RESULTS

Acute Toxicity

4.1 Oral Acute toxicity

Mortality in the acute toxicity test of MC was not seen in the limit test at the dose of 5000 mg/kg. Therefore $1/20^{th}$ and $1/10^{th}$ of the dose (250 & 500mg/kg p.o) were selected for the study.

Mortality in the acute toxicity test of MD was also not seen in the limit test at the dose of 5000 mg/kg. Therefore $1/20^{th}$ and $1/10^{th}$ of the dose (250 & 500mg/kg p.o) were selected for the study.

Mortality in the acute toxicity test of saponins of MC was seen in the limit test at the dose of 5000 mg/kg. Mortality was not seen in the main test up to dose of 1750 mg/kg and hence $1/10^{th}$ and $1/20^{th}$ of 1750mg/kg (87.5 & 175 mg/kg) were selected for the study.

Mortality in the acute toxicity test of saponins of MD was seen in the limit test at the dose of 5000 mg/kg. Mortality was not seen in the main test up to dose of 550 mg/kg, hence $1/10^{th}$ and $1/20^{th}$ of 550mg/kg (27.5 & 55 mg/kg) were selected for the study.

Effect on Metabolic Disorder ...

4.2.1 Diabetes Mellitus

4.2.1.1. Effect on streptozotocin induced Type 1 diabetes

Effect of ethanolic extract roots of MC (250&500 mg/kg, p.o., /day/30days) on serum glucose, insulin, creatinine, BUN, and liver glycogen

Effect on Serum glucose level: Administratrion of streptozotocin (65mg/kg, i.p. single dose) to Wistar rats significantly ($p<0.001$) increased glucose level when compared with normal control rats. Streptozotocin induced diabetic rats treated with MC (250 & 500mg/kg, p.o, /day/30days) showed a significant reduction in serum glucose when compared to diabetic control rats (Table 1.1).

There was a significant reduction in serum glucose in diabetic rats treated with MD (250 & 500mg/kg, p.o, /day/30days) when compared to diabetic control rats (Table 1.3) Diabetic rats treated with saponin fractions of MC (87.5&175mg/kg, p.o/day/30days) showed significant ($p<0.001$), ($p<0.01$) antihyperglycemic effect when compared with diabetic control rats (Table 1.3).

Saponin fractions of MD (27.5&55mg/kg, p.o/day/30days) also showed a significant ($p<0.001$), ($p<0.01$) antihyperglycemic effect when compared to diabetic control rats on 31st day (Table 1.4).

Effect on Serum Insulin level: Rats treated with streptozotocin (65mg/kg, i.p.) had a significant ($p<0.001$) decrease in serum insulin level when compared to normal control rats. Diabetic rats treated with MC (250 & 500mg/kg p.o/day/30days) showed a significant ($p<0.05$, $p<0.001$) increase in serum insulin level when compared to diabetic control rats (Table 1.1).

Serum insulin levels were significantly higher ($p<0.05$, $p<0.001$) in diabetic rats treated with MD (250 & 500mg/kg, p.o/day/30days) when compared to diabetic control rats (Table1.3).

Diabetic rats treated with saponin fractions of MC (175 & 87.5mg/kg, p.o/day/30days) showed a significant ($p<0.05$, $p<0.001$) increase in serum insulin level when compared to diabetic control rats. Saponin fractions of MD (27.5 & 55mg /kg, p.o, /day/30days) also showed significant ($p<0.05$), ($p<0.001$) increase in serum insulin level when compared to diabetic control animals (Table 1.2 and 1.4).

Effect on Serum Cholesterol level: Streptozotocin (65mg/kg, i.p./single dose) treatment to Wistar rats significantly ($p<0.001$) increased serum cholesterol level when compared with normal control rats on (Table 1.1).

Diabetic rats treated with MC (250 & 500mg/kg, p.o/day/30days) and MD (250 & 500mg/kg, p.o/day/30days) significantly ($p<0.001$) decreased the serum cholesterol level when compared to the diabetic control animals. (Table 1.1and 1.2).

Saponin fractions of MC (87.5 & 175mg/kg, p.o, /day/30days) and Saponin fractions of MD (27.5 & 55mg/kg, p.o/day/30days) also showed a significant ($p<0.001$) cholesterol lowering effect (Table 1.3 and 1.4).

Effect on Serum Triglyceride level: Rats treated with streptozotocin (65mg/kg, i.p./single dose) significantly ($p<0.001$) increased serum triglyceride level when compared with normal control rats (Table 1.1).

Diabetic rats treated with MC (250 & 500mg/kg, p.o/day/30days) and MD (250 & 500mg/kg, p.o/day/30days) significantly ($p<0.001$) decreased the serum triglyceride level when compared to the diabetic control rats (Table 1.1and 1.2).

Saponin fractions of MC (87.5 & 175mg /kg, p.o, /day/30days) and saponin fractions of MD (27.5 & 55mg /kg, p.o /day/30days) also showed a significant ($p<0.001$) serum triglyceride lowering effect when compared to diabetic control rats (Table 1.3 and 1.4).

Effect on Serum Creatinine level: Streptozotocin (65mg/kg i.p./single dose) induced diabetic rats showed a significant ($p<0.001$) increase in serum creatinine level when compared to normal rats (Table 1.1).

Diabetic rats treated with MC (250&500mg/kg, p.o/day/30days) significantly ($p<0.001$) decreased serum creatinine level (Table 1.1).

Diabetic rats treated with MD (250&500mg/kg, p.o/day/30days) also showed a significant ($p<0.05$ & $p<0.01$) decrease in the creatinine level when compared to diabetic control rats. (Table 1.2)

Saponin fractions of MC (87.5 & 175 mg/kg, p.o/day/30days) and saponin fraction of MD (55mg/kg, p.o/day/30days) also showed a significant ($p<0.01$) decrease in the serum creatinine level when compared to diabetic control rats. Saponin fractions of MD (27.5 mg/kg p.o, /day/30days) did not show a significant creatinine lowering effect (Table 1.3, 1.4).

Effect on Serum Blood urea nitrogen (BUN) Level: Serum Blood Urea Nitrogen (BUN) level showed a significant ($p<0.001$) increase in streptozotocin induced

diabetic rats when compared to normal rats. Treatment with MC (250 & 500mg/kg, p.o/day/30days) significantly ($p<0.01$) decreased BUN level when compared to diabetic control rats (Table1.1).

Treatment with MD (500mg/kg, p.o/day/30days) showed a significant ($p<0.01$) decrease in BUN when compared to diabetic control rats. Lower dose of MD (250mg/kg, p.o, /day/30days) did not show a significant BUN lowering effect (Table 1.3).

Effect on liver glycogen level: A significant ($p<0.001$) decrease in hepatic glycogen level was seen in the streptozotocin (65mg/kg i.p./single dose) induced diabetic rats when compared with normal rats. Treatment with MC (250&500mg/kg, p.o/day/30days) significantly ($p<0.001$) increased hepatic glycogen level when compared with diabetic control rats (Table 1.1)

Treatment with MD (250&500mg/kg, p.o/day/30days) also showed a significant ($p<0.05$) increase in the hepatic glycogen level when compared to diabetic control rats (Table 1.2).

Saponin fractions of MC (87.5&175 mg/kg, p.o/day/30days) showed a significant ($p<0.001$) increase in the hepatic glycogen level when compared to diabetic control rats (Table 1.3).

The saponin fractions of MD (27.5&55 mg/kg, p.o /day/30days) also showed a significant ($p<0.05$ & $p<0.001$) increase in the hepatic glycogen level when compared to diabetic control rats (Table 1.4).

Effect on HMG CoA reductase enzyme: HMG CoA reductase activity is expressed as the of ratio HMG CoA vs Mevalonate. Lower ratio indicates higher enzyme activity and vice-versa. Significant ($p<0.001$) decrease in the ratio of HMG CoA vs Mevalonate was seen in streptozotocin diabetic rats indicating increase in the HMG CoA reductase activity when compared to normal rats (Table 1.3).

Saponin fraction of MC (175 mg/kg, p.o/day/30days) showed a significant ($p<0.01$) increase in the ratio of HMG CoA vs Mavalonate when compared with diabetic control rats indicating HMG CoA reductase enzyme inhibitory activity. Lower dose of saponin fractions of MC (87.5 mg/kg, p.o/day/30days) did not show a significant increase in the HMG CoA reductase activity when compared to normal rats (Table 1.3)

The saponin fractions of MD (55 mg/kg, p.o/day/30days) also showed a significant ($p<0.01$) increase in the ratio of HMG CoA vs Mavalonate when compared

with diabetic control rats indicating HMG CoA reductase enzyme inhibitory activity where as lower dose of Saponin fractions of MD (27.5 mg/kg, p.o/day/30days) did not show significant increase in the ratio of HMG CoA vs Mavalonate (Table 1.4).

Effect on Heart (histopathology): Histopathological sections of the heart of diabetic rats showed infiltration of the inflammatory cells without continuity in muscle fibers suggesting an irreversible cell injury (Fig1.1b).

Histopathological sections of the MC (500mg/kg p.o/day/30days) treated rats showed normal regenerative changes with striations, branched appearance and continuity with the adjacent myofibrils (Fig1.1c).

Effect on Pancreas (histopathology): Histopathological sections of the pancreatic islets of diabetic rats showed irregularly shaped, small and scanty islets. Severe vacuolation and degranulation were present in the β-cells of a number of islets. (Fig 1.2b).

Histopathological sections of the pancreatic islets in MC (500mg/kg p.o/day/30days) treated rats showed increase in the number of pancreatic cell islets (Fig 1.2c) which were similar to that of healthy pancreatic islets.

Effect on Kidney (histopathology): Histopathological sections of the diabetic rats showed severe glomerular sclerosis, arteriolar hyalinization, cortical interstitial fibrosis and increased number of vacuoles which may be due to glycogen deposition (pas positive) (Fig 1.3b).

Histopathological sections of the MC (500mg/kg p.o./day/30days) treated rats showed a minimal glomerular sclerosis and vacuolization (Fig 1.3c).

Histopathological sections of the Insulin (6IU/kg p.o /day/30days) treated rats also showed minimal glomerular sclerosis and vacuolization (Fig 1.1d).

4.2.1.2 Insulin sensitizing activity of MC and MD on fructose rich diet (FRD) induced hyperinsulinemia

Effect on Insulin level: Feeding FRD for 15 days significantly ($p<0.001$) increased the serum insulin level when compared to normal diet fed control rats.

FRD rats treated with MC (250 & 500 mg/kg p.o./day/15days) significantly ($p<0.01$ & $p<0.001$) decreased the insulin levels when compared to FRD control rats. (Table 1.5)

FRD rats treated with MD (250&500 mg/kg p.o.day/15days) also significantly ($p<0.01$ & $p<0.001$) decreased the serum insulin level when compared to FRD control rats (Table 1.7).

The saponin fractions of MC (175 mg/kg, p.o/day/15days) and saponin fractions of MD (55 mg/kg, p.o/day/15days) showed a significant ($p<0.001$) fall in serum insulin level when compared to FRD control rats, where as the saponins of MC (87.5 mg/kg, p.o/day/15days) and MD (27.5 & 55mg/kg, p.o /day/15days) did not show a significant decrease in serum insulin level when compared to FRD control rats. (Table 1.6 and 1.8)

Effect on serum triglyceride level: Feeding FRD to rats for 15 day significantly ($p<0.001$) increased the serum triglyceride level when compared to normal diet fed control rats. Treating the FRD rats with MC (250 & 500 mg/kg p.o/daily/15days) the serum triglyceride level showed a significant ($p<0.001$) decrease when compared to FRD control rats (Table 1.5).

MD (250 & 500 mg/kg p.o/daily/15days) also significantly ($p<0.001$) decreased the serum triglyceride level in FRD rats when compared to FRD control rats (Table 1.7).

The saponin fractions of MC (87.5 & 175 mg/kg, p.o/day/15days) and saponin fractions of MD (27.5 & 55 mg/kg, p.o/day/15days) showed a significant ($p<0.001$) decrease in serum triglyceride level in FRD rats when compared to FRD control rats (Table 1.6, 1.8).

Effect on serum glucose level: Serum glucose levels increased significantly ($p<0.001$) in rats after feeding FRD for 15 days when compared to the normal diet fed control rats (Table1.5).

Treating the FRD fed rats with MC (250 & 500 mg/kg p.o/day/15days), the serum glucose level significantly ($p<0.05$ & $p<0.001$ respectively) decreased when compared to FRD control rats.

Treating the FRD rats with MD (250 & 500 mg/kg p.o/day/15days) also significantly ($p<0.05$ & $p<0.001$ respectively) decreased the serum glucose levels when compared to FRD control rats (Table 1.7).

Saponin fraction of MC (87.5 &175 mg/kg, p.o/day/15days) significantly ($p<0.05$ & $p<0.001$ respectively) decreased the serum glucose level in FRD rats when compared to FRD control rats (Table 1.6).

Saponin fractions of MD (27.5 & 55mg/kg, p.o/day/15days) also showed a significant ($p<0.05$ & $p<0.001$respectively) decrease in serum glucose level in FRD rats when compared with FRD control rats (Table 1.8).

Effect on serum Cholesterol level: Feeding FRD to rats for 15 day significantly ($p<0.001$) increased the serum cholesterol level when compared to normal diet fed control rats.

Treating FRD rats with MC (250 & 500 mg/kg p.o/day/15days), serum cholesterol level significantly ($p<0.001$) decreased in FRD rats when compared with FRD control rats (Table1.5).

Treating FRD rats with MD (250 & 500 mg/kg p.o/day/15days), serum cholesterol level significantly ($p<0.001$) decreased in FRD rats when compared to FRD control rats (Table1.7).

Saponin fraction of MC (87.5 & 175 mg/kg, p.o/day/15days) also significantly (($p<0.05$ & $p<0.001$) reduced the serum cholesterol level in FRD rats when compared to FRD control rats (Table 1.6).

Saponin fractions of MD (27.5 &55 mg/kg, p.o/day/15days) showed a significant ($p<0.01$ & $p<0.001$ respectively) decrease in serum cholesterol level when compared with FRD control rats (Table 1.8).

Effect on Hepatic HMG CoA reductase level: HMG CoA activity is expressed as ratio HMG CoA vs Mevalonate. Lower ratio indicates higher enzyme activity and vice-versa. Significant ($p<0.001$) decrease in the ratio of HMG CoA vs Mevalonate was seen in FRD rats when compared to normal control rats, indicating increase in the HMG CoA reductase activity (Table 1.6).

Saponin fractions of MC (175 mg/kg, p.o/day/15days) (Table 1.6) administered to FRD rats showed a significant ($p<0.01$) increase in the ratio of HMG CoA vs Mevalonate when compared to FRD control rats indicating HMG CoA reductase enzyme inhibitory activity. Saponin fractions of MC (87.5 mg/kg, p.o, /day/15days) did not show significant increase.

Saponin fractions of MD (55 mg/kg, p.o,/day/15days) also showed a significant ($p<0.05$) increase in the ratio of HMG CoA vs Mevalonate in FRD rats when compared to FRD control rats indicating HMG CoA reductase enzyme inhibitory activity; where as lower dose of saponin fractions of MD (27.5 mg/kg, p.o/day/15days) administered to FRD rats did not show significant increase in the ratio of HMG CoA vs Mavalonate when compared to FRD control rats. (Table 1.4)

4.2.1.3. Effect of MC& MD on serum Cholesterol, Triglyceride, HDL, LDL, VLDL and Glucose in 26 days atherogenic diet (AD) fed rats.

Effect on serum triglyceride: Feeding AD to rats for 26 days, the triglyceride level increased significantly ($p<0.001$) when compared to the normal control group. Treatment with MC (250&500 mg/kg p.o/day/26days) or MD (250&500mg/kgp.o/day/26days) to AD rats showed a significant ($p<0.001$) decrease in the triglyceride level when compared to AD control rats. MC (250 & 500 mg/kg, p.o/day/26days) or MD (250 & 500 mg/kg p.o/day/15days) did not show significant change in triglyceride level in normal rats. (Table 3.1and 3.2)

Effect on serum Cholesterol: AD feeding for 26 days to the rats significantly ($p<0.001$) increased the cholesterol level when compared with control group. Treatment with MC (250&500 mg/kg, p.o/day/26days) or MD (250&500mg/kg, p.o/day/26days) to AD rats significantly ($p<0.001$) decreased the cholesterol level when compared to AD control rats (Table 3.1).

MC (250&500 mg/kg, p.o/day/26days) or MD (250&500 mg/kg po) did not show significant change in cholesterol level in normal rats (Table: 3.1 and 3.2).

Effect on serum HDL: AD feeding for 26 days significantly ($p<0.001$) lowered HDL cholesterol level when compared to normal control rats. (Table 3.1)
Treatment with MC (250&500 mg/kg, p.o/day/26days) or MD (250&500 mg/kg, p.o/day/26days) to AD rats significantly ($p<0.001$) increased the serum HDL level when compared to AD control group (Table 3.1 and 3.2).

Both MC and MD did not have any effect on the level of HDL in normal rats (Table 3.1and 3.2).

Effect on serum LDL: AD feeding for 26 days significantly ($p<0.001$) increased LDL cholesterol level when compared to the normal control group

Treatment with MC (250&500 mg/kg p.o/day/26days) or MD (250&500 mg/kg p.o/day/26days) to AD rats significantly [($p<0.001$), ($p<0.01$) respectively] decreased the serum LDL level when compared to AD control group (Table 3.1& 3.2).

Both MC and MD did not have any effect on the level of LDL in normal rats

Effect on serum VLDL: VLDL level increased significantly ($p<0.001$) after feeding atherogenic diet to the rats for 26 days when compared to the normal rats.

MC (250&500 mg/kg p.o/day/26days) or MD (250&500 mg/kg p.o/day/26days) treatment to AD rats significantly ($p<0.001$) decreased the serum VLDL level when compared to AD group (Table 3.1& 3.2)

Both MC and MD did not have any effect on the level of VLDL in normal rats

Effect on serum Glucose: Feeding atherogenic diet for 26 days significantly ($p<0.001$) increased serum glucose level when compared to the normal control group (Table 3.1)

Treatment with MC (250 & 500 mg/kg p.o/day) or MD (250 & 500 mg/kg p.o/day/26days) to AD rats significantly [($P<0.001$), ($P<0.01$) respectively] decreased the serum glucose level when compared to AD control group (Table 3.1& 3.2).

Both MC and MD did not have any effect on the serum glucose level in normal rats

Effect on aorta (Histopathology): Transverse section of the aorta in the AD group exhibited thickening of vascular wall of aortic musculature with fatty tissue and formation of neointima containing vascular smooth muscle cells of tunica media that indicates atherosclerotic plaque formation (Fig3.1b)

Treatment with MC (500mg/kg p.o/day/26days) to AD rats did not show formation of neointima containing vascular smooth muscle cells of tunica media and had almost normal architecture very similar to lovostatin treated group (Fig3.1c& 1e).

3.2.1.4. Effect of saponin fraction of MC and MD on serum Triglyceride, Cholesterol, LDL, VLDL and HMG CoA reductase activity in High Cholesterol Diet (HCD) fed rabbits for 35 days.

Effect on serum total cholesterol: Feeding Standard Rabbit Chow (SRC) rabbits with HCD for 35 days significantly ($p<0.001$) increased cholesterol level when compared to SRC control rabbits.

Saponin fractions of MC (175mg/kg p.o/daily/35days) or Saponin fractions of MD (55mg/kg p.o /day/35days) significantly ($p<0.001$) decreased the cholesterol level in HCD rabbits when compared to HCD control rabbits.

But saponin fractions of MC (175mg/kg p.o/daily/35days) or Saponin fractions of MD (55mg/kg p.o/daily/35days) did not show significant change in the cholesterol levels in SRC rabbits when compared to SRC control rabbits (Table 3.5, 3.6).

Effect on serum Triglyceride: Feeding Standard Rabbit Chow (SRC) rabbits with HCD for 35 days significantly ($p<0.001$) increased serum triglyceride level when compared to SRC control rabbits.

Treatment with saponin fractions of MC (175mg/kg p.o/daily/35days) or MD (55mg/kg p.o/daily/35days) significantly ($p<0.001$) decreased the triglyceride level in HCD rabbits when compared to HCD control group (Table 3.5 & 3.6).

But saponin fractions of MC (175mg/kg p.o/daily/35days) or Saponin fractions of MD (55mg/kg p.o/daily/35days) did not show significant change in the triglyceride levels in SRC rabbits when compared to SRC control rabbits (Table 3.5, 3.6).

Effect on serum LDL: Feeding HCD to SRC rabbits for 35 days significantly ($p<0.001$) increased the LDL level when compared to the SRC control rabbits.

Saponin fractions of MC (175mg/kg p.o/daily/35days) or Saponin fractions of MD (55mg/kg p.o/daily/35days) significantly ($p<0.001$) decreased the LDL level in HCD rabbits when compared to the HCD control rabbits (table 3.5 & 3.6).

But saponin fractions of MC (175mg/kg p.o/daily/35days) or Saponin fractions of MD (55mg/kg p.o/daily/35days) did not show significant change in the serum LDL levels in SRC rabbits when compared to SRC control rabbits (Table 3.5, 3.6).

Effect on serum VLDL: Serum VLDL level also significantly ($p<0.001$) increased by feeding HCD to rabbits for 35 days when compared to the SRC control rabbits.

Saponin fractions of MC (175mg/kg p.o/day/35days) significantly ($p<0.001$) decreased the VLDL level in HCD rabbits when compared to HCD control rabbits. Saponin fractions of MD (55mg/kg p.o/day/35days) also significantly ($p<0.001$) decreased the serum VLDL level in HCD rabbits when compared to HCD control rabbits. Saponin fractions of MC or MD did not make any significant change in the triglyceride levels in standard rabbit chow fed rabbits when compared to SRC control rabbits.

