tables 4.27 and Tables 4.28).

Table 4.26: Effect of green tea extract on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in doxorubicin induced testicular toxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	2.06±0.12***	2.68±0.15***	2.37±0.07**	3.72±0.2***
Group 3	1.81±0.08 NS	3.09±0.07 NS	2.84±0.18NS	4.06±0.26 NS
Group 4	1.73±0.08 NS	3.56±0.29 NS	3.8±0.13 NS	5.35±0.22 NS
Group 5	1.67±0.09*	3.9±0.15**	4.06±0.21 NS	5.74±0.27*
F value	**11.23**	**7.28**	**4.84**	**12.18**
P value	**P<0.001**	**P=0.0005**	**P=0.005**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.27: Effect of melatonin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in doxorubicin induced testicular toxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	2.06±0.12***	2.68±0.15***	2.37±0.07**	3.72±0.2***
Group 3	1.78±0.08NS	2.99±0.04 NS	2.61±0.19 NS	4.18±0.23 NS
Group 4	1.57±0.06**	3.33±0.19 NS	3.67±0.26 NS	5.38±0.29 NS
F value	**17.21**	**9.55**	**5.097**	**12.97**
P value	**P<0.0001**	**P=0.0004**	**P=0.0088**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.28: Effect of lovastatin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in doxorubicin induced testicular toxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	2.06±0.12***	2.68±0.15***	2.37±0.07**	3.72±0.2***
Group 3	1.89±0.07 NS	2.91±0.11 NS	2.86±0.11 NS	4.16±0.19 NS
Group 4	1.7±0.08 NS	2.97±0.25 NS	3.22±0.2 NS	5.19±0.17 NS
F value	**16.39**	**7.92**	**5.20**	**15.10**
P value	**P<0.0001**	**P=0.0003**	**P=0.0034**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.3.1.3.3 Effect on membrane bound enzymes

4.3.3.1.3.3.1 Effect on Sodium Potassium ATPase

In the testes of doxorubicin (10 mg/kg i.v.) treated rats (Group 2) the activity of Na^+K^+ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not alter the Na^+K^+ATPase levels; but at higher dose (100 mg/kg), it significantly ($P<0.01$) increased these levels as compared to doxorubicin treated group (Table 4.29).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not produce significant increase in Na^+K^+ATPase levels as compared to doxorubicin treated group (Table 4.30 and Table 4.31).

4.3.3.1.3.3.2 Effect on Calcium ATPase

Acute treatment with doxorubicin (10 mg/kg i.v.) resulted in a significant ($P<0.05$) decrease in the Ca^{2+}ATPase activity as compared to the control (Group 1).

Pretreatment of green tea extract (25 50 and100 mg/kg/day p.o. for 30 day), melatonin (3 and 6 mg/kg/day p.o. for 30 day) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not alter the Ca^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.29,4.30,4.31).

4.3.3.1.3.3.3 Effect on Magnesium ATPase

In the testes of doxorubicin (10 mg/kg i.v.) treated rats (Group 2) the activity of Mg^{2+}ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Pretreatment of green tea extract (25,50 and 100 mg/kg/day p.o. for 30 day), melatonin (3 and 6 mg/kg/day p.o. for 30 day) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration did not affect the Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.29,4.30,4.31).

Table 4.29: Effect of green tea extract on membrane bound enzymes in the testes of rats in doxorubicin induced testicular toxicity (acute study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	4.77±0.24***	2.75±0.16*	4.13±0.25***
Group 3	4.9±0.17NS	2.94±0.17 NS	4.08±0.11 NS
Group 4	5.91±0.23 NS	3.55±0.17 NS	4.63±0.15 NS
Group 5	6.24±0.22**	3.82±0.34 NS	4.89±0.22 NS
F value	**35.65**	**3.51**	**16.22**
P value	**P<0.0001**	**P=0.0208**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day)

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.30: Effect of melatonin on membrane bound enzymes in the testes of rats in doxorubicin induced testicular toxicity (acute study) in rats

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	4.77±0.24***	2.75±0.16*	4.13±0.25***
Group 3	5.05±0.12^{NS}	3.23±0.19 ^{NS}	4.32±0.29 ^{NS}
Group 4	5.73±0.2^{NS}	3.74±0.33 ^{NS}	4.62±0.24 ^{NS}
F value	**49.10**	**3.20**	**13.57**
P value	**P<0.0001**	**P=0.045**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day)

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.31: Effect of lovastatin on membrane bound enzymes in the testes of rats in doxorubicin induced testicular toxicity (acute study) in rats

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	4.77±0.24***	2.75±0.16*	4.13±0.25***
Group 3	4.85±0.18NS	2.99±0.17 NS	4.5±0.15 NS
Group 4	5.21±0.29 NS	3.6±0.14 NS	4.62±0.16 NS
F value	**35.64**	**4.45**	**14.79**
P value	**P<0.0001**	**P<0.0075**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (10 mg/kg, i.v.on 30th day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by doxorubicin (10 mg/kg i.v.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.3.1.1.4 HISTOPATHOLOGY

Control rat (Fig 4.114-A) showed normal histoarchitecture of testes. Seminiferous tubules reflects the organized arrangement and well-defined boundaries

Acute doxorubicin (10 mg/kg, i.v.) administration to rats causes vacuolization and fibrinoid debris in the seminiferous tubules. Shrunken seminiferous tubules showed disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also observed in interstitial tissues. (Fig 4.114-B)

Pretreatment of green tea extract followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration exhibited decreased degree of vacuolization, fibrinoid debris and disorganization of germinal epithelium in the seminiferous tubules with increasing doses [Fig. 4.114(C)-4.114(E)].

Similarly, Pretreatment of melatonin followed by acute doxorubicin (10 mg/kg i.v.on 30th day) administration exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium at the dose of 6 mg/kg [Fig. 4.115(C)-4.115(D)].

The degree of testicular damage in lovastatin treated group was similar to doxorubicin (10 mg/kg i.v.on 30th day) treated group in regard to morphological changes showing vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium with disorganization of germinal epithelium [Fig. 4.116(C)-4.116(D)].

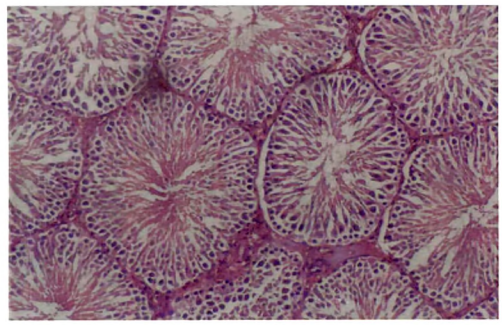
Fig. 4.114: Photomicrographs showing effect of green tea extract on the testes of doxorubicin treated rats (acute study).

(Magnification 10 X)

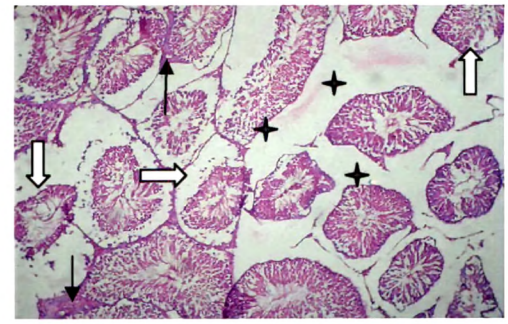
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

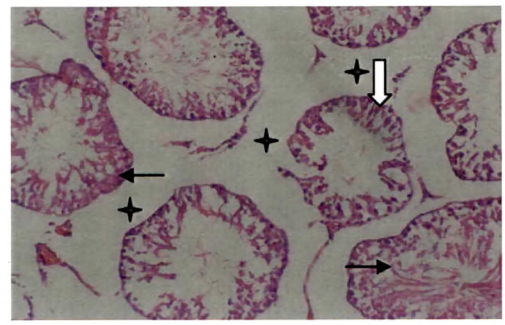
A: Normal control



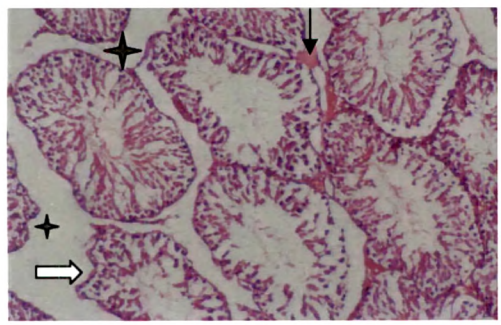
B: Doxorubicin (10 mg/kg, i.v.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

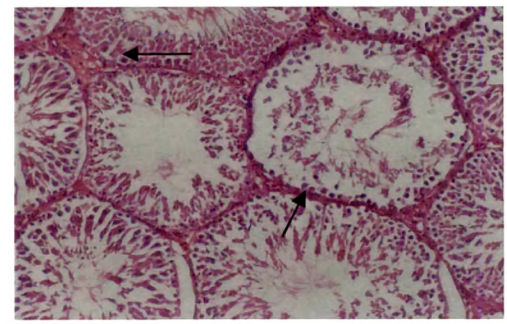


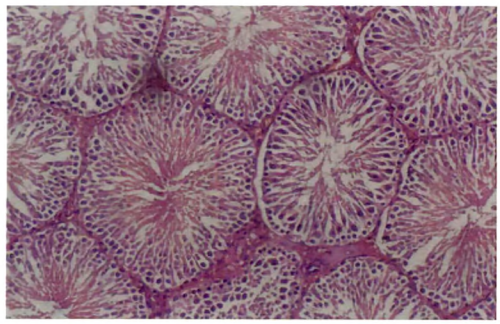
Fig. 4.115: Photomicrographs showing effect of melatonin on the testes of doxorubicin treated rats (acute study).

(Magnification 10 X)

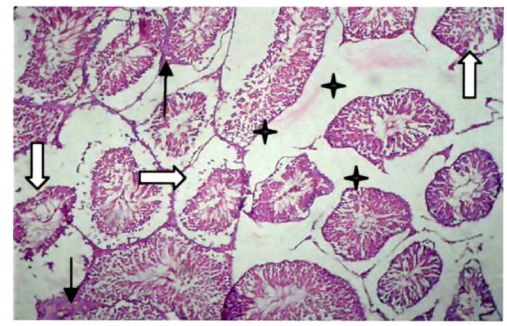
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

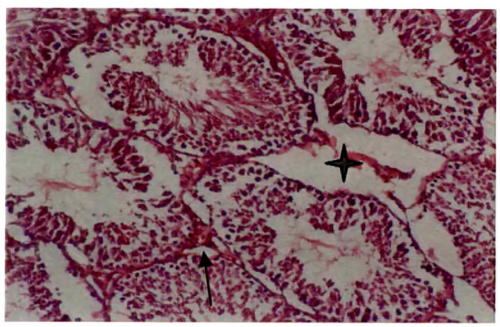
A: Normal control



B: Doxorubicin (10 mg/kg, i.v.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

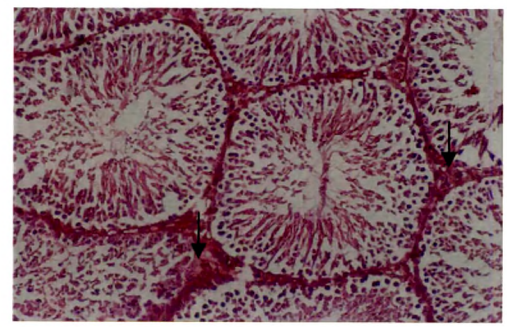


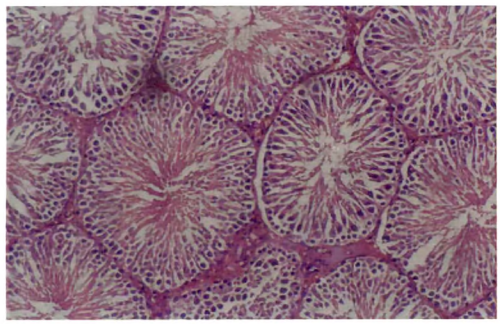
Fig. 4.116: Photomicrographs showing effect of lovastatin on the testes of doxorubicin treated rats (acute study).

(Magnification 10 X)

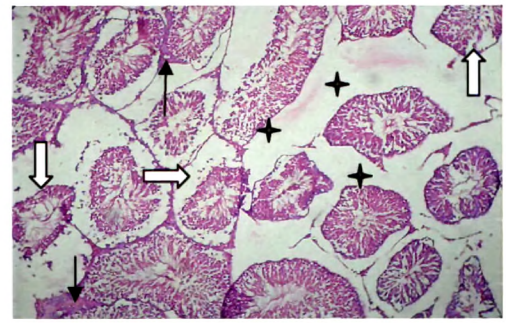
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

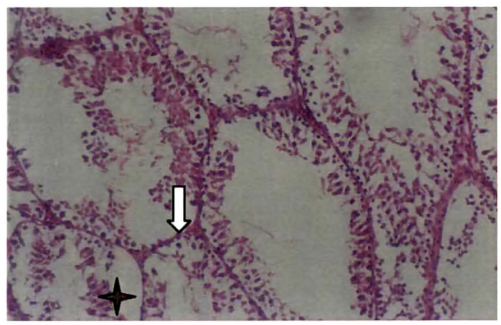
A: Normal control



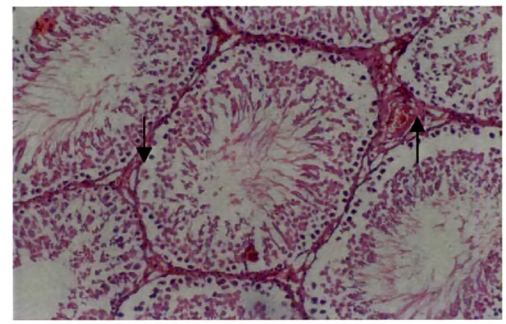
B: Doxorubicin (10 mg/kg, i.v.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.3 DOXORUBICIN INDUCED TESTICULAR TOXICITY

4.3.3.2: CHRONIC STUDY IN RATS

4.3.3.2.1 Effect of drugs on Testosterone

Chronic administration of doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) did not produce significant ($P<0.001$) decrease in the serum concentration of testosterone as compared to control group.

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in the level of serum testosterone; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the testosterone level as compared to doxorubicin treated group (Fig.4.117).

Treatment of melatonin (3 and 6 mg/kg) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) did not produce significant increase in the level of serum testosterone as compared to doxorubicin treated group (Fig.4.118).

Treatment of lovastatin (3 and 6 mg/kg) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in the level of serum testosterone as compared to doxorubicin treated group (Fig.4.119).

Fig.4.117: Effect of green tea extract on the serum testosterone in doxorubicin induced testicular toxicity (chronic study) in rats.

Fig.4.118: Effect of melatonin on the serum testosterone in doxorubicin induced testicular toxicity (chronic study) in rats.

