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Lagenaria siceraria fruit extract ameliorate fat amassment and serum TNF- α in high-fat diet-induced obese rats

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ABSTRACT

Objective: To investigate the effects of ethanolic extract of *Lagenaria siceraria* fruit (ELSF) on fat amassment and serum TNF- α in high-fat diet-induced obese rats. **Methods:** The high fat diet induced obese rats were orally treated with orlistat (50 mg/kg) and ELSF (100, 200, 300 mg/kg/day) to the respective treatment groups. The body weight, fasting blood glucose level, lipid profile, serum levels of tumor necrosis factor- α (TNF- α) in rats were measured after 30 days of treatment and compared to the obese control animals. **Results:** ELSF significantly ($P < 0.001$) reduced the body weight gain, fasting blood glucose, total cholesterol, triglyceride, total protein and TNF- α . **Conclusions:** These encouraging findings suggest that *Lagenaria siceraria* has excellent pharmacological potential to prevent fat amassment.

1. Introduction

Obesity, which results from a prolonged energy imbalance during intake exceeds expenditure, constitutes one of the most widespread metabolic disorders in contemporary society. It is closely related to hypertension, type 2 diabetes, coronary heart disease, cancer, respiratory complications and osteoarthritis[1,2]. The regulation of energy homeostasis for metabolic diseases is one of the most rapidly advancing subjects in biomedical research today. Breakthroughs in understanding of the molecular mechanisms regulating body weight have also provided potential opportunities for therapeutic intervention and brought renewed hope and vitality for the development of antiobesity drugs[3–6].

The potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective antiobesity drugs. It has shown that some phytochemicals in traditional medicinal plants present an exciting opportunity

for the development of newer treatment of obesity and other metabolic diseases.

Lagenaria siceraria fruit (Cucurbitaceae), popularly known as Bottle gourd, is a common vegetable for Indians. *Lagenaria siceraria* is a pubescent or trailing herb with bottle-shaped fruits. In India, *Lagenaria siceraria* fruits are frequently used to treat a large variety of ailments and symptoms of hyperlipidemia and atherosclerotic impasse. *Lagenaria siceraria* fruits are traditionally used for its cardioprotective, cardiotonic and diuretic properties[7]. It also cures pain, ulcers, jaundice and fever, and used to treat pectoral cough, asthma and other bronchial disorders[8–12]. Some studies have reported that *Lagenaria siceraria* fruit is effective on decreasing the levels of total lipids[7]. *Lagenaria siceraria* fruit also shows antioxidant activity[13]. Lagenin—a novel protein is isolated and characterized from *Lagenaria siceraria* fruits for its anticancer activity[14]. Early work showed that semipurified dietary fibers isolated from *Lagenaria siceraria* fruits affects fecal excretion of steroids[15].

It is well-established that diet rich in vegetables and fruits can reduce cardiovascular diseases[16,17]. However, it has not yet been reported whether *Lagenaria siceraria* fruit affects on fat amassment and serum tumor necrosis factor- α (TNF- α) in high fat diet (HFD) induced obese rats.

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Therefore, an attempt has been made to investigate the effect of ethanolic extract of *Lagenaria siceraria* fruit (ELSF) on the fat amassment and serum TNF- α in high-fat diet-induced obese rats.

2. Materials and methods

2.1. Collection and authentication of plant

The fresh fruits of *Lagenaria siceraria* were collected in the month of March 2010 from the Wardha District of Maharashtra, India. The fruits were authenticated by Dr.Thakare, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur and voucher specimen LP-09011is lodged in our research laboratory for the future reference.

2.2. Animals

Albino Wistar rats of either sex weighing between 150 and 180 g were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee (Reg. no.784/03/C/CPCSEA/5). The guidelines of CPCSEA, India, were strictly followed during the maintenance and experiment.

2.3. Chemicals

Biochemical kits for the estimation of glucose (GOD-POD), total triglycerides (GPO-POD), total cholesterol (CHOD-POD), high density lipoprotein (PEG), and total protein (Biuret) were purchased from Crest Biosystems Kits (India). Serums TNF- α level in rats were measured by use of commercially available rat enzyme-linked immunosorbent assay kit. Normal diet (ND) and HFD were obtained from ACP, Wardha.

2.4. Preparation of crude extract

The fresh fruits of *Lagenaria siceraria* were sliced, shade dried, coarsely powdered and cold macerated with ethanol to obtain ethanolic extract. The solvent extract was evaporated *in vacuo* (40 °C). The yield recorded was 24.7% (w/w). The extract (1%, w/v) was dissolved in distilled water for oral administration to experimental animals.

