

Anti-obese activity of *Butea monosperma* (Lam) bark extract in experimentally induced obese rats

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To study the efficacy of ethanolic extract of *B. monosperma* bark in cafeteria and atherogenic diet fed rats and monosodium glutamate (MSG) obese rats, different doses (200, 400 and 800 mg/kg) of ethanolic extract of *B. monosperma* bark showed dose dependent decrease in body weight, daily food intake, glucose, lipids, internal organs' weight and fat pad weight in cafeteria and atherogenic diet fed rats and monosodium glutamate obese rats. The results suggested that *B. monosperma* has significant anti-obese activity.

Keywords: Atherogenic diet, *Butea monosperma*, Cafeteria diet, Monosodium glutamate, Obesity

The prevalence of obesity has been increasing worldwide, and is reaching epidemic proportions in developed countries. Among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, lack of exercise, psychological factors, and the consumption of energy rich diets are the major ones^{1,2}. Adipose tissue plays a role in energy storage and insulation from environmental temperature and trauma and also functions as an endocrine organ. In the long run, white fat mass reflects the net balance between energy expenditure and energy intake. The adipocytes store energy as triacylglycerols. Fat storage occurs both by the direct uptake of circulating lipoprotein triacylglycerols, which are hydrolyzed by lipoprotein lipase to non-esterified free fatty acids, and also by local lipogenic pathways, i.e. the *de novo* synthesis from glucose and other precursors. On the other hand, this tissue can release both free fatty acids and glycerol, providing circulating substrates for other tissues, according to their energy needs.

In addition to serving as an energy store site, adipocytes secrete hormones (e.g. leptin, adiponectin, resistin) that regulate energy balance, metabolism,

and neuroendocrine response to altered nutrition. The hormone leptin stimulates energy expenditure and inhibits food intake by acting *via* hypothalamic receptors, and also has peripheral effects, such as inhibition of liver and white adipose tissue lipogenesis rate and lipolysis stimulation in adipocyte. The expression of leptin in adipocytes and its plasma concentration are both positively correlated with total adiposity.

Drug treatment of obesity has often seen as controversial, largely because of failure to understand how it should be used. Due to paucity of data, no particular strategy or drug can yet be recommended for routine use. Currently approved drugs are best, when used in conjunction with diet, exercise and behaviour change regimens. They do not cure obesity when they are discontinued and weight regain occurs. There is a demand for search of new and safer ones³. A large section of world's population relies on tradition remedies to various diseases. Medicinal herb is an indispensable part of tradition medicine practised all over the world due to low costs, easy access and ancestral experience⁴. The main reason for this popularity is the belief that most herbal medicine, due to their natural origin, are harmless and without side effects⁵. *Butea monosperma* Lam (Syn. *Butea frondosa*; Family Fabaceae), is a common plant throughout the world. Plant mainly contains flavonoids and steroids and tetramers of leucocynidin

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are isolated from stem bark⁶. *B. monosperma* is reported to have anti-diabetic⁷ anti-diarrheal⁸, anti-inflammatory⁹, anti-hyperglycemic and anti-hyperlipaemic¹⁰ activities. However, the effect of its bark on experimentally induced obesity is not scientifically documented. Hence, the present study has been undertaken to study the effect of ethanolic extract of *B. monosperma* bark on cafeteria and atherogenic diet induced obesity in rats.

Materials and Methods

Plant material—*B. monosperma* bark was collected from the rural area of Bangalore, India during July 2008 and was authenticated by Dr. Shiddamallayya N, Regional Research Institute, Bangalore. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2008-09, 242) of bark has been deposited in the Department of Pharmacology, Acharya & B.M. Reddy College of Pharmacy, Bangalore.

Preparation of extract—The bark was dried in shade at room temperature. The dried bark was powdered by using grinder, to coarse powder and this powder was packed into Soxhlet column and extracted with petroleum ether (60-80 °C) for 24 h. The same marc was successively extracted with chloroform (50-60 °C) and afterwards with ethanol for 24 h (yield: 43.70%; w/w). The extract was concentrated under reduced pressure (bath temperature 50 °C). The dried extract was stored in airtight container at 4 °C until used. The chemical constituents of the ethanolic extract were identified by qualitative analysis¹¹.

