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Anti obesity activity and beneficial effects of methanolic extract of *Desmostachya bipinnata, Canthium dicoccum, Sebestiania chaemelea* in HFD and Progesterone induced obesity in rats and mice

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ABSTRACT

Obesity is very serious and concerned problem these days. From the first human civilization, research is going to find the drugs to treat obesity and its complications. Despite availability of many drugs in market to treat obesity, no single drug is ideal for treating all sorts of problems caused by obesity. So the research is going on finding perfect drug. Prior going to evaluating drugs on humans, it is necessary to go for preclinical evaluation and usually the rodents are suitable models. The ideal obesity models available for obesity are induced by using chemicals and high fat diet. Methanolic extract of aerial parts of *Desmostachya bipinnata, Canthium dicoccum, Sebestiania chaemelea* plant was studied for its Anti-obesity activity in animal experimental models. Wistar albino rats, albino mice were used to study anti-obesity activity of methanolic extract of *D.bipinnata* plant aerial parts at doses 200 mg/kg p.o. and 400 mg/kg p.o. against the standard orlistat 50 mg/kg p.o. in models of anti-obesity activity viz. High fat induced obesity, Progesterone induced obesity model. The induction of obesity is done by diet (20 grams/animal/day) and progesterone (subcutaneous) in High fat induced obesity, Progesterone induced obesity models respectively. The study period is 28 days for both models. In both models, the plant showed anti-obesity activity significantly through the biochemical and behavioral parameters.

Keywords: Desmostachya Bipinnata, Canthium Dicoccum, Sebestiania Chaemelea, High Fat Diet, Orlistat, Progesterone, Anti-Obesity Activity

INTRODUCTION

Based on Ayurveda, Siddha, Unani systems traditional treatments for various diseases by plant extracts and products is on practice. But there is no sufficient preclinical evaluation studies are present to claim the plants are good at activity. From previous studies done on *Desmostachya bipinnta* and due to the presence of effective phytochemical constituents, methanolic extract of the plant aerial parts were selected for evaluating anti-obesity activity. The drug available in market, frequently prescribed and used for treating obesity is orlistat was kept as standard drug. And for obesity, various drugs are available in market and some are under clinical and preclinical phases. Emerging approach for treating obesity is on based on herbal and plant products [1-2]. From literature survey it was found that *Desmostachya bipinnata, Canthium dicoccum, Sebestiania chaemelea,* effective as diuretic, stimulants, aphrodisiac and used in dysentery and

menorrhagia, jaundice, asthama, uropathy and skin eruptions, Antiulcerogenic, Antipyretic, analgesic, anti-inflammatory, antihelicobacter activity [3-12]. The study period was 28 days for both models viz. High fat induced obesity, Progesterone induced obesity model. Animals used are male wistar rats and female albino mice in High fat induced obesity, Progesterone induced obesity models respectively. Before performing the anti-obesity activity of methanolic extract of the plant aerial parts, phytochemical evaluation was done. Progesterone is female reproductive hormone and neuro steroid [13]. Reports are demonstrated that it changes patho physiology and behavior of organism. It causes excess fat deposition in body. So progesterone was taken as disease control in progesterone induced obesity model. Epidemiological, preclinical studies suggest that there is a direct relationship between amount of diet consumed and obesity occurrence [14]. So the high fat diet was taken as disease control in High fat induced obesity model. The parameters evaluated in studies are biochemical and behavioral in both models.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Male wistar rats and female albino mice of 150-200 grams and 20-25 grams weighed were used for present study. The animals were housed in polypropylene cage (5 animals per cage), the standard conditions were maintaned (12 hours light and 12 hours dark cycle, 23 ± 5° C and 40-60% humidity). The standard rat and mice pellet, water were provided ad libitum. All the animals were collected from the central animal house SICRA Labs Pvt Ltd, IDA- Kukatpally, Hyderabad and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical Committee IAEC reg.no. (l82l/PO/Re/S/l5/CPCSEA).

EXPERIMENTAL PROCEDURE INDUCTION OF OBESITY

By High Fat Diet Method

The obesity in this model was induced by providing high fat diet 20 grams/day/animal. The study period is 28 days and high fat diet was provided daily.

By Progesterone

The obesity induced by injection of progesterone through sub cutaneous route for 28 days at dorsal neck region. The dose required for induction is 10 mg/kg and it was prepared by dissolving in arachis oil. 30 minutes prior to the administration of progesterone, test drugs were administered.

Preparation of Test Drug

The test drugs were prepared by 2% tween 80 . both standard and test drugs were given by oral gavage i.e. per oral route at a dose of 0.4 ml/kg body weight. All drugs were prepared freshly before administration.

EXPERIMENTAL PROCEDURE

In High Fat Diet Model

Rats were divided into five groups containing 6 animals in each group

Group I: Normal Control fed with normal diet and 2% tween 80 per oral
Group II: Negative Control fed with High Fat diet and 2% tween 80 per oral
Group III: Positive Control fed with High Fat diet and Orlistat 50 mg/kg B.W. per oral
Group IV: Test group (T1) fed with High Fat Diet and 200 mg/kg B.W. MEDB per oral
Group V: Test group (T2) fed with High Fat Diet and 400 mg/kg B.W. MEDB per oral

In Progesterone Induced Obesity Model

Mice were divided into five groups containing 6 animals in each group

Group I: Normal Control fed with normal diet and 2% tween 80 per oral
Group II: Negative Control treated with Progesterone in arachis oil sub cutaneously
Group III: Positive Control treated with Progesterone in arachis oil sub cutaneously and Orlistat 50 mg/kg B.W. per oral
Group IV: Test group (T1) treated with Progesterone in arachis oil sub cutaneously and 200 mg/kg B.W. MEDB per oral

Group V: Test group (T2) treated with Progesterone in arachis oil sub cutaneously and 400 mg/kg B.W. MEDB per oral

The study was carried out for 28 days. After completion of studies rats and mice were sacrificed, before sacrification of animals the blood was collected for biochemical estimation.

Assessment of Food Consumption Behaviour in Mice

In Progesterone induced obesity, it is important to observe food intake. It was observed on 7, 14, 21, 28th days. On these 4 days 30 min after last drug administration, 10 grams of sweetened chow was presented to each group of mice in petridishes and food take was recorded at 0.5, 1, 2 hrs time intervals. Rearing, grooming and ambulatory movements were recorded [15].

Biochemical parameters

On 29th blood was collected from mice and rats by retro orbital puncture method and subjected to TC, TG, LDL-c, VLDL-c, HDL-c, SGOT, SGPT, ALP [16-24] estimations by using prietest biochemical kits by ROBONIK biochemical analyzer.

Histopathology of Liver

After blood collection, the animals were sacrificed and livers were isolated for histopathology. After isolation organs were fixed in 10% formalin for prevent damage and stored for further histopathological process.

Statistical Analysis

The obtained results were expressed as Mean ± SEM. Comparison between control and treatment groups were performed by one way analysis of variance (ANOVA) followed by Dunnets test. The statistical significance criterion was $p< 0.05$ (95% level). $P<0.05$ is considered as significant.

RESULTS

In Progesterone induced obesity model, rearing, grooming, ambulatory movements were recorded and estimated serum glucose, TC, TG, LDL-c, VLDL-c, HDL-c, SGOT, SGPT, ALP. In High Fat Diet model TC, TG, LDL-c, VLDL-c, HDL-c, SGOT, SGPT, ALP estimations were performed and treatment groups are compared with disease control i.e. obesity control groups. And the statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnets test and results were found significant.

In Progesterone Induced Obesity Model

There was no significant change in the exploratory behavior of Progesterone control animals as compared to the control group animals but coadministration of MEDB, MECD, MESC 200 and 400 mg/kg significantly increased the number of ambutations and grooming but not the number of rearing.

Mice treated with Progesterone showed impairment in normal lipid profile, leading to increased total cholesterol, triglyceride, LDL-C, VLDL-C while HDL-C was decreased. MEDB, MECD, MESC at 200mg/kg bw showed significant reduction ($p<0.01$), while, MEDB, MECD, MESC at 400mg/kg bw significantly decreased ($p<0.05$) the total cholesterol levels were highly significant reduction of $p<0.01$ was observed with orlistat at 10 mg/kg bw.

Significant reduction of triglycerides, $p<0.05$ was seen with MEDB, MECD, MESC 200 mg/kg bw and the values were found to be <0.05 with MEDB, MECD, MESC 400 mg/kg bw whereas highly significant reduction $p<0.01$ was seen with orlistat at 10 mg/kg bw.

LDL and VLDL were significantly reduced $p<0.01$ with MEDB, MECD, MESC at 200 mg/kg bw but with MEDB, MECD, MESC 400 mg/kg bw and orlistat at 10 mg/kg bw the value of LDL was found to be $p<0.05$.Whereas HDL-C levels were significantly increased with MEDB, MECD, MESC 400 mg/kg bw and orlistat at 10 mg/kg bw $p<0.05$ when compared to normal and untreated groups.

In High Fat Diet Model

Rats fed with high fat diet (HFD) showed impairment in normal lipid profile, leading to increased total cholesterol, triglyceride, LDL-C, VLDL-C while HDL-C was decreased. MEDB, MECD, MESC at 200 mg/kg bw showed significant reduction ($p<0.05$), while, MEDB, MECD, MESC at 400 mg/kg bw significantly decreased ($p<0.01$) the total cholesterol levels were highly significant reduction of $p<0.001$ was observed with orlistat at 50 mg/kg bw.

