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EVALUATION OF ETHANOLIC LEAF EXTRACT OF *CEIBA PENTANDRA* FOR ANTI-OBESITY AND HYPOLIPIDAEMIC ACTIVITY IN CAFETERIA DIET (CD) TREATED WISTAR ALBINO RATS

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ABSTRACT

Ceiba pentandra (*C. pentandra*) has been traditionally used in the ayurveda system of medicine for the treatment of obesity and atherosclerosis. The present study was undertaken to evaluate anti-obesity and hypolipidaemic activity of the ethanolic leaf extract of *C. pentandra* in Cafeteria diet (CD) treated Wistar albino rats. Obesity was induced in Wistar albino rats by feeding CD daily for twelve weeks. Body weight was measured initially and then every week thereafter. Food and water intake was measured daily. On day 85th, the serum biochemical parameters were estimated and the animals were sacrificed with over dose of anesthetic ether. The liver and fat pads (perirenal, mesenteric and epididymis) were removed and weighed immediately. The intestinal activity of enzyme alkaline phosphatase (ALP) was determined. Administration of *C. pentandra* (125 mg/kg) prevented the increase in body weight in cafeteria diet treated animals. Administration of *C. pentandra* showed significant decrease in body weight gain in CD treated obese rats. The significant decrease in ALP level was observed in *C. pentandra* treated rats. Administration of *C. pentandra* showed significant decrease in the weight of liver and fat pads as compared to CD control and BMI (g/cm2) was lowered. Results of this study showed that *C. pentandra* has anti-obesity activity which may partly be mediated via inhibition of intestinal lipid absorption and thermogenesis. In conclusion, the results of this study showed that *C. pentandra* has therapeutic potential in the management of obesity.

INTRODUCTION: Obesity is a medical condition involving an excess accumulation of body fat. The prevalence of obesity has increased steadily over the past five decades not only in adults, but also among children and adolescents and had a significant impact on the quality adjusted life years [1].

The rapid increase in the incidence of obesity during the past several decades is a matter of great concern [2]. The drugs used in the treatment of obesity acts either on central nervous system by suppressing appetite or inducing thermogenisis therefore leading to lipolysis e.g. Phentermine and Sibutramine, or acts on peripheral nervous system, which decreases fat absorption by preventing the breakdown of dietary fat in the gastrointestinal tract e.g. Orlistat [3].

Each of these promotes 5 to 10% loss of body weight and has their own limitations and side effects. An endocannibinoid receptor antagonist, Rimonabant was withdrawn from the market due to concerns about its safety, including risk of seizures and suicidal tendencies [4]. However, these drugs do not cure obesity; weight rebounds when discontinued.

Thus, there is great demand for the search of new and safer antiobesity drugs [5]. It is well documented that overeating and obesity can be produced in experimental animals like rats by offering them diets that are high in fat, sugar, or both. The most pronounced effects are obtained when the animals are offered an assortment of tasty fat and sugar-rich foods marketed for human consumption, which are referred to as the ''supermarket'' or ''cafeteria'' diet [6, 7].

Some herbs act on digestion, metabolism, or appetite to impact weight loss. Certain substances can increase thermogenesis (generation of heat), or metabolism, which may lead to weight loss. Herbal weight loss products are a great safe option for the people who want to lose weight naturally. Various herbal supplements have proved to be active against obesity like *Allium sativum* L., *Citrus aurantium* L., *Cyperus Rotundus* L., *Glycyrrhiza glabra* Linn, etc and its related diseases [8]. Hence, there is an intense need to explore the anti obesity activity on different plant/ herbal extract [9, 10].

Ceiba pentandra (*C. pentandra* Syn: Kapok tree) belonging to family Bombaceae is tall tree; trunk prickly when young; branches horizontal grown wildly, found throughout India. The juice obtained from roots is considered a most valuable cure for diabetes and antidote for scorpion venom. The gum and leaves has a bitter sharp taste; removes “kapha” and “vata” hot, cures disease of liver, spleen, blood; removes tumors, fat, pain, alexitermic (Ayurveda) [11, 12].

However, its antiobesity profile has not been scientifically documented. Hence, the objective of present study is to investigate the antiobesity and hypolipidaemic activity of ethanolic leaf extract of *C. pentandra* on MSG induced obese rats.