Animals—Female Albino Wistar rats weighing between 130-180 g were used for the cafeteria and atherogenic diets induced obese model and one day old neonates (pups) (immediately after birth and usually 4-6 g body weight) were used for the monosodium glutamate induced obesity model. Animals were housed under standard conditions of temperature (24 ± 2 °C) and relative humidity (50-60%) with a 12:12 light: dark cycle. The rats were allowed free access to feed and water. The animals' protocols were approved by the Institutional Animal Ethical committee (IAEC/PP/04/2008-09) and experiments were performed according to CPCSEA (Reg no. 97/c/06/CPCSEA) norms and conformed to the guidelines of care and use of animals in research and teaching.

Diets—Cafeteria and atherogenic diets: The cafeteria diet consisted of 3 diets (condensed milk, 48 g + bread, 48 g), (chocolate, 18 g + biscuits, 36 g +

dried coconut, 36 g) and (cheese, 40 g + boiled potatoes, 60 g). The three diets were presented to group of 6 rats on day 1, 2 and 3 respectively and then repeated in same succession for 40 days. The atherogenic diet consisted of cholesterol 1%, cholic acid 0.5%, and olive oil 5%. The atherogenic diet was prepared freshly for every day. These diets were provided in addition to normal pellet chow for 40 days and rats were eating daily^{12,13}.

Chemicals—Cholesterol and cholic acid were purchased from Sigma Chem Co., U.S.A. Monosodium glutamate and olive oil were purchased from Loba Chemie, Bangalore, India. Glucose, cholesterol and triglycerides kits were purchased from Swemed Diagnostic, Bangalore, India. Sibutramine was a gift sample obtained from Cipla India Ltd, Bangalore.

Experimental design

Cafeteria diet induced obesity—Rats were randomly divided into following 6 groups; of 6 each. Gr I: was served as vehicle control (received 1 mL/100 g, po, normal saline solution); Gr II: served as cafeteria diet control, Gr III, IV and V: received ethanolic extract of *B. monosperma* bark orally at doses of 200, 400 and 800 mg/kg/day respectively. Gr VI: was served reference standard drug (Sibutramine 5 mg/kg/day, po). The treatment was continued for 40 days.

Atherogenic diet induced obesity—Anti-obese effect of *B. monosperma* was tested in female Wistar albino rats by feeding atherogenic diet. It was provided in addition to normal pellet (laboratory pellets) chow to rats every day for 40 days. Rats were randomly divided into 6 groups of 6 each. Group I was served as vehicle control (received 1 mL/100 g, po, normal saline solution). Group II served as atherogenic diet control, Groups III, IV and V received ethanolic extract of *B. monosperma* bark orally at doses of 200, 400 and 800 mg/kg/day respectively. Group VI was served reference standard drug (Sibutramine 5 mg/kg/day, po). The treatment was continued for 40 days^{12,13}.

Monosodium glutamate induced obesity—Induction of neurotoxicity with monosodium glutamate was performed as per Alarcon-Aguilar *et al*¹⁴. On the day of delivery, pups were randomly divided into two groups: Gr. I (control, received normal saline) and Gr. II (monosodium glutamate obese rats). Pups in Gr. II received a dose of 2 mg/g monosodium glutamate, sc, once in a day, on the 2nd and 4th postnatal days and 4 mg/g, sc, monosodium glutamate

once in a day on 6th, 8th, and 10th postnatal days. The injection volume was 8 μ L/g body weights. Pups in Gr. I were injected with equivalent volume of normal saline. The animals were weaned at the 21st postnatal day and housed under control condition. At the age of 65 days the rats in Grs I and II were divided in to two subgroups. Subgroups I and II of monosodium glutamate obese rats received normal saline and ethanolic extract of *B. monosperma* bark orally at doses of 200, 400 and 800 mg/kg/day respectively. Subgroup I and II of control received normal saline solution and 800 mg/kg of ethanolic extract of *B. monosperma* bark respectively.

