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Research Article

ANTI-OBESITY AND ANTI-HYPERLIPIDEMIC ACTIVITY OF TERMINALIA CATAPPA *LINN. IN HIGH-FAT-DIET INDUCED OBESE RATS*

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ABSTRACT

Obesity is recognized as a social problem, associated with serious health risks and increased mortality. The incidence of obesity has increased at an alarming rate in recent years, becoming a worldwide health problem with incalculable social costs. Numerous trials have been conducted to find and develop new anti-obesity drugs through herbal sources to minimize side effects associated with the present anti-obesity drugs in the market.

The present study was designed to evaluate the anti-obesity and anti-hyperlipidemic activity of leaves of Terminalia catappa Linn. Preliminary phytochemical screening has also been performed on the powdered leaf. Phytochemical analysis indicated presence of alkaloids, tannins, saponins and flavonoids in methanolic leaf extract of Terminalia catappa Linn. The oral administration of methanolic leaf extract of Terminalia catappa Linn. at high dose of 400 mg/kg body weight resulted in decrease total cholesterol, triglycerides, LDL in high fat diet treated obese animals. The results showed that treatment with Atorvastatin a standard anti obesity drug reduced the triglycerides of HFD fed obese rats by 19% while 200 mg/kg and 400 mg/kg body weight of methanol leaf extract of Terminalia catappa Linn. caused a reduction of 9% and 17% respectively in the high fat diet treated animals. Atorvastatin reduced body weight of high fat diet fed obese rats by 16% while 200 mg/kg and 400 mg/kg body weight of methanol leaf extract of Terminalia catappa caused a reduction of 8% and 11% respectively in body weight during the treatment period.

Key words: *Terminalia catappa Linn., high-fat-diet, anti-obesity, anti-hyperlipidemia*

INTRODUCTION

Obesity is a complex interplay between environmental and genetic factors and is associated with significant morbidity and mortality [1]. Obesity is difficult to define in quantitative term. Obesity refers to the above average amount of fat contained in the body, this in turn is dependent on the lipid content of each fat cell and on the total number of fat cells [2]. By 2005, obesity had affected 400 million adults, and since 1997, WHO has cited obesity as a global epidemic. More than half of the adult population in OECD countries is overweight (body mass index [BMI] $\geq$25 kg/m^2).

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The obesity incidence has increased at an alarming rate in recent years, becoming a worldwide health problem, with incalculable social costs. According to WHO, obesity is related to cardiovascular diseases, hypertension, diabetes mellitus, cancer, osteoarthritis, pulmonary diseases, as well as psychological issues, including social bias, prejudice, discrimination, and overeating [3,4]. Economically, obesity and its health consequences contribute to enormous costs now and for future health care. Therefore, prevention and treatment of obesity are important for a healthy life. However, owing to the adverse side effects associated with many synthetic anti-obesity drugs, more recent trials have focused on screening natural sources that have been reported to reduce body weight with minimal side effects. This may be an excellent alternative strategy for developing

effective and safe anti-obesity drugs in the future [5,6]. Single and mixed anti-obesity medicinal plant preparations may have different effects. The botanical sources, route of administration, presence of various bioactive components and their respective functions, experimental methods used, treatment dosage, study design, treatment duration, and safety and efficacy of the plant are also factors. A variety of natural products, including crude extracts and isolated compounds from plants, have been widely used traditionally to treat obesity. Literature review reveals that flavonoids, sterols, tannins, and alkaloids have shown positive effects to tackle obesity by various mechanisms [7-9].

Terminalia catappa Linn. (Combretaceae) is found in the warmer parts of India. Due to their large size, old leaves may be considered a nuisance to some people. *Terminalia catappa,* is an important medicinal plant with diverse pharmacological spectrum [10]. The leaves, trunk, bark and fruits have been used in folk medicine for the treatment of dermatitis, and for antipyretic and homeostatic purposes. There are number of phytochemicals presents in this plant such as gallic acid, ellagic acid, corilagin and some unidentified flavonoids. These compounds found to be responsible for many of the pharmacological activities. Leaves of this plant have shown the presence of phenols, flavonoids, alkaloids and saponins and others in extracts. Due to the presence of these types of phytoconstituents, the different extracts may show antimicrobial, antioxidant, antibacterial, antidiabetic, anthelmintic, antitumor, hematological activities [11,12].