Significant reduction of triglycerides, $p<0.05$ was seen with MEDB, MECD, MESC 200 mg/kg

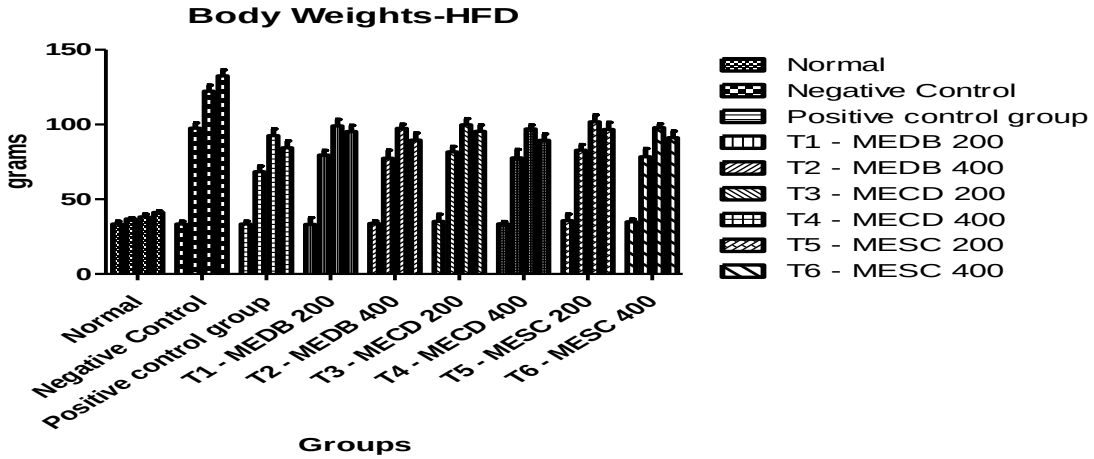
bw and the values were found to be <0.01 with MEDB, MECD, MESC 400 mg/kg bw whereas highly significant reduction p<0.001 was seen with orlistat at 50 mg/kg bw.

LDL and VLDL were significantly reduced p<0.05 with MEDB, MECD, MESC at 200 mg/kg bw but with MEDB, MECD, MESC 400 mg/kg bw and orlistat at 50 mg/kg bw the value of LDL was found to be p<0.01.Whereas HDL-C levels were significantly increased with MEDB, MECD, MESC 400 mg/kg bw and orlistat at 50 mg/kg bw p<0.01 when compared to normal and untreated groups.

Table 1: Effect of MEDB, MECD & MESC on body weights of rats (HFD Model)

	Differences in body weights (gm) (Mean ± SEM)			
Group (n=6)	Week	Week 2	Week 3	Week 4
Group I Normal control group	33.2 ± 1.92	36.8 ± 0.9	38.2 ± 1.9	41.20 ± 1.0
Group II Negative control group HFD	33.4 ± 1.89	97.6 ± 3.5	122.3 ± 4.0	132.6 ± 3.9
Group III Positive control group Orlistat 50mg/kg b.w. p.o	33.4 ± 1.86	68.4 ± 3.8	92.6 ± 4.5	84.4 ± 4.6
Group IV T_1 – MEDB 200mg/kg b.w. p.o	33.2 ± 4.5	79.6 ± 3.1	99.1 ± 4.3	95.3 ± 4.1
Group V T_2 – MEDB 400mg/kg b.w. p.o	33.8 ± 1.6	77.4 ± 5.4	97.4 ± 2.8	89.54 ± 4.8
Group VI T_3 – MECD 200mg/kg b.w. p.o	35.2 ± 4.7	81.7 ± 3.6	99.8 ± 4.1	95.39± 4.3
Group VII T_4 – MECD 400mg/kg b.w. p.o	33.6 ± 1.4	77.7 ± 5.6	97.1 ± 2.6	89.44 ± 4.2
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	35.7 ± 4.3	82.8 ± 3.8	101.8 ± 4.6	96.7 ± 4.7
Group IX T_6 – MEDB 400mg/kg b.w. p.o	34.9 ± 1.8	78.4 ± 5.5	97.9 ± 2.5	91.24 ± 4.3

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to untreated groups.



Graph 1: Effect of MEDB, MECD & MESC on body weights of rats

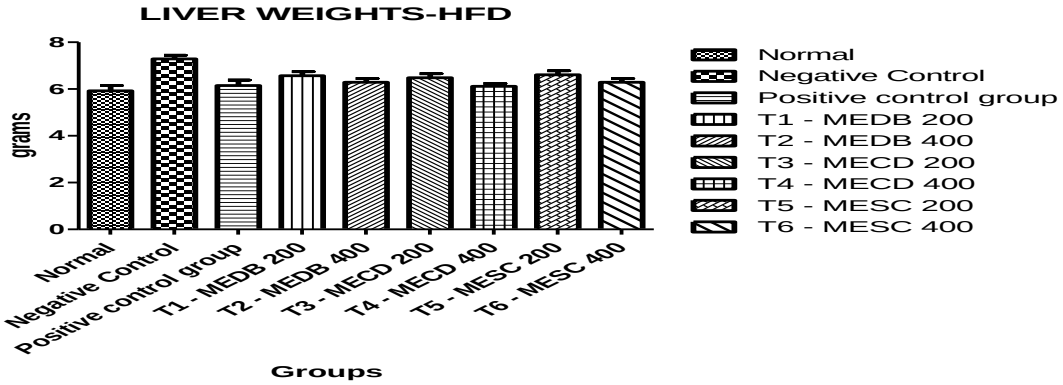
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to untreated groups

Table 2: Effects of MEDB, MECD & MESC on liver weights of rats (HFD Model)

Groups (n = 6)	Liver weights (g) (Mean ± SEM)
Group I Normal control group	5.92 ± .23
Group II Negative control group HFD	7.29 ± 0.15
Group III Positive control group Orlistat 50mg/kg b.w. p.o	6.15 ± 0.23***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	6.57 ± 0.17*
Group V T_2 – MEDB 400mg/kg b.w. p.o	6.29 ± 0.16**
Group VI T_3 – MECD 200mg/kg b.w. p.o	6.48 ± 0.18*
Group VII T_4 – MECD 400mg/kg b.w. p.o	6.12 ± 0.12***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	6.61 ± 0.17
Group IX T_6 – MEDB 400mg/kg b.w. p.o	6.29 ± 0.16**

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to untreated groups.



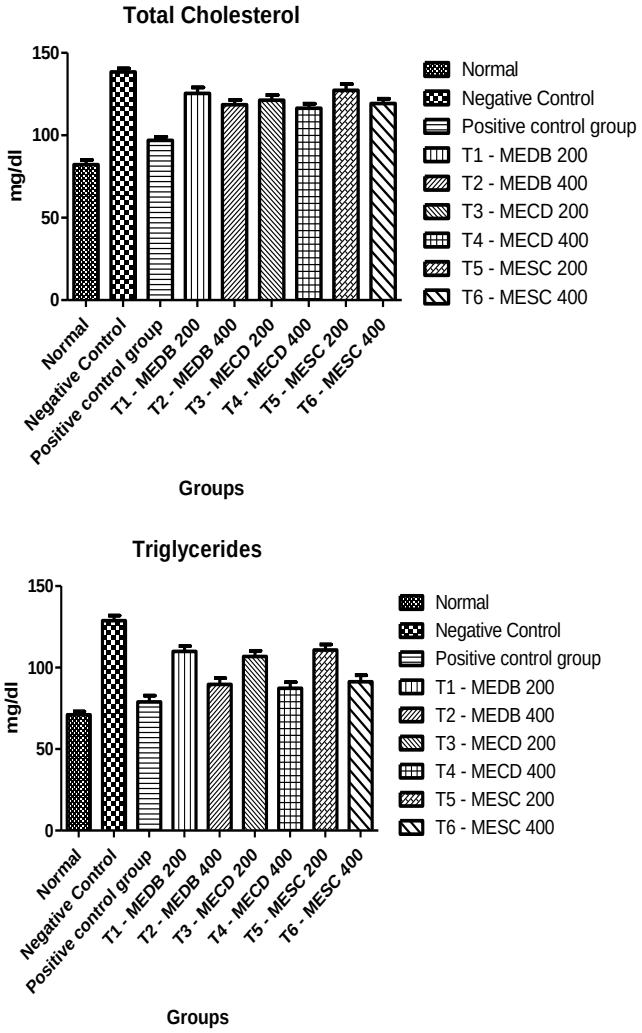
Graph 2: Effect of MEDB, MECD & MESC on Liver weights of rats (HFD MODEL)

ANOVA followed by Dunnet's t-test Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to untreated groups

Table 3: Effect of MEDB, MECD & MESC on Total Cholesterol and Triglyceride levels in HFD rats

Groups (n = 6)	Total Cholesterol (mg/dl) Mean ± SEM	Triglycerides (mg/dl) Mean ± SEM
Group I Normal control group	82.13 ± 2.98	71.05 ± 1.98
Group II Negative control group HFD	158.43 ± 2.13	168.87 ± 3.12
Group III Positive control group Orlistat 50mg/kg b.w. p.o	96.98 ± 2.04***	78.91 ± 3.89***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	125.43 ± 3.65*	109.98 ± 3.16***
Group V T_2 – MEDB 400mg/kg b.w. p.o	118.5 ± 2.91**	89.63 ± 3.87
Group VI T_3 – MECD 200mg/kg b.w. p.o	121.25 ± 3.22*	106.85 ± 3.45***
Group VII T_4 – MECD 400mg/kg b.w. p.o	116.43 ± 2.78***	87.32 ± 3.69
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	127.33 ± 3.72	110.82 ± 3.34***
Group IX T_6 – MEDB 400mg/kg b.w. p.o	119.3 ± 2.93**	91.34 ± 3.91

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to Untreated groups.