Effect on Hepatic HMG CoA reductase level: HMG CoA reductase activity is expressed as ratio HMG CoA vs Mevalonate. Lower ratio indicates higher enzyme activity and vice-versa. Significant ($p<0.001$) decrease in the ratio of HMG CoA vs Mavalonate was seen in HCD rabbits when compared to standard rabbit chow fed control rabbits indicating increase in the HMG CoA reductase activity. Saponin fractions of MC (175 mg/kg, p.o, /day/35days) showed a significant ($p<0.01$) increase in the ratio of HMG CoA vs Mavalonate ratio in HCD rabbits compared to HCD control rabbits indicating HMG CoA reductase inhibitory activity. Saponin fractions of MC (175mg/kg, p.o, /day/35days) did not show significant change in HMG CoA reductase activity in standard rabbit chow fed rabbits compared to standard rabbit chow fed control rabbits. (Table 3.5)

Saponin fractions of MD (55 mg/kg, p.o, /day/35days) (Table 3.6) did not show change in HMG CoA reductase activity in HCD rabbits compared to HCD control rabbits.

Effect on aorta (histopathology): Aortic sections of standard rabbit chow fed control rabbits showed normal histology of the tunica intima, media and adventia. The intima was composed of a continuous layer of endothelial cells. Feeding rabbits with HCD thickened vascular wall of aortic musculature with fatty tissue there was also formation of neointima containing vascular smooth muscle cells of tunica media. Saponin fractions of MC&MD (175 and 55mg/kg p.o/day/35days respectively) treatment to HCD rabbits did not show formation of neointima containing vascular smooth muscle cells of tunica media and almost normal architecture.

1.1. Effect of MC in Streptozotocin treated rats in serum Glucose, Insulin, Cholesterol, Triglyceride,Creatinine, Blood Urea Nitrogen (BUN) and hepatic glycogen after 30 days treatment.

Groups	Treatment	Serum Glucose (mg/dl)	Serum Insulin (μu/ml)	Liver Glycogen (mg/gm of Tissue)	Serum total cholesterol (mg /dl)	Serum Triglyceride (mg / dl)	Serum Creatinine (mg/dl)	Serum BUN (mg/dl)
I.	Sodium citrate buffer(control)	83.38 ±1.85	20.00 ± 0.13	17.22 ±0.12	120.85 ±0.21	130.56 ±0.13	1.02 ±0.26	20.07 ±0.25
II.	STZ (control) (65 mg /kg, i.p)	282.36 ±2.08***	6.43 ± 0.06***	9.26± 0.16 ***	240.12± 0.28 ***	185.2± 0.06 ***	2.22± 0.16***	30.22± 0.29 ***
III.	STZ+ Insulin (6U/kg, i.p,)	126.94 ±1.15†††	17.1 ± 0.68†††	15.38 ± 0.17†††	138.21± 0.16†††	140.56± 0.71†††	01.12± 0.09†††	24.3± 0.13††
IV.	STZ + MC(250mg/kg p.o/30days)	129.8 ±1.59†††	8.50 ± 0.06†	13.31 ± 0.16†††	209.8± 0.21†††	170.5± 2.15††	1.23± 0.28†††	24.04± 0.23††
IV.	STZ + MC(500mg/kg p.o/30days)	115. 6 ±2.15†††	14.71 ± 0.14†††	16.7 ± 0.13†††	140.5± 0.21†††	152.2± 0.34†††	0.98± 0.16†††	21.43± 0.17†††

Values expressed as mean ±SEM, n=6,

***p<0.001 When compared to normal control group

†††p<0.001,††p<0.01 When compared to STZ control group

Observation:

- Streptozotocin (65mg/kg i.v./single dose) has developed highly significant hyperglycemia
- MC (250&500 mg/kg p.o/day/30days) significantly reduced elevated serum glucose, cholesterol, triglyceride, creatinine and BUN
- MC (250&500 mg/kg p.o/day/30days) significantly increased the serum insulin level

Table 1.2 Effect of saponin fractions of MC (87.5 &175 mg/kg, p.o,/daily) in Streptozotocin treated rats on serum Glucose, Insulin, Cholesterol, Triglyceride, Creatinine and hepatic glycogen after 30 days treatment.

Groups	Treatment	glucose (mg/dl)	TG (mg/dl)	Chole sterol (mg/dl)	HDL (mg/dl)	creatinine (µmol/l)	Liver glycogen (mg/ 100gm tissue)	HMG CoA activity*	Serum Insulin (µu/ml)
G1	Sodium citrate buffer (Control)	83.8 ±1.984	64.33 ±1.22	55.68 ±1.80	42.1 ±1.49	1.12 ±0.44	1330.33 ±5.17	6.91 ±0.09	20.41 ± 0.19
G2	STZ-Control (65mg/kg i.p.single dose)	274.08± 7.27†††	88.11± 2.47†††	128.58± 2.85†††	30.36± 1.46††	2.45± 0.93††	779.0± 8.11†††	3.9± 0.09†††	6.91 ± 0.23***
G3	STZ+ Saponin fraction of MC(87.5mg/kg)	172.1 ±2.33**	76.53 ±2.37	126.4 ±1.71	43.34 ±2.24	1.89 ±0.27	990.83 ±4.84***	3.95 ±0.15	8.98 ± 0.29'
G4	STZ+ Saponin fraction of MC (175mg/kg)	131.63 ±1.09***	66.8 ±1.35***	73.18 ±1.84***	55.98 ±3.15***	1.29 ±0.76**	1115.0 ±17.87***	5.71 ±0.10**	13.45 ±0.12 '''
G5	STZ+ insulin (6 I.U)	122.13 ±1.23 ***	69.5 ±0.67***	67.0 ±1.17***	55.96 ±1.69***	1.18 ±0.38***	1074.66 ±5.60***	5.85 ±0.14***	14.91 ± 0.26'''

Values expressed as mean ±SEM for six animals

$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.

***p<0.001, **p<0.01, *p<0.05 when compared to STZ control group

*expressed as HMG CoA vs Mevalonate ratio

Observation:

- Saponins of MC also shows similar antidiabetic activity as that of MC
- Saponins have significantly decreased the HMG CoA reductase activity. Hence antihyperlipidemic activity of the drug may be by inhibiting HMG CoA reductase enzyme

Figure 1.1 Pictograms of rat cardiac sections showing the effect of MC (500 mg/kg p.o/day/30days) in Streptozotocin (65mg/kg i.p single dose) treated rats after 30 days of treatment (H & E =100), n=6

Fig1.1a. T S of normal heart

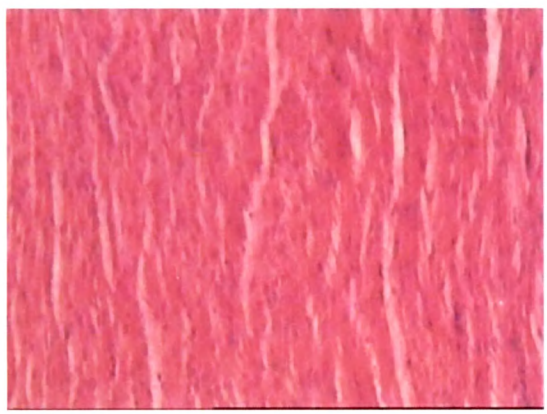


Fig 1.1b. T S of diabetic heart

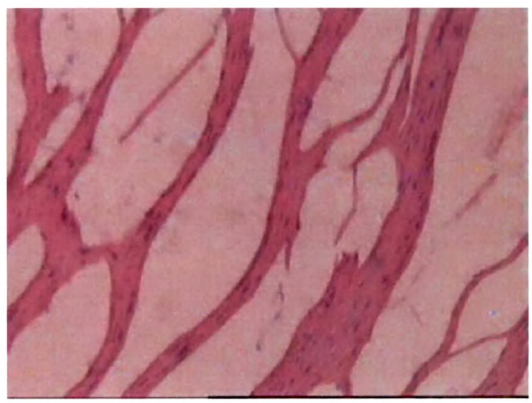


Fig1.1c. T S of MC (500 mg/kg) treated diabetic heart

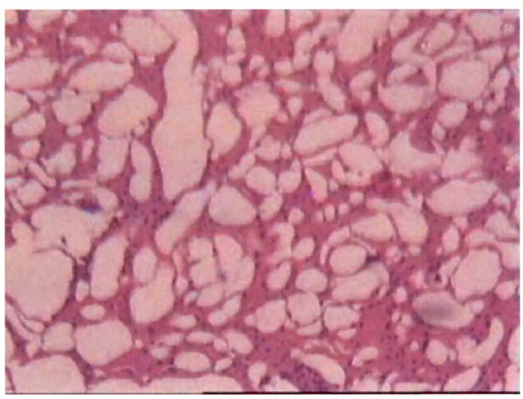


Fig1.1d. T S of Insulin(6U/kg) treated diabetic heart

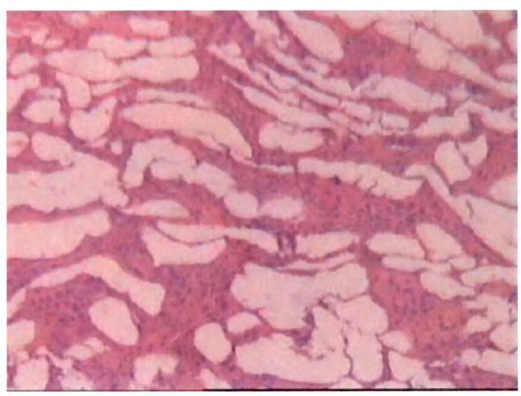
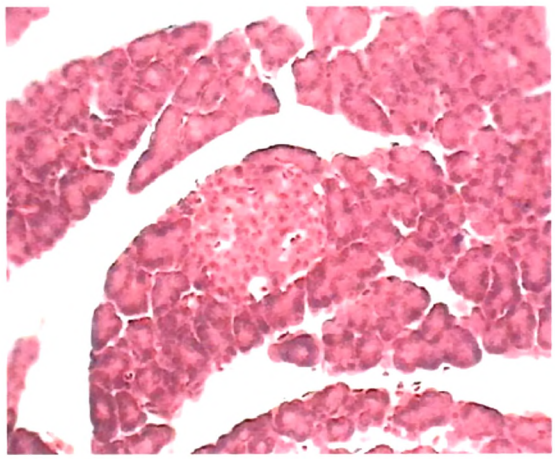
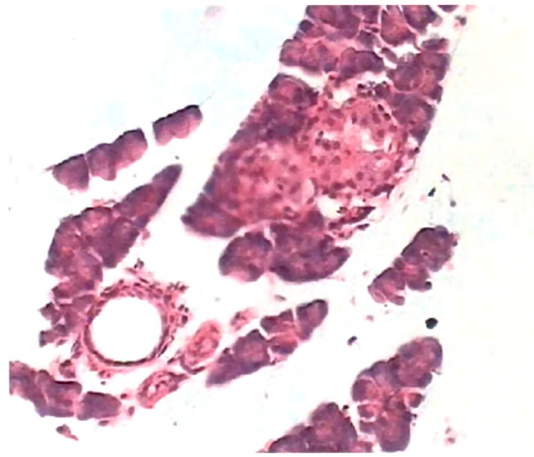


Figure 2.2 Pictograms of rat Pancreatic sections showing the effect of MC (500 mg/kg p.o/day/30days) in Streptozotocin (65mg/kg i.p/single dose) treated rats after 30 days of treatment (H & E =100), n=6

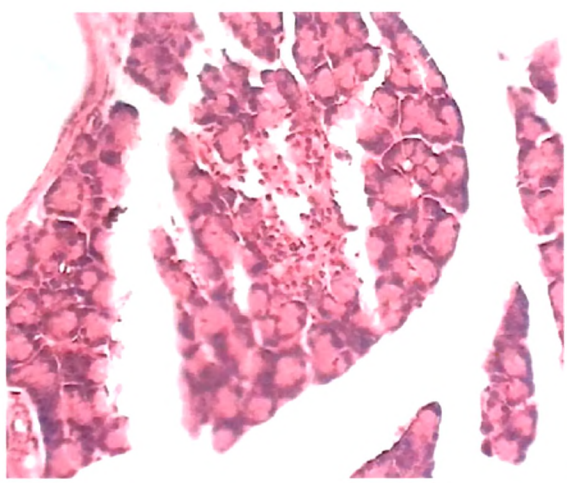
2.2a sections of pancreas of normal control rat



2.2b sections of diabetic control rat



2.2c sections of pancreas of MC (250mg/kg) treated diabetic rats



2.2d sections of pancreas of MC (500mg/kg) treated diabetic rats

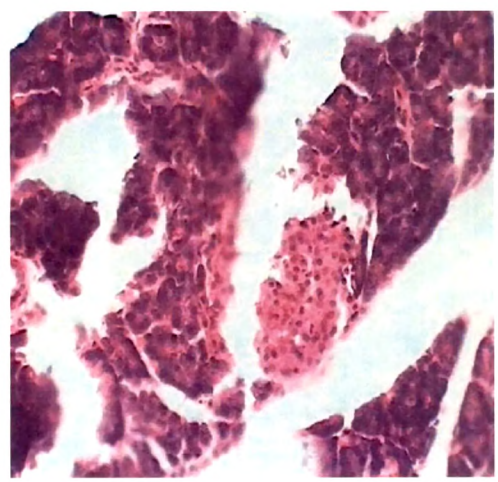
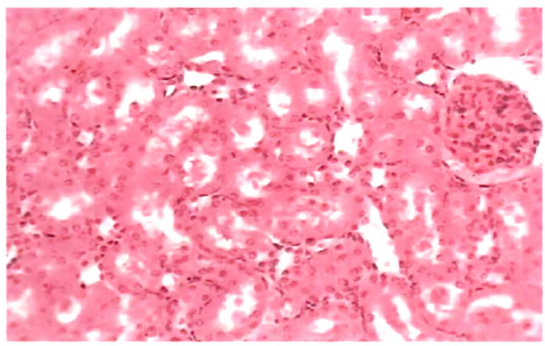
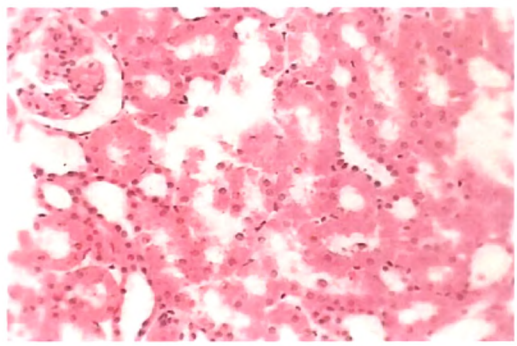


Figure 2.3 Pictograms of rat Cortex of Kidney sections showing the effect of MC (500 mg/kg p.o/day/30days) in Streptozotocin (65mg/kg i.p) treated rats after 30 days of treatment(H & E =100), n=6

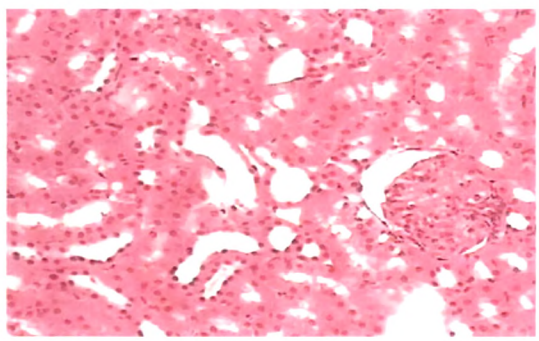
2.3a sections of kidney of normal control rat



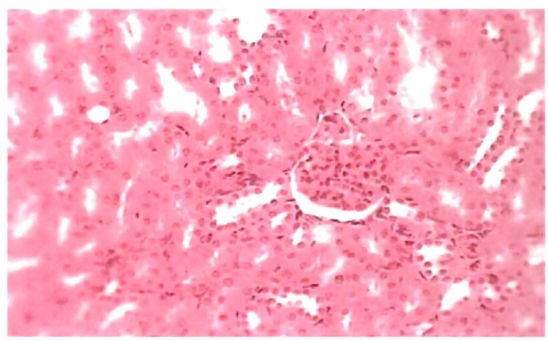
2.3b sections of kidney of diabetic control rat



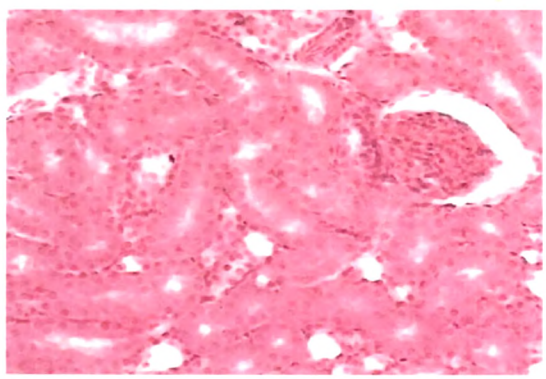
2.3c sections of kidneys of MC (250/kg) treated diabetic rats



2.3d sections of kidneys of MC (500mg/kg) treated diabetic rats



2.3d kidneys section of insulin (6 I.U/kg)treated diabetic rats



1.3 Effect of MD on Streptozotocin treated rats in serum Glucose, Insulin, Cholesterol, Triglyceride, Creatinine and hepatic glycogen after 30 days treatment

Groups	Treatment	Glucose (mg/dl)	Insulin (µu/ml)	Liver Glycogen (mg/gm of Tissue)	cholesterol (mg /dl)	Triglyceride (mg / dl)	Creatinine (mg/dl)
I.	Sodium citrate buffer (Control)	83.38 ±1.85	20.00 ± 0.13	17.22 ±0.12	120.85 ±0.21	120.56 ±0.13	1.02 ±0.26
II.	STZ-Control (65mg/kg, i.p/single dose)	282.36 ±2.08***	6.43 ± .06***	9.26 ±0.16***	240.12 ±0.28***	185.2 ±0.06***	2.22 ±0.16***
III.	STZ+ Insulin 6U/kg, i.p/day)	126.94 ±1.15'''	17.1 ± 0.68'''	15.38 ± 0.17'''	138.21 ±0.16'''	115.56 ±0.71'''	01.12 ±0.09'''
IV.	STZ+ MD (250mg/kg p.o/day)	214.06 ±4.28''	7.49 ± 0.12'	10.28 ± 0.12'	201.56 ±0.09'''	173.9 ±0.29'	1.98 ±0.21'
IV.	STZ+ MD (500mg/kg p.o./day)	155.5 ±4.30'''	10.18 ±0.07'''	11.51 ±0.13'	149.2 ±0.25'''	160.6 ±0.15''	1.45 ±0.41''

Values expressed as mean ±SEM, n=6,

***p<0.001 compared to normal control group.

'''p<0.001, 'p<0.05 compared to STZ control group.

Observation:

- Streptozotocin (65mg/kg i.p./single dose) has developed highly significant hyperglycemia
- MC (250 and 500mg/kg, p.o./day/30days) has significantly reduced elevated serum glucose, cholesterol, triglyceride, creatinine and BUN
- MC (500mg/kg p.o./ day/30days) has significantly increased the serum insulin level

Table 1.4 Effect of administration of saponins of MD (27.5 & 55 mg/kg, p.o,/day) in Streptozotocin treated rats on serum Glucose, Insulin, Cholesterol, Triglyceride, Creatinine and hepatic glycogen after 30 days treatment.

Groups	Treatment	glucose (mg/dl)	TG (mg/dl)	cholesterol (mg/dl)	HDL (mg/dl)	creatinine (µmol/l)	Liver glycogen (mg/100gm tissue)	HMG CoA activity*
G1	Sodium citrate buffer (Control)	83.8 ±1.984	64.33 ±1.22	55.68 ±1.80	42.1 ±1.49	1.12 ±0.44	1330.33 ±5.17	6.91 ±0.09
G2	STZ-Control (65mg/kg i.p.)	274.08 ±7.27†††	98.11 ±2.47†††	128.58 ±2.85†††	30.36 ±1.46††	2.45 ±0.93††	779.0 ±8.11†††	3.9 ±0.09†††
G3	STZ + Saponins MD (27.5mg/kg)	176.1 ±2.43**	81.53 ±2.37	126.4 ±1.71	40.34 ±2.24	1.99 ±0.27	890.13 ±4.84*	3.95 ±0.15
G4	STZ + Saponins MD (55 mg/kg)	141.63 ±1.19***	76.8 ±1.35***	76.11 ±1.84***	46.98 ±3.15***	1.39 ±0.76**	1195.0 ±14.87***	4.71 ±0.10**
G5	insulin (6 IU/kgs.c).	122.13 ±1.23***	69.5 ±0.67***	67.0 ±1.17***	45.96 ±1.69***	1.18 ±0.38***	1094.66 ±5.60***	5.85 ±0.14***

Values expressed as mean ±SEM for six animals

$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$when compared to normal control group.

***p<0.001, **p<0.01, *p<0.05 when compared to STZ control group.

*expressed as HMG CoA vs Mevalonate ratio

Observation:

- Saponins of MD also shows similar antidiabetic activity as that of MD
- Saponins have significantly decreased the HMG CoA reductase activity. Hence antihyperlipidemic activity of the drug may be by inhibiting HMG CoA reductase enzyme

Table 1.5 Effect of MC on fasting Plasma Triglyceride, Cholesterol, Glucose, and Insulin in rats fed with Fructose Rich Diet (FRD) after 15 days treatment

Groups	Treatment	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	Insulin (μU/ml)
I	Normal Diet (Control)	67.9 ±3.41	78.6 ±6.17	80.8 ±6.95	28.2 ±4.93
II	FRD-Control	298.8 ±15.60†††	134.6 ±4.65†††	126.8 ±9.41††	94.2 ±7.16†††
III	FRD + MC (250 mg/kg,p.o/day/15day)	101.4 ±4.53***	99.2 ±2.51***	94.6 ±7.26*	47.4 ±6.52**
IV	FRD + MC (500 mg/kg p.o/day/15days)	79.3 ±2.64***	89.4 ±3.35***	85.4 ±5.73**	23 ±8.78***

Values are in mean±SEM. n=06

* p<0.05, ** p<0.01, *** p<0.001 when compared with FRD control

† p<0.05, ††† p<0.001 when compared with normal control

Observation:

- Fructose rich diet has significantly increased the serum insulin and glucose level and thus has developed an insulin resistant state
- High fructose diet had also developed hypercholesteremic and hypertriglyceridemic state
- MC (250 & 500mg/kg p.o/day/15days) has decreased both insulin and glucose level
- MC (250 & 500mg/kg p.o/day/15days) has also decrease triglyceride and cholesterol levels

Table 1.6 Effect of saponins of MC in fasting Plasma Triglyceride, Cholesterol, Glucose, Insulin and HMG CoA reductase on rats fed with Fructose Rich Diet (FRD) after 15 days treatment

Groups	Treatment	glucose (mg/dl)	TGS (mg/dl)	Cholesterol (mg/dl)	Insulin (mU/l)	HMG CoA reductase*
G1	Normal Diet (Control)	80.06 ±0.90	64.5 ±1.67	62.56 ±1.06	28.5 ±1.19	3.11 ±0.03
G2	FRD-Control	133.45 ±1.06†††	319.23 ±3.62†††	132.8 ±1.52†††	69.25 ±1.10†††	2.03 ±0.02†††
G3	FRD + Saponins MC(87.5 mg/kg p.o)	125.55 ±1.77*	145.45 ±1.84***	113.75 ±1.48**	63.0 ±0.70	1.98 ±0.02
G4	FRD + Saponins MC(175 mg/kg p.o)	98.23 ±2.99***	84.28 ±1.70***	82.01 ±1.50***	37.75 ±1.93***	2.44 ±0.07**
G5	FRD + Metformin (200 mg/kg p.o)	88.7 ±2.57***	78.78 ±1.78***	72.4 ±2.61***	33.25 ±0.75***	2.99 ±0.02***

Values expressed as mean ±SEM for six animals,

$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.