The methanolic and aqueous extracts of fruit/leaves exhibited significant anti-hyperglycemic activities in alloxan induced diabetic rats and improvement in parameters like body weight and lipid profile. Till date there is no anti-obesity study has been reported on *Terminalia catappa*. With the broad aim, our present study was to investigate the anti-obesity activity of leaf extracts of *Terminalia catappa* in high fat diet induced obese rats.

MATERIALS AND METHODS

Drugs and Chemicals

All the drugs used in this study were of pharmaceutical grade. Atorvastatin (Atvas, Zydus cadila Healthcare), diagnostic kits like Triglycerides (Biolab diagnostic pvt ltd), Cholesterol (Span diagnostic pvt ltd). All Solvents and chemicals were of analytical grade like pertroleum ether, methanol, ethanol, chloroform (Ranbaxy Fine chemicals) and formalin (Merk, Mumbai).

Plant Material

Terminalia catappa Linn. leaves were collected in the month of June from Mulund, Maharastra, India. The specimen plant was (voucher no.16063) was identified with the help of literature and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. The fresh plant material were cleaned with distilled water to dry at 35 to 40ºC for 15 days, pulverized in electric grinder and the powder was passed through sieve No. 60 and used for further extraction.

Preparation of plant extract

The Soxhlet extraction of dried and powdered plant material (500g) was performed successively with methanol for period of 1 week. The trace of methanolic solvent was removed by reduced pressure distillation and then vacuum dried. The dark semi solid mass was obtained [13]. It was stored at 4ºC until further used. When needed, the extract were suspended in the desired solvent and used.

Preliminary Phytochemical analysis

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate and Harborne [14,15].

Animal

Female Wistar strain albino rats (body weight: 250-300 g) were procured form Bombay Veterinary College, Parel (Mumbai), India. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) and animal care was taken as per guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA). Experimental animals were housed in groups, six per cage, (polycarbonate cage) under laboratory conditions with alternating light and dark cycle of 12 h each. Animals were fed on

standard commercial pelleted diet and water at. libitum. Animals were acclimatized one week before the experiment was carried out. Toxicity study of *Terminalia catappa*. leaf powder extract was carried out using Female Albino Swiss mice (25-30 g) as experimental model.

Toxicity Study

The acute oral toxicity test of methanolic leaf powder extract was determined prior to the efficacy study as per the OECD (Organization for Economic Co-operation and Development) 420 Guidelines. Female Albino Swiss mice were administered the *Terminalia catappa* Leaf powder (TCLP) as a single dose of 2000 mg/kg body weight. The treated animals were observed for 14 days for mortality, clinical signs and symptoms. There was no mortality even at higher dose of 2000 mg/kg body weight [16,17].

Anti-obesity study

Induction of Obesity:

Obesity was induced in overnight fasted Female Wistar rats of weighing around 250-300g by simplified high fat diet (HFD) which induces marked obesity in rats. An increase in food consumption and water intake accompanies the body weight gain which is the characteristic features of HFD. The study was carried out for a period of one month. The animals were obese on 7th day of study and were screened for anti-obesity study. All doses were started seven days after the induction of obesity. Required quantity of pellets were crushed and 2% cholesterol, and 1% of coconut oil was added. The mixture was made into round balls with required quantity of saline water. Parachute oil was chosen because of its high saturated fat content which aggravates the antherogenic profile in experimental rats.

Experimental design

Animals were divided into five groups with of six animals in each group. The animals were identified by picric acid marking. The experimental design was as follows.

Group 1: Normal control treated with saline only (Saline water)

Group 2: Positive control treated with High Fat Diet (HFD)

Group 3: Treated with Standard drug (Atorvastatin) (10 mg/kg body weight) + HFD

Group 4: Treated with methanolic extract of *Terminalia catappa* (200 mg/kg body weight) + HFD

Group 5: Treated with methanolic extract of *Terminalia catappa* (400 mg/kg body weight) + HFD

At the end of the study all the rats were decapitated for collection of blood and liver samples to carry out biochemical assays. Body weights of the rats were recorded on 7th, 14th, and 28th day of study by standard weighing machine. Blood samples were drawn from retro-orbital sinus into eppendorff tubes at weekly intervals till end of study (30 days) and used for estimation of various biochemical parameters such as serum total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol. Histopathology of liver and kidney were performed using the standard procedure

Statistical Analysis

The values were expressed as Mean ± SD. The statistical analysis was carried out by One way Analysis of Variance using SPSS v.19 (IBM, India). Differences between groups were considered statistically significant at $p<0.05$.

