CHAPTER 5
RESULTS

5.1. Pilot study for dose fixation

The effect of administration of Vit.E (25, 50 and100mg/kg), GT (10, 30 and 100mg/kg), LYP (5, 10 and 15 mg/kg), LSFJ (100, 200 and 400mg/kg) and PGFE (25, 50 and 100mg/kg) at different doses for 30 days on the activities of serum LDH, CK-MB, tissue LPO and GSH levels in ISO induced myocardial infarction in rats is shown in Fig.5.1 & 5.2. ISO injected rats showed a significant ($P<0.001$) increase in serum LDH, CK-MB, tissue LPO levels and a significant ($P<0.001$) decrease in GSH levels as compared to control group.

Treatment with Vit.E (50 and 100mg/kg) showed a significant ($P<0.05$, $P<0.001$) decrease in serum LDH levels. It was further found that Vit.E (100mg/kg) showed a significant ($P<0.001$) decrease in CK-MB, LPO levels and a significant ($P<0.01$) increase in GSH levels as compared to ISO injected rats (Fig.5.1 & 5.2).

Treatment with GT (100mg/kg) showed a significant ($P<0.001$) decrease in LDH, LPO levels and a significant ($P<0.01$) increase in GSH levels as compared to ISO injected rats. GT (50 and 100mg/kg) significantly ($P<0.05$, $P<0.001$) reduced the elevated levels of serum CK-MB as compared to ISO injected rats (Fig.5.1 & 5.2).

Treatment with LYP (10 and 15mg/kg) showed a significant ($P<0.01$, $P<0.001$) decrease in serum LDH, CK-MB levels and a significant increase in GSH levels as compared to ISO injected rats. LYP (5, 10 and 15mg/kg) showed a significant ($P<0.01$, $P<0.001$) decrease in tissue LPO levels as compared to ISO injected rats (Fig.5.1 & 5.2).

Treatment with LSFJ (400mg/kg) significantly ($P<0.05$) decreased the elevated levels of serum LDH, CK-MB, tissue LPO and significantly ($P<0.05$) increased GSH levels as compared to ISO injected rats (Fig.5.1 & 5.2).

Treatment with PGFE (50 and 100mg/kg) significantly ($P<0.05$, $P<0.001$) reduced the elevated level of LDH as compared to ISO injected rats. PGFE (100mg/kg)

significantly ($P<0.001$) reduced the elevated levels of CK-MB, LPO and significantly ($P<0.001$) increased the GSH levels as compared to ISO injected rats (Fig.5.1 & 5.2).

From this study, it was found that Vit.E (100mg/kg), GT (100mg/kg), LYP (10mg/kg), LSFJ (400mg/kg) and PGFE (100mg/kg) significantly reduced the elevated levels of LDH, CK-MB, LPO and significantly increased the GSH levels as compared to ISO injected rats. Since the above doses exhibited maximum protection at minimum concentration, they were selected alone and in combination for evaluating different parameters in ISO induced myocardial infarction in rats.

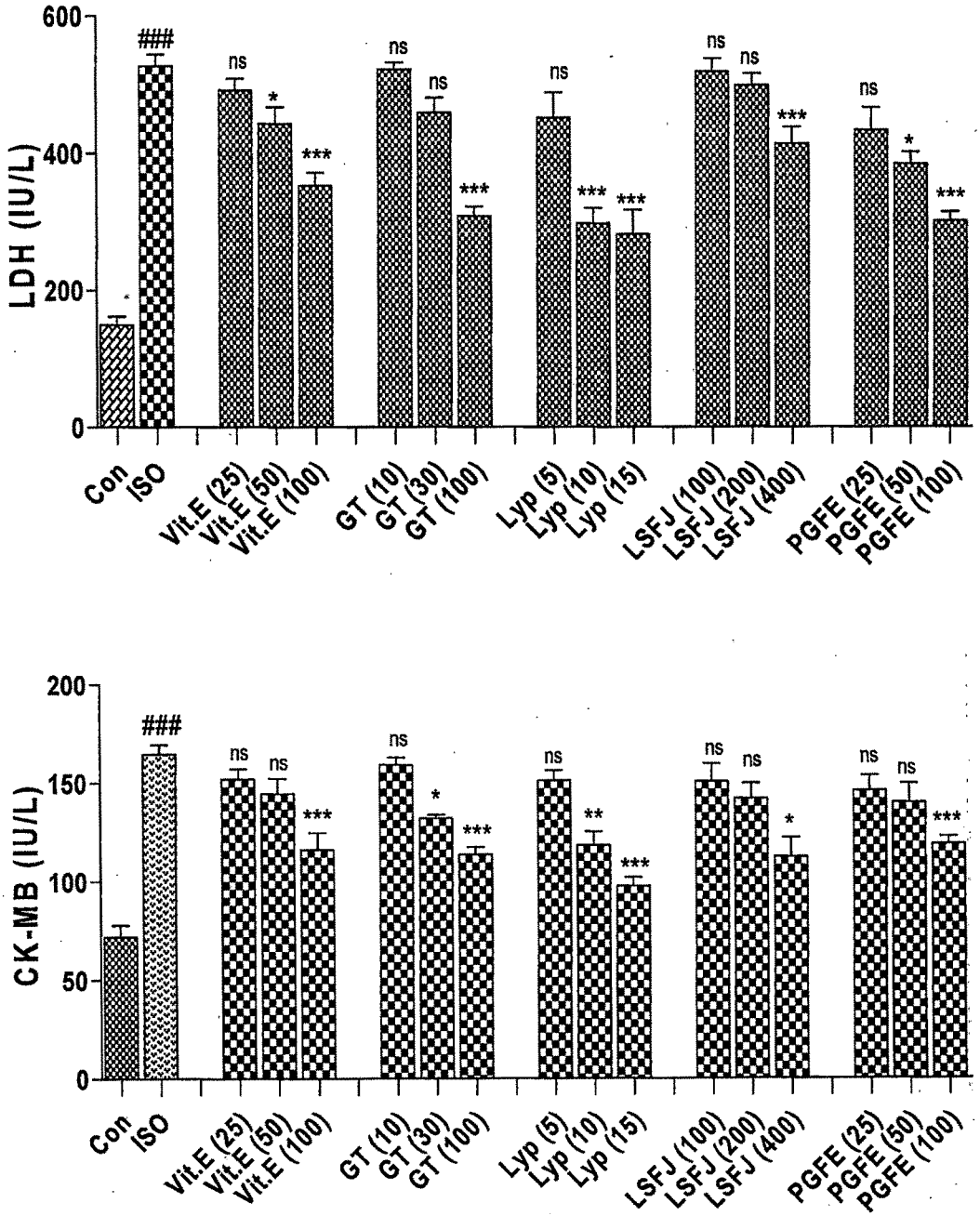


Fig. 5.1 Effect of Vit.E, GT, LYP, LSFJ and PGFE at different doses on serum LDH and CK-MB levels in ISO injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. P>0.05 was considered as non-significance (ns).

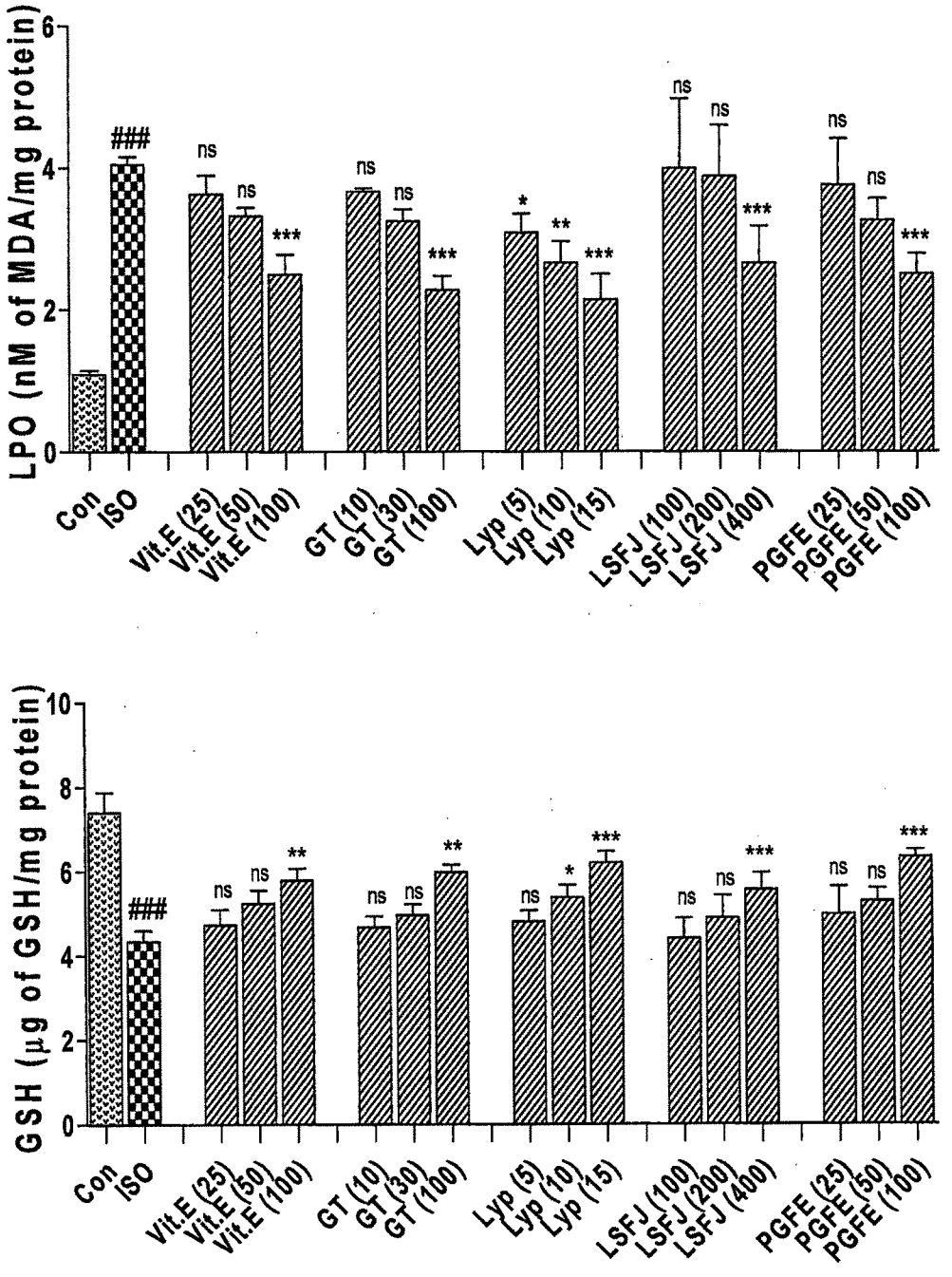


Fig. 5. 2 Effect of Vit.E, GT, LYP, LSFJ and PGFE at different doses on tissue LPO and GSH levels in ISO injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group.

5.2. Effect of Vitamin E (100 mg/kg/day, p.o) alone and its combination with Green tea (100 mg/kg/day, p.o) for 30 days in ISO (200 mg/kg, s.c) induced MI

5.2.1. Effect of Vit. E and GT on body weight, heart weight and heart/body weight ratio

Rats injected with isoproterenol (ISO) for two consecutive days showed a significant ($P<0.01$) decrease in body weight, significant ($P<0.01$, $P<0.001$) increase in heart weight and heart to body weight ratio as compared to control rats. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<0.05$) increase in body weight and a significant ($P<0.05$, $P<0.01$) decrease in heart weight and heart to body weight ratio as compared to ISO injected rats. Co-administration of Vit. E and GT in ISO injected rats (Vit.E+GT+ISO) did not show significant changes in body weight, heart weight and heart to body weight ratio as compared to Vit.E+ISO or GT+ISO treated groups (Table 5.1).

5.2.2. Effect of Vit. E and GT on Electrocardiographic changes

The ECG pattern of control and experimental groups are shown in Fig. 5.3 and Table 5.2. ISO injected rats showed a significant ($P<0.001$, $P<0.01$) elevation of ST-interval, QT interval along with a significant ($P<0.001$, $P<0.01$) decrease in P wave, QRS complex and RR interval as compared to control group. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<0.001$, $P<0.01$, $P<0.05$) decrease in ST-segment, QT interval along with a significant ($P<0.001$, $P<0.01$, $P<0.05$) increase in QRS complex and RR interval as compared to ISO injected rats. The co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) showed a significant ($P<0.001$, $P<0.01$, $P<0.05$) decrease in ST and QT interval along with a significant ($P<0.001$, $P<0.01$, $P<0.05$) increase in P wave, QRS complex and RR interval as compared to ISO, Vit.E+ISO

or GT+ISO treated groups. Heart rate did not significantly differ in control and injected groups.

5.2.3. Effect of Vit. E and GT on systolic, diastolic and mean blood pressure by tail cuff method

ISO injected rats showed a significant ($P<0.01$, $P<0.001$) decrease in diastolic and mean blood pressure as compared to control group. Treatment with Vit.E alone and in combination with GT in ISO injected rats (Vit.E+GT+ISO) slightly improved the diastolic blood pressure as compared to ISO injected rats. However, this combination showed significantly ($P<0.05$) improvement in mean blood pressure as compared to ISO injected rats (Fig.5.4).

5.2.4 .Effect of Vit. E and GT on serum cardiac marker enzymes

The activities of cardiac marker enzymes such as AST, ALT, ALP, LDH and CK-MB in serum of control and experimental animals are shown in Fig. 5.5 and 5.6. Rats injected with ISO showed a significant ($P<0.001$) increase in the activities of AST, ALT, ALP, LDH and CK-MB as compared to control group. Treatment of Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<0.001$) decrease in the activities of AST, ALT, ALP, LDH and CK-MB as compared to ISO injected rats. Further it was found that the combination of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) significantly ($P<0.05$) decreased the activities of AST, ALT, ALP, LDH and CK-MB as compared to Vit. E+ISO or GT+ISO treated groups. ISO injected rats showed a significant ($P < 0.001$) increase in serum uric acid level and a significant ($P<0.001$) decrease in total protein level as compared to control rats. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) showed a significant ($P<0.01$, $P<0.001$) decrease in the level of serum uric acid and a significant ($P<0.01$, $P<0.001$) increase in the level of total protein as compared to ISO, Vit.E+ISO or GT+ISO treated groups (Fig. 5.6b).

5.2.5. Effect of Vit. E and GT on lipid peroxidation, markers of oxidative stress and vitamin E level

The effect of Vit.E and GT on lipid peroxidation and markers of oxidative stress are shown in Fig.5.7 and Table 5.3. Rats injected with ISO showed a significant ($P<0.001$) increase in LPO level as compared to control rats. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) significantly ($P<0.05$) decreased the elevated LPO level as compared to Vit.E+ISO or GT+ISO treated groups (Fig.5.7).

ISO injected rats showed a significant ($P<0.001$) decrease in the activities of heart GSH, GPx, GST, SOD, CAT and Vit.E level as compared to control group. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<005$, $P<0.01$, $P<0.001$) increase in the activities of GSH, GPx, GST, SOD, CAT and Vit. E level as compared to ISO injected rats. Co-administration of Vit. E and GT in ISO injected rats (Vit.E+GT+ISO) showed a significant ($P<0.001$, $P<0.05$) increase in the activities of GSH, GPx, GST, SOD, CAT and Vit. E level as compared to ISO, Vit.E+ISO or GT+ISO treated group. Combination Vit.E and GT in ISO injected rats showed better effects in maintaining these antioxidant enzymes towards normal as compared to Vit.E+ISO or GT+ISO treated groups (Table 5.3).

5.2.6. Effect of Vit. E and GT on membrane bound phosphatases (ATPases) and electrolytes levels in heart

The effect of Vit.E and GT on membrane bound phosphatases and electrolyte levels in the heart of normal and ISO injected rats are shown in Fig.5.8 and Table 5.4. ISO injected rats showed a significant ($P<0.001$) decrease in the activities of Na^{+}/K^{+}-ATPase, Mg^{+2}-ATPase with a significant ($P<0.001$) increase in Ca^{+2}-ATPase activity as compared to control group. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) showed a significant ($P<0.001$, $P<0.05$) increase in the activities of Na^{+}/K^{+} ATPase, Mg^{+2} ATPase along with a significant ($P<0.001$,

$P<0.05$) decrease in Ca^{+2} ATPase activity compared to ISO, Vit.E+ISO or GT+ISO treated groups. Vit.E+ISO treated rats showed a non-significant rise in Mg^{+2} ATPase activity as compared to ISO injected rats (Fig. 5.8).

Rats injected with ISO showed a significant ($P<0.001$) increase in sodium and calcium levels along with significant ($P<0.001$) decrease in potassium level as compared to control group. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) significantly ($P<0.001$, $P<0.05$) decreased the sodium, calcium levels and significantly ($P<0.001$, $P<0.05$) increased the potassium level as compared to ISO, Vit.E+ISO or GT+ISO treated groups (Table 5.4).

5.2.7. Effect of Vit. E and GT on LDH isoenzyme pattern

The pattern of LDH isoenzymes in serum of normal and experimental groups of rats (as separated by agarose gel electrophoresis) are shown in Fig.5.9. ISO injected rats showed an increased expression of LDH-1 and LDH-2 isoenzyme bands as compared to control rats. Treatment with Vit. E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) significantly decreased the intensity of LDH-1 and LDH-2 isoenzyme bands as compared to ISO injected rats. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) further reduced the intensity of LDH1 and LDH2 isoenzymes towards normal as compared to Vit. E+ISO or GT+ISO treated groups.

5.2.8. Effect of Vit. E and GT on serum and heart tissue lipid profile

ISO injected rats showed a significant ($P<0.001$, $P<0.01$) increase in the levels of serum TC, TG, LDL, VLDL, FFA, PL and a significant ($P<0.01$) decrease in HDL level as compared to control rats. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) significantly ($P<0.001$, $P<0.01$) decreased the elevated levels of TC, TG, LDL, VLDL, FFA and PL as compared to ISO injected rats. The level of HDL was also found to be significantly ($P<0.01$) increased with Vit.E+ISO and GT+ISO treated rats. Co-administration of Vit.E and GT in ISO injected rats

(Vit.E+GT+ISO) did not produce significant effect on serum lipid profile as compared to Vit.E+ISO or GT+ISO treated groups (Fig.5.10 & 5.11).

ISO injected rats showed a significant increase ($P<0.01$, $P<0.001$) in the levels of heart TC, TG, FFA and significant decrease ($P<0.01$) in the PL level as compared to control group. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<0.01$, $P<0.05$) decrease in the levels of TC, TG, FFA and a significant ($P<0.05$) increase in PL level as compared to ISO injected rats. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) did not show significant effect on heart lipid profile as compared to Vit. E+ISO or GT+ISO treated groups (Table 5.5).

5.2.9. Effect of Vit. E and GT on myocardial Lipid metabolizing enzymes

The effect of Vit.E and GT on the activities of tissue lipid metabolizing enzymes in control and ISO injected rats is shown in Fig.5.12. ISO injected rats showed a significant ($P<0.01$, $P<0.001$) decrease in the activities of LCAT, LPL and a significant ($P<0.001$) increase in the activity of CES as compared to control group. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) significantly ($P<0.01$, $P<0.001$) increased the activities of LCAT, LPL, significantly ($P<0.01$) decreased the activity of CES as compared to ISO injected rats. Although Vit.E+ISO treatment increased LCAT activity, it was found to be statistically non-significant. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) did not produce significant effects on lipid metabolizing enzymes as compared to Vit.E+ISO or GT+ISO treated groups (Fig. 5.12).

5.2.10. Effect of Vit. E and GT on Serum CRP and tissue MPO activity

Rats injected with ISO showed a significant ($P<0.001$) increase in serum CRP level and tissue MPO activity compared to control group. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) significantly ($P<0.001$) decreased the CRP level and MPO activity as compared to ISO injected group. Co-administration

of Vit. E and GT in ISO injected rats (Vit.E+GT+ISO) showed significant ($P<0.001$, $P<0.05$) decrease in serum CRP level and tissue MPO activity as compared to Vit.E+ISO or GT+ISO treated groups (Fig.5.13 & 5.14).

5.2.11. Effect of Vit. E and GT on tissue nitrite levels

ISO injected rats showed a significant increase ($P < 0.001$) in tissue nitrite level as compared to control rats. Treatment of GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<0.01$) decrease in tissue nitrite level compared to ISO injected rats. Treatment with Vit. E alone and in combination with GT in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO) did not produce significant effect on tissue nitrite level as compared to Vit.E+ ISO or GT+ISO treated groups (Fig.5.15).

5.2.12. Effect of Vit.E and GT on Histopathological (H & E staining) changes

The effect of Vit.E and GT on the degree of histological changes in myocardial tissues of normal and ISO injected rats is shown in Fig.5.16 and Table 5.6. Histopathological findings of ISO injected rats showed infarcted zone with edema, inflammatory cells and separation of muscle fibers (Fig. 5.16B, Table 5.6). Treatment with Vit.E in ISO injected rats (Vit.E+ISO) showed necrosis with less edema and inflammatory cells (Fig. 5.16C, Table 5.6). Treatment with GT in ISO injected rat (GT+ISO) showed moderate degree of edema with mild changes in necrosis and inflammatory cells infiltrations (Fig. 5.16D, Table 5.6). Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) showed mild degree of edema without necrosis and inflammatory cells infiltration (Fig.5.16E, Table 5.6).

5.2.13. Effect of Vit. E and GT on Periodic acid Schiff's staining of cardiac tissue

There was an arbitrary increase in the amount of glycoproteins or glycoconjugates in ISO treated rats (Fig. 5.17B) as compared to control rats (Fig. 5.17A). Treatment with Vit. E in combination with GT in ISO treated rats (Vit.E+ISO or GT+ISO)

resulted in normal architecture of membrane and maintenance of membrane bound glycoconjugates (Fig. 5.17E). Vit.E+ISO and GT+ISO treated groups also showed significant reduction in glycoconjugates (Fig. 5.17C and 5.17D).

5.2.14. Effect of Vit. E and GT on Masson's trichrome staining of cardiac tissue

ISO injected rats showed muscle cell necrosis with disruption in arrangement of collagen fibers (Fig. 5.18B) as compared to control rats (Fig.5.18A). Cardiac tissue sections of rats treated with the co-administration of Vit.E and GT in ISO treated rats (Vit.E+GT+ISO) showed minimally damaged collagen fibers as compared to Vit.E+ISO (Fig.5.18C) or GT+ISO (Fig.5.18D) treated groups (Fig. 5.18E).

5.2.15. Effect of Vit. E and GT on macroscopic enzyme assay (TTC test) and area of infarction

A high percentage of mean infarct size with increased staining was observed in ISO injected rats. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) showed a significant ($P<0.05$, $P<0.001$) decrease in infarct size and staining compared to ISO injected rats. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) showed further reduction in infarct size and staining as compared to Vit.E+ISO or GT+ISO treated groups (Fig.5.19).

5.2.16. Effect of Vit.E and GT on DNA damage by gel electrophoresis

Rats injected with ISO showed an increase in DNA damage as compared to control group (Lane A and B). Treatment with Vit.E+ISO or GT+ISO showed significant reduction in the intensity of DNA damage compared to ISO injected rats. Co-administration of Vit. E and GT in ISO injected rats (Vit.E+GT+ISO) showed active prevention in the severity of DNA damage (Lane E) as compared to Vit.E+ISO or GT+ISO treated rats (Lane C and D) (Fig. 5.20).

5.2.17. Effect of Vit.E and GT on Caspase-3 activity

The effect of Vit.E and GT on Caspase-3 activity in normal and ISO injected rats is shown in Fig.5.21. ISO injected rats showed a significant ($P<0.01$) increase in caspase-3 activity as compared to control rats. Treatment with Vit.E or GT in ISO injected rats (Vit.E+ISO or GT+ISO) significantly reduced ($P<0.05$) caspase-3 activity compared to ISO injected rats. Co-administration of Vit.E and GT in ISO injected rats (Vit.E+GT+ISO) did not show further reduction in caspase-3 activity as compared to Vit.E+ISO or GT+ISO treated groups.

Table 5.1. Effect of Vit. E (100 mg/kg/day, p.o) and GT (100 mg/kg/day, p.o) for 30 days on body weight, heart weight and heart to body weight ratio in normal and ISO (200 mg/kg, s.c) injected rats

Groups	Final body weight (g)	Heart weight (g)	HW/BW ratio
Control	232.5 ± 3.39	0.667 ± 0.031	0.00286 ± 0.0091
ISO	214.2 ± 3.48$^{\#\#}$	0.920 ± 0.054$^{\#\#}$	0.00429 ± 0.0155$^{\#\#\#}$
Vit.E+GT	228.2 ± 4.88	0.661 ± 0.055	0.00289 ± 0.0112
Vit.E+ISO	227.0 ± 3.29*	0.705 ± 0.030*	0.00309 ± 0.0091**
GT+ISO	228.5 ± 2.88*	0.710 ± 0.040*	0.00310 ± 0.0138**
Vit.E+GT+ISO	228.9 ± 2.38*	0.703 ± 0.041*	0.00307 ± 0.0172**

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group.

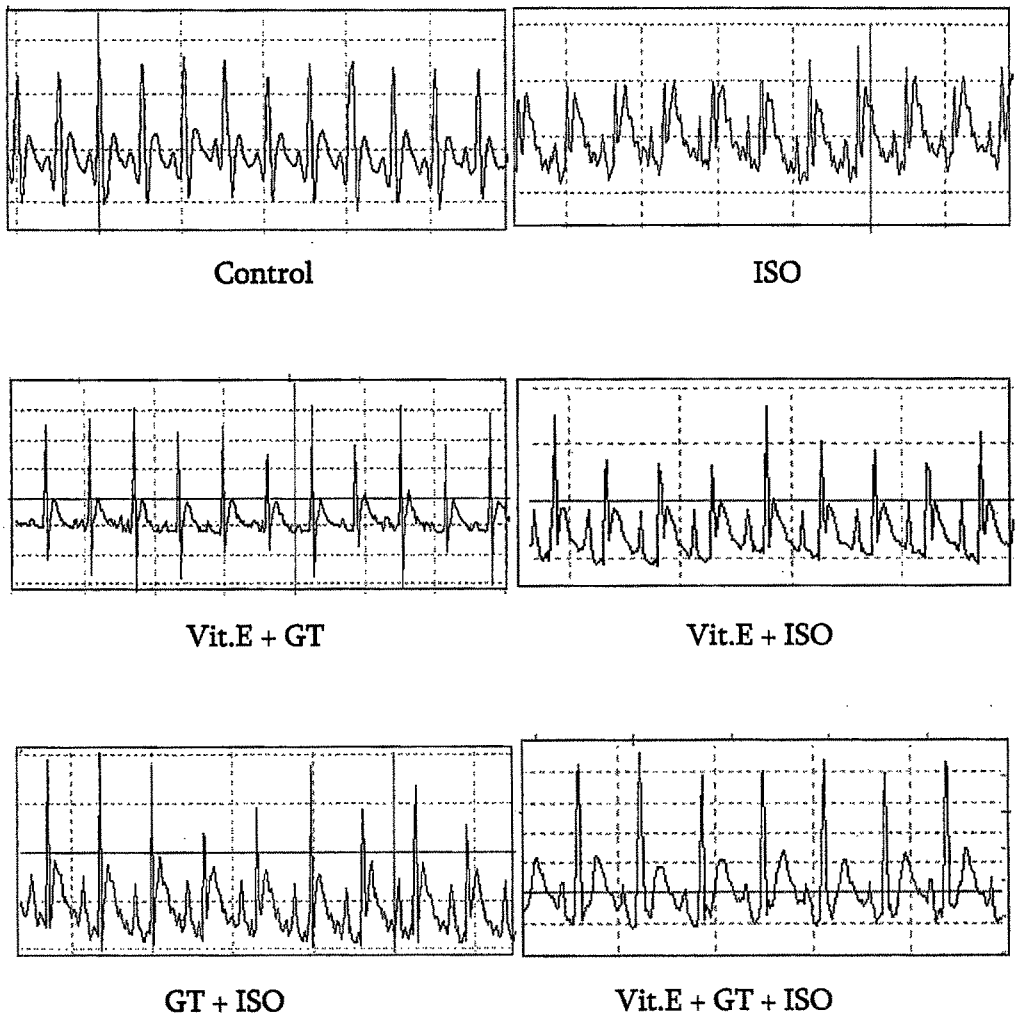


Fig. 5.3: Effect of Vit. E (100 mg/kg/day, p.o) and GT (100 mg/kg/day, p.o) for 30 days on ECG changes in normal and ISO (200 mg/kg, s.c) injected rats

Table 5.2: Effect of Vit. E (100 mg/kg/day, p.o) and GT (100 mg/kg/day, p.o) for 30 days on ECG changes and heart rate in normal and ISO (200 mg/kg, s.c) injected rats

Groups	ST elevation	P wave	QRS complex	QT interval	R-R interval	Heart rate
Control	0.184 ± 0.0022	0.028 ± 0.00037	0.0416 ± 0.005	0.071 ± 0.0006	0.170 ± 0.0007	338.30 ± 12.88
ISO	0.302 ± 0.0040$^{\#\#\#}$	0.024 ± 0.00030$^{\#\#}$	0.0285 ± 0.007$^{\#\#\#}$	0.081 ± 0.0012$^{\#\#}$	0.158 ± 0.0017$^{\#\#}$	400.09 ± 14.32^{ns}
Vit.E+GT	0.189 ± 0.0011	0.029 ± 0.00061	0.0421 ± 0.008	0.071 ± 0.0009	0.171 ± 0.0006	339.90 ± 08.23
Vit.E+ISO	0.251 ± 0.0023***	0.025 ± 0.00049^{ns}	0.0326 ± 0.001*	0.074 ± 0.0076*	0.158 ± 0.0008*	372.80 ± 16.52
GT+ISO	0.227 ± 0.0031***	0.025 ± 0.00025^{ns}	0.0350 ± 0.007**	0.077 ± 0.0012*	0.164 ± 0.0011**	364.00 ± 12.28
Vit.E+GT+ISO	0.207 ± 0.0041^{***ab}	0.027 ± 0.00054^{*b}	0.0388 ± 0.009***	0.073 ± 0.0005^{**a}	0.168 ± 0.0010^{**a}	356.70 ± 13.23

Values are expressed as mean ± SEM (n= 6). The ECG parameters are expressed in seconds (sec), heart rate as Beats per minutes (BPM), ST elevation in milivolt (mv). $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 values compared to control group. *P<0.05, **P<0.01, ***P<0.001 values compared to ISO injected group. ^{a}P<0.05 compared to Vit.E+ISO and ^{b}P<0.05 compared to GT+ISO group.

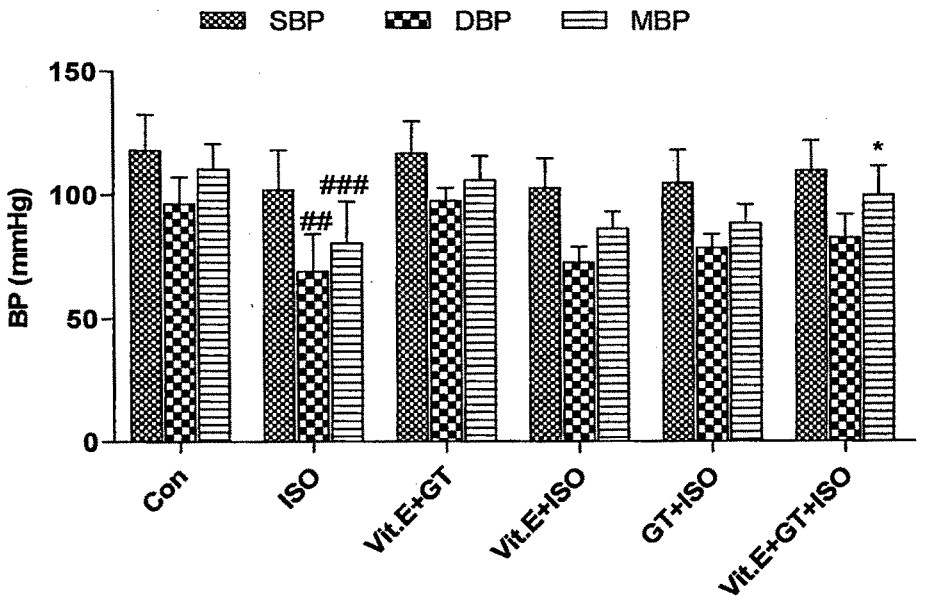


Fig. 5. 4. Effect of Vit. E (100 mg/kg/day, p.o) and GT (100 mg/kg/day, p.o) for 30 days on systolic, diastolic and mean blood pressure in normal and ISO (200 mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group.