MATERIALS AND METHOD:

Experimental animals: The experimental protocol was approved by the Institutional Animal Ethics Committee. Animals were obtained from the Animal house facility of Smt. Kashibai Navale College of Pharmacy, Pune. Wistar albino rats (50-70 g) of either sex were used in the study. They had free access to food and water ad libitum throughout the duration of the study. The rats were maintained at an ambient temperature between 28-30^{o}C, humidity of 55±5%, and standard (natural) photoperiod of approximately 12 h of light (06:30-18:30 h) alternating with approximately 12 h of darkness (18:30-06:30 h).

Preparation of Ethanolic leaf extract of *C. pentandra*: The leaves of *C. pentandra* were collected from the regions surrounding Pune and were authentified by Botanical Survey of India, Pune, Maharashtra. Voucher specimen was submitted at the herbarium for future reference. The leaves were air dried, powered, and then extracted with 70% ethanol by using Soxhlet method. The extract was filtered with filter paper and then the solvent was evaporated at reduced pressure by using Rotavapor (Heidolph) apparatus to get viscous mass. The yield of extract obtained was 12.85%

Cafeteria diet induced Obesity: The animals were divided into three groups (n=6) and individually housed in cages. All the extracts were freshly prepared in distilled water and were administered in the form of suspension. Group I received normal standard feed, group II received cafeteria diet and group III received cafeteria diet along with *C. pentandra* in dose of (125mg/kg, p.o) body weight, for a period of 12 weeks. The cafeteria diet composed of (a) Boiled Potatoes (4 g) + (b) Biscuits (1 g) + (c) Groundnut oil (1 g) + (d) Chow (4 g) was given.

Acute Toxicity Studies: Evaluation of acute oral toxicity of *C. pentandra* was carried out according to the OECD guidelines for testing of chemicals – 425 (OECD, 2001). The dose was found safe up to 5000 mg/kg as no behavioral changes and mortality was observed till 14 days.

Measurement of Food and Water intake: The food (g) and water (ml) intake of each group was determined daily by measuring the difference between the pre-weighed chows and the weight of the food that remained after 24 hours.

Blood Biochemical Analysis: On day 85th, blood was collected by retro-orbital puncture from the ether-anesthetized rats and subjected to centrifugation to obtain the serum. The serum levels of glucose, total cholesterol, triglycerides (TGs), high density lipoprotein (HDL), LDL and VLDL were estimated using the biochemical kits (Diagnostics). The atherogenic index of plasma (AIP) was calculated by using: AIP = log (TGs/HDL).

Estimation of organ (Liver) and fat pads (Perirenal, epididymis and mesentric) weight: The animals were sacrificed with an overdose of anesthetic ether. The liver and fat pads (perirenal, epididymis and mesentric) were quickly removed and weighed.

Measurement of intestinal Alkaline Phosphatase (ALP): On day 85th, the animals were killed by overdose of anesthetic ether after an overnight fast between 08:00 and 09:00 h. Small (5cm) segments of the proximal duodenum and middle part of the jejunum were immediately removed. The intestinal mucosa was scraped, transferred in saline solution (5 ml) and centrifuged as per the modified method of [13]. The intestinal ALP was measured using autoanalyser (Erba CHEM-5 Plus V2).

Statistical Analysis: All data is represented as Mean ± SE (n=6). Comparison between groups was done by One-way ANOVA followed by Bonferroni's multiple comparison test. $P < 0.05$ was considered significant.

RESULTS:

Phytochemical test: Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponin, phenol, protein, carbohydrate, fat, vitamin A, vitamin C and vitamin E compounds in *C. pentandra.*

Effect of *C. pentandra* on body weight: Table 1 shows the changes in body weight in different group of animals, during experiment. Animals fed with cafeteria diet for twelve weeks produced a significant ($P<0.05$) increase in body weight compared to negative control and *C. pentandra* treated. Treatment with *C. pentandra* (125 mg/kg) prevented the increase in body weight as compared to cafeteria diet control group in (76.58%). Ethanolic extract of *C. pentandra* showed significant ($P<0.05$) decrease in body weight as compared to CD group.

Effect of *C. pentandra* on food and water intake: Administration of *C. pentandra* (125 mg/kg) produces no significant reduction in food and water intake when compared to cafeteria diet control (**Fig. 1**).

