

Hypolipidemic and weight reducing activity of the ethanolic extract of *Tamarindus indica* fruit pulp in cafeteria diet- and sulpiride-induced obese rats

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ABSTRACT

Objectives: To investigate the effect of ethanolic extract of fruit pulp of *Tamarindus indica* Linn. (Family: Caesalpiniaceae) on obesity in rats using cafeteria diet-induced obesity and antipsychotic drug (sulpiride)-induced obesity. **Materials and Methods:** Cafeteria diet was administered for 40 successive days to male Wistar rats and sulpiride (20 mg/kg, i.p.) was administered for 28 successive days to female Wistar rats. In separate groups of animals, the ethanolic extract (50 and 100 mg/kg p.o.) of *Tamarindus indica* fruit was administered along with cafeteria diet for 40 successive days to Wistar male rats and along with sulpiride for 28 successive days to Wistar female rats. **Results:** Cafeteria diet alone significantly increased body weight, serum total cholesterol, triglycerides, and glucose levels and decreased HDL cholesterol in male rats as compared to control. Sulpiride per se significantly increased the levels of glucose, triglycerides, cholesterol and there was no significant effect on HDL-cholesterol in female rats as compared to control. Ethanolic extract showed a significant decrease in body weight, serum cholesterol, and triglycerides and a significant increase in HDL-cholesterol in cafeteria diet- and sulpiride-induced obese rats as compared to their respective control groups. **Conclusions:** Thus, the ethanolic extract of *Tamarindus indica* fruit pulp showed a significant weight-reducing and hypolipidemic activity in cafeteria diet- and sulpiride-induced obese rats.

Key words: Cafeteria diet, hyperlipidemia, obesity, sulpiride, *Tamarindus indica*

INTRODUCTION

Obesity is defined as an increase in total fat mass and it occurs when unilocular adipocytes show hyperplasia or hypertrophy following macrophage infiltration of fat tissue.^[1] Although a number of pharmacological approaches for treatment of obesity

have been investigated, but only few are safe and most of these have adverse effects.^[2] So alternative is to discover antiobesity drugs from plants. So aim of the present study was to evaluate antiobesity activity of *Tamarindus indica*.

T. indica Linn. (Family: Caesalpiniaceae) is a well-known plant of the Indian medicinal system. Seeds of the plant have antidiabetic,^[3] antsnake venom,^[4] hepatoregenerative^[5] properties. Pulp of fruits have hypolipidemic, antioxidant,^[6] antifluorosis,^[7] and analgesic,^[8] hepatoregenerative,^[5] and spasmolytic^[9] activities. Its leaves have antiemetic,^[10] antibacterial,^[11] and hepatoregenerative^[5] activities. The stem bark of the plant has analgesic^[12] and spasmogenic^[13] activities. The fruit pulp has been reported to contain tartaric acid, lactic acid, citric acid, and malic acid.^[14] The aqueous pulp extract

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of *T. indica* administered for 28 days significantly decreased body weight, serum cholesterol, and LDL and increased HDL and triglycerides of normal rats fed with a normal chow diet.^[15] But there are no reports regarding weight reducing and hypolipidemic activities of the ethanolic extract of *T. indica* fruit pulp in normal and obese rats, so we evaluated the antiobesity activity of this extract and also explored the role of the dopaminergic system in this activity.

MATERIALS AND METHODS

Experimental animals

Wistar albino rats of either sex, 4-5 weeks old and weighing around 30-40 g were purchased from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana). Male and female animals were housed in separate cages under 12-12 h day light cycle, 25±3°C temperature and 55-65% humidity condition. The animals had free access to food (freshly cooked dalia) and water. The animals were kept fasted 2 h before and 2 h after drug administration. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and chemicals

Sulpiride (Sigma-Aldrich, St. Louis, USA), rectified ethanol, diethyl ether, and glacial acetic acid (Sd Fine-Chem Ltd, Mumbai) and kits for estimation of serum glucose, cholesterol, HDL, triglycerides (Crest Biosystems, Division of Coral Clinical Systems, Goa) were used in the present study.