Graph 3, 4: Effect of MEDB, MECD & MESC on Total Cholesterol, Triglycerides of rats (HFD MODEL)

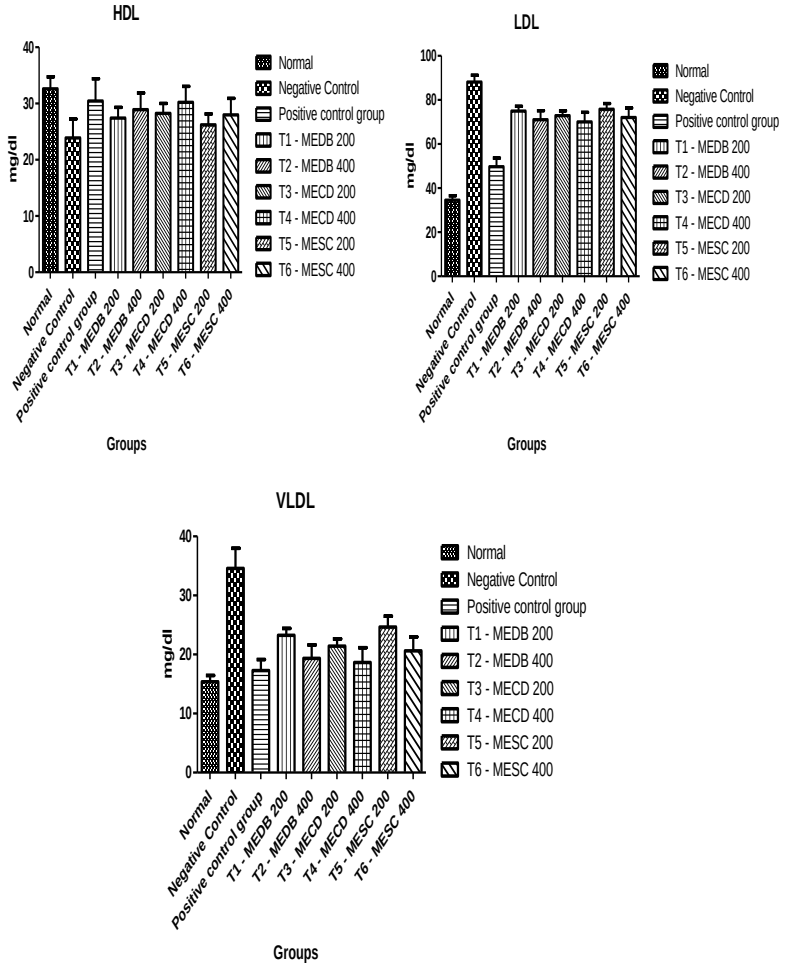
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to Untreated groups

Table 4: Effect of MEDB, MECD & MESC on HDL, LDL AND VLDL levels in rats

Groups (n = 6)	HDL (mg/dl) Mean ± SEM	LDL (mg/dl) Mean ± SEM	VLDL (mg/dl) Mean ± SEM
Group I Normal control group	32.62 ± 2.12	34.54 ± 2.01	15.39 ± 1.07
Group II Negative control group HFD	23.87 ± 3.39	88.09 ± 3.12	27.59 ± 3.39
Group III Positive control group Orlistat 50mg/kg b.w. p.o	30.45 ± 3.97**	49.67 ± 3.96***	17.29 ± 1.87***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	27.42 ± 1.89*	74.98 ± 2.12***	23.24 ± 1.18**
Group V T_2 – MEDB 400mg/kg b.w. p.o	28.91 ± 2.98**	71.02 ± 4.14***	19.36 ± 2.25***
Group VI T_3 – MECD 200mg/kg b.w. p.o	28.26 ± 1.76*	72.85 ± 2.23***	21.43 ± 1.21***
Group VII T_4 – MECD 400mg/kg b.w. p.o	30.21 ± 2.83**	70.02 ± 4.34***	18.64 ± 2.52***
Group VIII T_5– MEDB 200mg/kg b.w. p.o	26.22 ± 1.93*	75.81 ± 2.53***	24.65 ± 1.82*
Group IX T_6 – MEDB 400mg/kg b.w. p.o	27.99 ± 2.94**	72.02 ± 4.32***	20.63± 2.36***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to normal and untreated groups



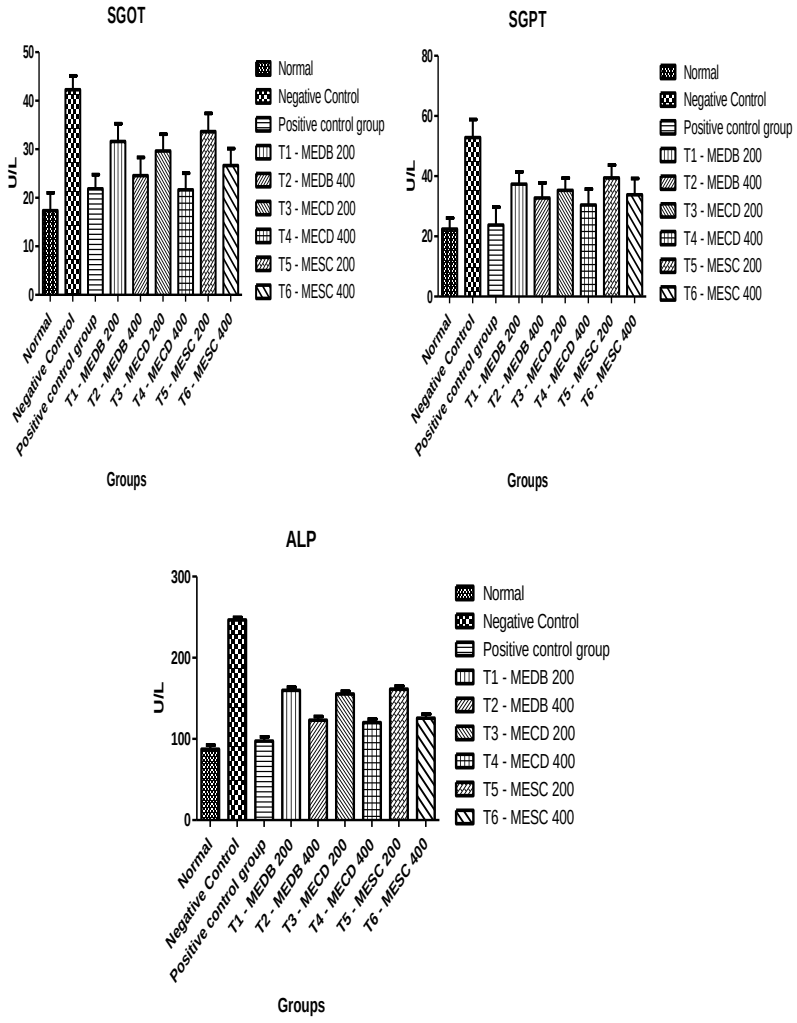
Graph 5, 6, 7: Effect of MEDB, MECD & MESC on HDL, LDL, VLDL of rats (HFD MODEL)

ANOVA followed by Dunnet's t-test *p<0.05, **p<0.01 was considered significant compared to normal and untreated groups

Table 5: Effect of MEDB, MECD & MESC on SGOT, SGPT AND ALP levels in rats

Groups (n =6)	**SGOT (IU/L) Mean ± SEM**	**SGPT (IU/L) Mean ± SEM**	**ALP (IU/L) Mean ± SEM**
Group I Normal control group	17.34 ± 3.67	22.42 ± 3.65	87.49 ± 4.93
Group II Negative control group HFD	42.28 ± 2.87	52.85 ± 5.98	246.59 ± 2.98
Group III Positive control group Orlistat 50mg/kg b.w. p.o	21.84 ± 2.91***	23.78 ± 5.92***	97.31 ± 5.24***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	31.59 ± 3.66	37.39 ± 4.03	159.93 ± 3.61***
Group V T_2 – MEDB 400mg/kg b.w. p.o	24.56 ± 3.75**	32.78 ± 5.02*	123.09 ± 4.63***
Group VI T_3 – MECD 200mg/kg b.w. p.o	29.64 ± 3.45	35.28 ± 4.12	155.34 ± 3.28***
Group VII T_4 – MECD 400mg/kg b.w. p.o	21.63 ± 3.43***	30.45 ± 5.23*	120.09 ± 4.38***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	33.62 ± 3.76	39.46 ± 4.23	161.49 ± 3.65***
Group IX T_6 – MEDB 400mg/kgb.w. p.o	26.65 ± 3.49*	33.84 ± 5.39	125.68 ± 4.93***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to normal and untreated groups