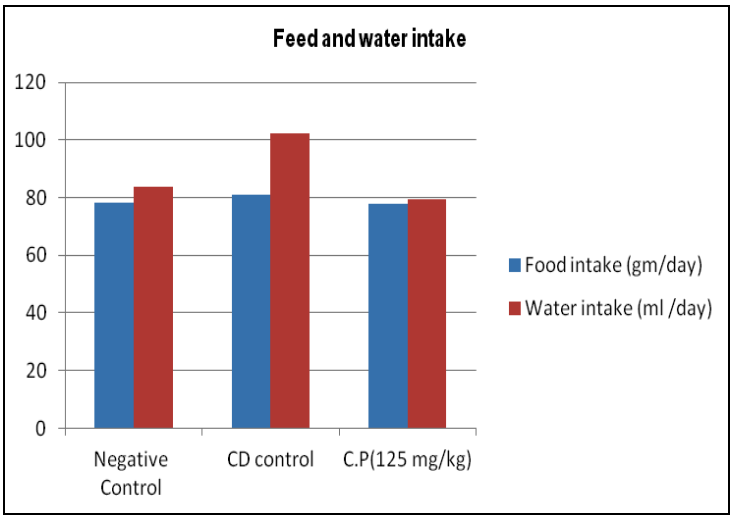

FIG. 1: EFFECT OF *C. PENTANDRA* ON FEED AND WATER INTAKE

Effect of *C. pentandra* on Serum Biochemical Parameters: *C. pentandra* at the dose (125 mg/kg) showed no significant decrease in level of serum total cholesterol (**Table 2**).

Activity of *C. pentandra* on intestinal ALP, SGOT and SGPT: Animals fed with cafeteria diet for twelve weeks produced significant ($P<0.05$) increase in intestinal ALP in CD control group. Treatment with *C. pentandra* (125 mg/kg) significantly reduced the intestinal ALP (**Table 3**).

Effect of *C. pentandra* on Liver weight and fat pads (perirenal, epididymis and mesentric): Administration of ethanolic extract of *C. pentandra* for twelve weeks, decreased the weight of liver and fat pads (perirenal, epididymis and mesentric) as compared to CD control (**Table 4**).

Effect of *C. pentandra* on Body mass index (BMI) (g/cm^2): Animals fed with cafeteria diet for twelve weeks increases the BMI of animals. Administration of *C. pentandra* for twelve weeks decreased the BMI (g/cm^2) as compared to CD control (**Table 5**).

DISCUSSION: Various animal models of obesity have been used to emulate obesity like condition in humans, in order to develop effective antiobesity treatments. Among the animal models of obesity, rats that are fed a high fat diet are considered useful; a high percentage of fat in their diet is considered to be an important factor in the development of obesity, leading to accumulation of fat into the body, even in the absence of an increase in calorie intake [14].

TABLE 1: EFFECT OF *C. PENTANDRA LEAVES* EXTRACT ON BODY WEIGHT (G) IN RATS TREATED WITH MSG (n=6)

Week	Negative control	Cafeteria diet control	*C. pentandra* 125 mg/kg
0	61.38 ± 2.09	58.7 ± 1.15	58.45 ± 1.006
1	75.02 ± 4.18	65.03 ± 1.19	67.6 ± 1.80
2	86.42 ± 4.70	71.58 ± 1.60	81.03 ± 4.05
3	97.42 ± 6.51	103 ± 3.43	97.58 ± 5.76
4	118.2 ± 7.19	118.3 ± 6.37	108.4 ± 5.64
5	138.9 ± 9.09	158.7 ± 4.93	124.5 ± 5.45
6	158.3 ± 10.86	182.8 ± 8.46	140 ± 6.34
7	179.1 ± 11.74	196.4 ± 9.68	163.3 ± 6.98
8	188.2 ± 11.25	222.3 ± 13.67	173.9 ± 7.03
9	213.3 ± 11.97	240.4 ± 13.83	189.4 ± 8.20
10	220.4 ± 11.71	260.5 ± 16.22	203.7 ± 8.84*
11	233.3 ± 12.17	284.2 ± 19.27	220.1 ± 10.33**
12	243.8 ± 11.06	307.1 ± 18.59	235.2 ± 10.4***

Values are mean ± SEM, *P < 0.05 considered significantly compared to CD control.

