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Evaluation of Anti-Obesity Potential of *Erythroxylon Monogynum* Against High Fat Diet Induced Obesity In Wistar Rats

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ABSTRACT

Obesity is an abnormal excessive growth of adipose tissue, results from the combined effects of excess energy intake and reduced energy expenditure. Ever growing research in herbal drugs from Indian system of medicine suggests that beneficial effect herbs or its phytoconstitiuents in various ailments since ancient time. Anti obesity potential of chloroform fraction of erythroxylon monogynum (CEM) in high fat diet induced obesity in wistar rats was evaluated. Female rats were fed with high fat diet for 8 weeks. CEM was administered at a dose of 250 mg/kg, p.o. and 500 mg/kg for last 3 weeks while high fat diet offering. Parameters like body weight, feed consumption were monitored throughout experimental period. On Day 57, various biochemical parameters like serum glucose, serum lipid profile e.g. total cholesterol and triglyceride , HDL-C, LDL-C, VLDL as well as other biochemical parameters like SGOT, SGPT and total protein were estimated. Effect of treatment on cardiovascular risk factor indicator i.e. atherogenic index was also calculated. Finally effect on vital organs like liver, heart and kidney as well as epididymal fat pad was also recorded. Due to treatment, there was a significant reduction in dose dependant manner in body weight and other elevated biochemical parameters like serum glucose, TG, TC, LDL-C, and VLDL levels. Decreased HDL- C level in HFD fed animals were significantly improved due to treatment of CEM. Atheroginic index as well as relative epididymal fat pad weight was found to be reduced due to CEM treatment.

Keywords: Chloroform fraction, Atheroginic index, Hypolipidemic, Erythroxylon monogynum.

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INTRODUCTION

Obesity is a chronic disease of increasing prevalence in most countries, which leads to substantial increase in morbidity, and mortality in association with insulin resistance, diabetes, hyperlipidaemia, hypertension, and other cardiovascular diseases[1]. Among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, lack of exercise, psycho-logical factors, and the consumption of energy rich diets are the major ones[2]. In brief, obesity is an abnormal excessive growth of adipose tissue, results from the combined effects of excess energy intake and reduced energy expenditure[3].

Although a multitude of pharmaceutical agents are available for the treatment of obesity, the long-term persistence rate with medications is poor probably due to adverse effects, modest efficacy and expense. However, adverse effects of these drugs are not negligible, and must be taken into consideration when it is time to prescribe one of these anti-obesity drugs[4].

There is a need for better tolerated anti-obesity drugs expanding the understanding of peripheral signals and CNS pathways involved in the regulation of adiposity to treat obesity in the near future [5]. In particular, many therapeutic herbs and nutrients have far fewer side effects and may provide an alternative treatment or could be used to enhance the effect of prescription medications. While extensive research has been conducted on the development of anti-obesity drugs, the phytoconstituents like flavonoids, saponin, alkoloids derived from plant sources, including caffeine in oolong tea[6], saponin in the roots of broad bellflower[7] and capsiate in sweet pepper[8] for preventing and ameliorating obesity have been investigated.

The leaves paste of *erythroxylon monogynum* is traditionally reported to taken orally for anti appetite action [9]. Moreover this plant is reported for presence of various phytochemicals like alkaloids and flavonoids, 3α-(3′, 4′, 5′-Trimethoxybenzoyloxy) tropane. Due to presence of these phytochemocals, various parts of plants are claimed to have medicinal benefits in various disorders like such as diaphoretic, Stomachic, Diuretic and anti bacterial and curing psoriasis reference need [10,11.]

However, effect of *erythroxylon monogynum* or its phytoconstituents have not scientifically reported for anti-obesity effect. In the present study, the anti-obesity effects of *erythroxylon monogynum* was investigated in rats fed with high fat (HF) diet by measuring changes in body weight, food consumption, serum lipid profile, changes in epididymal fat pad weights.