$^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared to FRD control group

*HMG CoA vs mevalonate

Observation:

- Saponins of MC also have insulin sensitizing activity like that of MC
- The antihyperlipidemic activity in this model also may be by inhibiting HMG CoA reductase enzyme

Table 1.7 Effect of MD on fasting Plasma Triglyceride, Cholesterol, Glucose, and Insulin in rats fed with fructose rich diet (FRD) after 15 days treatment

Groups	Treatment	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	Insulin (μU/ml)
I	Normal diet (Control)	63.9 ±3.41	84.6 ±6.17	85.8 ±2.90	24.2 ±3.93
II	FRD-Control	262.8 ±11.60†††	144.6 ±3.65†††	136.8 ±5.41††	114.2 ±7.16†††
III	FRD + MD (250 mg/kg p.o)	171.4 ±5.53***	101.2 ±3.51***	104.6 ±4.26*	57.4 ±3.52**
IV	FRD + MD (500 mg/kg p.o)	119.3 ±3.64***	99.4 ±2.15***	95.4 ±3.73**	32 ±2.78***

Values are in mean±SEM. n=06

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared with FRD control

† $p<0.05$, ††† $p<0.001$ when compared with normal control

Observation:

- Fructose rich diet significantly increased the serum insulin and glucose level and thus has developed an insulin resistant state
- High fructose diet had also developed hypercholesteremia and hypertrigylceridemia state
- MD (250 & 500mg/kg p.o/day/15days) has decreased both insulin and glucose level
- MD(250 & 500mg/kg p.o/day/15days) has also decrease triglyceride and cholesterol levels

Table 1.8 Effect of saponins of MD on fasting Plasma Triglyceride, Cholesterol, Glucose, Insulin and HMG CoA reductase in rats fed with fructose rich diet (FRD) after 15 days treatment

Groups	Treatment	Serum glucose (mg/dl)	Serum TGS (mg/dl)	Total Cholesterol (mg/dl)	Serum Insulin (mU/l)	HMG CoA reductase*
G1	Normal diet(Control)	80.06 ±0.90	64.5 ±1.67	62.56 ±1.06	28.5 ±1.19	3.11 ±0.03
G2	FRD-Control	133.45 ±1.06†††	319.23 ±3.62†††	132.8 ±1.52†††	69.25 ±1.10†††	2.03 ±0.02†††
G3	FRD + Saponin MD(27.5mg/kg p.o/day15days)	129.55 ±1.97*	155.45 ±1.94***	123.75 ±1.48**	64.11 ±0.70	1.58 ±0.02
G4	FRD + Saponin MD(55mg/kg p.o/day15days)	99.43 ±1.99***	86.28 ±1.90***	89.01 ±1.50***	39.75 ±1.93***	2.49 ±0.07*
G5	FRD + Metformin (200 mg/kg)	88.7 ±2.57***	78.78 ±1.78***	72.4 ±2.61***	33.25 ±0.75***	2.99 ±0.02**

Values expressed as mean ±SEM n=6,

$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.

$^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared to FRD control group

*HMG CoA vs mevalonate

Observation:

- Saponins of MD also have insulin sensitizing activity like that of MD
- The antihyperlipidemic activity in this model also seems to be by inhibiting HMG CoA reductase enzyme

3.1. Effect of MC on Serum Cholesterol, Triglyceride, HDL, LDL, VLDL and Glucose after 26 days Atherogenic Diet (AD) fed rats

Gp no	Treatment	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Glucose (mg/dl)
I	Normal Control	59.00 ± 3.67	63.00 ± 3.86	53.40 ± 4.56	23.20 ± 3.83	16.80 ± 3.10	87.00 ± 6.92
II	AD control	175.80 ± 4.80***	136.80 ± 5.20***	14.00 ± 2.00***	81.00 ±7.89***	36.40 ± 4.97***	106.00 ± 4.78***
III	Atherogenic diet + Lovastatin(6mg/kg p.o/day/26days)	128.20 ± 6.30†††	95.40 ± 2.01†††	38.40 ± 2.13†††	60.60 ± 1.72	22.20 ± 4.39†††	89.80 ± 2.74†††
IV	Atherogenic diet + MC (250 mg/kg p.o/day/26days)	131.20 ± 6.60†††	92.40 ± 5.32†††	38.80 ± 4.91†††	52.40 ± 2.97†††	17.20 ± 4.0†††	90.00 ± 1.87†††
V	Atherogenic diet + MC (500 mg/kg p.o/day/26days)	103.40 ± 5.10†††	84.80 ± 3.20†††	42.80 ± 3.89†††	47.00 ± 3.57††	19.20 ± 2.65†††	86.40 ± 3.57†††
VI	Lovastatin (6mg/kg p.o/day/26days)	49.60 ± 5.15	47.20 ± 6.07	66.60 ± 6.72	25.40 ± 3.75	9.60 ± 2.61	71.00 ± 5.31
VII	MC (250mg/kg p.o/day/26days)	50.4 ± 3.41	55 ± 3.4	51.6 ± 5.15	27.2 ± 3.89	9.4 ± 2.42	83.2 ±4.86
VIII	MC (500 mg/kg p.o/day/26days)	47.4 ± 5.74	45.6 ± 7.0	54 ± 7.24	26.6 ± 3.82	10.00 ± 2.42	75.2 ± 3.18

Values are expressed as mean ± *SEM* n= 6
*** p<0.001 compared to normal control
††† p<0.001, †† p<0.01 compared to AD control

Observation

- MC (500mg/kg) has significantly decreased serum triglyceride, Cholesterol, LDL VLDL and glucose in atherogenic diet induced hyperlipidemic rats
- MC (500mg/kg) has not affected the serum lipid levels of normal rats

3.2. Effect of MD on serum Cholesterol, Triglyceride, HDL, LDL, VLDL and Glucose after 26 days Atherogenic Diet (AD) fed rats

Gp no	Treatment	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Glucose (mg/dl)
I	Normal Control	69.00 ± 3.67	65.00 ± 3.86	53.40 ± 4.56	23.20 ± 3.83	16.80 ± 3.10	87.00 ± 6.92
II	AD control	185.80 ± 4.80***	144.80 ± 4.20***	13.00 ± 2.00***	91.00 ±7.89***	39.40 ± 4.97***	136.00 ± 4.78***
III	AD+ Lovastatin (6mg/kg p.o/day/26days)	118.20 ± 5.30†††	91.40 ± 2.01†††	39.40 ± 2.13†††	50.60 ± 1.42	21.20 ± 4.39	99.80 ± 2.74†††
IV	AD+ MD (250 mg/kg p.o/day/26days)	141.20 ± 6.60†††	112.40 ± 5.32†††	30.80 ± 4.91†††	82.40 ± 2.97†††	24.20 ± 4.00††	119.00 ± 1.87†††
V	AD + MD (500 mg/kg p.o/day/26days)	113.40 ± 4.10††	104.80 ± 3.20†††	38.80 ± 3.89†††	67.00 ± 3.47††	21.10 ± 2.65†††	102.40 ± 3.57†††
VI	Lovastatin (6mg/kg p.o/day/26days)	49.60 ± 5.15	57.20 ± 6.07	67.60 ± 6.72	25.40 ± 3.75	9.60 ± 2.61	71.00 ± 5.31
VII	MD (250mg/kg p.o/day/26days)	61.4 ± 3.41	65 ± 3.4	52.6 ± 5.15	23.2 ± 3.89	9.9 ± 2.42	88.2 ±4.86
VIII	MD (500 mg/kg p.o/day/26days)	57.4 ± 5.74	63.6 ± 7.0	55.1 ± 7.24	23.6 ± 3.82	10.01 ± 2.42	79.2 ± 3.18

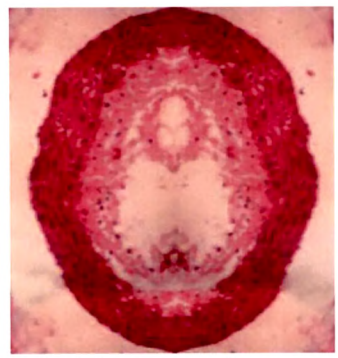
Values are expressed as mean ± *SEM* N= 6;
*** $p<0.001$ compared to normal control
††† $p<0.001$, †† $p<0.01$ compared to Atherogenic diet control

Observation:

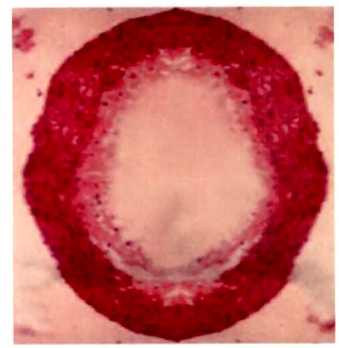
- MD (500mg/kg) has significantly decreased serum triglyceride, Cholesterol, LDL VLDL and glucose in atherogenic diet induced hyperlipidemic rats
- MD (500mg/kg) has not affected the serum lipid levels of normal rats

Fig 3.1 Pictograms of Rat Aortic Sections showing the effect of MC (250 & 500 mg/kg p.o/day/26days) after 26 days atherogenic diet fed rats

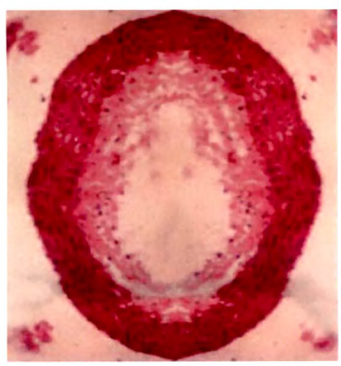
3.1a Normal control group



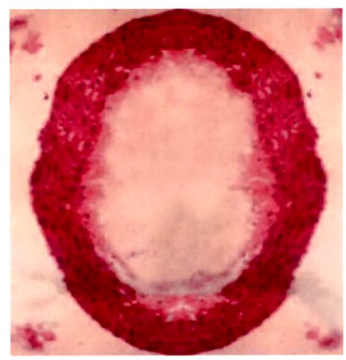
3.1b Atherogenic diet control group



3.1c Lovastatin 6 mg/kg in atherogenic diet treated group



3.1d Momordica cymbalaria 250mg/kg in Atherogenic diet treated group



3.1e Momordica cymbalaria 500mg/kg in Atherogenic diet treated group

Observation:

- Transverse section of the aorta in the atherogenic diet group exhibited thickening of vascular wall of aortic musculature with fatty tissue and formation of neointima containing vascular smooth muscle cells of tunica media that indicates atherosclerotic plaque formation.
- MC (500mg/kg) did not have formation of neointima containing vascular smooth muscle cells of tunica media and almost normal architecture.

Table 3.3 Effect of saponin fraction of MC (175 mg/kg, p.o./day/35days) on serum Triglyceride, Cholesterol, LDL, VLDL and HMG CoA reductase activity after High Cholesterol Diet (HCD) fed rabbits for 35 days.

Groups	Treatment	Serum TC	Serum TG	Serum LDL-C	Serum VLDL-C	HMG CoA reductase*
1.	Standard Rabbit Chow(Control)	53.53 ±5.39	50.07 ±2.84	20.26 ±4.22	10.06 ±1.21	3.60 ±0.038
2.	HCD-Control	557.07 ±21.74***	344.78 ±12.10***	420.92 ±19.20***	65.03 ±1.12***	2.53 ±0.06***
3.	HCD + Saponin of MC (175mg/kg p.o/day/35days)	124.02 ±5.85†††	109.49 ±4.60†††	58.94 ±7.10†††	21.66 ±0.74†††	2.86 ±0.03††
4.	HCD + Lovastatin (6 mg/kg p.o/day/35days)	100.90 ±5.44†††	85.28 ±2.83†††	48.20 ±7.52†††	17.64 ±0.91†††	3.26 ±0.03†††
5.	SRC + Lovastatin (6 mg/kg p.o/day/35days)	40.63 ±2.78	47.06 ±3.56	12.15 ±1.86	9.87 ±0.83	4.12 ±0.06
6.	SRC + Saponin of MC (175 mg/kg p.o/day/35days)	47.02 ±4.81	49.46 ±3.31	14.02 ±2.24	9.95 ±1.47	3.91 ±0.03

Values expressed as mean ±SEM for six animals,
$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.
***p<0.001, **p<0.01, *p<0.05 when compared to HCD Control group.
*HMG CoA vs mevalonate

Observation:

- Serum cholesterol that had increased 10 times upon cholesterol rich diet decreased significantly (***P<0.001) upon saponins of MC treatment.
- HMG CoA activity inhibited significantly upon saponins of MC treatment but the inhibitory activity was less than lovastatin.

Table 3.4 Effect of saponin fraction of MD (55 mg/kg, p.o.,/day/35days) on serum Triglyceride, Cholesterol, HDL, LDL, VLDL and HMG CoA reductase activity after High Cholesterol Diet (HCD) fed rabbits for 35 days.

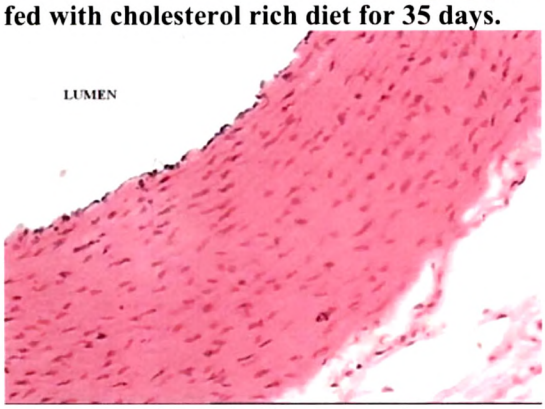
Groups	Treatment	Serum TC	Serum TG	Serum HDL-C	Serum LDL-C	Serum VLDL-C	HMG CoA reductase*
1	Standard Rabbit Chow (Control)	53.53 ±5.39	50.07 ±2.84	28.00 ±1.31	20.26 ±4.22	10.06 ±1.21	3.60 ±0.038
2	HCD-Control	557.07 ±21.74***	344.78 ±12.10***	67.36 ±3.06***	420.92 ±19.20***	65.03 ±1.12***	2.53 ±0.06***
3	HCD + Saponin of (MD 55 mg/kg p.o/day/35days)	156.00 ±2.67†††	190.67 ±4.34†††	57.31 ±0.84†	61.17 ±1.40†††	38.13 ±0.81†††	2.55±0.010
4	HCD + Lovastatin 6 mg/kg p.o/day/35days	100.90 ±5.44†††	85.28 ±2.83†††	35.64 ±2.78†††	48.20 ±7.52†††	17.64 ±0.91†††	3.26 ±0.03†††
5	SRC + Lovastatin 6 mg/kg p.o/day/35days)	40.63 ±2.78	47.06 ±3.56	19.15 ±2.028	12.15 ±1.86	9.87 ±0.83	4.12 ±0.06
6	SRC + Saponin of MD 55 mg/kg .p.o/day/35days	56.93±2.94	59.25±0.13	28.48±0.8	13.54±0.80	11.84±0.24	3.613±0.009

Values expressed as mean ±SEM, n=6,
$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, $^{\dagger}p<0.05$ when compared to normal control group.
***p<0.001, **p<0.01, *p<0.05 when compared to HCD control group
*HMG CoA vs mevalonate

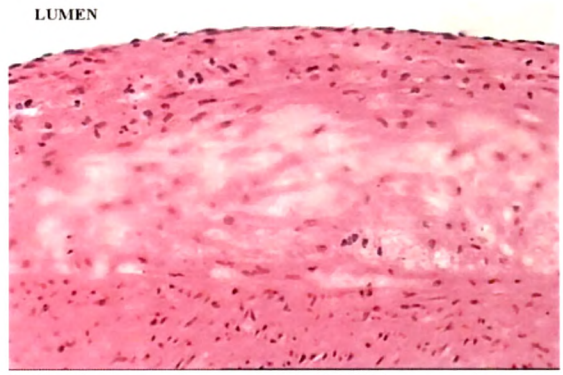
Observation:

- Serum cholesterol that had increased 10 times upon cholesterol rich diet decreased significantly (***p<0.001) upon saponins of MD treatment.
- HMG CoA activity inhibited significantly upon saponins of MD treatment but the inhibitory activity was less than lovastatin.

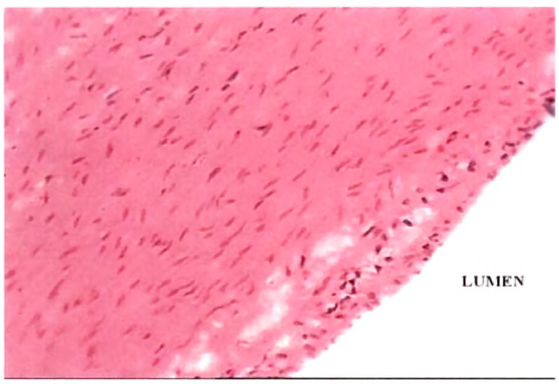
Fig: 3.2 Pictograms of rabbit aortic sections showing the effect of saponin fractions of MC&MD (175 & 55mg/kg p.o/day/35days respectively) after rabbits fed with cholesterol rich diet for 35 days.



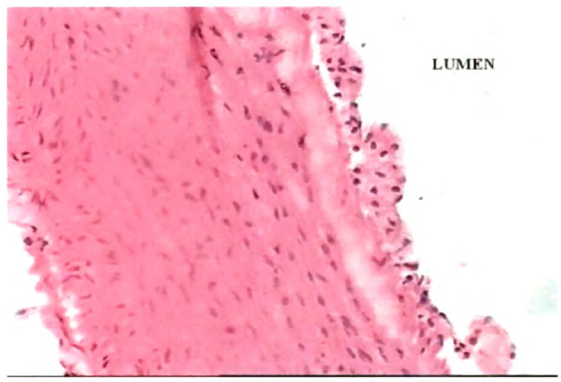
3.1a Standard Rabbit Chow control group



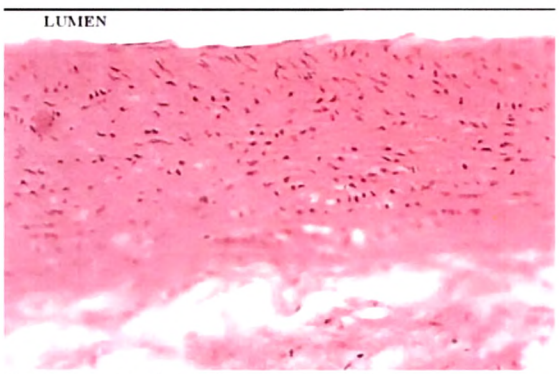
3.1b High Cholesterol diet(HCD) control group



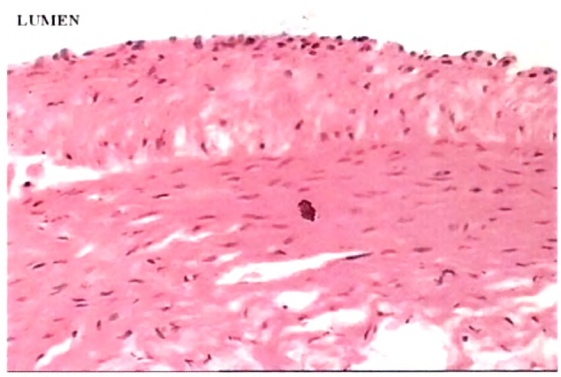
HCD + Saponins of MC (175mg/kg po)



HCD + Lovastatin (6mg/kg)



SRC + saponins of MC(175mg/kg)



HCD + Saponins of MD (55mg/kg po)

Observations: HCD control group showed thickening of vascular wall of aortic musculature with fatty tissue. Saponins of MC treatment showed mild formation of neointima containing vascular smooth muscle cells of tunica media and almost there is an appearance of normal architecture.

Effect on Cardiovascular Disorders...

4.2.1.7 Effect of MC or MD (10mg/kg, i. v) on vascular reactivity

Pressor response to Adr (1µg/kg, i.v), NA (1µg/kg, i.v) and PE (1µg/kg, i.v) significantly ($p<0.001$) decreased after MC (10mg/kg, i.v) administration as compared to control response (before MC10mg/kg, i.v, administration). Pressor response was not altered significantly to ANGII (1µg/kg/i.v) and depressor response to Iso (1µg/kg/i.v), 5-HT (1µg/kg/i.v) and histamine (1µg/kg/i.v) were not altered significantly after administration of MC (10mg/kg, i.v) (Fig1, 2).

Pressor response to Adr (1µg/kg/i.v), NA (1µg/kg/i.v), PE (1µg/kg/i.v), ANGII (1µg/kg/i.v) and depressor response to Iso (1µg/kg/i.v), 5-HT (1µg/kg/i.v)and histamine (1µg/kg/i.v) was not altered significantly after MD (10mg/kg) as compared to control response (before MD 10mg/kg, i.v, administration) (Fig 3).

4.2.1.8 Effect on systolic blood pressure in HFD induced hypertensive rats by non invasive (tail cuff) method

HFD induced a significant ($p<0.001$) rise in systolic blood pressure in male Wister rats when compared to normal control rats. HFD induced hypertensive rats treated with MC (500mg/kg p.o/5 weeks) showed a significant ($p<0.05$) decrease in the systolic blood pressure.