Fig.4.119: Effect of lovastatin on the serum testosterone in doxorubicin induced testicular toxicity (chronic study) in rats.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with control.
Groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
Groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
Groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.117

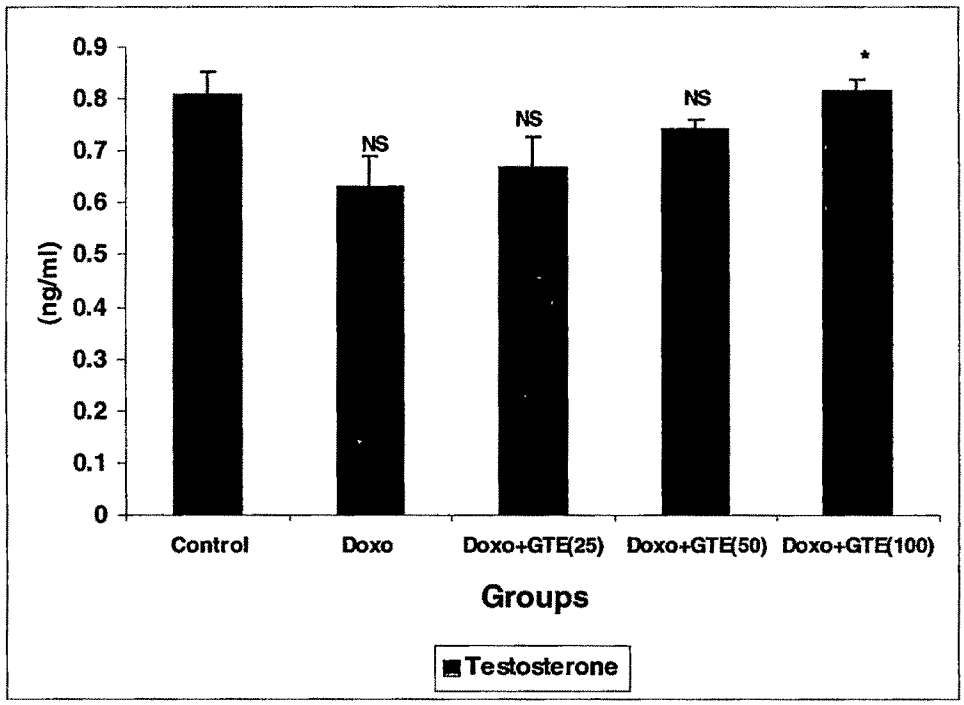


Fig.4.118

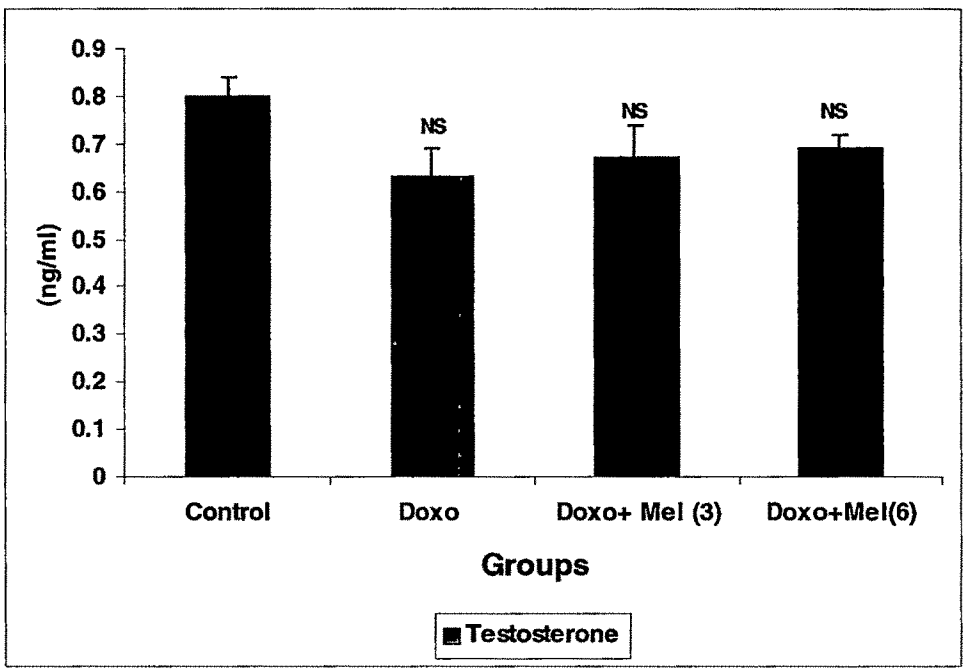
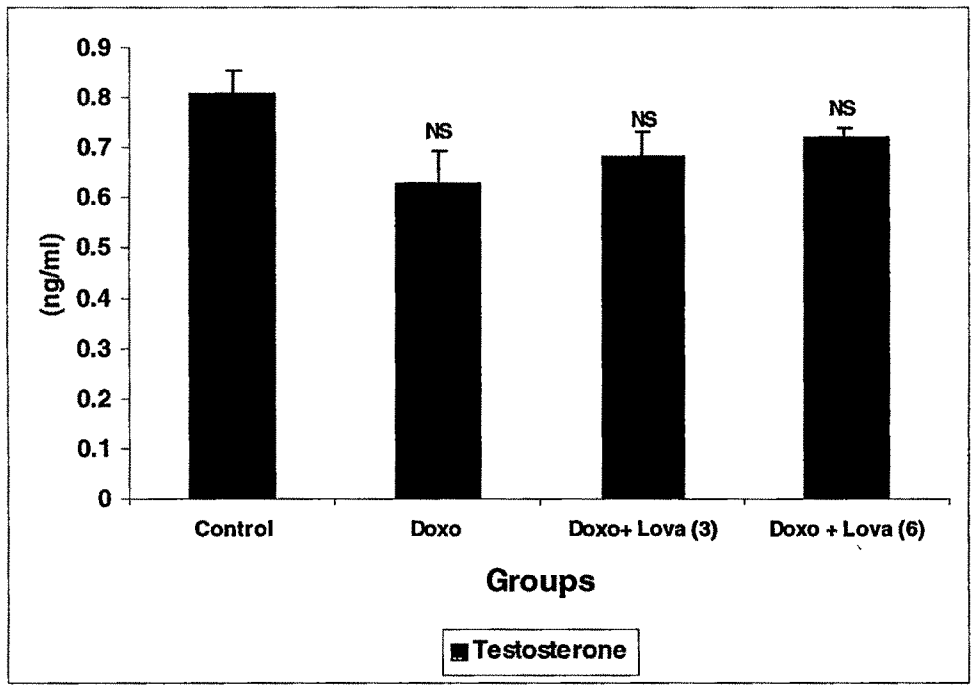


Fig.4.119


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(ng/ml)
NS
NS
NS
Control
Doxo
Doxo+ Lova (3)
Doxo + Lova (6)
Groups
Testosterone

4.3.3.2.2 Effect of drugs on Sperm count

Chronic administration of doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) produced a significant ($P<0.01$) decrease in the sperm count of rats (Group 2) as compared to control group (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increased in the sperm count; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the sperm count as compared to doxorubicin treated group (Fig.4.120).

Treatment of melatonin (3 and 6mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration produced a significant ($P<0.05$) increase in the sperm count as compared to doxorubicin treated group (Fig.4.121).

Treatment of lovastatin (3 and 6mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant increase in the sperm count as compared to doxorubicin treated group (Fig.4.122).

Fig.4.120: Effect of green tea extract on sperm count in doxorubicin induced testicular toxicity (chronic study) in rats.

Fig.4.121: Effect of melatonin on sperm count in doxorubicin induced testicular toxicity (chronic study) in rats.

Fig.4.122: Effect of lovastatin on sperm count in doxorubicin induced testicular toxicity (chronic study) in rats.

Values are expressed as mean ± SEM.
The group treated with doxorubicin alone (Doxo) was compared with Control.
Groups treated with green tea extract {Doxo + GTE (25), Doxo + GTE (50) and Doxo + GTE (100)} were compared with Doxo alone group.
Groups treated with melatonin {Doxo + Mel (3) and Doxo + Mel (6)} were compared with Doxo alone group.
Groups treated with lovastatin {Doxo + Lova (3) and Doxo + Lova (6)} were compared with Doxo alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.120

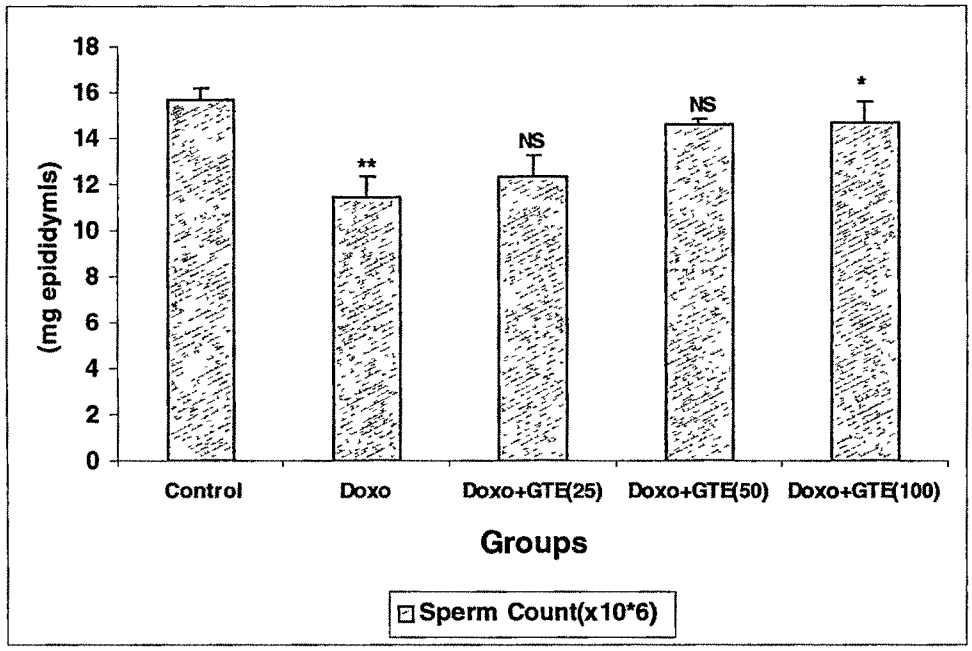


Fig.4.121

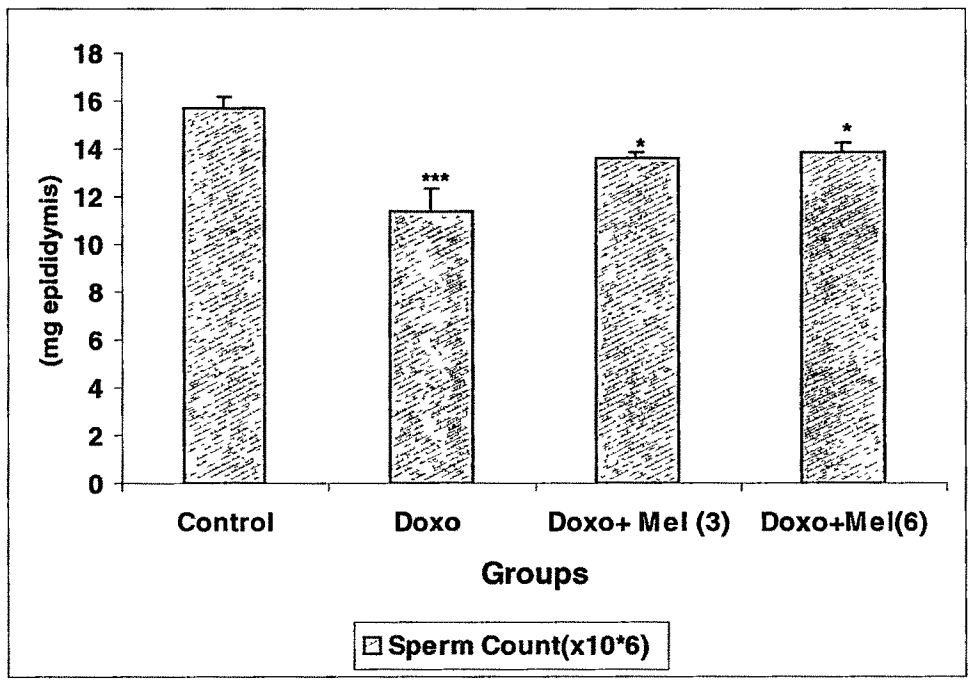
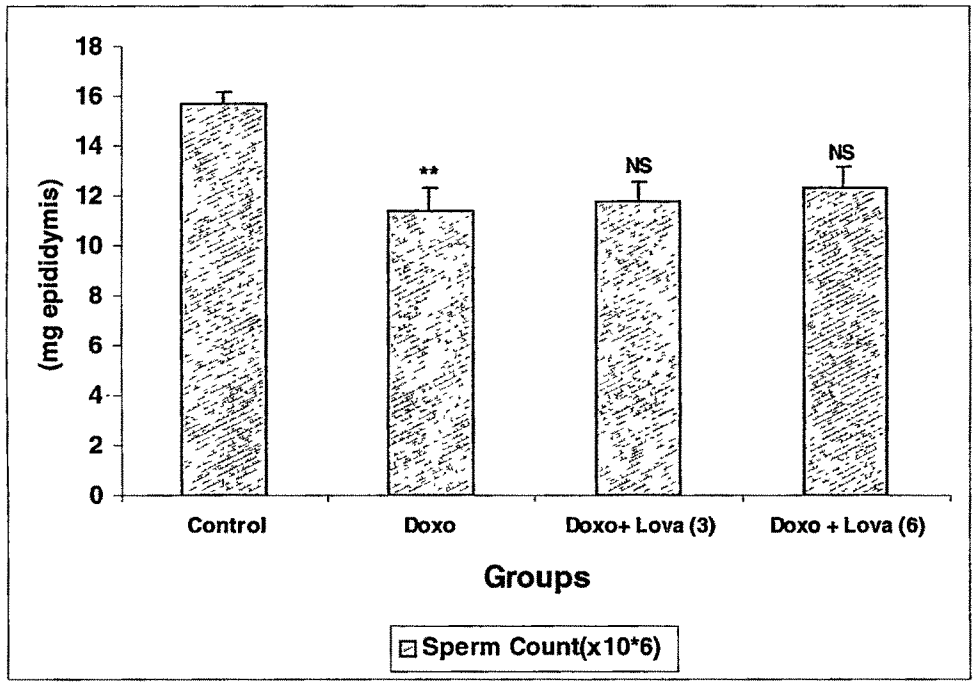


Fig.4.122


18
16
14
12
10
8
6
4
2
0
(mg epididymis)
**
NS
NS
Control
Doxo
Doxo+ Lova (3)
Doxo + Lova (6)
Groups
Sperm Count(x10*6)

4.3.3.2.3 TISSUE PARAMETERS

4.3.3.2.3.1 Effect on lipid peroxidation

Chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration to rats led to a significant ($P<0.01$) increase in lipid peroxidation or MDA content in testes of rats as compared to the control group.

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce any significant change in the level of lipid peroxidation (MDA content); but at the higher dose (100 mg/kg), It significantly ($P<0.05$) decreased these levels as compared to doxorubicin treated group (Table 4.32).

Treatment of melatonin (3mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant change in the level of lipid peroxidation (MDA content); but the higher dose (6 mg/kg) of melatonin significantly ($P<0.05$) decreased these levels when compared with doxorubicin treated group (Table 4.33).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant change in the level of lipid peroxidation (MDA content) when compared with doxorubicin treated group (Table 4.34).

4.3.3.2.3.2 Effect on endogenous antioxidants

4.3.3.2.3.2.1 Effect on Superoxide dismutase

Chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration reduced the SOD activity significantly ($P<0.05$) in testes of rats as compared to control (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant changes in the level of SOD content; but at the higher dose (100 mg/kg), it significantly ($P<0.05$)

decreased these levels as compared to doxorubicin treated group (Table 4.32).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant changes in the level of SOD content as compared to doxorubicin treated group (Table 4.33).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant changes in the level of SOD content as compared to doxorubicin treated group (Table 4.34).

4.3.3.2.3.2.2 Effect on Catalase

The catalase activity in doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treated rats (Group 2) was significantly ($P<0.001$) reduced as compared to control rats (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce any significant change in the level of catalase; but at the higher dose (50 and 100 mg/kg), it significantly ($P<0.05$) increased these levels as compared to doxorubicin treated group (Table 4.32).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce any significant change in the level of catalase; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased these levels as compared to doxorubicin treated group (Table 4.33).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce any significant changes in the level of catalase as compared to doxorubicin treated group (Tables 4.34).

4.3.3.2.3.2.3 Effect on Reduced glutathione

A significant ($P<0.001$) reduction in reduced glutathione concentration was observed in doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treated rats (Group 2) as compared to control animal (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce any significant change in the level of GSH; but at the higher dose (50 and 100 mg/kg), it significantly ($P<0.05$) increased these levels as compared to doxorubicin treated group (Table 4.26).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant change in the level of GSH; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased these levels as compared to doxorubicin treated group (Table 4.33).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce significant changes in the level of GSH as compared to doxorubicin treated group (Tables 4.34).

Table 4.32: Effect of green tea extract on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in doxorubicin induced testicular toxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	1.82±0.10**	2.63±0.15**	2.55±0.15*	3.90±0.27***
Group 3	1.725±0.14NS	3.26±0.18NS	2.68±0.19NS	4.42±0.32NS
Group 4	1.67±0.081 NS	3.96±0.43*	4.40±0.35NS	5.99±0.35*
Group 5	1.395±0.08*	3.98±0.06*	4.58±0.17*	6.03±0.44*
F value	**6.51**	**5.79**	**5.37**	**8.40**
P value	**P=0.0010**	**P=0.0019**	**P=0.0029**	**P=0.0002**

Group 1: Normal control

Group 2: Doxorubicin treated group (3 mg/kg/week i.p.on days 1,7,14,21and 28).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.33: Effect of melatonin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in doxorubicin induced testicular toxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.04	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	1.82±0.10**	2.63±0.15***	2.55±0.15*	3.90±0.27***
Group 3	1.65±0.11NS	3.14±0.21NS	2.76±0.22NS	5.87±0.37NS
Group 4	1.44±0.1*	3.68±0.06*	3.32±0.16NS	6.38±0.36*
F value	**7.29**	**9.81**	**4.35**	**8.64**
P value	**P=0.0017**	**P=0.0003**	**P=0.0163**	**P=0.0007**

Group 1: Normal control

Group 2:Doxorubicin treated group (3 mg/kg/week i.p.on days 1,7,14,21and 28).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.34: Effect of lovastatin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in doxorubicin induced testicular toxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.04	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	1.82±0.10**	2.63±0.15***	2.55±0.15*	3.90±0.27***
Group 3	1.7±0.12NS	3.13±0.1 NS	2.888±0.2 NS	4.23±0.28 NS
Group 4	1.69±0.13 NS	3.26±0.15 NS	3.07±0.28 NS	4.31±0.29 NS
F value	**5.74**	**8.72**	**3.83**	**10.46**
P value	**P=0.0020**	**P=0.0001**	**P=0.0146**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (3 mg/kg/week i.p.on days 1,7,14,21and 28).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with Lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.3.2.3.3 Effect on membrane bound enzymes

4.3.3.2.3.3.1 Effect on Sodium Potassium ATPase

In the testes of doxorubicin (3 mg/kg/week i.p.on days 1,7,14,21and 28) treated rats (Group 2), the activity of Na^+K^+ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not alter the Na^+K^+ATPase levels; but at the higher dose (50 and 100 mg/kg), it significantly ($P<0.05$,$P<0.01$) increased these levels as compared to doxorubicin treated group (Table 4.35).