Fig.5.5 (a)

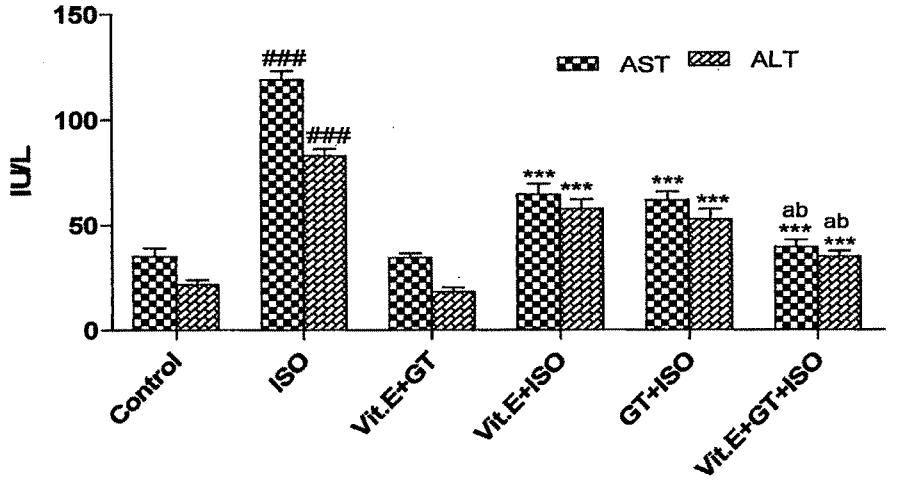


Fig. 5.5 (b)

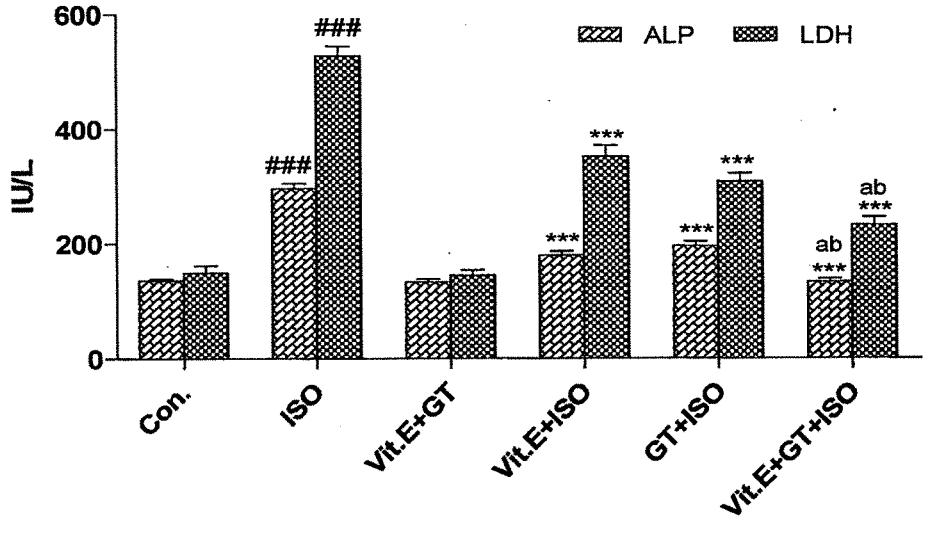


Fig. 5. 5: Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on serum (a) AST and ALT (b) ALP and LDH levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

Fig.5.6 (a)

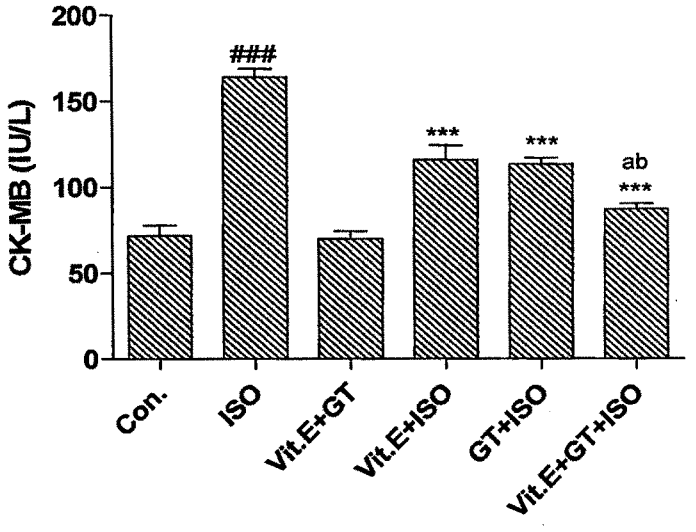


Fig.5.6 (b)

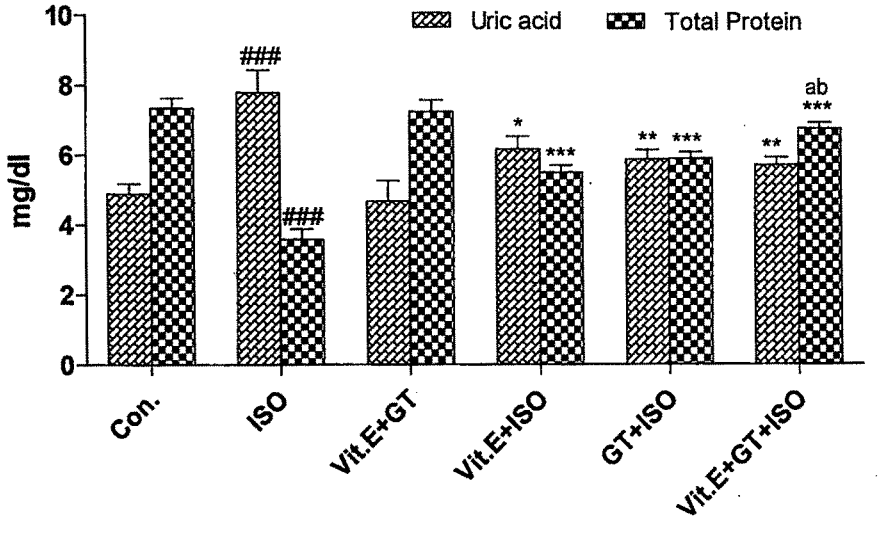


Fig. 5. 6: Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on serum (a) CK-MB (b) Uric acid and total protein levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

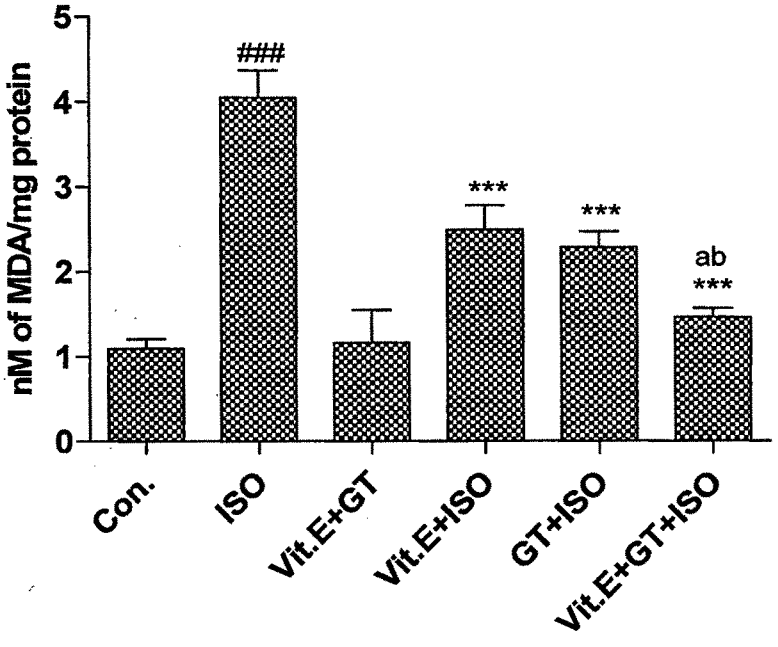


Fig. 5.7: Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on myocardial Lipid peroxidation in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

Table 5.3: Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on endogenous antioxidants and vitamin E levels in normal and ISO (200mg/kg, s.c) injected rats

Groups	GSH	GPx	GST	SOD	CAT	Vitamin E
Con.	7.401 ± 0.480	6.102 ± 0.206	110.40 ± 5.712	4.496 ± 0.203	6.290 ± 0.331	2.02 ± 0.047
ISO	4.338 ± 0.260$^{\#\#\#}$	4.032 ± 0.231$^{\#\#\#}$	68.91 ± 4.829$^{\#\#\#}$	2.272 ± 0.166$^{\#\#\#}$	3.687 ± 0.219$^{\#\#\#}$	1.09 ± 0.043$^{\#\#\#}$
Vit.E+GT	6.164 ± 0.390	6.664 ± 0.390	111.23 ± 3.760	4.564 ± 0.391	6.464 ± 0.390	2.17 ± 0.081
Vit.E+ISO	5.785 ± 0.288**	5.635 ± 0.336**	95.55 ± 4.089***	3.566 ± 0.390*	5.188 ± 0.167**	1.84 ± 0.035***
GT+ISO	5.972 ± 0.186**	5.840 ± 0.371***	97.11 ± 2.904***	3.552 ± 0.291*	5.375 ± 0.207**	1.77 ± 0.036***
Vit.E+GT+ISO	7.320 ± 0.254^{***ab}	6.933 ± 0.254^{***ab}	122.20 ± 7.407^{***ab}	4.322 ± 0.284^{***ab}	6.783 ± 0.180^{***ab}	2.09 ± 0.035^{***b}

Values are expressed as mean±SEM, (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

GSH: (µg of GSH /mg protein), GPx: (µmoles of glutathione oxidized/min/mg protein), GST: (µmoles of CDNB conjugated/min/mg protein), SOD: (units/ mg protein), CAT: (µmoles of H_2O_2 consumed/min/mg protein), Vitamin E: (mmole/mg protein).

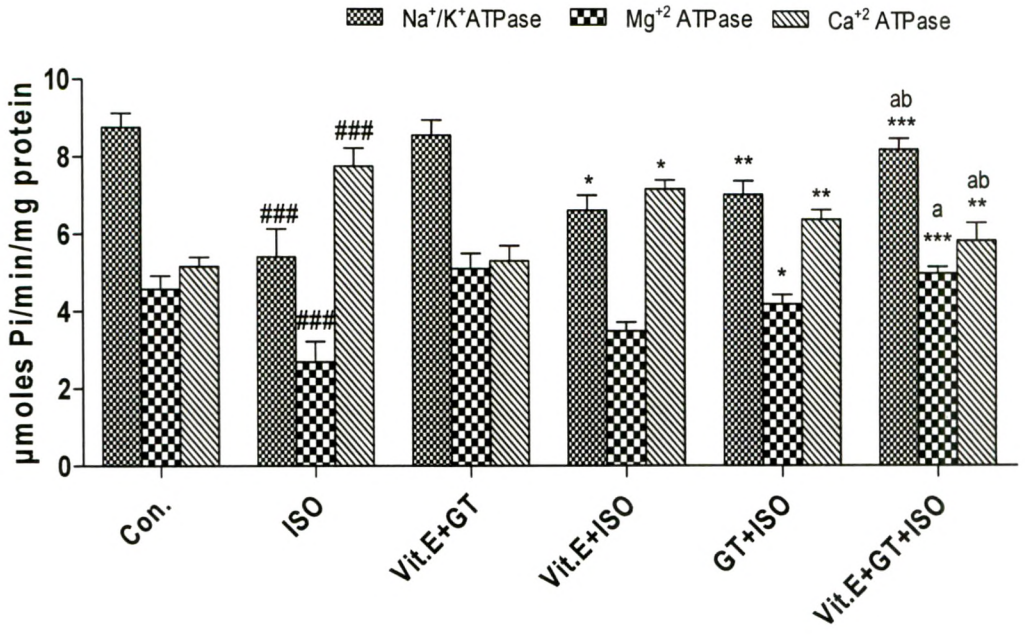


Fig. 5. 8. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on Na^{+}/K^{+} ATPase, Mg^{+2} ATPase and Ca^{+2} ATPase activities in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

Table 5.4: Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on the levels of sodium, potassium and calcium in normal and ISO (200mg/kg, s.c) injected rats

Groups	Sodium (Na^+)	Potassium (K^+)	Calcium (Ca^{++})
Control	6.088 ± 0.125	8.978 ± 0.164	10.81 ± 0.107
ISO	7.728 ± 0.312$^{###}$	6.162 ± 0.216$^{###}$	13.83 ± 0.344$^{###}$
Vit.E+GT	5.982 ± 0.101	9.028 ± 0.133	10.53 ± 0.132
Vit.E+ISO	7.198 ± 0.156*	7.462 ± 0.189***	12.63 ± 0.291**
GT+ISO	6.788 ± 0.115**	7.938 ± 0.149***	11.94 ± 0.149***
Vit.E+GT+ISO	6.228 ± 0.122^{***a}	8.725 ± 0.152^{***ab}	11.04 ± 0.099^{***ab}

(Units for Na^+, K^+ and Ca^{+2} are expressed as nmol/mg protein)

Values are expressed as mean±SEM (n=6). $^{#}P<0.05$, $^{##}P<0.01$, $^{###}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

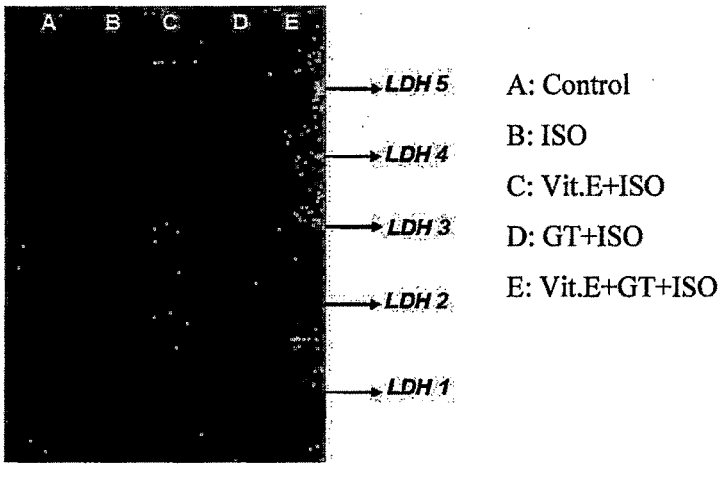


Fig. 5.9. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on serum LDH isoenzyme pattern in normal and ISO (200mg/kg, s.c) injected rats

Fig.5.10 (a)

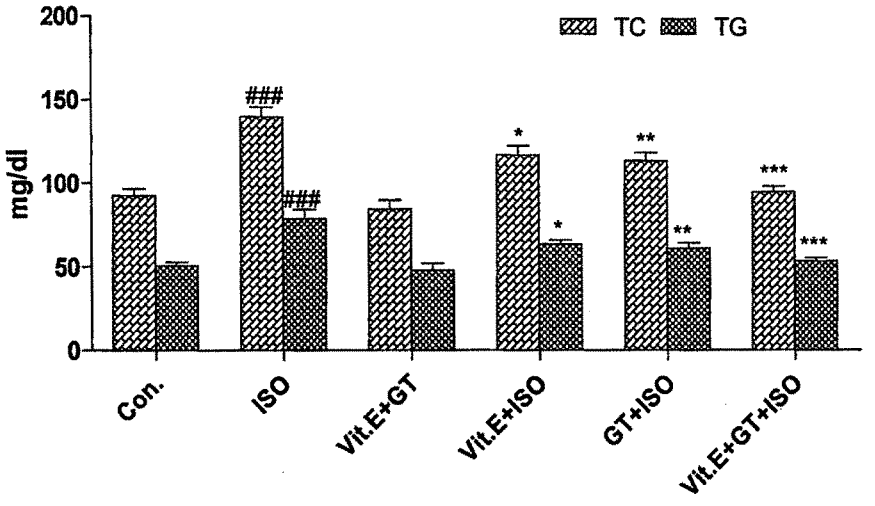


Fig.5.10 (b)

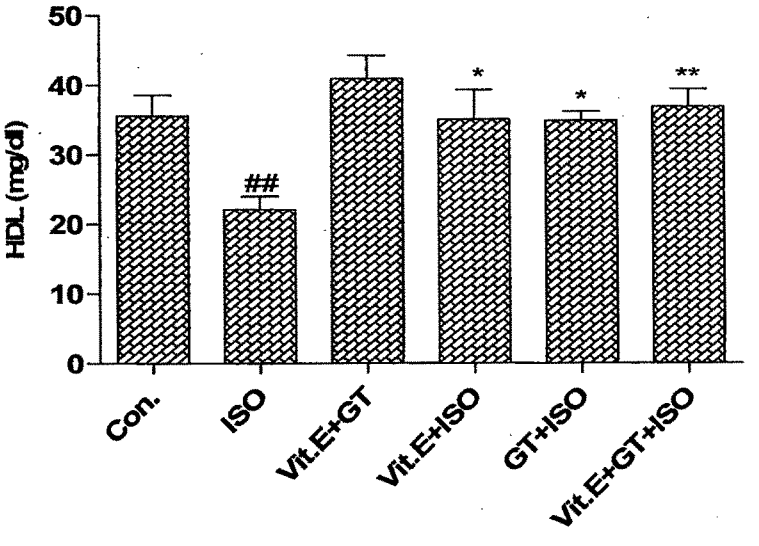


Fig. 5.10. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on serum a) TC and TG, b) HDL levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

Fig. 5.11 (a)

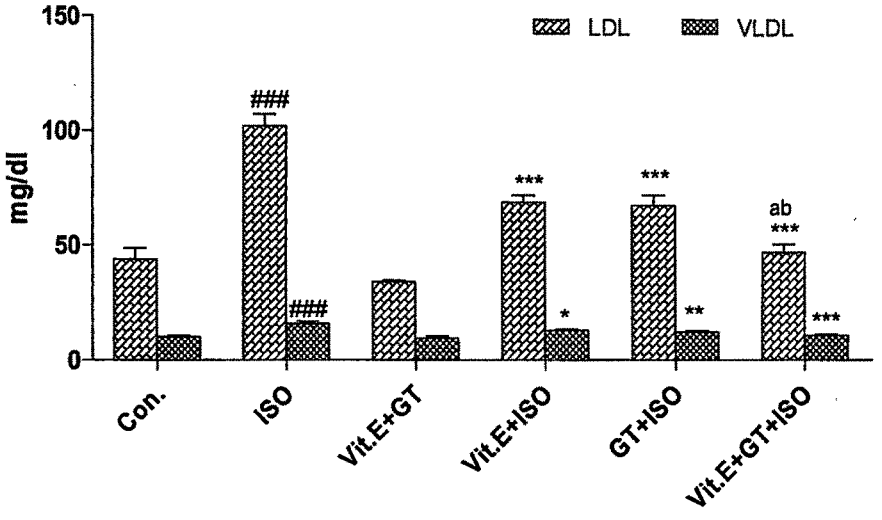


Fig. 5.11 (b)

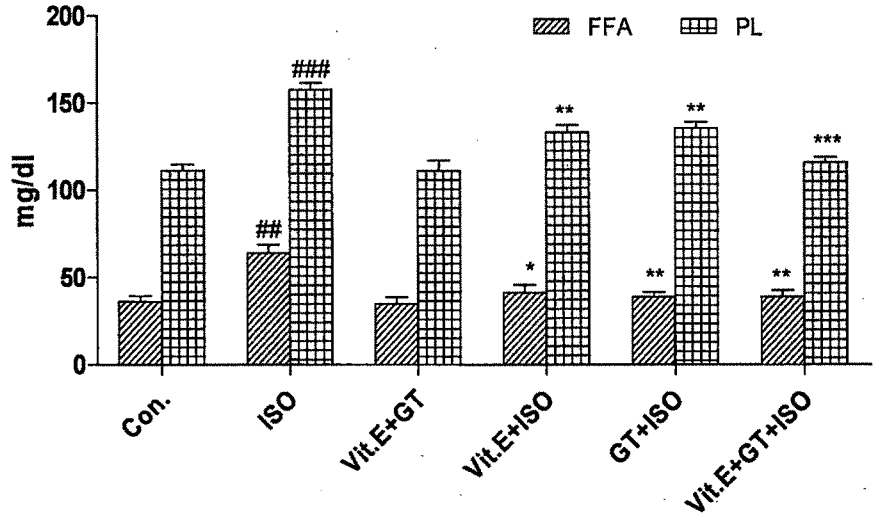


Fig. 5. 11 Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on a) LDL and VLDL, b) FFA and PL levels in normal and ISO (200mg/kg, s.c) injected rats

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

Table 5.5: Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on myocardial lipid profile in normal and ISO (200mg/kg, s.c) injected rats

Groups	TC	TG	FFA	PL
Con.	8.938 ± 0.590	6.337 ± 0.721	0.945 ± 0.075	25.391 ± 1.750
ISO	13.690± 1.340$^{\#\#}$	11.260± 0.840$^{\#\#\#}$	1.450± 0.117$^{\#\#}$	16.620± 1.902$^{\#\#}$
Vit.E+GT	7.314 ± 0.390	5.999 ± 0.390	0.888 ± 0.190	25.230 ± 1.330
Vit.E+ISO	10.080 ± 0.483*	7.337 ± 0.823**	1.102 ± 0.078	23.640 ± 1.982*
GT+ISO	10.015 ± 0.624*	7.825 ± 0.628*	1.032 ± 0.202*	23.600 ± 2.882*
Vit.E+GT+ISO	9.148 ± 0.670**	7.502 ± 0.488**	0.976 ± 0.098*	24.201 ± 2.431*

(Units for TC, TG, FFA and PL are expressed as mg/g wt. tissue)

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

Fig. 5.12 (a)

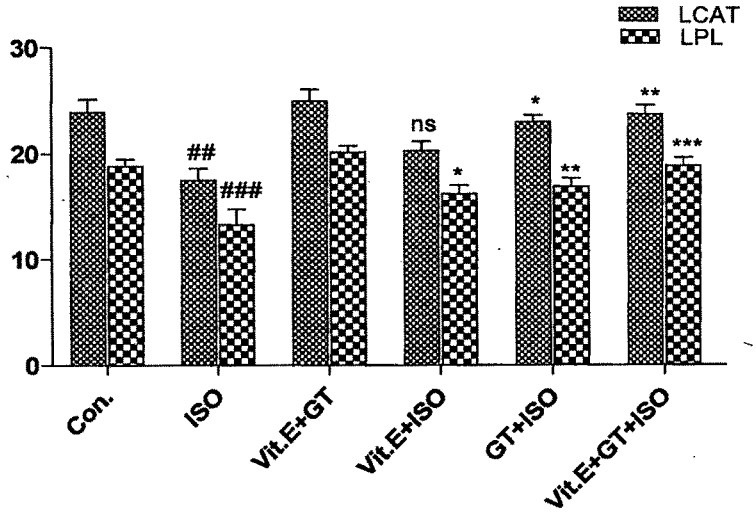


Fig. 5.12 (b)

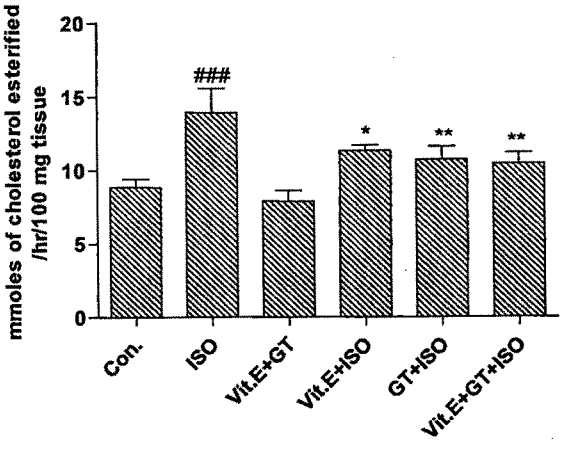


Fig. 5.12. Effect of Vit.E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on (a) LCAT and LPL (b) CES levels in normal and ISO (200mg/kg, s.c) injected rats

Units: LCAT (μmoles of cholesterol esterified/hr/100mg tissue); LPL (μmoles of fee fatty acids liberated/100mg tissue)

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

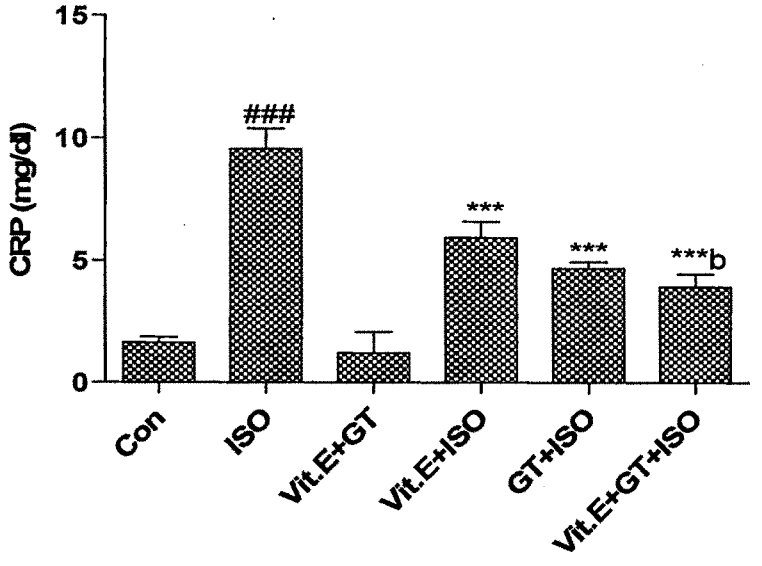


Fig. 5.13. Effect of Vit.E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on serum CRP level in normal and ISO (200mg/kg, s.c) injected rats

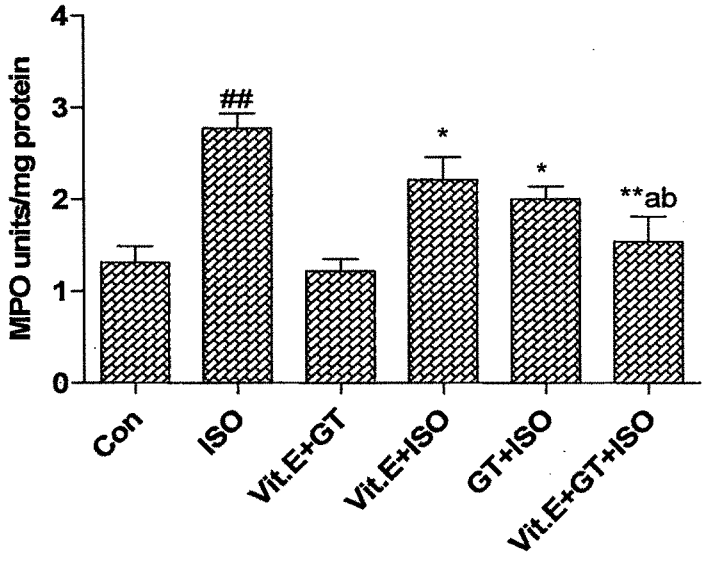


Fig. 5.14 Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on tissue MPO activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

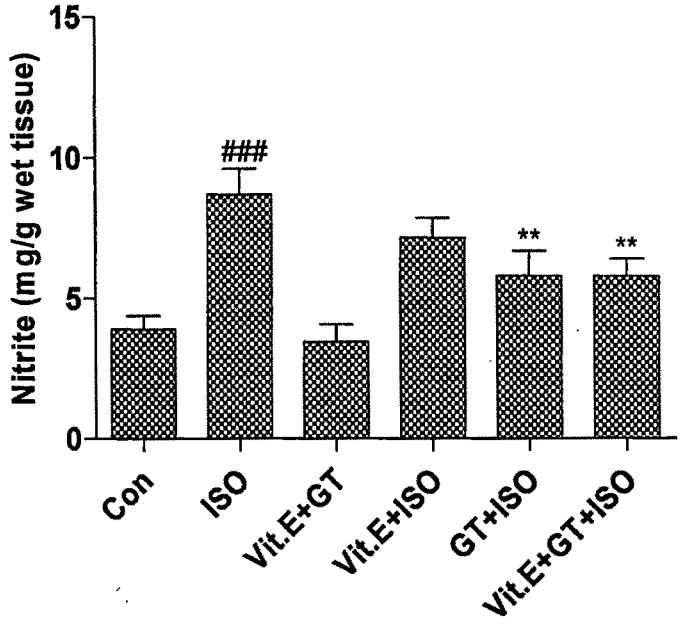
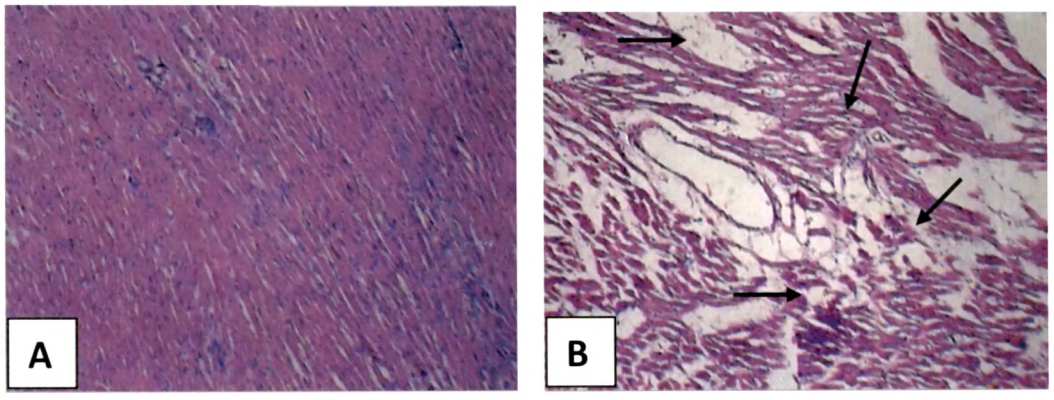
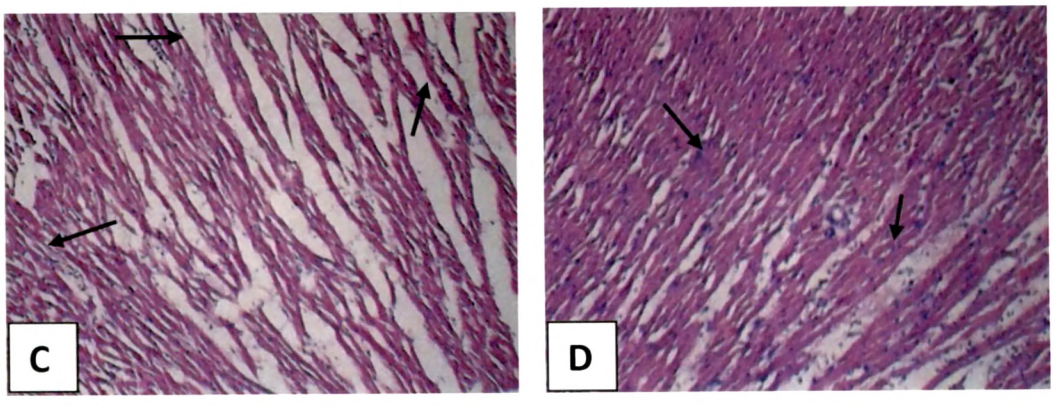


Fig. 5.15. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on tissue nitrite levels in normal and ISO (200mg/kg, s.c) injected rats

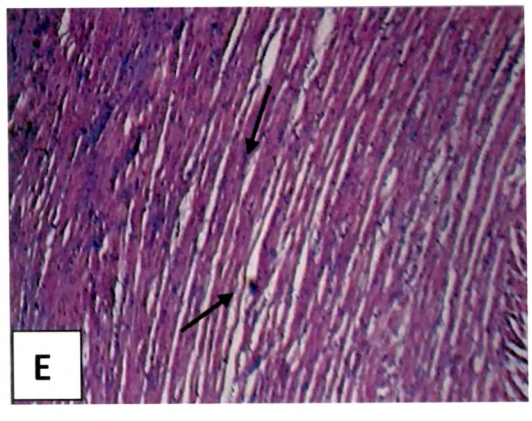
Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.



Control ISO



Vit. E+ISO GT+ISO



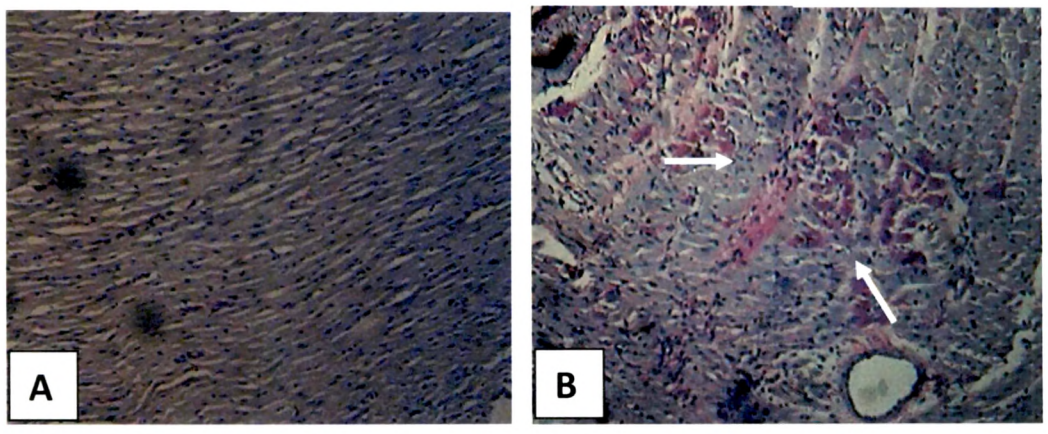
Vit.E+GT+ISO

Fig. 5.16. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on Histopathological (H & E) alteration in normal and ISO (200mg/kg, s.c) injected rats

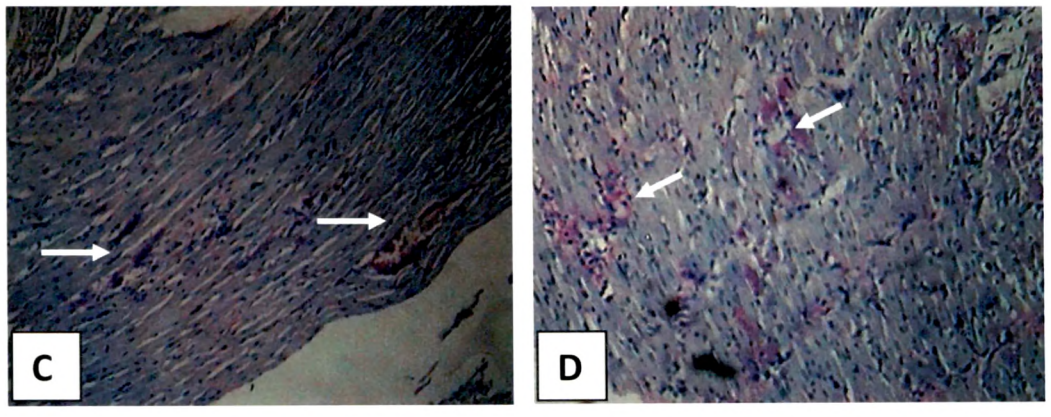
Table 5.6. Effect of Vit.E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on the degree of histological changes in normal and ISO (200mg/kg, s.c) injected rats

Groups	Necrosis	Oedema	Inflammatory cells
Control	A	A	A
ISO	+++	+++	++
Vit.E+ISO	++	++	+
GT+ISO	+	++	+
Vit.E+GT+ISO	A	+	A

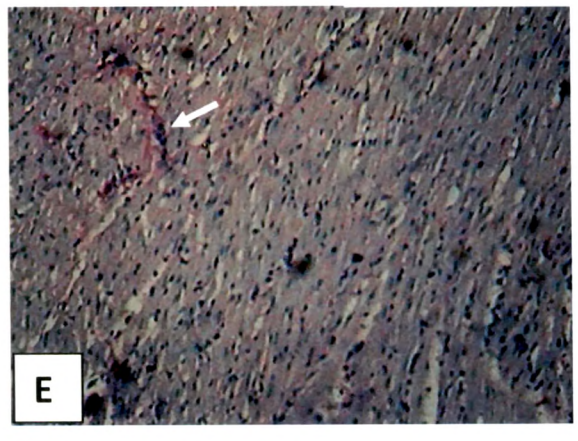
Photomicrographs were used to evaluate the damage in the heart tissues: (A) no change, (+++) marked changes, (++) moderate changes, (+) mild changes



Control ISO



Vit.E+ISO GT+ISO



Vit.E+GT+ISO

Fig. 5.17. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on Periodic acid Schiff's staining in normal and ISO (200mg/kg, s.c) injected rats (10X)

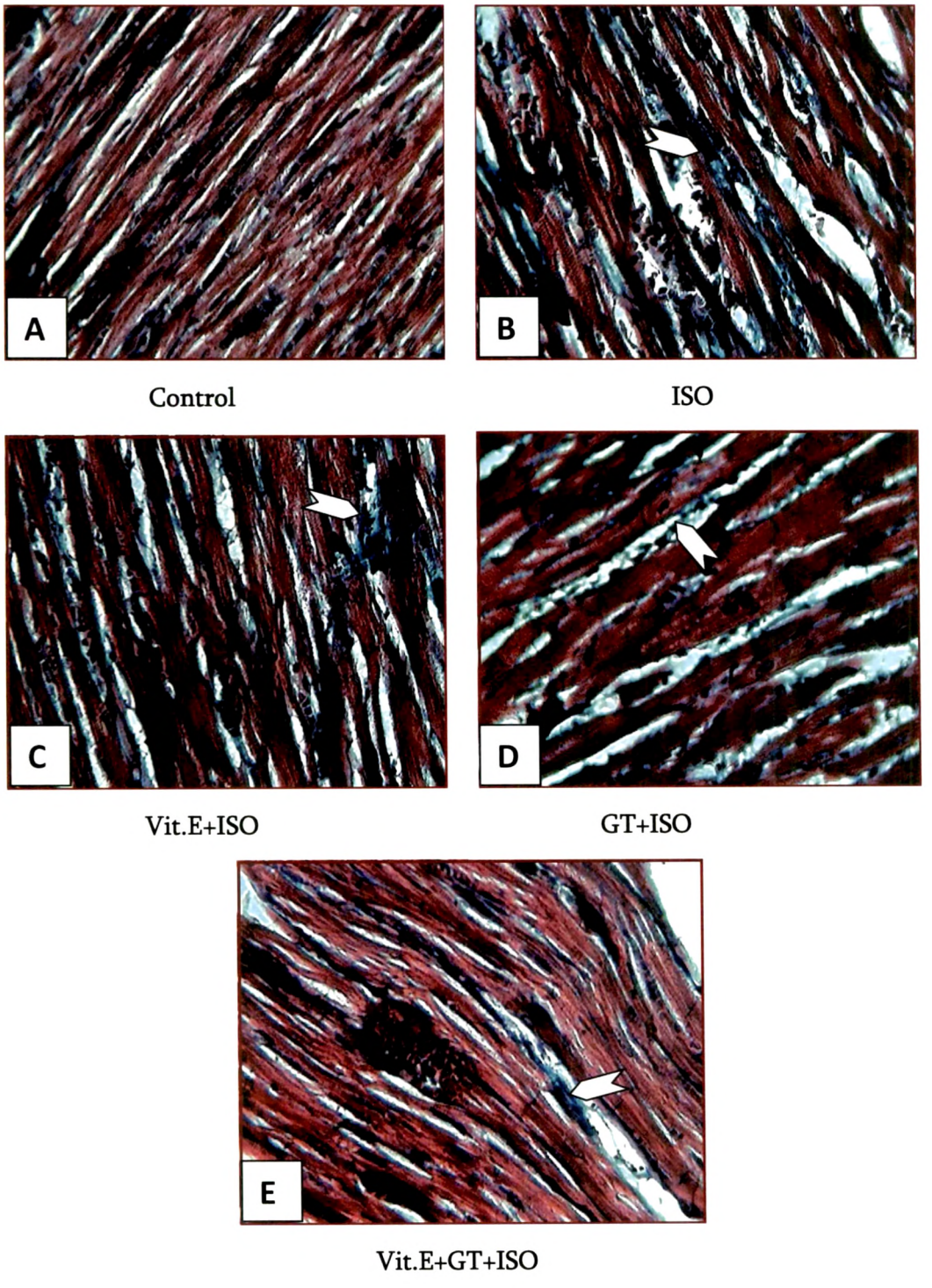
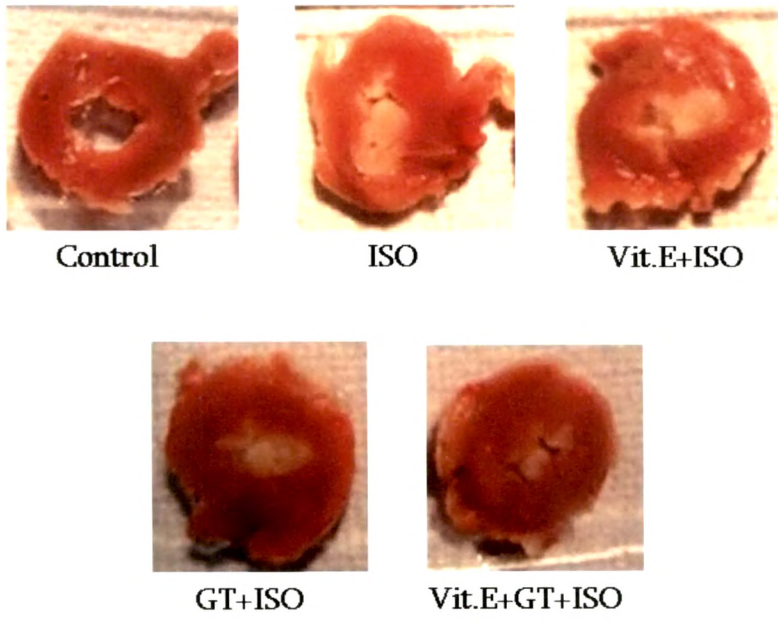


Fig. 5.18. Effect of Vit.E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on Masson's Trichrome staining of cardiac tissue in normal and ISO (200mg/kg, s.c) injected rats (40X)



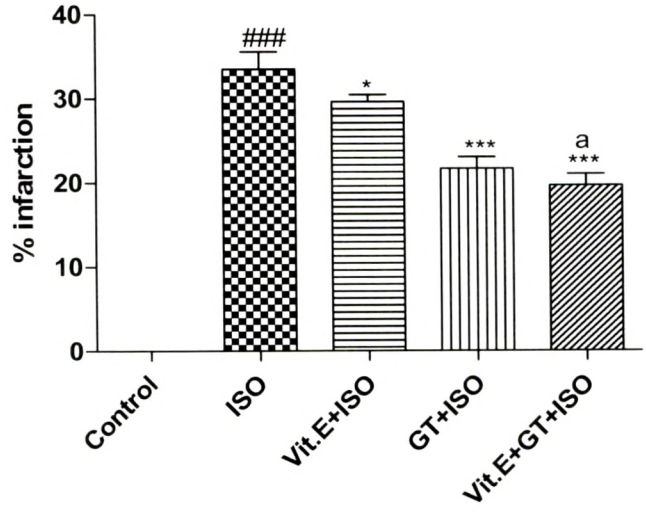
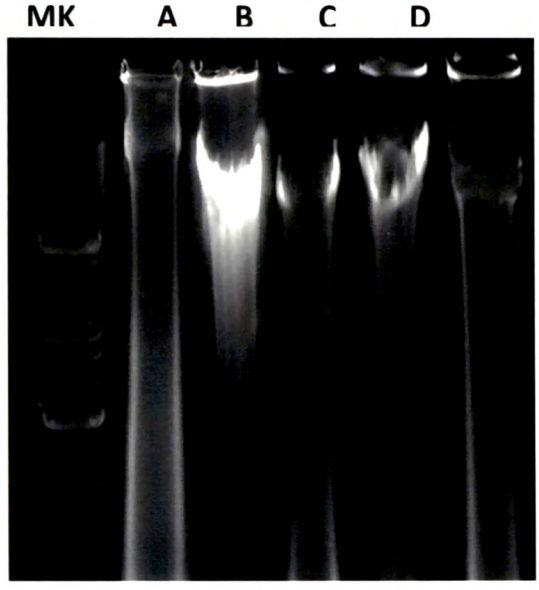


Fig. 5.19. Effect of Vit. E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on macroscopic enzyme mapping (TTC) assay and area of infarction in normal and ISO (200mg/kg, s.c) injected rats.