The mechanism of HF diet-induced obesity is still unclear, but long-term exposure to a HF diet can increase body weight and adiposity in human and animals. Fat not only increases the

palatability of food but also is converted into body fat far more efficiently than carbohydrates. Another characteristic of HF diet-induced obese rodents is that they have a low sympathetic activity, which in turn results in decreased energy expenditure[5].

MATERIALS AND METHOD

Collection of Plant material, Extraction and fractionation

Fresh leaves of *erythroxylon monogynum* was obtained from local area of kadapa A.P. India & authentified by Sri Madhava Chetty; Dept of Botany; S.V University, Tirupati, (A.P, India). The specimen voucher of same is kept in department of pharmacology; PRRM Collage of Pharmacy; Kadapa.

The collected plant material of *erythroxylon monogynum* was washed thoroughly in water, and air dried for two weeks. The 500 gm of air dried and coarsely powdered material of plants were extracted with 95% of ethanol by cold maceration method for 72 hrs. Then the extract was filtered with muslin cloth and filtrate was evaporated under reduced pressure and vacuum dried. This yielded a greenish residue of 20- 25% W/W extract with reference to dry starting material. Further this alcoholic extract was fractioned by successive solvent fractionation method by using non polar solvents to polar solvents system (Pet ether – Chloroform - Ethyl acetate – n Butenol) and all fractions were tested for preliminary phytochemical tests. Chloroform fraction of the plant showed presence of maximum reported phytochemicals and hence this fraction is labeled as chloroform fraction of *erythroxylon monogynum* (CEM)

Experimental Animals

Healthy adult female albino rats were procured from Raghavendra enterprises, Bangalore weighing between 100-150 g were used. They were housed under standard laboratory conditions and food and water were provided *ad libitum*. The temperature was kept at 22 ± 2º c. The animals were maintained under a 12 h light / 12 h darkness cycle. All animal procedures were approved by the Institutional Animal Ethical Committee of P. Rami Reddy Memorial college of pharmacy, Kadapa (Ref No: 1423/PO/a/11/CPCSEA/001).

Chemicals

Most of Ingredients for High fat diet obtained from local market Kadapa. All other chemicals were procured from SD fine chemicals Ltd. India and were of analytical grade.

Acute toxicity study and gross behavior[12]

Acute toxicity study was performed according to Organization for Economic Co-operation and Development guidelines. Two groups of rats (n = 3 in each group) were taken for the study. One

group was treated with CEM separately 5000 mg/kg p.o. Another group was treated as control group (administered with vehicle 1% CMC). After oral ingestion animals are observed continuously for 2h under the following profiles like alertness, restlessness, irritability, fearfulness spontaneous activity, reactivity, touch response, pain response, defecation and urination. After periods of 24 and 72 h, animals were observed for signs of lethality or for death. No mortality or morbidity was observed in CEM treated animals even at 5000 mg/kg. Hence 500 mg/kg and 250 mg/kg doses were taken for efficacy studies.

Composition of High fat diet and Induction of obesity [1, 13]

The composition of high fat diet contains 23% whole wheat, 23% yellow corn, 11% barley, 17% milk powder, 1% bone meal, 1 % calcium chloride, 1% sodium chloride, 11% coconut oil, 11% butter and one multivitamin capsule. The diet was prepared daily morning freshly and offered in form of ball to animals. The rats were rendered obese by a HF diet, while the control rats had access to a normal (N) diet for the total period of 8 weeks. After 5 weeks of HF diet, the rats received daily treatment with test and standard drugs for another 3 weeks.

Experimental design

The animals offered with High fat diet (HFD) for 5 weeks were divided into four groups (n=6). All groups were treated with test and standard drugs for further 3 weeks as fallows. Normal control group offered with normal diet was maintained separately and received vehicle for treatment period. Chloroform fraction of *erythroxylon monogynum* (CEM) and the standard drug atorvastatin were freshly suspended in 1% CMC daily and administered to animals by oral feeding needle immediately. Grouping and treatment were done as shown in Table 1.