HFD induced hypertensive rats treated with MD (500mg/kg p./5 weeks) did not show significant change in the systolic blood pressure when compared to normal control rats. (Fig 4).

4.2.1.9 Cardiopretective activity in Isoproterenol - myocardial infarction rats

Effect of pretreatment with MC or MD for 45 days followed by Isoproterenol (ISO) (60mg / kg s.c./day/2days) on serum AST, ALT, LDH, ALP, CPK, Cholesterol, Triglyceride, HDL, LDL, VLDL uric acid levels in heart and it's histopathology in rats

Effect on AST: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased AST level when compared with normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45days respectively) showed a significant ($p<0.001$) reduction in the serum AST level in ISO rats when compared to ISO control rats.

Effect on ALT: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased ALT level when compared to normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45 days respectively) showed a significant ($p<0.001$) reduction in the serum AST level in ISO rats when compared to ISO control rats.

Effect on LDH: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.01$) increased LDH level when compared to normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45days respectively) showed a significant ($p<0.05$) reduction in the serum LDH level in ISO rats when compared to ISO control rats.

Effect on ALP: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased ALP level when compared to normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.001$) reduction in the serum ALP level in ISO rats when compared to ISO control rats.

Effect on CPK: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased CPK level when compared to normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/60days respectively) showed a significant ($p<0.001$) reduction in the serum CPK level in ISO rats when compared to ISO control rats.

Effect on Uric Acid: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased uric acid level when compared to normal control rats. Pretreatment with MC (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.05$& $p<0.001$ respectively) reduction in the serum uric acid level in ISO rats when compared to ISO control rats. Pretreatment with MD (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.05$& $p<0.001$respectively) reduction in the serum uric acid level when compared to ISO control rats.

Effect on Cholesterol: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased Cholesterol level when compared to normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.001$) reduction in the serum Cholesterol level in ISO rats when compared to ISO control rats.

***Effect on* Triglycerides:** Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased serum triglyceride level when compared to

normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.001$) reduction in the serum Triglyceride level in ISO rats when compared to ISO control rats.

Effect on HDL: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.05$) increased HDL level when compared with normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.05$) reduction in the serum HDL level in ISO rats when compared to ISO control rats.

Effect on LDL: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased LDL level when compared to normal control rats. Pretreatment with MC or MD (250 & 500 mg/kg, p.o/day/60days) showed a significant ($p<0.001$) reduction in the serum LDL level in ISO rats when compared to ISO control rats.

Effect on VLDL: Administration of ISO (60mg/kg s.c/day/2days) to Wister rats significantly ($p<0.001$) increased VLDL level when compared with normal control rats. Pretreatment with MC (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.05$& $p<0.001$) reduction in the serum VLDL level when compared to ISO control rats. Pretreatment with MD (250 & 500 mg/kg, p.o/day/45days) showed a significant ($p<0.05$& $p<0.001$) reduction in the serum VLDL level in ISO rats when compared to ISO control rats.

Effect on heart (histopathology): The cardiac sections of the ISO control group revealed degenerative changes in the muscle fiber, showing a coagulative necrosis characterized by more homogenous esocinophillic cytoplasm. The nuclei of myofibril revealed pyknotic nucleus. Interstitial edema was present in the connective tissue spaces. Pretreatment with MC or MD (250/500 mg/kg, p.o/day/45days) showed a normal myofibrillar structures with striations.

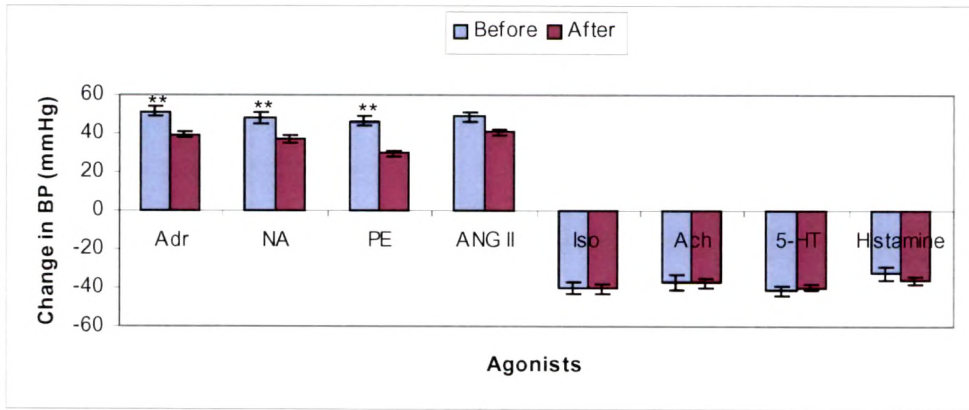


Figure 7.1: Effect of MC (10mg/kg/i.v) on vascular reactivity to various agonists in normotensive rats. Changes in systolic blood pressure (mmHg) before and after the administration of MC (10mg/kg/i.v) to Adr (1µg/kg/i.v), NA (1µg/kg/i.v), PE(1µg/kg/i.v), ANGII(1µg/kg/i.v), Iso (1µg/kg/i.v), 5-HT(1µg/kg/i.v)and Histamine(1µg/kg/i.v). Values are mean ± SEM. n=6, ** $p<0.01$ when compared with control.

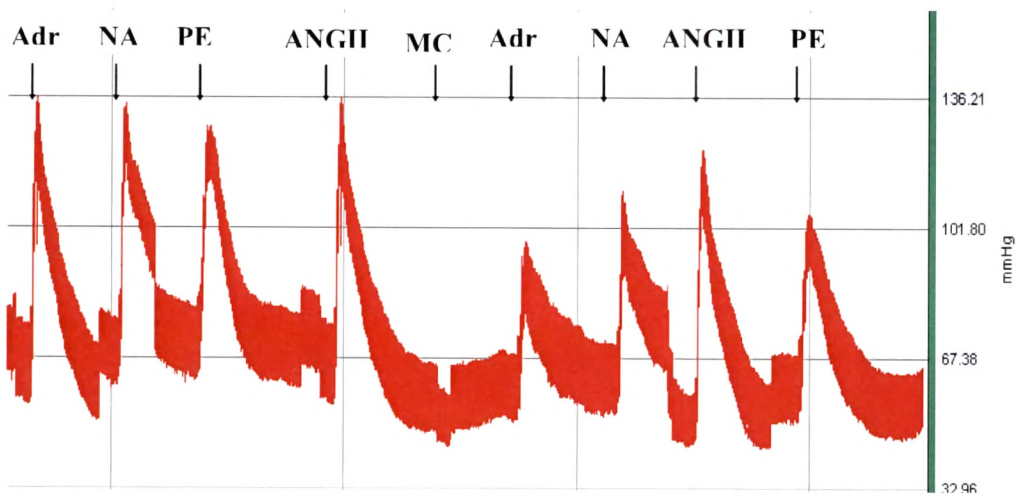


Figure 7.2: Tracings of the arterial blood pressure of anaesthetized rat (urethane 120mg/kg/i.p) using BIPOAC DATA acquisition system after and before administration of MC (10mg/kg/i.v).

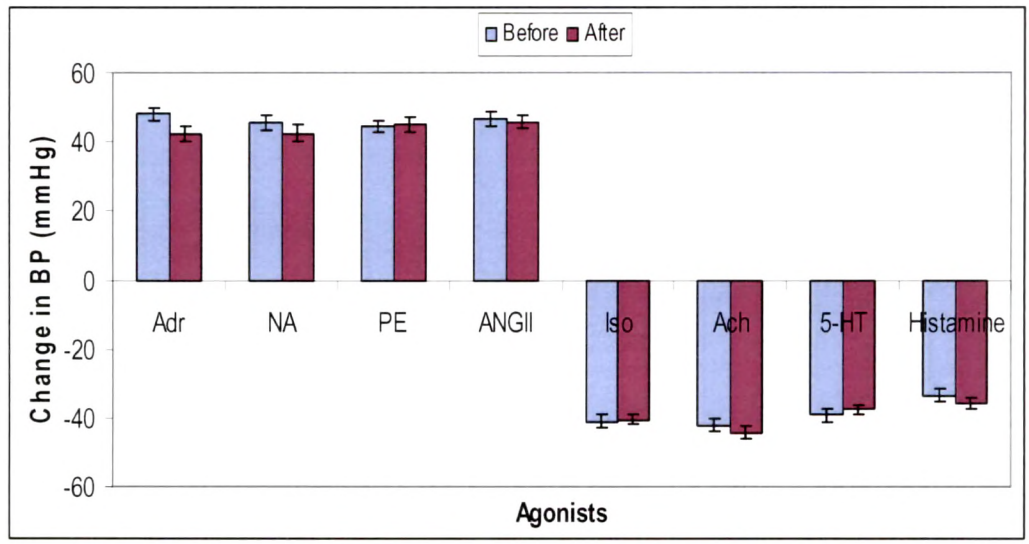


Figure 7.3: Effect of MD (10mg/kg/i.v) on vascular reactivity to various agonists in normotensive rats. Changes in systolic blood pressure (mmHg) before and after the administration of MD (10mg/kg/i.v) to Adr (1µg/kg/i.v), NA (1µg/kg/i.v), PE(1µg/kg/i.v), ANGII(1µg/kg/i.v), Iso (1µg/kg/i.v), 5-HT(1µg/kg/i.v)and Histamine(1µg/kg/i.v).

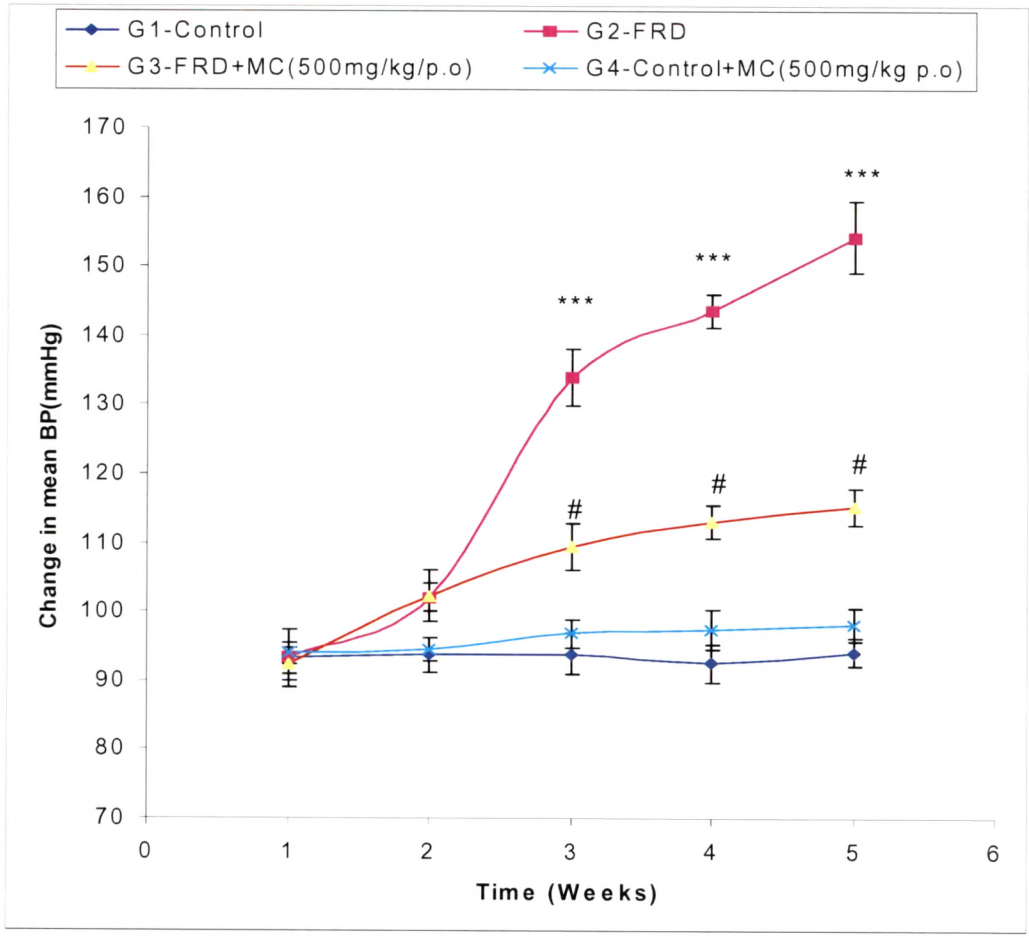


Figure 8.1:Time course of changes of mean systolic blood pressure (mmHg) during 5 weeks in control, FRD, FRD+ MC 500mg/kg p.o./5weeks)and control rats +MC 500mg/kg p.o./5weeks) $^{\#}$ $p<0.05$ when compared to FRD control, $^{***}p<0.001$ when compared to control rats. Vertical lines represents SEM. n=6.

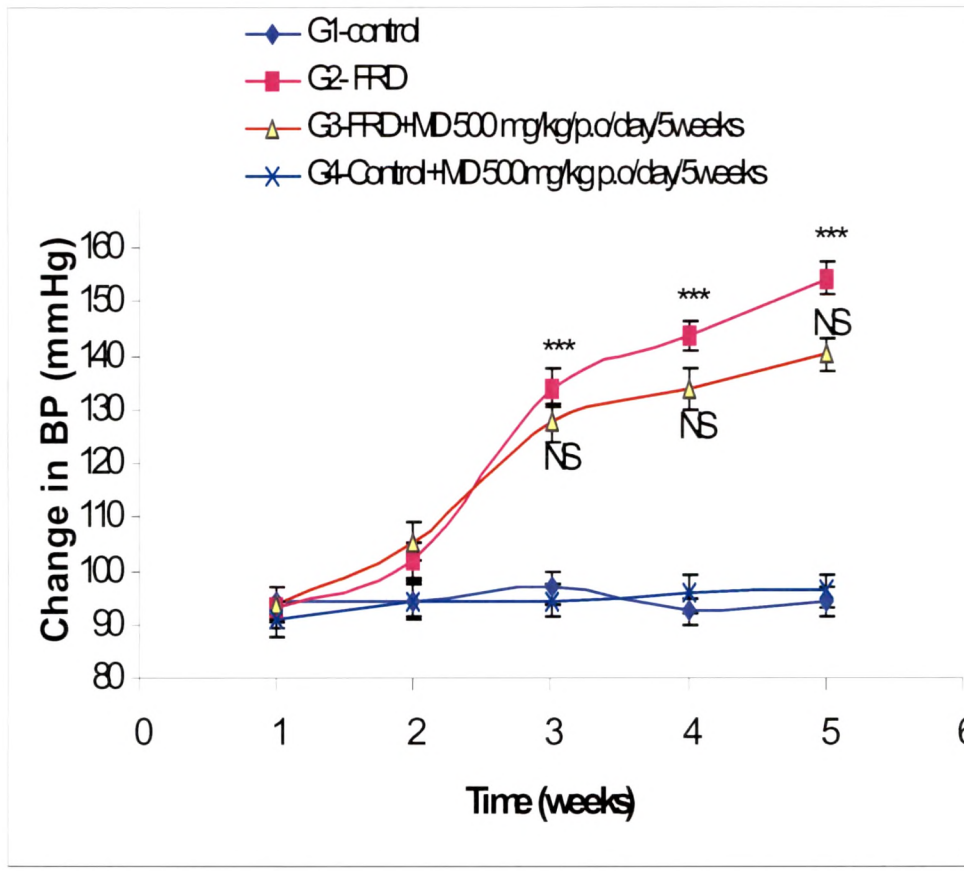


Figure 8.2: Time course of changes of mean systolic blood pressure (mmHg) during 5 weeks in control, FRD, FRD+ MD (500mg/kg p.o./5weeks),and control rats +MD (500mg/kg p.o./5weeks), $^{***}p<0.001$ when compared to control rats, NS –Not Significant. Vertical lines represent SEM n=6.

Table 9.1.Effect of pretreatment with MC or MD for 45 days followed by Isoproterenol (ISO) (60mg / kg s.c./day/2days)on serum AST, ALT, LDH, ALP, CPK and uric acid levels in rats

Group	Treatment	AST U/L	ALT U/L	LDH U/L	ALP U/L	CPK U/L	Uric acid mg / dl
I	Normal control	75.91 ± 1.82	27.68 ± 0.86	48.03 ± 1.48	113.32 ± 2.22	260.07 ± 2.45	6.11 ±0.72
II	ISO- control	171.76 ± 2.81$^{\#\#\#}$	57.87 ±0.45$^{\#\#\#}$	58.15 ± 2.84$^{\#\#}$	197.21 ± 3.03$^{\#\#\#}$	324.57 ± 2.08$^{\#\#\#}$	10.51 ±0.28$^{\#\#\#}$
III	ISO+MC (250mg/kg p.o.)	123.64 ± 2.01***	38.86 ± 1.23***	51.84 ± 2.19*	150.54 ± 2.79***	281.4 ± 3.13***	7.75 ±0.84*
IV	ISO+MC (500mg/kg p.o.)	78.5 ±1.66***	30.22 ± 1.49***	49.34 ± 1.08*	116.37 ± 3.66***	274.5 ± 2.14***	6.26 ±0.45***
V	ISO+MD (250mg/kg p.o)	123.64 ± 2.01***	41.37 ± 1.87***	53.15 ± 1.16*	150.54 ± 2.79***	281.4 ± 3.13***	7.75 ±0.84*
VI	ISO+MD (500mg/kg p.o	86.12 ±2.23***	34.73 ± 1.28***	51.16 ± 1.72*	116.37 ± 3.66***	274.5 ± 2.14***	6.26 ±0.45***

Values expressed as mean ±SEM, n=6

$^{\#\#\#}p<0.001$,$^{\#\#}p<0.0$,compared to group normal control

$^{***}p<0.001$,$^{*}p<05$, compared to ISO control

Table 9.2 Effect of pretreatment with MC or MD for 45 days followed by Isoproterenol (ISO) (60mg / kg s.c./day/2days)on serum Cholesterol, Triglyceride, HDL,VLDL in rats

Group	Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Normal control	120.39 ±2.18	139.46 ±2.28	42.92 ±1.14	80.19 ±1.12	40.82 ±1.86
II	ISO- control	205.8 ±3.56†††	177.20 ±2.51†††	36.22 ±1.63†	109.10 ±2.34†††	58.16 ±1.72†††
III	ISO+MC (250mg/kg p.o)	141.87 ±2.67***	152.94 ±1.93***	38.36 ±1.18*	86.66 ±1.02***	50.32 ±1.23**
IV	ISO+MC (500mg/kg p.o)	122.38 ±1.04***	141.16 ±1.39***	41.28 ±1.83*	80.80 ±1.82***	41.63 ±1.12***
V	ISO+MD (250mg/kg p.o)	149.17 ±2.37***	162.94 ±1.93***	34.16 ±1.18*	96.66 ±1.02***	53.32 ±1.23*
VI	ISO+MD (500mg/kg p.o)	129.48 ±2.04***	144.16 ±1.79***	40.28 ±1.33*	87.50 ±1.32***	43.63 ±1.42***

Values expressed as mean ±SEM, n=6

$^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger}p<0.01$, compared to normal control group

$^{***}p<0.001$, $^{*}p<0.05$, compared to ISO group

Figure 9.3 Effect of pretreatment with MC&MD(500mg/kg p.o/day/45days) followed by Isoproterenol (ISO) (60mg / kg s.c./day/2days)on the histological pictures of cardiac sections(H x E 40X)

Fig 1a. control group

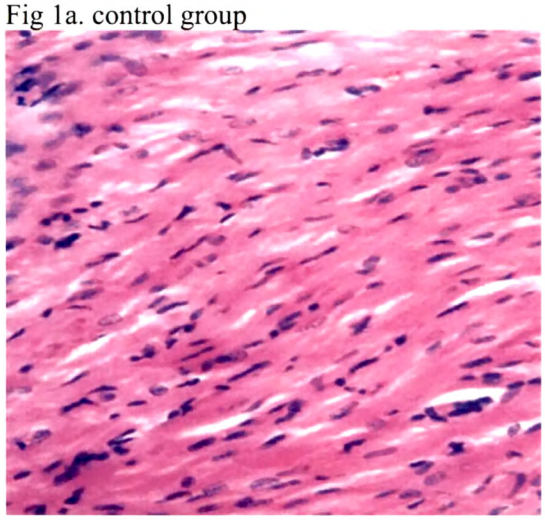


Fig 1b. ISO control group

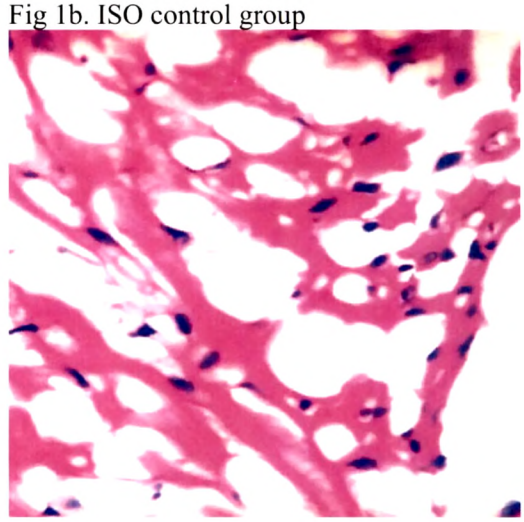


Fig 1c. ISO + MC(500mg/kg p.o/day/45days) treated group

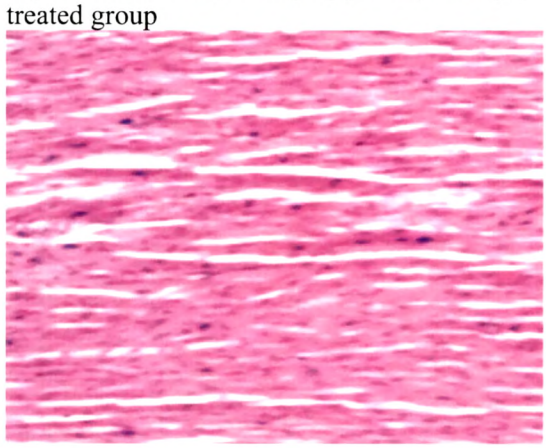
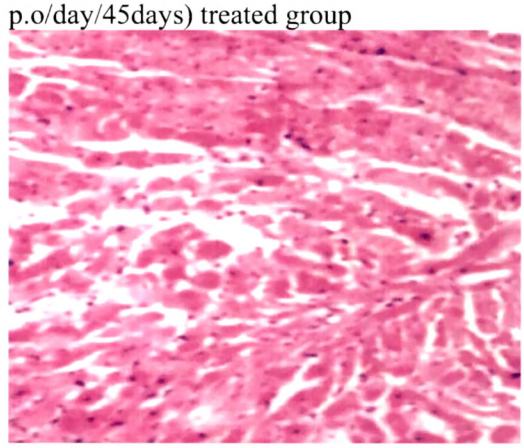
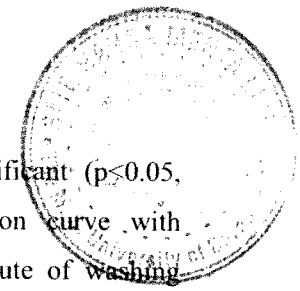


Fig 1d. ISO + MD(500mg/kg p.o/day/45days) treated group



Effect on Isolated Tissues...