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.



Lane A- Control

Lane B- ISO

Lane C- Vit. E +ISO

Lane D- GT +ISO

Lane E- Vit. E +GT+ISO

Fig. 5. 20. Effect of Vit.E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on DNA damage (gel electrophoresis) in normal and ISO (200mg/kg, s.c) injected rats

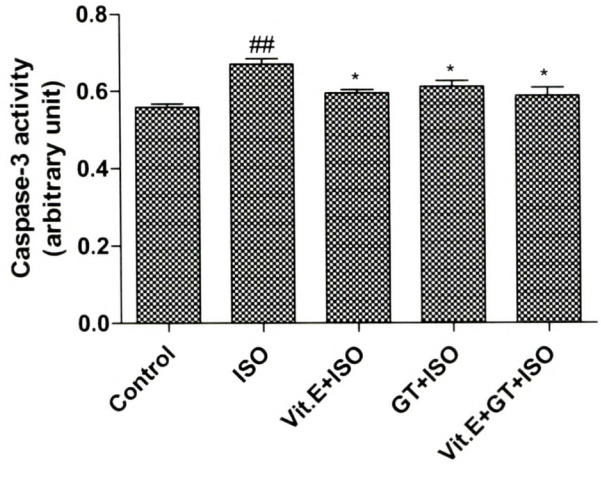


Fig. 5. 21. Effect of Vit.E (100mg/kg/day, p.o) and GT (100mg/kg/day, p.o) for 30 days on Caspase-3 activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. . $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to GT+ISO group.

5.3. Effect of Vitamin E (100 mg/kg/day, p.o) alone and its combination with Lycopene (10 mg/kg/day, p.o) for 30 days in ISO (200 mg/kg, s.c) induced MI

5.3.1. Effect of Vit.E and LYP on body weight, heart weight and heart/body weight ratio

Rats injected with ISO showed a significant ($P<0.01$) decrease in body weight with a significant ($P<0.01$, $P<0.001$) increase in heart weight and heart to body weight ratio as compared to control rats. Treatment of LYP in ISO injected rats (LYP+ISO) significantly ($P<0.05$) increased the body weight and a significantly ($P<0.05$, $P<0.01$) decreased the heart weight and heart to body weight ratio as compared to ISO injected groups. Co-administration of Vit. E and LYP in ISO injected rats (Vit.E+LYP+ISO) did not show significant changes in body weight, heart weight and heart to body weight ratio as compared to Vit.E+ISO or LYP+ISO treated group (Table 5.7).

5.3.2 .Effect of Vit.E and LYP on Electrocardiographic changes

The effects of Vit.E and LYP on electrocardiographic changes in normal and ISO injected rats are shown in Fig. 5.22 and Table 5.8. ISO injected rats showed a significant ($P<0.001$) increase in ST-interval, QT interval along with a significant ($P<0.001$) decrease in P wave, QRS complex and RR interval. Treatment of LYP alone and in combination with Vit.E in ISO injected rats (LYP+ISO, Vit.E+LYP+ISO) significantly ($P<0.01$, $P<0.001$, $P<0.05$) decreased ST- interval, QT interval and significantly ($P<0.01$, $P<0.001$, $P<0.05$) increased P wave, QRS complex and RR interval as compared to ISO, Vit.E+ISO or LYP+ISO treated group. It was found that this combination showed better effect in maintaining the ECG changes towards normal as compared to Vit.E+ISO or LYP+ISO treated groups (Table 5.8).

5.3.3. Effect of Vit.E and LYP on systolic, diastolic and mean blood pressure

ISO injected rats showed a significant ($P<0.01$, $P<0.001$) decrease in diastolic and mean blood pressure as compared to control group. Treatment with LYP in ISO injected rats (LYP+ISO) showed a significant ($P<0.05$) increase in mean blood pressure compared to ISO injected rats. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) did not show significant improvement in diastolic and mean blood pressure as compared to Vit.E alone treated group (Vit.E+ISO) (Fig. 5.23).

5.3.4. Effect of Vit.E and LYP on serum cardiac marker enzymes

A significant ($P<0.001$) increased in the activities of AST, ALT, ALP, LDH and CK-MB were observed in ISO injected rats. Vit.E+ISO or LYP+ISO treated rats showed a significant ($P<0.001$) decrease in these cardiac marker activities compared to ISO injected rats. Co-administration of Vit.E or LYP in ISO injected rats (Vit.E+LYP+ISO) showed significant ($P<0.05$) reduction in the activities of AST, ALT, ALP, LDH and CK-MB as compared to Vit.E+ISO or LYP+ISO treated groups (Fig. 5.24 and 5.25).

ISO injected rats showed a significant increase ($P < 0.001$) in serum uric acid levels and significant decrease in total protein ($P < 0.001$) levels as compared to control rats. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed significant ($P < 0.05$) decrease in uric acid with significant ($P < 0.001$) increase in total protein level as compared to Vit.E+ISO or LYP+ISO treated group. The combination showed better effects in maintaining cardiac marker enzymes towards normal as compared to Vit.E+ISO or LYP+ISO treated group (Fig. 5.25b).

5.3.5. Effect of Vit.E and LYP on lipid peroxidation (LPO) and biomarkers of oxidative stress

The effect of Vit.E and LYP on LPO and markers of oxidative stress in normal and ISO injected rats are shown in Fig. 5.26 and Table 5.9. Rats injected with ISO

showed a significant (P<0.001) increase in LPO along with a significant (P<0.001) decrease in GSH, GPx, GST, SOD, CAT activities and Vit. E levels as compared to control group. Treatment of Vit.E or LYP in ISO treated rats (LYP+ISO or Vit.E+ISO) significantly (P<0.001) decreased LPO level and significantly (P<0.001) increased the activities of markers of oxidative stress as compared to ISO injected rats. Further it was found that co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed significant (P<0.001, P<0.05) decrease in LPO level along with a significant (P<0.001, P<0.05) increase in GSH, GPx, GST, SOD, CAT activities and Vit. E level as compared to Vit.E+ISO, LYP+ISO or ISO treated rats.

5.3.6. Effect of Vit.E and LYP on membrane bound ATPases and electrolytes level

ISO injected rats showed significant (P < 0.001) decrease in Na^{+}/K^{+}-ATPase, Mg^{+2}-ATPase activities with significant (P<0.001) increase in Ca^{+2}-ATPase activity. Treatment of Vit.E or LYP in ISO injected rats (Vit.E+ISO or LYP+ISO) showed significant (P<0.01, P<0.001) increase in Na^{+}/K^{+}-ATPase, Mg^{+2}-ATPase activities with significant (P<0.001) decrease in Ca^{+2}-ATPase activity as compared to ISO injected rats. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed significant (P<0.001, P<0.05) improvements in maintaining membrane bound ATPases towards normal as compared to Vit.E+ISO or LYP+ISO treated group (Fig. 5.27).

The levels of sodium and calcium were significantly (P<0.001) increased and potassium was significantly (P<0.001) decreased in ISO injected rats. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly (P<0.001, P<0.05) decreased the levels of sodium, calcium and significantly (P<0.001, P<0.05) increased the potassium levels compared to Vit.E + ISO or LYP+ISO treated rats (Table 5.10).

5.3.7. Effects of Vit.E and LYP on LDH isoenzyme pattern

Agarose gel electrophoretic separation of serum LDH-isoenzyme patterns of normal and ISO injected rats are shown in Fig.5.28. ISO injected rats showed an increase intensity of LDH-1 and LDH-2 isoenzyme as compared to control rats. Co-administration of Vit. E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly decreased the intensity of LDH-1 and LDH-2 isoenzyme bands towards normal compared to Vit.E+ISO or LYP+ISO treated rats (Fig. 5.28).

5.3.8. Effect of Vit. E and LYP on serum and heart tissue lipid profile

Levels of various lipids in serum of control and ISO injected animals were recorded (Fig. 5.29 & Fig. 5.30). Rats injected with ISO showed a significant ($P<0.001$, $P<0.01$) increase in serum TC, TG, LDL, VLDL, FFA and PL levels with a significant ($P<0.01$) decrease in HDL level. Co-administration of Vit. E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly ($P<0.001$, $P<0.05$) decreased the elevated levels of TC, TG, LDL, VLDL, FFAs, PL and significantly ($P<0.001$, $P<0.05$) increased the HDL levels as compared to Vit.E+ISO or LYP+ISO treated groups.

ISO injected rats showed a significant increase ($P < 0.01$, $P < 0.001$) in the levels of heart TC, TG and FFAs with a significant decrease ($P < 0.01$) in PL level compared to control group. Co-administration of Vit.E and LYP to ISO injected rats (Vit.E+LYP+ISO) significantly ($P<0.01$, $P<0.05$) decreased TC, TG, FFA level and significantly ($P<0.05$) increased PL level compared to rats treated with Vit.E+ISO or LYP+ISO treated groups (Table 5.11).

5.3.9. Effect of Vit. E and LYP on myocardial Lipid metabolizing enzymes

The activities of myocardial LCAT, LPL and CES in control and ISO injected rats were observed (Fig.5.31a-b). A significant ($P<0.01$, $P<0.001$) decreased in the activities of LCAT, LPL and a significant ($P<0.001$) increased in the activity of CES was observed in ISO injected rats. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly ($P<0.001$, $P<0.05$) increased the

activities of LCAT, LPL and significantly ($P<0.001$, $P<0.05$) decreased the CES activity as compared to Vit.E+ISO or LYP+ISO treated groups.

5.3.10. Effect of Vit. E and LYP on Serum CRP and tissue MPO activity

Rats injected with ISO showed a significant ($P<0.001$, $P<0.01$) increase in CRP level and MPO activity as compared to control group. Treatment with Vit. E or LYP alone and with combination in ISO injected rats (Vit.E+ISO or LYP+ISO Vit.E+LYP+ISO) showed a significant ($P<0.001$, $P<0.05$) decrease in CRP level and significant ($P<0.01$, $P<0.05$) increase in MPO activity as compared to Vit.E+ISO or LYP+ISO treated group. The combination of Vit.E and LYP was found to be more effective in maintaining the level of CRP and MPO activity towards normal compared to Vit.E and LYP alone treated group (Fig. 5.32 and 5.33).

5.3.11. Effect of Vit. E and LYP on tissue nitrite levels

Tissue nitrite level was found to be significantly ($P<0.001$) increased in ISO injected compared to control rats. Rats injected with LYP+ISO showed a significant ($P<0.01$) decrease in nitrite level as compared to ISO injected rats. Further it was found that co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) significantly reduced tissue nitrite level compared to Vit. E +ISO or LYP+ISO treated groups (Fig.5.34).

5.3.12. Effect of Vit. E and LYP on Histopathological alteration

Histopathological sections of ISO injected rats showed necrosis of muscle fibers with inflammatory cell infiltration, edema and separation of muscle fibers (Fig. 5.35B, Table 5.12). Treatment with Vit.E + ISO showed myonecrosis with less edema and inflammatory cells (Fig. 5.35C). LYP+ISO treated rat showed mild degree of edema, inflammatory cells and separation of muscle fibers (Fig.5.35D). Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed near normal architecture of heart tissue with mild edema, muscle fragmentation along with absence of necrosis and inflammatory cells (Fig. 5.35E, Table 5.12).

5.3.13. Effect of Vit. E and LYP on Periodic acid Schiff's staining

PAS staining of ISO injected rats showed an arbitrary increase in the amount of glycoproteins or glycoconjugates as compared to normal control rats (Fig. 5.36A and 5.36B). Co-administration of Vit. E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed normal architecture of membrane with maintenance of membrane bound glycoconjugates (Fig. 5.36E). Vit.E+ISO or LYP+ISO treated groups also showed reduction in glycoconjugates (Fig. 5.36C and 5.36D).

5.3.14. Effect of Vit. E and LYP on Masson's trichrome staining

Masson's trichrome staining of cardiac tissue of control and ISO injected groups were carried out Fig. 5.37. Normal architecture of muscle fibres primarily confined to the intramuscular fasciculi was observed in cardiac tissue of normal rats (Fig. 5.37A). ISO injected rats showed muscle cell necrosis with disruption in arrangement of collagen fibers (Fig. 5.37B). Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed less damaged collagen fibers exhibiting significant protection from collagen disruption (Fig. 5.37E).

5.3.15. Effect of Vit. E and LYP on macroscopic enzyme assay (TTC test) and area of infarction

The percentage of mean infarct size with increased staining was observed in ISO injected rats when compared to control group. Treatment with Vit.E+ISO and LYP+ISO showed a significant decrease in infarct size and staining as compared to ISO injected rats. Further co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed reduction in infarct size and staining as compared to Vit.E+ISO or LYP+ISO treated groups (Fig. 5.38).

5.3.16. Effect of Vit.E and LYP on the DNA damage by gel electrophoresis

ISO injected rats showed severity of DNA damage as compared to control rats (Lane B). Treatment of Vit.E+ISO or LYP+ISO showed active prevention in the severity of DNA damage (Lane C and D) compared to alone ISO injected rats. Co-

administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed further prevention of DNA damage compared to Vit.E+ISO and LYP+ISO treated group (Fig. 5.39).

5.3.17. Effect of Vit.E and LYP on Caspase-3 activity

The effects of Vit.E and LYP on caspase-3 activity in normal and ISO injected rats are shown in Fig. 5.40. ISO injected rats showed a significant ($P<0.01$) increase in caspase-3 activity which was significantly ($P<0.05$) reduced after treatment with Vit. E+ISO or LYP+ISO. Co-administration of Vit.E and LYP in ISO injected rats (Vit.E+LYP+ISO) showed further reduction in caspase-3 activity as compared to Vit. E+ISO and LYP+ISO treated group (Fig. 5.40).

Table 5.7. Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on body weight, heart weight and heart to body weight ratio in normal and ISO (200mg/kg, s.c) injected rats

Groups	Final body weight (g)	Heart weight (g)	HW/BW ratio
Control	232.5 ± 3.39	0.667 ± 0.031	0.00286 ± 0.0091
ISO	214.2 ± 5.88$^{\#\#}$	0.920 ± 0.054$^{\#\#}$	0.00429 ± 0.0155$^{\#\#\#}$
Vit.E+LYP	231.2 ± 6.77	0.664 ± 0.032	0.00289 ± 0.0047
Vit.E+ISO	227.7 ± 3.39*	0.705 ± 0.030*	0.00309 ± 0.0091**
LYP+ISO	222.9 ± 4.26*	0.717 ± 0.065*	0.00321 ± 0.0152**
Vit.E+LYP+ISO	228.4 ± 5.65*	0.691 ± 0.082**	0.00302 ± 0.0145***

Values are expressed as mean±SEM (n=6), $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

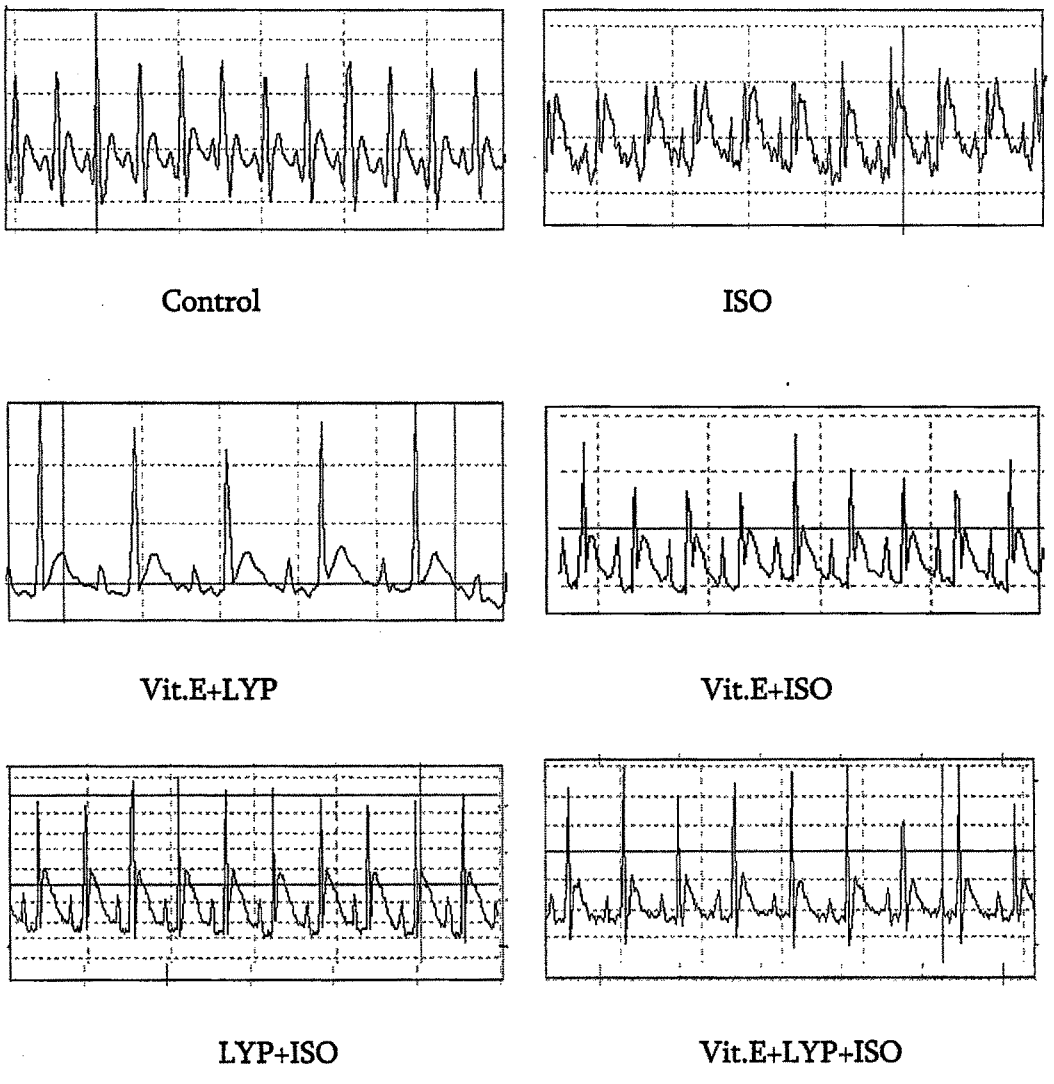


Fig. 5. 22 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on ECG changes in normal and ISO (200mg/kg, s.c) injected rats

Table 5.8: Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on ECG changes and heart rats in normal and ISO (200mg/kg, s.c) injected rats

Groups	ST elevation	P wave	QRS complex	QT interval	R-R interval	Heart rate
Control	0.184 ± 0.0022	0.028 ± 0.00037	0.0416 ± 0.005	0.071 ± 0.0006	0.170 ± 0.0007	338.30 ± 12.88
ISO	0.302 ± 0.0040$^{\#\#\#}$	0.024 ± 0.00030$^{\#\#}$	0.0285 ± 0.007$^{\#\#\#}$	0.081 ± 0.0012$^{\#\#}$	0.158 ± 0.0017$^{\#\#}$	400.09 ± 14.32^{ns}
Vit.E+LYP	0.178 ± 0.0013	0.029 ± 0.00054	0.0418 ± 0.006	0.070 ± 0.0009	0.170 ± 0.0005	340.90 ± 10.54
Vit.E+ISO	0.251 ± 0.0023***	0.025 ± 0.00049^{ns}	0.0326 ± 0.001*	0.074 ± 0.0076*	0.164 ± 0.0008*	362.80 ± 16.52
LYP+ISO	0.232 ± 0.0025***	0.026 ± 0.00011*	0.0364 ± 0.004**	0.075 ± 0.0013*	0.161 ± 0.0016**	359.00 ± 10.11
Vit.E+LYP+ISO	0.193 ± 0.0033^{***ab}	0.027 ± 0.00012^{*b}	0.0400 ± 0.003***	0.072 ± 0.0002^{**a}	0.169 ± 0.0009^{**a}	342.82 ± 9.55

Values are expressed as mean±SEM for 6 animals in each group. The ECG parameters are expressed in seconds (sec), heart rate as Beats per minutes (BPM), ST elevation in milivolt (mv). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO group.

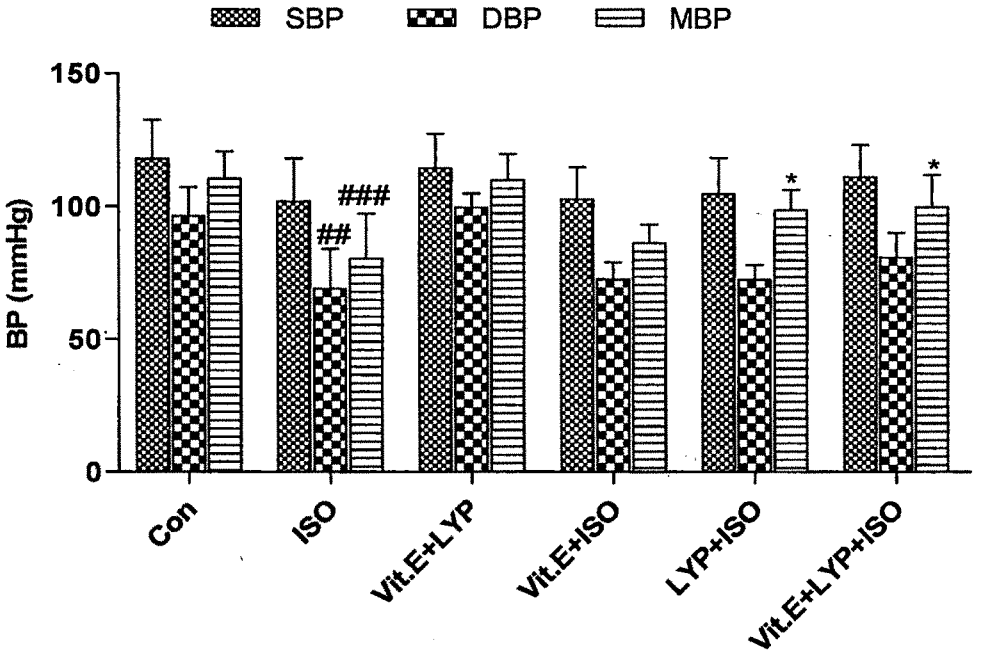


Fig. 5. 23 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on systolic, diastolic and mean blood pressure in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Fig. 5.24 (a)

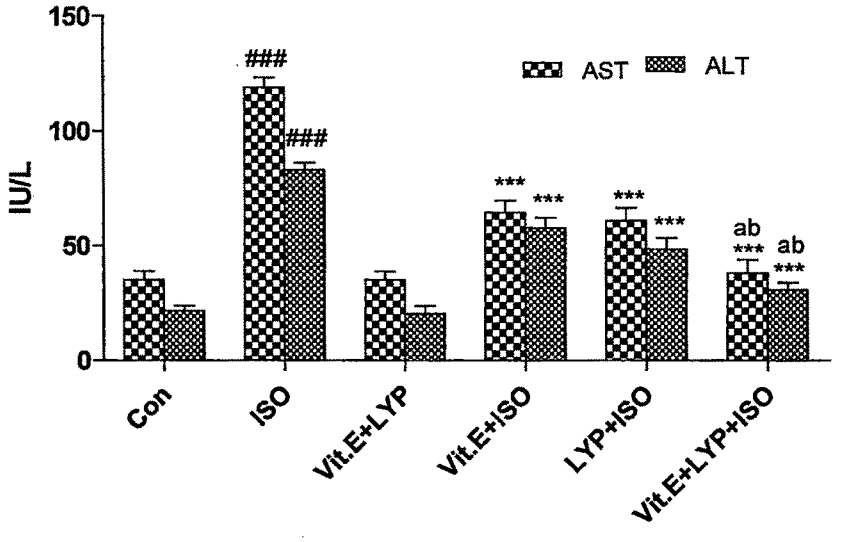


Fig. 5.24 (b)

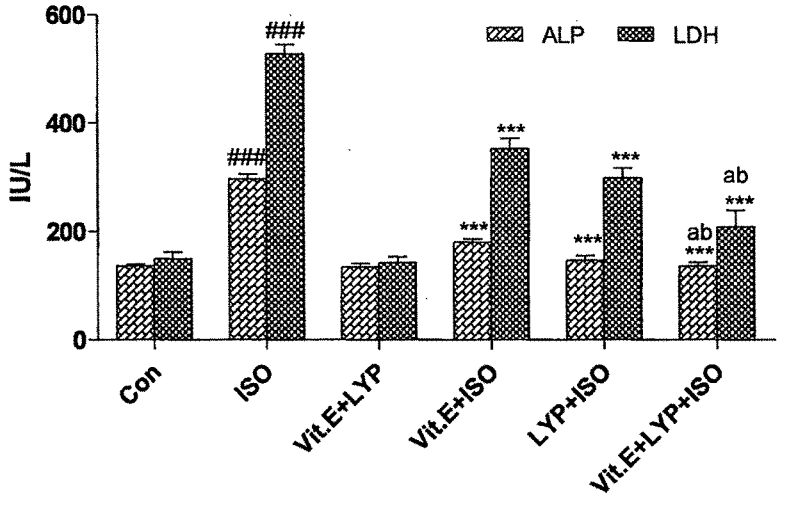


Fig. 5. 24 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on serum (a) AST and ALT (b) ALP and LDH levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Fig. 5.25 (a)

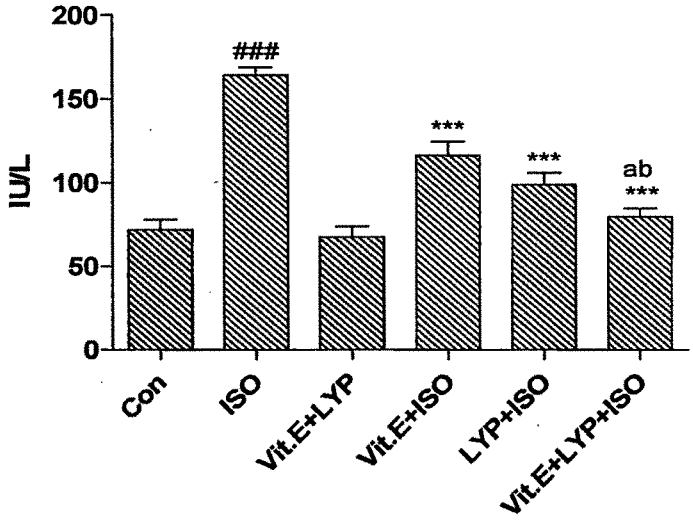


Fig. 5.25 (b)

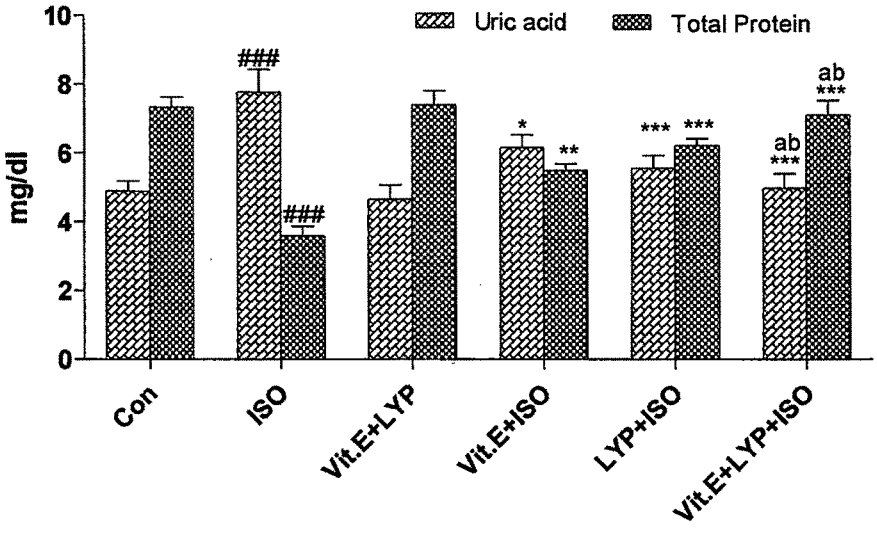


Fig. 5. 25 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on serum (a) CK-MB (b) Uric acid and Total protein levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM, (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

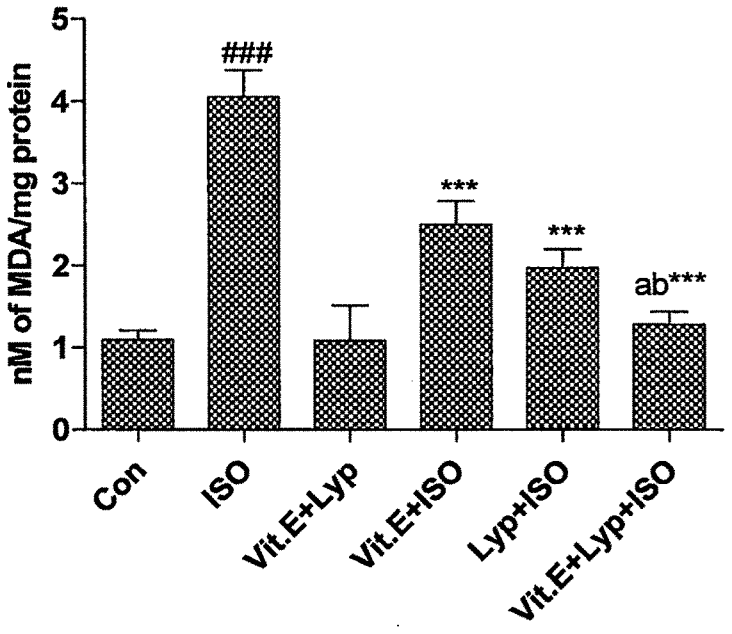


Fig. 5.26 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day, p.o) for 30 days on myocardial Lipid peroxidation in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6).

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups,

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

$^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Table 5.9: Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on endogenous antioxidants and vitamin E level in normal and ISO (200mg/kg, s.c) injected rats

Groups	GSH	GPx	GST	SOD	CAT	Vitamin E
Con.	7.401 ± 0.480	6.102 ± 0.206	110.40 ± 5.712	4.496 ± 0.203	6.290 ± 0.331	2.02 ± 0.047
ISO	4.338 ± 0.260$^{\#\#\#}$	4.032 ± 0.231$^{\#\#\#}$	68.91 ± 4.829$^{\#\#\#}$	2.272 ± 0.166$^{\#\#\#}$	3.687 ± 0.219$^{\#\#\#}$	1.09 ± 0.043$^{\#\#\#}$
Vit.E+LYP	7.648 ± 0.491	6.801 ± 0.299	118.33 ± 4.920	4.882 ± 0.463	6.763 ± 0.288	2.22 ± 0.031
Vit.E+ISO	5.785 ± 0.288**	5.635 ± 0.336**	95.55 ± 4.089***	3.566 ± 0.390*	5.188 ± 0.167**	1.84 ± 0.035***
LYP+ISO	6.208 ± 0.255***	5.502 ± 0.499**	93.75 ± 5.891***	4.212 ± 0.216***	5.042 ± 0.219**	1.99 ± 0.022***
Vit.E+LYP+ISO	7.600± 0.213^{***a}	6.423 ± 0.382^{***ab}	132.20 ± 5.721^{***ab}	5.122 ± 0.332^{***a}	6.622 ± 0.214^{***ab}	2.18 ± 0.017^{***ab}

Values are expressed as mean±SEM, (n=6). $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 values compared to control group. *P<0.05, **P<0.01, ***P<0.001 values compared to ISO injected group. ^{a}P<0.05 compared to Vit.E+ISO and ^{b}P<0.05 compared to LYP+ISO group.

GSH: (µg of GSH /mg protein), GPx: (µmoles of glutathione oxidized/min/mg protein), GST: (µmoles of CDNB conjugated/min/mg protein), SOD: (units/mg protein), CAT: (µmoles of H_2O_2 consumed/min/mg protein), Vitamin E: (mmole/mg protein).