Treatment of melatonin (3 mg/kg/day p.o. for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not produce any significant increase in Na^+K^+ATPase levels; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased these levels as compared to doxorubicin treated group (Table 4.36).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) did not alter the Na^+K^+ATPase levels as compared to doxorubicin treated group (Table 4.37).

4.3.3.2.3.3.2 Effect on Calcium ATPase

Chronic administration of doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) did not produce significant changes in the Ca^{2+}ATPase activity as compared to the control (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day), melatonin (3 mg/kg/day p.o. for 30 day) and lovastatin (3 and 6 mg/kg/day p.o. for 30 day) along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not alter the Ca^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.35,4.36,4.37).

4.3.3.2.3.3.3 Effect on Magnesium ATPase

In the testes of doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treated rats (Group 2), the activity of Mg^{2+}ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration did not alter the Mg^{2+}ATPase levels; but at the higher dose (50 and 100 mg/kg), it significantly ($P<0.05$) increased these levels as compared to doxorubicin treated group (Table 4.35).

Treatment of melatonin (3 and 6 mg/kg/day p.o. for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) significantly ($P<0.05$) increased Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.36).

Treatment of lovastatin (3 and 6 mg/kg/day p.o. for 30 day) with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) did not alter the Mg^{2+}ATPase levels as compared to doxorubicin treated group (Table 4.37).

Table 4.35: Effect of green tea extract on membrane bound enzymes in the testes of rats in doxorubicin induced testicular toxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	5.01±0.30***	3.08±0.22^{NS}	4.01±0.26***
Group 3	5.02±0.31^{NS}	3.18±0.25^{NS}	4.25±0.19^{NS}
Group 4	6.31±0.10*	3.63±0.21^{NS}	5.16±0.23*
Group 5	6.76±0.34**	3.71±0.30^{NS}	5.25±0.22*
F value	**23.49**	**1.81**	**13.37**
P value	**P<0.0001**	**P=0.1571**	**P<0.0001**

Group 1: Normal control

Group 2:Doxorubicin treated group (3 mg/kg/week i.p.on days 1,7,14,21 and 28).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.36: Effect of melatonin on membrane bound enzymes in the testes of rats in doxorubicin induced testicular toxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.5±0.34	4.24±0.57	6.56±0.4
Group 2	5.01±0.3***	3.08±0.22 NS	4.01±0.26***
Group 3	6.21±0.4NS	3.97±0.28 NS	5.37±0.31*
Group 4	6.82±0.42*	3.98±0.31 NS	5.49±0.23*
F value	**15.06**	**1.80**	**11.39**
P value	**P<0.0001**	**P=0.178**	**P=0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (3 mg/kg/week i.p.on days 1,7,14,21 and 28).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.37: Effect of lovastatin on membrane bound enzymes in the testes of rats in doxorubicin induced testicular toxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	5.01±0.30***	3.08±0.22 NS	4.01±0.26***
Group 3	5.21±0.25 NS	3.2±0.24 NS	4.13±0.15 NS
Group 4	6.14±0.10 NS	3.5±0.17 NS	4.7±0.25 NS
F value	**29.42**	**2.47**	**16.06**
P value	**P<0.0001**	**P=0.072**	**P<0.0001**

Group 1: Normal control

Group 2: Doxorubicin treated group (3 mg/kg/week i.p.on days 1,7,14,21 and 28).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) along with doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) treatment.

Values are expressed as mean ± SEM.

The group treated with doxorubicin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with doxorubicin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.3.2.4 HISTOPATHOLOGY

Control rat (Fig 4.123-A) showed normal histoarchitecture of testes. Seminiferous tubules reflect the organized arrangement and well-defined boundaries.

Chronic administration of doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) causes vacuolization and fibrinoid debris in the seminiferous tubules. Shrunken seminiferous tubules showed disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also observed in interstitial tissues. (Fig 4.123-B)

Treatment of green tea extract along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules with increasing doses and disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also decreased as compared to doxorubicin treated rats [Fig. 4.123(C)-4.123(E)].

Similarly, Treatment of melatonin along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium at the dose of 6 mg/kg [Fig. 4.124(C)-4.124(D)].

The degree of testicular damage in lovastatin treated group along with chronic doxorubicin (3 mg/kg i.p. on days 1, 7, 14, 21 and 28) administration was similar to doxorubicin treated group in regard to morphological changes showing vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium with disorganization of germinal epithelium [Fig. 4.125(C)-4.125(D].

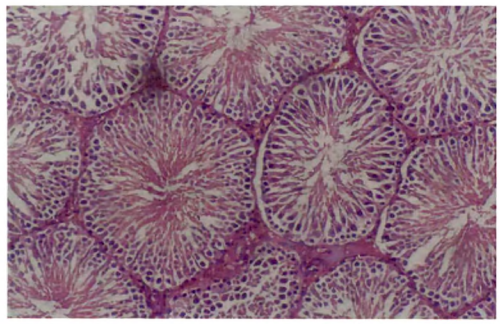
Fig. 4.123: Photomicrographs showing effect of green tea extract on the testes of doxorubicin treated rats (chronic study).

(Magnification 10 X)

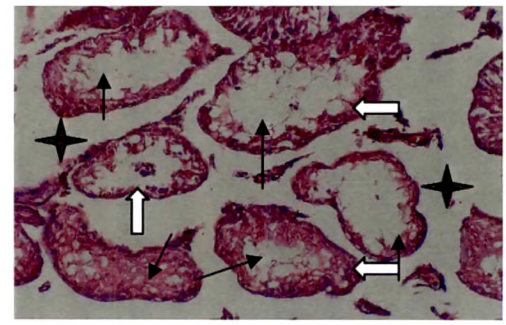
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

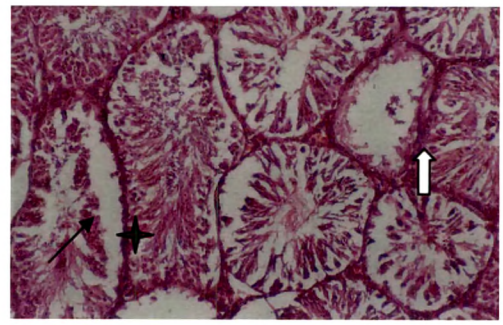
A: Normal control



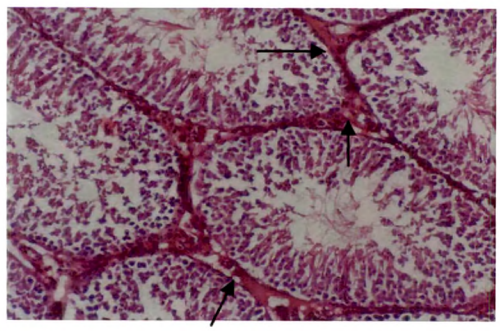
B: Doxorubicin (3 mg/kg, i.p.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

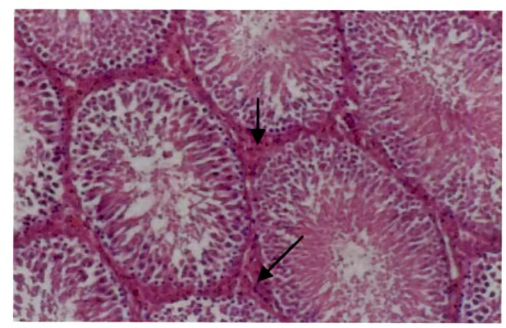


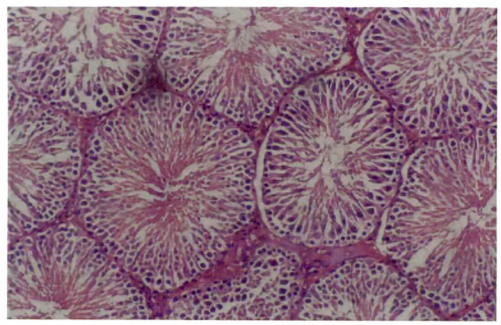
Fig. 4.124: Photomicrographs showing effect of melatonin on the testes of doxorubicin treated rats (chronic study).

(Magnification 10 X)

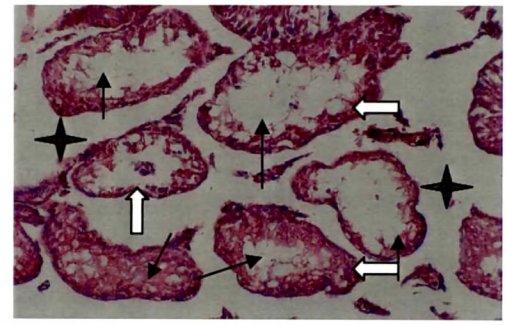
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

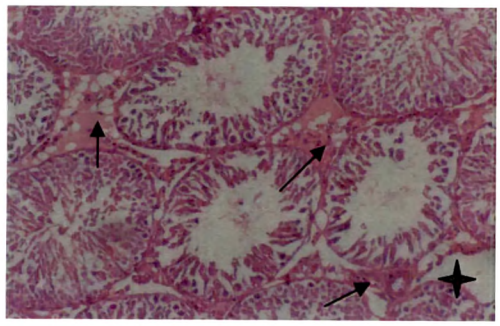
A: Normal control



B: Doxorubicin (3 mg/kg, i.p.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

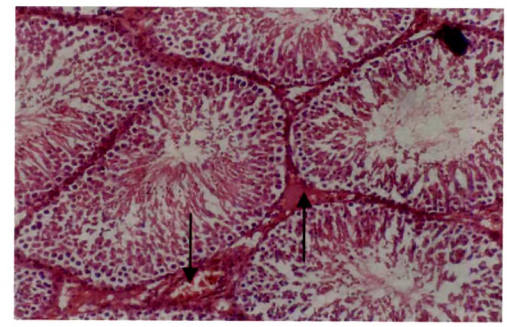


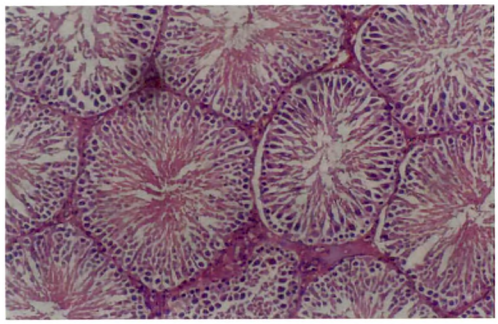
Fig. 4.125: Photomicrographs showing effect of lovastatin on the testes of doxorubicin treated rats (chronic study).

(Magnification 10 X)

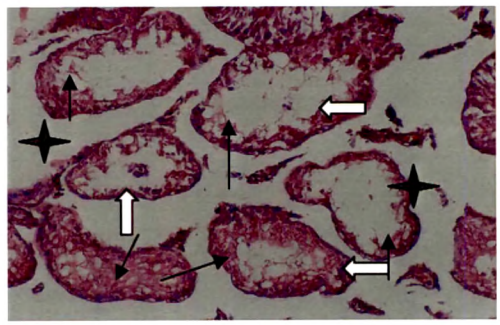
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

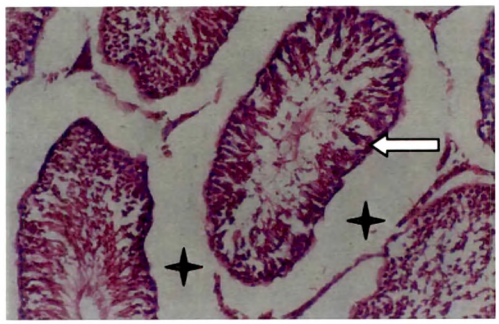
A: Normal control



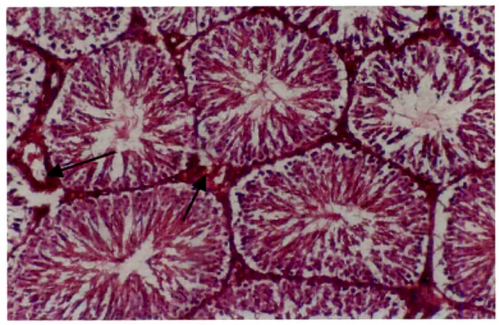
B: Doxorubicin (3 mg/kg, i.p.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.4 CISPLATIN INDUCED TESTICULAR TOXICITY

4.3.4.1: ACUTE STUDY IN RATS

4.3.4.1.1 Effect of drugs on Testosterone

Acute administration of cisplatin (5 mg/kg i.p.) produced a significant ($P<0.001$) decrease in the activity of testosterone in serum of rats (Group 2) as compared to control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce significant increase in the level of serum testosterone; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the testosterone level as compared to cisplatin treated group (Fig.4.126).

Pretreatment of melatonin (3 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant increase in the level of serum testosterone; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased the testosterone level as compared to cisplatin treated group (Fig.4.127).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce significant increase in the level of serum testosterone as compared to cisplatin treated group (Fig.4.128).

Fig.4.126: Effect of green tea extract on the serum testosterone in cisplatin induced testicular toxicity (acute study) in rats.

Fig.4.127: Effect of melatonin on the serum testosterone in cisplatin induced testicular toxicity (acute study) in rats.

Fig.4.128: Effect of lovastatin on the serum testosterone in cisplatin induced testicular toxicity (acute study) in rats.

Values are expressed as mean ± SEM.
The group treated with cisplatin alone (Cis) was compared with control.
Groups treated with green tea extract {Cis + GTE (25), Cis + GTE (50), Cis + GTE (100)} were compared with Cis alone group.
Groups treated with melatonin {Cis + Mel (3) and Cis + Mel (6)} were compared with Cis alone group.
Groups treated with lovastatin {Cis + Lova (3) and Cis + Lova (6)} were compared with Cis alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.126