2.5. Phytochemical screening of ELSF

ELSF was subjected to phytochemical screening^[18] for the detection of various phyto-constituents.

2.6. Toxicity studies

Toxicity studies were carried out on normal healthy rats. The ELSF (50, 100, 150, 200, 300 mg/kg/day) was administered orally for 4 days of six groups of rats ($n=6$) and the animals

were kept under observation for mortality as well as any behavioral changes.

2.7. Experimental models

2.7.1. Induction of obesity

After 7 days of acclimatization, animals were randomly divided into 6 groups ($n=6$): normal control group, HFD control group and remaining III–VI as treatment groups. Animals in normal control group were fed with ND while the other groups were fed with HFD^[19,20] *ad libitum*, throughout the experiment.

2.7.2. Experimental design and treatment protocol

The animals were divided into six groups of six animals each as follows:

Group I : Non-obese control (NOB) rats fed with ND throughout the course of study;

Group II : Obese control (OB) rats fed with HFD for 30 days;

Group III : Obese rats given orlistat suspension prepared with saline 50 mg/kg, *p.o.* for 30 days;

Groups IV : Obese rats given ELSF 100 mg/kg, *p.o.* for 30 days;

Groups V : Obese rats given ELSF 200 mg/kg, *p.o.* for 30 days;

Groups VI : Obese rats given ELSF 300 mg/kg, *p.o.* for 30 days.

2.7.3. Determination of body weight

During the experimental period, change in body weight of each rat was measured once in a week of ELSF treatment.

2.7.4. Estimation of plasma glucose, serum levels of TNF- α and lipid profile

Plasma glucose, serum levels of TNF- α and lipid profiles of all the rats were determined on 30 day (post-treatment). The blood samples were collected from each rat by retro-orbital venepuncture of the overnight fasted rats into micro centrifuge tubes containing heparin (10 μ L, 1 000 IU/mL). Biochemical parameters were estimated using commercially available diagnostic kits.

2.8. Statistical analysis

Statistical analysis was carried out by using Graph-Pad Instat statistical package (Graphpad Software Inc.). Values are expressed as mean \pm SEM. For multiple comparisons, one way ANOVA was used followed by Tukey test *P* value less than 0.05 was regarded to be significant.

3. Results

3.1. Phytochemical screening of ELSF

Phytochemical screening revealed that ELSF showed the presence of flavonoids, saponins, steroids and polyphenolic compounds.

3.2. Effect of ELSF on body weight

One-way ANOVA followed by Tukey's test revealed that body weight was slightly increased in non-obese control rats compared to initial body weight whereas HFD induced obese rats shows marked increase of body weight [(268.50±1.57) g] after 30 days as compared with initially weight of obese rats [(258.40±1.56) g]. The weight gain effect evidenced in orlistat (50 mg/kg; *p.o.*) treated rats was [(187.00±1.67) g] and treatment with ELSF (300 mg/kg; *p.o.*) groups showed most significant ($P < 0.001$) reduction in weight gain [(189.80±1.62) g] as compared to OB group (Table 1).

Table 1

Effect of ELSF on body weight (g) in normal and obese rats (mean±SEM, $n = 6$).

Group	Initial body weight	Final body weight
Non-obese control	162.60±1.22	167.80±1.42
Obese control	258.40±1.56	268.50±1.57 [#]
Orlistat (50 mg/kg; <i>p.o.</i>)	195.50±1.40	185.00±1.67 ^{***}
ELSF (100 mg/kg; <i>p.o.</i>)	209.70±1.06	198.80±1.60 ^{**}
ELSF (200 mg/kg; <i>p.o.</i>)	200.20±1.38	192.70±1.63 ^{***}
ELSF (300 mg/kg; <i>p.o.</i>)	198.80±1.63	189.80±1.62 ^{***}

Values are statistically significant at [#] $P < 0.001$ vs. nonobese group; ^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ vs. obese control group respectively (One-way ANOVA followed by Tukey's *post hoc* test).

3.3. Effect of ELSF on fasting blood glucose

One-way ANOVA indicated the significant influence of ELSF on elevation of fasting blood glucose. On 30 days of treatment with ELSF 300 mg/kg; *p.o.* [(92.67±3.58) mg/dL] as compared to OB group [(157.70±3.21) mg/dL] exhibited significant ($P < 0.001$). Orlistat (50 mg/kg, *p.o.*) also showed significant ($P < 0.001$) reduction of fasting blood glucose levels

[(90.33±3.92) mg/dL] as compared to OB group (Table 2).