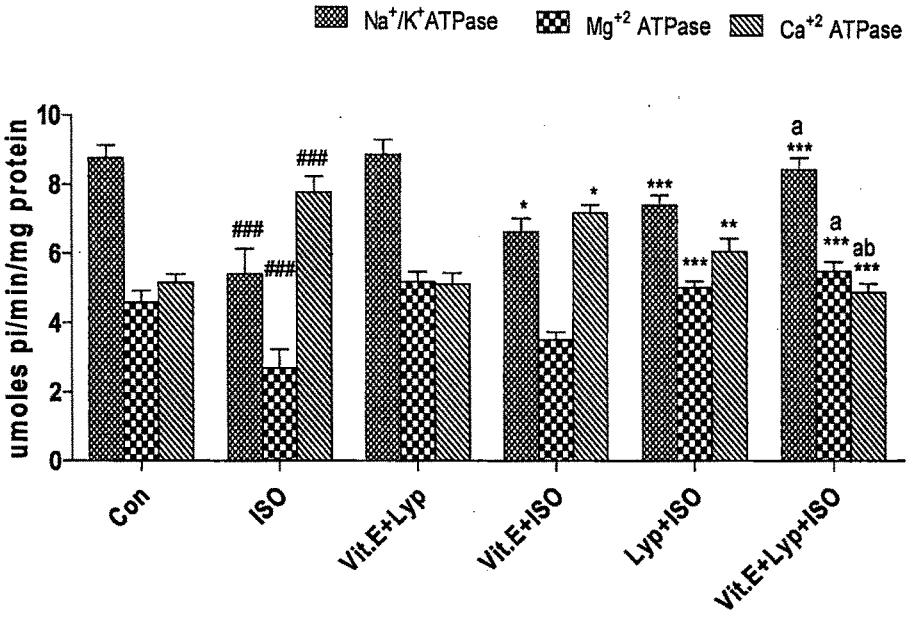


Fig. 5.27 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on the activity of Na^{+}/K^{+} ATPase, Mg^{+2} ATPase and Ca^{+2} ATPase in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6).

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Table 5.10 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on the levels of sodium, potassium and calcium in the heart of normal and ISO (200mg/kg, s.c) injected rats

Groups	Sodium (Na^+)	Potassium (K^+)	Calcium (Ca^{++})
Control	6.088 ± 0.125	8.978 ± 0.164	10.81 ± 0.107
ISO	7.728 ± 0.312$^{\#\#\#}$	6.162 ± 0.216$^{\#\#\#}$	13.83 ± 0.344$^{\#\#\#}$
Vit.E+LYP	6.082 ± 0.122	8.784 ± 0.121	10.77 ± 0.083
Vit.E+ISO	7.198 ± 0.156*	7.462 ± 0.189***	12.63 ± 0.291**
LYP+ISO	6.542 ± 0.109**	8.332 ± 0.136***	11.32 ± 0.143***
Vit.E+LYP+ISO	6.186 ± 0.131^{***a}	8.884 ± 0.201^{***ab}	10.90 ± 0.155^{***ab}

All the values are expressed as nmol/mg protein

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

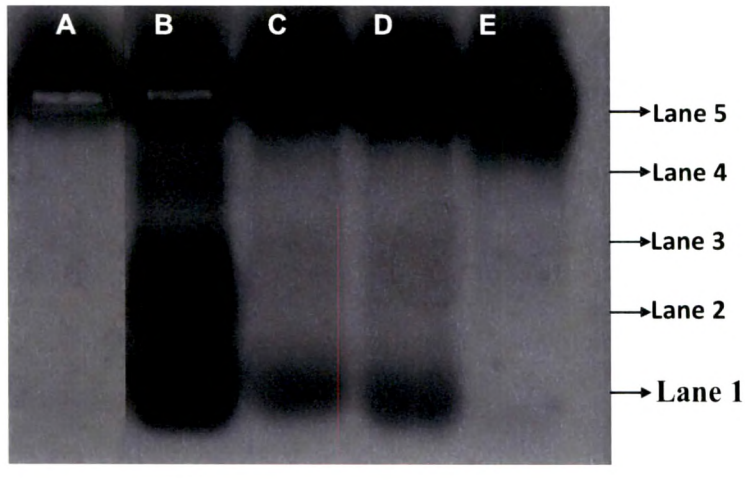


Fig. 5.28 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on LDH isoenzyme pattern in normal and ISO (200mg/kg, s.c) injected rats

A – Control, B – ISO, C – Vit. E +ISO, D - LYP +ISO, E – Vit. E +LYP+ISO

Fig. 5.29 (a)

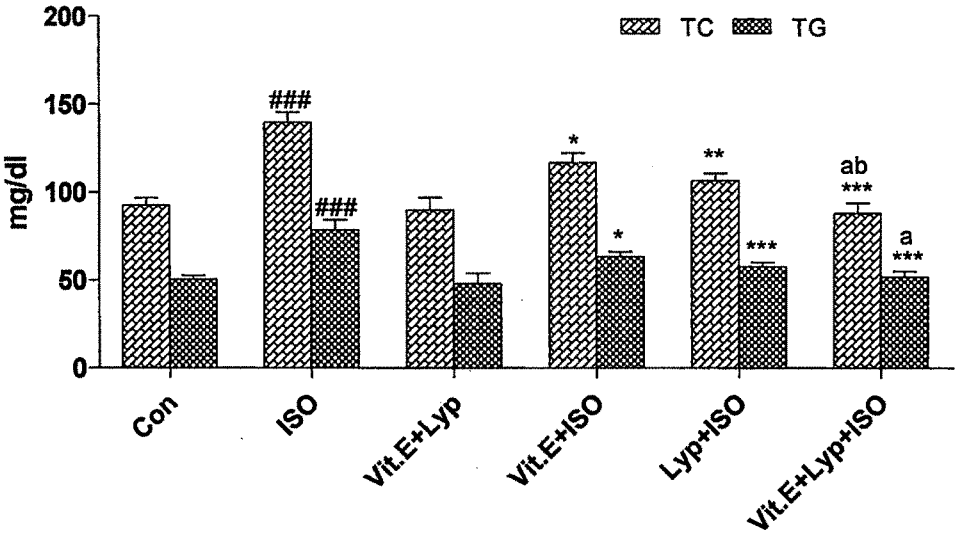


Fig. 5.29 (b)

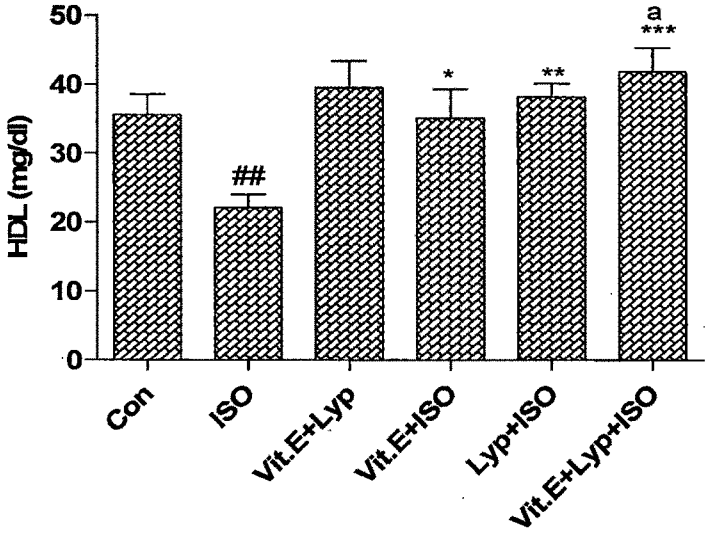


Fig. 5.29 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on serum a) TC and TG, b) HDL levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as Mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Fig. 5.30 (a)

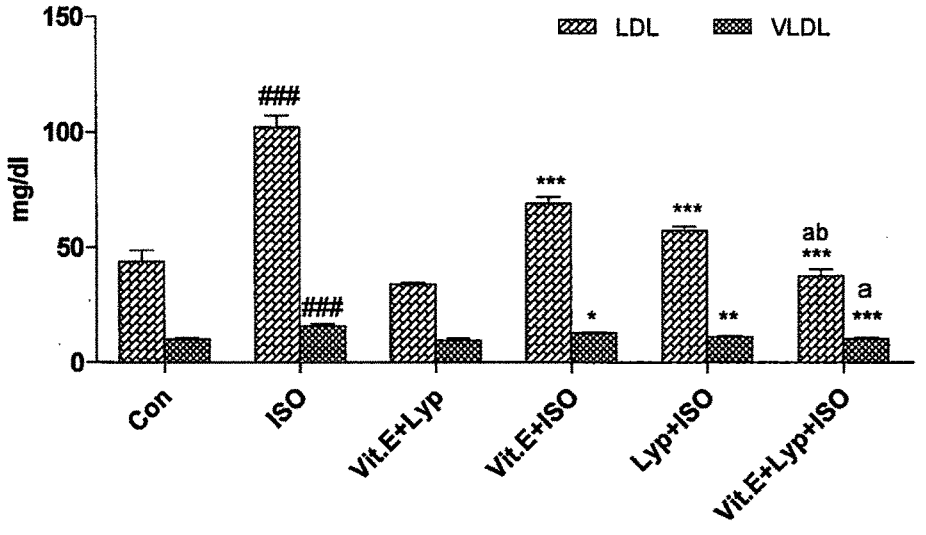


Fig. 5.30 (b)

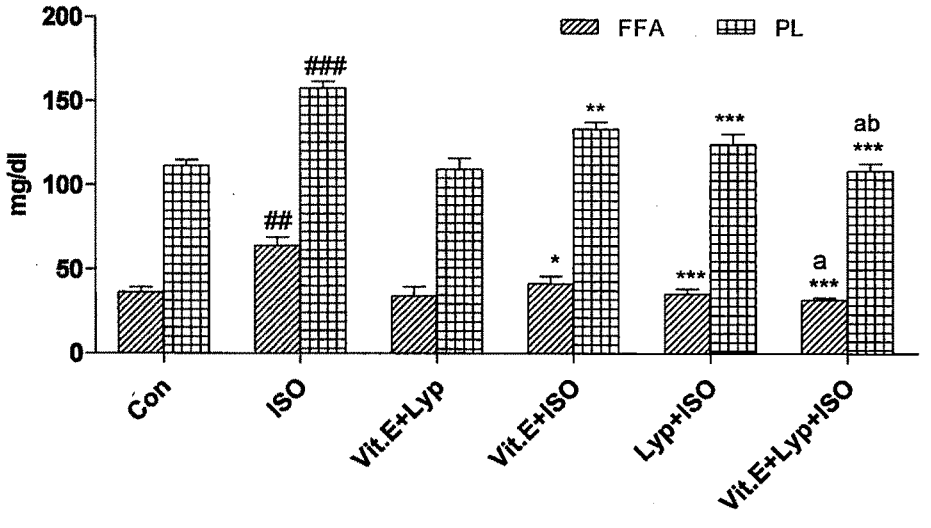


Fig. 5.30 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on a) LDL and VLDL, b) FFA and PL levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Table 5. 11 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on tissue lipid profile in normal and ISO (200mg/kg, s.c) injected rats

Groups	TC	TG	FFA	PL
Con.	8.230 ± 0.637	6.147 ± 0.637	0.871 ± 0.077	25.620 ± 1.988
ISO	13.690 ± 1.340$^{\#\#}$	11.260 ± 0.840$^{\#\#\#}$	1.450 ± 0.117$^{\#\#}$	16.620 ± 1.902$^{\#\#}$
Vit.E+LYP	7.832 ± 0.435	6.176 ± 0.422	0.868 ± 0.222	24.879 ± 1.883
Vit.E+ISO	10.080 ± 0.483*	7.337 ± 0.823**	1.102 ± 0.077	23.640 ± 1.982*
LYP+ISO	9.342 ± 0.732**	7.158 ± 0.710**	0.881 ± 0.122**	24.576 ± 3.111**
Vit.E+LYP +ISO	8.315 ± 0.572^{***a}	5.411 ± 0.529^{***ab}	0.877 ± 0.194^{**a}	26.872± 2.996^{**a}

Values are expressed as mg/g wt tissue

Values are expressed as mean ± SEM (n=6).

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups,

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

$^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

Fig. 5.31 (a)

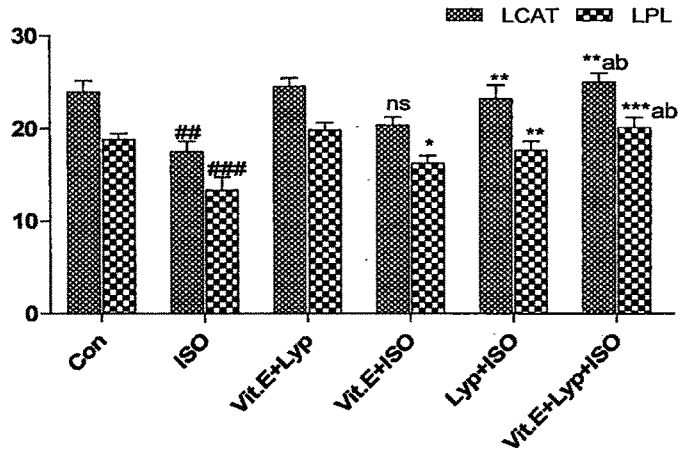


Fig. 5.31 (b)

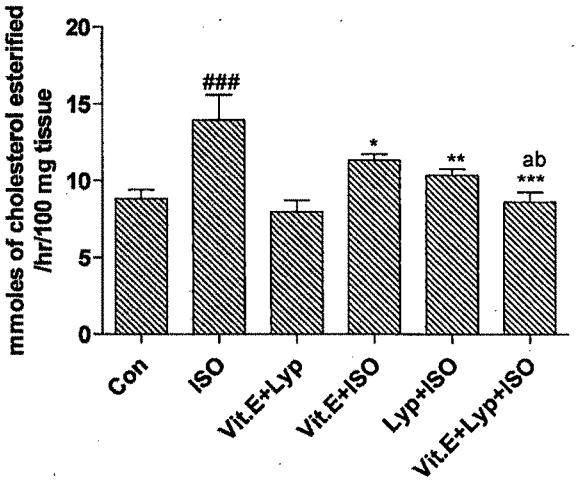


Fig. 5.31 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day, p.o) for 30 days on (a) LCAT and LPL (b) CES levels in normal and ISO (200mg/kg, s.c) injected rats

Units: LCAT (μmoles of cholesterol esterified/hr/100mg tissue)

LPL (μmoles of fee fatty acids liberated/100mg tissue)

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

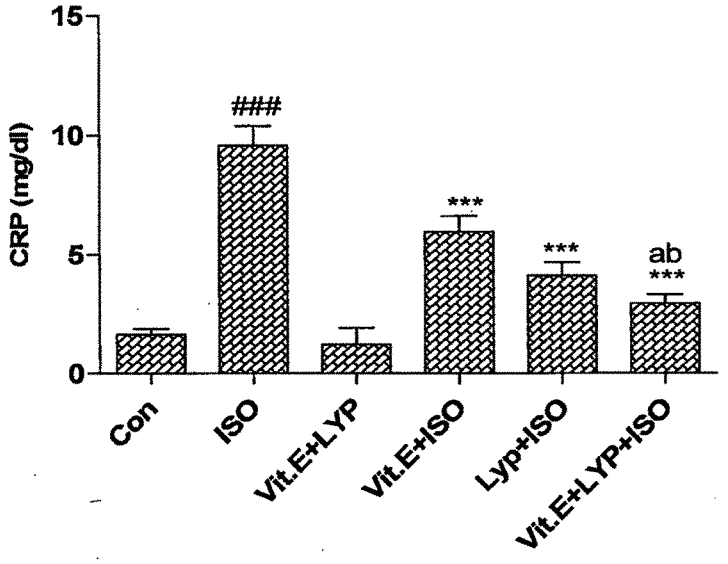


Fig. 5.32 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on serum CRP in normal and ISO (200mg/kg, s.c) injected rats

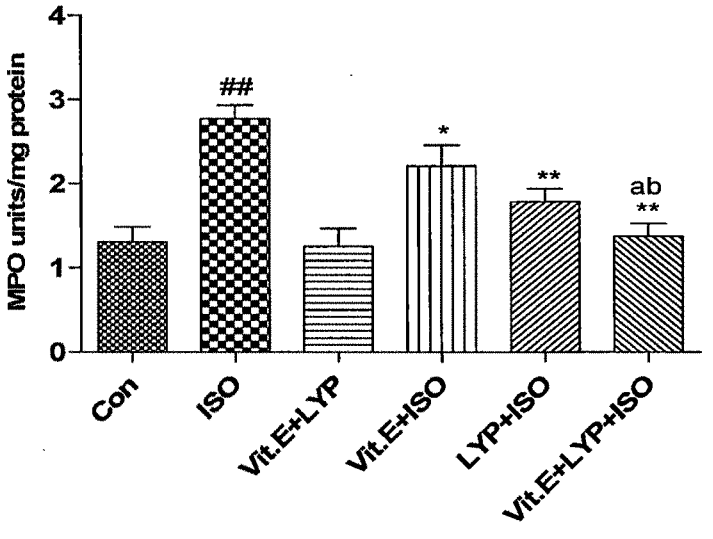


Fig. 5.33 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on tissue Myloperoxidase activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

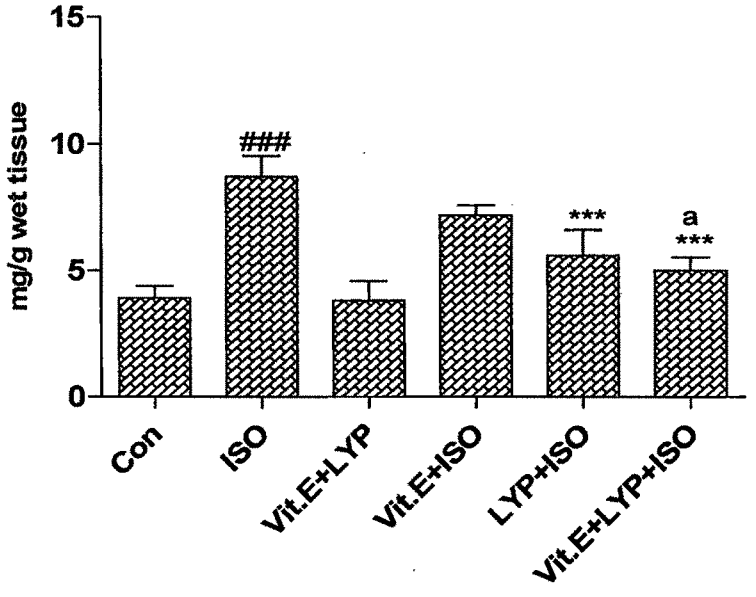


Fig. 5.34 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on tissue nitrite levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

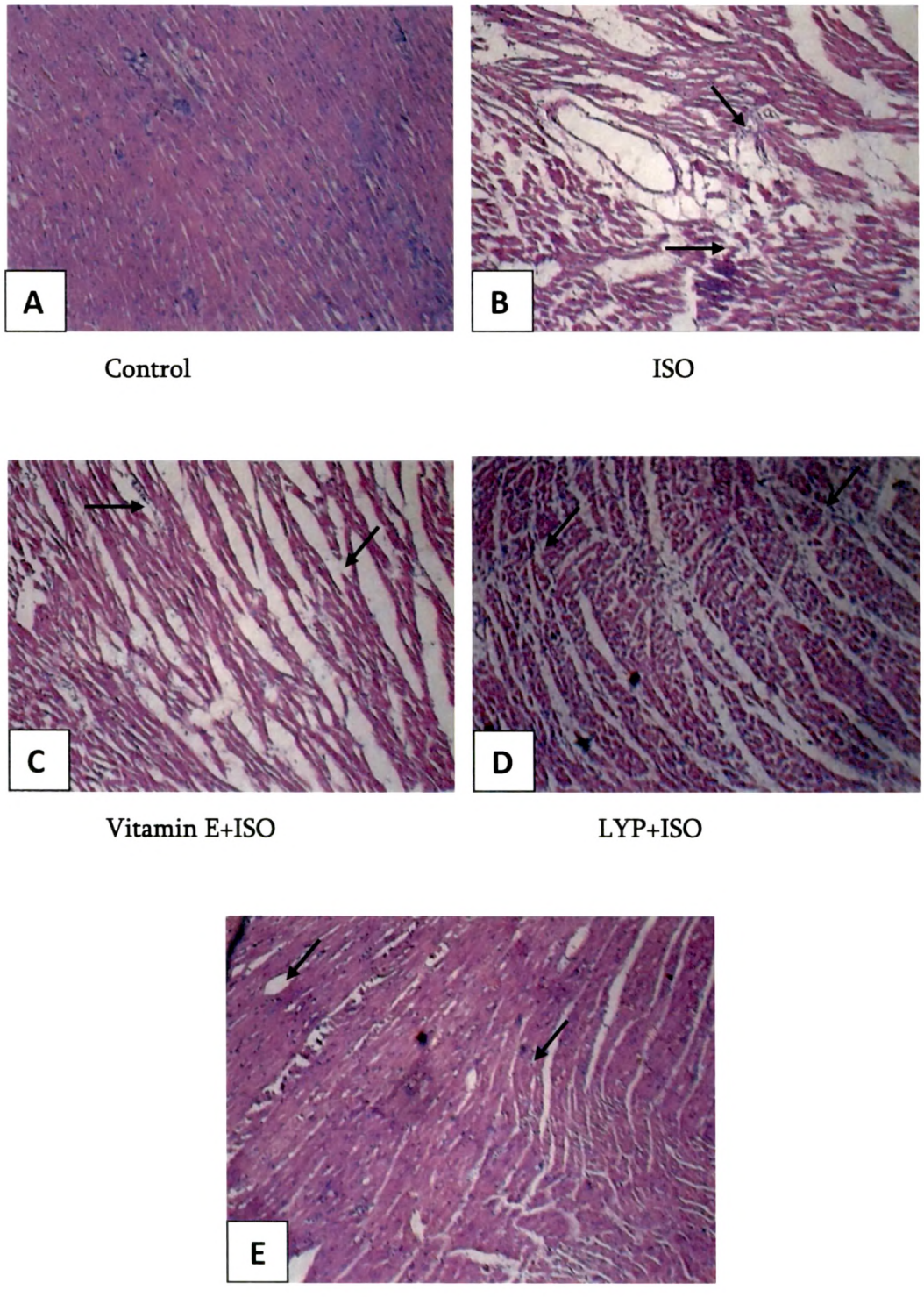


Fig. 5. 35 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on Histopathological alteration in normal and ISO (200mg/kg, s.c) injected rats (10X)

Table 5.12 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on the degree of histological changes in normal and ISO (200mg/kg, s.c) injected rats

Groups	Necrosis	Oedema	Inflammatory cells
Control	A	A	A
ISO	+++	+++	++
Vit.E+ISO	++	++	+
LYP+ISO	+	+	+
Vit.E+LYP+ISO	+	+	A

Photomicrographs were used to evaluate the damage in the heart tissues: (A) no change, (+++) marked changes, (++) moderate changes, (+) mild changes

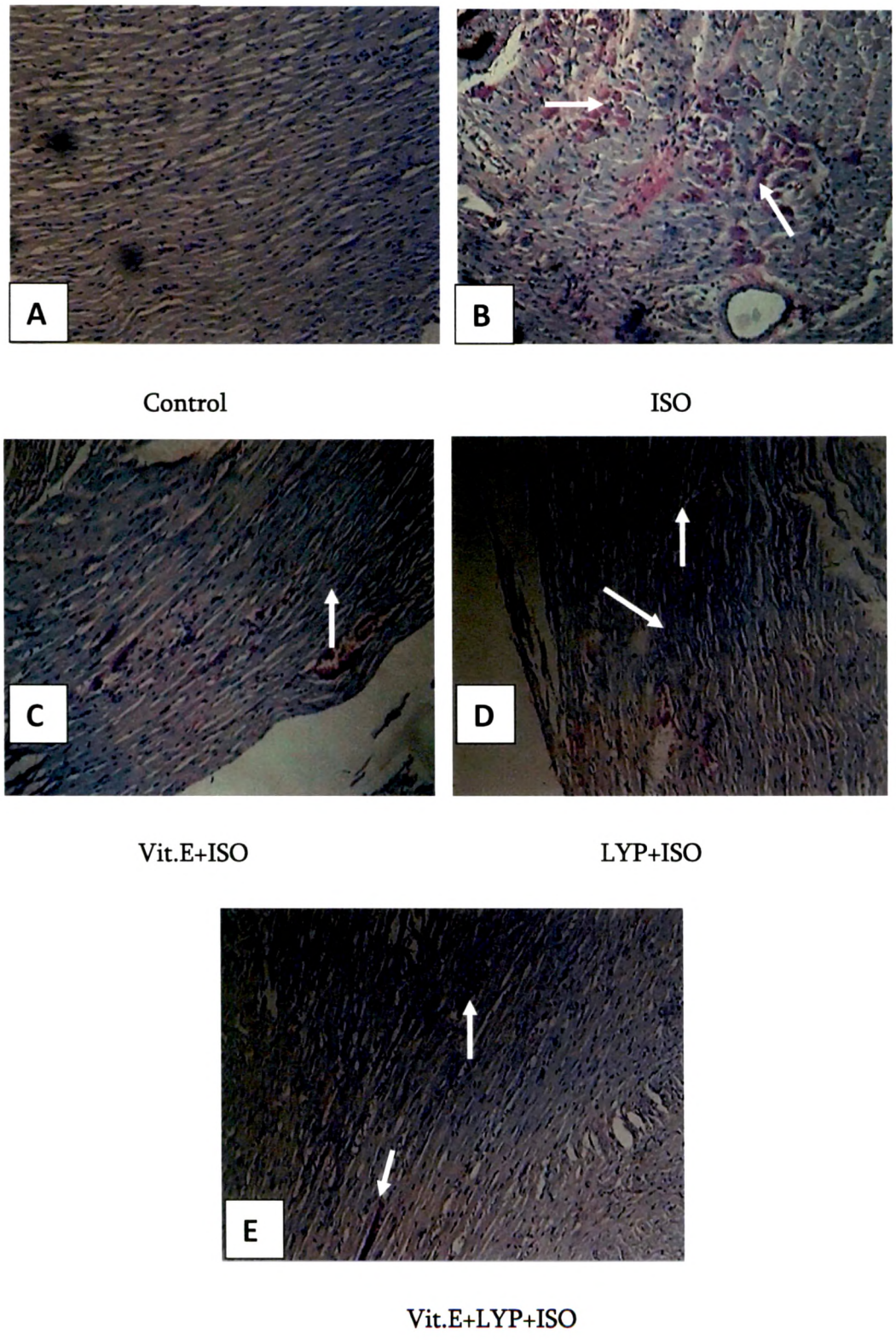
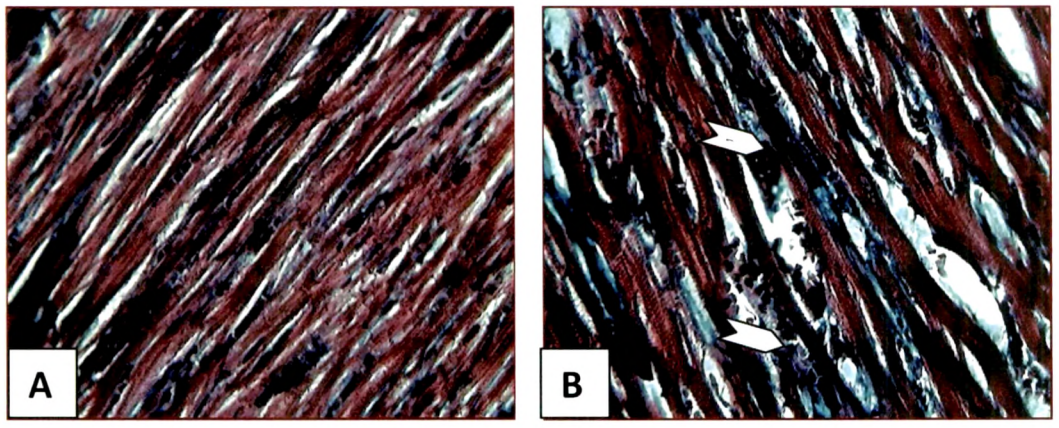
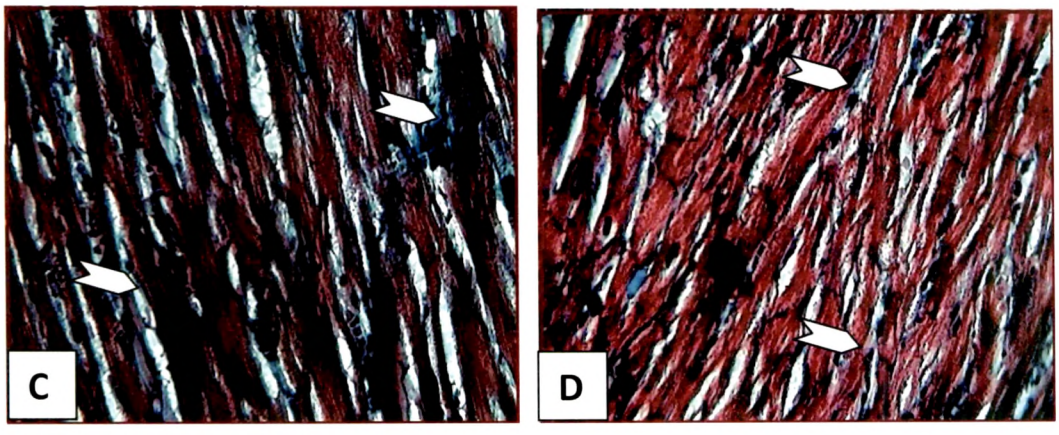


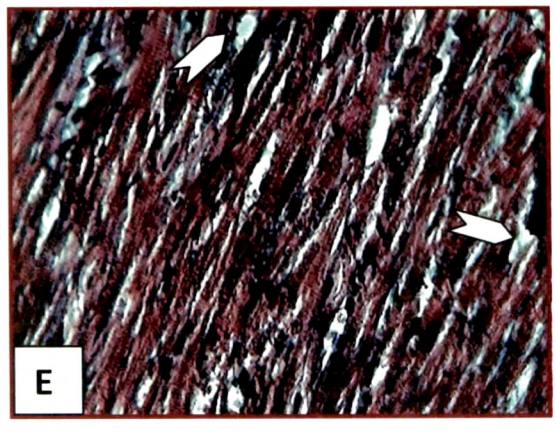
Fig. 5. 36 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day, p.o) for 30 days on Periodic acid Schiff's staining in normal and ISO (200mg/kg, s.c) injected rats (10X)



Control ISO

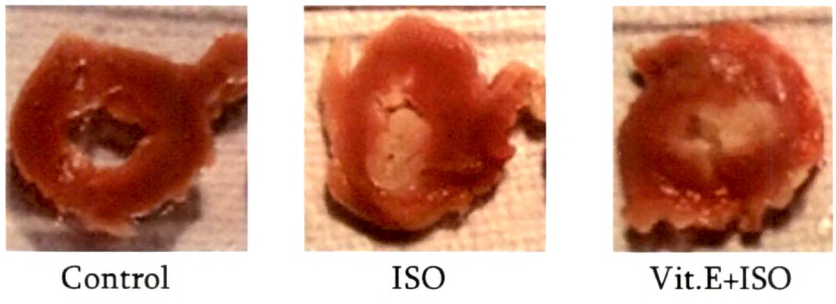


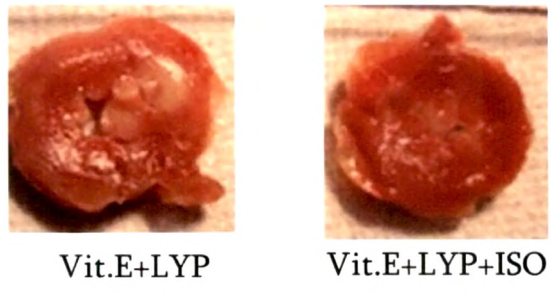
Vit.E+ISO LYP+ISO



Vit.E+LYP+ISO

Fig. 5.37 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on Masson's Trichrome staining of cardiac tissue in normal and ISO (200mg/kg, s.c) injected rats (40X)





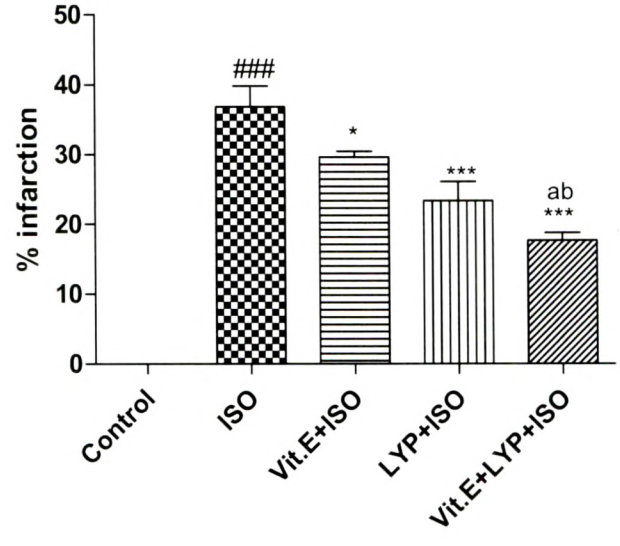
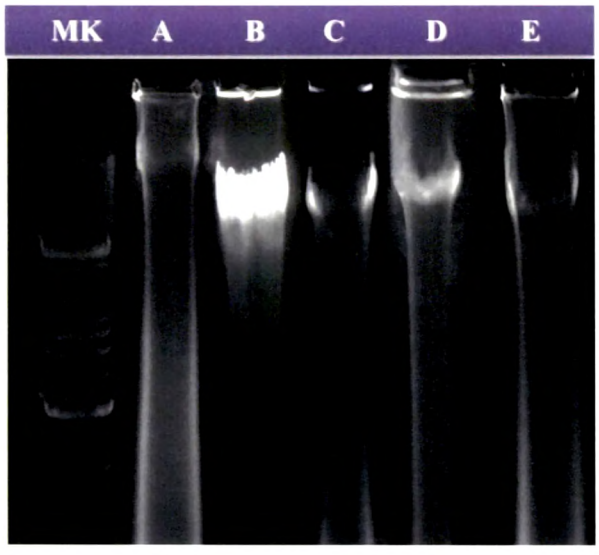


Fig. 5.38 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on macroscopic enzyme mapping assay (TTC) and area of infarction in normal and ISO (200mg/kg,s.c) injected rats

Values are expressed as Mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO



Lane A- Control

Lane B- ISO

Lane C- Vit. E +ISO

Lane D- LYP +ISO

Lane E- Vit. E +LYP+ISO

Fig. 5.39 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on DNA damage in normal and ISO (200mg/kg,s.c) injected rats

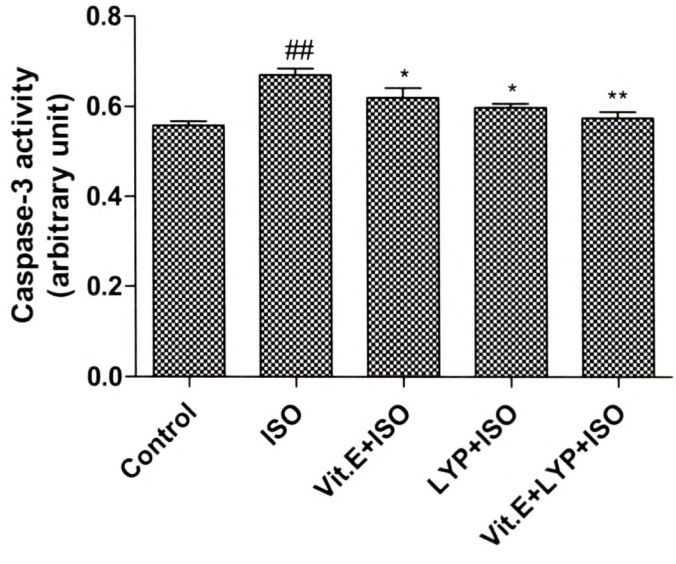


Fig. 5.40 Effect of Vit. E (100mg/kg/day, p.o) and LYP (10mg/kg/day,p.o) for 30 days on Caspase-3 activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as Mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LYP+ISO.

5.4 Effect of Vitamin E (100 mg/kg/day, p.o) alone and its combination with Pomegranate fruit extract (100 mg/kg/day, p.o) for 30 days in ISO (200 mg/kg, s.c) induced MI

5.4.1 Effect of Vit.E and PGFE on body weight, heart weight and heart/body weight ratio

ISO injected rats showed a significant ($P<0.01$) decrease in body weight and a significant ($P<0.01$, $P<0.001$) increase in heart weight and heart to body weight ratio as compared to control rats. Treatment of PGFE in ISO injected rats (PGFE+ISO) showed a significant ($P<0.05$) increase in body weight. However, Heart weight and heart to body weight ratio were reduced but was found to be non-significant. Co-administration of Vit. E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed significant ($P<0.05$) increase in body weight and significant ($P<0.05$, $P<0.05$) decrease in heart weight and heart to body weight ratio as compared to ISO injected rats (Table 5.13).