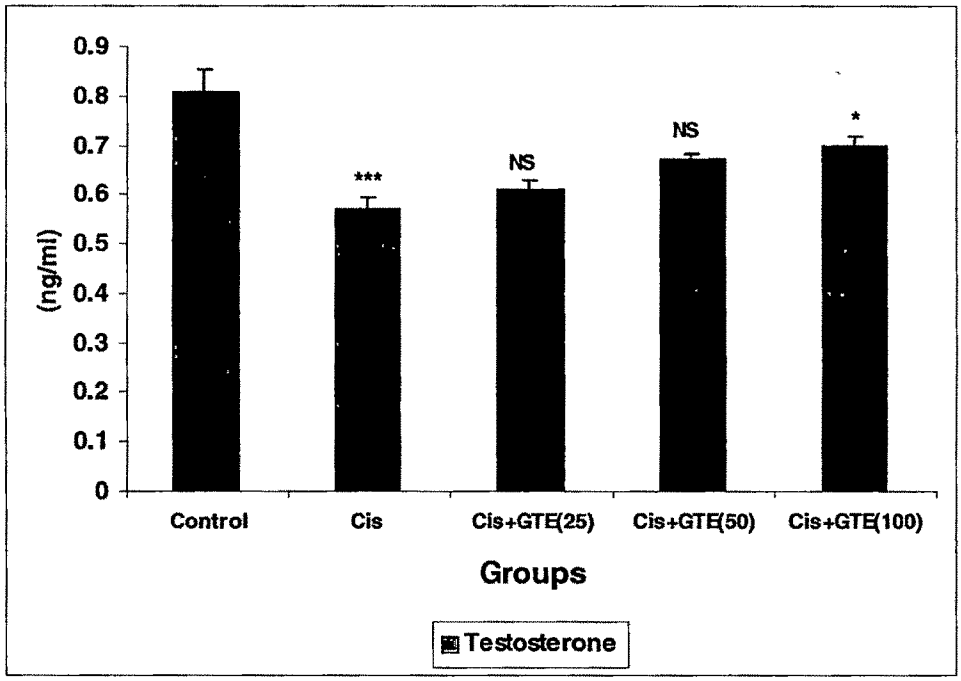


Fig.4.127

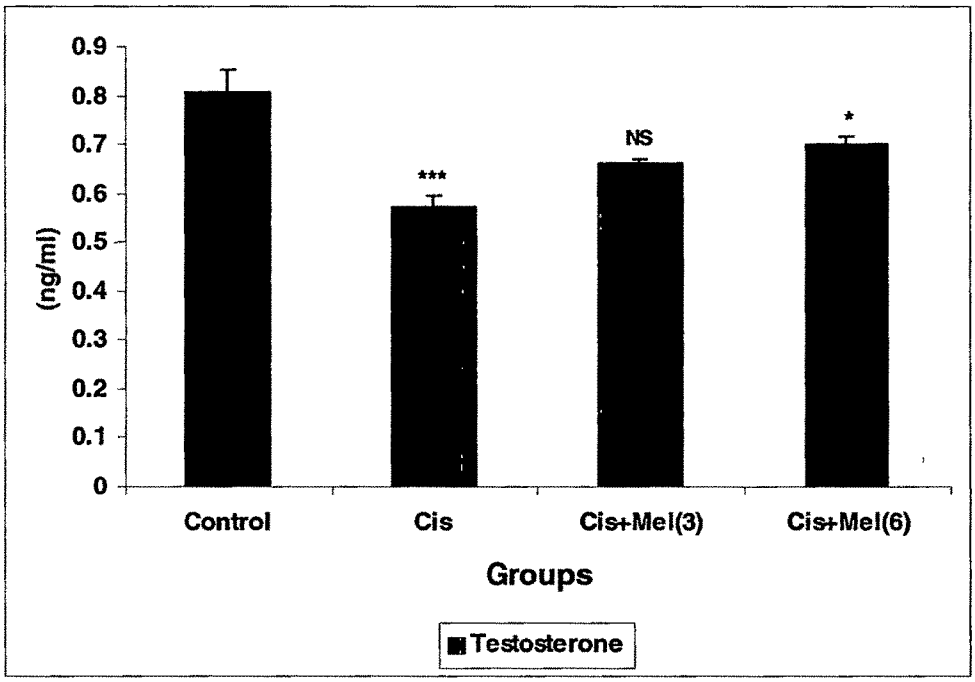
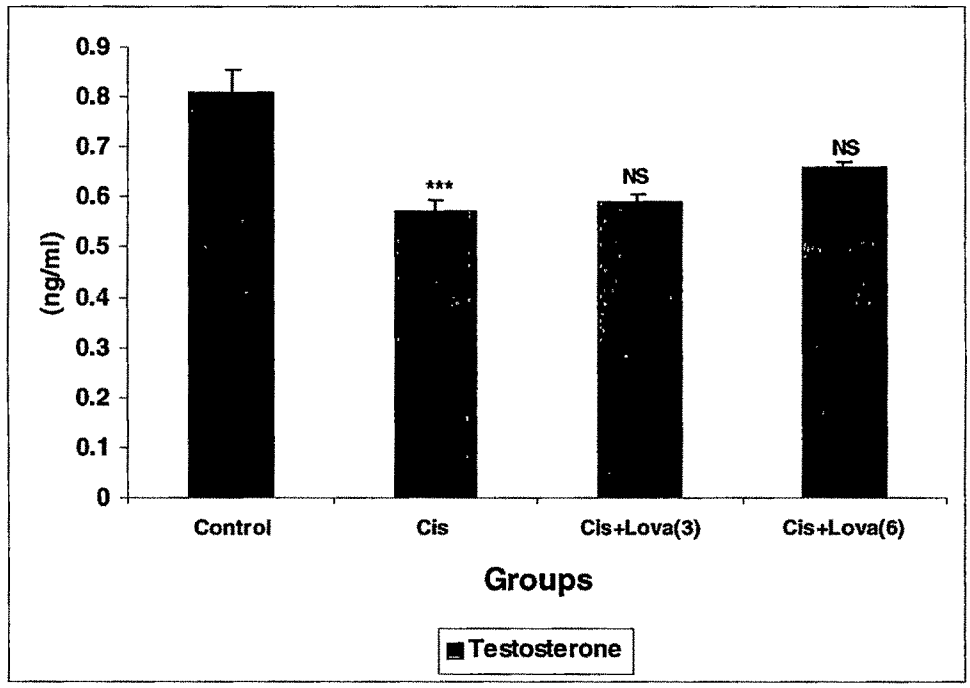


Fig.4.128


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(ng/ml)
***
NS
NS
Control
Cis
Cis+Lova(3)
Cis+Lova(6)
Groups
Testosterone

4.3.4.1.2 Effect of drugs on Sperm count

Acute cisplatin (5 mg/kg i.p.) administration produced a significant ($P<0.001$) decrease in the sperm count of rats (Group 2) as compared to control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce significant increase in the sperm count; but the higher dose (100 mg/kg) of green tea extract significantly ($P<0.05$) increased the sperm count as compared to cisplatin treated group (Fig.4.129).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce significant increase in the sperm count as compared to cisplatin treated group (Fig.4.130).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce significant increase in the sperm count as compared to cisplatin treated group (Fig.4.131).

Fig.4.129: Effect of green tea extract on sperm count in cisplatin induced testicular toxicity (acute study) in rats.

Fig.4.130: Effect of melatonin on sperm count in cisplatin induced testicular toxicity (acute study) in rats.

Fig.4.131: Effect of lovastatin on sperm count in cisplatin induced testicular toxicity (acute study) in rats.

Values are expressed as mean ± SEM.
The group treated with cisplatin alone (Cis) was compared with control.
Groups treated with green tea extract {Cis + GTE (25), Cis + GTE (50) and Cis + GTE (100)} were compared with Cis alone group.
Groups treated with melatonin {Cis + Mel (3), Cis + Mel (6)} were compared with Cis alone group.
Groups treated with lovastatin {Cis + Lova (3) and Cis + Lova (6)} were compared with Cis alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.129

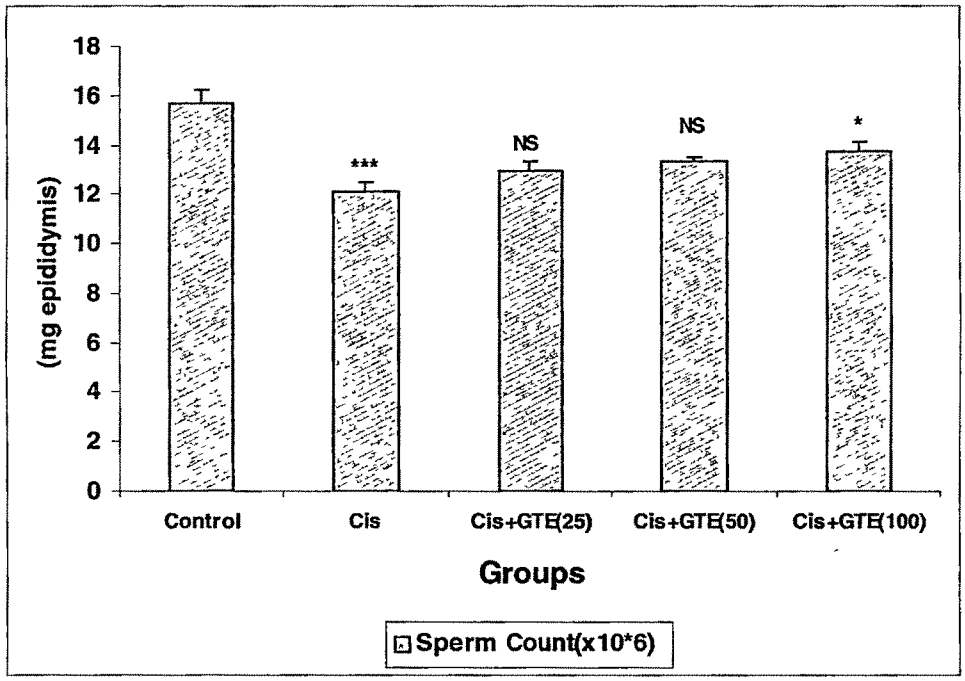


Fig.4.130

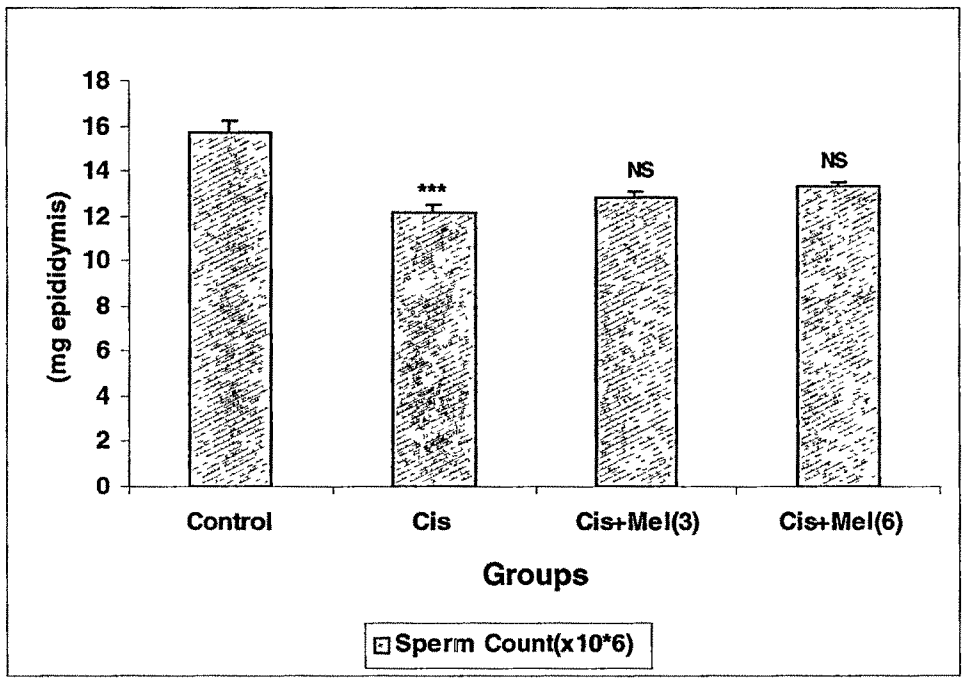
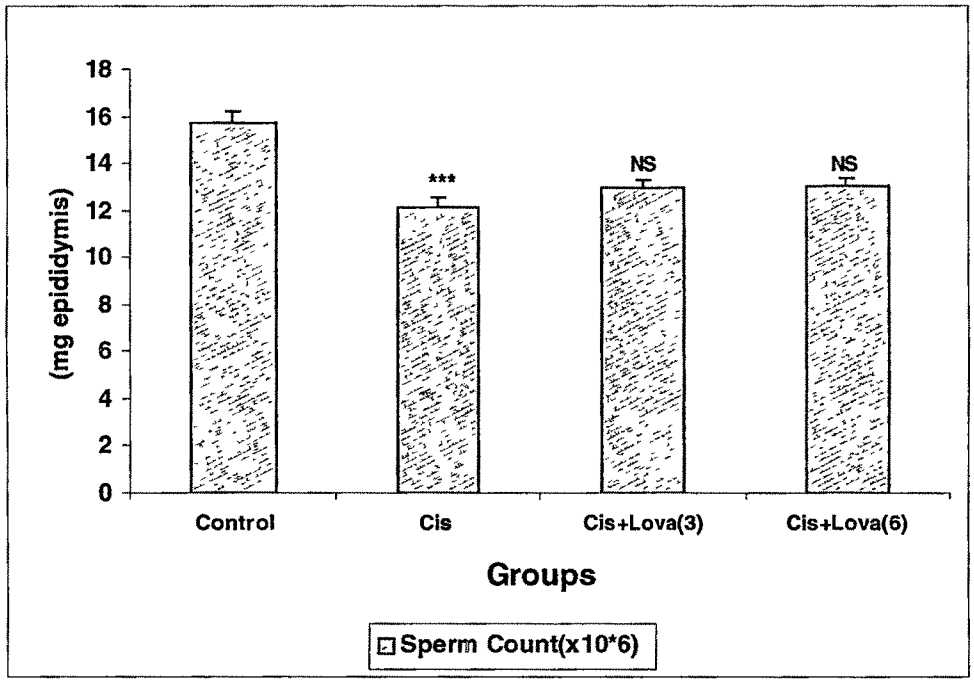


Fig.4.131


18
16
14
12
10
8
6
4
2
0
(mg epididymis)
***
NS
NS
Control
Cis
Cis+Lova(3)
Cis+Lova(6)
Groups
Sperm Count(x10*6)

4.3.4.1.3 TISSUE PARAMETERS

4.3.4.1.3.1 Effect on lipid peroxidation

Acute cisplatin (5 mg/kg i.p.) administration (Group 2) to rats led to a significant (P<0.001) increase in lipid peroxidation or MDA content in testes of rats as compared to the control group (Group 1).

Pretreatment of green tea extract (25 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant change in the level of lipid peroxidation (MDA content); but the higher doses (50 and100 mg/kg) of green tea extract significantly (P<0.05,P<0.01) decreased these levels as compared to cisplatin treated group (Table 4.38).

Pretreatment of melatonin (3mg/kg/day p.o.for 30 day) did not produce significant change in the level of lipid peroxidation (MDA content); but at the higher dose (6 mg/kg), it significantly (P<0.01) decreased these levels as compared to cisplatin treated group (Table 4.39).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant change in the level of lipid peroxidation (MDA content) as compared to cisplatin treated group (Table 4.40).

4.3.4.1.3.2 Effect on endogenous antioxidants

4.3.4.1.3.2.1 Effect on Superoxide dismutase

Acute cisplatin (5 mg/kg i.p.) administration (Group 2) reduced the SOD activity significantly (P<0.001) in testes of rats as compared to control (Group 1).

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant changes in the level of SOD content as compared to cisplatin treated group (Table 4.38).

Pretreatment of Melatonin at all the doses (3 and 6mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) fails to produce any significant changes in the level of SOD content as compared to cisplatin treated group (Table 4.39).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant changes in the level of SOD content as compared to cisplatin treated group (Table 4.40).

4.3.4.1.3.2.2 Effect on Catalase

The catalase activity in cisplatin (5 mg/kg i.p.) treated group (Group 2) was significantly ($P<0.001$) reduced as compared to control group (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant change in the level of catalase; but at the higher dose (100 mg/kg), it significantly ($P<0.05$) increased these levels as compared to cisplatin treated group (Table 4.38).

Pretreatment of Melatonin (3 and 6mg/kg/day p.o.for 30 day) and lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce significant changes in the level of catalase content as compared to cisplatin treated group (Tables 4.39 Table 4.40).

4.3.4.1.3.2.3 Effect on Reduced glutathione

A significant ($P<0.001$) reduction in reduced glutathione concentration was observed in cisplatin (5 mg/kg i.p.) treated rats (Group 2) as compared to control animal (Group 1).

Pretreatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant changes in the level of GSH content as compared to cisplatin treated group (Table 4.38).

Pretreatment of melatonin (3 mg/kg and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin treatment fails to produce significant changes in the level of GSH content as compared to cisplatin treated group (Tables 4.39).

Pretreatment of lovastatin (3 mg/kg and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin treatment did not produce any significant changes in the level of GSH as compared to cisplatin treated group (Table 4.40).

Table 4.38: Effect of green tea extract on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in cisplatin induced testicular toxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.04	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	3.13±0.28***	2.44±0.22***	1.63±0.25***	3.51±0.3***
Group 3	2.84±0.12^{NS}	2.47±0.22^{NS}	1.87±0.32 ^{NS}	3.82±0.12^{NS}
Group 4	2.24±0.19*	3.27±0.21^{NS}	2.59±0.29 ^{NS}	4.16±0.2^{NS}
Group 5	2.14±0.14**	3.31±0.23^{NS}	3.66±0.13 ^{NS}	5.23±0.31*
F value	**17.51**	**8.19**	**7.53**	**13.97**
P value	**P<0.0001**	**P=0.0002**	**P=0.0004**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p.).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.39: Effect of melatonin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in cisplatin induced testicular toxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	3.13±0.28***	2.44±0.22***	1.63±0.25**	3.51±0.3***
Group 3	2.61±0.14^{NS}	2.63±0.11^{NS}	2.15±0.26^{NS}	4.29±0.15^{NS}
Group 4	2.3±0.11*	3.06±0.2^{NS}	2.71±0.17^{NS}	4.93±0.18^{NS}
F value	**22.68**	**11.27**	**7.47**	**14.18**
P value	**P<0.0001**	**P=0.0002**	**P=0.0015**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg i.p.on 30th day)

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.40: Effect of lovastatin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in cisplatin induced testicular toxicity (acute study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	3.13±0.28***	2.44±0.22***	1.63±0.25***	3.51±0.3***
Group 3	3±0.18^{NS}	2.44±0.14 ^{NS}	1.96±0.18 ^{NS}	3.67±0.17 ^{NS}
Group 4	2.57±0.27^{NS}	2.86±0.18 ^{NS}	2.27±0.27 ^{NS}	3.78±0.17 ^{NS}
F value	**9.61**	**11.52**	**8.46**	**18.78**
P value	**P<0.0001**	**P<0.0001**	**P=0.0002**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg i.p.on 30th day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.4.1.3.3 Effect on membrane bound enzymes

4.3.4.1.3.3.1 Effect on Sodium Potassium ATPase

In the testes of cisplatin (5 mg/kg i.p.on 30th day) treated rats (Group 2) the activity of Na^+K^+ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not alter the Na^+K^+ATPase levels; but at higher dose (100 mg/kg), it significantly ($P<0.001$) increased these levels as compared to cisplatin treated group (Table 4.41).