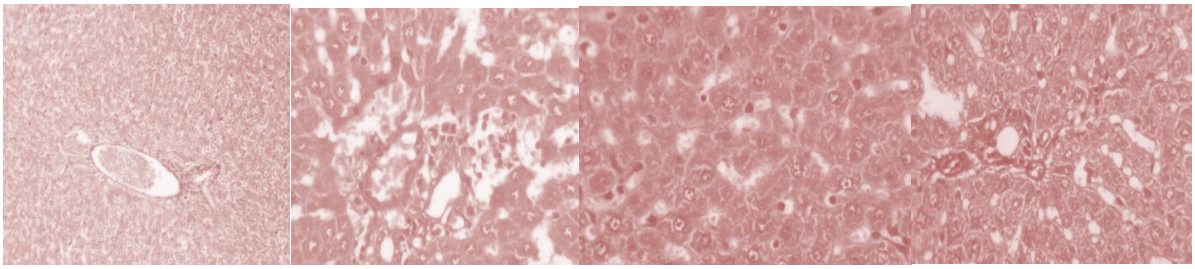


Graph 8, 9, 10: Effect of MEDB, MECD & MESC on SGOT, SGPT AND ALP of rats (HFD MODEL)

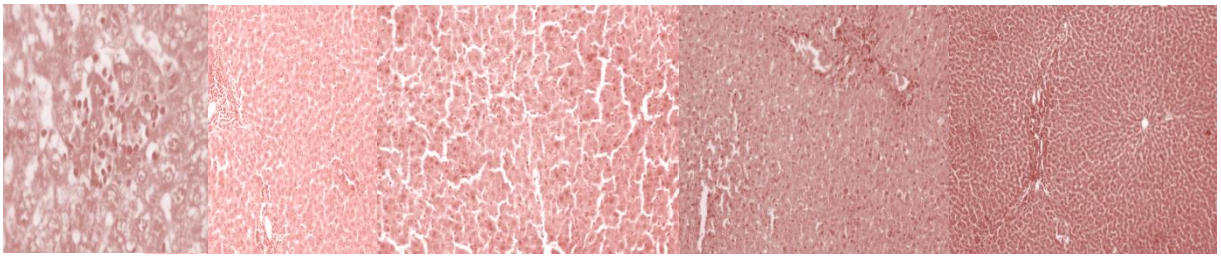
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to normal and untreated groups

Histopathology-HFD model



Normal Control | Disease Control (HFD) | MEDB-T_1 200mg/kg | MEDB-T_2 400mg/kg



Standard T_3 MECD200mg/kg T_4 MECD 400mg/kg T_5 MESC 200mg/kg T_6 MESC 400mg/kg

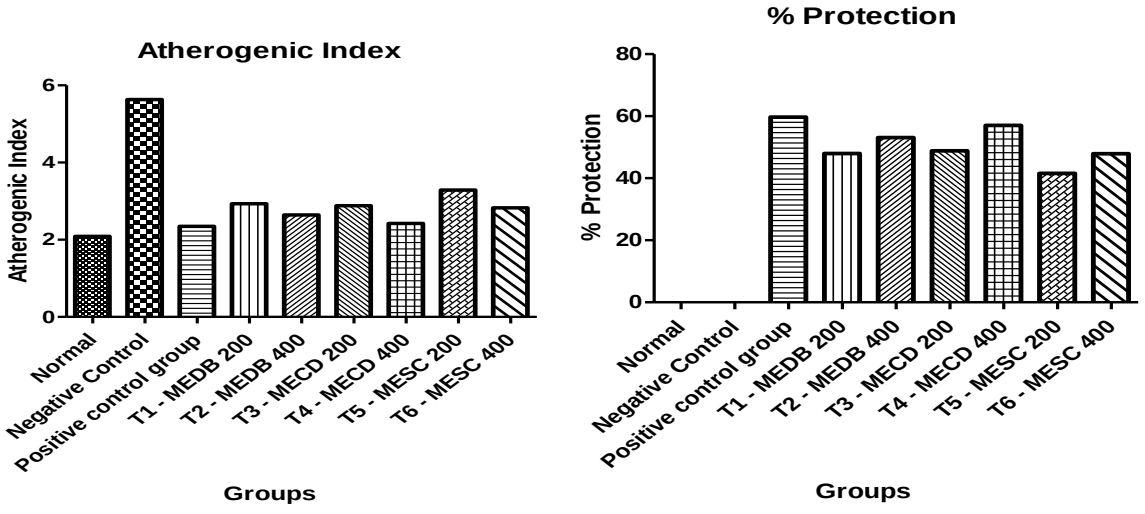
Figure 1 Histopathology of Liver-High Fat Diet

Table 6: Atherogenic index and percentage protection with MEDB, MECD & MESC: (HFD MODEL)

Groups (n =6)	Atherogenic index of plasma (AIP)	Percentage protection
Group I Normal control group	2.09	
Group II Negative control group HFD	5.63	
Group III Positive control group Orlistat 50mg/kg b.w. p.o	2.35	59.7 %
Group IV T_1 – MEDB 200mg/kg b.w. p.o	2.93	47.95 %
Group V T_2 – MEDB 400mg/kg b.w. p.o	2.64	53.10 %
Group VI T_3 – MECD 200mg/kg b.w. p.o	2.88	48.84 %
Group VII T_4 – MECD 400mg/kg b.w. p.o	2.42	57.01 %
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	3.29	41.56 %
Group IX T_6 – MEDB 400mg/kg b.w. p.o	2.83	49.73 %

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated group

EFFECT OF MEDB, MECD & MESC ON ATHEROGENIC INDEX IN HFD MODEL



Graph 11, 12: Effect of MEDB, MECD & MESC on AI, % protection of rats (HFD MODEL)

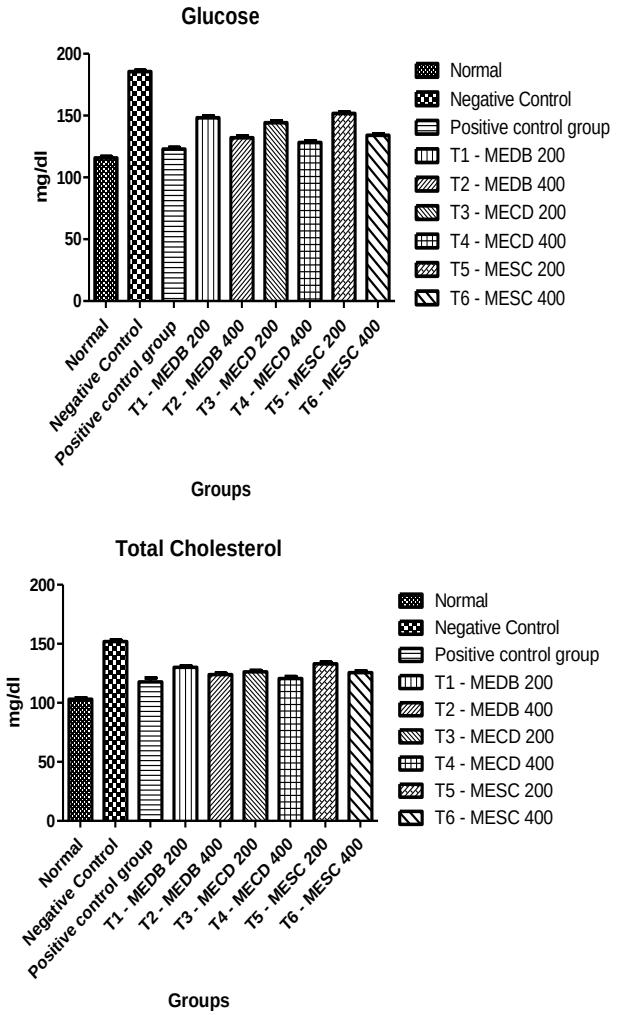
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05,**p<0.01 was considered significant compared to untreated groups

Table 7: Effect of MEDB, MECD, and MESC on Glucose and Total cholesterol levels in mice

Groups (n =6)	Glucose (mg/dl) Mean ± SEM	TC (mg/dl) Mean ± SEM
Group I Normal control group	115.81 ± 1.16	103.03 ± 1.19
Group II Negative control group HFD	185.50 ± 1.09	151.92 ± 1.12
Group III Positive control group Orlistat 50mg/kg b.w. p.o	122.93 ± 1.31***	117.83 ± 3.3***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	148.33 ± 1.47***	130.06 ± 1.16***
Group V T_2 – MEDB 400mg/kg b.w. p.o	132.1 ± 1.42***	123.91 ±1.1***
Group VI T_3 – MECD 200mg/kg b.w. p.o	144.21 ± 1.47***	126.24 ± 1.24***
Group VII T_4 – MECD 400mg/kg b.w. p.o	128.23 ± 1.35***	120.63 ±1.56***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	151.67 ± 1.25***	133.06 ± 1.36***
Group IX T_6 – MEDB 400mg/kg b.w. p.o	134.1 ± 1.22***	125.62 ±1.45***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to Untreated groups



Graph 13, 14: Effect of MEDB, MECD, and MESC on Glucose and Total cholesterol of Mice (Progesterone induced Model)