5.4.2 Effect of Vit.E and PGFE on ECG changes and heart rate

Effects of Vit.E and PGFE on ECG changes in normal and ISO injected rats were recorded (Fig. 5.41, Table 5.14). ISO injected rats showed a significant ($P<0.01$, $P<0.001$) elevation of ST-interval, QT interval along with significant decrease in P wave, QRS complex and RR interval as compared to control group. Treatment with PGFE in ISO injected rats (PGFE+ISO) showed significant ($P<0.01$) effects on ST interval, QRS complex and RR interval compared to ISO injected rats. However, co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed a significant ($P<0.001$, $P<0.01$, $P<0.05$) decrease in ST interval, QT interval and a significant increase in P wave, QRS complex and RR interval compared to ISO, Vit.E+ISO or PGFE+ISO treated group.

5.4.3 Effect of Vit.E and PGFE on systolic, diastolic and mean blood Pressure

ISO injected rats showed a significant (P<0.01, P<0.001) decrease in diastolic and mean blood pressure as compared to control group. Treatments with Vit.E alone and its combination with PGFE in ISO injected rats (Vit.E+ISO, Vit.E+PGFE+ISO) showed slight improvement in blood pressure, which was found to be non-significant as compared to ISO injected rats (Fig. 5.42).

5.4.4 Effect of Vit.E and PGFE on serum cardiac marker enzymes

The activities of cardiac merker enzymes such as AST, ALT, ALP, LDH and CK-MB were found to be significantly (P<0.001) increase in ISO injected rats compared to control rats. Treatment of PGFE alone and in combination with Vit.E in ISO injected rats (PGFE+ISO, Vit.E+PGFE+ISO) showed a significant (P<0.001, P<0.05) decrease in the activities of AST, ALT, ALP, LDH and CK-MB as compared to ISO injected rats (Fig. 5.43 and 5.44a).

The level of serum uric acid was significantly (P < 0.001) increased and total protein was significantly (P < 0.001) decreased in ISO injected rats as compared to control rats. Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed significant (P < 0.01) decrease in the level of serum uric acid and significant (P< 0.001, P< 0.05) increase in the level of total protein as compared to ISO injected rats (Fig.5.46b).

5.4.5 Effect of Vit.E and PGFE on lipid peroxidation, markers of oxidative stress and vitamin E level

The effects of Vit.E and PGFE on LPO and markers of oxidative stress in the heart of normal and ISO injected rats are shown in Fig. 5.45 and Table 5.15. ISO injected rats showed a significant (P<0.001) increase in heart LPO level along with a significant (P<0.001) decrease in GSH, GPx, GST, SOD, CAT activities and Vitamin E level as compared to control group. Treatment of Vit.E or PGFE in ISO

injected rats (Vit.E+ISO or PGFE+ISO) showed a significant (P<0.001) decrease in LPO level and significant (P<0.001, P<0.01) increase in GSH, GPx, GST, SOD and Vitamin E level as compared to ISO injected rats. Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) significantly (P<0.001, P<0.05) decrease LPO level and significantly (P<0.001, P<0.05) increased the activities of GSH, GPx, GST, SOD , CAT and Vitamin E level compared to ISO or Vit.E+ISO treated groups.

5.4.6 Effect of Vit.E and PGFE on membrane bound ATPase and electrolytes levels

ISO injected rats showed a significant (P < 0.001) decrease in Na^{+}/K^{+}-ATPase, Mg^{+2}-ATPase activities and significantly (P<0.001) increase in Ca^{+2}-ATPase activity. Co-administration of Vit. E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed significant (P < 0.001) increase in the activities of Na^{+}/K^{+} and Mg^{+2} ATPase with a significant (P < 0.001) decrease in Ca^{+2} ATPase compared to ISO injected rats (Fig. 5.46).

The electrolyte levels in control and ISO injected rats are shown in Table 5.16. ISO injected rats showed a significant (P<0.001) increase in sodium, calcium levels and significant (P<0.001) decrease in potassium level. Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) significantly (P<0.001) decreased the levels of sodium, calcium and significantly (P<0.001, P<0.05) increased the level of potassium as compared to ISO injected rats (Table 16). This combination did not produce further improvement in ATPase activities and electrolyte levels as compared to Vit.E+ISO and PGFE+ISO treated groups.

5.4.7 Effect of Vit.E and PGFE on LDH isoenzyme pattern

The pattern of LDH isoenzymes in serum of normal and ISO injected groups (as separated by agarose gel electrophoresis) are shown in Fig. 5.47. ISO injected rats showed an increase intensity of LDH-1 and LDH-2 isoenzyme bands. PGFE +ISO

treatment decreased the intensity of LDH1 and LDH2 isoenzyme compared to ISO injected rats. Co-administration of Vit. E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed further reduction in the intensity of LDH-1 and LDH-2 isoenzyme bands towards normal compared ISO injected rats.

5.4.8 Effect of Vit. E and PGFE on serum and tissue lipid profile

Rats injected with ISO showed a significant ($P<0.001$, $P<0.01$) increase in the levels of serum TC, TG, LDL, VLDL, FFA and PL with a significant ($P<0.01$) decrease in HDL level. Treatment with PGFE in ISO injected rats (PGFE+ISO) significantly ($P<0.001$, $P<0.01$) decreased the elevated levels of TC, TG, LDL, VLDL, FFAs, PLs and significantly ($P<0.01$) increased the level of HDL as compared to ISO injected rats.

ISO injected rats showed a significant increase ($P < 0.01$, $P < 0.001$) in the levels of tissue TC, TG, FFA with a significant decrease ($P < 0.01$) in the PL level as compared to control groups. Rats treated with PGFE+ISO showed a significant ($P<0.01$, $P<0.05$) decrease in TC, TG, FFA levels and a significant ($P<0.05$) increase in PL level as compared to ISO injected rats (Table 5.17). Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) did not show statistically significant effect in maintaining serum and tissue lipid profile towards normal compared to Vit.E+ISO and PGFE+ISO treated groups (Fig.5.48 and 5.49).

5.4.9 Effect of Vit. E and PGFE on myocardial Lipid metabolizing enzymes

The activities of myocardial LCAT, LPL and CES in control and ISO injected rats are shown in Fig. 5.50a & 5.50b. ISO injected rats showed a significant ($P<0.01$, $P<0.001$) decrease in the activities of LCAT, LPL and a significant ($P<0.001$) increase in the activity of CES as compared to control group. Treatment with PGFE in ISO injected rats (PGFE+ISO) significantly ($P<0.001$) increased LCAT, LPL activities and significantly ($P<0.001$) decreased in CES activity as compared to ISO injected rats. Howerver co-administration of PGFE with Vit.E in ISO injected

rats (Vit.E+PGFE+ISO) did not show further improvement in LCAT, LPL and CES activities as compared to Vit.E + ISO and PGFE+ISO treated groups.

5.4.10. Effect of Vit. E and PGFE on Serum CRP level and tissue MPO activity

Rats injected with ISO showed a significant ($P<0.001$) increase in serum CRP level and tissue MPO activity compared to control group. Treatment of PGFE in ISO injected rats (PGFE+ISO) significantly ($P<0.001$) reduced CRP level and MPO activity compared to ISO injected rats. However, co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed better improvement in maintaining the levels of CRP and MPO compared to Vit.E+ISO or PGFE+ISO treated groups (Fig. 5.51 & 5. 52).

5.4.11 Effect of Vit. E and PGFE on tissue nitrite levels

Rats injected with ISO showed a significant increase ($P < 0.001$) in tissue nitrite level as compared to control rats. Treatment with Vit.E or PGFE in ISO injected rats (Vit.E+ISO and PGFE+ISO) decreased the elevated tissue nitrite level as compared to ISO injected rats, it was found to be non-significant compared to ISO injected rats. Co-administration of Vit.E and PGFE in ISO treated rats (Vit.E+PGFE+ISO) further reduced the elevated nitrite level. However it was found to be statistically non significant compared to ISO injected rats (Fig. 5.53).

5.4.12 Effect of Vit. E and PGFE on Histopathological (H&E staining) changes

Histopathological sections of ISO injected rats showed necrosis of muscle fibers, inflammatory cell infiltration and edema with fragmentation of muscle fibers (Fig. 5.54B, Table 5.18) as compared to control group (Fig. 5.54A,Table 5.18). Treatment with PGFE in ISO injected rat (PGFE+ISO), showed absence of inflammatory cells with mild edema, necrosis and fragmentation of muscle fibers (Fig. 5.54D, Table 5.18). Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO)

showed near normal architecture of heart tissue with absence of edema and inflammatory cells with mild necrosis (Fig. 5.54E, Table 5.18).

5.4.13. Effect of Vit. E and PGFE on Periodic acid Schiff's staining

Periodic acid Schiff's staining of control and experimental groups of rats are shown in Fig. 5.55. There was an arbitrary increase in the amount of glycoproteins or glycoconjugates in ISO injected rats (Fig. 5.55B) as compared to control rats (Fig. 5.55A). Co-administration of Vit. E and PGFE in ISO injected rats (Vit.E+ PGFE+ISO) resulted in normal architecture of membrane with maintenance of membrane bound glycoconjugates (Fig. 5.55E).

5.4.14 Effect of Vit.E and PGFE on Masson's trichrome staining

Normal architecture of muscle fibres primarily confined to the intramuscular fasciculi was observed in cardiac tissue of normal rats (Fig. 5.56A). ISO injected rats showed muscle cell necrosis with disruption in arrangement of collagen fibers (Fig. 5.56B). Cardiac tissue sections of rats treated with the combination of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) showed minimally damaged collagen fibers exhibiting near normal structure of heart tissue (Fig. 5.56E).

5.4.15 Effect of Vit. E and PGFE on macroscopic enzyme assay (TTC test) and area of infarction

ISO injected rats showed a high percentage of infarct size with increase staining of cardiac tissue as compared to control group. Vit.E+ISO treated rats showed a significant ($P<0.05$) decrease in infarct size and staining as compared to ISO injected rats. Treatment with PGFE in ISO injected rats (PGFE+ISO) showed significant ($P<0.01$) reduction in infarction size compared to ISO injected rats. The co-administration of Vit.E and PGFE further reduced the infarction size compared to individual drug treatment groups (Fig.5. 57).

5.4.16 Effect of Vit.E and PGFE on DNA damage by gel electrophoresis

Rats injected with ISO (Lane B) showed severity in DNA damage as compared to control animals (Lane A). Treatment with PGFE+ISO showed active prevention in the severity of DNA damage (Lane D) compared to ISO injected rats. Co-administration of Vit.E and PGFE in ISO injected rats (Vit.E+PGFE+ISO) did not produce further prevention in DNA damage compared to Vit.E+ISO and PGFE+ISO treated group (Lane E) (Fig. 5.58).

5.4.17 Effect of Vit.E and PGFE on the activity of Caspase-3 activity

ISO injected rats showed a significant ($P<0.01$) increase in caspase-3 activity as compared to control group. Treatment with PGFE alone and in combination with Vit.E in ISO injected rats (PGFE+ISO, Vit.E+PGFE+ISO) did not produce significant effects on caspase-3 activity as compared to ISO, Vit.E+ISO or PGFE+ISO treated groups (Fig. 5.59).

Table 5.13. Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on body weight, heart weight and heart to body weight ratio in normal and ISO (200mg/kg, s.c) injected rats

Groups	Body weight (g)	Heart weight (g)	HW/BW
Control	232.5 ± 3.39	0.667 ± 0.031	0.00286 ± 0.0091
ISO	214.2 ± 3.48$^{\#\#}$	0.920 ± 0.054$^{\#\#}$	0.00429 ± 0.0155$^{\#\#\#}$
Vit.E+PGFE	240.3 ± 6.32	0.671 ± 0.043	0.00279 ± 0.0680
Vit.E+ISO	227.7 ± 3.29*	0.705 ± 0.030*	0.00309 ± 0.0091**
PGFE+ISO	225.7 ± 6.55*	0.843 ± 0.032^{ns}	0.00374 ± 0.0488^{ns}
Vit.E+PGFE+ISO	224.2 ± 5.35*	0.702 ± 0.076*	0.00324 ± 0.0110*

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

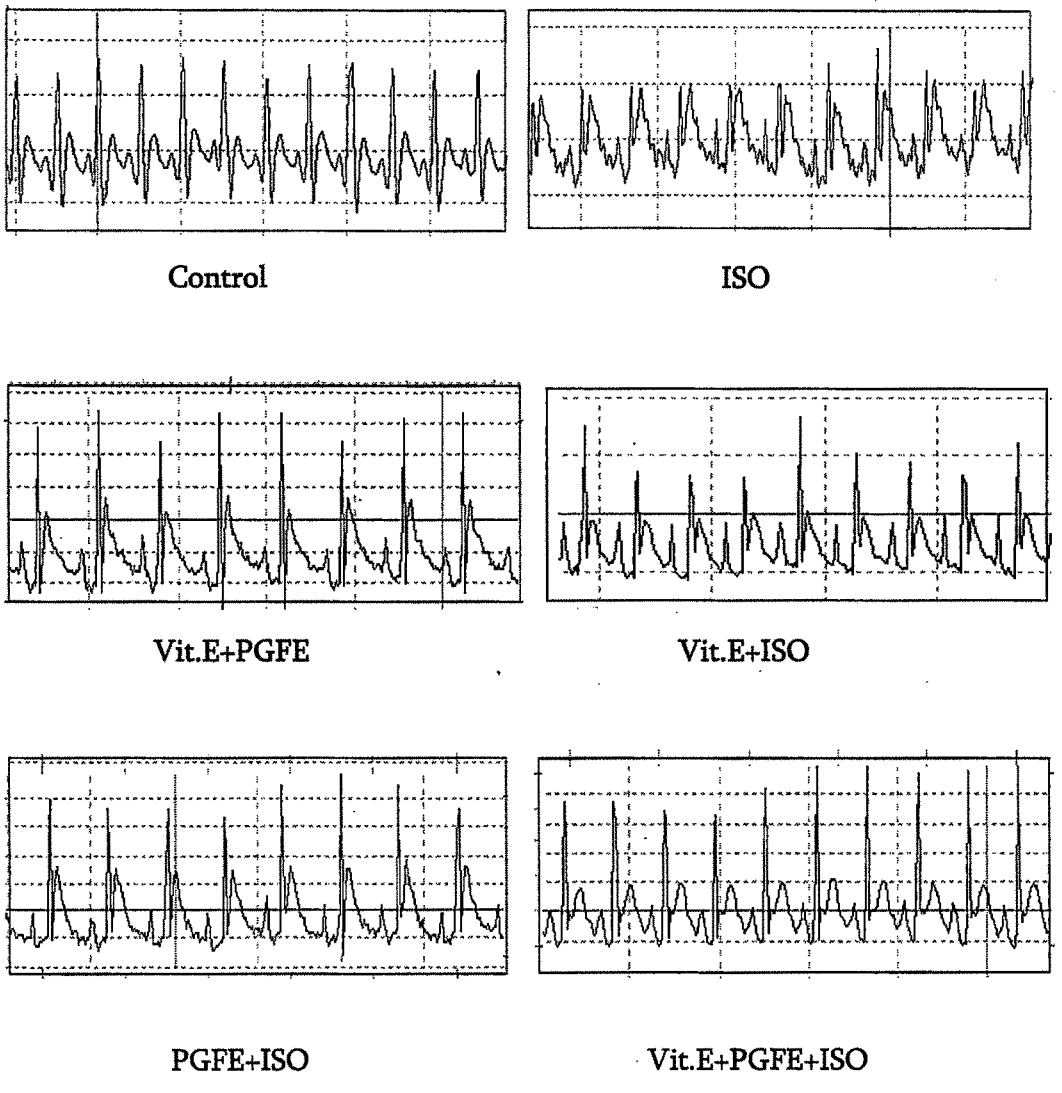


Fig. 5.41 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on ECG changes in normal and ISO (200mg/kg,s.c) injected rats

Table 5.14: Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on ECG changes and heart rats in normal and ISO (200mg/kg, s.c) injected rats

Groups	ST elevation	P wave	QRS complex	QT interval	R-R interval	Heart rate
Control	0.184 ± 0.0022	0.028 ± 0.00037	0.0416 ± 0.005	0.071 ± 0.0006	0.170 ± 0.0007	338.30 ± 12.88
ISO	0.302 ± 0.0040$^{\#\#\#}$	0.024 ± 0.00030$^{\#\#}$	0.0285 ± 0.007$^{\#\#\#}$	0.081 ± 0.0012$^{\#\#}$	0.158 ± 0.0017$^{\#\#}$	400.09 ± 14.32^{ns}
Vit.E+PGFE	0.182 ± 0.0032	0.030 ± 0.00044	0.0410 ± 0.004	0.070 ± 0.0021	0.170 ± 0.0005	336.88 ± 10.54
Vit.E+ISO	0.251 ± 0.0023***	0.025 ± 0.00049^{ns}	0.0326 ± 0.001*	0.074 ± 0.0076*	0.164 ± 0.0008*	362.80 ± 16.52
PGFE+ISO	0.280 ± 0.0044**	0.025 ± 0.00067^{ns}	0.0358 ± 0.009**	0.079 ± 0.0038^{ns}	0.158 ± 0.0090**	370.00 ± 9.97
Vit.E+PGFE+ISO	0.222 ± 0.0056^{***b}	0.026 ± 0.00032^{*b}	0.0390 ± 0.006^{***a}	0.074 ± 0.0017^{ab}	0.166 ± 0.0011^{**a}	355.30 ± 10.12

Values are expressed as mean±SEM for 6 animals in each group. The ECG parameters are expressed in seconds (sec), heart rate as Beats per minutes (BPM), ST elevation in milivolt (mv). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO group.

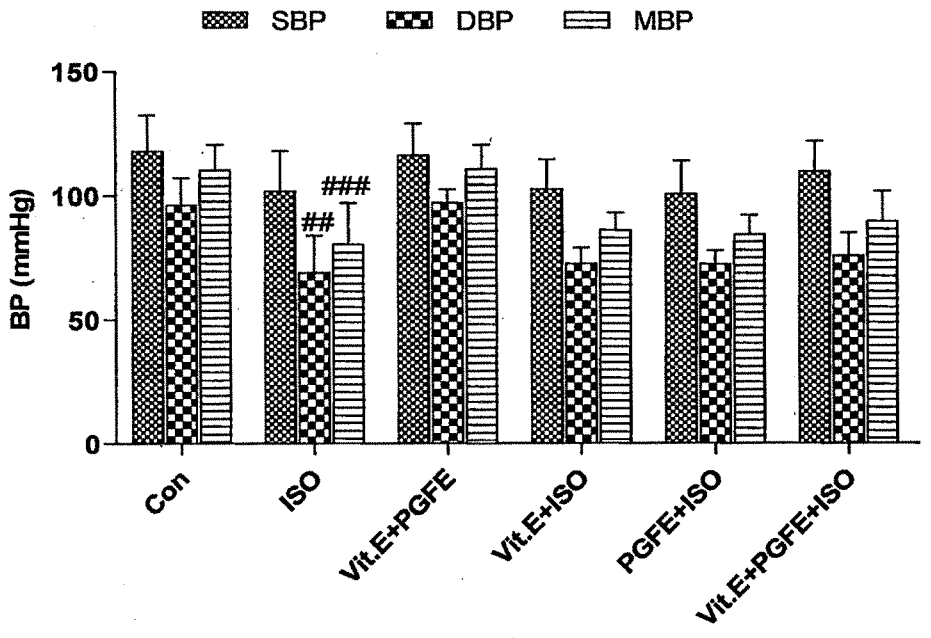


Fig. 5.42 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on systolic, diastolic and mean blood pressure in normal and ISO (200mg/kg,s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group.

Fig. 5.43 (a)

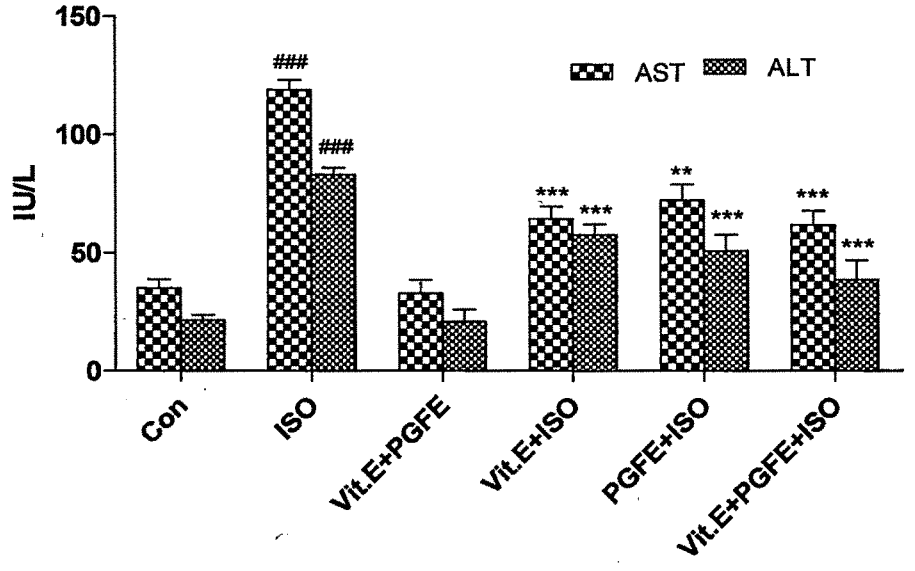


Fig. 5.43 (b)

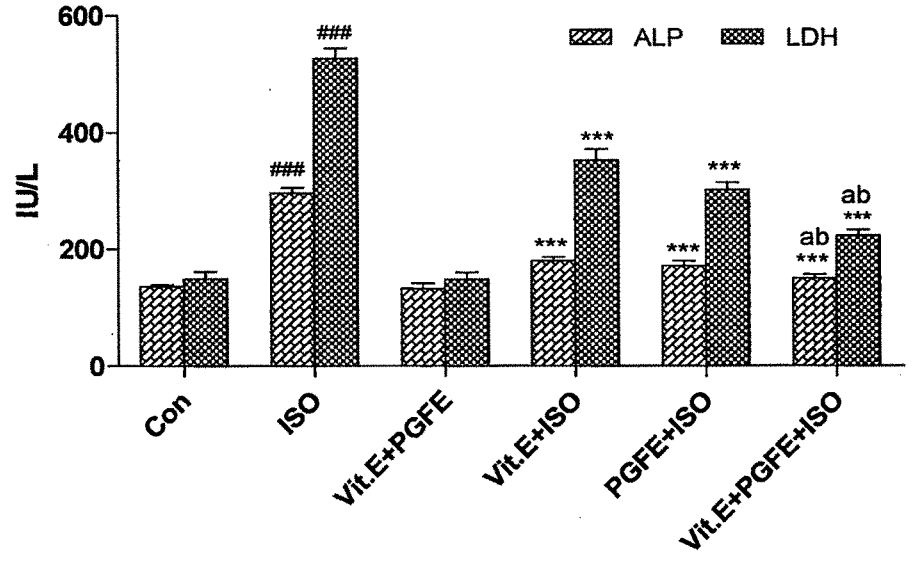


Fig. 5.43 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on serum (a) AST and ALT (b) ALP and LDH levels in normal and ISO (200mg/kg,s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

Fig. 5.44(a)

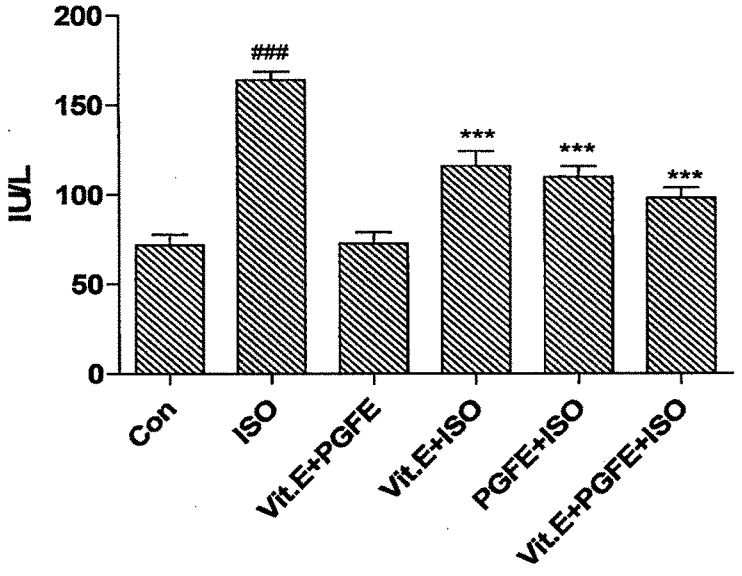


Fig. 5.44(b)

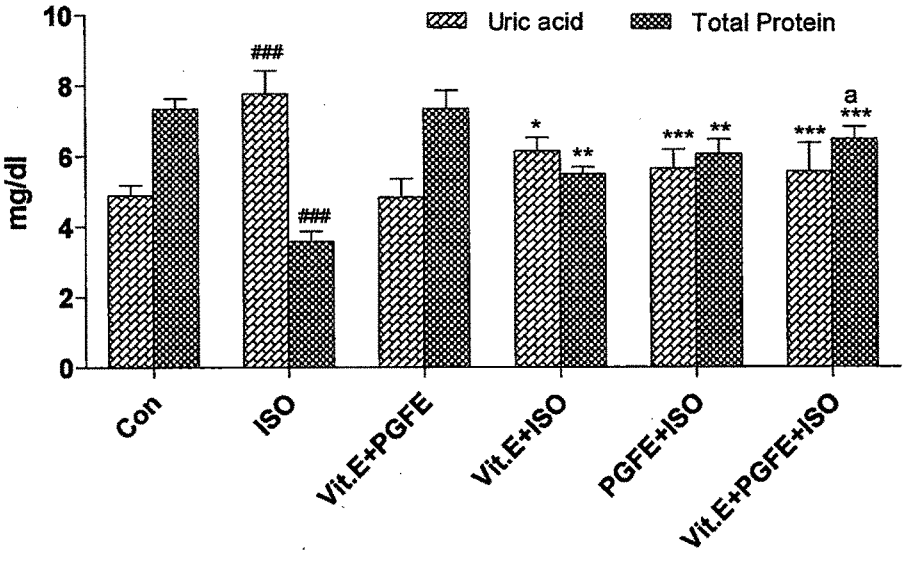


Fig. 5.44 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on serum (a) Ck-MB (b) Uric acid and Total protein levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

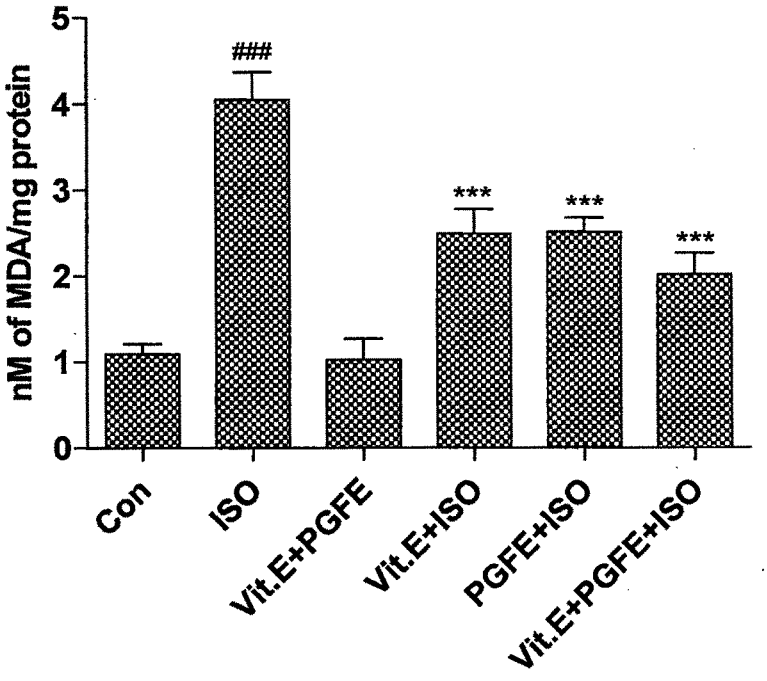


Fig. 5.45 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on myocardial Lipid peroxidation in normal and ISO (200mg/kg,s.c) injected rats

Values are expressed as Mean ± SEM (n=6).

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group,

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group,

$^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

Table 5.15: Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on endogenous antioxidants activities and vitamin E level in normal and ISO (200mg/kg, s.c) injected rats

Groups	GSH	GPx	GST	SOD	CAT	Vitamin E
Con.	7.401 ± 0.480	6.102 ± 0.206	110.40 ± 5.712	4.496 ± 0.203	6.290 ± 0.331	2.02 ± 0.047
ISO	4.338 ± 0.260$^{\#\#\#}$	4.032 ± 0.231$^{\#\#\#}$	68.91 ± 4.829$^{\#\#\#}$	2.272 ± 0.166$^{\#\#\#}$	3.687 ± 0.219$^{\#\#\#}$	1.09 ± 0.043$^{\#\#\#}$
Vit.E+PGFE	7.211 ± 0.633	6.444 ± 0.512	114.21 ± 5.121	4.408 ± 0.333	6.117 ± 0.486	2.11 ± 0.062
Vit.E+ISO	5.785 ± 0.288**	5.635 ± 0.336**	95.55 ± 4.089***	3.566 ± 0.390*	5.188 ± 0.167**	1.84 ± 0.035***
PGFE+ISO	6.332 ± 0.409***	5.774 ± 0.511***	82.43 ± 1.763**	3.652 ± 0.541**	4.883 ± 0.763^{ns}	1.64 ± 0.066**
Vit.E+PGFE+ISO	6.884 ± 0.333^{***a}	5.886 ± 0.601^{***a}	102.55 ± 3.708***	4.022 ± 0.321***	5.905 ± 0.294***	1.92 ± 0.032^{***b}

Values are expressed as mean±SEM, (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO group.

GSH: (µg of GSH /mg protein), GPx: (µmoles of glutathione oxidized/min/mg protein), GST: (µmoles of CDNB conjugated/min/mg protein), SOD: (units/mg protein), CAT: (µmoles of H_2O_2 consumed/min/mg protein), Vitamin E: (mmole/mg protein).

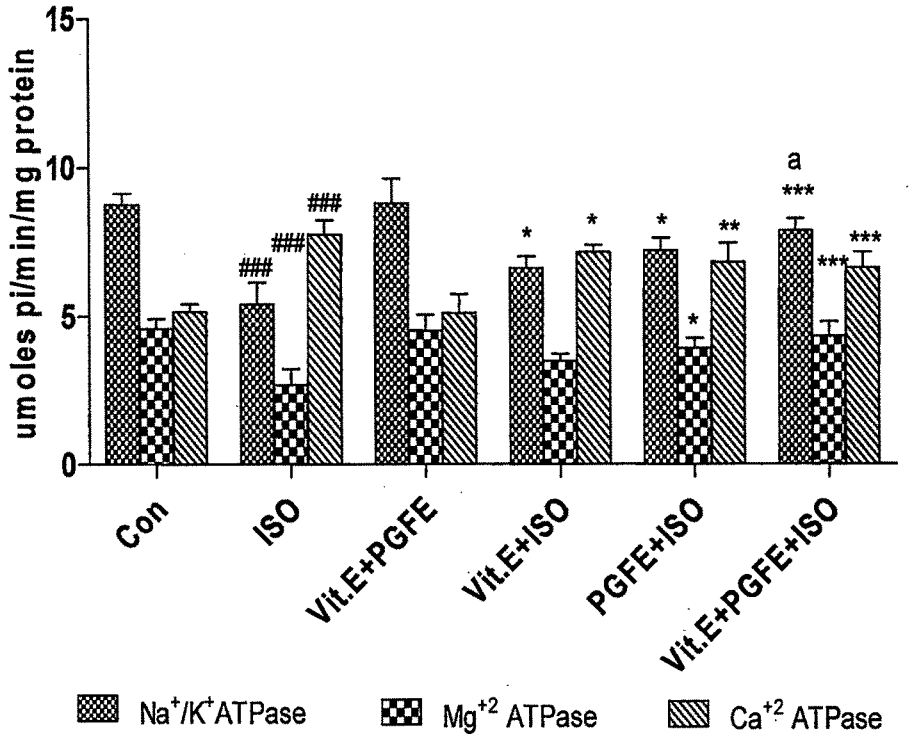


Fig. 5.46 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on the activity of Na^{+}/K^{+} ATPase, Mg^{+2} ATPase and Ca^{+2} ATPase in normal and ISO (200mg,kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

Table 5.16 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on the levels of sodium, potassium and calcium in the heart of normal and ISO (200mg/kg, s.c) injected rats

Groups	Sodium (Na^+)	Potassium (K^+)	Calcium (Ca^{++})
Control	6.088 ± 0.125	8.978 ± 0.164	10.81 ± 0.107
ISO	$7.728 \pm 0.312^{\#\#\#}$	$6.162 \pm 0.216^{\#\#\#}$	$13.83 \pm 0.344^{\#\#\#}$
Vit.E+PGFE	6.111 ± 0.103	8.832 ± 0.108	10.93 ± 0.132
Vit.E+ISO	$7.198 \pm 0.156^{*}$	$7.462 \pm 0.189^{***}$	$12.63 \pm 0.291^{**}$
PGFE+ISO	$6.832 \pm 0.211^{**}$	$7.232 \pm 0.223^{**}$	$12.56 \pm 0.303^{**}$
Vit.E+PGFE+ISO	$6.599 \pm 0.203^{***}$	$8.126 \pm 0.108^{****}$	$11.84 \pm 0.210^{***}$

All the values are expressed as nmol/mg protein

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO .

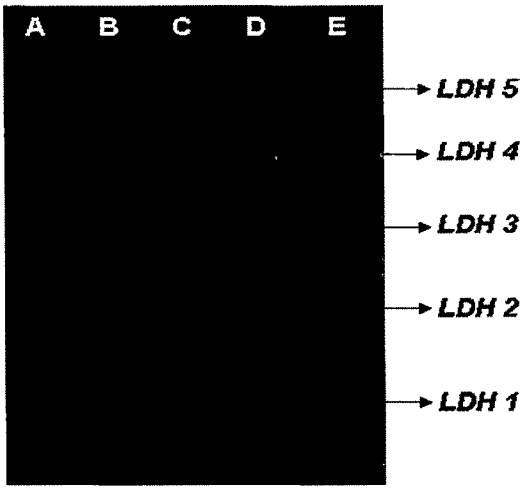


Fig. 5.47 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on LDH isoenzyme pattern in normal and ISO (200mg/kg, s.c) injected rats

A: Control, B: ISO, C: Vit.E + ISO, D: PGFE + ISO, E: Vit.E + PGFE + ISO

Fig. 5.48 (a)

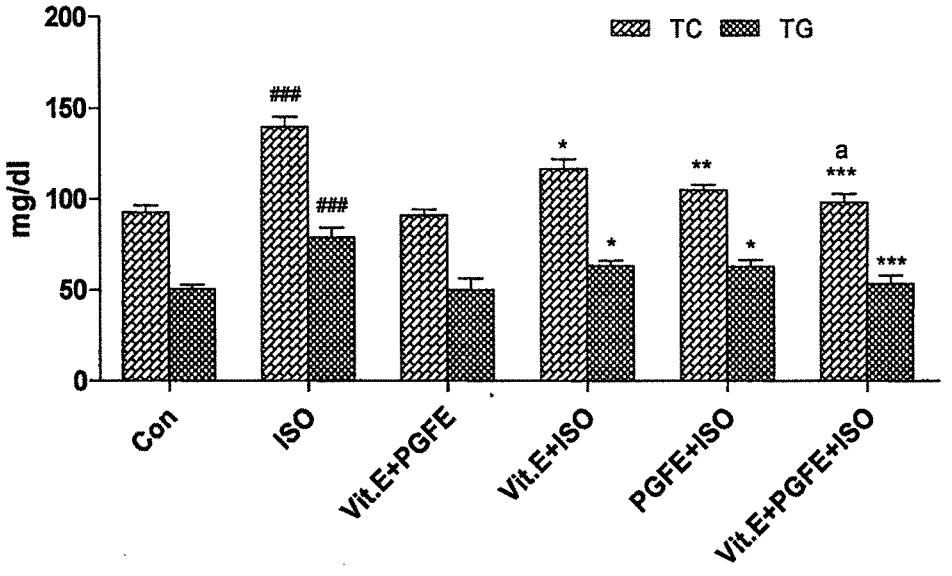


Fig. 5.48 (b)

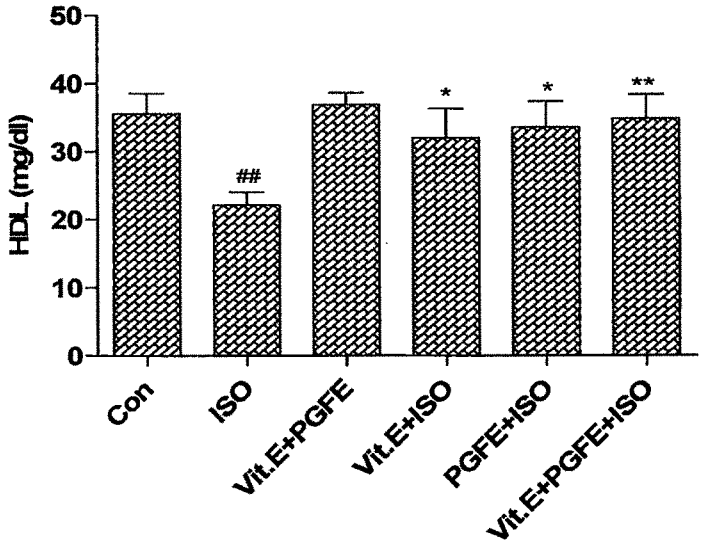


Fig. 5.48 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on serum a) TC and TG, b) HDL-c levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

Fig. 5.49 (a)

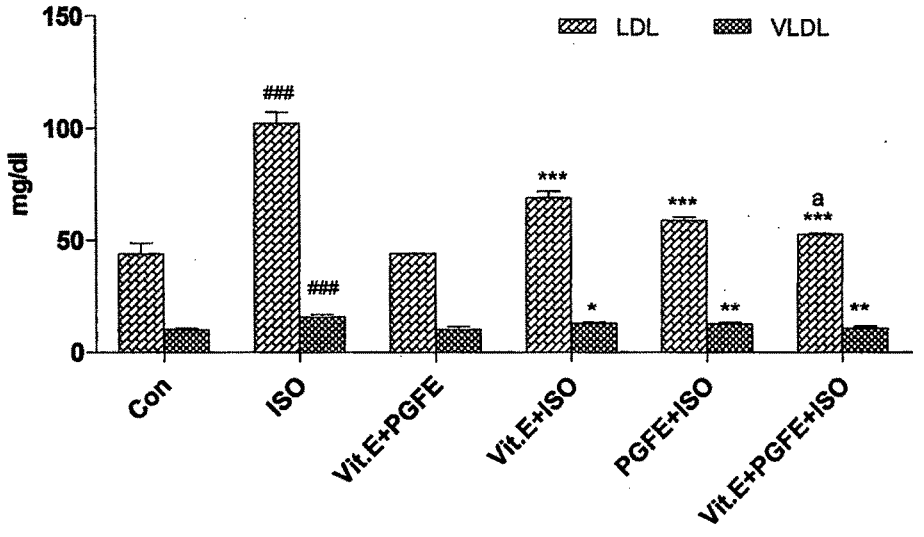


Fig. 5.49 (b)

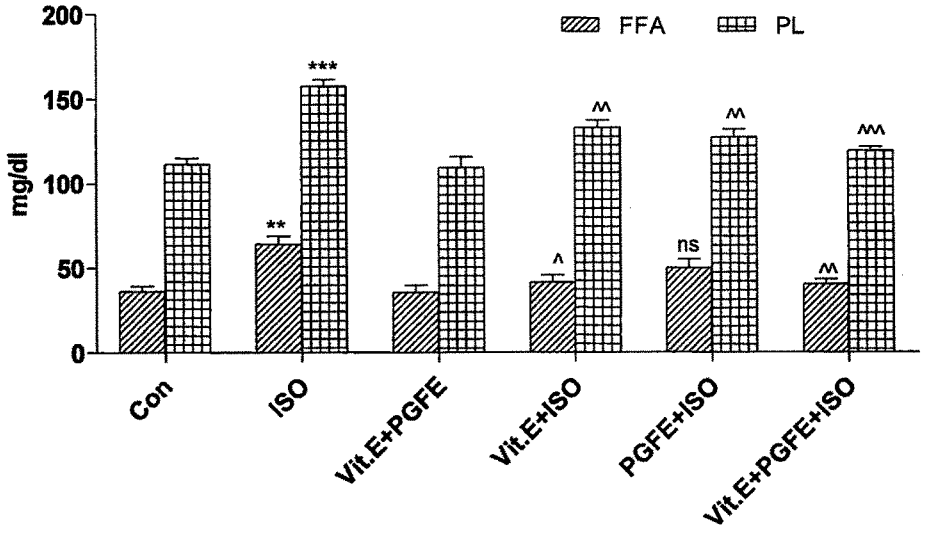


Fig. 5.49 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on a) LDL and VLDL b) FFA and PL levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

Table 5.17. Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on tissue lipid profile in normal and ISO (200mg/kg, s.c) injected rats

Groups	TC	TG	FFA	PL
Con.	8.230 ± 0.637	6.147 ± 0.637	0.871 ± 0.077	25.620 ± 1.988
ISO	13.690 ± 1.340$^{\#\#}$	11.260 ± 0.840$^{\#\#\#}$	1.450 ± 0.117$^{\#\#}$	16.620 ± 1.902$^{\#\#}$
Vit.E+PGFE	8.144 ± 1.523	6.108 ± 0.854	0.863 ± 0.121	25.055 ± 1.223
Vit.E+ISO	10.080 ± 0.483*	7.337 ± 0.823**	1.102 ± 0.077^{ns}	23.640 ± 1.982*
PGFE+ISO	9.675 ± 0.883*	7.423 ±1.553**	1.001 ± 0.321*	22.003 ± 1.564*
Vit.E+PGFE +ISO	8.902 ± 1.043**	7.029 ± 0.887**	0.901 ± 0.332*	24.97 ± 1.406*

Values are expressed as (mg/g wt tissue) for TC, TG, FFA and PL

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group.

Fig. 5.50 (a)

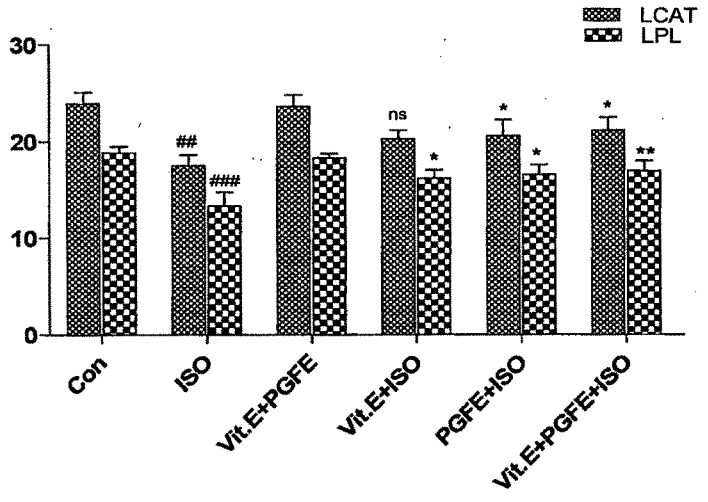


Fig. 5.50 (b)

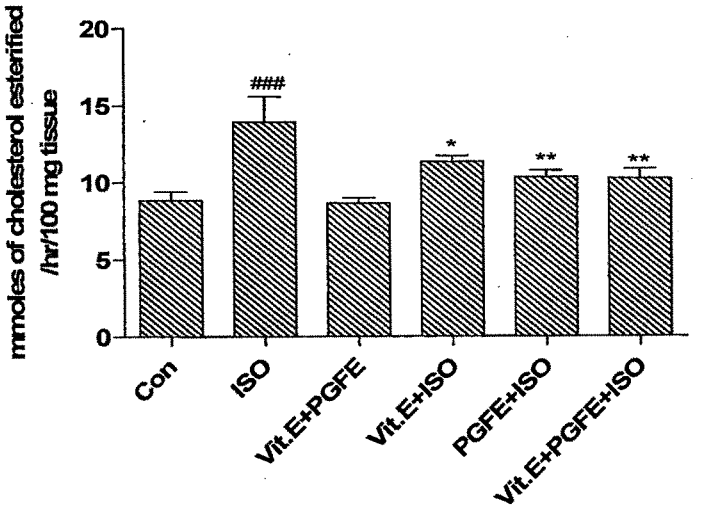


Fig. 5.50 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on (a) LCAT and LPL (b) CES levels in normal and ISO (200mg/kg, s.c) injected rats

Units: LCAT (μmoles of cholesterol esterified/hr/100mg tissue). LPL (μmoles of fee fatty acids liberated/100mg tissue)

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group.

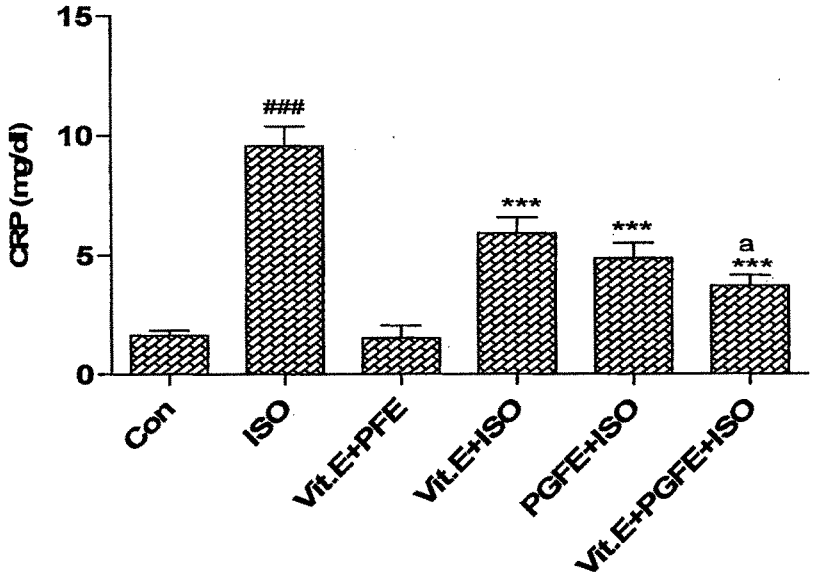


Fig. 5.51 Effect of Vit. E (100mg/kg, p.o) and PGFE (100mg/kg, p.o) for 30 days on serum CRP levels in normal and ISO (200mg/kg, s.c) injected rats

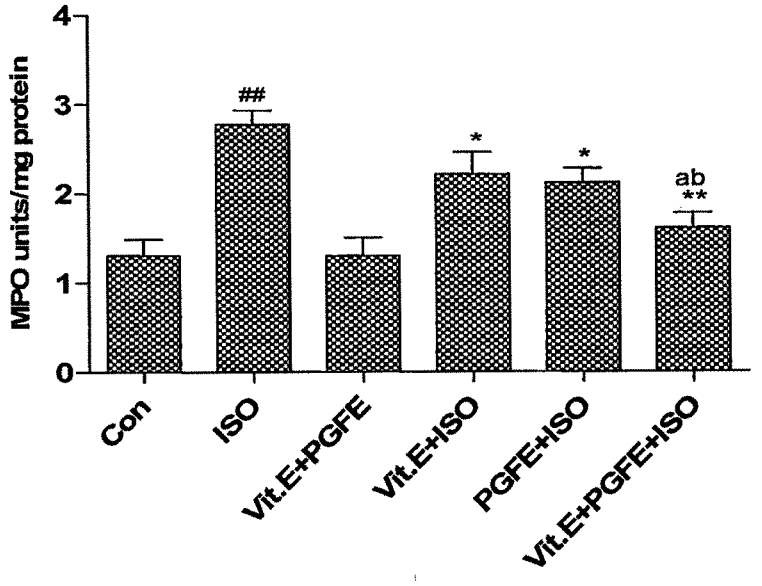


Fig. 5.52 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on tissue MPO activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

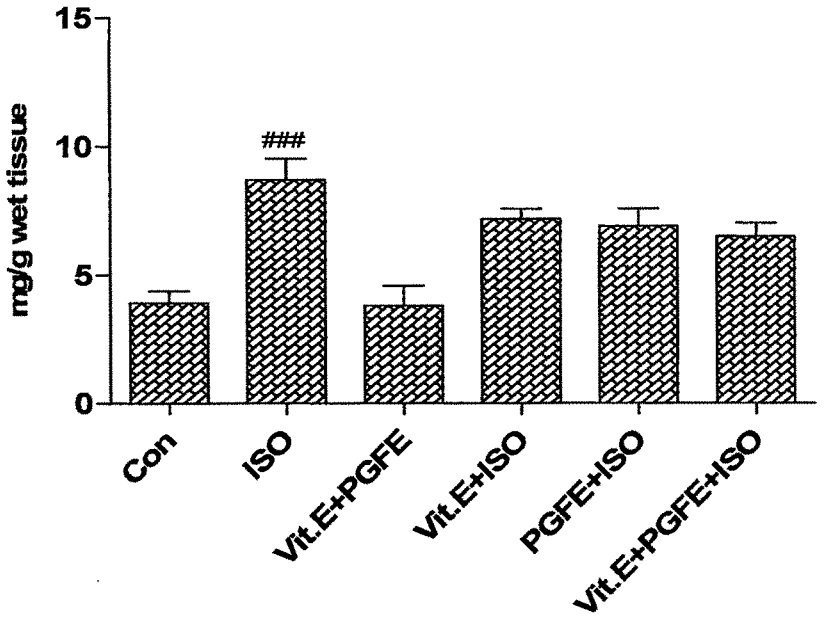


Fig. 5. 53 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on tissue nitrite levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

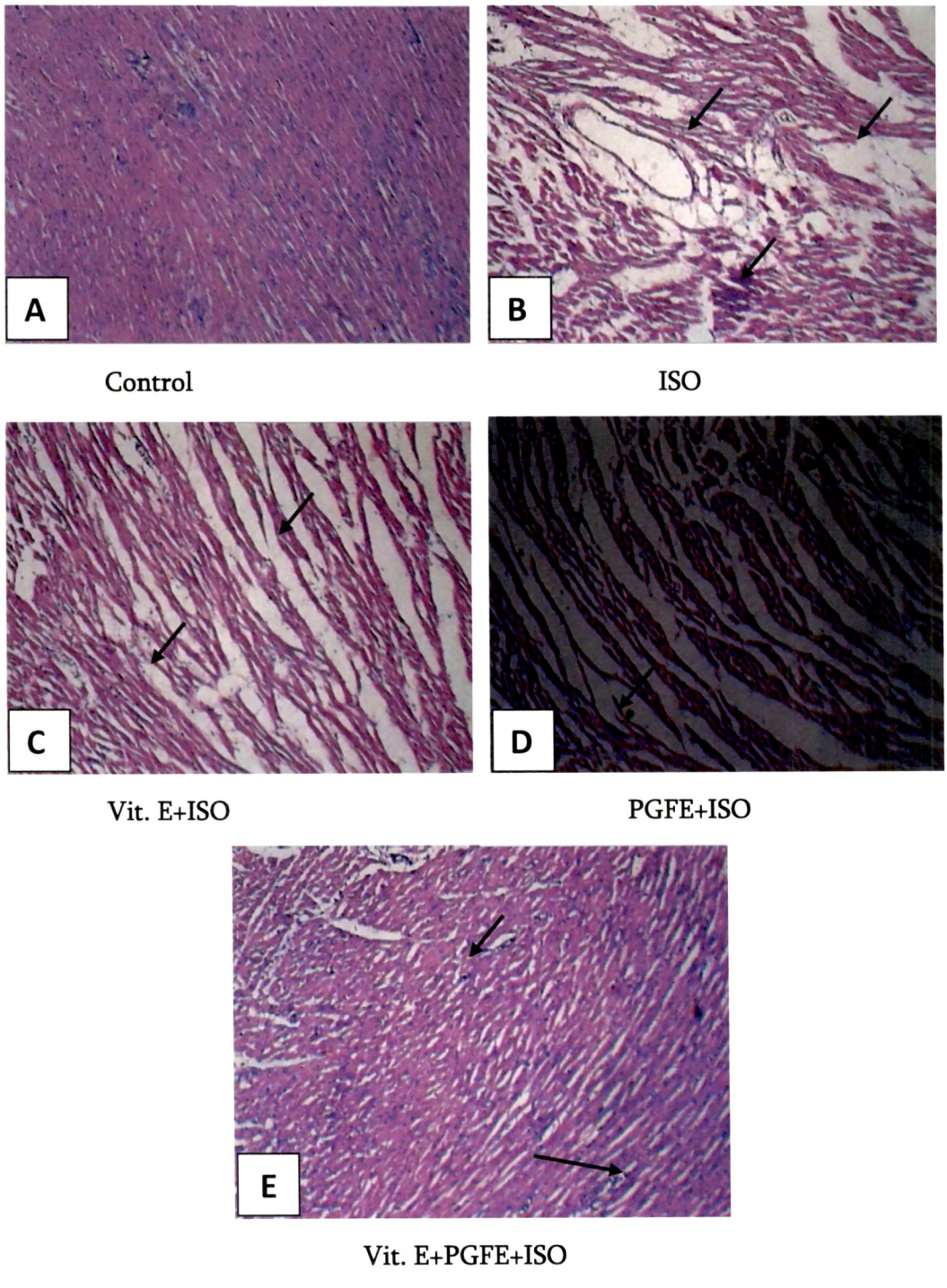


Fig. 5.54 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on Histopathological alteration in normal and ISO (200mg/kg, s.c) injected rats

Table 5.18 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on the degree of histological changes in myocardial tissues in normal and ISO (200mg/kg, s.c) injected rats

Groups	Necrosis	Oedema	Inflammatory cells
Control	A	A	A
ISO	+++	+++	++
Vit.E+ISO	++	++	+
PGFE+ISO	++	++	A
Vit.E+PGFE+ISO	+	A	A

Photomicrographs were used to evaluate the damage in the heart tissues: (A) no change, (+++) marked changes, (++) moderate changes, (+) mild changes

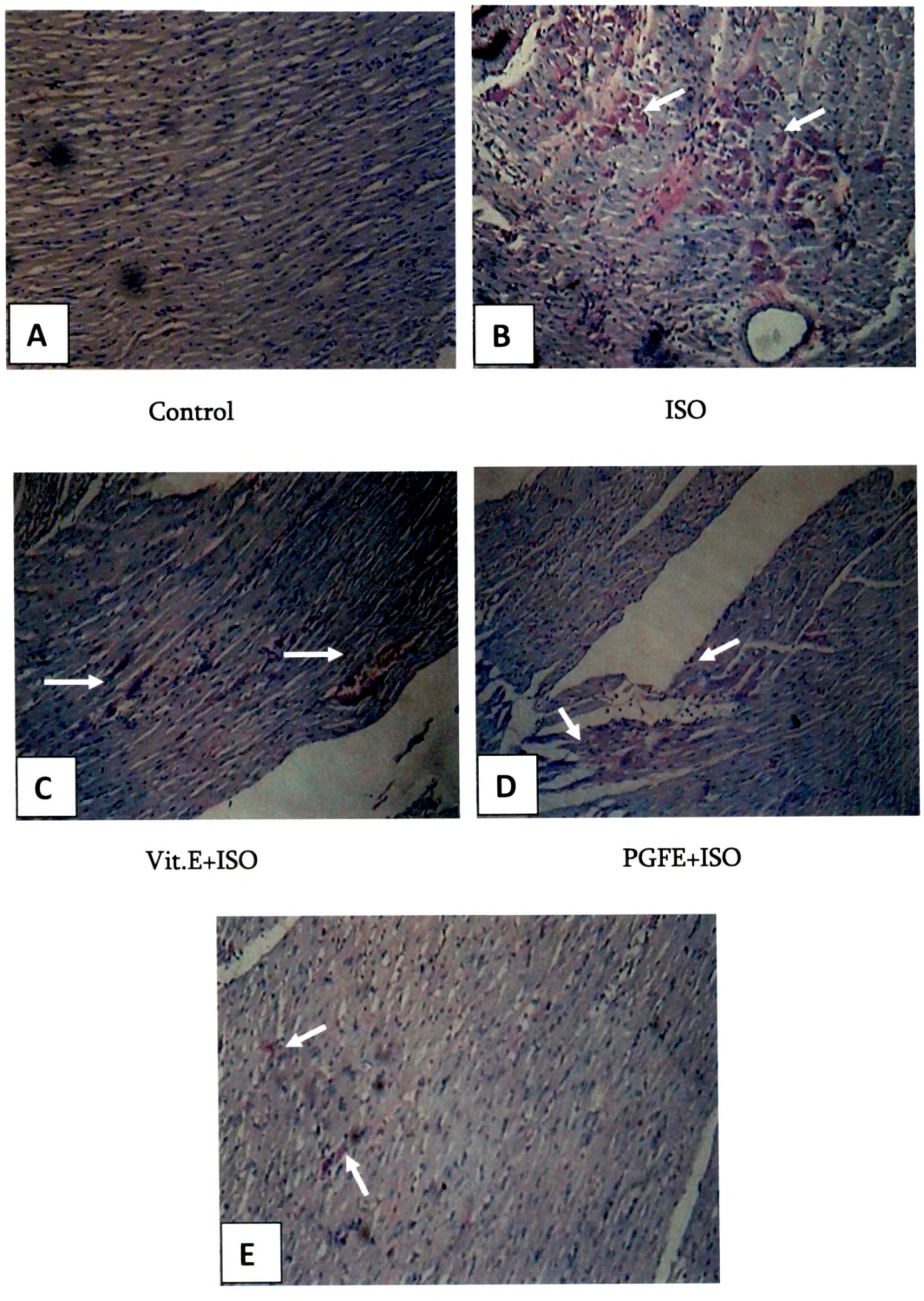


Fig. 5.55 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on Periodic acid Schiff's staining in normal and ISO (200mg/kg, s.c) injected rats (10X)

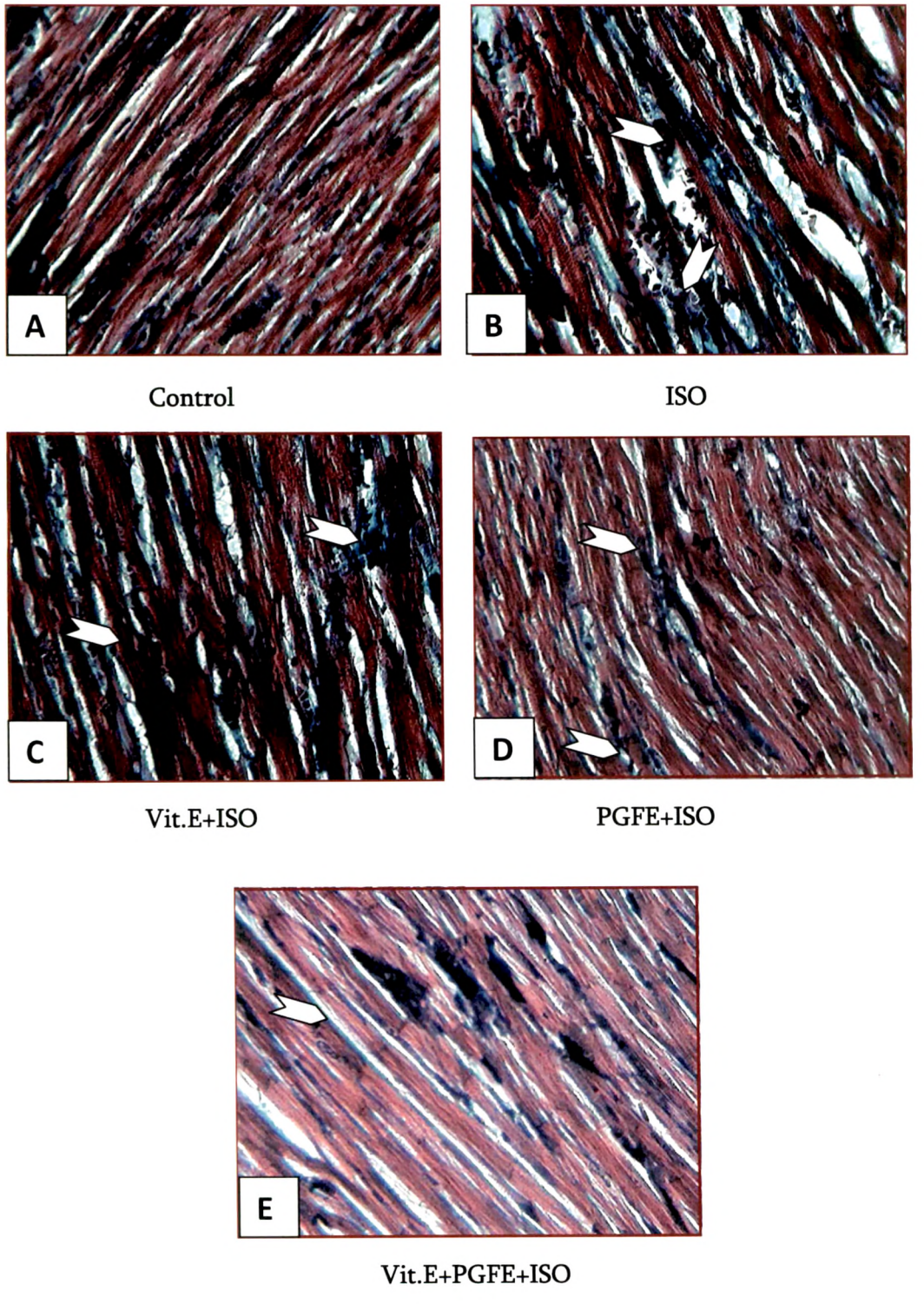


Fig. 5.56 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on Masson's trichrome staining of cardiac tissue in normal and ISO (200mg/kg, s.c) injected rats (40X)

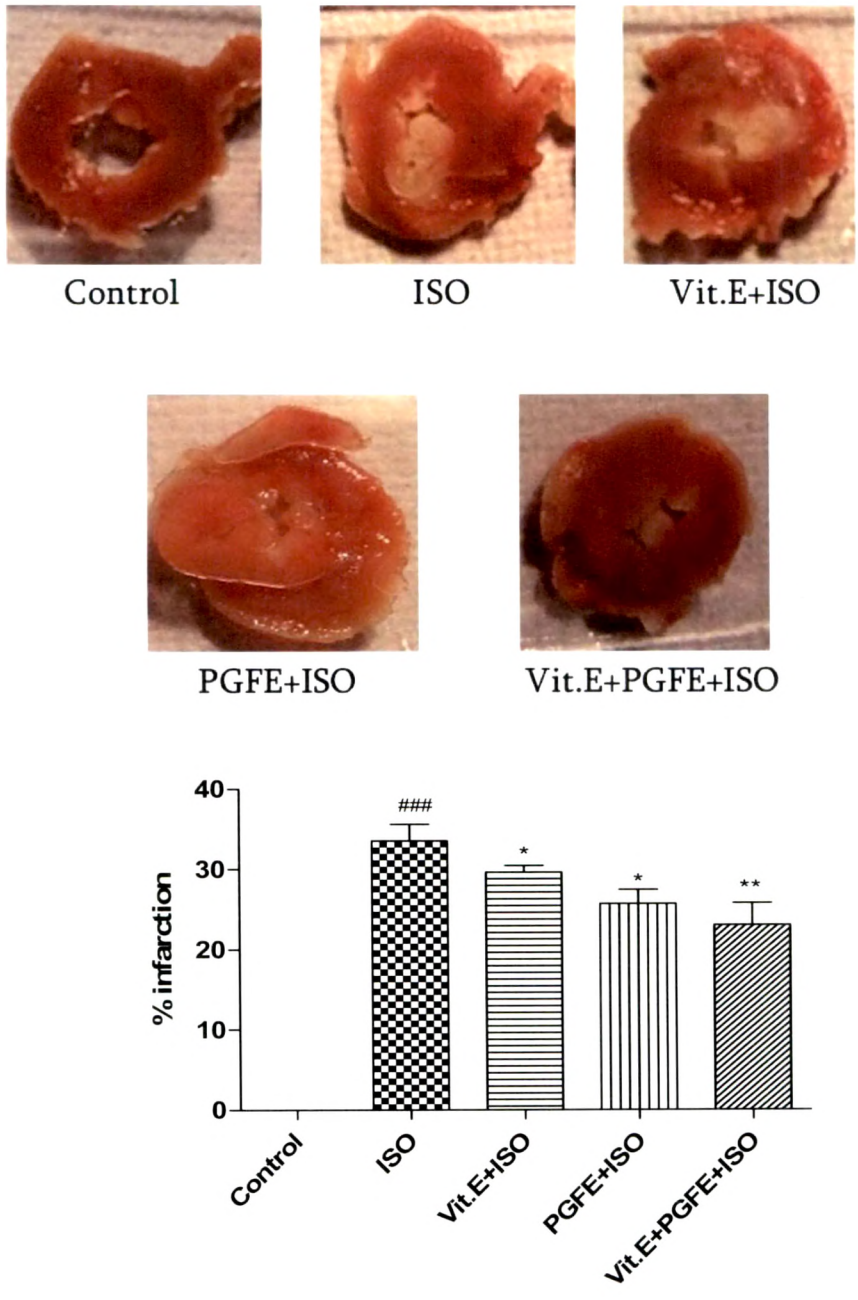
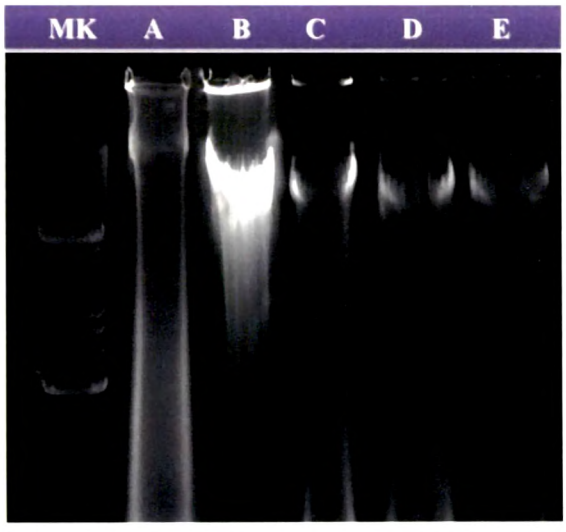


Fig. 5.57 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on macroscopic enzyme assay and area of infarction in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.



Lane A- Control

Lane B- ISO

Lane C- Vit. E +ISO

Lane D- PGFE +ISO

Lane E- Vit. E +PGFE+ISO

Fig. 5.58 Effect of Vit. E (100mg/kg/day, p.o) and PGFE (100mg/kg/day, p.o) for 30 days on DNA damage (gel electrophoresis) in normal and ISO (200mg/kg,s.c) injected rats

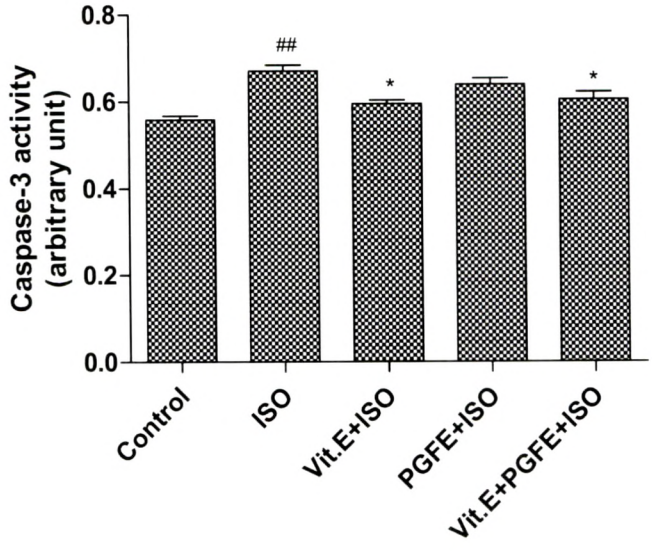


Fig. 5.59 Effect of Vit. E (100mg/kg, p.o) and PGFE (100mg/kg, p.o) for 30 days on Caspase-3 protease activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group, $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to PGFE+ISO.

5.5. Effect of Vitamin E (100 mg/kg/day, p.o) alone and its combination with LSFJ (400 mg/kg/day, p.o) for 30 days in ISO (200 mg/kg, s.c) induced MI

5.5.1. Effect of Vit.E and LSFJ on body weight, heart weight and heart/body weight ratio

Rats injected with ISO for two consecutive days showed a significant ($P<0.01$) decrease in body weight ($P<0.01$) and a significant ($P<0.01$, $P<0.001$) increase in heart weight and heart to body weight ratio as compared to control rats. Treatment with LSFJ in ISO injected rats (LSFJ+ISO) did not show significant improvement in body weight, heart weight and heart to body weight ratio as compared to ISO injected rats. Co-administration of Vit. E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) showed significant increase ($P<0.05$) in body weight. However heart weight and heart to body weight ratio was found to be non significant compared to Vit.E alone treated rats (Table 5.19).

5.5.2. Effect of Vit.E and LSFJ on ECG changes

The effect of Vit.E and LSFJ on ECG changes in normal and ISO injected rats is shown in Fig. 5.60. ISO injected rats showed a significant ($P<0.01$) increase in ST-interval, QT interval and a significant ($P<0.01$, $P<0.001$) decrease in P wave, QRS complex and RR interval as compared to control group. Treatment with LSFJ in ISO injected rats (LSFJ+ISO) showed a significant ($P<0.01$) decrease in ST-elevation compared to control rats. Further it was found that LSFJ in combination with Vit.E in ISO injected rats (Vit.E+LSFJ+ISO) significantly ($P<0.001$, $P<0.05$) reduced ST interval compared to Vit.E alone treated rats (Table 5.20).

5.5.3. Effect of Vit.E and LSFJ on systolic, diastolic and mean blood pressure

ISO injected rats showed a significantly ($P<0.001$) decrease in diastolic and mean blood pressure. Treatment of LSFJ alone and in combination with Vit.E in ISO

injected rats (LSFJ+ISO, Vit.E+LSFJ+ISO) did not show significant improvement in blood pressure as compared to ISO or Vit.E+ISO treated groups (Fig. 5.61).

5.5.4. Effect of Vit.E and LSFJ on serum cardiac marker enzymes

A significant ($P<0.001$) increased in the activities of serum AST, ALT, ALP, LDH and CK-MB were observed in ISO injected rats. Treatment with LSFJ in ISO injected rats (LSFJ+ISO) showed significant ($P<0.01$) decrease in the activities of these markers enzymes compared to ISO injected rats. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show any further improvement in the activities of AST, ALT, ALP, LDH and CK-MB compared to Vit.E+ISO treatment groups (Fig. 5.62 and 5.63a). Co-administration of LSFJ and Vit.E in ISO injected rats (Vit.E+LSFJ+ISO) significantly increased the level of total protein ($P<0.001$) and significantly decreased the level of uric acid ($P<0.001$) as compared to ISO injected rats (Fig. 5.63b).

5.5.5. Effect of Vit.E and LSFJ on lipid peroxidation, markers of oxidative stress and Vitamin E level

ISO injected rats showed a significant ($P<0.001$) increase in LPO level and significant decrease in GSH, GPx, GST, SOD, CAT activities and Vitamin E level as compared to control group. Treatment with LSFJ in ISO treated rats (LSFJ+ISO) showed a significant ($P<0.05$) decrease in LPO level and significant ($P<0.05$) increase in GSH, GPx activities and Vitamin E level as compared to ISO injected rats. LSFJ in combination with Vit.E in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant improvement in LPO and markers of oxidative stress as compared to Vit.E+ISO treated group (Fig. 5.64, Table 5.21).

5.5.6. Effect of Vit.E and LSFJ on membrane bound ATPases and electrolytes level

The effect of Vit.E and LSFJ on membrane bound ATPase and electrolyte levels in normal and ISO injected rats are shown in Fig.5.65 and Table 5.22. Rats injected

with ISO showed a significant ($P<0.001$) decrease in Na^{+}/K^{+}-ATPase, Mg^{+2}-ATPase and a significant ($P<0.001$) increase in Ca^{+2}-ATPase activity as compared to control rats. Treatment with LSFJ in ISO injected rats (LSFJ+ISO) showed a significant ($P<0.05$) decrease in Ca^{+2} ATPase as compared to ISO injected group. Co-administration of Vit.E and LSFJ in ISO treated rats (Vit.E+LSFJ+ISO) significantly ($P<0.01$) reduced Ca^{+2}-ATPase level and significantly ($P<0.05$) increased Mg^{+2}-ATPase level as compared to ISO injected rats. Further it was found that this combination did not produce significant effects on membrane bound ATPase and electrolyte levels as compared to Vit.E alone treated groups (Table 5.22).