Table 1:- Grouping and Treatment schedule

S.NO	Groups	Diet and Treatment (3 weeks)
I	Normal	Normal feed + 1 % CMC (p.o.)
II	Control	High fat diet + 1 % CMC (p.o)
III	Standard	High fat diet + Atorvastatin (30 mg/kg ,p.o)
IV	Low dose	High fat diet + CEM (250 mg/kg, p.o.)
V	High dose	High fat diet + CEM (500 mg/kg, p.o.)

Measurements and sample collection.

Body weights and food intake were recorded twice a week throughout experimental period. At the end of experiment, the blood samples were collected from the retro orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood into micro centrifuge tubes it is kept aside for 30 min at room temperature and then serum was separated by centrifugation at 2000 rpm for 15 min and stored at -80º C until analyzed for various biochemical parameters.

After blood collection, animals were sacrificed by deep anesthesia and vital organs like Liver, kidney, heart as well as epididymal fat pads were isolated and weighted immediately.

Estimation of Biochemical Parameter

Various biochemical parameter like serum glucose, total cholesterol (TC) , HDL, Triglycerides (TG), SGOT, SGPT and total protein were estimated by using Erba kit and semi auto analyzer (Maxlyzer, Avecon model no: NB-201). Other parameters like LDL, VLDL and atherogenic index were calculated by using equitation-

- VLDL= TG/5
- LDL = {TC – (HDL + VLDL)}
- Atherogenic index = LDL + VLDL/ HDL

Statistical analysis

All the data was expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dunnet's test using computer based fitting program (Prism graph pad.). Statistical significance was taken as $P< 0.05$.

RESULTS AND DISCUSSION

The present study demonstrates the beneficial effect of Chloroform fraction of *Erythroxylon monogynum* (CEM) in high fat diet induced obesity model. Chloroform fraction of *Erythroxylon monogynum* was administered at 250 mg/kg p.o. and 500 mg/kg p.o. treatment. These doses were selected from acute toxicity study. In acute toxicity study, the rats treated with chloroform fraction of erythroxylon monogynum were well tolerated and exhibited normal behavior up to 5000 mg/kg orally. All animals were alert with normal grooming, touch response, pain response and there was no sign of passivity, stereotypy, and vocalization. There was no abnormal change in motor activity, secretary signs as well as their body weight and water intake during drug administration. Therefore 1/10 of this tested dose were taken as high dose (500 mg/kg) and 1/20 of this dose was taken as low dose 250 mg/kg[14].

High fat diet induced obesity is commonly used animal model to screen anti-obesity potential of the drug. The outcome of the study clearly indicates the offering of High fat diet for period of 8 week to the animals resulted into drastic body weight gain as well as alteration of various biochemical parameters. The body weight of each group was recorded twice a week throughout the study period. The results obtained where shown as Mean ± SEM in Figure 1. Animals offered with high fat diet showed gradual body weight gain with time as compared to animals maintained on normal diet.