Parameters tested

Food intake—The daily food intake for group of 6 rats was measured daily for 40 days in cafeteria and atherogenic diet induced obese rats and for 60 days in monosodium glutamate obese rats in each group.

Body weight—The body weight (g) was recorded on day 1 and then on alternate days for 40 days in cafeteria and atherogenic diet induced obese rats. The body weight was recorded weekly for 60 days in monosodium glutamate obese rats in each group.

Body temperature—The body temperature was recorded on day 39 in cafeteria and atherogenic diet fed rats and on day 59 in monosodium glutamate obese rats using rectal telethermometer before and after drug administration at 30, 60, 90, 120 and 180 min time interval with a contact time of 1 min.

Locomotor activity—Locomotor activity was recorded on day 40 in cafeteria and atherogenic diet fed rats and on day 60 in monosodium glutamate obese rats using actophotometer with 10 min observation time after drug administration to treatment groups.

Biochemical parameter—Blood was collected from retro-orbital plexus in anaesthetised rats on day 41 in cafeteria and atherogenic diet fed rats and on day 61 in monosodium glutamate obese rats, and was subjected to centrifugation to obtain serum. Changes in glucose, total cholesterol and triglyceride levels were measured from serum sample using the biochemical kits.

Organs and uterine fat pad weights—The animals were sacrificed by excess of ether anaesthesia and then different organs (heart, kidney, liver, spleen) and uterine fat pad were removed, rinsed in cold saline, patted between paper towels, and weighed^{12,13}.

Statistical analysis—The results are expressed as mean \pm SE. Comparisons between the treatment

groups and control were performed by analysis of variance (ANOVA) followed by Dunnet's multiple test. In all tests the criterion for statistical significance was $P < 0.05$.

Results

Preliminary phytochemical screening of ethanolic extract revealed the presence of flavonoids, triterpenes, saponins, arotinoids, alkaloids, glycosides and carbohydrates.

Effect of on food intake—Cafeteria diet and atherogenic diet fed rats showed increase in daily food intake when compared to vehicle control group fed with normal diet. Treatment with ethanolic extract of *B. monosperma* bark at a dose of 400 and 800 mg/kg caused significant ($P < 0.01$) decrease the daily intake of food in cafeteria and atherogenic diet fed rats as compared to their respective control group (Tables 1 and 2). In monosodium glutamate induced rats increase daily food intake when compared to vehicle control rats fed with normal diet. Doses of 400 and 800 mg/kg caused significant ($P < 0.05$, < 0.01) decrease in the daily intake of food in monosodium glutamate induced rats and also decrease the daily intake of food in normal rats which received 800 mg/kg ethanolic extract of *B. monosperma* bark when compared to vehicle control rats (Table 3).

Effect of body weight—Figure 1 shows the changes in body weights of the groups during the experiments. *B. monosperma* extract treatment has significantly ($P < 0.01$) suppressed the increase in body weight compared to the high fat diet control group during treatment period.

B. monosperma extract (800 mg/kg) administered to vehicle control group (Gr. II) slightly decreased body weight compared to Gr. I (vehicle control group). Monosodium glutamate obese rats showed significant ($P < 0.01$) increase in body weight as compared to vehicle control rats. Treatment of ethanolic extract of *B. monosperma* bark at a dose of 800 mg/kg elicited a significant ($P < 0.01$) reduction in body weight of monosodium glutamate obese rats when compared with control group.

Effect on body temperature—Treatment of ethanolic extract of *B. monosperma* bark caused slight decrease in body temperature in cafeteria, atherogenic diet fed rats and monosodium glutamate obese rats as compared to their respective vehicle control group. Treatment of ethanolic extract of at a dose of 800 mg/kg caused significant ($P < 0.01$) decrease body

temperature in monosodium glutamate obese rats when compared to control group (Tables 1-3).