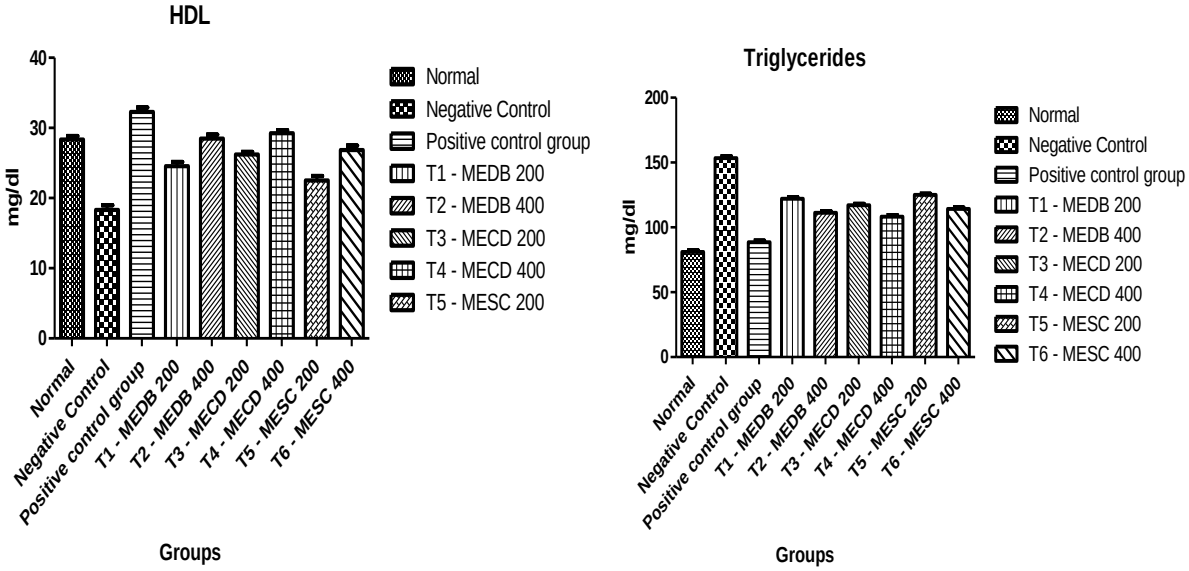
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05,**p<0.01 was considered significant compared to untreated groups

Table 8: Effect of MEDB, MECD, and MESC on HDL and Triglycerides levels in mice

Groups (n =6)	HDL (mg/dl) Mean ± SEM	TG (mg/dl) Mean ± SEM
Group I Normal control group	28.34 ± .52	81.09 ± 1.25
Group II Negative control group HFD	18.31 ± .67	153.46 ± 1.55
Group III Positive control group Orlistat 50mg/kg b.w. p.o	32.29 ± .61***	88.62 ± 1.24***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	24.56 ± .59***	122.06 ± 1.43***
Group V T_2 – MEDB 400mg/kg b.w. p.o	28.48 ± .57***	111.22 ±1.34***
Group VI T_3 – MECD 200mg/kg b.w. p.o	26.23 ± .36***	117.06 ± 1.22***
Group VII T_4 – MECD 400mg/kg b.w. p.o	29.26 ± .43***	108.25 ±1.26***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	22.52 ± .64***	125.06 ± 1.07***
Group IX T_6 – MEDB 400mg/kg b.w. p.o	26.86 ± .63***	114.22 ±1.26***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to Untreated groups



Graph 15, 16: Effect of MEDB, MECD, and MESC on HDL, TG of Mice (Progesterone induced Model)

ANOVA followed by Dunnet's t-test

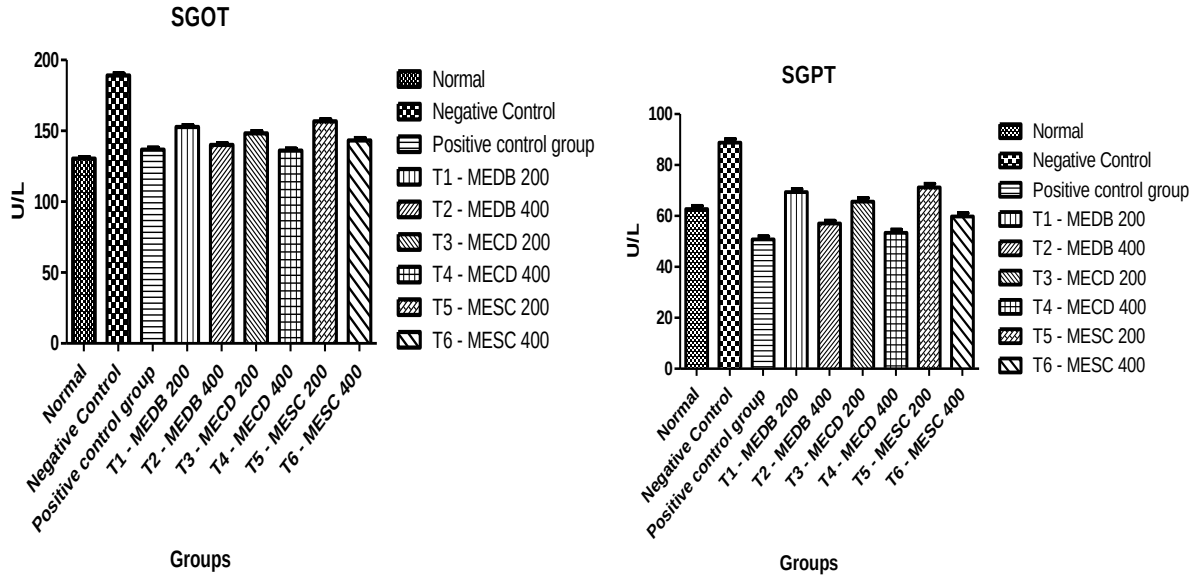
Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to Untreated groups

Table 9: Effect of MEDB, MECD, and MESC on SGOT, SGPT in mice

Groups (n =6)	SGOT (mg/dl) Mean ± SEM	SGPT (mg/dl) Mean ± SEM
Group I Normal control group	130.43 ± .92	62.67 ± 1.26
Group II Negative control group HFD	189.08 ± 1.26	88.83 ± 1.36

Group III Positive control group Orlistat 50mg/kg b.w. p.o	136.8 ± 1.21***	50.83 ±1.14***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	152.68 ± 1.17***	69.36 ±1.19***
Group V T_2 – MEDB 400mg/kg b.w. p.o	140.01 ±1.19***	57.04 ±1.10***
Group VI T_3 – MECD 200mg/kg b.w. p.o	148.28 ± 1.5***	65.62 ±1.38***
Group VII T_4 – MECD 400mg/kg b.w. p.o	136.12 ±1.28***	53.36 ±1.28***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	156.82 ± 1.28***	71.24 ±1.36***
Group IX T_6 – MEDB 400mg/kg b.w. p.o	143.29 ±1.64***	59.81 ±1.29***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated groups



Graph 17, 18: Effect of MEDB, MECD, and MESC on SGOT, SGPT of Mice (Progesterone induced Model)

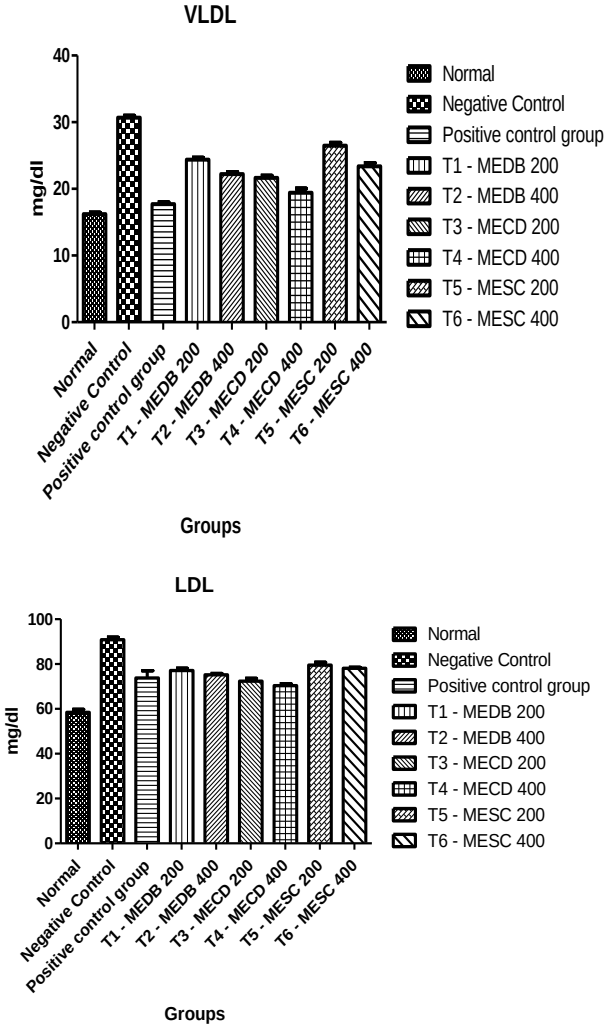
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05,**p<0.01 was considered significant compared to untreated Groups