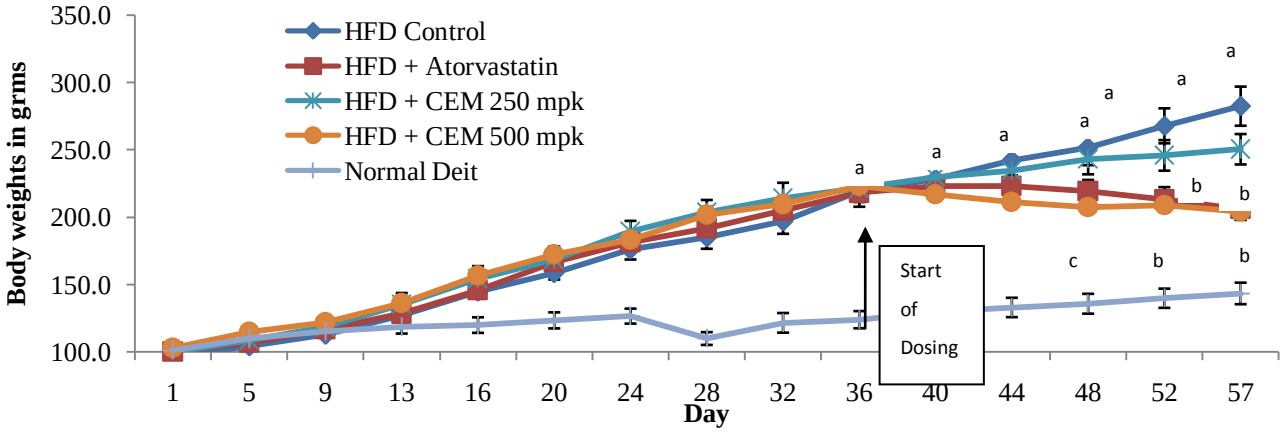

Figure 1:- Effect of CEM on body weight gain

Day wise body weight for experimental group. Values are represented as mean ± SEM. Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- p<0.0001vs Normal Diet group; b- p<0.0001 vs HFD control group; c- p<0.001 vs HFD control group

After 5 week offering of high fat diet (HFD), animal treated with standard and test group for next 3 weeks showed significant reduction of body weight gain. High dose of chloroform fraction of erythroxylon monogynum (CEM) showed reduction in body weight from Day 48 (p<0.001) to Day 57 (p<0.0001) when compared to HFD control group. On other hand, reduction in body weight shown by low dose of CEM was not statically significant. Animals treated with slandered drug i.e. Atorvastatin 30 mg/ kg P.O also showed statistically significant (p<0.0001) reduction in body weight gain.

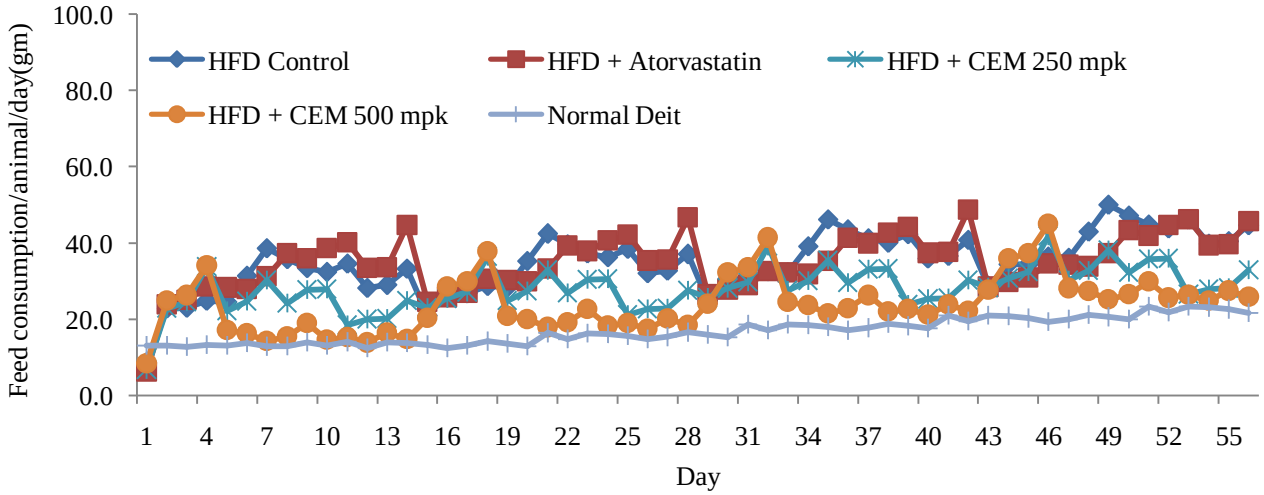

Figure 2:- Feed consumption

Feed consumption data for each group. Values are represented as feed consumed per animal per day in gram.