Effect on locomotor activity—In cafeteria, atherogenic and monosodium glutamate treated groups, *B. monosperma* bark showed not significantly

increase in locomotor activity but slightly dose dependent increase in locomotor activity (Tables 1-3).

Effect on biochemical parameters—In cafeteria diet fed rats, the ethanolic extract treatment *per se* significantly ($P < 0.01$) decreased triglycerides level

Table 1—Effect of ethanolic extract of *B. monosperma* bark on average food intake, body temperature, locomotor activity and biochemical parameters in cafeteria diet induced obese rats.
[Values are mean \pm SE from 6 rats in each group]

Treatment Dose (mg/kg)	Average food intake (g/day)	Body temperature (°C)	Locomotor Activity (10 min)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Vehicle control (1 ml/100 g)	59.55 \pm 1.14	33.19 \pm 0.04	738.83 \pm 39.64	69.77 \pm 3.54	69.37 \pm 2.89	70.70 \pm 1.58
Cafeteria diet control	82.47 \pm 1.81	33.36 \pm 0.05	490.67 \pm 56.75	174.37 \pm 7.68	207.57 \pm 8.39	228.01 \pm 7.83
Cafeteria diet + <i>B. monosperma</i> (200)	77.87 \pm 1.48	33.43 \pm 0.08	454.83 \pm 32.82	179.34 \pm 6.80	193.81 \pm 19.56	141.37 \pm 13.99**
Cafeteria diet + <i>B. monosperma</i> (400)	71.45 \pm 0.96**	33.38 \pm 0.06	480.67 \pm 40.77	152.22 \pm 7.40	190.16 \pm 16.00	113.21 \pm 6.06**
Cafeteria diet + <i>B. monosperma</i> (800)	66.27 \pm 1.01**	33.31 \pm 0.04	570.00 \pm 34.57	117.61 \pm 6.02**	162.42 \pm 9.50**	105.06 \pm 9.40**
Cafeteria diet + Sibutramine (5)	61.92 \pm 0.51**	33.32 \pm 0.08	593.50 \pm 45.13	104.76 \pm 4.81**	119.95 \pm 10.05**	100.56 \pm 8.03**

P values * <0.05 , ** <0.01 as compared to cafeteria diet induced control group.

Table 2—Effect of ethanolic extract of *B. monosperma* bark on average food intake, body temperature, locomotor activity and biochemical parameters in atherogenic diet induced obese rats.
[Values are mean \pm SE from 6 rats in each group]

Treatment Dose (mg/kg)	Average food intake (g/day)	Body temperature (°C)	Locomotor Activity (10 min)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Vehicle control (1 ml/100 g)	59.55 \pm 1.14	33.19 \pm 0.04	738.83 \pm 39.64	69.77 \pm 3.54	69.37 \pm 2.89	70.70 \pm 1.58
Atherogenic diet control	92.00 \pm 0.95	33.57 \pm 0.07	622.83 \pm 72.04	189.74 \pm 9.77	231.66 \pm 7.92	249.01 \pm 13.38
Atherogenic diet + <i>B. monosperma</i> (200)	89.15 \pm 0.84	33.47 \pm 0.07	621.50 \pm 62.61	177.42 \pm 10.57	206.67 \pm 19.77	229.39 \pm 13.84
Atherogenic diet + <i>B. monosperma</i> (400)	82.60 \pm 0.75**	33.36 \pm 0.11	660.83 \pm 52.82	160.00 \pm 7.96*	171.67 \pm 11.37**	192.67 \pm 11.98**
Atherogenic diet + <i>B. monosperma</i> (800)	71.12 \pm 0.56**	33.29 \pm 0.10	684.33 \pm 72.90	113.18 \pm 7.59**	135.00 \pm 9.91**	154.90 \pm 10.26**
Atherogenic diet + Sibutramine (5)	61.22 \pm 0.56**	33.15 \pm 0.15	724.67 \pm 59.93	97.84 \pm 3.78**	98.33 \pm 7.49**	111.26 \pm 10.03**

P values * <0.05 , ** <0.01 as compared to atherogenic diet induced control group.