4.2.1.10 Effect on isolated rat's aortic strip

MC (10µg /ml, 30µg/ml and 100µg/ml) displayed a significant ($p<0.05$, $p<0.01$, $p<0.01$ respectively) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No10.1).

The MD (10µg /ml, 30µg /ml and 100µg /ml) displayed significant ($p<0.05$) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No 10.2).

Saponins of MC (1µg /ml, 3µg /ml and 10µg /ml) displayed significant ($p<0.05$, $p<0.01$, $p<0.01$ respectively) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No10.3).

Saponins of MD (1µg /ml, 3µg /ml and 10µg /ml) displayed significant ($p<0.05$) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No10.4).

4.2.1.11 Effect on rat's isolated anconccygeous muscle

MC (100µg/ml, 200µg/ml and 400µg/ml) displayed significant ($p<0.05$, $p<0.01$, $p<0.01$ respectively) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No11.1).

MD (100µg/ml, 200µg/ml and 400µg/ml) displayed significant ($p<0.05$) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No11.2).

Saponin fractions of MC (10µg/ml, 20µg/ml and 40µg/ml) displayed significant ($p<0.05$, $p<0.01$, $p<0.01$ respectively) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No11.3).

Saponin fractions of MD (10µg/ml, 20µg/ml and 40µg/ml) displayed significant ($p<0.05$) rightward shift of NA concentration curve with suppression of maxima. The effect was not reversed after 30 minute of washing period (Fig No11.4).

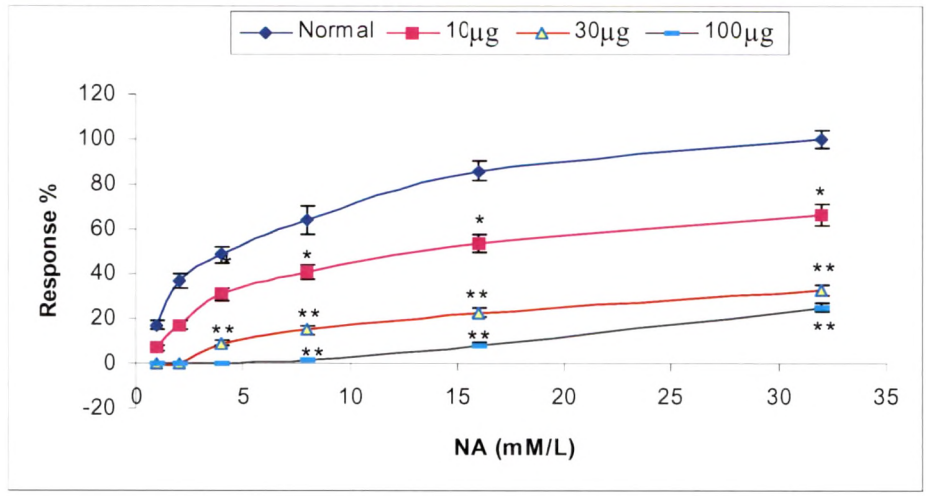


Figure10.1: The concentration response curve of NA on the isolated rat aortic strip in presence and absence of MC 10µg/ml, 30µg/ml and 100µg/ml .*P<0.05, ** P<0.01 when compared to normal control response (before MC administration), Vertical lines represents SEM. N=6.

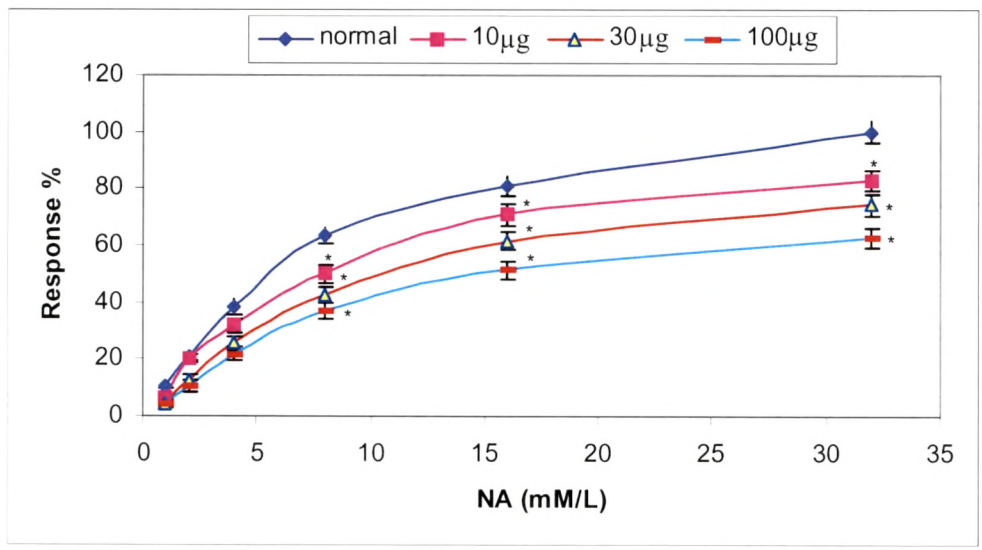


Figure 10.2: The concentration response curve of NA on the isolated rat aortic strip in presence and absence of MD10µg/ml, 30µg/ml and 100µg/ml.*P<0.05 when compared to control response (before MD administration), Vertical lines represents SEM. N=6.

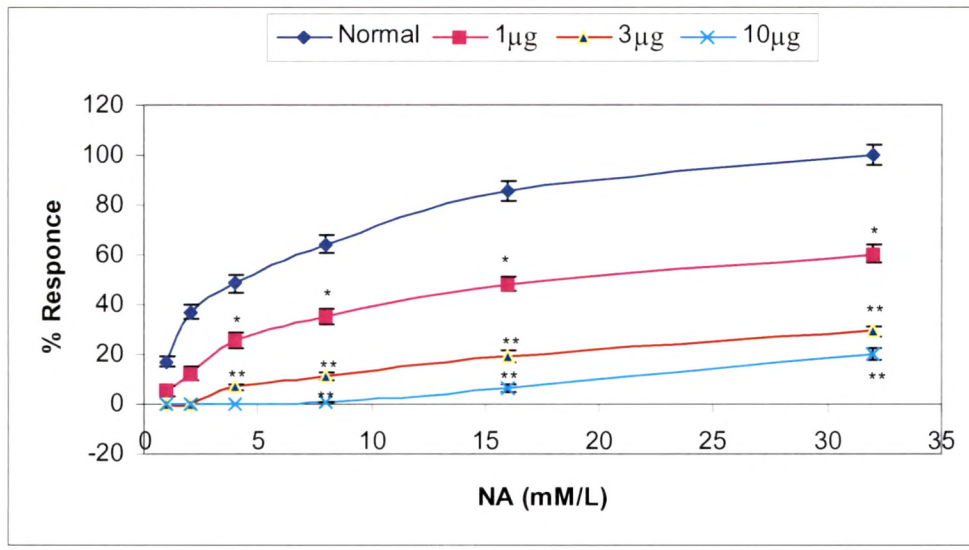


Figure 10.3: The concentration response curve of NA on the isolated rat aortic strip in presence and absence of saponins fractions of MC1µg/ml, 3µg/ml and 10µg/ml.*P<0.05, ** P<0.01 when compared to normal control response (before administration of saponin fractions of MC), Vertical lines represent SEM. N=6.

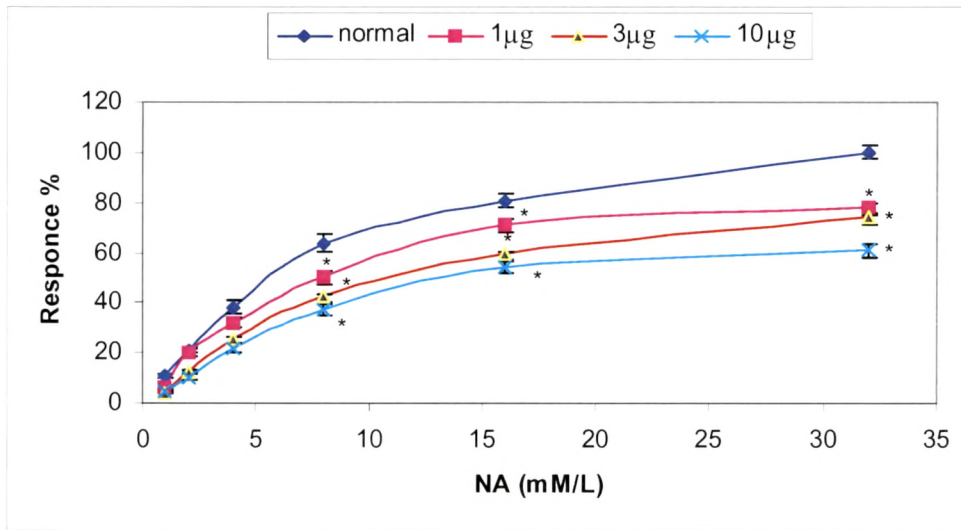


Figure 10:4. The concentration response curve of NA on the isolated rat aortic strip in presence and absence of saponins fractions of MD1µg/ml, 3µg/ml and 10µg/ml.*P<0.05, when compared to normal control response (before administration of saponin fractions of MD) Vertical lines represents SEM. N=6.

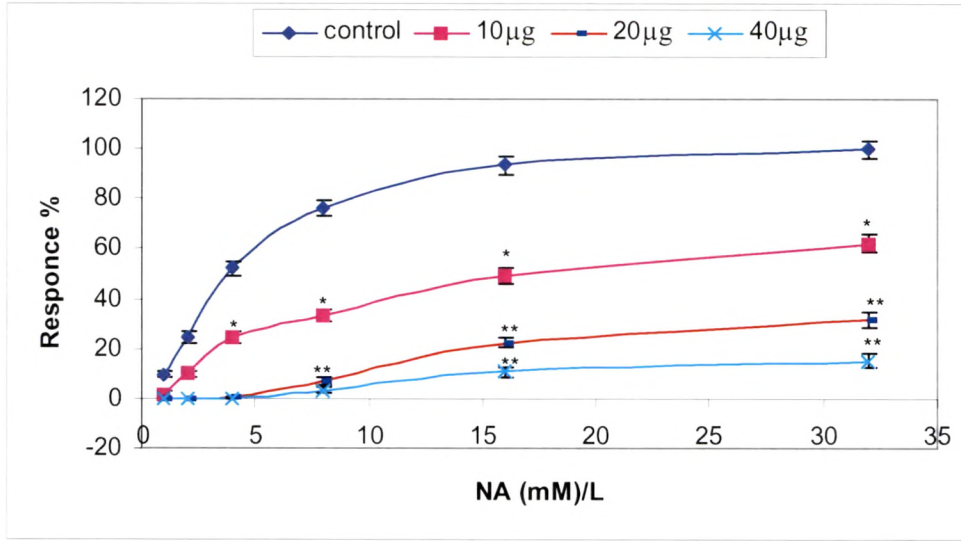


Figure 11.1: The concentration response curve of NA on the rat anconccygeous muscle in presence and absence of Saponin fractions of MC10µg/ml, 20µg/ml and 40µg/ml. *P<0.05, **P<0.01 when compared to control response (before administration of saponin fraction of MC) vertical lines represent SEM. N=6

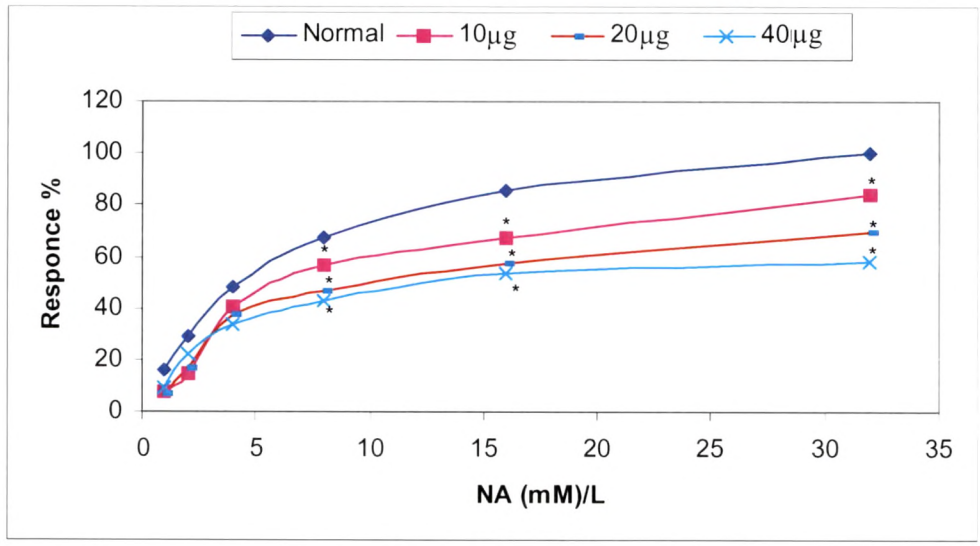


Figure 11.2: The concentration response curve of NA on the rat anconccygeous muscle in presence and absence of Saponin fractions of MD10µg/ml, 20µg/ml and 40µg/ml. *P<0.05, when compared to control (before administration of saponin fraction of MD), Vertical lines represent SEM. N=6

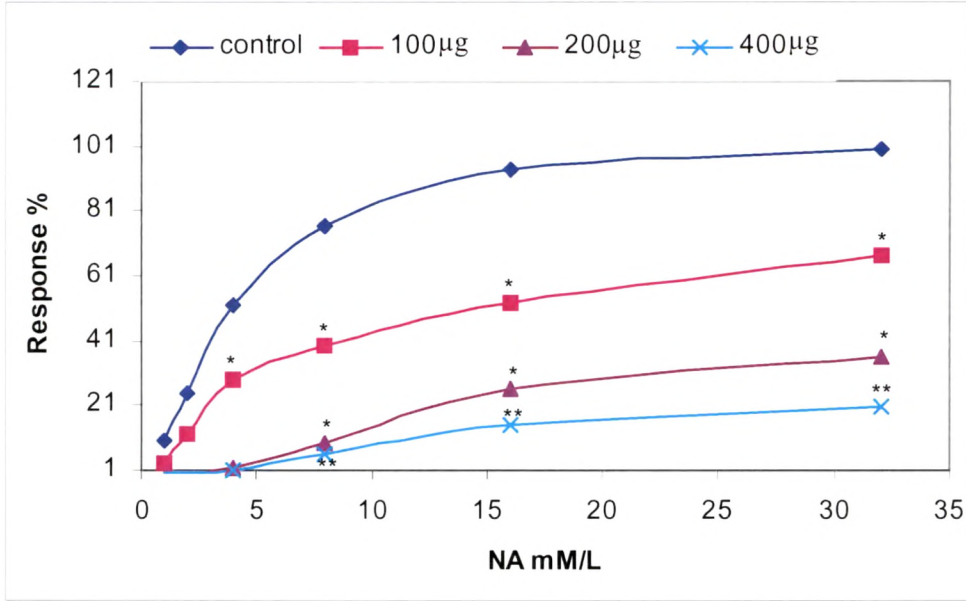


Figure 11.3: The concentration response curve of NA on the rat anconccygeous muscle in presence and absence of MC100µg/ml, 200µg/ml and 400µg/ml.*P<0.05, **P<0.01 when compared to control (before administration of MC), Vertical lines represent SEM. N=6

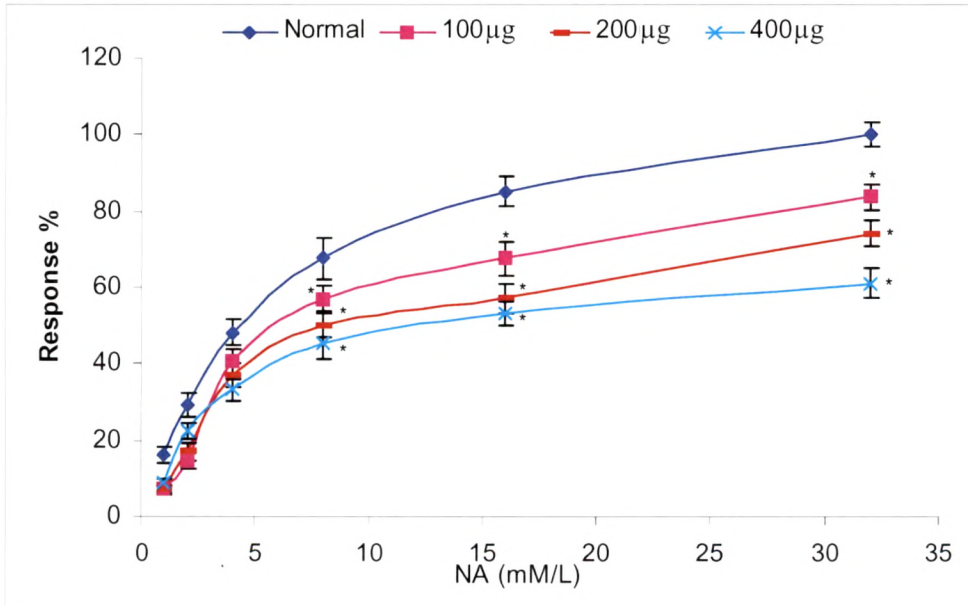


Figure 11.4: The concentration response curve of NA on the rat anconccygeous muscle in presence and absence of MD100µg/ml, 200µg/ml and 400µg/ml.*P<0.05 when compared to control (before administration of MD), Vertical lines represent SEM. N=6

Effect on Antioxidant parameters

4.2.1.12 Hepatic antioxidant parameters in Carbon tetrachloride (CCl_4) induced hepatic injury in rats

Effect on lipid peroxidation: CCl_4 (1.25mg/kg, i.p/alternate day/14days) to Wistar rats significantly ($p<0.001$) increased MDA content in the liver as compared to control group.

Treatment with MC or MD (250mg/kg p.o/day/14days) in CCl_4 treated rats significantly ($p<0.05$) reduced MDA content in the liver as compared to CCl_4 control group.

Treatment with MC or MD (500mg/kg p.o/day/14days) in CCl_4 treated rats also significantly ($p<0.001$) reduced lipid peroxidation or MDA content in the liver as compared to CCl_4 control group (Table 12.1)

Effect on reduced Glutathione: Significant ($p<0.001$) reduction in the reduced glutathione concentration was observed in CCl_4 (1.25mg/kg, i.p/alt day/14days) treated rats as compared to normal control rats.

Treatment with MC or MD (250mg/kg p.o/day/14days) in CCl_4 treated rats significantly ($p<0.05$) increased the reduced glutathione content in the liver as compared to CCl_4 control group

Treatment with MC or MD (500mg/kg p.o/day/14days) in CCl_4 treated rats also significantly ($p<0.001$) increased the reduced glutathione content in the liver as compared to CCl_4 control group (Table 12.1).

Effect on Catalase: The catalase activity in CCl_4 (1.25mg/kg, i.p/alt day/14days) treated rats significantly ($p<0.001$) decreased in the liver as compared to normal control rats.

Treatment with MC or MD (250mg/kg p.o/day/14days) in CCl_4 treated rats significantly ($p<0.05$) increased the catalase activity in the liver as compared to CCl_4 control group.

Treatment with MC or MD (500mg/kg p.o/day/14days) also significantly ($p<0.001$) increased the catalase activity in the liver as compared to CCl_4 control group (Table 12.1).

Effect on Superoxide Dismutase: The SOD activity in CCl_4 (1.25mg/kg, i.p/alt day/14days) treated rats significantly ($p<0.001$) decreased in the liver as compared to normal control rats.

Treatment with MC or MD (250/500mg/kg p.o/day/14days) in CCl_4 treated rats significantly ($p<0.05$) increased the SOD activity in the liver as compared to CCl_4 control group (Table 12.1)

4.2.1.13 Cardiac antioxidant parameters in streptozotocin induced Type 1 diabetes

Effect on lipid peroxidation: Streptozotocin (65mg/kg i.p /single dose) to Wister rats significantly increased lipid peroxidation or MDA content in the heart as compared to normal control groups (Table 12.2).

Treatment with MC or MD (250mg/kg p.o/day/30days) in STZ rats significantly ($p<0.05$) reduced lipid peroxidation or MDA content in the heart as compared to Streptozotocin control group (Table 12.2)

Treatment with MC or MD (500mg/kg p.o/day/30daysdays) in STZ rats also significantly ($p<0.001$) reduced lipid peroxidation or MDA content in the heart as compared to Streptozotocin control group (Table 12.2)

Effect on reduced Glutathione: Significant ($p<0.001$) reduction in the cardiac reduced glutathione concentration was observed in streptozotocin (65mg/kg i.p /single dose) treated Wister rats as compared to normal control group(Table 12.2).

Treatment with MC or MD (250mg/kg p.o/day/30days) in STZ rats significantly ($p<0.05$) increased the reduced glutathione content in the heart as compared to Streptozotocin control group (Table 12.2).

Treatment with MC or MD (500mg/kg p.o/day/30days) in STZ rats also significantly ($p<0.001$) increased the reduced glutathione content in the heart as compared to Streptozotocin control group (Table 12.2).