Feed consumption was calculated from feed offered and feed left to next day (including wastage of feed in cage). Feed consumption of all the groups were recovered every day throughout experimental period. The results were expressed as feed consumed per animal per day in gram. The total feed consumption during whole experimental period was not remarkably different among the groups. Feed consumption pattern for all the treated and control group was found simillar (figure 2)

High fat diet has been often used to elevate serum or tissue cholesterol levels to assess the hypercholesterolemia- related metabolic disturbances in animals [15]. The present study showed that dietary treatment of rats with high-cholesterol diets caused elevated serum lipid profile and other biochemical parameters along with increase in epididymal fat pad. Serum glucose and lipid profile was estimated by using Erba glucose kit. The results obtained where shown as Mean ± SEM in Figure 3 and Figure 4. The animals fed with high fat diet showed significant increase in glucose level ($P<0.0001$) when compared to the normal group animals. The treatment with CEM showed a dose dependent decrease in elevated glucose level in high fat feed rats. CEM 250 mg/kg b.w. p.o and 500 mg/kg b.w. p.o. showed significant decrease ($p<0.01$, $p<0.0001$ respectively) in glucose level when compared with HFD control group. Similar results were obtained in case of lipid profile. The animals fed with high fat diet showed significant increase ($P<0.0001$) in lipid parameters like Cholesterol (TC) , Triglycerides (TG), VLDL and LDL level when compared to the normal group animals. HDL level of high fat diet control animals were found be significantly decreased ($P<0.0001$) as compared to the normal group animals. The treatment with CEM showed a dose dependent improvement in towards normalizing lipid parameters. CEM 250 mg/kg b.w. p.o and 500 mg/kg b.w. p.o. showed significant decrease ($p<0.001$, $p<0.0001$ respectively) in Cholesterol (TC) , Triglycerides (TG), VLDL and LDL level when compared with HFD control group. CEM 500 mg/kg treatment was able to normalized HDL level and showed significant increased level ($p<0.01$) when compared with HFD control group, However CEM 250 mg/kg treatment was unable to produce significant improvement in HDL levels. Standard drug treatment i.e Atorvastatin showed significant result in all parameters which were comparable to results shown by high dose treatment of CEM. These findings are in accordance with earlier several reported studies [2,7,13]. Atorvastatin was used because it is a potent hypolipidemic drug known to exert its action by inhibiting HMG CoA reductase, the rate- limiting step in cholesterol biosynthesis. In humans, the statin family has been well-accepted as cholesterol- lowering drug 16. Thus, the standard group was used to compare the effectiveness of the drug in reducing elevated plasma parameters.