3.4. Effect of ELSF on serum TNF- α level

One-way ANOVA indicated the significant influence serum TNF- α level were increased in obese control rats fed with HFD compared with NOB group (Table 2) [Group I, (2.60±0.62) pg/mL; group II, (4.37±1.14) pg/mL; $P < 0.001$]. In contrast, TNF- α level were decreased in ELSF (300 mg/kg; *p.o.*) treated groups compared with OB group [(2.05±1.16) pg/mL; $P < 0.001$]. On the other hand, orlistat (50 mg/kg, *p.o.*) showed significant [(1.95±1.37) pg/mL; $P < 0.001$] effect in serum TNF- α level as compared to the OB (Table 2).

3.5. Effect of ELSF on lipid profile

HFD treatment resulted in total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), total protein (TP) and reduction in high density-lipoprotein level (HDL) as compared to the NOB rats as noted at end of the study (Table 2). When OB rats treated with ELSF (300 mg/kg; *p.o.*) for 30 days showed most significant ($P < 0.001$) reduction in TC level [(101.90±3.89) mg/dL], TG level [(197.30±7.70) mg/dL], LDL level [(47.90±1.01) mg/dL], VLDL level [(39.40±1.54) mg/dL], TP level [(6.14±0.30) g/dL]. Conversely, HDL cholesterol level were significantly ($P < 0.001$) increased [(45.80±4.38) mg/dL] in the ELSF treated groups at the 30 days of treatment as compared to OB group. However, the standard drug orlistat (50 mg/kg, *p.o.*) markedly exerted the most significant ($P < 0.001$) effects as: TC level [(99.40±1.82) mg/dL], TG level [(193.30±3.01) mg/dL], LDL level [(46.40±2.71) mg/dL], VLDL level [(37.00±0.60) mg/dL], TP level [(5.68±0.17) g/dL] and HDL level [(47.90±2.32) mg/dL] on 30 days as compared to the OB (Table 2).

Table 2

Effect of ELSF on plasma glucose rate, serum TNF- α level and lipid profile in normal and obese rats (mean±SEM, $n = 6$).

Biochemical parameters	Days	Group I	Group II	Group III	Group IV	Group V	Group VI
Fasting blood sugar (mg/dL)	0	86.23±5.16	112.33±4.03	98.35±5.84	110.00±6.24	98.23±4.08	99.50±5.76
	30	84.00±6.03	157.70±3.21 [#]	90.33±3.92 ^{***}	99.00±10.37 [*]	94.50±4.03 ^{***}	92.67±3.58 ^{***}
TC(mg/dL)	0	71.30±1.60	119.00±1.30	140.10±1.67	149.30±0.81	154.10±0.87	131.30±0.60
	30	78.60±2.05	158.30±2.90 [#]	99.40±1.82 ^{***}	134.30±4.46 [*]	123.00±6.21 ^{**}	101.90±3.89 ^{***}
TG(mg/dL)	0	164.40±0.54	212.50±0.49	224.50±0.80	223.40±0.75	221.30±0.55	228.60±1.47
	30	172.60±1.69	244.90±2.65 [#]	193.30±3.01 ^{***}	219.20±0.85 [*]	218.30±2.70 [*]	197.30±7.70 ^{***}
HDL(mg/dL)	0	32.50±0.80	35.00±0.61	39.03±1.02	36.00±0.77	35.40±0.75	34.70±0.89
	30	35.70±0.54	32.80±0.72 ^{ns}	47.90±2.32 ^{***}	42.00±1.01 [*]	43.90±2.13 [*]	45.80±4.38 ^{**}
LDL(mg/dL)	0	15.70±0.83	63.50±0.92	60.30±0.89	67.70±1.04	62.70±1.07	61.90±0.97
	30	17.80±3.60	69.50±1.76 [#]	46.40±2.71 ^{***}	53.40±5.16 [*]	49.60±5.06 ^{**}	47.90±1.01 ^{***}
VLDL(mg/dL)	0	31.30±0.71	41.70±0.66	51.40±0.76	54.70±0.71	57.70±0.65	54.50±0.79
	30	34.50±0.33	48.90±0.53 [#]	37.00±0.60 ^{***}	44.40±0.54 [*]	44.80±0.17 [*]	39.40±1.54 ^{***}
TP(g/dL)	0	3.69±0.18	8.45±0.34	8.67±0.12	8.88±0.23	8.98±0.49	8.20±0.27
	30	5.29±0.13	10.42±0.24 [#]	5.68±0.17 ^{***}	8.48±0.22 [*]	8.16±0.56 ^{**}	6.14±0.30 ^{***}
TNF- α (pg/mL)	0	2.98±1.55	2.92±2.10	2.89±3.11	3.18±0.56	3.29±3.36	2.97±3.67
	30	2.60±0.62	4.37±1.14 [#]	1.95±1.37 ^{***}	2.78±1.47 [*]	2.68±2.22 [*]	2.05±1.16 ^{***}