Table 3—Effect of ethanolic extract of *B. monosperma* bark on average food intake, body temperature and locomotor activity and biochemical parameters in monosodium glutamate induced obese rats.
[Values are mean \pm SE from 6 rats in each group]

Treatment Dose (mg/kg)	Average food intake (g/day)	Body temperature (°C)	Locomotor Activity (10 min)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Vehicle control (1 ml/100 g)	58.86 \pm 0.41	31.42 \pm 0.06	716.83 \pm 87.24	72.14 \pm 1.39	67.35 \pm 3.30	76.99 \pm 3.75
Vehicle control + <i>B. monosperma</i> (800)	51.35 \pm 0.61	31.34 \pm 0.05	625.33 \pm 64.52	69.12 \pm 2.15	62.41 \pm 4.40	68.88 \pm 1.74
MSG control	55.71 \pm 0.93	31.71 \pm 0.05	668.20 \pm 54.88	115.35 \pm 1.97	96.66 \pm 3.58	139.19 \pm 4.87
MSG + <i>B. monosperma</i> (200)	54.46 \pm 0.88	31.53 \pm 0.08	681.20 \pm 64.34	111.02 \pm 2.81	94.16 \pm 9.18	133.07 \pm 4.56
MSG + <i>B. monosperma</i> (400)	52.51 \pm 0.81*	31.50 \pm 0.04	627.17 \pm 48.73	99.44 \pm 2.10**	82.64 \pm 7.86	118.89 \pm 5.65*
MSG + <i>B. monosperma</i> (800)	50.45 \pm 0.71**	31.26 \pm 0.06**	647.00 \pm 55.89	87.80 \pm 1.77**	75.00 \pm 6.08	104.17 \pm 5.66**

P values * $<$ 0.05, ** $<$ 0.01, as compared to monosodium glutamate control group.

but glucose and cholesterol levels were not changed in 200 and 400 mg/kg doses. Bark extract at a dose of 800 mg/kg significantly changed glucose, cholesterol and triglycerides levels when compared to control group (Table 1).

In atherogenic diet control group significant ($P<$ 0.01) increase in serum glucose, total cholesterol and triglycerides was observed when compared with vehicle control group. Treatment of ethanolic extract at doses of 400 and 800 mg/kg elicited significant reduction in serum glucose, cholesterol and triglycerides levels when compared to control group (Table 2).

Table 3 shows the effect on glucose, total cholesterol and triglycerides produced by daily administration of the ethanolic extract of *B. monosperma* bark. Difference in initial glucose, total cholesterol and triglycerides were statistically significant between vehicle control and monosodium glutamate control group ($P<$ 0.01). The extract (400 and 800 mg/kg) significantly reduced glucose and triglycerides levels in monosodium glutamate obese rats on the 60th day, but did not change the cholesterol level in monosodium glutamate obese rats. In vehicle control treated with *B. monosperma* extract there was a slight but not statistically significant reduction in glucose, total cholesterol and triglycerides levels on 60th day in comparison with vehicle control group.

Effect on organs and fat pad weights—There was significant increase in weights of internal organs like heart, kidney, liver, spleen and uterine fat pad in cafeteria, atherogenic diet fed rats and monosodium

glutamate obese rats compared to vehicle control group. Furthermore, the rat fed with high fat diet for a long period of time developed fatty liver, and an increase in liver, heart, kidney, spleen weights and the accumulation of hepatic triglycerides and total cholesterol compared to the vehicle control group. Administration of *B. monosperma* extract (800 mg/kg) produced significant ($P<$ 0.01) reduction in weights of heart, kidney, liver, spleen and uterine fat pad in cafeteria, atherogenic diet fed rats and monosodium glutamate obese rats when compared with their respective control group (Fig. 2).