Collection of plant material

The pulp of *T. indica* was purchased from local market in Hisar, Haryana, and was identified as *T. indica* by Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources, New Delhi (Reference number NISCAIR/RHMD/Consult/2010-11/1479/77).

Preparation of extract of *T. indica*

Ethanolic extract was prepared by cold maceration process. Around 100 g of the fruit pulp were placed in a conical flask and soaked for 3 days in 400 ml of 95% v/v of ethanol at room temperature. Then, the extract was filtered and filtrate was evaporated to dryness at a low temperature (40-60°C) using water bath. The dried extract was reddish-brown in color and the yield was 9.2%w/w.

Experimental protocols

The ethanolic extract of *T. indica* was dissolved in distilled water. Sulpiride was dissolved in normal saline followed by the addition of one drop of glacial acetic acid. Cafeteria diet was

prepared using bread (25 g) + boiled potato (25 g)/ condensed milk (25 g) + biscuits (25 g)/ potato chips (25 g) + rice polish (25 g).^[16] These diets were fed along with normal diet for one week in rotation for a total period of 6 weeks to male rats. In another model, sulpiride (20 mg/kg/day, i.p.) was given for 28 days to induce obesity in female rats. Sulpiride is known to induce weight gain, hyperphagia, hyperprolactinemia, hypogonadism, and perhaps increased insulin sensitivity in rats.^[17]

Experimental protocols

- In cafeteria diet-induced model, the animals were divided into four groups and each group comprised of five animals. Animals were grouped as follows:

Group I : Vehicle-treated group

Group II : Cafeteria diet control

Group III : Cafeteria diet + Ethanolic extract of *T. indicus* (50 mg/kg p.o.)

Group IV : Cafeteria diet + Ethanolic extract of *T. indicus* (100 mg/kg p.o.)

All the treatments were carried out for 40 days. Before and after the treatment, the animals were fasted for 2 h to improve the absorption rate.

- In the sulpiride-induced obesity model, the female rats were divided into four groups of six animals each as follows:

Group I : Vehicle-treated group

Group II : Sulpiride control

Group III : Sulpiride + Ethanolic extract of *T. indicus* (50 mg/kg p.o.)

Group IV : Sulpiride + Ethanolic extract of *T. indicus* (100 mg/kg p.o.)

All the treatments were carried out for 28 days. Before and after the treatment the animals were fasted for 2 h to improve the absorption rate. In both the models, the weekly body weight analyses were carried out for entire duration of the study. At end of the study, the blood samples were collected from all the groups of the animals through the orbital sinus and the lipid profile was estimated.

Statistical analysis

All values were expressed as mean ± SEM. Data in Tables 1 and 3 were analyzed using the repeated measures ANOVA followed by the Tukey-Kramer multiple comparison test. Data in Tables 2 and 4 were analyzed by one-way ANOVA followed by the Tukey's multiple comparison test. A *P* value <0.05 was considered significant.

RESULTS

Cafeteria diet significantly increased the body weight of rats as compared to vehicle-treated control after 1 week of

Table 1: Effect of ethanolic extract of *T. indica* on body weight of rats in the cafeteria diet-induced obesity model

Group no.	Treatment	Body weight (g)						
		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
I	Vehicle control	34.2±0.7	36±0.7	37.8±0.9	42.2±1.9	46.2±1.9	52.6±1.4	52.2±1.0
II	Cafeteria diet	34.4±0.7	63.4±2.0 ^a	78.6±3.5 ^a	95.2±3.7 ^a	103.2±4.0 ^a	114±5.0 ^a	113.6±3.9 ^a
III	Ethanolic extract (50 mg/kg) + cafeteria diet	34.2±0.9	66.6±3.0	84.2±5.1	96.4±2.2	92.2±2.3	86.6±4.5 ^b	83.6±4.4 ^b
IV	Ethanolic extract (100 mg/kg) + cafeteria diet	34.6±1.4	67.2±2.1	82±3.6	99±3.4	107.4±3.9	97.4±1.2 ^b	90.6±2.7 ^b

n = 5 in each group; values are in mean ± SEM. Data were analyzed by repeated measures ANOVA followed by the Tukey-Kramer multiple comparison test;