TABLE 2: EFFECT OF *C. PENTANDRA LEAVES* EXTRACT ON SERUM BIOCHEMICAL PARAMETERS IN RATS TREATED WITH MSG (n=6)

Obesity markers	Negative control	CD control	*C. pentandra (*125mg/kg)
Total cholesterol (mg %)	53.15 ± 0.006	111 ± 7.63	113.3 ± 4.06
Serum triglycerides (mg %)	61.66 ± 3.15	90.67 ± 8.58	105.3 ± 12.04
HDL-C (mg %)	33 ± 0.62	17.5 ± 2.91	16.33 ± 1.05
LDL-C (mg %)	7.83 ± 1.14	75.03 ± 5.94	75.93 ± 3.45
VLDL-C (mg %)	12 ± 0.62	18.13 ± 1.71	21.05 ± 2.41

Values are mean ± SEM, *P < 0.05, considered significantly compared to CD control.

TABLE 3: EFFECT OF *C. PENTANDRA LEAVES* EXTRACT ON SERUM BIOCHEMICAL PARAMETERS AND INTESTINAL ALP ACTIVITY IN RATS TREATED With MSG (n=6)

Parameters	Negative control	CD control	*C. pentandra (*125 mg/kg)
ALP (IU/L)	128.33 ± 8.69	999 ± 120.4	601± 63.66 *

Values are mean ± SEM, **P < 0.01considered statistically significant compared to CD control

TABLE 4: EFFECT OF *C. PENTANDRA* ON WEIGHT OF LIVER AND FAT PADS (PERIRENAL, EPIDIDYMIS AND MESENTERIC)

Group	Perirenal (gm)	% Decrease	Epididymis (gm)	% Decrease	Mesentric (gm)	% Decrease	Liver (gm)	% Decrease
CD (control)	4.99 ±1.0	100%	6.92 ± 0.29	100%	3.95 ± 0.38	100%	14.54 ± 0.61	100%
CP 125	3.52 ± 1.05	70.54%	2.45 ± 0.22*	35.40%	3.75 ± 0.75	94.93%	9.70 ± 0.91**	66.71%

Values are mean ± SEM, *P < 0.05, considered significantly compared to CD control

TABLE 5: EFFECT OF *C. PENTANDRA* ON BODY MASS INDEX (g/cm^2)

Sr. No	Group	BMI (g/cm^2)
1	CD control	0.20
2	Negative control	0.17
3	*C. pentandra* (125mg/kg)	0.16

The present study showed that administration of a CD for twelve weeks, in Wistar albino rats produces obesity with increase in body weight, parametrial adipose tissue weight and serum lipid levels. The ethanolic extract of *C. pentandra* showed significant decrease in body weight as compared to CD control group. Upon administration of ethanolic extract of *C. pentandra* no decrease in total cholesterol, serum triglycerides, LDL and VLDL and no increase in HDL level were observed which reveals that the drug does not have any effect on the metabolism of fats in liver. The significant decrease in level of intestinal ALP in *C. pentandra* treated group indicates that it may decrease fat absorption by preventing the breakdown of dietary fat in the gastrointestinal tract. It was also observed that ethanolic extract of *C. pentandra* slightly decreases the food and water intake measured for 84 days, indicating that it might not suppress the appetite.

Administration of *C. pentandra* for twelve weeks, showed significant decrease in the weight of liver and fat pads (perirenal, epididymis and mesentric) as compared to CD control. The body mass index (g/cm2) was decreased indicating the sign of anti-obesity activity.

CONCLUSION: The ethanolic extract of *C. pentandra* may exert its effect via central nervous system, acting on the thermoregulation centre in the hypothalamus, causing thermogenisis thereby leading lipolysis and contributing to weight loss. Also by decreasing the level of intestinal ALP, it may decrease fat absorption by preventing the breakdown of dietary fat in the gastrointestinal tract. When the extract treated group was compared to CD control, it does not produce promising hypolipidaemic effect. The dose of *C. pentandra* (125 mg/kg) can be the optimal dose showing anti-obesity activity. In conclusion, the results of this study showed that *C. pentandra* has therapeutic potential in the management of obesity.

CONFLICT OF INTEREST: No conflict of interest declared.

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