



Anti-obesity effect of *Tamarindus indicus* seed extract against a high-fat diet-induced obese model in rats

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ABSTRACT

The present study was aimed to evaluate the anti-obesity property of *Tamarindus indica* seed extract (TSE) on high fat-fed obese rats. TSE was prepared by cold maceration method and qualitative phytochemical studies had been carried out. In vitro cell viability assay (MTT assay) and oil red staining for evaluating the lipid accumulation in cells was carried out using 3T3-L1 cells, and leptin levels was evaluated by ELISA. In-vivo Obesity was induced in experimental rats by administration of a high-fat diet for 04 weeks. The anti-obesity effect was screened by oral administration of TSE at two different dose levels i.e., 250 and 500mg/kg b. Wt. Along with a high-fat diet for a period of 04 weeks. The anti-obesity activity is estimated in terms of body weight gain, serum triglycerides (TG), Total cholesterol (TC). In -vitro studies revealed that the TSE has no cytotoxic effect, Administration of a high-fat diet for 04 weeks significantly increased the body weight, serum triglycerides, cholesterol. Upon treatment with TSE, a significant dose-dependent alteration in body weight, triglycerides, cholesterol levels were observed, inferring the anti-obesity property of *Tamarindus* seed extract.



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INTRODUCTION

Obesity is characterized by excessive fat deposition in adipose tissue (Zimmet et al., 2005), which is due

to the disorder of lipid metabolism leading to the disarray of energy balance (Wang and Lim, 2012). It is a major global public health problem as it's prevalence, cutting across all age groups, sex, and race (Kreidieh et al., 2018). The rates of obesity are increasing exponentially over time and leading to other complications like diabetes, cardiovascular problems, and sleeping disorders, etc. (Westphal, 2008). At present obesity is being treated by behaviour therapy, modifying food habits, calorie-based diet restriction (Harris et al., 2009; Luttkhuis et al., 2009) and so on, but a herbal treatment to reduce the fat deposits are also warranted as it is a safe alternative to other drug moieties (Hasani-Ranjbar et al., 2013).

A large number of Indigenous drugs have been claimed to possess an anti-obesity effect (Hasani-

Ranjbar *et al.*, 2013; Roh and Jung, 2012; Kim *et al.*, 2006). *Tamarindus indica* Linn. (Family: Caesalpiniaceae) is a well-known plant of the Indian medicinal system. Seeds of the plant have antidiabetic, anti-snake venom, hepatoregenerative properties. The pulp of fruits has hypolipidemic, antioxidant, antidiarrhoeal, and analgesic, hepatoregenerative, and spasmolytic activities. Its leaves have antiemetic, antibacterial, and hepatoregenerative activities. The stem bark of the plant has analgesic and spasmogenic activities. The fruit pulp has been reported to contain tartaric acid, lactic acid, citric acid, and malic acid (Maiti *et al.*, 2004; Escalona-Arranz *et al.*, 2010; Doughari, 2007; Siddhuraju, 2007; Tsuda *et al.*, 1994; Suralkar *et al.*, 2012; Martinello *et al.*, 2006). However, there were no scientific claims relating to the anti-obesity property of this plant. Ours is the first study aimed to report the anti-obesity property of the seeds of this plant.

MATERIALS AND METHODS

Collection and authentication of plant material

Tamarindus Indica seeds were procured from Gundelpet Taluk, Mysuru, Karnataka. It was authenticated by Dr. Naganandini, Assistant Professor, Department of Pharmacognosy, JSS College of Pharmacy, Mysuru- 570015, Karnataka (Certificate No:289/09/2015/JSSCPM).

Preparation of *Tamarindus Indica* Seed Extract (TSE)

The procured seeds were washed, debris was removed, and shade dried. After 48 hrs, the seeds were grounded to a coarse powder by mechanical means. The coarse powder was weighed (1.6 kg), and in a large round bottom flask of 5L capacity, it was kept macerated with alcohol 90% (2L). The mixture was kept for 72 hours while being stirred vigorously every day at 3-time intervals. On the 3rd day, the marc is pressed for the macerate, and the fresh solvent is added. This process was continued for up to 4 cycles. The macerate obtained was pooled, and the solvent was evaporated out using a rotary vacuum evaporator, and the percentage yield was recorded and stored until further use (Gupta *et al.*, 2012).