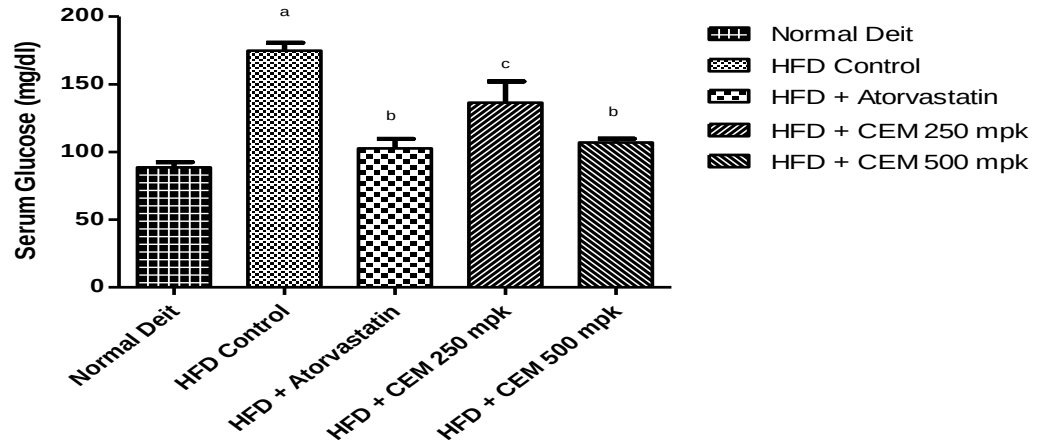

Figure 3:- Effect on serum glucose level

Effect of CEM on serum glucose level. Values are represented as mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- p<0.0001vs Normal Diet group; b- p<0.0001 vs HFD control group; c- p<0.01 vs HFD control group.

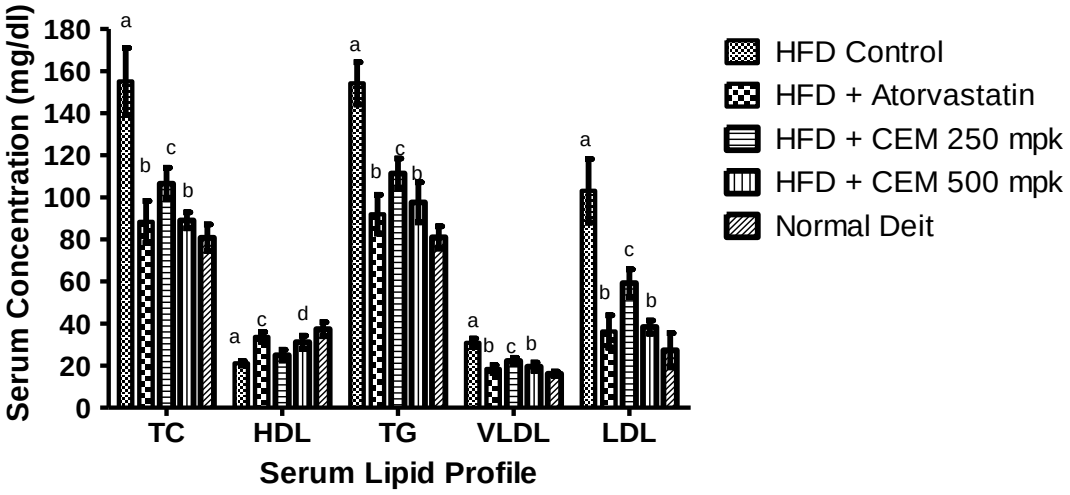

Figure 4:- Effect on serum Lipid Profile

Effect of CEM on serum Lipid profile. Values are represented as mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- p<0.0001vs Normal Diet group; b- p<0.0001 vs HFD control group; c- p<0.001 vs. HFD control group ; d- p<0.01 vs. HFD control group.

It is well established that elevated cholesterol and LDL-cholesterol levels in serum promote obesity and other associated cardiovascular disease [17]. High density lipoproteins (HDL) collect cholesterol from the body's tissues, and bring it back to the liver for catabolism. Hence, HDLs are referred to as the "good cholesterol" lipoprotein [18]. A low level of HDL-C is associated with

high risk of obesity and associated coronary artery disease [19]. Reduction in Total cholesterol , LDL- cholesterol and increase in HDL-concentration are significantly related to lipid-lowering therapy. Three week treatment with CEM 250mg/kg p.o. as well as 500 mg/kg p.o. to high fat diet offered animals resulted into reduction of total cholesterol and LDL – Cholesterol as well as increase in HDL – cholesterol indicates its potential in lipid lowering therapy.

Triglycerides (TG) circulate in the blood are stored in the body fat, and are used when the body needs extra energy. Excessive TG is related to the occurrence of obesity. In this study, feeding the rats with a high fat diet (control group) increased the plasma TG level as compared to normal diet animals. Animals treated with Atorvastatin (ATR) and CEM at both doses shows significant decrease in Triglyceride level.