Pretreatment of melatonin (3 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not produce any significant increase in Na^+K^+ATPase levels; but at higher dose (6 mg/kg), it significantly ($P<0.05$) increased these levels as compared to cisplatin treated group (Table 4.42).

Pretreatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not alter the Na^+K^+ATPase levels as compared to cisplatin treated group (Table 4.43).

4.3.4.1.3.3.2 Effect on Calcium ATPase

Acute treatment with cisplatin (5 mg/kg i.p.on 30th day) resulted in a significant ($P<0.05$) decrease in the Ca^{2+}ATPase activity as compared to the control (Group 1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day), melatonin (3 and 6 mg/kg/day p.o.for 30 day) and lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not alter the Ca^{2+}ATPase levels as compared to cisplatin treated group (Table 4.41,4.42,4.43).

4.3.4.1.3.3.3 Effect on Magnesium ATPase

In the testes of cisplatin (5 mg/kg i.p.on 30th day) treated rats (Group 2) the activity of Mg^{2+}ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Pretreatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30th day) did not alter the

Mg^{2+}ATPase levels; but at higher dose (100 mg/kg), it significantly ($P<0.001$) increased these levels as compared to cisplatin treated group (Table 4.41).

Pretreatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) and lovastatin (3 and 6 mg/kg/day p.o.for 30 day) followed by acute cisplatin (5 mg/kg i.p.on 30^{th} day) did not alter the Mg^{2+}ATPase levels as compared to cisplatin treated group (Table 4.42 and 4.43).

Table 4.41: Effect of green tea extract on membrane bound enzymes in the testes of rats in cisplatin induced testicular toxicity (acute study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	4.85±0.39***	2.7±0.33*	3.54±0.26***
Group 3	5.46±0.29NS	3.05±0.096 NS	3.75±0.1 NS
Group 4	6.07±0.26NS	3.5±0.25 NS	4.21±0.23 NS
Group 5	7.3±0.15***	3.77±0.32 NS	5.63±0.2***
F value	**23.56**	**2.89**	**24.94**
P value	**P<0.0001**	**P=0.042**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg, i.p.).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with Cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.42: Effect of melatonin on membrane bound enzymes in the testes of rats in cisplatin induced testicular toxicity (acute study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	4.85±0.39***	2.7±0.33*	3.54±0.26***
Group 3	5.67±0.19NS	2.97±0.11NS	3.84±0.15NS
Group 4	6.47±0.37*	3.22±0.13NS	3.99±0.24NS
F value	**21.68**	**3.77**	**24.63**
P value	**P<0.0001**	**P=0.0268**	**P<0.001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg i.p.on 30th day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.43: Effect of lovastatin on membrane bound enzymes in the testes of rats in cisplatin induced testicular toxicity (acute study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	4.85±0.39***	2.7±0.33*	3.54±0.26***
Group 3	5.1±0.24NS	3.13±0.13 NS	3.68±0.16 NS
Group 4	5.76±0.14 NS	3.22±0.32 NS	3.97±0.25 NS
F value	**25.02**	**2.96**	**22.06**
P value	**P<0.0001**	**P=0.0391**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin treated group (5 mg/kg i.p.on 30th day) .

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) followed by cisplatin (5 mg/kg i.p.on 30th day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.4.1.4 HISTOPATHOLOGY

Control rat (Fig 4.132-A) showed normal histoarchitecture of testes. Seminiferous tubules reflect the organized arrangement and well-defined boundaries.

Acute administration of cisplatin (5 mg/kg i.p.on 30th day) causes vacuolization and fibrinoid debris in the seminiferous tubules. Shrunken seminiferous tubules showed disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also observed in interstitial tissues. (Fig 4.132-B)

Pretreatment of green tea extract followed by acute cisplatin (5 mg/kg i.p.on 30th day) exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules with increasing doses and disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also decreased as compared to cisplatin treated rats [Fig. 4.132(C)-4.132(E)].

Similarly, pretreatment of melatonin followed by acute cisplatin (5 mg/kg i.p.on 30th day) exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium at the dose of 6 mg/kg [Fig. 4.133(C)-4.133(D)].

The degree of testicular damage in lovastatin treated group (3 mg/kg) was similar to cisplatin (5 mg/kg i.p.on 30th day) treated group in regard to morphological changes showing vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium with disorganization of germinal epithelium [Fig. 4.134(C)-4.134(D)].

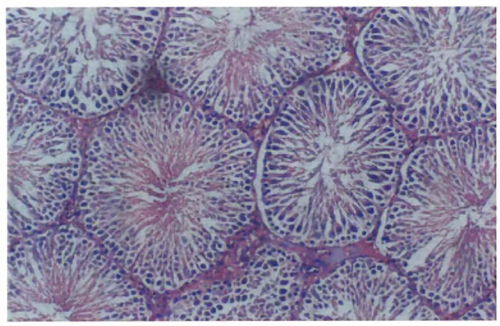
Fig. 4.132: Photomicrographs showing effect of green tea extract on the testes of cisplatin-treated rats (acute study).

(Magnification 10 X)

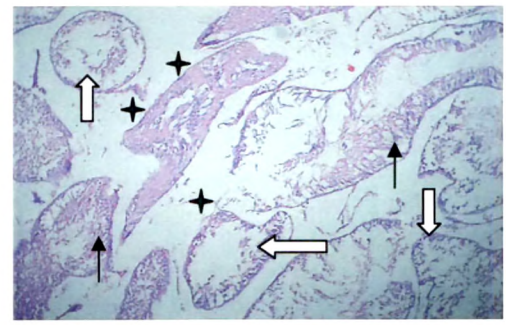
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

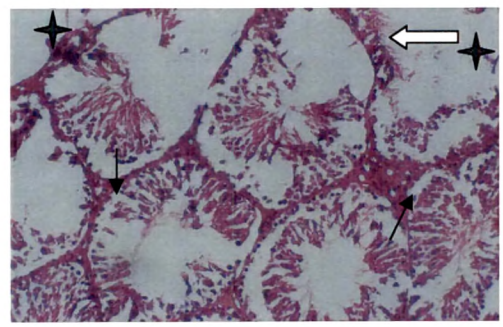
A: Normal control



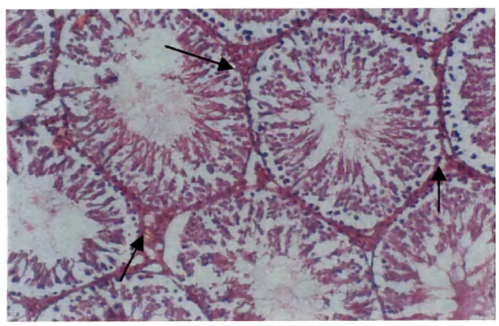
B: Cisplatin (5mg/kg, i.p.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

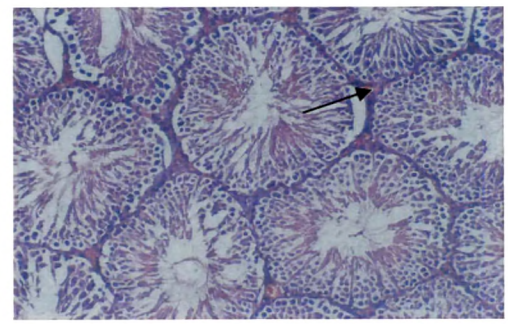


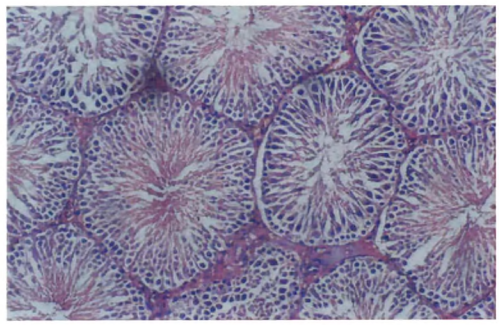
Fig. 4.133: Photomicrographs showing effect of melatonin on the testes of cisplatin treated rats (acute study).

(Magnification 10 X)

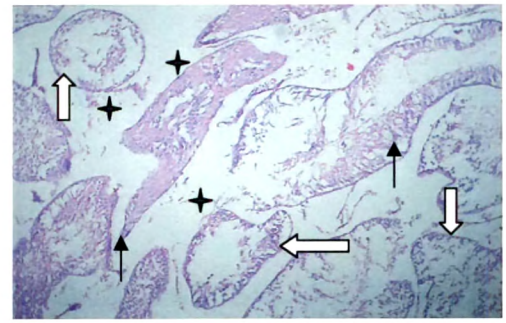
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

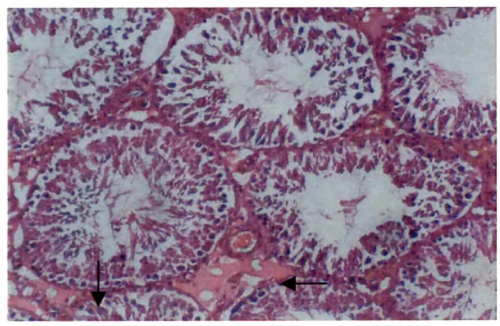
A: Normal control



B: Cisplatin (5mg/kg, i.p.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

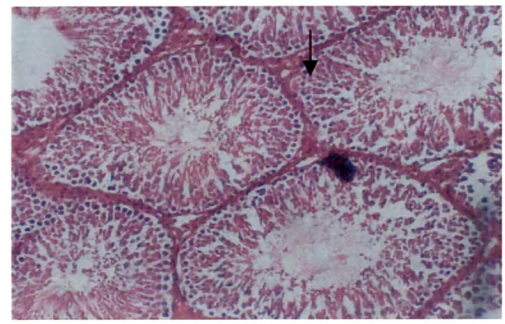


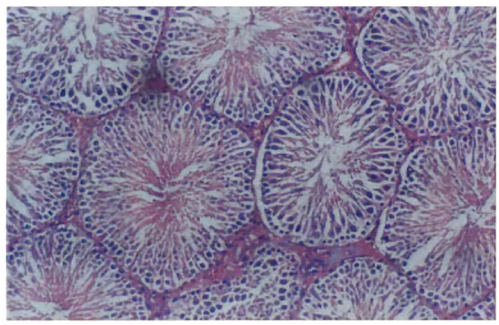
Fig. 4.134: Photomicrographs showing effect of lovastatin on the testes of cisplatin treated rats (acute study).

(Magnification 10 X)

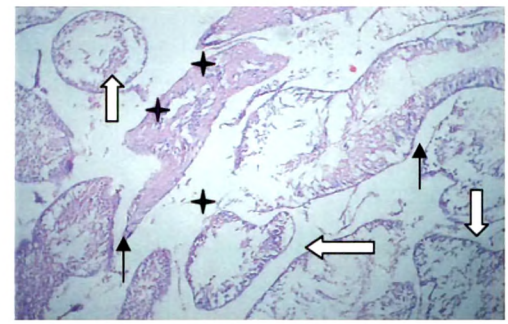
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

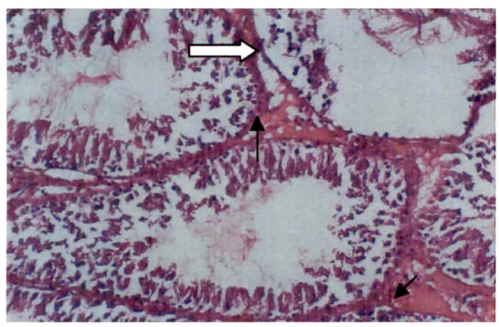
A: Normal control



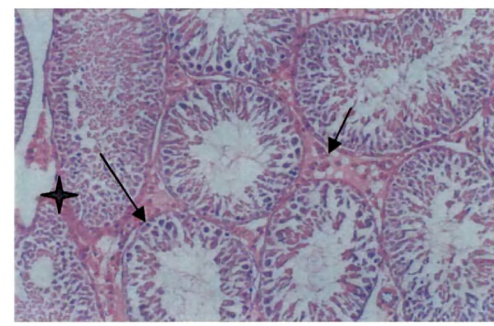
B: Cisplatin (5mg/kg, i.p.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.4 CISPLATIN INDUCED TESTICULAR TOXICITY

4.3.4.2: CHRONIC STUDY IN RATS

4.3.4.2.1 Effect of drugs on Testosterone

Chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration produced a significant (P<0.001) decrease in the activity of testosterone in serum of rats (Group 2) as compared to control group (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant increase in the level of serum testosterone; but the higher dose (50 and 100 mg/kg) of green tea extract significantly (P<0.05,P<0.001) increased the testosterone level as compared to cisplatin treated group (Fig.4.135).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) with chronic cisplatin (3mg/kg i.p.on 1, 7, 14, 21 and 28day) significantly (P<0.05,P<0.01) increased the testosterone level as compared to cisplatin treated group (Fig.4.136).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) did not produce significant increase in the level of serum testosterone as compared to cisplatin treated group (Fig.4.137).

Fig.4.135: Effect of green tea extract on the serum testosterone in cisplatin induced testicular toxicity (chronic study) in rats.

Fig.4.136: Effect of melatonin on the serum testosterone in cisplatin induced testicular toxicity (chronic study) in rats.

Fig.4.137: Effect of lovastatin on the serum testosterone in cisplatin induced testicular toxicity (chronic study) in rats.

Values are expressed as mean ± SEM.
The group treated with cisplatin alone (Cis) was compared with control.
Groups treated with green tea extract {Cis + GTE (25), Cis + GTE (50) and Cis + GTE (100)} were compared with Cis alone group.
Groups treated with melatonin {Cis + Mel (3) and Cis + Mel (6)} were compared with Cis alone group.
Groups treated with lovastatin {Cis + Lova (3) and Cis + Lova (6)} were compared with Cis alone group.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.135