Preliminary Phytochemical Screening of TSE

Preliminary phytochemical studies of TSE had been carried out by adapting standard procedures (Kumarawa *et al.*, 2007; Aiyegoro and Okoh, 2010) to identify the presence of alkaloids, glycosides, flavonoids, saponins, phenolic compounds, tannins, etc

In vitro anti-obesity activity in 3T3-L1 cell lines

Cell culture and induction of differentiation

Choi *et al.* (2014) 3T3-L1 preadipocytes mouse fibroblast cell line was obtained from National Centre for Cell Sciences, Pune India. 3T3-L1 preadipocytes were grown in high glucose DMEM supplemented with 10% FBS at 37°C in an atmosphere containing 5% CO₂. Adipocyte differentiation was induced in post-confluence 3T3-L1 preadipocytes (day 0) by incubating with (10 µg/mL insulin, 2.5 µM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine) along with plant extract for 48 hr (day 2). Then 10 µg/mL insulin was added up to (day 4), and media was changed every alternate day with plant extracts up to 8 days (Kanda *et al.*, 2012).

Cell Viability and Oil Red O Staining Intracellular Triglycerides

Cell viability was determined by MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide). The 3T3 L1 cells were plated at 5 X 10⁴ cells/well in 24 well plates after 24 hours. Cells were subjected to a different concentration of beverage mix and cell damage. After 24 hours incubation, MTT 0.5mg/ml was added to each well for 2-hour incubation at 37°C, 100 µl of DMSO was added to dissolve formazan crystals. The absorbance was measured at 540nm. Results were expressed as a percentage of control (Riss *et al.*, 2016).

Lipid accumulation in differentiated adipocyte was measured by oil red staining. Cells were washed with phosphate-buffered saline, and cells were fixed with 10% formalin for 1hour and stained for 30 minutes with 0.5% oil red stain in 100% isopropanol. Images were captured by an Olympus microscope (Tzeng and Liu, 2013).

Triglyceride and free glycerol release estimation

Triglycerides in the cell were estimated using a commercially available triglyceride kit (Agappe Diagnostics Ltd, Ernakulam Kerala India) according to manufacturer instructions. The cells were washed with PBS, scraped and lysed in homogenizing buffer (42mM KCl, 1mM EDTA, and 50mM Tris pH 7.4), and cell lysate was centrifuged at 3000g for 10 minutes at 4°C. The supernatant was assessed for triglyceride content. Lipolysis was measured by commercially available free glycerol reagent after 24 hr adipocyte differentiation with and without plant extracts. 50 µl of the cell incubation medium was taken and incubated with free glycerol reagent for 15 minutes. The glycerol content was measured at 540 nm (Vaidya *et al.*, 2013).

ELISA Analysis of Leptin

Leptin and Adiponectin concentration was measured using leptin EIA kit (SEA084mu), and leptin concentrations were expressed in ng/ml.

***In-vivo* anti-obesity activity**

Animals

Male Wistar albino rats weighing 150- 200 gm was procured from the in-house animal facility and housed in individual cages at a constant temperature (25°C) under a 12-h light-dark cycle. All animals were maintained by providing standard animal feed and potable water ad libitum until the commencement of the experiment.

Composition of high-fat diet (HFD)

Composition of normal pellet, as well as High Fat Diet, were represented in Table 1 All the constituents were either available in powdered form or eventually powdered, butter was melted in a low heat and double distilled water was used to mix the constituents and prepare the dough, which was then rolled into pellet size and baked in a hot air oven to form the final pellets. 12g of the above diet was fed to each rat of the respective groups during the experimentation period. (Srinivasan *et al.*, 2005)

Grouping and Treatment

Animals were divided into four groups (n=6). Animals of all groups except group1 (Normal) were fed with HFD for a period of 4 weeks, where group 1 received a normal diet. Group 2 served as control and received only HFD. Group 3 and 4 received test extract of TSE at 250 & 500 mg/kg b.wt, p.o for a period of 4 weeks.

Bodyweight

Weekly Body weight was assessed during the study period.