Generally, it is difficult to detect the CVD risk factor based on individual lipoproteins (TC, LDL-C, HDL-C) and TG levels. Consequently, the Atherogenic index (TC to HDL-C ratio) was used for this purpose. This ratio is a good marker for identifying and minimizing the risk of CVD [20]. An increase in this ratio increases the risk of cardiovascular diseases. Atherogenic index (AI) was calculated for each animal and the results obtained where shown as Mean ± SEM in Figure 5. Mean AI for animals fed with normal deit were found to be 1.25 ± 0.27. Animals offered with High fat deit showed significant increase ($p<0.0001$) in AI (6.48 ± 0.97). Mean AI for Atorvastatin 30 mg/kg ,p.o., CEM 250 mg/kg p.o and 500 mg/kg p.o. treatment groups were found to be 1.64 ± 0.18, 3.42 ± 0.43 and 1.99 ± 0.28 respectively. It clearly indicates high fat diet offered animals treated with CEM 250mg/kg p.o. as well as 500 mg/kg p.o. for 3 week treatment resulted into reduction of atherogenic index.

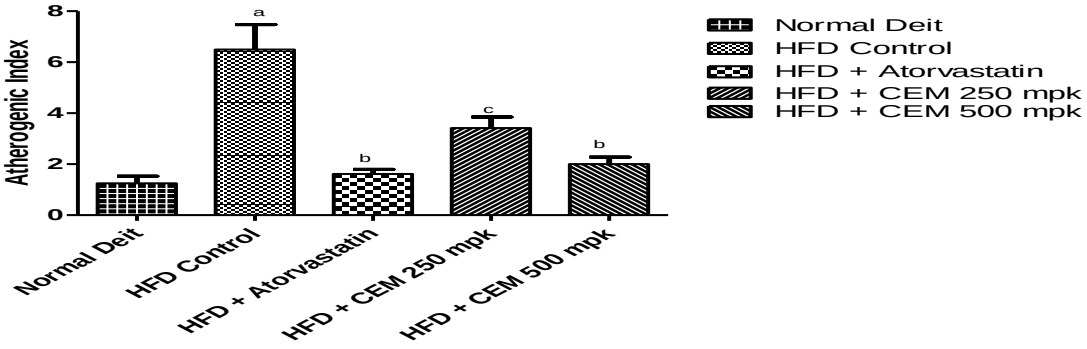

Figure 5:- Atherogenic Index

Effect of CEM on Atherogenic Index. Values are represented as mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- $p<0.0001$vs Normal Diet group; b- $p<0.0001$ vs HFD control group; c- $p<0.001$ vs HFD control group

Other Serum parameters like SGOT, SGPT and Total Protein were estimated by using Erba kits. The results obtained where shown as Mean ± SEM in Table 2. The animals fed with high fat diet showed significant increase in SGOT and SGPT level (P<0.0001) when compared to the normal group animals. Standard drug treatment i.e Atorvastatin showed significant (p<0.0001) decrease in SGOT and SGPT level when compared to control group. The high dose of CEM i.e. 500 mg/kg treatment was able to produce significant reduction in SGOT (p<0.0001) and SGPT (p<0.001) level, However treatment with low dose of CEM (250mg/kg) was unable to produce any significant achievement. Total protein levels were neither affected by HFD diet nor modulated by standard and test drug treatment.