Table 10: Effect of MEDB, MECD, and MESC on VLDL, LDL levels in mice

Groups (n =6)	**VLDL (mg/dl) Mean ± SEM**	**LDL (mg/dl) Mean ± SEM**
Group I Normal control group	16.2 ± .25	58.48 ± 1.32
Group II Negative control group HFD	30.69 ± .30	90.91 ± 1.03
Group III Positive control group Orlistat 50mg/kg b.w. p.o	17.72 ± .25***	73.81 ±3.24***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	24.39 ± .28***	77.11 ±1.05***
Group V T_2 – MEDB 400mg/kg b.w. p.o	22.23 ±.27***	75.19±.62***
Group VI T_3 – MECD 200mg/kg b.w. p.o	21.65 ± .34***	72.36 ±1.23***
Group VII T_4 – MECD 400mg/kg b.w. p.o	19.43 ±.63***	70.37±.84***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	26.47 ± .46***	79.54 ±1.22***
Group IX T_6 – MEDB 400mg/kg b.w. p.o	23.37 ±.51***	78.14±.41***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant Compared to untreated groups



Graph 19, 20: Effect of MEDB, MECD, and MESC on VLDL, LDL of Mice (Progesterone induced Model)

ANOVA followed by Dunnet's t-test

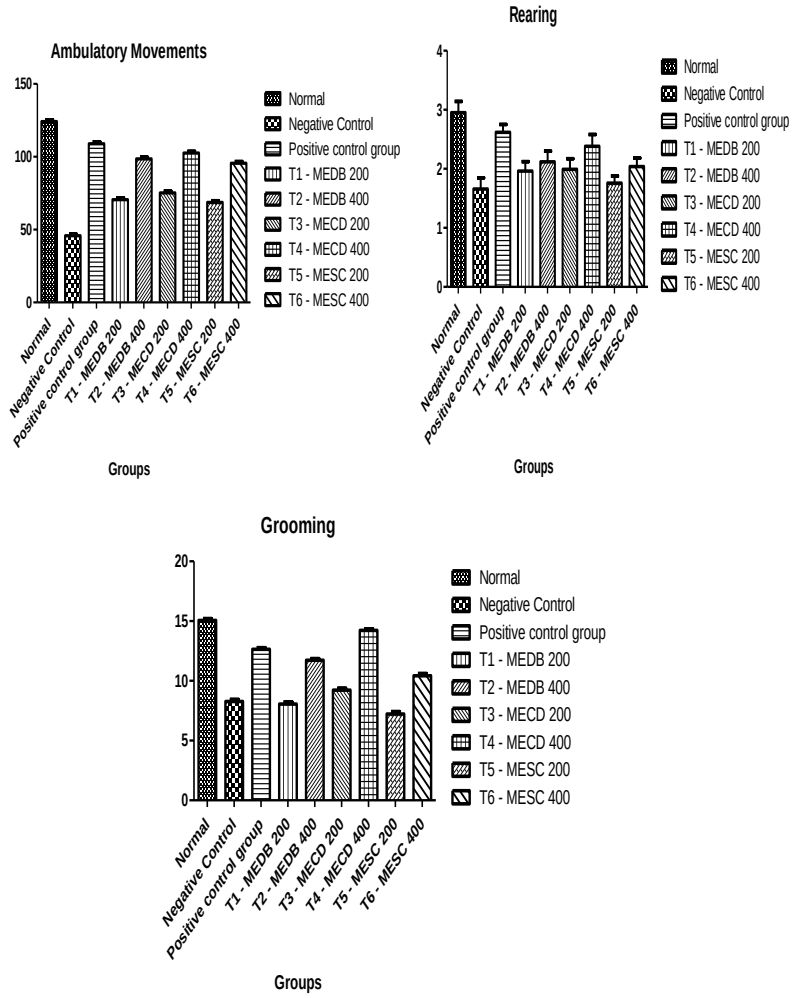
Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01 was considered significant compared to untreated Groups

Table 11: Effect of MEDB, MECD, and MESC on Ambulatory movements, Rearing and Grooming in ice

Groups (n =6)	Ambulatory movements Mean ± SEM	Rearing Mean ± SEM	GROOMING Mean ± SEM
Group I Normal control group	124.18 ±1 .25	2.95 ± .19	15.06 ± .13
Group II Negative control group HFD	45.77 ±1.12	1.66 ± .19	8.27 ±.17
Group III Positive control group Orlistat 50mg/kg b.w. p.o	108.94 ± 1.31***	2.62 ±.13**	12.64± .14***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	70.57 ± 1.21***	1.96 ±.16 NS	8.05 ±.18NS
Group V T_2 – MEDB 400mg/kg b.w. p.o	98.56 ±1.21***	2.12±.18NS	11.72 ±.14***
Group VI T_3 – MECD 200mg/kg b.w. p.o	75.23 ± 1.26***	1.99 ±.18 NS	9.22 ±.16**
Group VII T_4 – MECD 400mg/kg b.w. p.o	102.56 ±1.28***	2.38±.20NS	14.23 ±.11***

Group VIII T_5 – MEDB 200mg/kg b.w. p.o	68.73 ± 1.19***	1.76 ±.12 NS	7.22 ±.21***
Group IX T_6 – MEDB 400mg/kg b.w. p.o	95.54 ±1.21***	2.04±.14NS	10.43 ±.18***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated groups



Graph 21, 22, 23: Effect of MEDB, MECD, and MESC on Ambulatory movements, Rearing of Mice (Progesterone induced Model)

ANOVA followed by Dunnet's t-test

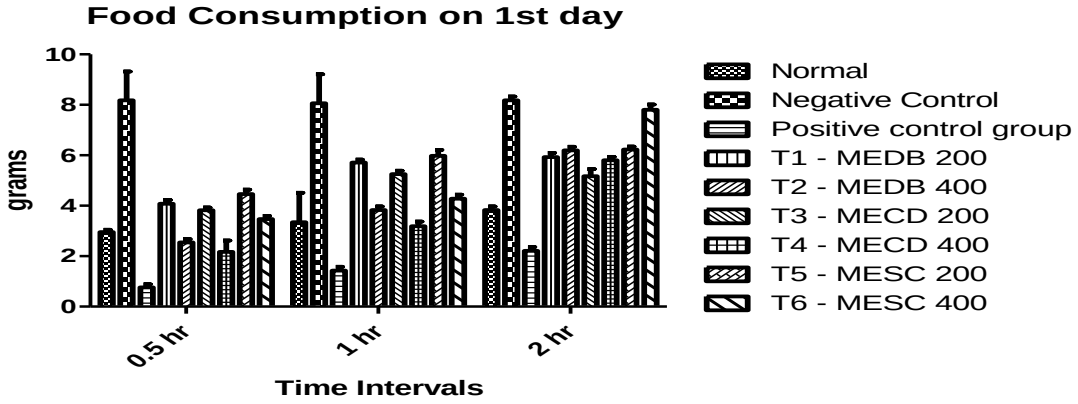
Values are expressed as Mean ± SEM (n=6) *p<0.05,**p<0.01 was considered significant compared to untreated Groups

Table 12: Effect of MEDB, MECD, and MESC on Food Consumption in mice on 1st day

Groups (n =6)	0.5hr	1hr	2hr
Group I Normal control group	2.95 ± .1	3.35 ± 1.16	3.83 ± .14
Group II Negative control group HFD	8.18 ± 1.14	8.06 ± 1.15	8.18 ± .15
Group III Positive control group Orlistat 50mg/kg b.w. p.o	.76 ± .13***	1.43 ± .15***	2.21 ± .15***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	4.08 ± .15**	5.71 ± .12**	5.93 ± .17*
Group V T_2 – MEDB 400mg/kg b.w. p.o	2.55 ± .13***	3.83 ± .14*	6.2 ± .14***
Group VI T_3 – MECD 200mg/kg b.w. p.o	3.82 ± .11**	5.25 ± .14***	5.17 ± .28**

Group VII T_4 – MECD 400mg/kg b.w. p.o	2.17 ± .45***	3.19 ± .18**	5.8 ± .14***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	4.46 ± .18*	5.98 ± .23*	6.23 ± .12*
Group IX T_6 – MEDB 400mg/kg b.w. p.o	3.47 ± .13***	4.28 ± .16***	7.8 ± .21***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated groups



Graph 24: Effect of MEDB, MECD & MESC on Food Consumption of Mice (Progesterone induced Model)-1st day

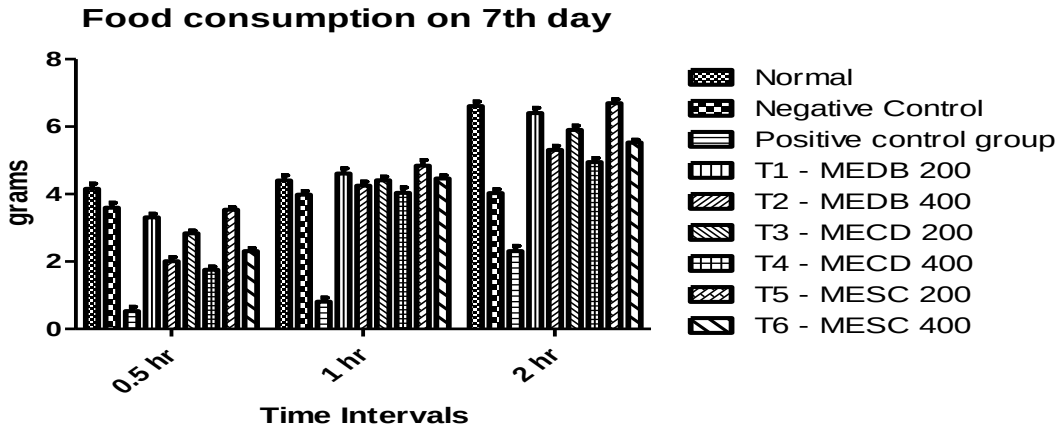
ANOVA followed by Dunnet's t-test

Values are expressed as Mean ± SEM (n=6) *p<0.05,**p<0.01 was considered significant compared to untreated groups