Group-I served as non-obese control. Group-II served as obese control. Group III received 50 mg/kg (*p.o.*) of orlistat (positive control). Group IV received 100 mg/kg (*p.o.*) of ELSF. Group V received 200 mg/kg (*p.o.*) of ELSF. Group VI received 300 mg/kg (*p.o.*) of ELSF. Values are statistically significant at [#] $P < 0.001$ vs. non-obese group; ^{ns}-nonsignificant ($P > 0.05$) vs. non-obese group; ^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ vs. obese control group.

4. Discussion

There is overwhelming evidence that obese individuals have a substantially higher risk of developing many diseases such as type 2 diabetes, hyperlipidemia, cardiovascular disease and hypertension^[21,22]. Thus the quest for possible compounds to aid in the treatment of obesity has intensified.

In the present study albino wistar rats, a widely known model for studying obesity and related metabolic disorders were used. The results indicated that the ELSF (100–300 mg/kg/day; *p.o.*) when administered for 30 days showed beneficial effects in fat amassment.

Several studies divulge that a significant reduction in fasting blood glucose level of obese rats. Treatment with ELSF (100, 200 and 300 mg/kg, *p.o.*) for 30 days significantly reduced TC, TG, TP, LDL and VLDL associated with significant increased in HDL level in obese rats. Orlistat (50 mg/kg; *p.o.*) has also been included in the study in order to understand how far activity of ELSF is comparable to that of a standard drug. Since HDL is responsible for the transportation of cholesterol from peripheral tissues to the liver for metabolism^[23]. The weight loss in obese rats may be associated with lipid lowering activity of ELSF or due to its influence on various lipid regulation system. Hence, treatment with ELSF (100, 200 and 300 mg/kg body weight) in rats have potential role to prevent formation of atherosclerosis disease^[24]. ELSF significantly reduced both the TC and LDL cholesterol. Recent studies also shows that triglycerides are directly or indirectly related to coronary heart diseases^[25]. TNF- α , a cytokine secreted by adipocytes, influences energy balance and glucose homeostasis. TNF- α causes insulin resistance and plays a major role in the pathogenesis of obesity-linked DM II ^[26,27]. Serum TNF- α level increased in obesity and may be the strong correlation between total cholesterol and serum TNF- α level^[28,29]. We also found increased serum TNF- α level in animals receiving a high-fat diet. In the absence of a high-fat diet, serum TNF- α level was lower to the NOB group. Serum TNF- α level was significant reduced in ELSF treated groups compared with obese control group.

The mechanisms by which ELSF causes a reduction in body weight gain are not yet clear. However, potential mechanisms include reductions in plasma cholesterol levels and triglyceride, inhibition of lipid droplet accumulation in fat and biochemical characteristics.

Phytochemical screening revealed that the presence of flavonoids, saponins, steroids and polyphenolic compounds in the ELSF. Supplementation of ELSF itself contributed to the reduced body weight gain when compared to the OB group, which suggests that other functional factor(s) could be present in the ELSF, for instance, polyphenolic compounds

and flavonoids. Generally, a high-fat diet significantly increases the total cholesterol level in serum and triglyceride level in rats. Supplementation of ELSF significantly lowered total cholesterol and triglyceride level, by compared to the OB group. This could be related to the high contents of saponins, steroids and flavonoids in ELSF. Several studies show that plant saponins and steroids are known to possess both hypolipidemic and antihyperlipidemic activities^[7]. Flavonoids and other polyphenolic compounds have also suggested for their hypocholesterolemic and hypolipidemic effects^[30]. In conclusion, our study demonstrates that ELSF inhibit fat amassment in high-fat diet-induced obese rats and related metabolic disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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