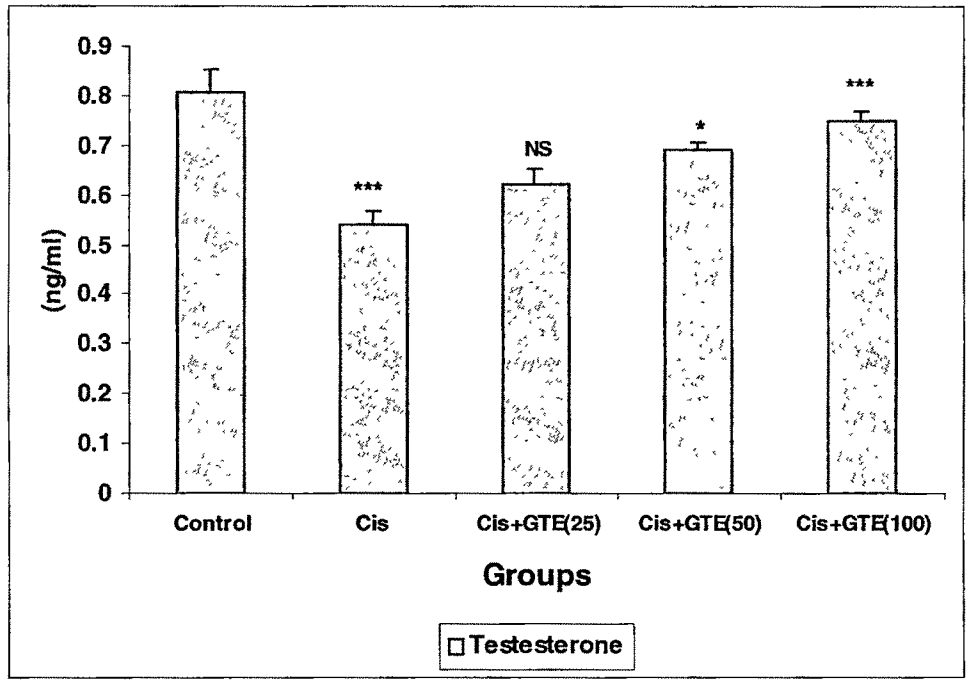


Fig.4.136

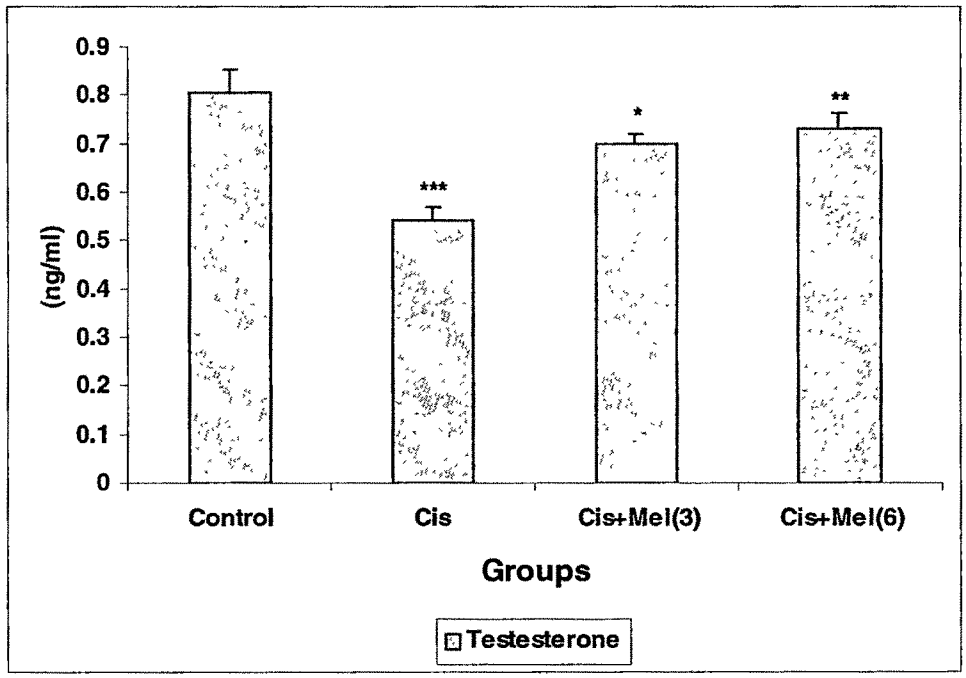
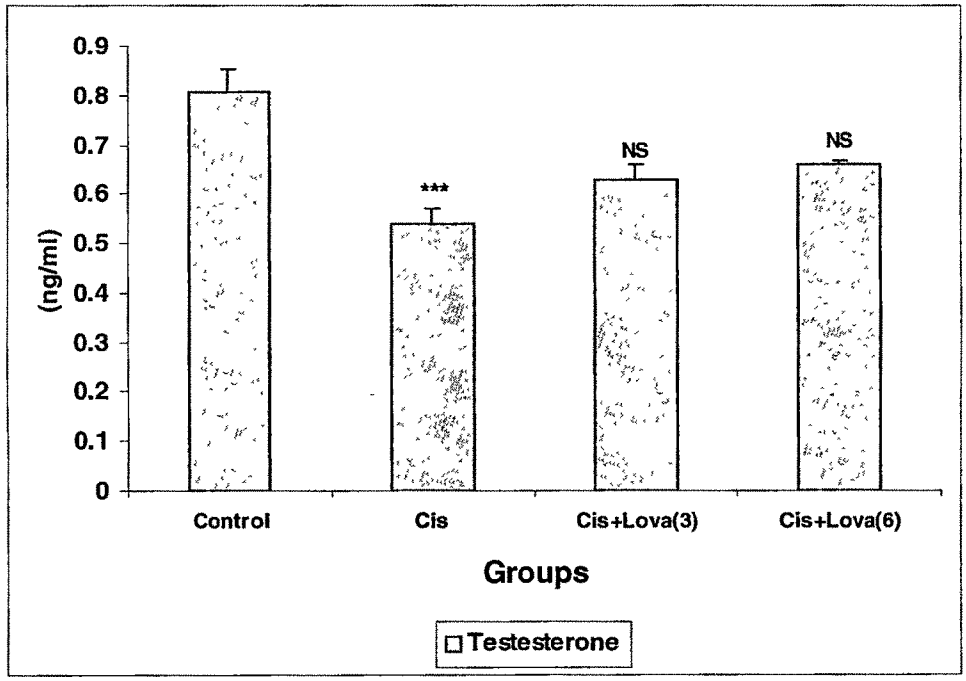


Fig.4.137


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(ng/ml)
***
NS
NS
Control
Cis
Cis+Lova(3)
Cis+Lova(6)
Groups
Testesterone

4.3.4.2.2 Effect of drugs on Sperm count

Chronic cisplatin (3mg/kgi.p.on1, 7,14,21and 28 day) administration produced a significant (P<0.001) decrease in the sperm count of rats (Group 2) as compared to control group (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant increase in the sperm count; but the higher dose (50 and 100 mg/kg) of green tea extract significantly (P<0.05,P<0.01) increased the sperm count as compared to cisplatin treated group (Fig.4.138).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) did not produce any significant increased in the sperm count; but at the higher dose (6 mg/kg), it significantly (P<0.01) increased the sperm count as compared to cisplatin treated group (Fig.4.139).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with cisplatin (3 mg/kg i.p.) did not produce significant increase in the sperm count as compared to cisplatin treated group (Fig.4.140).

Fig.4.138: Effect of green tea extract on sperm count in cisplatin induced testicular toxicity (chronic study) in rats.

Fig.4.139: Effect of melatonin on sperm count in cisplatin induced testicular toxicity (chronic study) in rats.

Fig.4.140: Effect of lovastatin on sperm count in cisplatin induced testicular toxicity (chronic study) in rats.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control.

Groups treated with green tea extract {Cis + GTE (25), Cis + GTE (50) and Cis + GTE (100)} were compared with Cis alone group.

Groups treated with melatonin {Cis + Mel (3) and Cis + Mel (6)} were compared with Cis alone group.

Groups treated with lovastatin {Cis + Lova (3) and Cis + Lova (6)} were compared with Cis alone group.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = Non Significant

Fig.4.138

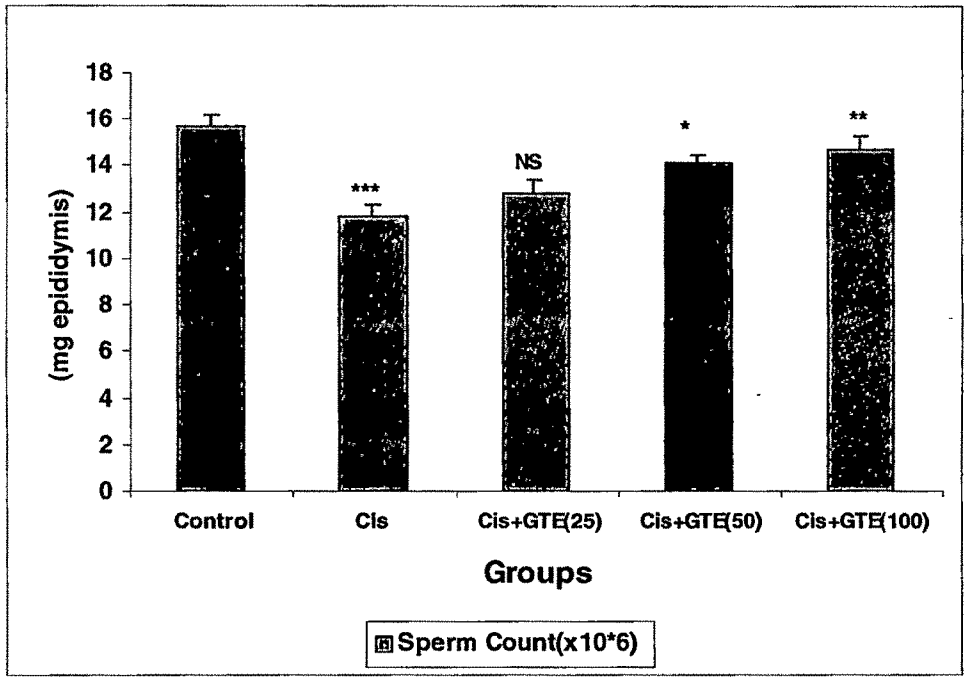


Fig.4.139

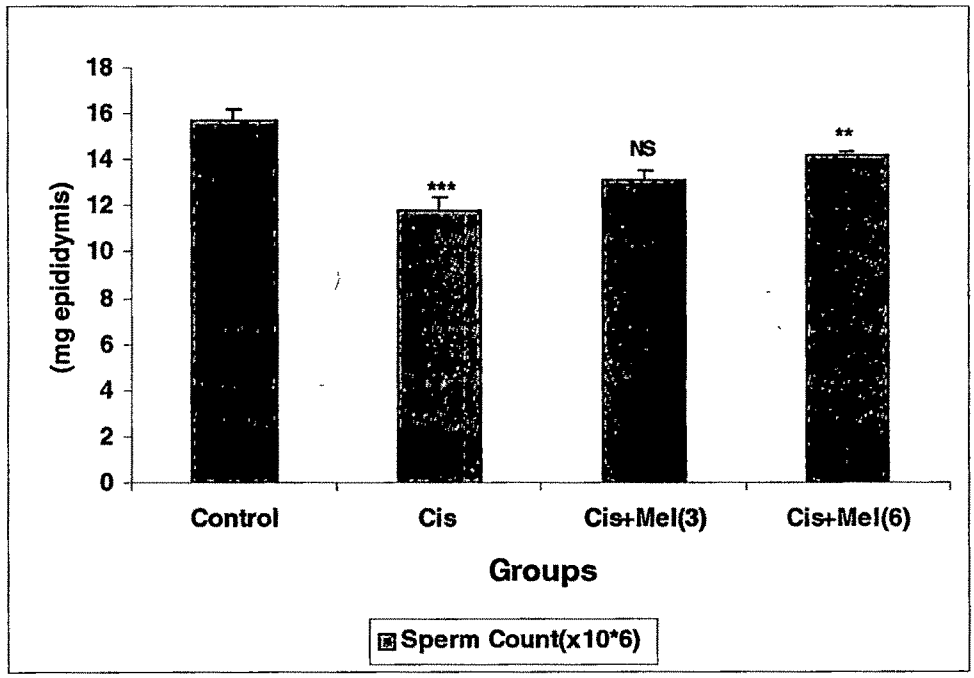
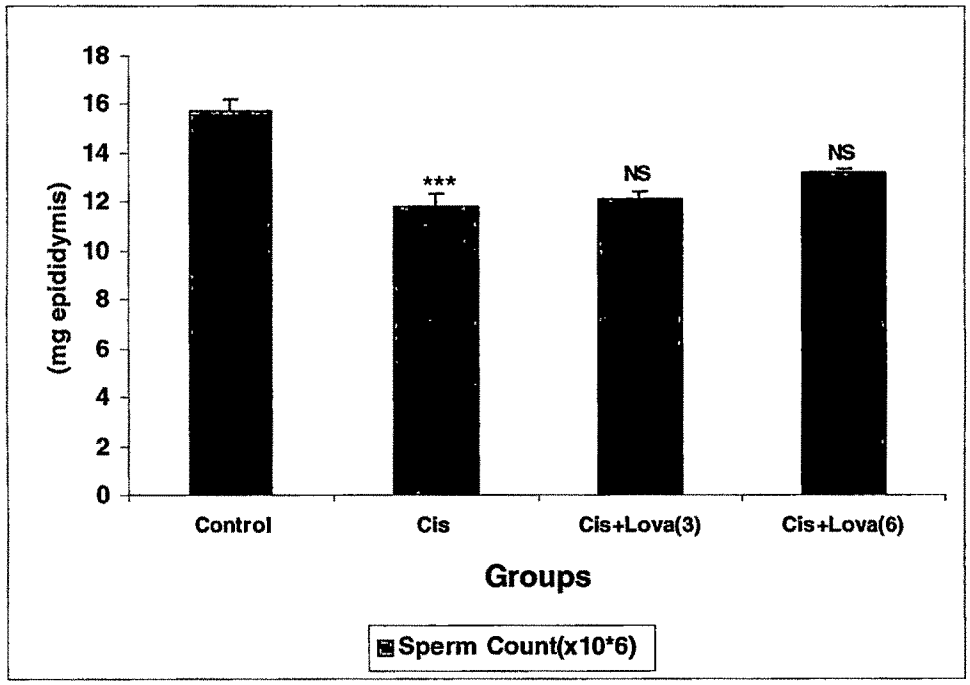


Fig.4.140


18
16
14
12
10
8
6
4
2
0
(mg epididymis)
***
NS
NS
Control
Cis
Cis+Lova(3)
Cis+Lova(6)
Groups
Sperm Count(x10*6)

4.3.4.2.3 TISSUE PARAMETERS

4.3.4.2.3.1 Effect on lipid peroxidation

Chronic cisplatin (3mg/kgi.p.on1, 7,14,21and 28 day) administration to rats led to a significant (P<0.001) increase in lipid peroxidation or MDA content in testes of rats as compared to the control group (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant change in the level of lipid peroxidation (MDA content); but the higher doses (50 and 100 mg/kg) of green tea extract significantly (P<0.01,P<0.001) decreased these levels as compared to cisplatin treated group (Table 4.44).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration produced a significant (P<0.01,P<0.001) decreased in lipid peroxidation (MDA content) as compared to cisplatin treated group (Table 4.45).

Treatment of lovastatin (3 mg/kg/day p.o. for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant change in the level of lipid peroxidation (MDA content); but at the higher dose (6 mg/kg), it significantly (P<0.01) decreased these levels as compared to cisplatin treated group (Table 4.46).

4.3.4.2.3.2 Effect on endogenous antioxidants

4.3.4.2.3.2.1 Effect on Superoxide dismutase

Chronic cisplatin (3mg/kgi.p.on1, 7,14,21and 28 day) administration reduced the SOD activity significantly (P<0.001) in testes of rats as compared to control (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant change in the level of SOD content; but at the higher doses (50 and 100 mg/kg), it significantly (P<0.05,P<0.01) increased these levels as compared to cisplatin treated group (Table 4.44).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration

produced a significant (P<0.05,P<0.01) increase in the level of SOD content as compared to cisplatin treated group (Tables 4.45).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant changes in the level of SOD content as compared to cisplatin treated group (Tables 4.46).

4.3.4.2.3.2.2 Effect on Catalase

The catalase activity in cisplatin (3mg/kgi.p.on1, 7,14,21and 28 day) treated group (Group 2) was significantly (P<0.001) reduced as compared to control group (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce any significant change in the level of catalase; but at the higher dose (50 and 100 mg/kg), it significantly (P<0.05,P<0.001) increased these levels as compared to cisplatin treated group (Table 4.44).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce significant changes in the level of catalase content; but at the higher dose (6 mg/kg), it significantly (P<0.01) increased these levels as compared to cisplatin treated group (Table 4.45).

Treatment of lovastatin (3 and 6mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce any significant changes in the level of catalase as compared to cisplatin treated group (Tables 4.46).

4.3.4.2.3.2.3 Effect on Reduced glutathione

A significant (P<0.001) reduction in reduced glutathione concentration was observed in cisplatin (3mg/kgi.p.on1, 7,14,21and 28 day) treated rats (Group 2) as compared to control animal (Group 1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce any significant change in the level of GSH; but at the higher dose (50 and 100 mg/kg), it significantly (P<0.05,P<0.01) increased these levels as compared to cisplatin treated group (Table 4.44).

Treatment of melatonin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration significantly ($P<0.05$,$P<0.01$) increased the level of GSH when compared with cisplatin treated group (Table 4.45).

Treatment of lovastatin (3 and 6mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce any significant changes in the level of GSH as compared to cisplatin treated group (Tables 4.46).

Table 4.44: Effect of green tea extract on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in cisplatin induced testicular toxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	2.61±0.11***	2.5±0.25**	0.59±0.125***	2.78±0.49***
Group 3	2.28±0.14^{NS}	3.08±0.28^{NS}	0.99±0.072^{NS}	3.55±0.62^{NS}
Group 4	2.05±0.18**	3.88±0.23*	2.79±0.426*	5.29±0.26*
Group 5	1.6±0.1***	4.05±0.3**	3.44±0.32**	6.95±0.66***
F value	**18.65**	**6.47**	**12.67**	**11.41**
P value	**P<0.0001**	**P=0.0010**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with Cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.45: Effect of melatonin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in cisplatin induced testicular toxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	2.61±0.11***	2.5±0.252***	0.59±0.125***	2.78±0.49***
Group 3	2.03±0.1**	3.49±0.18*	2.67±0.16*	4.44±0.21^{NS}
Group 4	1.71±0.14***	3.91±0.17**	3.24±0.18**	5.32±0.22**
F value	**28.93**	**9.22**	**12.39**	**14.97**
P value	**P<0.0001**	**P=0.0005**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.46: Effect of lovastatin on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the testes of rats in cisplatin induced testicular toxicity (chronic study) in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	1.22±0.043	4.15±0.32	4.92±0.97	7.29±0.77
Group 2	2.61±0.11***	2.5±0.25***	0.59±0.125***	2.78±0.49***
Group 3	2.34±0.11NS	2.67±0.16 NS	1.14±0.097 NS	3.32±0.5 NS
Group 4	2.08±0.11**	3.01±0.14 NS	1.41±0.15 NS	4.4±0.35 NS
F value	**37.34**	**7.28**	**15.79**	**13.56**
P value	**P<0.0001**	**P=0.0005**	**P<0.0001**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day).