5.5.7. Effect of Vit.E and LSFJ on LDH isoenzyme pattern

Agarose gel electrophoretic separation of serum LDH-isoenzyme patterns of normal and ISO injected rats are shown in Fig. 5.66. Rats injected with ISO showed an increase in the intensity of LDH-1 and LDH-2 isoenzyme bands compared to control rats. Treatment of LSFJ in ISO injected rats (LSFJ+ISO) showed decrease in the intensity of LDH1 isoenzymes. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show further reduction in the intensity of LDH1 isoenzyme compared to Vit.E+ISO and LSFJ+ISO treated groups (Fig. 5.66).

5.5.8. Effect of Vit. E and LSFJ on serum and tissue lipid profile

Levels of various lipids in serum of control and experimental animals were recorded (Fig. 5.67 and 5.68). Treatment with LSFJ in ISO injected rats (LSFJ+ISO) significantly ($P<0.01$, $P<0.05$) decreased the elevated levels of TC, TG, LDL, VLDL, FFA, PL and significantly ($P<0.05$) increased the level of HDL as compared to ISO injected rats. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) showed further decrease in the levels of TC, TG, LDL, VLDL, FFAs, PL and increase the level of HDL as compared to ISO injected rats. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not

produce significant effect on serum and heart lipid profile as compared to Vit.E alone treated group (Vit.E+ISO) (Table 5.23).

5.5.9. Effect of Vit. E and LSFJ on myocardial Lipid metabolizing enzymes

A significant ($P<0.01$, $P<0.001$) decreased in the activities of LCAT, LPL and a significant ($P<0.001$) increased in the activity of CES were observed in ISO injected rats as compared to control group. Treatment of LSFJ in ISO treated rats (LSFJ+ISO) showed a significant ($P<0.05$) increase in LCAT and LPL activities compared to ISO injected rats. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show further improvement in the activities of lipid metabolizing enzymes compared to Vit.E alone treated group (Fig. 5.69).

5.5.10. Effect of Vit. E and LSFJ on Serum CRP level and tissue MPO activity

ISO injected rats showed a significant ($P<0.001$) rise in CRP level and tissue MPO activity as compared to control group. Treatment with LSFJ in ISO injected rats (LSFJ+ISO) significantly ($P<0.05$) decreased the level of CRP and the activity of MPO remain unchanged. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not produce significant protection against CRP and MPO activity as compared to Vit.E alone treated groups (Vit.E+ISO) (Fig. 5.70 and 5.71).

5.5.11. Effect of Vit. E and LSFJ on tissue nitrite levels

Rats injected with ISO showed a significant increase ($P < 0.001$) in tissue nitrite level as compared to control rats. Treatment with LSFJ alone and in combination with Vit.E in ISO injected rats (LSFJ+ISO, Vit.E+LSFJ+ISO) did not show significant effect on tissue nitrite level as compared to ISO, LSFJ+ISO or Vit.E+ISO treated groups (Fig.5.72).

5.5.12. Effect of Vit. E and LSFJ on Histopathological alteration

Light micrograph of ISO injected rats showed necrosis of muscle fibers, inflammatory cell infiltration and edema with fragmentation of muscle fibers (Fig. 5.73B, Table 5.24) as compared to control group (Fig. 5.73A, Table 5.24). Treatment with LSFJ in ISO treated rats (LSFJ+ISO) showed moderate degree of edema, necrosis and inflammatory cells (Fig. 5.73D, Table 5.24). Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) showed mild changes in necrosis, edema and inflammation (Fig. 5.73E, Table 5.24).

5.5.13. Effect of Vit. E and LSFJ on Periodic acid Schiff's staining

PAS staining of ISO injected rats showed an arbitrary increase in the amount of glycoproteins or glycoconjugates (Fig. 5.74B) as compared to normal control rats (Fig. 5.74A). Co-administration of Vit. E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) showed decrease in membrane bound glycoconjugates (Fig. 5.74E) as compared to Vit.E alone treated groups (Fig. 5.74C and 5.74D).

5.5.14. Effect of Vit. E and LSFJ on Masson's trichrome staining

Masson's trichrome staining of cardiac muscle of control and ISO injected groups are shown in Fig. 5.75. ISO injected rats showed muscle cell necrosis with disruption in arrangement of collagen fibers (Fig. 5.75B). Cardiac tissue sections of rats injected with Vit.E and LSFJ in combination (Vit.E+LSFJ+ISO) did not show any protective effect on collagen degradation (Fig. 5.75E) as compared to Vit.E alone treated group (Vit.E+ISO).

5.5.15. Effect of Vit. E and LSFJ on macroscopic enzyme assay (TTC test) and area of infarction

The histochemical approach to detect the myocardial changes in the heart of control and experimental group of rats through macroscopic enzyme mapping assay are shown in Fig. 5.76. A high percentage of mean infarct size with increased staining was observed in ISO injected rats as compared to control group. Co-

administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant improvement in infarct size compared to Vit.E alone treated group (Vit.E+ISO).

5.5.16. Effect of Vit.E and LSFJ on DNA damage

LSFJ alone and in combination with Vit.E in ISO injected rats (LSFJ+ISO and Vit.E+LSFJ+ISO) did not show significant effects on the severity of DNA damage as compared to ISO injected rats and Vit.E alone treated rats (Lane A-E) (Fig.5.77).

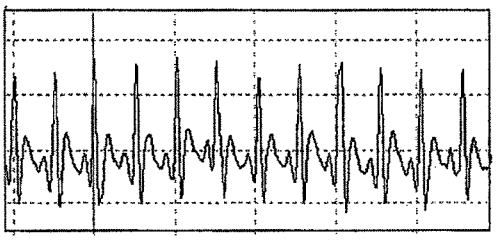
5.5.17. Effect of Vit.E and LSFJ on the activity of Caspase-3 activity

ISO injected rats showed a significant ($P<0.01$) increase in caspase-3 activity as compared to control rats. Treatment with LSFJ alone and in combination with Vit.E in ISO injected rats (LSFJ+ISO, Vit.E+LSFJ+ISO) did not produce significant effect on caspase-3 activity as compared to ISO injected rats and Vit.E alone treated rats (Vit.E+ISO)(Fig. 5.78).

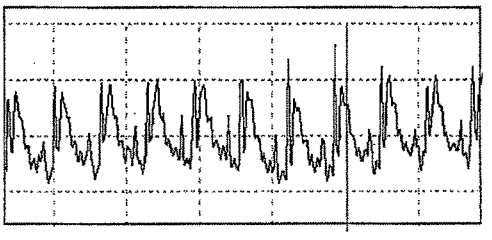
Table. 5.19. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on body weight, heart weight and heart to body weight ratio in normal and ISO (200mg/kg, s.c) injected rats

Groups	Body weight (g)	Heart weight (g)	HW/BW
Control	232.5 ± 3.39	0.667 ± 0.031	0.00286 ± 0.0091
ISO	214.2 ± 3.48$^{\#\#}$	0.920 ± 0.054$^{\#\#}$	0.00429± 0.0155$^{\#\#\#}$
LSFJ	229.3 ± 7.21	0.669 ± 0.072	0.00291 ± 0.0099
Vit.E+LSFJ	233.6 ± 5.21	0.663 ± 0.063	0.00283 ± 0.0120
Vit.E+ISO	227.7 ± 3.29*	0.705 ± 0.030*	0.00309 ± 0.0091**
LSFJ+ISO	221.3 ± 7.21^{ns}	0.806 ± 0.092^{ns}	0.00364 ± 0.0127^{ns}
Vit.E+LSFJ+ISO	225.8 ± 6.87*	0.732 ± 0.076^{ns}	0.00324 ± 0.0110^{ns}

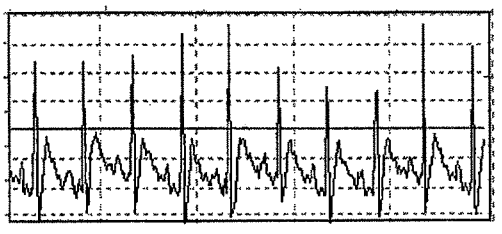
Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO groups. P>0.05 was considered as non-significance (ns).



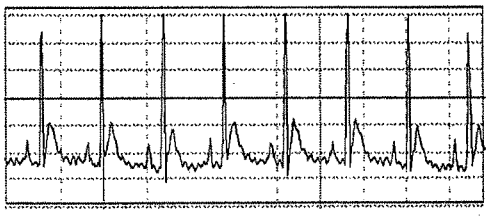
Control



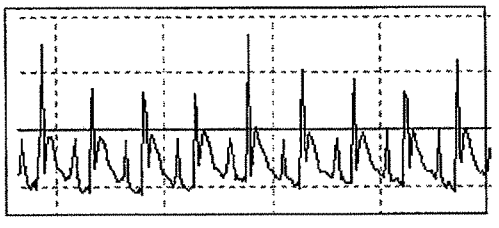
ISO



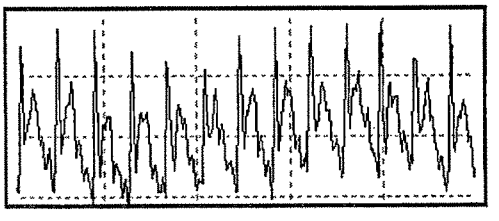
LSFJ



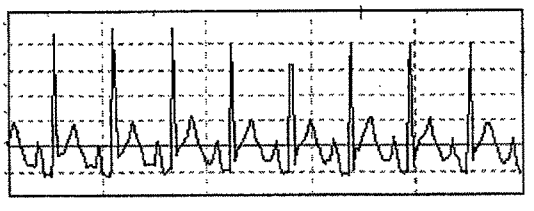
Vit.E+LSFJ



Vit.E+ISO



LSFJ+ISO



Vit.E+LSFJ+ISO

Fig. 5.60. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on ECG changes in normal and ISO (200mg/kg, s.c) injected rats

Table 5.20: Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on ECG changes and heart rats in normal and ISO (200mg/kg, s.c) injected rats

Groups	ST elevation	P wave	QRS complex	QT interval	R-R interval	Heart rate
Control	0.184 ± 0.0022	0.028 ± 0.00037	0.0416 ± 0.005	0.071 ± 0.0006	0.170 ± 0.0007	338.30 ± 12.88
ISO	$0.302 \pm 0.0040^{\#\#\#}$	$0.024 \pm 0.00030^{\#\#}$	$0.0285 \pm 0.007^{\#\#\#}$	$0.081 \pm 0.0012^{\#\#}$	$0.158 \pm 0.0017^{\#\#}$	400.09 ± 14.32^{ns}
LSFJ	0.181 ± 0.0012	0.028 ± 0.00039	0.0418 ± 0.009	0.070 ± 0.0023	0.170 ± 0.0008	335.10 ± 12.66
Vit.E+LSFJ	0.184 ± 0.0021	0.028 ± 0.00032	0.0419 ± 0.008	0.071 ± 0.0019	0.170 ± 0.0011	335.90 ± 12.23
Vit.E+ISO	$0.251 \pm 0.0023^{***}$	0.025 ± 0.00049^{ns}	$0.0326 \pm 0.001^{*}$	$0.074 \pm 0.0076^{*}$	$0.164 \pm 0.0008^{*}$	362.80 ± 16.52
LSFJ+ISO	$0.272 \pm 0.0081^{**}$	0.025 ± 0.00037^{ns}	0.0312 ± 0.010^{ns}	0.078 ± 0.0032^{ns}	0.160 ± 0.0009^{ns}	371.00 ± 14.08
Vit.E+LSFJ+ISO	$0.249 \pm 0.0022^{***b}$	0.026 ± 0.00066^{ns}	$0.0338 \pm 0.009^{*}$	$0.074 \pm 0.0011^{*}$	$0.166 \pm 0.0009^{*a}$	360.63 ± 12.65

Values are expressed as mea ±SEM for 6 animals in each group. The ECG parameters are expressed in seconds (sec), heart rate as Beats per minutes (BPM), ST elevation in milivolt (mv). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LSFJ+ISO group.

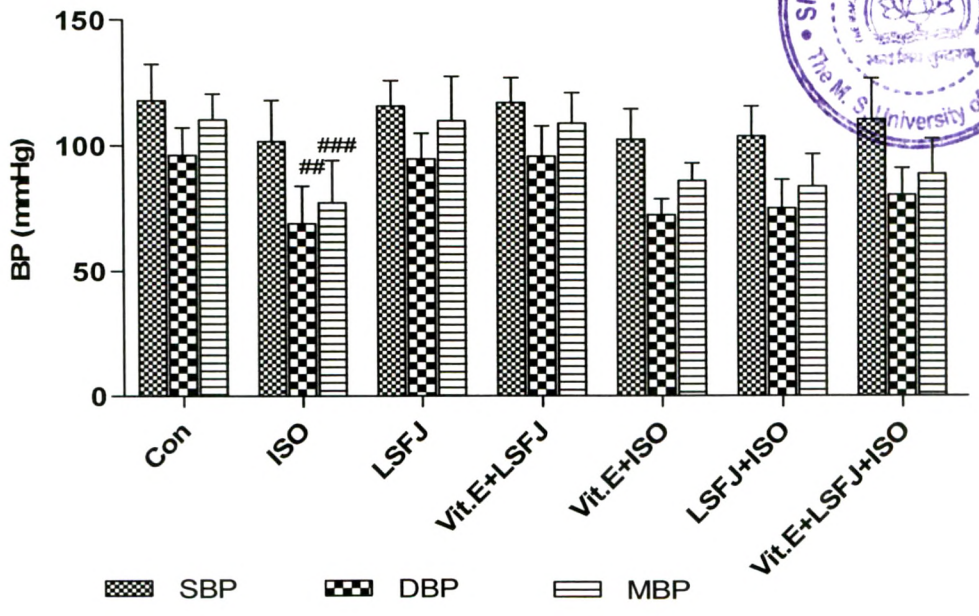


Fig. 5.61. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on systolic, diastolic and mean blood pressure in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

Fig. 5.62(a)

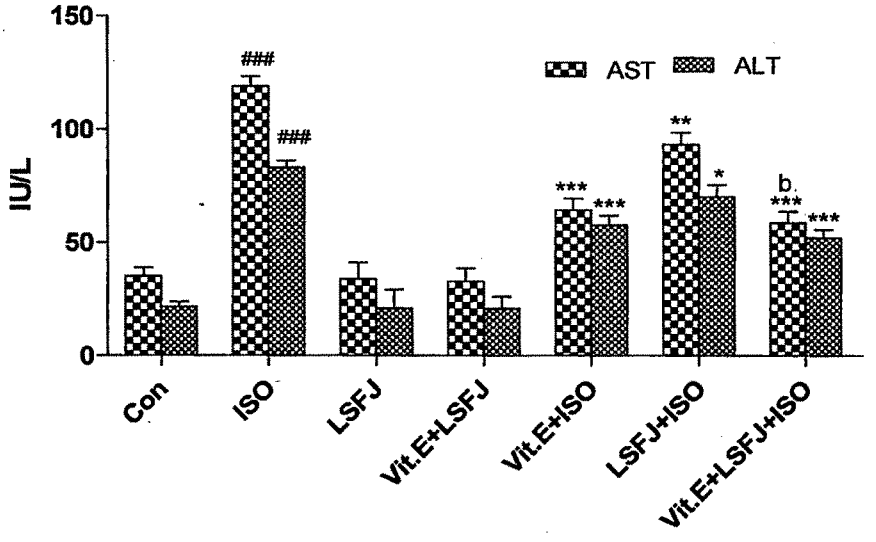


Fig. 5.62(b)

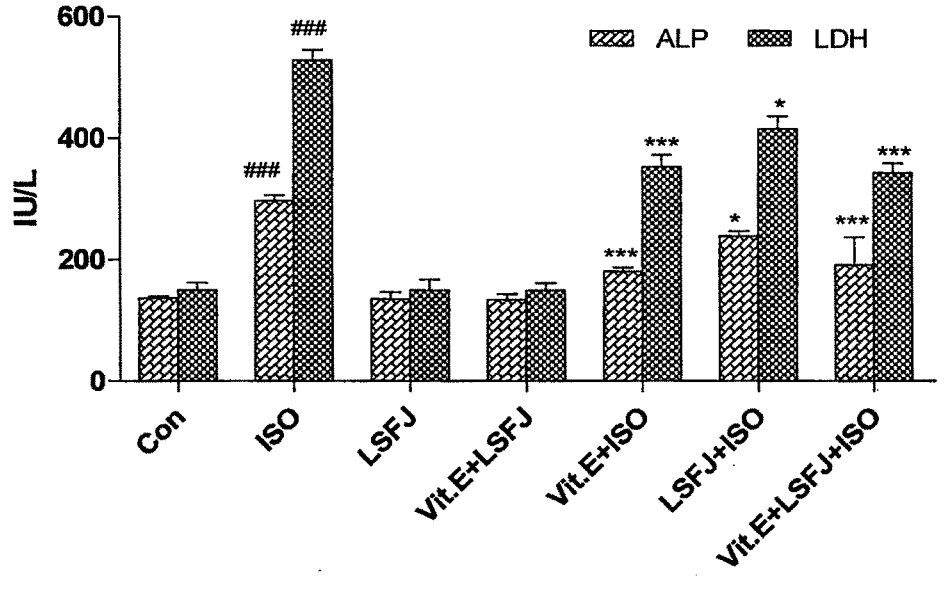


Fig. 5.62: Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on serum (a) AST and ALT (b) ALP and LDH levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LSFJ+ISO.

Fig. 5.63(a)

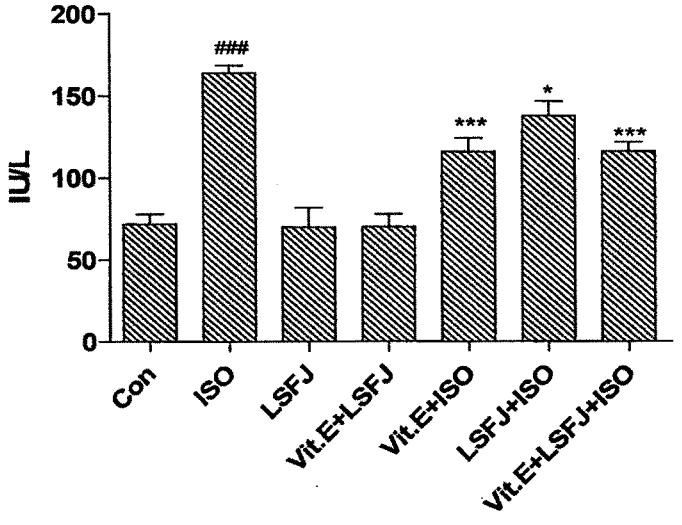


Fig. 5.63(b)

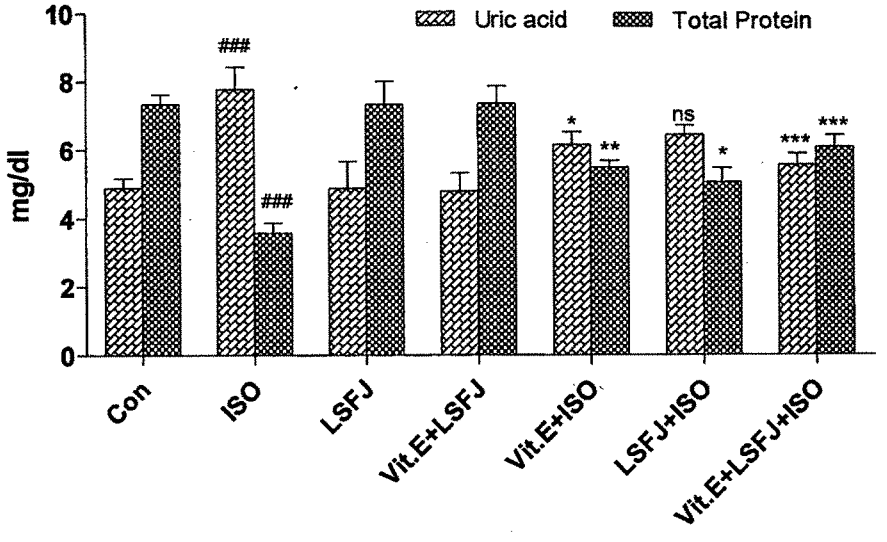


Fig. 5.63: Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on serum (a) CK-MB (b) Uric acid and Total protein levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LSFJ+ISO.

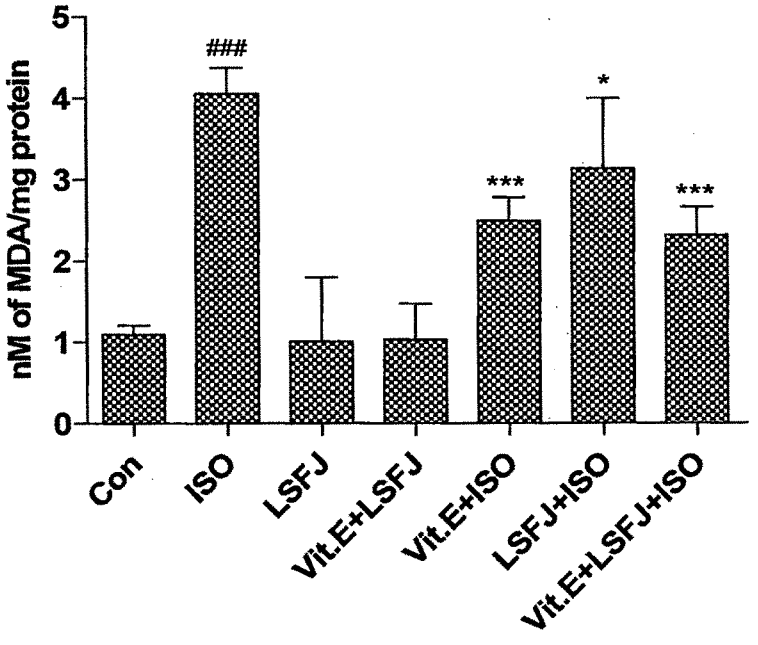


Fig. 5.64: Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on myocardial Lipid peroxidation in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean ± SEM (n=6).

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups,

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

$^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LSFJ+ISO.

Table 5.21: Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on endogenous antioxidants activities and vitamin E level in normal and ISO (200mg/kg, s.c) injected rats

Groups	GSH	GPx	GST	SOD	CAT	Vitamin E
Con.	7.401 ± 0.480	6.102 ± 0.206	110.40 ± 5.712	4.496 ± 0.203	6.290 ± 0.331	2.02 ± 0.047
ISO	4.338 ± 0.260***	4.032± 0.231***	68.91 ± 4.829***	2.272 ± 0.166***	3.687 ± 0.219***	1.09 ± 0.043***
LSFJ	7.101 ± 0.476	6.460 ± 0.654	112.43 ± 4.226	4.102 ± 0.621	6.122 ± 0.821	2.11 ± 0.052
Vit.E+LSFJ	7.001 ± 0.653	6.332 ± 0.734	112.09 ± 5.367	4.221 ± 0.725	6.003 ± 0.563	2.17 ± 0.081
Vit.E+ISO	5.785 ± 0.288**	5.635 ± 0.336**	95.55 ± 4.089***	3.566 ± 0.390*	5.188 ± 0.167**	1.84 ± 0.035***
LSFJ+ISO	5.555 ± 0.178*	5.007 ± 0.292*	79.11 ± 2.424	2.952 ± 0.220	4.208 ± 0.366	1.27 ± 0.036*
Vit.E+LSFJ+ISO	6.002 ± 0.329**	5.767 ± 0.468**	95.34 ± 5.281***	3.922 ± 0.264**	5.450 ± 0.405**	1.89 ± 0.035^{***b}

Values are expressed as mean±SEM, (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected group. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LSFJ+ISO group.

GSH: (µg of GSH /mg protein), GPx: (µmoles of glutathione oxidized/min/mg protein), GST: (µmoles of CDNB conjugated/min/mg protein), SOD: (units/mg protein), CAT: (µmoles of H_2O_2 consumed/min/mg protein), Vitamin E: (mmole/mg protein).

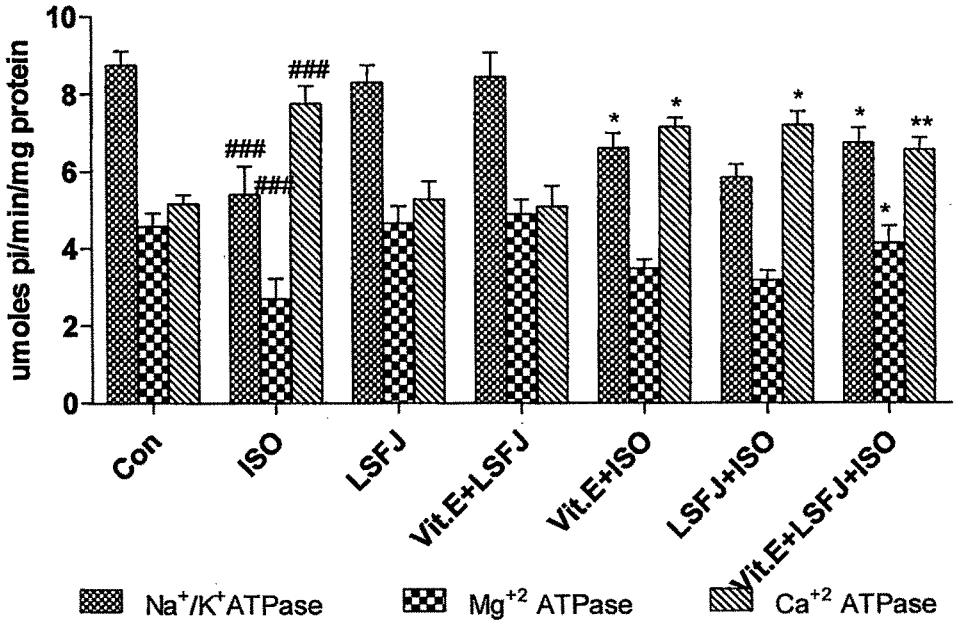


Fig. 5. 65. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on the activity of Na^{+}/K^{+} ATPase, Mg^{+2} ATPase and Ca^{+2} ATPase in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

Table 5.22: Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on the levels of sodium, potassium and calcium in the heart of normal and ISO (200mg/kg, s.c) injected rats

Groups	Sodium (Na^+)	Potassium (K^+)	Calcium (Ca^{++})
Control	6.088 ± 0.125	8.978 ± 0.164	10.81 ± 0.107
ISO	$7.728 \pm 0.312^{\#\#\#}$	$6.162 \pm 0.216^{\#\#\#}$	$13.83 \pm 0.344^{\#\#\#}$
LSFJ	6.087 ± 0.131	8.962 ± 0.118	10.72 ± 0.221
Vit.E+LSFJ	5.982 ± 0.101	9.028 ± 0.133	10.53 ± 0.132
Vit.E+ISO	$7.198 \pm 0.156^{*}$	$7.462 \pm 0.189^{***}$	$12.63 \pm 0.291^{**}$
LSFJ+ISO	7.573 ± 0.115^{ns}	$6.838 \pm 0.149^{*}$	13.02 ± 0.133^{ns}
Vit.E+LSFJ+ISO	$6.788 \pm 0.082^{**b}$	$7.762 \pm 0.121^{***b}$	$12.14 \pm 0.099^{**}$

All the values are expressed as nmol/mg protein

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups. $^{a}P<0.05$ compared to Vit.E+ISO and $^{b}P<0.05$ compared to LSFJ+ISO.

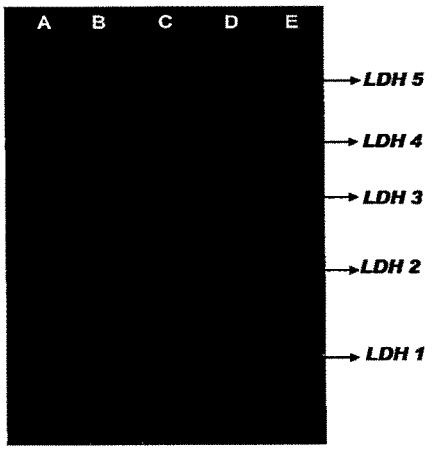


Fig. 5.66. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on LDH isoenzyme pattern in normal and ISO (200mg/kg, s.c) injected rats

A: Control, B: ISO C: Vit.E +ISO, D: LSFJ +ISO, E: Vit.E +LSFJ+ISO

Fig.5.67 (a)

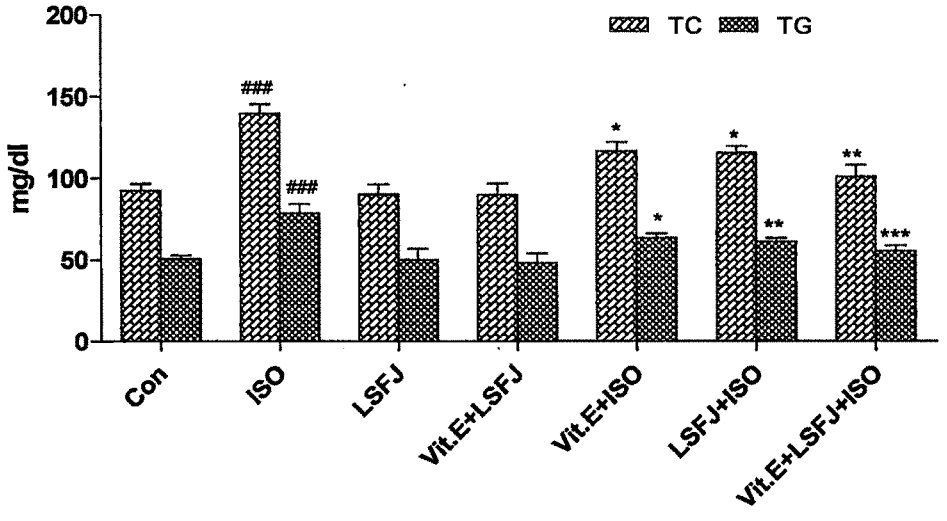


Fig.5.67 (b)

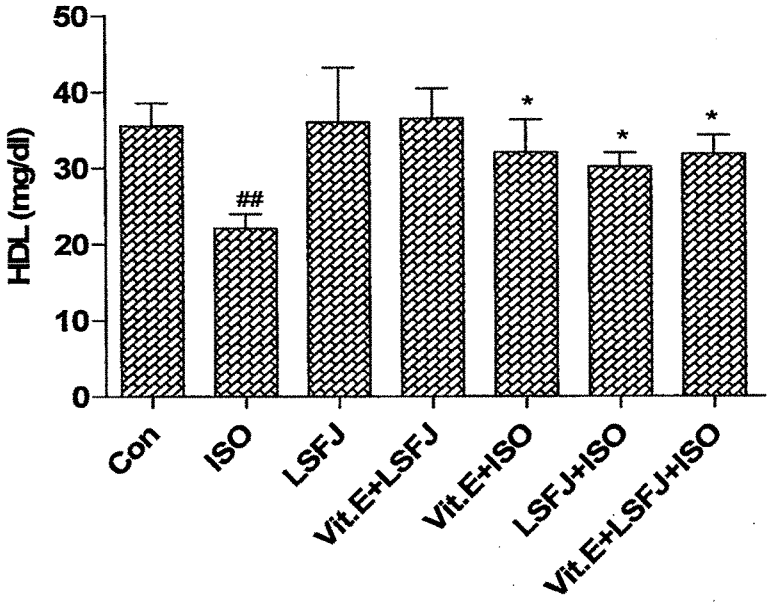


Fig. 5.67. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on serum a) TC and TG, b) HDL levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

Fig.5.68 (a)

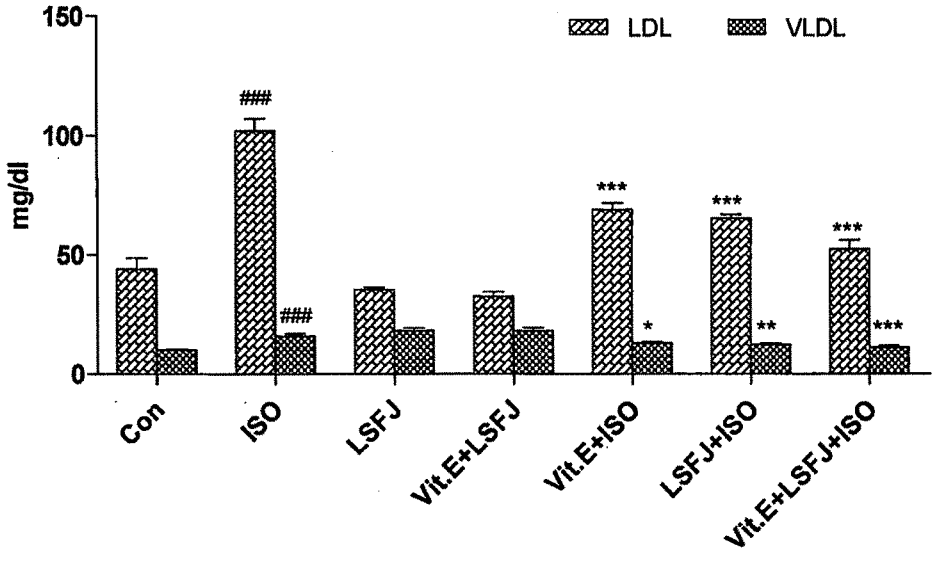


Fig.5.68 (b)

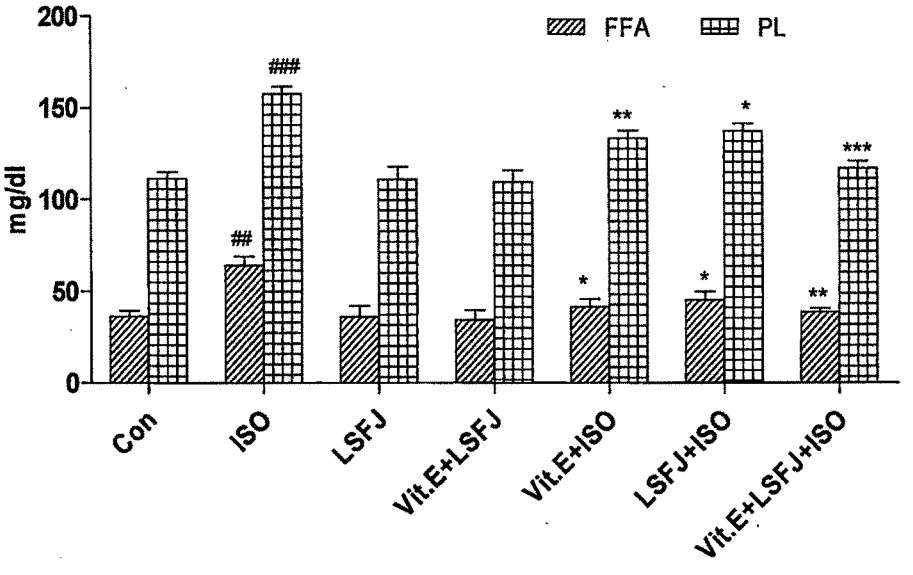


Fig. 5.68. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on a) LDL and VLDL b) FFA and PL levels in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

Table 5.23. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on tissue lipid profile in normal and ISO (200mg/kg, s.c) injected rats

Groups	TC	TG	FFA	PL
Con.	8.230 ± 0.637	6.147 ± 0.637	0.871 ± 0.077	25.620 ± 1.988
ISO	13.690 ± 1.340$^{\#\#}$	11.260 ± 0.840$^{\#\#\#}$	1.450 ± 0.117$^{\#\#}$	16.620 ± 1.902$^{\#\#}$
LSFJ	8.116 ± 2.087	6.001 ± 1.302	0.884 ± 0.208	24.891 ± 3.211
Vit.E+LSFJ	8.435 ± 1.761	6.119 ± 0.921	0.842 ± 0.112	24.785 ± 2.187
Vit.E+ISO	10.080 ± 0.483*	7.337 ± 0.823**	1.102 ± 0.077^{ns}	23.640 ± 1.982*
LSFJ+ISO	11.181 ± 1.004^{ns}	8.825 ±1.221*	1.302 ± 0.321^{ns}	18.112 ± 2.088^{ns}
Vit.E+LSFJ +ISO	9.881 ± 0.876*	7.502 ± 0.643**	1.001 ± 0.119*	22.109 ± 1.092*