Table 2:- Effect on Serum SGOT, SGPT and Total Protein

Effect on other biochemical parameters	Values (Mean ± SEM)				
	Normal Diet	HFD Control	HFD + Atorvastatin	HFD + CEM 250 mpk	HFD + CEM 500 mpk
SGOT (mg/dl)	81.0 ± 6.09	144.13 ± 5.54[a]	92.83 ± 5.02[b]	129.53 ± 8.97	99.17 ± 6.90[b]
SGPT (mg/dl)	18.83 ± 1.86	42.96 ± 5.23[a]	20.83 ± 2.4[b]	35.17 ± 4.87	24.64 ± 2.58[c]
Total Protein (g/dl)	5.9 7± 0.42	6.66 ± 0.39	6.33 ± 0.29	6.67 ± 0.39	6.50 ± 0.43

Table 2:- Effect of CEM on serum SGOT, SGPT and Total Protein. Values are represented as mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- p<0.0001vs Normal Diet group; b- p<0.0001 vs HFD control group; c- p<0.001 vs. HFD control group

End of the experiment, all animals were humanely sacrificed and vital organs like Liver, Kidney and Heart as well as epididymal fat pads were isolated and weighted immediately. The relative organ weights (gm/ Kg b.w) for each animal were calculated as per following formulae and results were represented in Mean ± SEM in table 3.

Table 3:- Effect on organ weights and fat pad weight

Groups	Normal diet	HFD control	HFD + Atorvastatin	HFD + CEM 250 mpk	HFD + CEM 500 mpk
Relative Liver Weight (gm/kg b.wt.)	41.39 ± 4.19	53.72 ± 2.34[a]	40.26 ± 3.22[c]	47.44 ± 2.97	41.39 ± 4.19[c]
Relative Kidney Weight (gm/kg b.wt.)	9.40 ± 0.55	9.52 ± 0.30	9.54 ± 0.25	9.25 ± 0.09	9.56 ± 0.34
Relative Heart Weight (gm/kg b.wt.)	3.63 ± 0.27	5.60 ± 0.48[b]	5.66 ± 0.37	5.05 ± 0.24	5.60 ± 0.42
Relative Epididymal Fat Pad Weight (gm/kg b.wt.)	10.20 ± 0.22	16.50 ± 0.99 [a]	11.51 ± 1.05[e]	12.80 ± 0.56[c]	11.54 ± 0.90[d]

Table 3:- Effect of CEM on organ weights and fat pad. Values are calculated as relative weight and represented as mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- $p<0.0001$vs Normal Diet group; b- $p<0.001$ vs Normal Diet group; c- $p<0.01$ vs. HFD control group; d- $p<0.001$ vs. HFD control group ; e- $p<0.0001$ vs. HFD control group

Relative Organ weight. (g/kg b. wt)	=	(Absolute organ weight in grams/body weight in grams of final day) X 1000

The animals fed with high fat diet showed significant increase in Liver ($P<0.0001$), Heart ($P<0.001$) and Epididymal fat pad ($P<0.0001$) weight (relative) when compared to the normal group animals. However Kidney weights were found unaltered due to High fat diet. Standard drug treatment i.e Atorvastatin showed significant decrease in Liver weight ($p<0.01$) and Epididymal fat pad weight ($p<0.0001$) when compared to control group. The treatment with CEM showed a dose dependent decrease in increased eipididymal fat pad weights in high fat feed rats. CEM 250 mg/kg b.w. p.o and 500 mg/kg b.w. p.o. showed significant decrease ($p<0.01$ and $p<0.001$) in eipididymal fat pad weights when compared with HFD control group. Moreover high dose of CEM was found to effective in decreasing increased liver weight. The increase in epididymal fat pad weight, Liver weight and Heart weight in HFD fed animals suggested tissue deposition of the fats. Results obtained were in accordance with the changes noticed in body weight pattern. Parallel to increased Body weight reduction, all the treatments were found to be effective to reduce regional epididymal fat pad weight.

CONCLUSION

Thus the present study indicates that the chloroform fraction of *Erythroxylon monogynum* (CEM) posses anti-obesity effect. The presence of various phytochemicals like essential oil, flavanoids, saponins and alkaloids in CEM may responsible for its effect. Further studies to understand actual underlying mechanism as well as studies on isolation and structural determination of active principles are in progress.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and

writing of the paper.

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