Group 3: Lovastatin (3 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 4: Lovastatin (6 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.4.2.3.3 Effect on membrane bound enzymes

4.3.4.2.3.3.1 Effect on Sodium Potassium ATPase

In the testes of cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treated rats (Group 2) the activity of Na^+K^+ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Treatment of green tea extract (25 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not alter the Na^+K^+ATPase levels; but the higher doses (50 and 100 mg/kg) of green tea extract significantly ($P<0.05$,$P<0.01$) increased these levels as compared to cisplatin treated group (Table 4.47).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not produce any significant increase in Na^+K^+ATPase levels; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased these levels as compared to cisplatin treated group (Table 4.48).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3 mg/kg i.p.on1, 7,14,21and 28 day) administration did not alter the Na^+K^+ATPase levels as compared to cisplatin treated group (Table 4.49).

4.3.4.2.3.3.2 Effect on Calcium ATPase

Chronic administration of cisplatin (3mg/kgi.p.on1, 7,14,21and 28 day) resulted in a significant ($P<0.05$) decrease in the Ca^{2+}ATPase activity as compared to the control (Group 1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not alter the Ca^{2+}ATPase levels; but at the higher dose (100 mg/kg), it significantly ($P<0.05$) increased these levels as compared to cisplatin treated group (Table 4.47).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) and lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not alter the Ca^{2+}ATPase levels as compared to cisplatin treated group (Table 4.48 and 4.49).

4.3.4.2.3.3.3 Effect on Magnesium ATPase

In the testes of cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treated rats (Group 2) the activity of Mg^{2+}ATPase enzyme was significantly ($P<0.001$) reduced as compared to the control (Group1).

Treatment of green tea extract (25 and 50 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not alter the Mg^{2+}ATPase levels; but at the higher dose (100 mg/kg), it significantly ($P<0.01$) increased these levels as compared to cisplatin treated group (Table 4.47).

Treatment of melatonin (3 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) did not produce any significant increase in Mg^{2+}ATPase levels; but at the higher dose (6 mg/kg), it significantly ($P<0.05$) increased these levels as compared to cisplatin treated group (Table 4.48).

Treatment of lovastatin (3 and 6 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) administration did not alter the Mg^{2+}ATPase levels as compared to cisplatin treated group (Table 4.49).

Table 4.47: Effect of green tea extract on membrane bound enzymes in the testes of rats in cisplatin induced testicular toxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	5.77±0.44**	2.8±0.22*	3.26±0.31***
Group 3	6.78±0.38NS	3.32±0.23NS	3.69±0.17NS
Group 4	7.8±0.49*	3.77±0.26NS	4.51±0.51NS
Group 5	8.43±0.51**	4.3±0.2*	5.48±0.45**
F value	**6.95**	**3.62**	**11.67**
P value	**P=0.0007**	**P=0.0183**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day).

Group 3: GTE (25 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 4: GTE (50 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 5: GTE (100 mg/kg/day p.o.for 30 days with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Cis) was compared with control while groups treated with green tea extract (Group 3, Group 4 and Group 5) were compared with cisplatin alone group.

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.48: Effect of melatonin on membrane bound enzymes in the testes of rats in cisplatin induced testicular toxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	5.77±0.44***	2.8±0.22*	3.26±0.31***
Group 3	6.81±0.23^{NS}	3.28±0.14^{NS}	3.92±0.14^{NS}
Group 4	7.45±0.3*	4.01±0.11^{NS}	4.85±0.45*
F value	**11.41**	**4.157**	**16.83**
P value	**P=0.0001**	**P=0.0193**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day).

Group 3: Melatonin (3 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 4: Melatonin (6 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with melatonin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Table 4.49: Effect of lovastatin on membrane bound enzymes in the testes of rats in cisplatin induced testicular toxicity (chronic study) in rats.

Groups	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated / min/mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg Protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/mg protein)
Group 1	8.50±0.34	4.24±0.57	6.56±0.40
Group 2	5.77±0.44***	2.8±0.22*	3.26±0.31***
Group 3	6.01±0.23 NS	3.23±0.16 NS	3.55±0.11 NS
Group 4	6.64±0.26 NS	3.75±0.08 NS	3.78±0.26 NS
F value	**14.15**	**3.556**	**26.25**
P value	**P<0.0001**	**P=0.0199**	**P<0.0001**

Group 1: Normal control

Group 2: Cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day).

Group 3:Lovastatin (3 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Group 4:Lovastatin (6 mg/kg/day p.o.for 30 days) with cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treatment.

Values are expressed as mean ± SEM.

The group treated with cisplatin alone (Group 2) was compared with control (Group 1) while groups treated with lovastatin (Group 3 and Group 4) were compared with cisplatin alone (Group 2).

* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.4.2.4 HISTOPATHOLOGY

Control rat (Fig 4.141-A) showed normal histoarchitecture of testes. Seminiferous tubules reflect the organized arrangement and well-defined boundaries.

Chronic administration of cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) causes vacuolization and fibrinoid debris in the seminiferous tubules. Shrunken seminiferous tubules showed disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also observed in interstitial tissues. (Fig 4.141-B)

Treatment of green tea extract (25, 50 and 100 mg/kg/day p.o.for 30 day) along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules with increasing doses and disorganization of germinal epithelium. Widening of the interstitial space and severe vacuolization were also decreased as compared to cisplatin treated rats [Fig. 4.141(C)-4.141(E)].

Similarly, Treatment of melatonin along with chronic cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) exhibited decreased degree of vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium at the dose of 6 mg/kg [Fig. 4.142(C)-4.142(D)].

The degree of testicular damage in lovastatin treated group (3 and 9 mg/kg) was similar to cisplatin (3mg/kg i.p.on1, 7,14,21and 28 day) treated group in regard to morphological changes showing vacuolization and fibrinoid debris in the seminiferous tubules and disorganization of germinal epithelium with disorganization of germinal epithelium [Fig. 4.143(C)-4.143(D)].

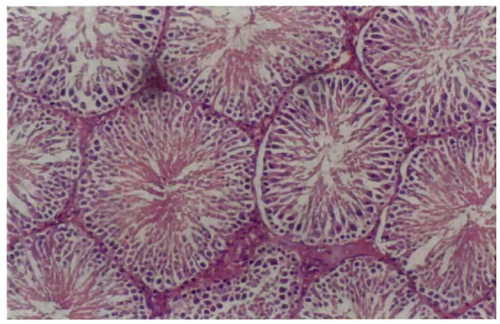
Fig. 4.141: Photomicrographs showing effect of green tea extract on the testes of cisplatin treated rats (chronic study).

(Magnification 10 X)

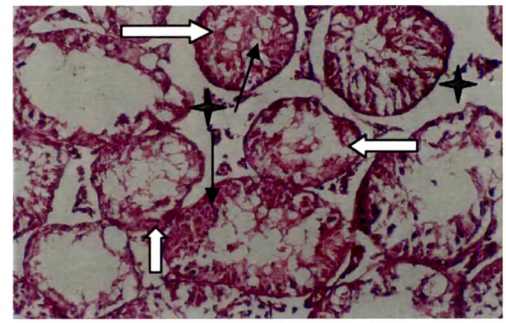
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

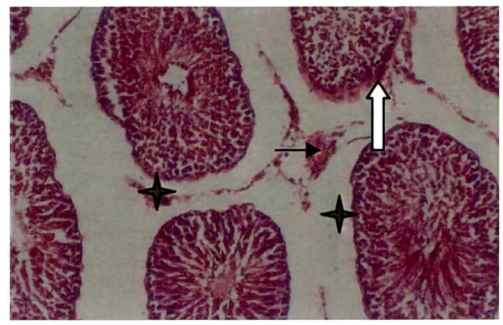
A: Normal control



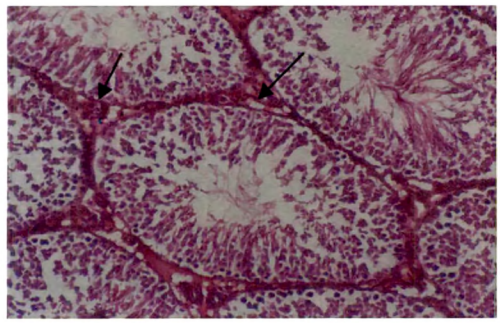
B: Cisplatin (3 mg/kg, i.p.)



C: GTE (25 mg/kg)



D: GTE (50 mg/kg)



E: GTE (100 mg/kg)

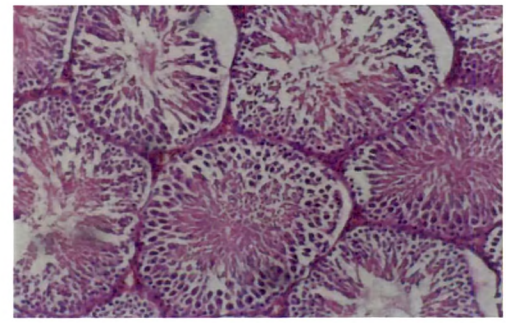


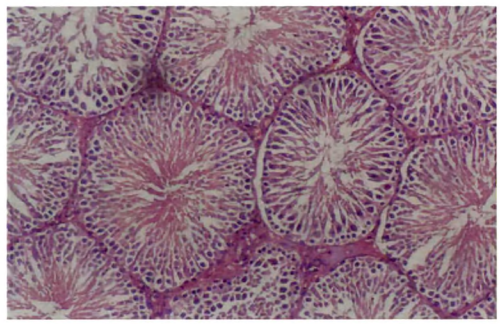
Fig. 4.142: Photomicrographs showing effect of melatonin on the testes of cisplatin treated rats (chronic study).

(Magnification 10 X)

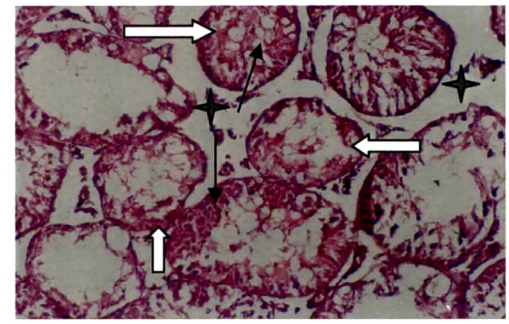
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

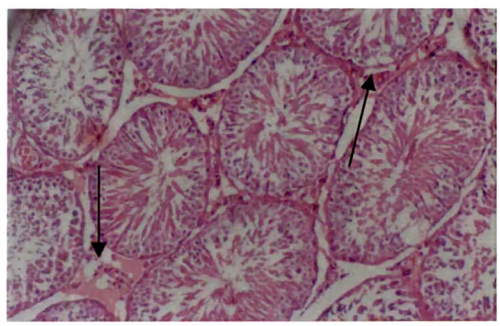
A: Normal control



B: Cisplatin (3 mg/kg, i.p.)



C: Melatonin (3 mg/kg)



D: Melatonin (6 mg/kg)

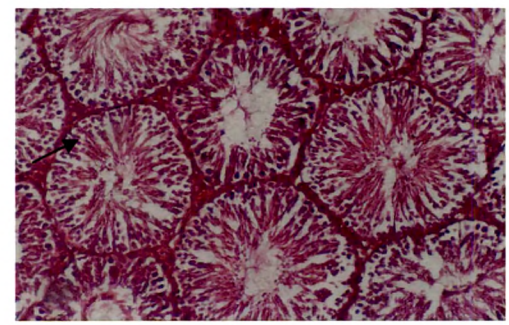


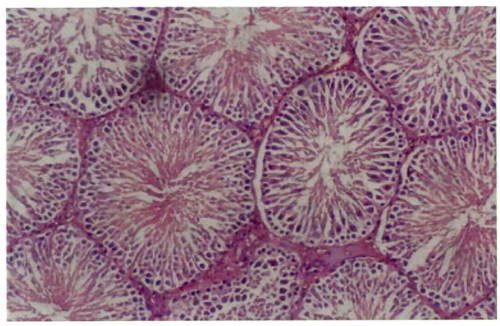
Fig. 4.143: Photomicrographs showing effect of lovastatin on the testes of cisplatin treated rats (chronic study).

(Magnification 10 X)

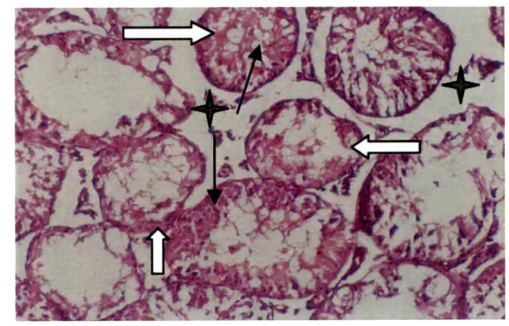
→ **Vacuolization** ✦ **Widening of the interstitial space**

⇨ **Shrunken seminiferous tubules**

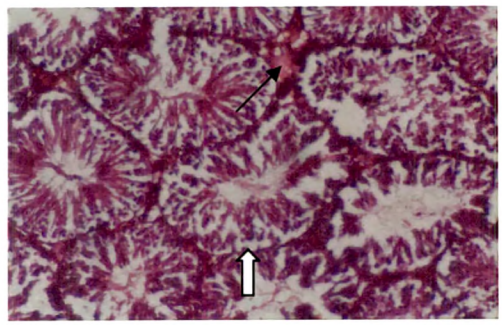
A: Normal control



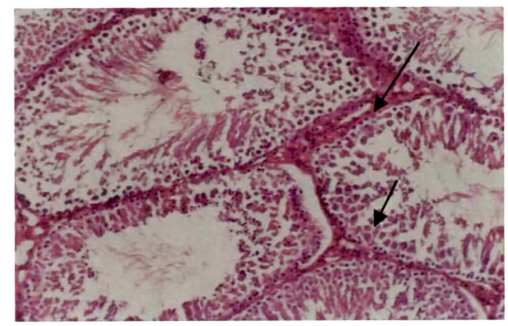
B: Cisplatin (3 mg/kg, i.p.)



C: Lova (3 mg/kg)



D: Lova (6 mg/kg)



4.3.5 DMBA INDUCED MAMMARY GLAND CANCER

4.3.5.1 SERUM PARAMETERS

4.3.5.1.1 Effect of drugs on Creatine Kinase

Administration of DMBA (15mg/rat i.g.) (Group 2) did not produce significant change in the serum concentration of creatine kinase as compared to control group (Group 1).

Chronic administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats (Group 2a) produced a significant ($P<0.001$) increase in the level of serum creatine kinase as compared to DMBA treated group (Group 2) (Fig.4.144).

Treatment of resveratrol (6mg/kg/day p.o. for 30 day) to the DMBA treated rats (Group 2b) did not alter serum creatine kinase when compared with DMBA treated group (Group 2) (Fig.4.144).

Treatment of resveratrol (6mg/kg/day p.o.for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats (Group 2c) produced a significant ($P<0.01$) decrease in the level of serum creatine kinase as compared to doxorubicin along with DMBA treated animals (Group 2a) (Fig.4.144).

4.3.5.1.2 Effect of drugs on Lactate dehydrogenase (LDH)

Administration of DMBA (15mg/rat i.g.) (Group 2) did not produce significant change in the serum concentration of LDH as compared to control group (Group 1).

Chronic administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated animals (Group 2a) produced a significant ($P<0.001$) increase in the level of serum LDH as compared to DMBA treated group (Group 2) (Fig.4.144).

Treatment of resveratrol (6mg/kg/day p.o. for 30 day) to the DMBA treated animal (Group 2b) did not produce significant change in serum LDH when compared with DMBA treated group (Group 2) (Fig.4.144).

Treatment of resveratrol (6mg/kg/day p.o.for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21,and 28) to the DMBA treated animals (Group 2 C) produced a significant ($P<0.05$) decrease in the level of

serum LDH as compared to doxorubicin along with DMBA treated animals (Group 2a) (Fig.4.144).

4.3.5.1.3 Effect of drugs on SGOT

Administration of DMBA (15mg/rat i.g.) (Group 2) produced significant ($P<0.01$) increase in the level of SGOT as compared to control group (Group 1).

Chronic administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated animals (Group 2a) produced a significant ($P<0.01$) increase in the level of SGOT when compared with DMBA treated group (Group 2) (Fig.4.145).

Treatment of resveratrol (6mg/kg/day p.o. for 30 day) with DMBA (Group 2B) produced a significant ($P<0.05$) decrease in SGOT when compared with DMBA treated group (Group 2) (Fig.4.145).

Treatment of resveratrol (6mg/kg/day p.o.for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21,and 28) to the DMBA treated animals (Group 2c) did not produce significant decrease in the level of SGOT as compared to doxorubicin along with DMBA treated animals (Group 2a) (Fig.4.145).

Fig.4.144: Effect of resveratrol on the serum levels of creatine kinase (CK) and Lactate dehydrogenase (LDH) in DMBA induced mammary cancer in rats.