^a*P*<0.05 significantly different compared to vehicle-treated control at similar weekly intervals; ^b*P*<0.05 significantly different compared to cafeteria diet-treated animals at similar weekly intervals

Table 2: Effect of ethanolic extract of *T. indica* on cholesterol, triglycerides, HDL, and glucose levels of rats in the cafeteria diet-induced obesity model

Group no.	Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	HDL-cholesterol (mg/dl)
I	Vehicle control	44.76±2.09	73.53±2.59	88.14±2.77	35.41±1.25
II	Cafeteria diet	136.54±1.99 ^a	169.02±2.33 ^a	133.38±1.74 ^a	18.44±1.25 ^a
III	Ethanolic extract (50 mg/kg) + cafeteria diet	70.55±1.72 ^b	98.65±2.04 ^b	132.33±2.12	30.49±1.53 ^b
IV	Ethanolic extract (100 mg/kg) + cafeteria diet	114.29±2.29 ^b	147.22±2.00 ^b	126.65±2.01	24.08±0.89 ^b

n = 5 in each group; values are in; ^a*P*<0.05 significantly different compared to vehicle-treated control at similar weekly intervals; ^b*P*<0.05 significantly different compared to cafeteria diet-treated animals at similar weekly intervals

Table 3: Effect of ethanolic extract of *T. indica* on body weight of rats in the sulpiride-induced obesity model

Group no.	Treatment	Body weight (g)				
		Week 0	Week 1	Week 2	Week 3	Week 4
I	Vehicle control	36.2±0.8	36.7±0.9	41.2±1.5	44.7±1.6	50.5±1.2
II	Sulpiride (20 mg/kg)	36.6±0.5	47.2±1.7 ^a	57.6±1.9 ^a	61.7±2.1 ^a	69.9±2.9 ^a
III	Ethanolic extract (50 mg/kg) + sulpiride	31.5±1.0	42.2±1.8	56.6±1.6	54.8±1.5	49.0±1.8 ^b
IV	Ethanolic extract (100 mg/kg) + sulpiride	32.5±1.2	48.41±2.82	64.0±1.5	58.9±1.3	52.8±1.4 ^b

n = 6 in each group; values are in mean ± SEM. Data were analyzed by repeated measures ANOVA followed by the Tukey-Kramer multiple comparison test;

^a*P*<0.05 significantly different compared to vehicle-treated control at similar weekly intervals; ^b*P*<0.05 significantly different compared to sulpiride-treated animals at similar weekly intervals

Table 4: Effect of ethanolic extract of *T. indica* on serum cholesterol, triglyceride, glucose, HDL-cholesterol of rats in the sulpiride-induced obesity model

Group no.	Treatment	Cholesterol (mg/dl ± SEM)	Triglyceride (mg/dl ± SEM)	Glucose (mg/dl ± SEM)	HDL-cholesterol (mg/dl ± SEM)
I	Vehicle control	45.06±1.73	72.59±2.31	85.43±2.27	35.34±1.02
II	Sulpiride control (20 mg/kg)	69.45±1.6 ^a	101.69±1.72 ^a	127.39±1.65 ^a	31.21±1.47
III	Ethanolic extract (50 mg/kg) + sulpiride	53.25±2.36 ^b	80.97±1.68 ^b	118.27±1.82 ^b	44.71±0.87 ^b
IV	Ethanolic extract (100 mg/kg) + sulpiride	57.10±1.13 ^b	90.84±1.89 ^b	120.39±1.60	27.49±0.66

n = 6 in each group; values are in mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test; ^a*P*<0.05 significantly different compared to vehicle-treated control at similar weekly intervals; ^b*P*<0.05 significantly different compared to sulpiride-treated animals at similar weekly intervals

treatment and continued up to 6 weeks. The ethanolic extract of *T. indica* (50 and 100 mg/kg p.o.) significantly decreased the body weight in cafeteria diet-induced obese rats after 5 and 6 weeks of treatment [Table 1]. Cafeteria diet significantly increased the level of glucose, triglycerides, cholesterol and significantly decreased HDL level as compared to vehicle treated control. Ethanolic extract (50 and 100 mg/kg p.o.) administered for 40 successive days to rats significantly decreased cholesterol and triglycerides; and significantly increased HDL-cholesterol level in cafeteria diet-induced

obese rats. There was no significant effect on serum glucose levels by the extract [Table 2].