Table 13: Effect of MEDB, MECD, and MESC on Food Consumption in mice on 7th day

Groups (n =6)	0.5hr	1hr	2hr
Group I Normal control group	4.16 ± .15	4.4 ± .16	6.61 ± .13
Group II Negative control group HFD	3.6 ± .14	3.98± .11	4.03 ± .11
Group III Positive control group Orlistat 50mg/kg b.w. p.o	.53 ± .13***	.81 ±.12***	2.31 ± .15***
Group IV T_1 – MEDB 200mg/kg b.w. p.o	3.31 ± .11	4.61 ± .15*	6.4 ± .16**
Group V T_2 – MEDB 400mg/kg b.w. p.o	2.01 ± .12**	4.25 ± .12	5.31 ± .12***
Group VI T_3 – MECD 200mg/kg b.w. p.o	2.84 ± .08	4.41 ± .11*	5.9 ± .13*
Group VII T_4 – MECD 400mg/kg b.w. p.o	1.76 ± .09**	4.04 ± .16	4.95 ± .12***
Group VIII T_5 – MEDB 200mg/kg b.w. p.o	3.54 ± .07	4.84 ± .17*	6.7 ± .11**
Group IX T_6 – MEDB 400mg/kg b.w. p.o	2.31 ± .09***	4.46 ± .10	5.53 ± .08***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant Compared to untreated groups

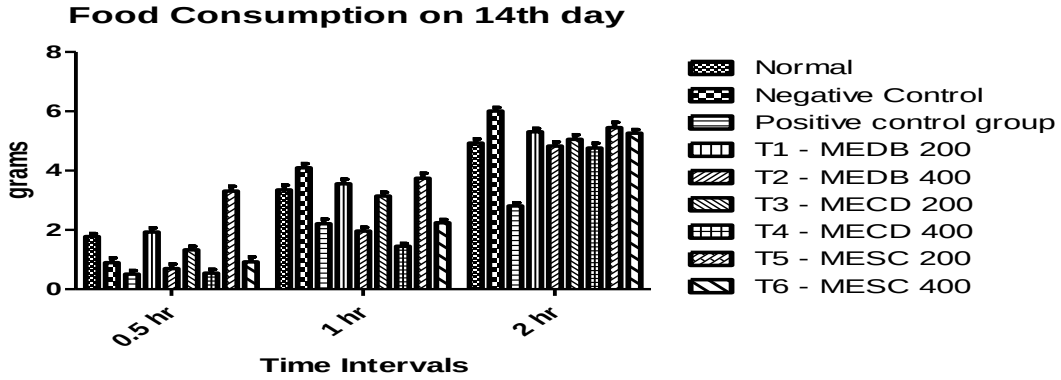


Graph 25: Effect of MEDB, MECD & MESC on Food Consumption of Mice (Progesterone induced Model)-7th days

Table 14: Effect of MEDB, MECD, and MESC on Food Consumption in mice on 14th day

Groups (n = 5)	0.5hr	1hr	2hr
Group I Normal control	1.78± .1	3.35 ± .16	4.93 ± .14
Group II Negative control (Progesterone)	.9 ± .16	4.1± .14	6.01 ± .12
Group III Positive control Orlistat 10mg/kg b.w. p.o	.51 ± .12***	2.21 ±.15***	2.81 ± .11***
Group IV T1 – MEDB 200mg/kg b.w. p.o	1.93 ± .14*	3.56 ± .15	5.31 ± .11**
Group V T2 – MEDB 400mg/kg b.w. p.o.	.7 ± .15	1.96 ± .13**	4.83 ± .14
Group VI T_3 – MECD 200mg/kg b.w. p.o	1.33 ± .12**	3.14 ± .13	5.06 ± .15**
Group VII T_4 – MECD 400mg/kg b.w. p.o.	.54 ± .13*	1.45 ± .09***	4.76 ± .16
Group VIII T_5 – MESC 200mg/kg b.w. p.o	3.31 ± .16**	3.74 ± .17	5.45 ± .18*
Group IX T_6 – MESC 400mg/kg b.w. p.o.	.92 ± .17***	2.24 ± .11**	5.26 ± .12**

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated group



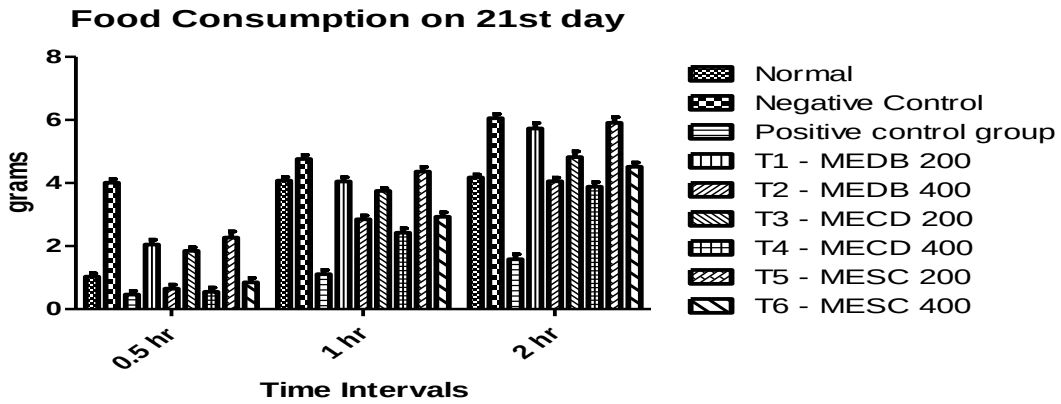
Graph 26: Effect of MEDB, MECD & MESC on Food Consumption of Mice (Progesterone induced Model)-14th days

Table 15: Effect of MEDB, MECD, and MESC on Food Consumption in mice on 21st day

Groups (n = 6)	0.5hr	1hr	2hr
Group I Normal control	1.03± .11	4.08 ± .11	4.18 ± .09
Group II Negative control (Progesterone)	4.01 ± .11	4.76± .13	6.06 ± .13
Group III Positive control	.46 ± .12***	1.11 ±.13***	1.58 ± .16***

Orlistat 10mg/kg b.w. p.o			
Group IV T1 – MEDB 200mg/kg b.w. p.o	2.05 ± .15***	4.05 ± .13**	5.73 ± .17
Group V T2 – MEDB 400mg/kg b.w. p.o.	.65± .13***	2.85 ± .12***	4.06 ± .11
Group VI T_3 – MECD 200mg/kg b.w. p.o	1.85 ± .11***	3.75 ± .09**	4.82 ± .18
Group VII T_4 – MECD 400mg/kg b.w. p.o.	.55± .13***	2.42 ± .14***	3.88 ± .15
Group VIII T_5 – MESC 200mg/kg b.w. p.o	2.27 ± .19***	4.36 ± .15**	5.91 ± .18
Group IX T_6 – MESC 400mg/kg b.w. p.o.	.85± .14***	2.93 ± .14***	4.52 ± .13

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated groups

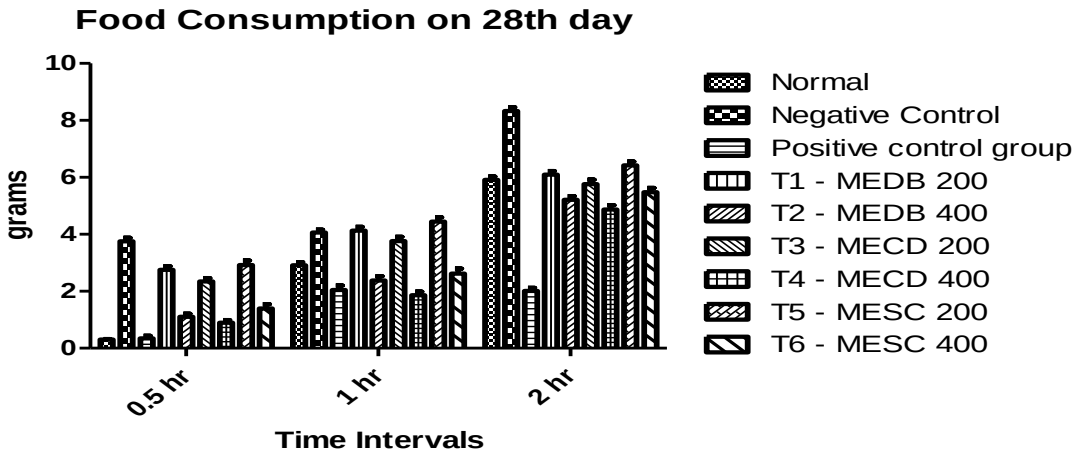


Graph 27: Effect of MEDB, MECD & MESC on Food Consumption of Mice (Progesterone induced Model)-21st days