Fig.4.145: Effect of resveratrol on the level of SGOT in DMBA induced mammary cancer in rats.

Group 1: Control animals received vehicle.
Group 2: received single intragastric dose of DMBA (15mg/rat).
Group 2a: received DMBA (15mg/rat) and doxorubicin (3 mg/kg i.p.on day1, 7, 14, 21, and 28).
Group 2b: received DMBA (15mg/rat) and resveratrol (6mg/kg/day orally for 30 days).
Group 2c: received DMBA (15mg/rat) and doxorubicin (3 mg/kg i.p.on day1, 7, 14, 21, and 28) and resveratrol (6mg/kg/day orally for 30 days).

Values are expressed as mean ± SEM.
Group 2 was compared with Group 1.
Group 2a and Group 2b were compared with Group 2.
Group 2c was compared with Group 2a.
* P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

Fig.4.144

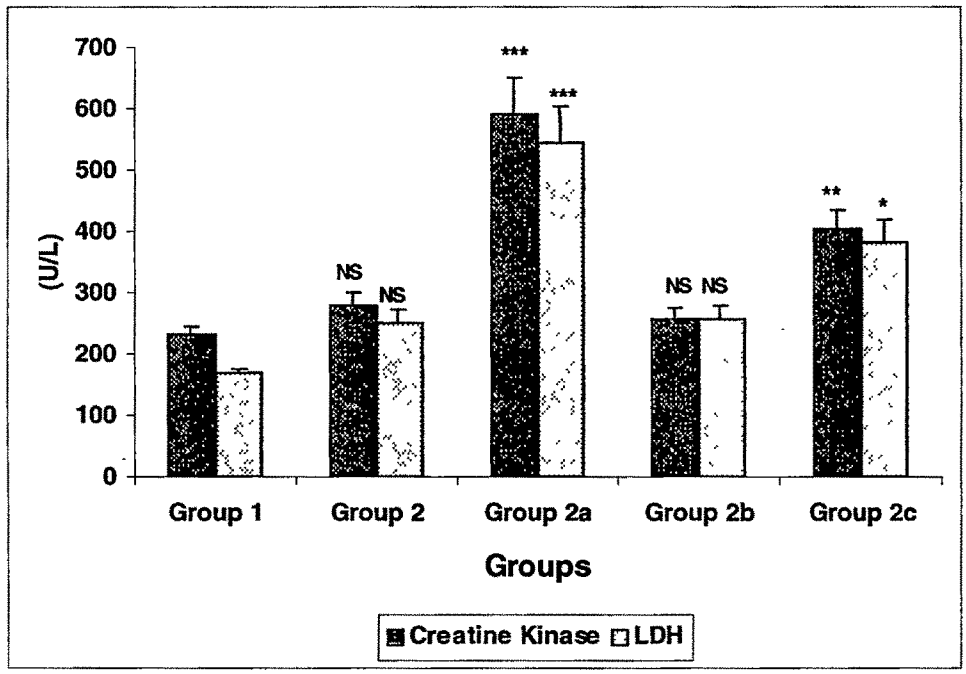
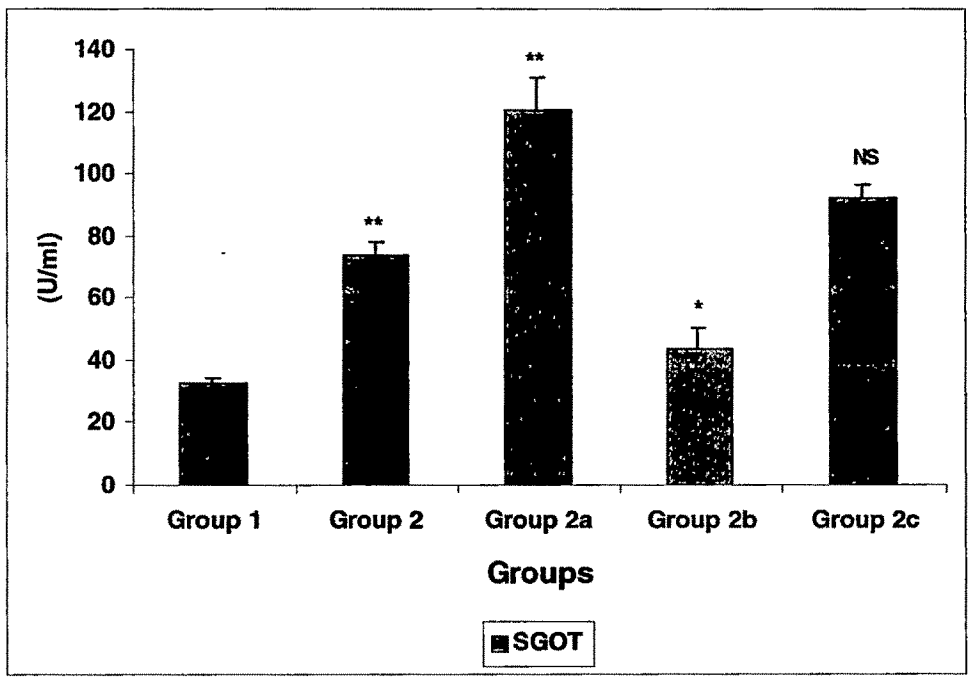


Fig.4.145



4.3.5.2 TISSUE PARAMETERS

4.3.5.2.1 Effect on lipid peroxidation

Administration of DMBA (15mg/rat i.g.) (Group 2) significantly ($P<0.0001$) increased the lipid peroxidation (MDA content) as compared to control group (Group 1) (Table 50).

Similarly, administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats (Group 2a) produced a significant ($P<0.01$) increase in the level of lipid peroxidation as compared to DMBA treated group (Group 2)(Table 50).

Resveratrol (6 mg/kg/day p.o.for 30 day) treatment to the DMBA treated animals (Group 2b) produced a significant ($P<0.01$) decrease in lipid peroxidation as compared to DMBA treated group (Group 2) (Table 50).

Administration of resveratrol (6mg/kg/day p.o.for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats (Group 2c) produced a significant ($P<0.05$) decrease in the level of lipid peroxidation as compared to doxorubicin along with DMBA treated animals (Group 2a) (Table 50).

4.3.5.2.2 Effect on endogenous antioxidants

4.3.5.2.2.1 Effect on Superoxide dismutase

Administration of DMBA (15mg/rat i.g.) (Group 2) did not produce significant change in the level of SOD activity as compared to control group (Group 1) (Table 50).

Administration of doxorubicin (3 mg/kg i.p. on day 1,7,14,21, and 28) to the DMBA treated group (Group 2a) produced a significant ($P<0.01$) decrease in the activity of SOD when compared with DMBA treated group (Group 2)(Table 50).

Resveratrol (6 mg/kg/day p.o.for 30 day) treatment to the DMBA treated animals (Group 2B) did not produce a significant change in SOD level when compared with DMBA treated group (Group 2) (Table 50).

Administration of resveratrol (6mg/kg/day p.o.for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1,7,14,21, and 28) to the DMBA treated rats (Group 2c) produced a significant ($P<0.05$) increase in the level of SOD

as compared to doxorubicin along with DMBA treated animals (Group 2a) (Table 50).

4.3.5.2.2.2 Effect on Catalase

Administration of DMBA (15mg/rat i.g.) (Group 2) did not produce significant change in the level of catalase activity as compared to control group (Group 1) (Table 50).

Administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated group (Group 2A) produced a significant ($P<0.001$) decrease in the activity of catalase when compared with DMBA treated group (Group 2)(Table 50).

Resveratrol (6mg/kg/day p.o.for 30 day) treatment to the DMBA treated animals (Group 2b) did not produce significant change in catalase level when compared with DMBA treated group (Group 2) (Table 50).

Administration of resveratrol (6mg/kg/day p.o.for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats (Group 2c) produced a significant ($P<0.05$) increase in the level of catalase as compared to doxorubicin along with DMBA treated animals (Group 2a) (Table 50).

4.3.5.2.2.3 Effect on Reduced glutathione

Administration of DMBA (15mg/rat i.g.) (Group 2) produced a significant ($P<0.05$) decrease the level of GSH as compared to control group (Group 1) (Table 50).

Administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated group (Group 2a) produced a significant ($P<0.05$) decrease in the activity of GSH when compared with DMBA treated group (Group 2)(Table 50).

Resveratrol (6mg/kg/day p.o.for 30 day) treatment to the DMBA treated animals (Group 2b) did not produce significant increase in GSH level when compared with DMBA treated group (Group 2) (Table 50).

Administration of resveratrol (6mg/kg/day orally for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats (Group 2c) produced a significant ($P<0.05$) increase in the level of GSH when compared with Group 2a (Table 50).

Table 4.50: Effect of resveratrol on lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione in the heart of rats in DMBA induced mammary cancer in rats.

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/ mg protein	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)
Group 1	3.06±0.16	9.45±1.21	2.33±0.36	4.02±0.32
Group 2	4.82±0.15***	6.72±0.26*	1.58±0.08^{NS}	3.73±0.13^{NS}
Group 2a	6.13±0.32**	3.97±0.40*	0.57±0.06**	2.20±0.17***
Group 2b	3.58±0.15**	7.73±0.33^{NS}	1.70±0.15^{NS}	3.96±0.18^{NS}
Group 2c	5.13±0.20*	6.54±0.23*	1.37±0.10*	3.27±0.17*
F value	**34**	**10.57**	**11.15**	**12.83**
P value	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Group 1:Control animals received vehicle.

Group 2: received single intragastric dose of DMBA (15mg/rat).

Group 2a: received DMBA (15mg/rat) and doxorubicin (3 mg/kg i.p.on day1, 7,14,21,and 28).

Group 2b: received DMBA (15mg/rat) and Resveratrol (6mg/kg/day orally for 30 days).

Group 2c: received DMBA (15mg/rat) and doxorubicin (3 mg/kg i.p.on day1, 7,14,21,and 28) and Resveratrol (6mg/kg/day orally for 30 days).

Values are expressed as mean ± SEM.

Group 2 was compared with Group 1.

Group 2a and Group 2b were compared with Group 2.

Group 2c was compared with Group 2a.

*P<0.05; ** P<0.01; *** P<0.001; NS = Non Significant

4.3.5.3 HISTOPATHOLOGY

4.3.5.3.1 Effect of resveratrol on the heart of rats in DMBA induced mammary cancer in rats.

Fig 4.146-A depicted the normal architecture of heart in rats of control group (Group 1) on histological examination. The figure reflects the organized arrangement, well-defined boundaries and distinct bundles of myocardial fibres.

Administration of DMBA (15mg/rat i.g.) exhibited normal architecture of heart with less fragmentation of fibres and well-defined boundaries or distinct bundles of myocardial fibres (Fig.4.146B).

Administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated group exhibited massive degenerative changes indicated by splaying and fragmentation of muscle fibers, edema and obvious infiltrations of inflammatory cells along with observation of cells with either pyknotic nuclei or anucleic cells and necrosis of heart muscle fibres was observed in Group 2A (Fig.4.146C).

Resveratrol (6mg/kg/day p.o.for 30 day) treatment to the DMBA treated animals exhibited normal architecture of heart with less fragmentation of fibres and well-defined boundaries or distinct bundles of myocardial fibres (Fig.4.146D).

Treatment of resveratrol (6mg/kg/day orally for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats exhibited decreased degree of necrosis with less fragmentation of fibres and well-defined boundaries or distinct bundles of myocardial fibres (Fig.4.146E).

4.3.5.3.2 Effect of Resveratrol on the tumors of rats in DMBA induced mammary cancer in rats.

Administration of DMBA (15mg/rat i.g.) induced ductal carcinomas and atypical ductal epithelial hyperplasia of breast tissue in rats (Fig.4.171B).

Administration of doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated group and treatment of resveratrol (6mg/kg/day orally for 30 day) along with doxorubicin (3 mg/kg i.p. on day 1, 7, 14, 21, and 28) to the DMBA treated rats exhibited lesser necrosis in breast tissue (Fig.4.171C-E).

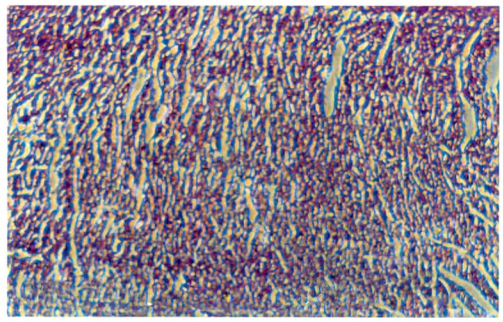
Fig. 4.146: Photomicrographs showing effect of resveratrol on the heart of rats in DMBA induced mammary cancer in rats.

(Magnification 10 X)

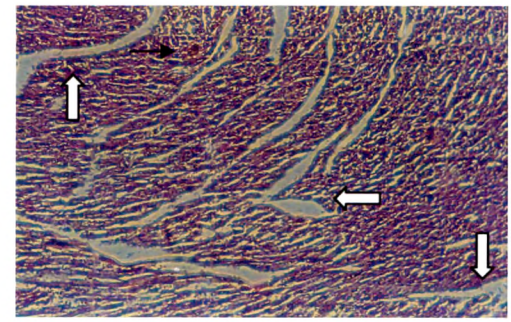
⇨ **Infiltration of inflammatory cell**

→ **Pyknotic nuclei**

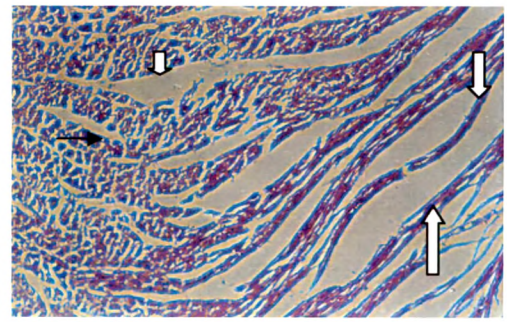
A: Group 1



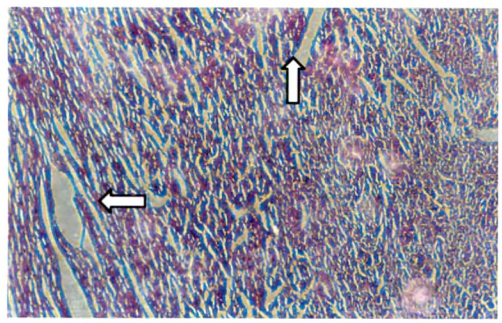
B: Group 2



C: Group 2a



D: Group 2b



E: Group 2c

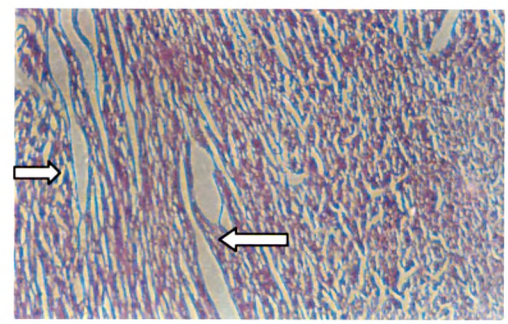
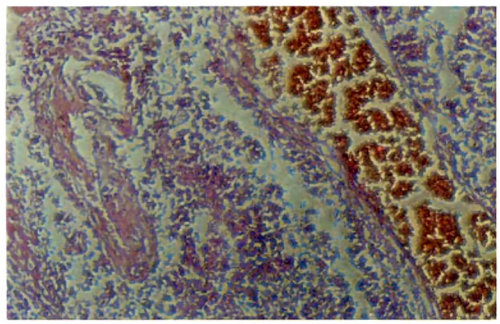


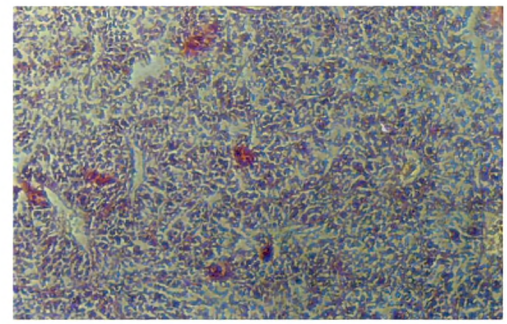
Fig. 4.147: Photomicrographs showing effect of resveratrol on the tumors of rats in DMBA induced mammary cancer in rats.

(Magnification 10 X)

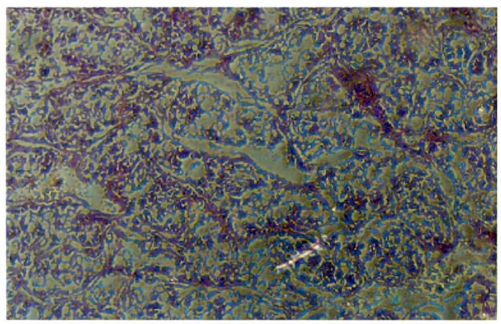
Group 2



Group 2A



Group 2B



Group 2C