Sulpiride significantly increased the body weight as compared to vehicle-treated control after 1 week of treatment and continued up to 4 weeks. Ethanolic extract (50 and 100 mg/kg; p.o.) significantly decreased the body weight in sulpiride-induced obese rats after 4 weeks of treatment [Table 3].

Sulpiride significantly increased the level of glucose,

triglycerides, cholesterol, but there was no significant effect on HDL-cholesterol as compared to vehicle-treated control. Lower dose (50 mg/kg p.o.) of ethanolic extract administered for 28 successive days to rats significantly decreased serum cholesterol, triglycerides, and glucose, and significantly increased serum HDL-cholesterol level in sulpiride-induced obese rats. On the other hand, higher dose (100 mg/kg p.o.) significantly decreased serum cholesterol and triglycerides levels without significantly affecting levels of glucose and HDL-cholesterol [Table 4].

DISCUSSION

In the present study, the ethanolic extract (50 and 100 mg/kg p.o.) of *T. indica* fruit pulp showed a significant decrease in body weight, serum cholesterol, and triglycerides and a significant increase in HDL-cholesterol in cafeteria diet- and sulpiride-induced obese rats as compared to their respective control groups. This is also supported by earlier study which shows the hypolipidemic activity of ethanolic extract of *T. indica* fruit pulp in hypercholesterolemic hamsters fed with atherogenic diet.^[6] There are also reports on the weight-reducing and hypolipidemic activities of the aqueous pulp extract of *T. indica* (2700 to 4500 mg/kg) administered for 28 days to normal rats fed with normal chow diet.^[15] Our study is different from these two reports in that we have employed cafeteria diet- and antipsychotic drug- induced obesity models in male and female rats, respectively.

A cafeteria diet-induced obesity model is the simplest obesity-induction model and possibly the one that most closely resembles the reality of obesity in humans.^[18] The results of the present study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate cafeteria foods elicited a significant increase in body weights and serum cholesterol, triglycerides, glucose, and a decrease in serum HDL-cholesterol. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans^[19] as well as animals.^[20] Further the composition^[21] and variety^[22] of cafeteria foods also exert synergistic effects on the development of obesity. The cafeteria diet has been reported to induce hyperphagia in rats^[23] which results in higher fat stores.^[24] Moreover, the down regulation of striatal D₂ receptor expression is a notable neuroadaptive response to over consumption of palatable food. Indeed, reductions in striatal D₂ receptor density are seen in overweight individuals.^[25]

Excessive body weight gain and hyperphagia is frequently observed during chronic administration (3-4 weeks) of antipsychotic drugs, such as sulpiride in female rats. Sulpiride does not affect bodyweight in male rats.^[26] In the present study, sulpiride administered for 4 weeks significantly increased body weights of female rats and also significantly increased

serum cholesterol, triglycerides and glucose levels. Sulpiride induces obesity by two mechanisms: (i) direct stimulation of feeding-related areas in the brain^[27] and (ii) metabolic and endocrine abnormalities secondary to hyperprolactinemia.^[28] Further, sulpiride is devoid of sedative and motor defects and induces hyperprolactinemia which may cause impairment in reproductive hormones that may promote weight gain.^[29]

Thus, the weight-reducing effect of the ethanolic extract of *T. indica* fruit pulp might be due to an increase in dopaminergic transmission, since the extract reversed sulpiride-induced as well as cafeteria diet-induced obesity. Flavonoids and polyphenolic compounds present in the ethanolic extract may be responsible for its antiobesity activity.^[6] However, a further study is required to find out the particular component(s) present in the ethanolic extract responsible for its weight reducing activity. Thus, the ethanolic extract of *T. indica* fruit pulp may be explored further for its potential in management of obesity.

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