Values are expressed as mg/g wt tissue for TC, TG, FFA and PL

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

Fig.5.69 (a)

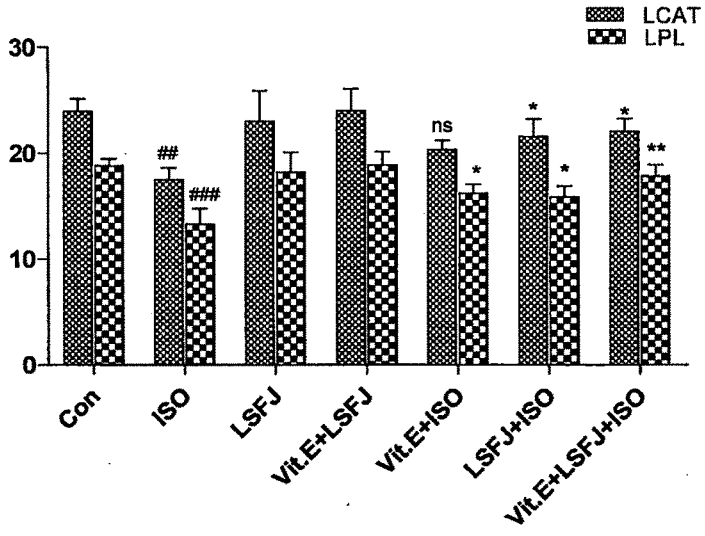


Fig.5.69 (b)

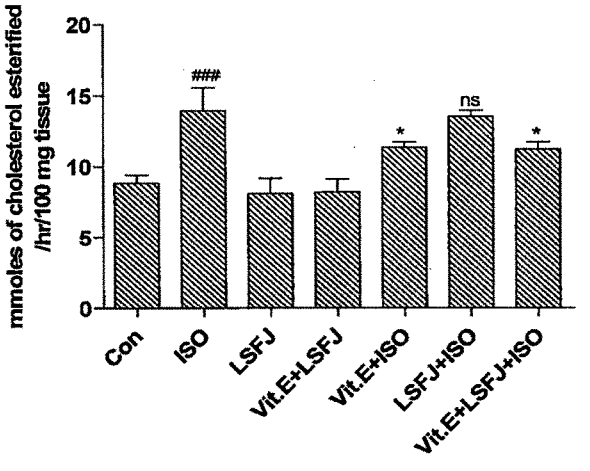


Fig.5. 69. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on (a) LCAT and LPL (b) CES levels in normal and ISO (200mg/kg, s.c) injected rats

Units: LCAT (μmoles of cholesterol esterified/hr/100mg tissue)

LPL (μmoles of fee fatty acids liberated/100mg tissue)

Values are expressed as Mean ± SEM(n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

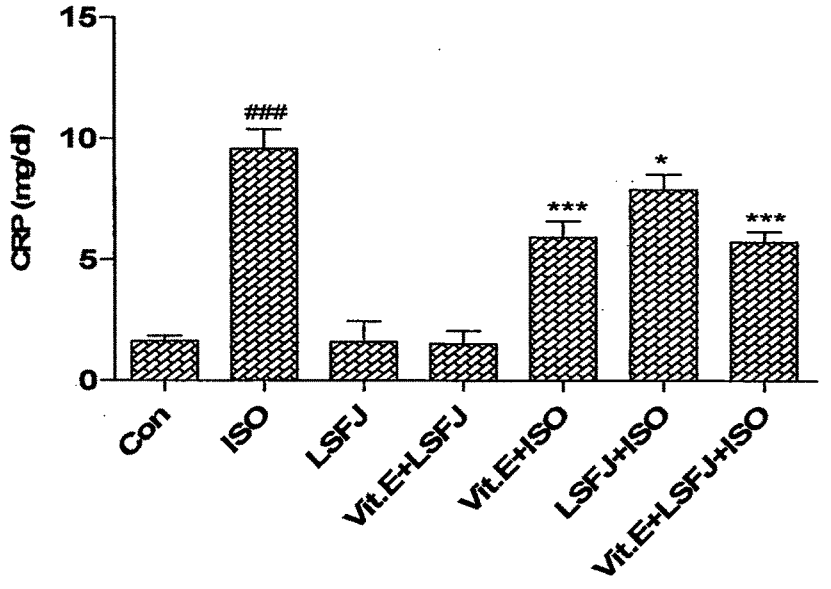


Fig. 5.70. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on serum CRP levels in normal and ISO (200mg/kg, s.c) injected rats

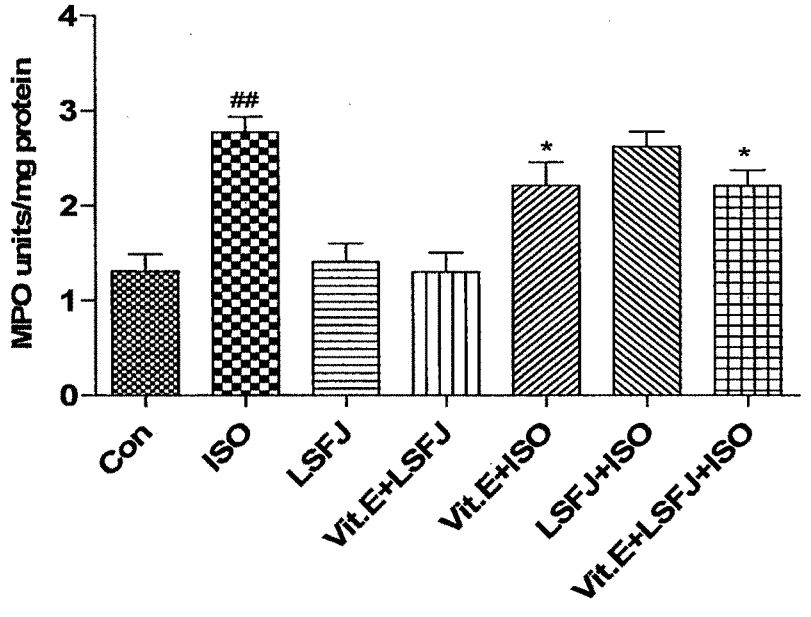


Fig. 5.71. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on tissue MPO activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

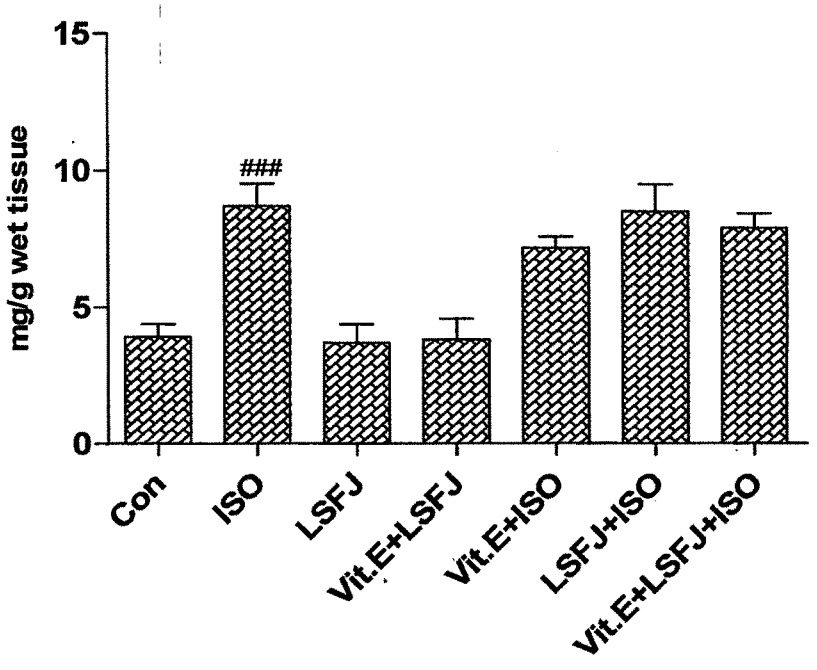
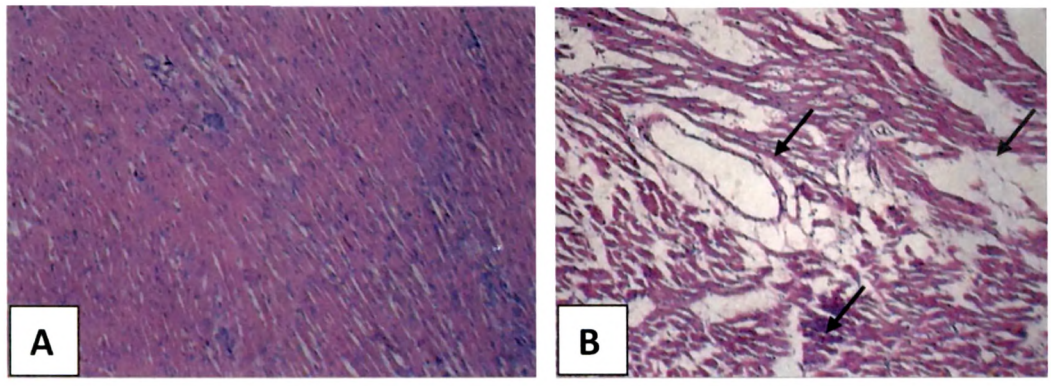
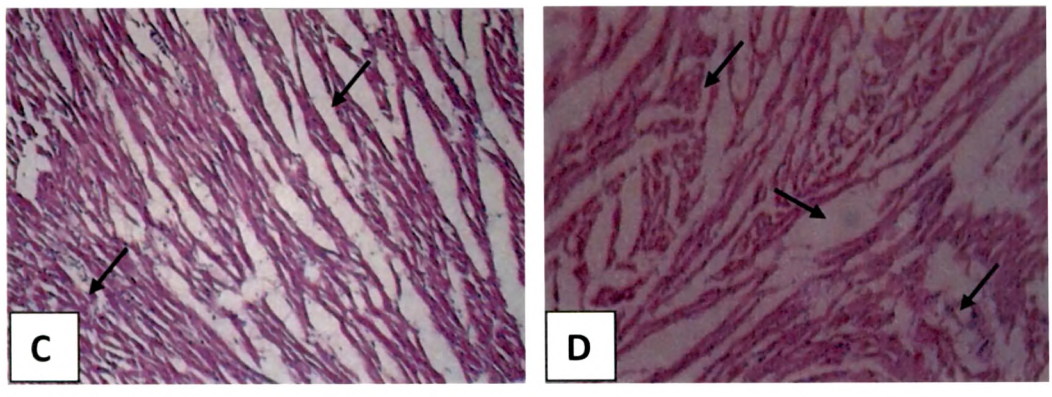


Fig. 5.72. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on tissue nitrite levels in normal and ISO (200mg/kg, s.c) injected rats

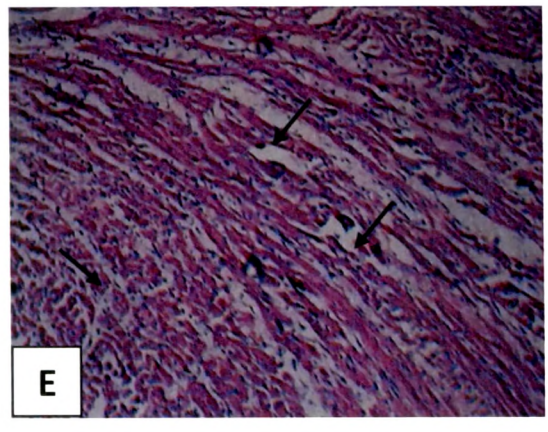
Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.



Control ISO injected



Vitamin E+ISO LSFJ+ISO



Vitamin E+LSFJ+ISO

Fig. 5.73. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on Histopathological alteration in normal and ISO (200mg/kg, s.c) injected rats

Table 5.24. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on the degree of histological changes in normal and ISO (200mg/kg, s.c) injected rats

Groups	Necrosis	Oedema	Inflammatory cells
Control	A	A	A
ISO	+++	+++	++
Vit.E+ISO	++	++	+
LSFJ+ISO	++	+++	+
Vit.E+LSFJ+ISO	+	++	++

Photomicrographs were used to evaluate the damage in the heart tissues: (A) no change, (+++) marked changes, (++) moderate changes, (+) mild changes

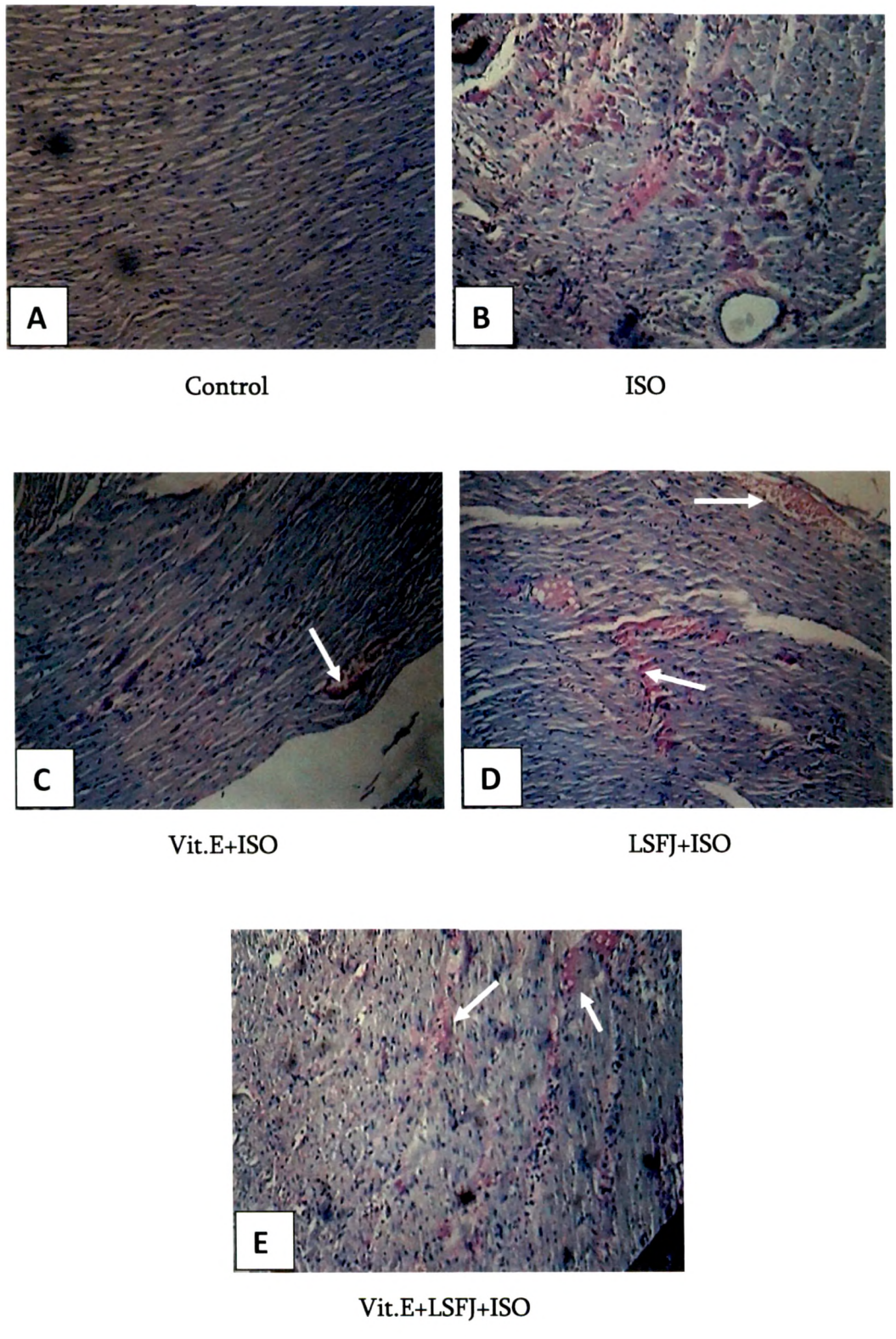


Fig. 5.74. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on Periodic acid Schiff's staining in normal and ISO (200mg/kg, s.c) injected rats (10X)

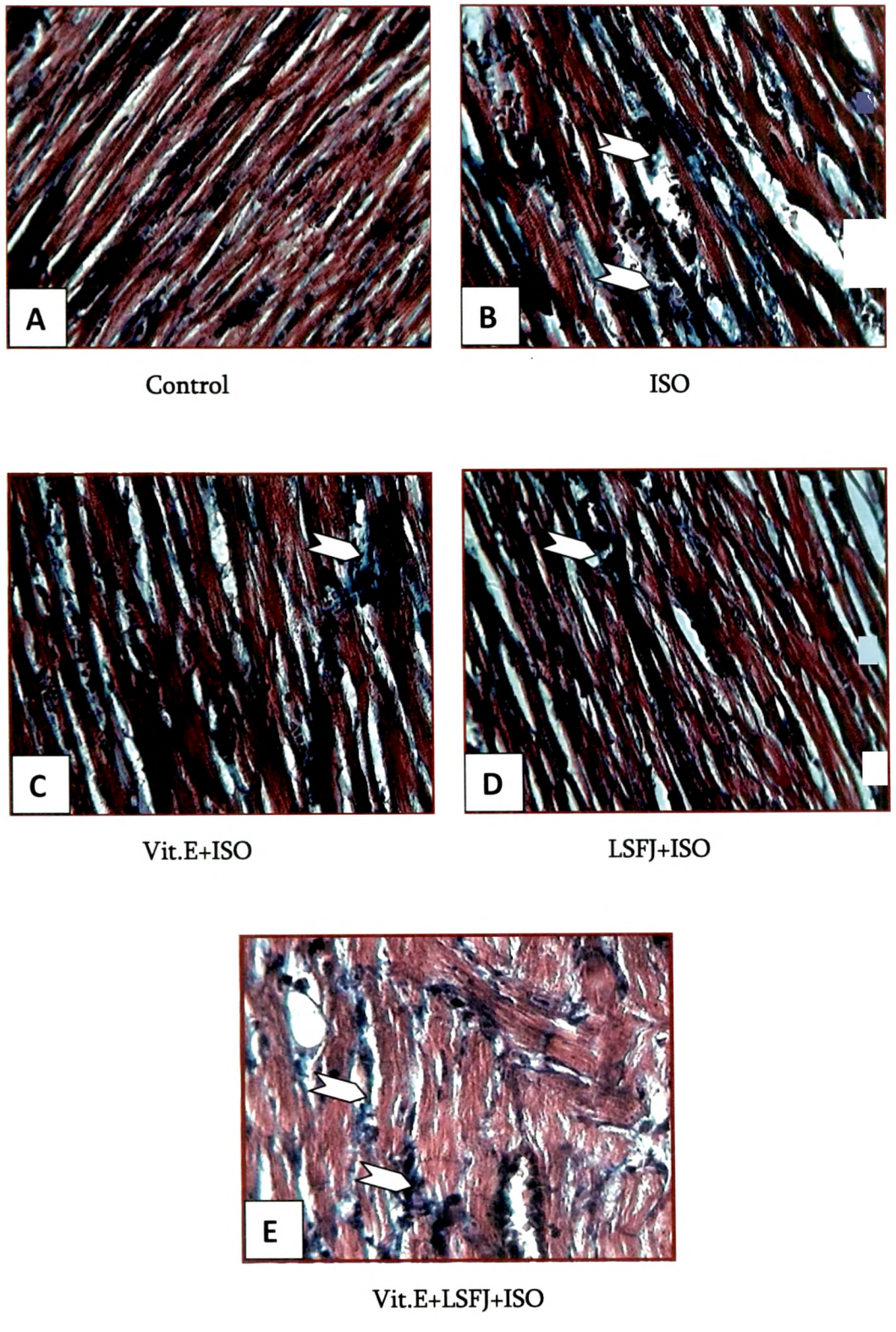


Fig. 5.75. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on Masson's trichrome staining of cardiac tissue in ISO (200mg/kg,s.c) injected rats (40X)

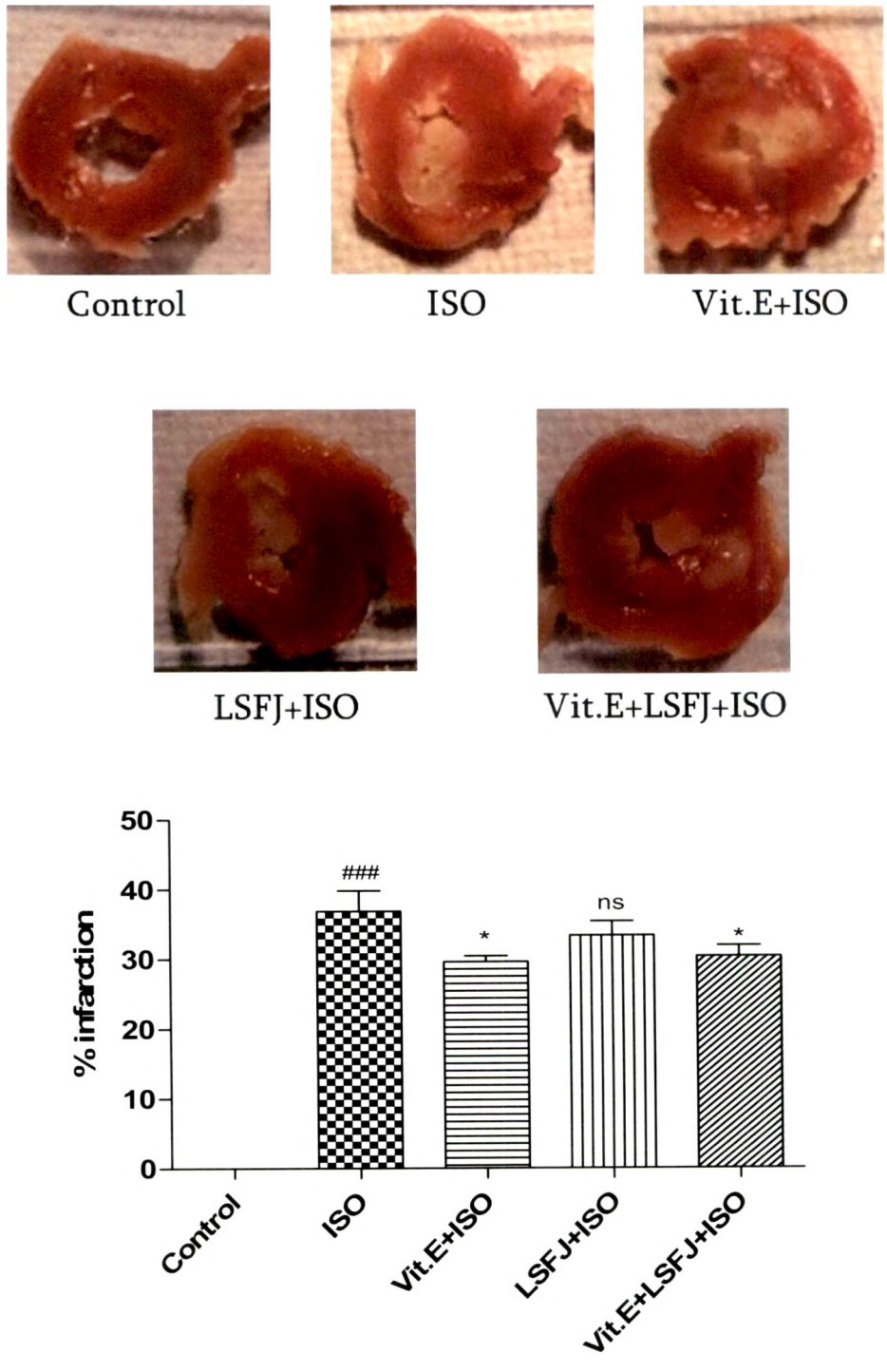


Fig. 5.76. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on area of infarction in normal and ISO (200mg/kg, s.c) injected groups

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

Lane A- Control

Lane B- ISO

Lane C- Vit. E +ISO

Lane D- LSFJ +ISO

Lane E- Vit. E +LSFJ+ISO

Fig. 5.77. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on DNA damage by gel electrophoresis in normal and ISO (200mg/kg, s.c) injected rats

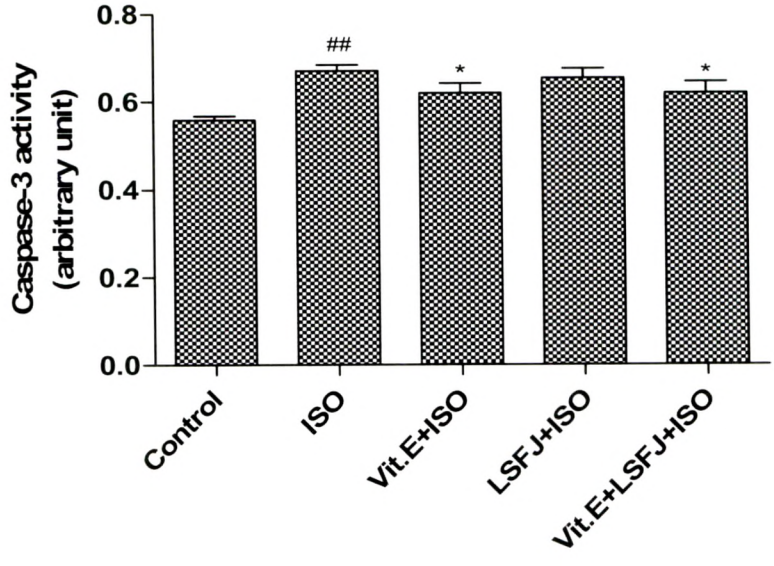


Fig.5.78. Effect of Vit. E (100mg/kg/day, p.o) and LSFJ (400mg/kg/day, p.o) for 30 days on Caspase-3 activity in normal and ISO (200mg/kg, s.c) injected rats

Values are expressed as mean±SEM (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ values compared to control groups, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ values compared to ISO injected groups.

5.6 Comparative *in vitro* antioxidant activities of Vit.E, Green tea, Lycopene, Pomegranate fruit extract and LS fruit juice

5.6.1 DPPH radical scavenging activity

The radical scavenging activities of Vit.E, GT, LYP, PGFE and LSFJ against DPPH at concentration ranging from 10-100 µg/ml are shown in Fig. 5.79. All the drugs showed significant DPPH radical scavenging activity at dose dependent manner. GT and LYP (100 µg/ml) was found to be equipotent in inhibiting DPPH radical. LSFJ (80 and 100 µg/ml) showed significant antioxidant activity and it was found to be less potent as compared to Vit.E, GT, LYP, and PGFE.

5.6.2 Superoxide radical scavenging activity

The comparative antiradical activities of Vit.E, GT, LYP, PGFE and LSFJ against superoxide radical scavenging assay at the concentration ranging from 10-100 µg/ml is shown in Fig.5.80. LYP was found to be more potent in scavenging superoxide radical at all the concentrations. Vit.E and GT (80 and 100 µg/ml) showed equipotent activity. LSFJ showed significant inhibition of superoxide radical at 80 and 100 µg/ml conc. LSFJ was found to be less potent in scavenging superoxide radical compared to Vit.E, GT, LYP, and PGFE.

5.6.3 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of Vit.E, GT, LYP, PGFE and LSFJ at different concentrations (10-100 µg/ml) is shown in Fig.5.81. GT showed maximum nitric oxide radical scavenging activity at higher concentration compared to Vit.E, LYP, PGFE and LSFJ. LSFJ at all the concentration did not show nitric oxide scavenging activity. PGFE showed greater activity as compared to Vit.E and LSFJ at all the concentrations.

5.6.4 Lipid peroxidation activity

The effect of Vit.E, GT, LYP, PGFE and LSFJ against iron induced lipid peroxidation in rats is shown in Fig.5.82. Iron induced rats showed a significant ($P<0.001$) increase in lipid peroxidation as compared to control animals. Vit.E, GT and LYP showed significant reduction in the level of lipid peroxidation at higher concentration as compared to iron induced group. PGFE showed significant effect at 100 µg/ml conc. However, LSFJ did not show significant inhibition on lipid peroxidation at all the concentration compared to iron induced group.

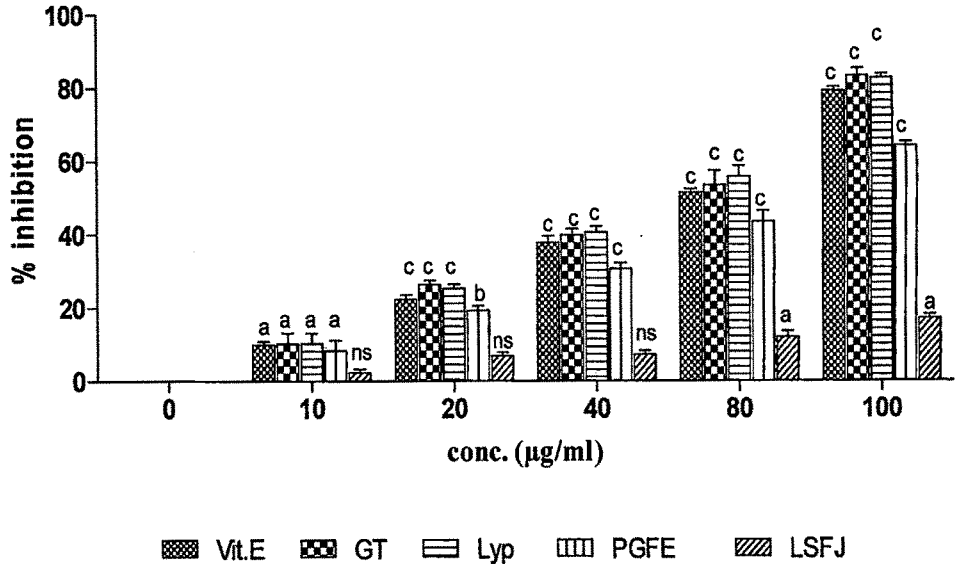


Fig. 5.79. Effect of Vit.E, GT, LYP, PGFE and LSFJ against DPPH radical scavenging assay

The results are mean ± SEM. of three parallel measurements. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P < 0.001$ vs. 0 µg/ml. $P>0.05$ considered as non significant (ns). GT: Green tea, Lyp: Lycopene, PGFE: pomegranate fruit extract, LSFJ: Lagenaria siceraria fruit juice.

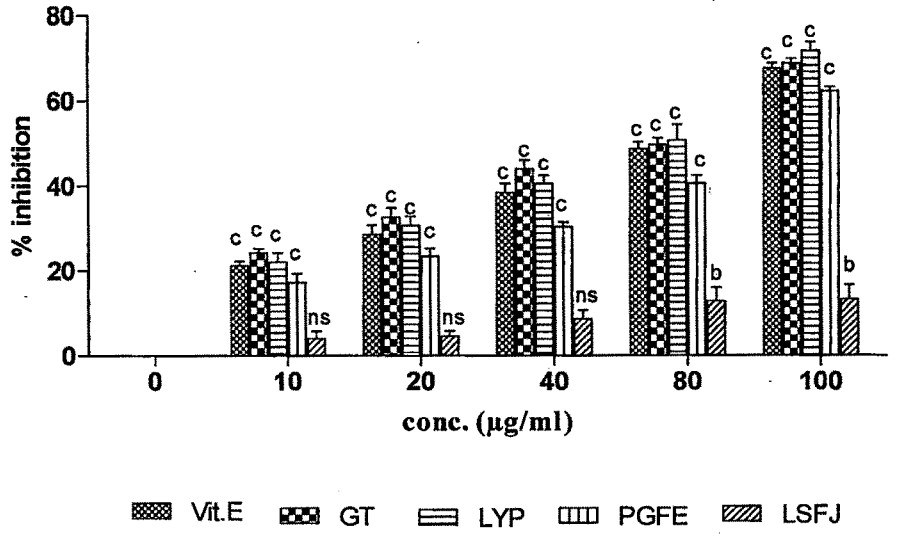


Fig. 5.80. Effect of Vit.E, GT, LYP, PGFE and LSFJ against superoxide radical scavenging assay

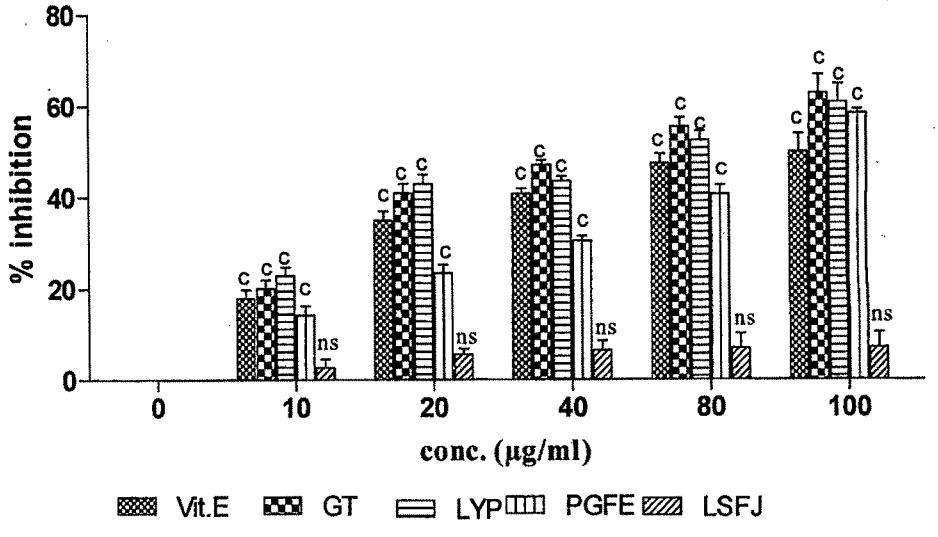


Fig. 5.81. Effect of Vit.E, GT, LYP, PGFE and LSFJ against nitric oxide radical scavenging assay

The results are mean ± SEM. of three parallel measurements. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P < 0.001$ vs. 0 µg/ml. $P>0.05$ considered as non-significant (ns).

GT: Green tea, Lyp: Lycopene, PGFE: pomegranate fruit extract, LSFJ: Lagenaria siceraria fruit juice.

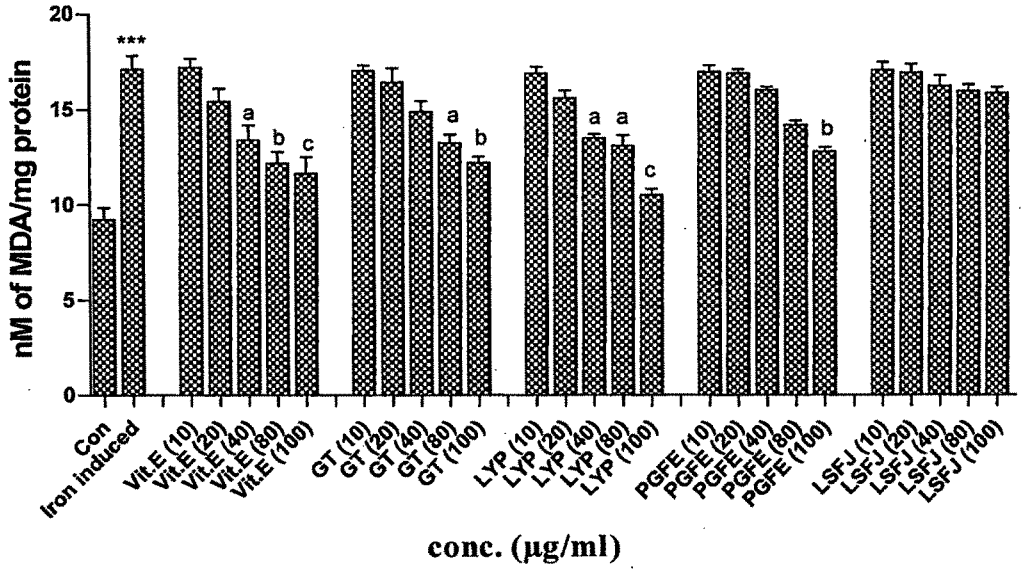


Fig. 5.82. Effect of Vit.E, GT, LYP, PGFE and LSFJ against iron induced lipid peroxidation in rat liver

The results are mean ± SEM. of three parallel measurements. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P < 0.001$ vs. Iron induces group, *** $P<0.001$ vs control.

GT: Green tea, Lyp: Lycopene, PGFE: pomegranate fruit extract, LSFJ: *Lagenaria siceraria* fruit juice.