Discussion

In the present study, the anti-obese effect of *B. monosperma* bark was studied using the dietary (cafeteria and atherogenic diets) animal models of obesity as they have been reported to bear close resemblance to human obesity¹⁵. The result of the present study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate cafeteria foods elicited significant increase in body weights and fat pad mass. Cafeteria diet has been previously reported to increase energy intake and cause obesity in human and animal¹⁵. Further the composition and variety of cafeteria foods also exert synergistic effect on development of obesity¹⁷⁻²⁰. In present study atherogenic diet fed rats also exhibited an increase the body weight along with corresponding rise in cholesterol level.

Treatment with *B. monosperma* resulted in reduction in body weight in cafeteria and atherogenic

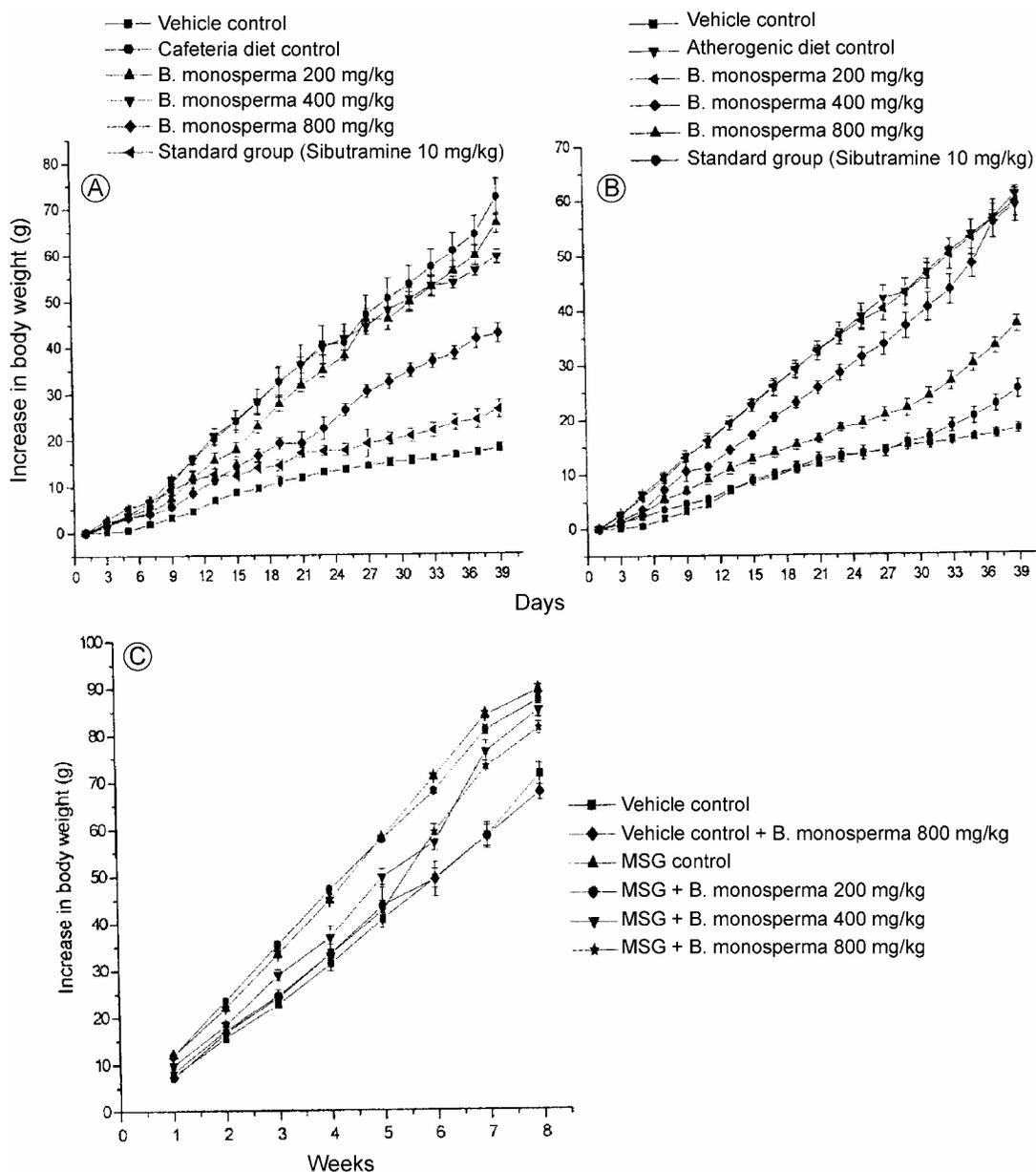


Fig. 1—Effect of ethanolic extract of *B. monosperma* bark on body weight in cafeteria diet (A), atherogenic diet (B) and monosodium glutamate (C) induced obese rats.

diets fed rats indicating that *B. monosperma* possess weight reducing property. Since obesity is associated with hyperphagia, in the present study cafeteria diet and atherogenic diet fed rats, consumed more food than normal diet fed rats. *B. monosperma* was effective in decreasing daily food intake in both cafeteria diet and atherogenic diet fed rats, indicating that it possesses hypophagic property. The extract showed significant reduction in serum levels of glucose, total cholesterol and triglycerides in cafeteria diet and atherogenic diet fed rats. These results are in

agreement with previously reported hypoglycaemic and hypolipidemic action of *B. monosperma*¹⁰. Since obesity is associated with increase in serum level of glucose (due to insulin resistant), total cholesterol and triglycerides.

Increase in rectal temperature and ambulatory activity by *B. monosperma* bark may be attributed to the overall stimulant and presence of thermogenic property in the plant. Since obesity associate with defective thermogenesis³.