Effect on Catalase: The cardiac catalase activity in Streptozotocin (65mg/kg i.p /single dose) treated rats significantly ($p<0.001$) decreased as compared to normal control rats.

Treatment with MC or MD (250/500mg/kg p.o/day/30days) in STZ rats significantly ($p<0.001$) increased the catalase activity in the heart as compared to streptozotocin control group.

Effect on Superoxide Dismutase: The cardiac SOD activity in Streptozotocin (65mg/kg I,p /single dose)treated Wister rats significantly ($p<0.001$) decreased as compared to normal control rats(Table 12.2)..

Treatment with MC or MD (250mg/kg p.o/day/30days) in STZ rats significantly ($p<0.05$) increased the SOD activity in the heart as compared to Streptozotocin control group (Table 12.2).

Treatment with MC or MD (500mg/kg p.o/day/30days) in STZ rats also significantly ($p<0.001$) increased the SOD activity in the heart as compared to Streptozotocin control group (Table 12.2)

4.2.1.14 Cardiac antioxidant parameters in Isoproterenol induced myocardial infarction in rats

Effect on lipid peroxidation: Isoproterenol (60mg / kg, s.c./daily/2days) to Wister rats significantly increased lipid peroxidation or MDA content in the heart as compared to normal control groups (Table 12.3).

Pretreatment with MC or MD (250mg/kg p.o/day/45daysdays) to ISO induced MI rats significantly ($p<0.05$) reduced lipid peroxidation or MDA content in the heart as compared to Isoproterenol control group (Table 12.3)

Pretreatment with MC or MD (500mg/kg p.o/day/45daysdays) to ISO induced MI rats also significantly ($p<0.001$) reduced lipid peroxidation or MDA content in the heart as compared to Isoproterenol control group (Table 12.3)

Effect on reduced Glutathione: Significant ($p<0.001$) reduction in the reduced glutathione concentration in heart was observed in Isoproterenol (60mg / kg, s.c./daily/2days) treated Wister rats as compared to normal control group(Table 12.3).

Pretreatment with MC or MD (250mg/kg p.o/day/45days) to ISO induced MI rats significantly ($p<0.05$) increased the reduced glutathione content in the heart as compared to Isoproterenol control group (Table 12.2).

Pretreatment with MC or MD (500mg/kg p.o/day/45days) to ISO induced MI rats also significantly ($p<0.001$) increased the reduced glutathione content in the heart as compared to Isoproterenol control group (Table 12.2)

Effect on Catalase: Significant ($p<0.001$) reduction in the catalase activity in heart was observed in Isoproterenol (60mg / kg, s.c./daily/2days) treated Wister rats as compared to normal control group(Table 12.3).

Pretreatment with MC or MD (250mg/kg p.o/day/45days) to ISO induced MI rats significantly ($p<0.05$) increased the catalase activity content in the heart as compared to Isoproterenol control group (Table 12.3).

Pretreatment with MC or MD (500mg/kg p.o/day/45days) to ISO induced MI rats also significantly ($p<0.001$) increased the catalase activity content in the heart as compared to Isoproterenol control group (Table 12.3).

Effect on Superoxide Dismutase: Significant ($p<0.001$) reduction in the Superoxide Dismutase activity in heart was observed in Isoproterenol (60mg / kg, s.c./daily/2days) treated Wister rats as compared to normal control group(Table 12.3). Pretreatment with MC or MD (250mg/kg p.o/day/45days) to ISO induced MI rats significantly ($p<0.05$) increased the Superoxide Dismutase activity in heart as compared to Isoproterenol control group (Table 12.3).

Pretreatment with MC or MD (500mg/kg p.o/day/45days) to ISO induced MI rats also significantly ($p<0.001$) increased the Superoxide Dismutase activity in heart as compared to Isoproterenol control group (Table 12.3).

Table 12.1 Effect of MC/MD on the hepatic LPO, GSH, CAT and SOD on Carbon Tetrachloride (CCl_4) (1.25mg/kg, ip/alt day/14days) treated rats

Groups	Treatment	LPO ηm of MDA / mg of protein	GSH µg/mg of protein	CAT µm H_2O_2/mg of protein	SOD U/mg of protein
I	Normal control	0.74 ±0.08	6.67 ±0.15	8.40 ±0.57	7.45 ±0.95
II	CCl_4-control	1.99 ±0.26†††	2.76 ±0.43†††	4.91 ±0.88†††	4.73 ±1.08†
III	MC 250mg/kg +CCl_4	1.40 ±0.17*	3.77 ±0.68*	6.8 ±0.75*	6.68 ±0.74*
IV	MC 500mg/kg +CCl_4	0.79 ±0.05***	6.89 ±0.26***	8.32 ±0.93***	7.39 ±0.83*
III	MD 250mg/kg +CCl_4	1.53 ±0.58*	3.91 ±0.72*	6.5 ±0.11*	6.90 ±0.83*
IV	MD 500mg/kg +CCl_4	0.84 ±0.18***	6.93 ±0.31***	8.60 ±0.75***	7.91 ±0.90*
V	Silymarin 100mg/kg +CCl_4	0.69 ±0.13***	6.59 ±0.47***	8.42 ±1.13***	7.59 ±0.64*

Values expressed as mean ±SEM, n=6, ††† P<0.001, †P<0.05 compared to normal control

***P<0.001,*P<0.05 compared to Carbon tetrachloride control

12.2 Effect of MC/MD on cardiac LPO, GSH, CAT and SOD on streptozotocin (STZ) induced diabetic rats

Group	Treatment	LPO ηm of MDA / mg of protein	GSH µg/mg of protein	CAT µm H_2O_2/mg of protein	SOD U/mg of protein
I	Normal control	0.616 ±0.008	5.36 ±0.11	10.43 ±0.16	12.7 ±0.16
II	STZ(65 mg/kg, i.p) - control	0.89 ±0.026*	2.66 ±0.08***	4.17 ±0.102***	8.33 ±0.08***
III	STZ+ Insulin (6U/kg, i.p 30days)	0.74 ±0.005†	4.44 ±0.17†††	8.55 ±0.09†††	10.58 ±0.11†††
IV	STZ +MC (250mg/kg p.o 30days)	0.86† ±0.01	2.59 ±0.09†	6.59††† ±0.12	8.09† ±0.29
V	STZ +MC (500mg/kg p.o. 30days.)	0.615 ±0.01†††	4.22 ±0.06†††	7.24 ±0.17†††	10.29 ±0.17†††
V	STZ +MD (250mg/kg p.o. 30days.	0.82 ±0.04†	2.42 ±0.13†	6.43 ±0.12†††	8.17† ±0.33
V	STZ +MD (500mg/kg p.o. 30days)	0.672 ±0.09††	3.91 ±0.01†††	7.01 ±0.09†††	9.98 ±0.23†††

Values expressed as mean ±SEM, n=6,
*** P<0.001 When compared to normal control group.
††† P<0.001 When compared to STZ control group

12.3 Effect of pretreatment of MC/MD, on cardiac LPO, GSH, CAT and SOD on Isoproterenol (ISO) (60mg / kg, s.c./daily/2days), induced myocardial infarction

Group	Treatment	LPO ηM of MDA / mg of protein	GSH µg /mg of protein	CAT µm H_2O_2/mg of protein	SOD U/mg of protein
I	Normal control	0.84 ±0.21	6.47 ±1.06	7.4 ±0.87	6.42 ±1.03
II	ISO- control	1.69 ±0.16†††	2.66 ±1.27†††	4.63 ±1.08†††	3.74 ±0.98†††
III	ISO + MC (250mg/kg p.o/daily/45 days).	1.40 ±0.07	3.27 ±1.31*	5.8 ±0.95*	4.48 ±0.74
IV	ISO + MC (500mg/kg p.o./daily/45 days)	0.89 ±0.23***	6.49 ±1.36***	7.52 ±1.13***	6.5 ±1.14***
V	ISO + MD (250mg/kg p.o/daily/45 days).	1.31 ±0.23	3.52 ±1.48*	5.9 ±0.12*	4.12 ±0.35*
VI	ISO + MD (500mg/kg p.o./daily/45 days)	0.81 ±0.14***	6.18 ±1.21***	7.01 ±1.24***	6.9 ±1.12***

Values expressed as mean ±SEM, n=6

† P<0.05 considered statistically significant as compared to normal control group.

*P<0.05 considered statistically significant as compared to ISO control group

Effect on Reproductive Parameters

4.2.1.15.1 Effect of MC/MD on implantation in rats at different stages of pregnancy

Effect on number of implantation sites: Treatment with MC or MD 250(mg/kg p.o/day/ day 1to7) significantly ($p<0.001$) decreased the number of implantation sites as compared the control rats.

Treatment with MC or MD 250(mg/kg p.o/day) from day 1 to 3, day 4&5 and day 6 to 9 showed significant ($p<0.01$) decrease in the number of implantation sites as compared to the control rats.

Treatment with MC or MD 500(mg/kg p.o/day/ day 1to7) significantly ($p<0.001$) decreased the number of implantation sites as compared the control rats.

Treatment with MC 500(mg/kg p.o/day) from day 1 to 3, day 4&5 and day 6 to 9 showed significant ($p<0.01$) decrease in the number of implantation sites as compared to the control rats.

Treatment with MD 500(mg/kg p.o/day) from day 1 to 3, day 4&5 and day 6 to 9 showed significant ($p<0.001$) decrease in the number of implantation sites as compared to the control rats (Table 13.1).

4.2.1.15.2 Estrogenic and antiestrogenic activity

Effect of MC on the histological changes in the diameter of uterus, thickness of endometrium and height of endometrial epithelium in 21 to 23 days old Wister female rats

Effect on the diameter of the Uterus: Ethinyl estradiol (1 µg/rat, s.c/day/7days) treatment significantly ($p<0.001$) increased the diameter of the uterus as compared with normal rats. MC (250 & 500 mg/kg p.o/day/7days) did not show change in the diameter of the uterus as compared to the control.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) did not show change in the diameter of the uterus as compared to the Ethinyl estradiol group (Table 13.2 & Figure 13.1).

Effect on the thickness of endometrium: Ethinyl estradiol (1 µg/rat, s.c/daily/7days) treatment significantly ($p<0.001$) increased the thickness of the endometrium as compared with normal rats. MC (250 & 500 mg/kg p.o/day/7days) did not show change in the thickness of the endometrium as compared to the control.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) did not show change in the thickness of the endometrium as compared to the Ethinyl estradiol group (Table 13.2 & Figure 13.1).

Effect on the height of endometrial epithelium: Ethinyl estradiol (1 µg/rat, s.c/day/7days) treatment significantly ($p<0.001$) increased the height of the endometrial epithelium as compared with normal rats. MC (250 & 500 mg/kg p.o/day/7days) did not show change in the height of the endometrial epithelium as compared to the control.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) did not show change in the height of the endometrial epithelium as compared to the Ethinyl estradiol group (Table 13.2 & Figure 13.1).

Effect of MC on the uterine weight, vaginal cornification, uterine glucose, Cholesterol and ALP in 21 to 23 days old Wister female rats

Effect of Uterine weight: Treatment with Ethinyl estradiol (1 µg/rat s.c/day/7days) significantly ($p<0.001$) increased the uterine weight as compared to the control rats. Treatment with MC (250 & 500 mg/kg p.o/day/7days) or MD (250 & 500 mg/kg p.o/day/7days) did not show any significant change in the uterine weight as compared to the control rats.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) or Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MD (250 & 500 mg/kg p.o/day/7days) did not show any change in the uterine weight as compared to the Ethinyl estradiol group. (Table 13.3 & 13.4)

Effect on Vaginal cornification: Treatment with Ethinyl estradiol (1 µg/rat) s.c/day/7days) significantly ($p<0.001$) increased the vaginal cornification and opening as compared to the control rats. Treatment with MC (250 & 500 mg/kg p.o/day/7days) or MD (250 & 500 mg/kg p.o/day/7days) did not show vaginal cornification and opening.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) or Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MD (250 & 500 mg/kg p.o/day/7days) showed vaginal opening similar to Ethinyl estradiol group. (Table 13.3 & 13.4)

Effect on uterine Glucose: Treatment with Ethinyl estradiol (1 µg/rat s.c/day/7days) significantly ($p<0.001$) increased the uterine glucose as compared to the control rats. Treatment with MC (250 & 500 mg/kg p.o/day/7days) or MD (250 & 500 mg/kg p.o/day/7days) did not show any significant change in the uterine glucose as compared to the normal control group.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) or Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MD (250 & 500 mg/kg p.o/day/7days) did not show any change in the uterine glucose level as compared to Ethinyl estradiol group. (Table 13.3 & 13.4)

Effect on uterine Cholesterol: Treatment with Ethinyl estradiol (1 µg/rat s.c/day/7days) significantly ($p<0.001$) increased the uterine glucose as compared to the control rats.

Treatment with MC (250 & 500 mg/kg p.o/day/7days) or MD (250 & 500 mg/kg p.o/day/7days) did not show any significant change in the uterine cholesterol level compared to the normal control group.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) or Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MD (250 & 500 mg/kg p.o/day/7days) did not show any change in the uterine cholesterol level as compared to Ethinyl estradiol group. (Table 13.3 & 13.4)

Effect on uterine Alkaline phosphatase: Treatment with Ethinyl estradiol (1 µg/rat) s.c/day/7days) significantly ($p<0.001$) increased the uterine alkaline phosphatase as compared to the control rats.

Treatment with MC (250 & 500 mg/kg p.o/day/7days) or MD (250 & 500 mg/kg p.o/day/7days) did not show any significant change in the uterine phosphatase level compared to the normal control group.

Treatment with Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MC (250 & 500 mg/kg p.o/day/7days) or Ethinyl estradiol (1 µg/rat, s.c/day/7days) + MD (250 & 500 mg/kg p.o/day/7days) did not show any change in the uterine phosphatase level as compared to Ethinyl estradiol group (Table 13.3 & 13.4).

4.2.1.15.1.3 Progestational and antiprogestational activity

Effect of MC or MD on the maintenance of pregnancy in the ovariectomized rats after treatment from 8^{th} to 19^{th} day of pregnancy.

Administration of Estradiol (0.1μg/rats.c/day/8^{th}to19^{th}day) to the ovariectomized pregnant rats from 8^{th} to 19^{th} day of pregnancy did not show any viable fetus on day 21 and this group was taken as normal control.

Administration of Estradiol (0.1μ g/rat s.c/day/8^{th} to 19^{th} day) and Progesterone 3mg/rat s.c/ h to 19^{th} day) to the ovariectomized pregnant rats from 8^{th} to 19^{th} day of pregnancy showed significant viable fetus as compared to normal control rats.

Administration of Estradiol (0.1μ g/rat s.c/day/8^{th} to 19^{th} day) and MC or MD (250 & 500 mg/kg p.o/day/8^{th} to 19^{th} day) to the ovariectomized pregnant rats from 8^{th} to 19^{th} day of pregnancy did not show any viable fetus on day 21.(Table 13.5).

4.2.1.16 Antiovulatory activity

Effect of MC or MD on the mean Proestrous, Estrous, Metaestrous and Diestrous days in female Wister rats after 15 days treatment

Administration of MC or MD (250 mg/kg p.o/day/15days) significantly ($p<0.05$) increased the Proestrous & Estrous days and significantly ($p<0.05$) decreased metestrous days as compared to control rats. There was no change in the diestrous days.

Administration of MC or MD (500 mg/kg p.o/day/15days) significantly ($p<0.01$) increased the Proestrous & Estrous days and significantly ($p<0.01$) decreased metestrous days as compared to control rats. There was no change in the diestrous days.

Effect of MC or MD on the ovarian weight and cholesterol in female Wister rats after 15 days treatment

Administration of MC or MD (250 & 500 mg/kg p.o/day/15days) significantly ($p<0.01$) decreased the ovarian weight and did not show any change in the ovarian cholesterol level as compared to normal rats (Table 13.7).

4.2.1.17 Abortifacient activity in rats

Administration of MC (250mg/kg p.o/day/6^{th} to 15^{th} day) showed a significant ($p<0.01$) decrease in the number of fetus as compared to control rats.

Administration of MC or MD (500mg/kg p.o/day/6^{th} to 15^{th} day) showed a significant ($p<0.001$) decrease in the number of fetus as compared to control rats.

Administration of MD (250mg/kg p.o/day/6^{th} to 15^{th} day) did not show a significant decrease in the number of fetus as compared to control rats.

4.2.1.18 Effect on male reproductive system in rats

Effect of MC and MD on Epididymal sperm density, Sperm motility, Serum Cholesterol, ALP activity and Testosterone level after 60 days of treatment male rats

Effect on Pregnancy rate: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the pregnancy rate as compared to control rats. (Table 13.9)

Effect on Number of viable fetus: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the Number of viable fetus as compared to control rats. (Table 13.9)

Effect on Sperm Density: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the sperm density as compared to control rats. (Table 13.9)

Effect on Sperm Motility: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the sperm motility as compared to control rats. (Table 13.9)

Effect on serum Cholesterol: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the serum cholesterol level as compared to control rats. (Table 13.9)

Effect on serum ALP activity: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the serum ALP activity as compared to control rats. (Table 13.9)

Effect on serum testosterone: Administration of MC (250mg/kg p.o/day/60 days) did not show any significant change in the serum testosterone level as compared to control rats.

Administration of MC (500mg/kg p.o/day/60 days) showed significant ($p<0.05$) change in the serum testosterone as compared to control rats.

Administration of MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the serum testosterone level as compared to control rats. (Table 13.9).

Effect of MC and MD on Body weight and weights of Testes, Epididymis, seminal vesicles and prostate glands after 60 days of treatment

Effect on Body weight: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the body weight as compared to control rats. (Table 13.10)

Effect on Testis weight: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the testis weight as compared to control rats. (Table 13.10)

Effect on Epididymis weight: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the Epididymis weight as compared to control rats. (Table 13.10)

Effect on seminal vesicles weight: Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the seminal vesicles weight as compared to control rats. (Table 13.10)

Effect on Prostate gland weight Administration of MC or MD (250 & 500mg/kg p.o/day/60 days) did not show any significant change in the prostate gland weight as compared to control rat (Table 13.10).

Table 13.1 Effect of MC/MD on implantation in rats at different stages of pregnancy

Treatment	Days of administration	No. of implantation sites	% of rats without implantation sites on day 10
Control (Distilled water5ml/kg p.o/day)	1 to 7	10.8 ± 0.94	0
MC 250 (mg/kg p.o/day)	1 to 7	0***	100
	1 to 3	2.6 ± 1.35**	50
	4 & 5	0***	100
	6 to 9	3 ± 1.61**	50
MC 500 (mg/kg p.o/day)	1 to 7	0***	100
	1 to 3	2.16 ± 1.51**	66.6
	4 & 5	1.66 ± 1.66**	83.3
	6 to 9	1.83 ± 1.32**	66.6
MD 250 (mg/kg p.o/day)	1 to 7	1.5±0.5**	66.6
	1 to 3	1.6±0.55**	66.6
	4 & 5	1.5±0.71**	50
	6 to 9	1.33±0.49**	66.6
MD 500 (mg/kg p.o/day)	1 to 7	0.16±0.16***	83.3
	1 to 3	0.67±0.33***	83.3
	4 & 5	0.33±0.71***	83.3
	6 to 9	0.5±0.34***	83.3

Values are mean ± SEM. n=6, ***P< 0.001, ** P<0.01 when compared with control

Table 13.2 Effect of MC on the histological changes in the diameter of uterus, thickness of endometrium and height of endometrial epithelium in 21 to 23 days old Wister female rats

Groups.	Treatment	Diameter of uterus (mm) H x E 40x	Thickness of endometrium (μm)H X E 100x	Height of endometrial epithelium (μm) H x E 100x
1.	Control Distilled water (5ml/kg p.o/day)+ Arachis oil (1ml/kg s.c/day)	0.50 ±0.09	17.58 ± 0.3	165.31 ± 2.26
2.	Ethinyl estradiol (1 μg/rat,s.c/daily/7days)	1.36 ± 0.04***	37.65 ± 1.04***	352.78 ±5.44***
3.	MC (250 mg/kg p.o/daily/7days)	0.49 ± 0.008	17.85 ± 0.31	166.48 ± 1.62
4.	MC (500 mg/kg p.o/daily/7days)	0.51 ± 0.02	17.71 ± 0.21	164.18 ± 2.82
5.	Ethinyl estradiol (1μg /rat,s.c/daily/7days) + MC(250 mg/kg p.o/daily/7days)	1.37 ± 0.02	38.68 ± 1.31	348.35 ± 5.53
6.	Ethinyl estradiol (1μg /rat,s.c/daily/7days) + MC(500 mg/kg p.o/daily/7days)	1.31 ± 0.03	38.68 ± 1.31	348.35 ± 5.53

Values are mean ± SEM. n=6; $p < 0.001$, when compared with control

Table 13.3 Effect of MC on the uterine weight, vaginal cornification, uterine glucose, Cholesterol and ALP in 21 to 23 days old Wister female rats

Groups	Treatment	Uterine weight (mg/100g body)	Vaginal cornification	Glucose (mg/100mg of uterus)	Cholesterol (mg/100mg of uterus)	Alkaline phosphatase (IU/100mg of uterus)
1.	Distilled water 5ml/kg /day p.o/7days+Arachis oil1ml/kg s.c/7days	39.31 ±0.53	Vaginal orifice closed	0.33 ± 0.02	0.60 ± 0.09	0.26 ± 0.02
2.	Ethinyl estradiol (1 µg/rat) s.c/day/7days)	130.51 ± 9.79***	Open (+++)	0.43 ± 0.03***	0.95 ± 0.07***	0.44 ± 0.04***
3.	MC (250mg/kg p.o/day/7days)	36.66 ± 3.08	Vaginal orifice closed	0.33 ± 0.01	0.59 ± 0.24	0.21 ± 0.04
4.	MC (500 mg/kg p.o/day/7days)	39.56 ± 1.95	Vaginal orifice closed	0.34 ± 0.03	0.55 ± 0.16	0.24 ± 0.01
5.	Ethinyl estradiol (1 µg /rat s.c/day/7days) + MC(250 mg/kg p.o/day/7days)	134.14 ± 3.41	Open (+++)	0.38 ± 0.07	0.85 ± 0.03	0.33 ± 0.02
6.	Ethinyl estradiol (1 µg /rat s.c/day/7days) + MC (500 mg/kg p.o/day/7days)	129.43 ± 6.68	Open (+++)	0.37 ± 0.03	0.83 ± 0.06	0.30 ± 0.03

Values are mean ± SEM. n=6; +++ = cornified cells. *** P< 0.001, when compared with control

Table 13.4 Effect of MD on the uterine weight, vaginal cornification, uterine glucose, Cholesterol and ALP in 21 to 23 days old Wister female rats

Groups	Treatment	Uterine weight mg/100g body	Vaginal cornification	Glucose mg/100mg of uterus	Cholesterol mg/100mg of uterus	Alkaline phosphatase IU/100mg of uterus
1.	Distilled water 5ml/kg /day p.o/7days+Arachis oil1ml/kg s.c/7days	29.51 ±0.77	Vaginal orifice closed	0.39 ±0.02	0.55 ±0.01	0.21 ±0.01
2.	Ethinyl estradiol (1 µg/rat s.c/day/7days)	125.76 ±0.63 *	Open (+++)	0.50 ±0.07 *	0.75 ±0.04 *	0.34 ±0.02 *
3.	MD (250 mg/kg p.o/day/7days)	31.56 ±1.32	Vaginal orifice closed	0.41 ±0.94	0.56 ±0.01	0.19 ±0.05
4.	MD (500 mg/kg p.o/day/7days)	28.76 ±0.56	Vaginal orifice closed	0.42 ±0.01	0.58 ±0.01	0.21 ±0.02
5.	Ethinyl estradiol (1 µg /rat s.c/day/7days) + MD(250 mg/kg p.o/day/7days)	127.61 ±0.41*	Open (+++)	0.49 ±0.05	0.77 ±0.06	0.34 ±0.03
6.	Ethinyl estradiol (1 µg /rat s.c/day/7days) + MD(500 mg/kg p.o/day/7days)	129.75 ±0.72	Open (+++)	0.50 ±0.04	0.79 ±0.06	0.35 ±0.09

Values are mean ± SEM. n=6; +++ = cornified cells.* P<.05 when compared with control

Table 13.5 Effect of MC or MD on the maintenance of pregnancy in the ovariectomized rats after treatment from 8^{th} to 19^{th} day of pregnancy.