Serum analysis

At the end of each experiment, the blood samples of animals of all the groups were collected through the tail vein and centrifuged at 1000×g for 15 min. The collected serum was used to determine Serum Cholesterol & Triglyceride levels. All the estimations were carried out using commercial kits obtained from M/s. Spinreact, Spain.

Statistical analysis

The result was expressed as the mean ± standard deviation (SD) of triplicate experiments(in-vitro). Statistical analysis was carried out using Graphpad Prism version 8.0. Statistically significant differences were determined using analysis of variance and Dunnett's post hoc test, and of p < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of the prepared extract was 11.85%

Preliminary Phytochemical Screening of the Extract

Preliminary phytochemical analysis of the prepared extract was positive for the presence of Sterols, Triterpenes, Saponins, Alkaloids, Carbohydrates, Tannins, Flavonoids, anthraquinone glycosides, Cardiac glycosides.

***In vitro* anti-obesity activity in 3T3-L1 cell lines**

Cell Viability

MTT assay

Analysis of cell viability through MTT assay in 3T3-L1 cells at 24 hours shows dose-dependent inhibition, in the lower doses 75mg has less effect 93.69±2.65 % viability and in higher doses 4800mg it has a viability of 32.81±1.95 %. Result obtained suggested plant extracts had no cytotoxic effect on the 3T3-L1 cells. IC₅₀ value was found to be 2.422 mg /ml (Table 2).

Oil red staining

It was observed that *Tamarindus indica* linn extracts were able to significantly reduce lipid accumulation dose-dependently when compared to the negative control group in the 3T3-L1 adipocytes suggesting anti-obesity activity (Figure 1).

Triglyceride and free glycerol release estimation

Treatment with TSE reduces the levels of triglycerides in a dose-dependent manner when compared to the negative controls (Figure 2).

Lipolysis was assessed through the measurement of glycerol released in the culture medium for 24 h incubation. The concentrations of glycerol release showed a dose-dependent increase in the media. It is indicative of conversion of adipocytes to normal cells which intern helps to reduce obesity (Figure 2).

ELISA Analysis

Leptin production is closely associated with adiposity, in the current study leptin levels in the treatment groups showed a dose-dependant reduction when compared to the negative control group (Figure 3).

***In-vivo* anti-obesity activity**

Bodyweight

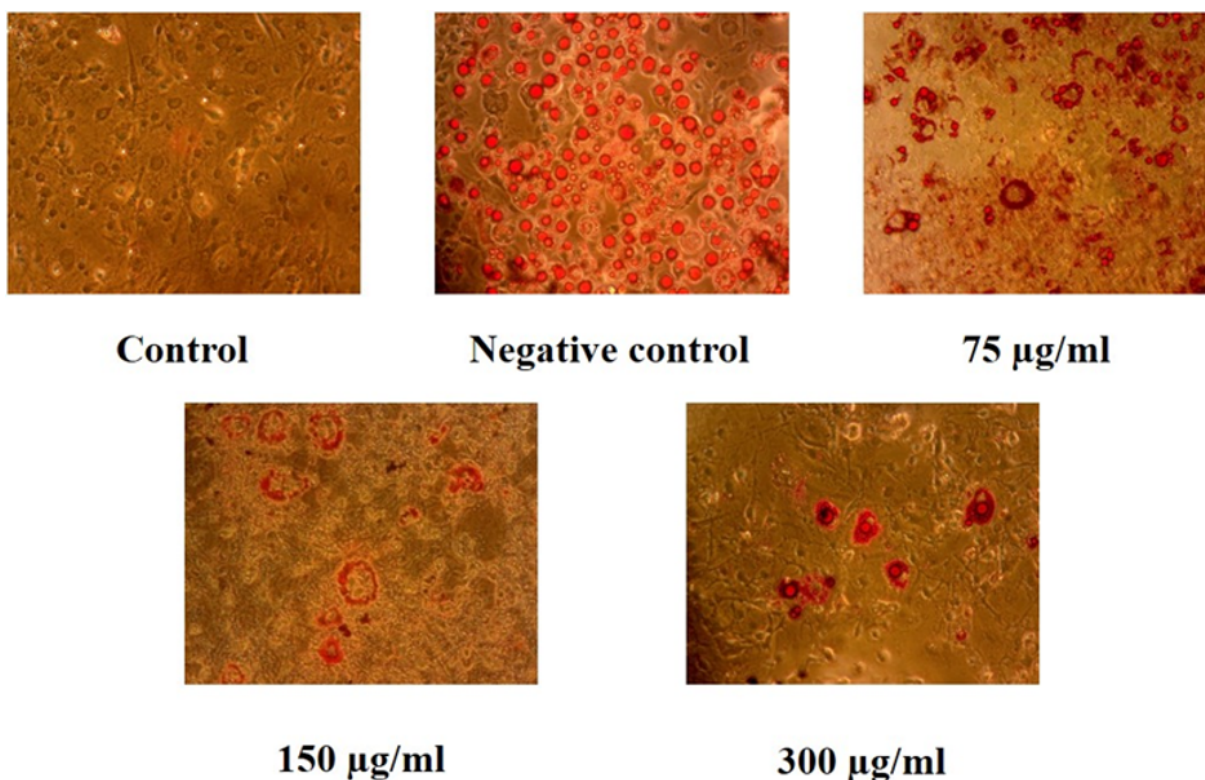
Animals fed with the high-fat diet for a period of 4 weeks exhibited increase their body weights, which is indicative of obesity. After treatment with the test