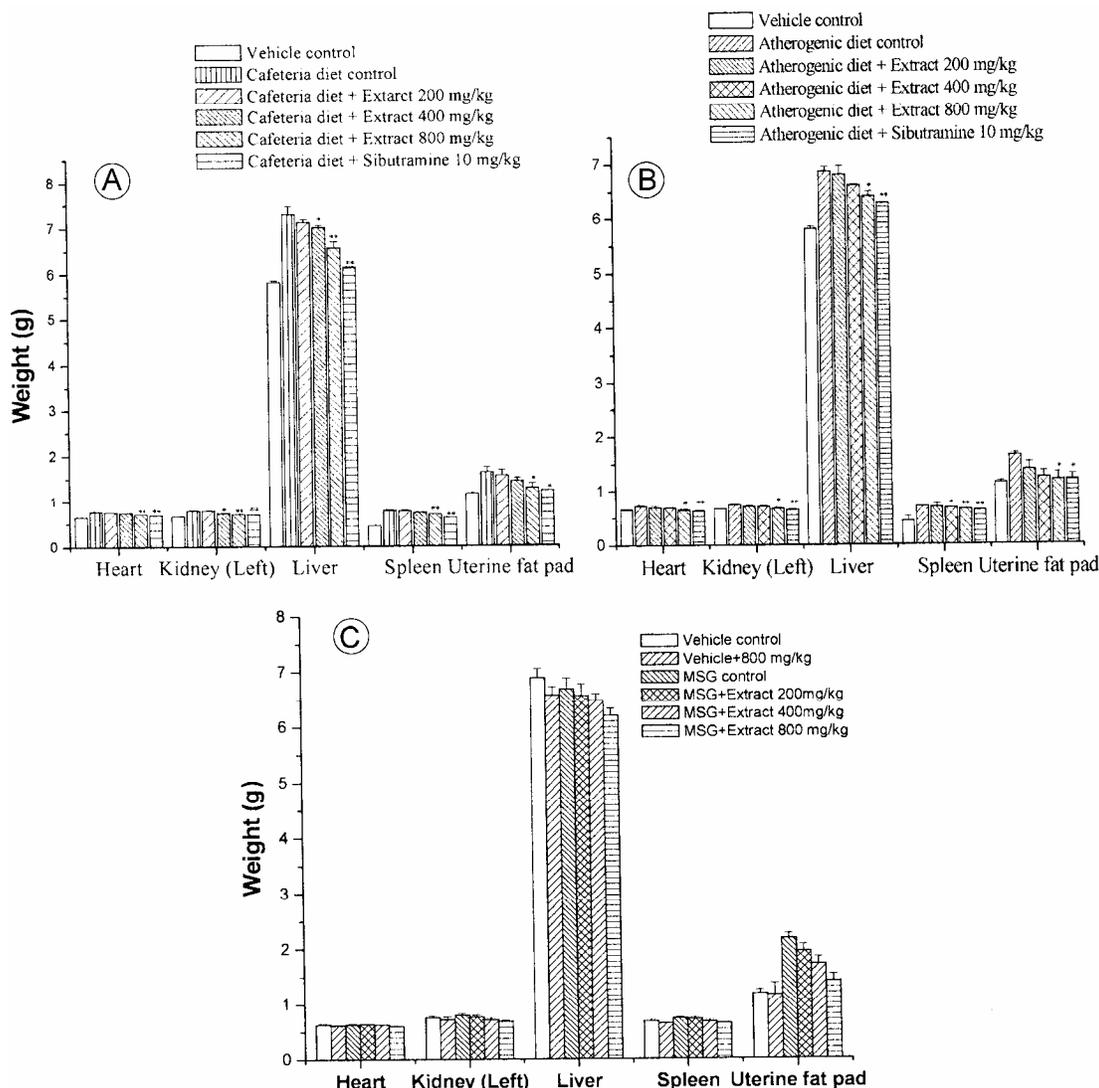


Fig. 2—Effect of ethanolic extract of *B. monosperma* bark on organs and fat pad weight (g) in cafeteria diet (A), atherogenic diet (B) and monosodium glutamate (C) induced obese rats.

It is well known that the parenteral administration of monosodium glutamate in newborn rodent produces adiposity when the animals reach adulthood. Monosodium glutamate obese animal resemble genetically obese animal in their greatly increased body lipid content, greatly decreased rates of hormone-stimulated lipolysis, and similar states of transient hyperglycemia.

The efficacy of new obesity treatments should be assessed by their effects on body weight. As such, a treatment should be considered successful if it prevents further weight gain, induces a 5–10% weight loss from the initial body weight, and allows long-term maintenance of the weight loss once it is

achieved. The present results showed that *B. monosperma* ethanolic extract suppresses body weight gain in monosodium glutamate obese rats by 9.4% and reduces glycemia. The monosodium glutamate rat model is characterized by glycosuria, hyperglycemia and hyperinsulinemia²¹. These data support the idea that *B. monosperma* extract contain agents that might be useful in the prevention and treatment of obesity and hyperglycaemia. Furthermore, food intake was not significantly modified by *B. monosperma* treatment in both healthy and obese rats.

The phytochemical analysis showed that major chemical constituents of *B. monosperma* were sterols, polyphenols, flavanoids, ascorbic acid and saponins.

It is well established that saponins are useful in treatment of obesity, phytosterols have beneficial effects on hyperlipidaemia, and polyphenols and flavanoids have potential antioxidant properties²¹⁻²⁴. Therefore, it could be possible that presence of these compounds is responsible for observed glucose and lipid lowering activity. Flavonoids possess a wide spectrum of biological actions including hypoazotemic, hypotensive, hypoglycemic, oestrogenous, spasmolytic, cholagogue, anti-inflammatory, antilipidemic and antioxidant activities²⁶. Further, antihyperlipidemic potential of *B. monosperma* extract was comparable with that of Sibutramine and can correlate with the previous research findings²⁷.

Treatment with *B. monosperma* bark also caused significant decrease in weights of different internal organs and fat pad in cafeteria diet and atherogenic diet fed rats, suggesting that *B. monosperma* bark reduce adipose tissue formation in rats.

The present investigation reveals that *B. monosperma* bark shown significant hypoglycemic and hypolipidemic effect on cafeteria, atherogenic diet fed rats and monosodium glutamate induced obese rats.

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