Treatment	Mean viable fetus	Net success index (%)
Group I: Estradiol 0.1µ g/rat s.c/day + distilled water	0	0
Group II: Estradiol 0.1µ g/rat s.c/day/8^{th} to 19^{th} day + Progesterone 3mg/rat s.c./day/8^{th} to 19^{th} day	5.67 ± 0.67***	51.51 ± 6.06***
Group III: Estradiol 0.1µg/rat s.c/day/8^{th} to 19^{th} day + MC 250mg/kg p.o /day/8^{th} to 19^{th} day	0	0
Group IV: Estradiol 0.1µg/rat s.c/day + MC 500mg/kg/ p.o/day/8^{th} to 19^{th} day	0	0
Group V: Estradiol 0.1µg/rat s.c/day + MD 250mg/kg/ p.o/day/8^{th} to 19^{th} day	0	0
Group VI: Estradiol 0.1µg/rat s.c/day + MD 500mg/kg/ p.o/day/8^{th} to 19^{th} day	0	0

Values are mean ± SEM. n=6;***P<0.001, when compared to control

Table 13.6 Effect of MC or MD on the mean Proestrous, Estrous, Metaestrous and Diestrous days in female Wister rats after 15 days treatment

Group	Treatment	Mean days of Proestrous	Mean days of Estrous	Mean days of Metestrous	Mean days of Diestrous
I	Control Distilled water(5ml/kg p.o/day/15days	2.08± 0.53	3.33± 0.33	4± 0.28	5.58± 0.37
II	MC(250mg/kg p.o/day/15days)	7.08± 0.5*	1.25± 0.28*	1.41± 0.2*	5.16± 0.47
III	MC(500mg/kg p.o/day/15days)	7.41± 0.8*	0.75± 0.4**	0.75± 0.35**	6.16± 1.23
IV	MD(250mg/kg p.o/day/15days)	7.0 ±0.78 *	2.05± 0.14*	1.75± 0.12*	5.42± 0.21
V	MD(500mg/kg p.o/day/15days)	7.22±0.33 **	1.00± 0.25*	0.62± 0.29**	5.85± 1.47

Values are mean ± SEM; n=6; *p<0.05, ** p< 0.01 when compared with control

Table 13.7 Effect of MC or MD on the ovarian weight and cholesterol in female Wister rats after 15 days treatment

Group	Treatment	Ovarian weight in mg/100g body weight	Mean cholesterol level in ovary (mg/50mg)
I	Control (distilled water 5ml/kg p.o/ day / 15days)	40.80 ± 1.13	0.33 ± 0.05
II	MC(250mg/kg p.o/day/15days)	31.88 ± 0.9**	0.40± 0.15**
III	MC(500mg/kg p.o/day/15days)	34.26 ± 1.16**	0.51 ± 0.03**
IV	MD(250mg/kg p.o/day/15days)	31.22 ±0.21**	0.47 ± 0.07**
V	MD(500mg/kg p.o/day/15days)	32.01 ±0.24**	0.38 ± 0.02*

Values are mean ± SEM; n=6; ** P< 0.01, when compared with control

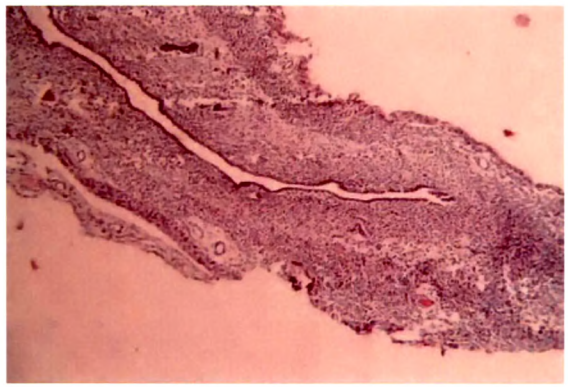
Table 13.8 Abortifacient effects of ethanolic and aqueous extracts of roots of Momordica cymbalaria in rats when fed orally between Days 6 to 15 of pregnancy

Treatment	No of foetus	Foetus weight	Abortion in %
Control **Distilled water(5ml/kg p.o/day/6th to 15th day)**	10.66 ± 0.16	1.35 ±0.03	0.0%
MC(250 mg/kg p.o/day/6th to 15th day)	6.16 ± 1.24**	1.32 ±0.04	0.0%
MC(500 mg/kg p.o/day/6th to 15th day)	0***	---	100%
MD(250 mg/kg p.o/day/6th to 15th day)	9.16 ±4.24	1.42 ±0.26	0.0%
MD(500 mg/kg p.o/day/6th to 15th day)	1.50 ±4.2***	0.95 ±0.17	84%

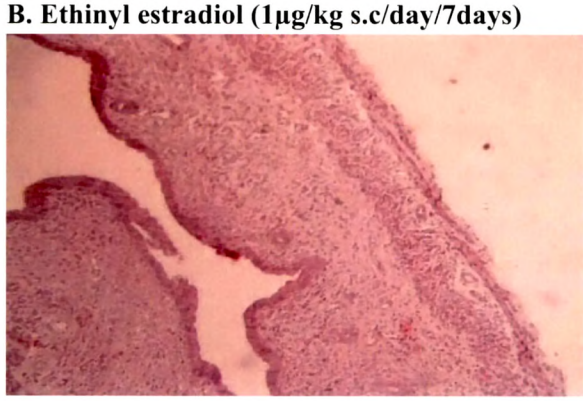
Values are mean ± SEM; n=6 *** P< 0.001, ** P< 0.05when compared with control,

Figure13. 1. Pictograms showing the effect of MC on the histological changes in the uterus in 21 to 23 days old Wister female rats, (H x E 100x), n=6

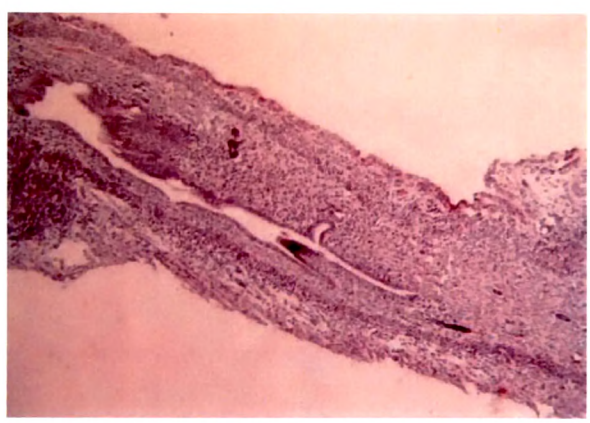
A. Normal Control



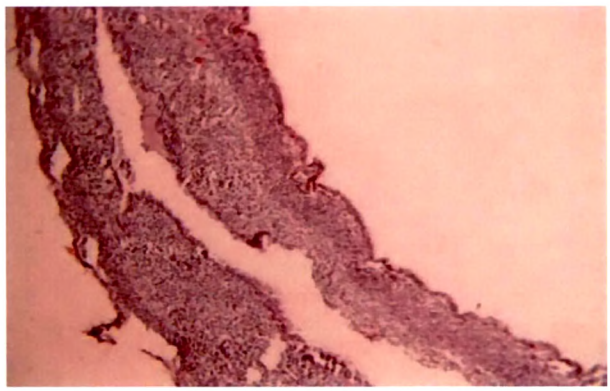
B. Ethinyl estradiol (1µg/kg s.c/day/7days)



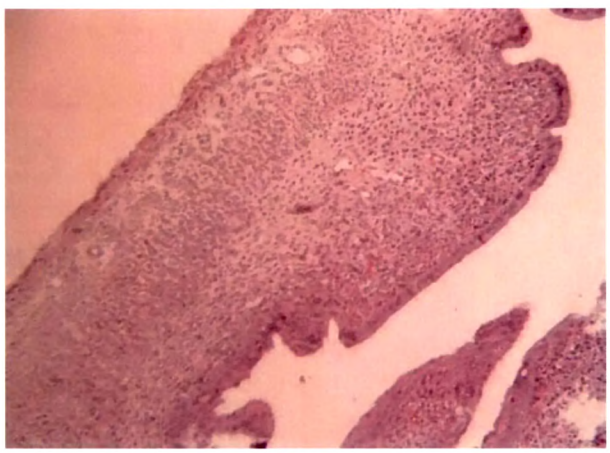
C. MC (250mg/kg p.o/daily/7days)



Ethinyl estradiol (1µg/kg s.c/day/7days)



E. Ethinyl estradiol (1µg/kg s.c/day/7days)
+ MC (250mg/kg p.o/daily/7days)



F. Ethinyl estradiol (1µg/kg s.c/day/7days)
+ MC (500mg/kg p.o/daily/7days)

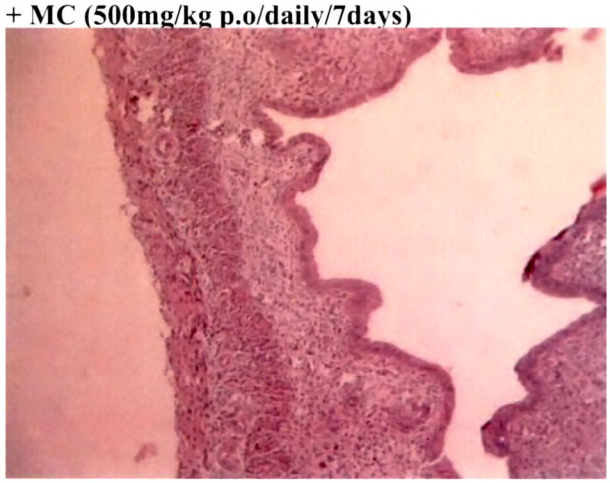
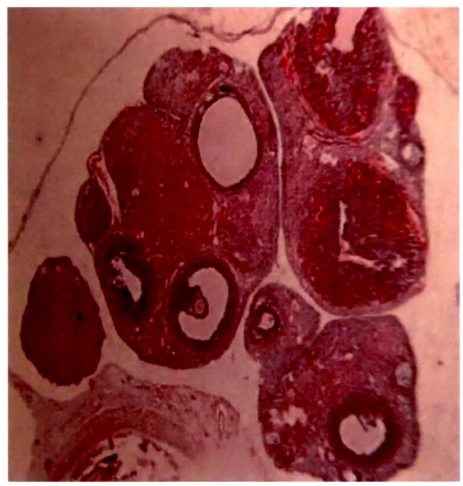
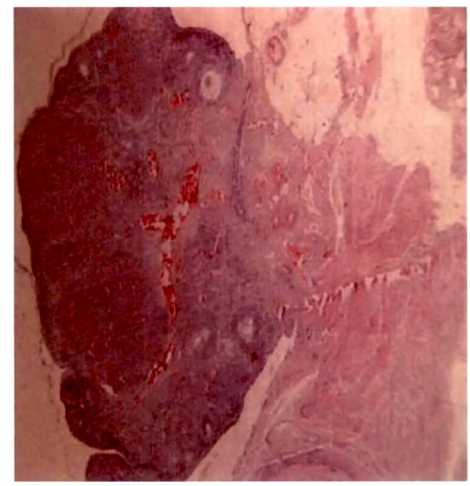


Figure 2.Pictograms showing the effect of MC or MD on the histological changes in the ovary in Wister female rats, treated for 15 days (H x E 100x), n=6

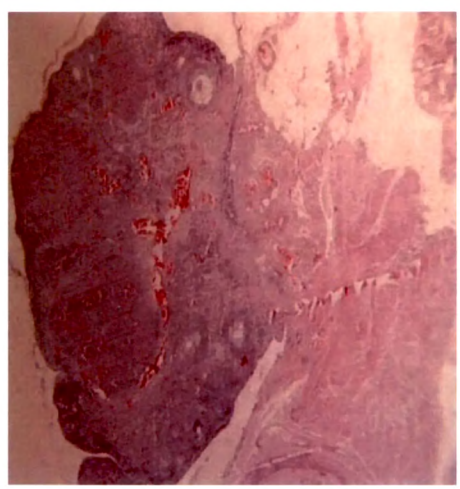
A. **Control**



C. MC (500mg/kg p.o/day/15days)



B. MC (250mg/kg p.o/day/1



D. MD (500mg/kg p.o/day/15days)

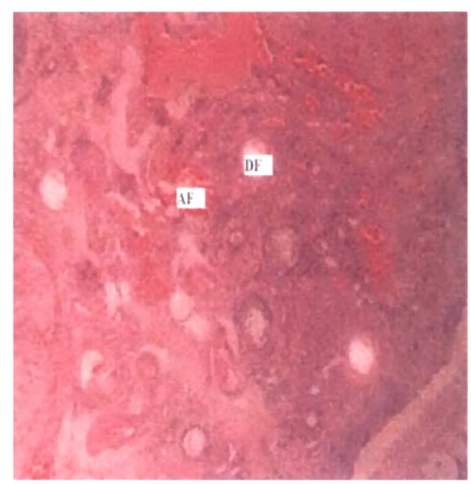
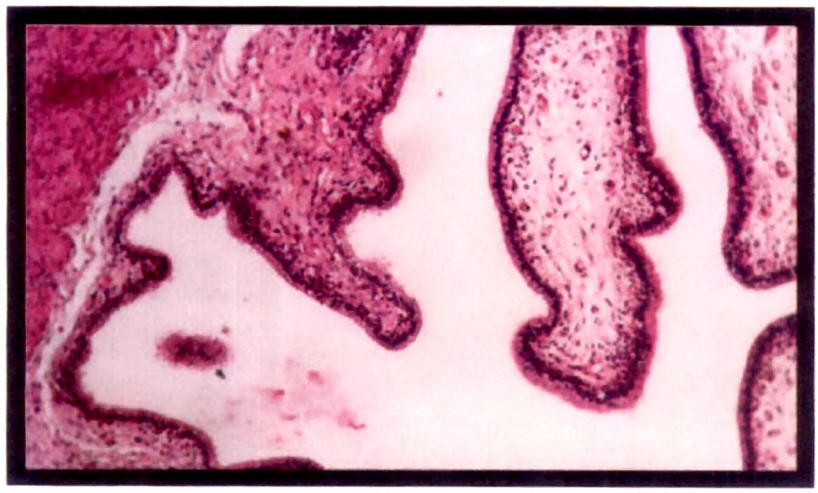
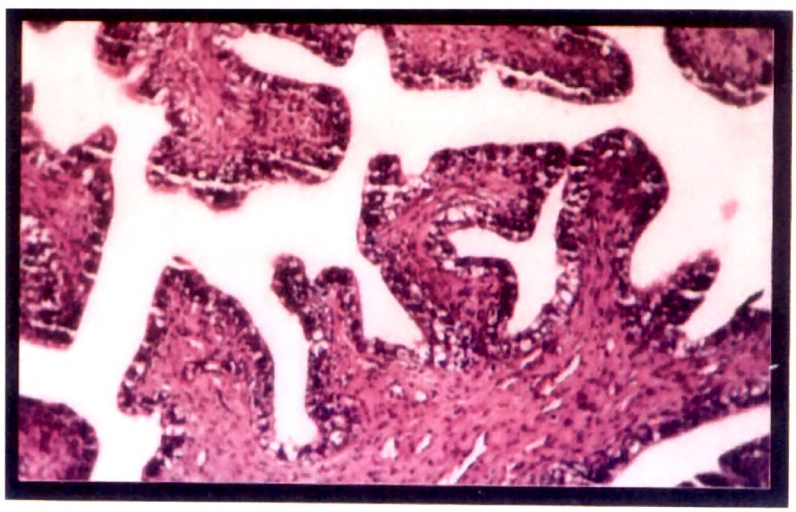


Figure3.Pictograms showing the effect of MC on the histological changes in the estrogen primed (estradiol valarate(8.3µg/kg s.c/day/6days))uterus in immature female rabbits, treated for 5 days (H x E 125x), n=6

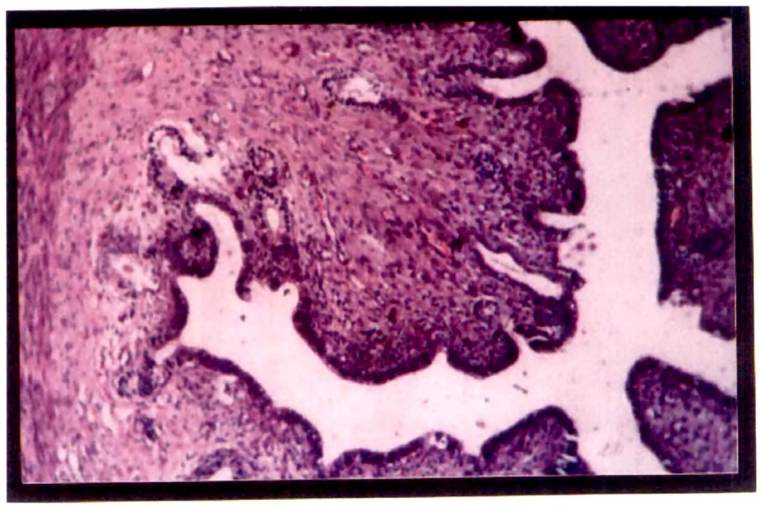
A. Control



B. MC (500mg/kg p.o/day/daily/5days)



C. Norethisterone (o.75mg/kg s.c/day/5days)



D. Norethisterone (o.75mg/kg s.c/day/5days)
+ MC (500mg/kg p.o/day/daily/5days)

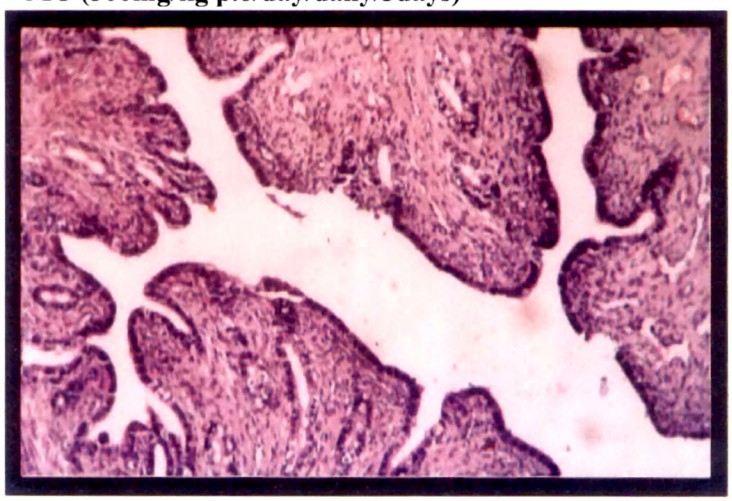


Table 13.9 Effect of MC and MD on Epididymal sperm density, Sperm motility, Serum Cholesterol, ALP activity and Testosterone level after 60 days of treatment male rats.