Table 1: Composition of normal and HFD

Normal Diet requirement per day per rat		High Fat Diet requirement per day per rat	
Constituents	Weight in gm	Constituents	Weight in gm
Whole Wheat	3.24	Whole Wheat	2.72
Yellow Corn	3	Yellow Corn	2.72
Barley	1.8	Barley	1.36
Milk Powder	1.8	Milk Powder	2.04
Pedigree	0.12	Pedigree	0.13
Calcium Chloride	0.12	Calcium Chloride	0.13
Salt	0.12	Salt	0.13
Oil	1.8	Oil	1.36
Vitamin B12 tablet	0.048	Vitamin B12 tablet	0.054
-	-	Butter	1.363

Table 2: Cell viability assay of *Tamarindus Indica* Linn extracts

Sample	IC ₅₀ mg/ml
75mg	93.69±2.65
150mg	82.74±2.42
300mg	77.39±2.26
600 mg	64.27±2.95
1200 mg	59.18±3.57
2400 mg	47.39±2.14
4800 mg	32.81±1.95

**Figure 1: Effects of *Tamarindus Indica* Linn extracts on fat droplet formation in 3T3-L1 cells. Stained with Oil Red O dye and examined using a light microscope**

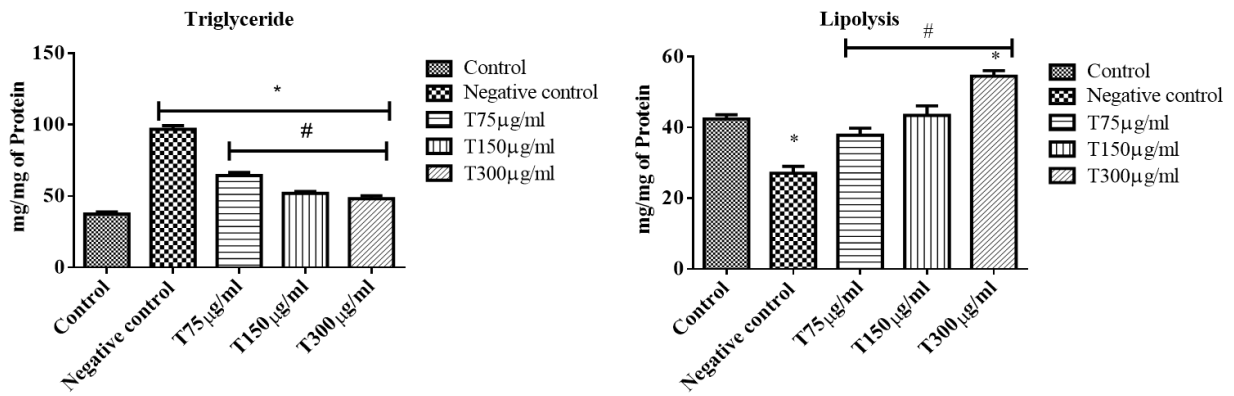


Figure 2: Effects of *Tamarindus Indica* Linn extracts on cellular triglycerides and cellular lipolysis