Table 16: Effect of MEDB, MECD, and MESC on Food Consumption in mice on 28th day

Groups (n = 5)	**0.5hr**	**1hr**	**2hr**
Group I Normal control	.3± .02	2.91 ± .11	5.91 ± .12
Group II Negative control (Progesterone)	3.75 ± .13	4.06± .10	8.33 ± .13
Group III Positive control Orlistat 10mg/kg b.w. p.o	.35 ± .09***	2.05 ±.15***	2.01 ± .11***
Group IV T1 – MEDB 200mg/kg b.w. p.o	2.75 ± .13***	4.13 ± .13	6.1 ± .12***
Group V T2 – MEDB 400mg/kg b.w. p.o.	1.1± .11***	2.38 ± .15***	5.21 ± .13***
Group VI T_3 – MECD 200mg/kg b.w. p.o	2.35 ± .11***	3.76 ± .15	5.76 ± .16***
Group VII T_4 – MECD 400mg/kg b.w. p.o.	.9± .09***	1.86 ± .13***	4.87 ± .15***
Group VIII T_5 – MESC 200mg/kg b.w. p.o	2.92 ± .16***	4.45 ± .15	6.42 ± .13***
Group IX T_6 – MESC 400mg/kg b.w. p.o.	1.4± .15***	2.62 ± .18***	5.48 ± .15***

Values are expressed as Mean ± SEM (n=6) *p<0.05, **p<0.01, ***p<0.001 was considered significant compared to untreated groups



Graph 28: Effect of MEDB, MECD & MESC on Food Consumption of Mice (Progesterone induced Model)-28th days

HEPATIC MORPHOLOGY AND HISTOPATHOLOGY:

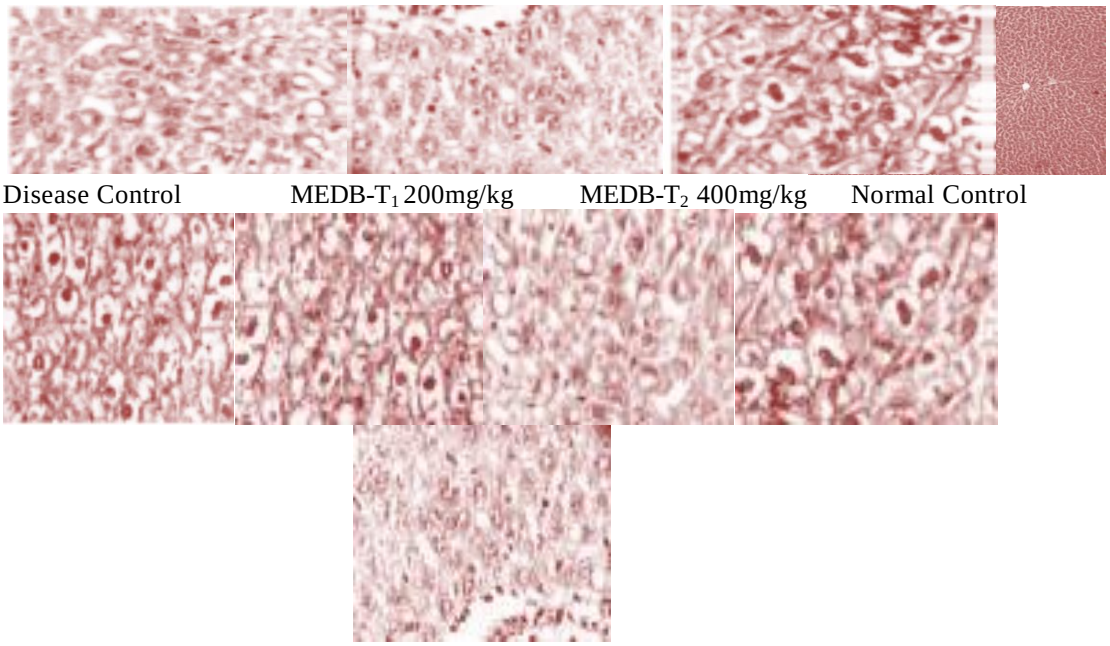


Figure 2 Histopathology of Liver-Progesterone Induced Model

DISCUSSION

In the present study, the anti-obesity activity of methanolic extract of aerial parts of *Desmostachya bipinnata, Canthium dicoccum, Sebestiania chaemelea* (MEDB, MECD, MESC) was studied using dietary animal's model of obesity. There was significant increase in the body weight in High fat diet (HFD) treated animals, which was significantly decreased by the administration of MEDB, MECD, MESC and Orlistat.

The investigation revealed that both models causes increase in serum lipid profiles: Total cholesterol, Triglycerides, LDL and VLDL with decrease in HDL and the liver function test also showed Increase in SGOT SGPT and ALP levels. However, there was significant decrease in TG, TC, LDL, VLDL, SGOT, SGPT and ALP with increase

in HDL levels. This may be attributed to the action of MEDB, MECD, MESC 400 mg/kg BW p.o and Orlistat 50 mg/kg BW p.o.

A significant increase in serum HDL levels in animals treated with MEDB, MECD, MESC 400 mg/kg B.W.p.o was observed. Considering the enhancement of cardioprotective lipid HDL, it can be concluded that the MEDB, MECD, MESC is not only anti obesity and anti hyperlipidemic agent but also a cardioprotective agent.

MEDB, MECD, MESC at 400mg/kg B.W .p.o showed cardioprotection by decreasing the atherogenic index and provided high % hyperlipidemia, which points out the reduction in risk against cardiovascular diseases. The livers of untreated rats were found to be yellow and bulky. Histopathological results revealed signs of fat accumulation indicating steatosis. Whereas, the condition was reversed in rats treated with MEDB, MECD, MESC 400 mg/kg b.w. p.o. and orlistat 50 mg/kg b.w. p.o.

It is believed that progesterone producing hyperphagia via progestin receptors which have been reported to be expressed on the serotonergic neurons & orlistat suppresses the progesterone induced hyperphagia by inhibiting reuptake of 5-HT (serotonin) at the hypothalamic site which regulate the food intake, which suggests the possible interaction exists between the neurosteroid and serotonin receptor system in regulating food intake and body weight. Further, these data implicate that disturbances in the ovarian hormone levels may predispose females to eating disorders by causing alterations in the serotonin level or serotonergic receptor function.

In this study administration of Progesterone to the control animals caused significant increase in the food consumption at 30 min, 1hr and 2hr which was significantly reduced by the administration of MEDB, MECD, MESC (200 mg/kg), MEDB, MECD, MESC at 1hr as compared to the disease control animals. Whereas MEDB, MECD, MESC (400 mg/kg) and the standard Orlistat significantly decreased the food consumption at 30 min, 1hr and 2hr as compared to both normal control as well as disease controls.

Saponin inhibits pancreatic lipase. Pancreatic lipase, a key enzyme, which is responsible for hydrolysis of a majority of dietary fats, may be targeted for the concerned obesity pandemic. It is responsible for hydrolysis of 50-60% of total dietary fats. The two main products formed by the hydrolysis of pancreatic lipase are fatty acids and 2-monoacylglycerols. These products combine with bile salts, dispersed as micelles and carried in this form to the site of absorption. Lipid absorption takes place in the apical part of the plasma membrane of epithelial cells, so inhibition of this enzyme may cause decrease in fat absorption. It can be anticipated that the lipid lowering mechanism may also enhanced removal or catabolism of lipoproteins, inhibition of HMG CO-A Reductase, and or inhibition of lysosomal lipid hydrolytic enzymes secreted by the liver.

Apart from being antioxidants, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 and glucose transport Isoform 2 (Glut 2), the intestinal transporter for vitamin C and glucose, leading to a decrease in the intestinal absorption of glucose, hence decrease in the blood glucose concentration [27]. Several researches have also demonstrated that flavonoids act as reducer of hyperglycemia by causing inhibition of renal glucose reabsorption through inhibition of the sodium-glucose symporters located in the proximal renal convulated tubule .[25-27]

Saponins are known antinutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion[28].

The administration of Progesterone caused significant increase in food intake compared to the control group animals which was significantly decreased by co-administration of MEDB, MECD, MESC 200 and 400 mg/kg Standard Orlistat was found to be more significant in this case

As far as effect on exploratory behaviour of Progesterone animals is concerned, there was no significant change in the exploratory behavior of Progesterone control animals as comparedto the control group animals but co-administration of MEDB, MECD, MESC 200 and 400 mg/kg significantly increased the number of ambutations and groomings but not the number of rearings.

By the phytochemical investigation it was found that *Desmostachya bipinnata*, *Canthium dicoccum*, *Sebestiania chaemelea* contains carbohydrates, flavanoids, glycosides and oils, saponins, alkaloids and tannins. It was reported that carbohydrates, flavanoids, glycosides and oils, saponins, alkaloids

and tannins reduces cholesterol levels and have antioxidants activity. The plant *Desmostachya bipinnata, Canthium dicoccum, Sebestiania chaemelea* was found to be useful in treatment of obesity and hyperlipidemia may be due to the presence of above mentioned phytoconstituents.

CONCLUSION

On evaluating behavioral and biochemical parameters, it was found that the methanolic extract of aerial parts of *Desmostachya bipinnata, Canthium dicoccum, Sebestiania chaemelea* showed anti-obesity activity in both High fat induced obesity, Progesterone induced obesity models by showing protective activity.

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