Treatment	Pregnancy rate	No of viable fetus	Sperm density (count/ ml)	Sperm motility (%)	Cholesterol mg/g in testes	ALP activity IU/g of testes	Testosterone level (ng/ml)
Control	6/6	11 ± 0.26	64.3 ± 0.95	67.33 1.76	1.85 ± 0.36	1.44 ± 0.12	3.38 ± 0.57
MC (250mg/kg p.o/day/60 days)	6/6	10.33 ± 0.33	64 ± 1.15	67 ±1.24	2.93 ± 0.24	4.56 ±0.18	3.06 ± 0.33
MC (500mg/kg p.o/day/60 days)	6/6	10.83 ± 0.40	64.3 ± 1.20	67 ±1.53	1.44 ± 0.12	3.66 ±0.22	5.34 ±0.37*
MD (250mg/kg p.o/day/60 days	6/6	9.13 ± 0.33	59.12 ±2.38	64.66 ±0.80	3.04 ±0.28	3.54 ±0.28	3.53 ±0.24
MD (500mg/kg p.o/day/60 days	6/6	11.81 ± 0.34	63.13 ±0.81	64.5 ±0.76	2.76 ±0.28	4.75 ±0.38	4.11 ±0.04

n=6. Mean ± SEM, * $p<0.05$

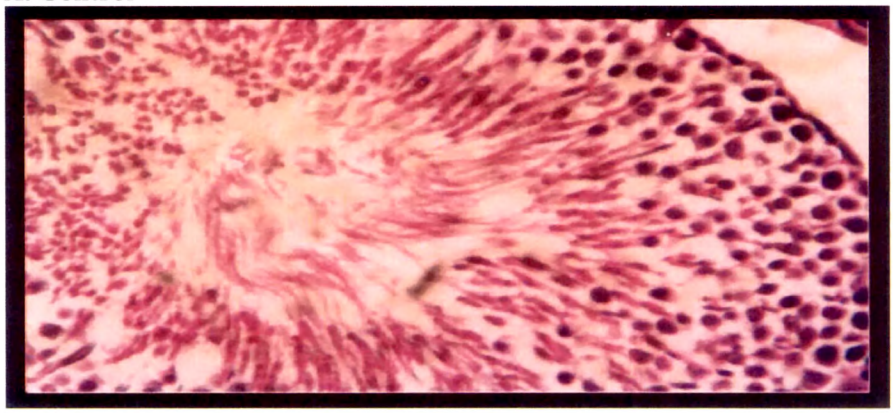
Table 13.10 Effect of MC and MD on Body weight and weights of Testes, Epididymis, seminal vesicles and prostate glands after 60 days of treatment

Treatment	Body weight Day 1	Body weight Day 61	Testis	Epididymis	Seminal vesicle	Prostate gland
Control	135 ± 3.42	220 ± 2.58	5.18 ± 0.15	2.28 ± 0.11	1.09 ± 0.06	1.53 ± 0.16
MC (250mg/kg p.o/daily/60days)	146.67 ± 4.22	231.67 ± 5.43	5.47 ± 0.13	2.34 ± 0.08	0.99 ± 0.08	1.42 ± 0.11
MC (500mg/kg p.o/daily/60days)	136.67 ± 3.42	220 ± 3.65	5.53 ± 0.21	2.14 ± 0.09	1.79 ± 0.06	1.68 ± 0.16
MC (250mg/kg p.o/daily/60days	122.63 ±2.27	213.6 ±6.12	4.83 ±0.17	2.15 ±0.07	1.16 ±0.06	1.49 ±0.13
MC (500mg/kg p.o/daily/60days)	123.66 ±4.01	218.66 ±16.92	5.02 ±0.15	2.28 ±0.08	1.30 ±0.13	1.47 ±0.02

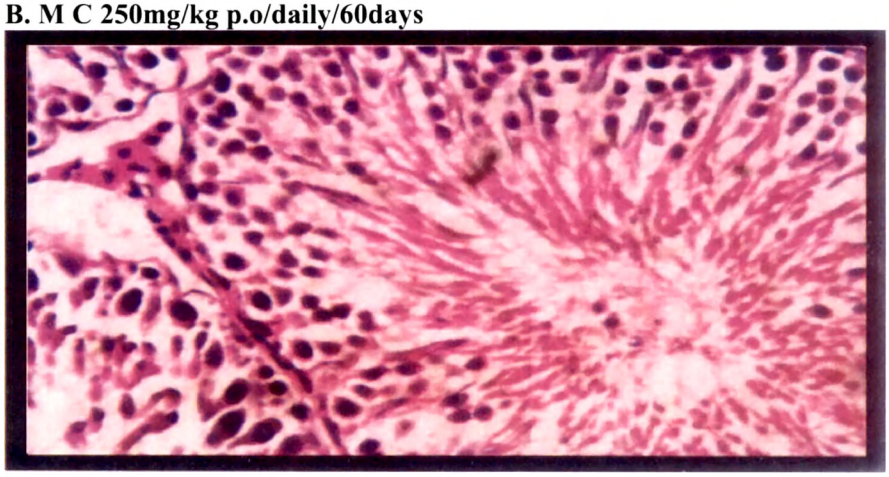
n=6. Mean ± SEM

Figure3. Pictograms showing the effect of MC on the seminiferous tubules in the testis of rat treated for 60 days (H x E 500x), n=6

A. Control



B. M C 250mg/kg p.o/daily/60days



C. M C 500mg/kg p.o/daily/60days

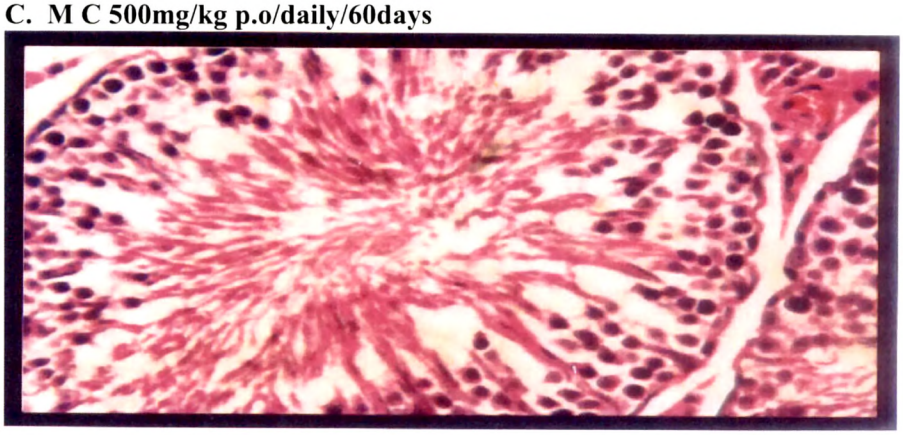
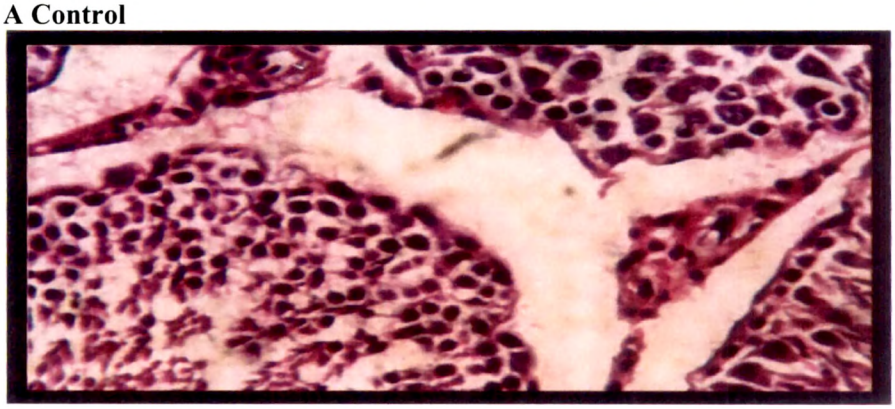
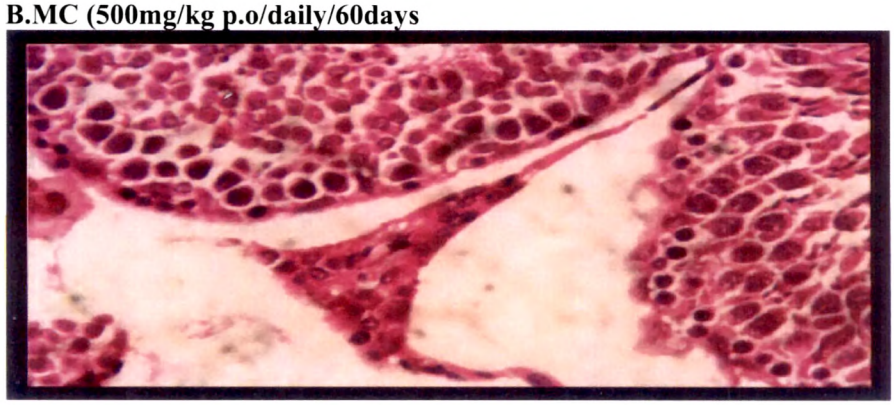


Figure 4 Pictograms showing the effect of MC on lyedig cells in the testis of rat treated for 60 days (H x E 500x), n=6

A Control



B.MC (500mg/kg p.o/daily/60days



Phytochemical Investigations

Preliminary Phytochemical investigations of MC, MD, Saponin fractions of MC, Saponin fractions of MD

Test for Alkaloids: MC and MD tested positive for alkaloids. Saponin fractions of MC and MD tested negative for alkaloids

Test for Carbohydrates: MC, MD, Saponins of MC and MD tested positive for carbohydrates

Tests for Glycosides: MC, MD, Saponins of MC and MD tested positive for glycosides

Test for Phytosterols: MC, MD, Saponins of MC and MD tested positive for phytosterols

Test for Saponins: MC, MD, Saponins of MC and MD tested positive for saponins

Test for Tannins: MC, MD, Saponins of MC and MD tested negative for tannins

Test for Proteins: MC, MD, Saponins of MC and MD tested negative for proteins

Test for Flavonoids *:* MC and MD tested positive for flavonoids. Saponin fractions of MC and MD tested negative for flavonoids.

PHYTOCHEMICAL INVESTIGATION (Khandelwal 2003)

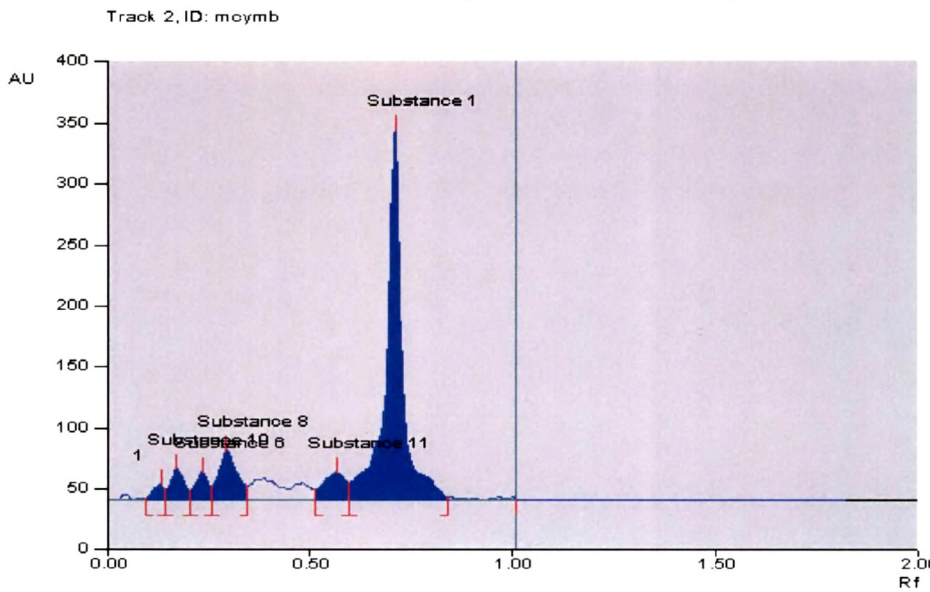
Sl. No.	Chemical Tests	MC	MD	Saponin fraction of MC	Saponin fraction of MD
I	Tests for Alkaloids				
	1.Hagner's Test	+ve	+ve	-ve	-ve
	2.Mayer's Test	+ve	+ve	-ve	-ve
	3.Wagner's Test	+ve	+ve	-ve	-ve
	4.Tannic acid Test	+ve	+ve	-ve	-ve
	5.Dragendroff's Test				
II	Tests for Carbohydrates				
	1.Molisch's Test	+ve	+ve	+ve	+ve
	2.Fehling's Test	+ve	+ve	+ve	+ve
	3.Benedict's Test	+ve	+ve	+ve	+ve
	4.Barfoed's Test	+ve	+ve	+ve	+ve
III	Tests for Glycosides				
	1.Libermann Burchard Test	+ve	+ve	+ve	+ve
	2.Legal's Test	+ve	+ve	+ve	+ve
IV	Tests for Phytosterols				
	1. Libermann Burchard Test	+ve	+ve	+ve	+ve
	2. Salkowski Test	+ve	+ve	+ve	+ve
V	Test for Saponins				
	1.Foam Test	+ve	+ve	+ve	+ve
	2.Hameolysis Test	+ve	+ve	+ve	+ve

VI	Test for Tannins				
	1.Ferric chloride Test	-ve	-ve	-ve	-ve
	2.Gelatin Test	-ve	-ve	-ve	-ve
	3.Lead acetate Test	-ve	-ve	-ve	-ve
	4.Aqueous bromine Test	-ve	-ve	-ve	-ve
VII	Test for Proteins/Amino acids	-ve	-ve	-ve	-ve
	1.Millon's Test	-ve	-ve	-ve	-ve
	2.Biuret's Test	+ve	+ve	-ve	-ve
	3.Ninhydrin Test	+ve	+ve	-ve	-ve
	4.Sodium bicarbonate Test	+ve	+ve	-ve	-ve
VIII	Test for Flavonoids				
	1.Ferric chloride Test	+ve	+ve	-ve	-ve
	2.Lead acetate Test	+ve	+ve	-ve	-ve
	3.Magenisum ribbon Test	+ve	+ve	-ve	-ve
	4.Boric acid hydrochloric acid Test	+ve	+ve	-ve	-ve

+ve = Present -ve = Absent

HPTLC plate of saponins of MC at 254 ηm

HPTLC Spectra of Saponins of MC at 254 ηm

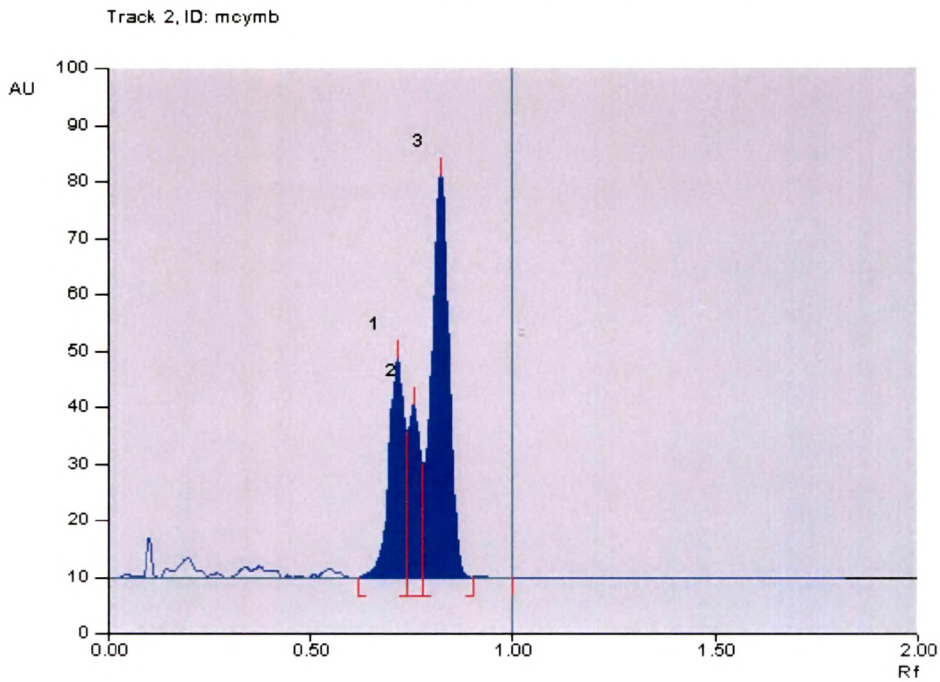


HPTLC Spectral Details of saponins of MC

Peak	Maximum Rf	% area
1	0.17	4.34
2	0.23	4.76
3	0.29	10.28
4	0.38	5.91
5	0.45	3.42
6	0.55	6.01
7	0.75	65.21

HPTLC plate of saponins of MC at 366 ηm

HPTLC Spectra of Saponins of MC at 336 ηm

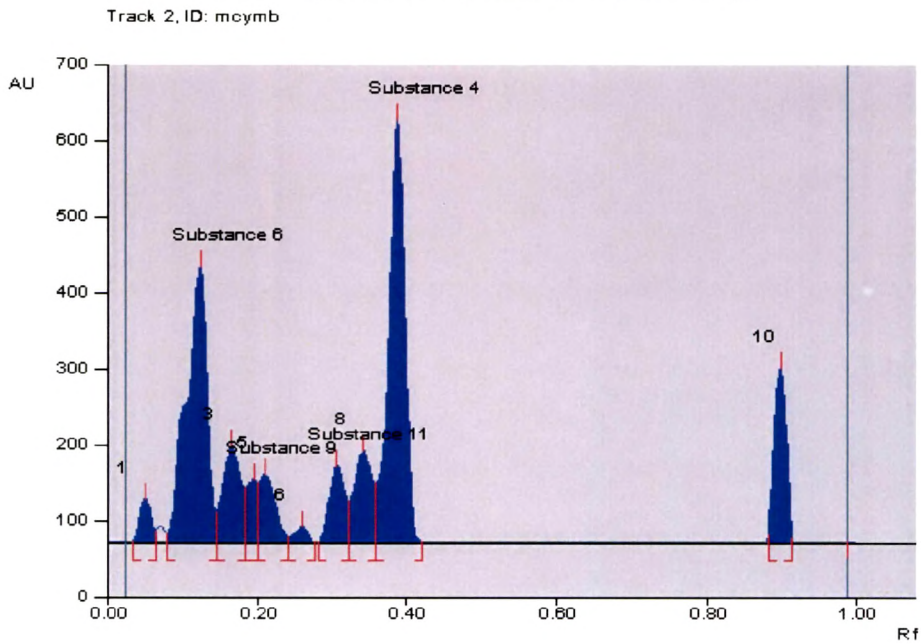


HPTLC Spectral Details of saponins of MC

Peak	Maximum Rf	% area
1	0.71	37.53
2	0.75	21.31
3	0.82	40.67

HPTLC plate of saponins of MC at 540 ηm

HPTLC Spectra of Saponins of MC at 540 ηm

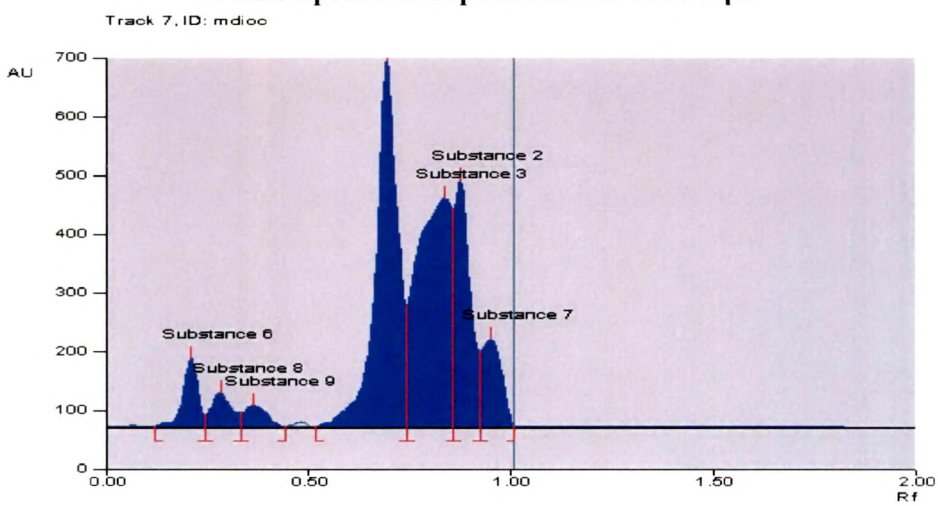


Spectral Details of saponins of MC at 540 ηm

Peak	Maximum Rf	% area
1	0.04	2.06
2	0.12	17.24
3	0.16	6.48
4	0.19	3.19
5	0.21	6.60
6	0.25	2.72
7	0.30	6.98
8	0.34	11.39
9	0.38	27.32
10	0.42	2.46
11	0.47	0.38
12	0.87	13.22

HPTLC plate of saponins of MD at 254 ηm

HPTLC Spectra of Saponins of MD at 254 ηm

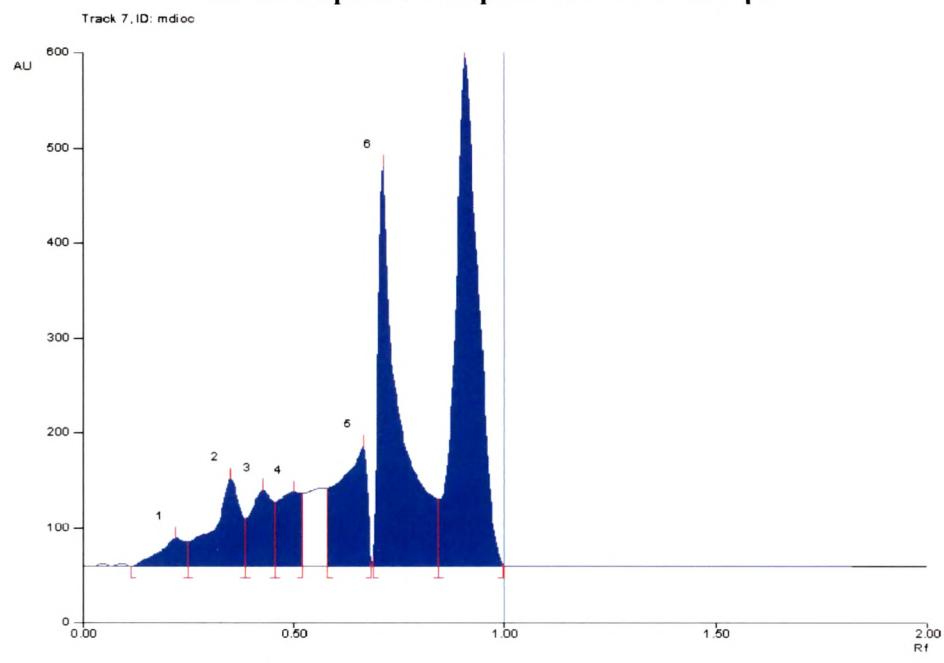


HPTLC Spectral Details of saponins of MD at 254 ηm

Peak	Maximum Rf	% area
1	0.20	3.69
2	0.28	2.33
3	0.37	1.38
4	0.70	36.09
5	0.78	28.36
6	0.82	22.29
7	0.90	5.87

HPTLC plate of saponins of MD at 366 ηm

HPTLC Spectra of Saponins of MD at 336 ηm



HPTLC Spectral Details of saponins of MD at 336 ηm

Peak	Maximum Rf	% area
1	0.23	3.12
2	0.35	8.90
3	0.43	7.47
4	0.50	6.79
5	0.66	9.97
6	0.71	29.91
7	0.94	33.84