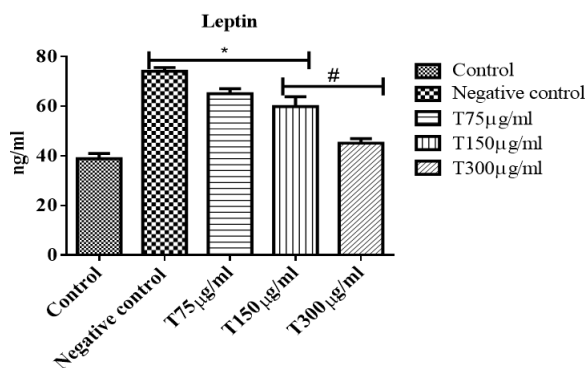


Figure 3: Effects of *Tamarindus Indica* Linn extracts on leptin levels

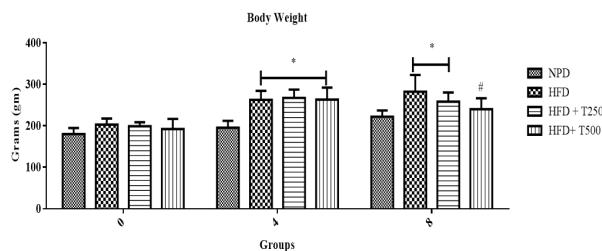


Figure 4: Effects of *Tamarindus Indica* Linn extracts on Bodyweight

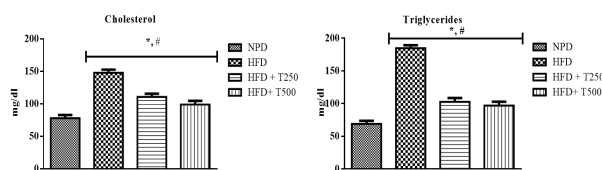


Figure 5: Effects of *Tamarindus Indica* Linn extracts on serum cholesterol and triglyceride

extract for a period of 4th weeks, a dose-dependent reduction in the body weights was observed (Figure 4).

Serum Cholesterol & Triglycerides

Serum cholesterol & triglycerides levels measured after 12 weeks of HFD and Treatment showed a dose-dependent decrease in their levels when compared to the HFD group (Figure 5).

Natural products having anti-obesity effects were reported to act through one of the following mechanisms (s) i.e., Decreased lipid absorption, energy intake, increased energy expenditure, Decreased pre-adipocyte differentiation, and proliferation and reduced lipogenesis and increased lipolysis (Westphal, 2008). The present study aimed at exploring the potential role of *Tamarindus Indica* seed extract on obesity induced by HFD. Data from the cell viability assay suggest that the TSE does not have any cytotoxic activity, and Oil red staining infers a significant reduction of lipid accumulation in a dose-dependent manner (Luttikhuis et al., 2009). In earlier studies, it was reported that HFD induced obesity in rats was a suitable model as they bear a close resemblance to human obesity. In view of our data, animals fed with HFD showed a significant increase in the obese group due to fat accumulation in the thoracic and abdominal regions due to the high cholesterol diet. In the present study, animals, which received TSE exhibited reduced body weight in a dose-dependent manner. Further, TSE has a beneficial effect on lipid profile through cholesterol and triglyceride reducing the effect, which is evident from the current data where a dose-dependent reduction in cholesterol and triglyceride levels were observed, which is indicative of the hypolipidemic activity.

Hypolipidemic mechanism of this extract is may be due to the presence of polyphenols in *Tamarindus* that are dominated by proanthocyanidins groups

such as procyanidin B2, apigenin, catechin, epicatechin, procyanidin dimers, procyanidin trimers, eriodictyol, taxifolin, and naringenin. Polyphenols are a group of antioxidants that are most abundant in plant metabolites and are an integral part of both human and animal diets, including the simple phenolic molecule (Kim *et al.*, 2006; Maiti *et al.*, 2004).

CONCLUSIONS

Obesity is a risk factor for metabolic syndromes. Our findings suggest that TSE treatment could repress adipogenesis by regulating lipid accumulation in HFD-induced obesity in rats. Thus, TSE has an anti-obesity property; thereby, it can be used as a potential therapeutic agent for obesity.

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