RESULTS

Preliminary phytochemical tests showed the presence of alkaloids, tannins, flavanoids, saponins, and antharoquinones. The results as shown in Table I. The composition of normal and HFD used during the study given as shown in Table II.

Table I: ***Preliminary phytochemical screening of leaf powder of Terminalia catappa*** **Linn.**

Test	*n-hexane*	*chloroform*	*methanol*	*aqueous*
Alkaloids				
Dragendroff's reagent	-	++	++	++
Mayer's reagent	-	-	++	+
Wagner's reagent	-	+	++	+
Tannins				
Ferric Chloride	+	++	+++	+++
Dilute Nitric acid	+	+	+++	++
Flavanoids	-	-	++	++
Steroids	-	-	-	-
Anthraquinones	-	-	+	+
saponins	+	+	++	+++

(+) present, (-)absent

Table II: The Composition of Normal and High Fat Diet (for100g)

Components	*High fat Diet (%)*	*Normal(%)*
Moisture	**7.4**	**7.35**
Protein	**25.9**	**25.87**
Fibre	**2.5**	**2.50**
Carbohydrate	**54.0**	**54.00**
Fat	**3.7**	**- - -**
Coconut oil	**1.0**	**- - -**
Cholestrol	**2.0**	**- - -**

Administration of methanolic leaf extract of *Terminalia catappa* at higher dose of 400 mg/kg body weight (Group V) and standard drug Atorvastatin (Group III) showed statistically significant decreased in total body weight of animals compared to positive control HFD (Group II) (Table III). The results reveals that feeding of high fat diet increased serum cholesterol, triglycerides, total protein and decreased high density lipoprotein levels compared to normal group animals (Group I) at over a period of 30 days as shown in Table IV.

Table III: ***Effects of the Terminalia catappa Linn. leaf powder extract and Atorvastatin on body weight in HDF induced obese rats***

	Group I	*Group II*	*Group III*	*Group IV*	*Group V*
Initial body weight (g)	237.50 ± 6.1	245.83 ± 13.5^{NS}	239.167 ± 9.1	246.66 ± 8.1	241.66 ± 9.8
Final body weight (g)	263.33 ± 7.5	356.83 ± 9.7$^{\#}$	272.67 ± 8.1**	318.50 ± 7.5*	298.33 ± 18.3*
Body weight gain (g)	25.8 ± 3.7	110.0 ± 10.0$^{\#}$	32.5 ± 2.7**	70.8 ± 13.5*	56.7 ± 15.0*

Data are mean ± SD values (n=6), NS (Not Significant). $^{\#}P$<0.001 versus the ND group; $^{*}P$<0.001 versus HD groups.

Table IV: ***The concentration of Lipid profile in serum of control and experimental groups (µg/dl)***

	TG	*TC*	*HDL*	*LDL*	*VLDL*
Group I	79.22 ± 17.3	91.61 ± 9.3	36.5 ± 6.6	39.26 ± 9.7	15.84 ± 3.4
Group II	122.55 ± 36.4$^{\#}$	116.44 ± 14.4$^{\#}$	34.72 ± 6.2$^{\#\#}$	57.21 ± 7.1$^{\#}$	24.51 ± 7.2$^{\#}$
Group III	99.11 ± 23.7***	102.11±11.5**	43.16 ± 11.1**	39.12 ± 8.0*	19.82 ± 4.7***
Group IV	111.05 ± 31.3^{NS}	109.66 ± 20.4^{NS}	38.33 ± 9.0^{NS}	49.12 ± 9.4**	22.21 ± 6.2^{NS}
Group V	101.16 ± 22.3***	104.44 ± 14.6***	41.11 ± 10.8***	43.1 ± 10.6*	20.23 ± 4.4***

Data are mean ± SD values (n=6), NS (Not Significant). $^{\#}P$<0.001, $^{\#\#}P$<0.05 versus the ND group; * P<0.001, $^{**}P$<0.01, $^{***}P$<0.05 versus HD groups.

Methanolic leaf extract of *Terminalia catappa* at higher dose of 400 mg/kg body weight (Group V) and standard drug Atorvastatin (Group III) showed statistically significant decreased in total cholesterol and triglycerides as compared to positive control HFD (Group

II). At this time increase in high density lipoprotein was also observed. The methanolic leaf extract of *Terminalia catappa* at high dose of 400 mg/kg body weight (Group V) showed better activity compared to extract of low dose of 200 mg/kg body weight (Group IV) as shown in Table 5. This is supported by histopathology of livers as shown in Figure 1.

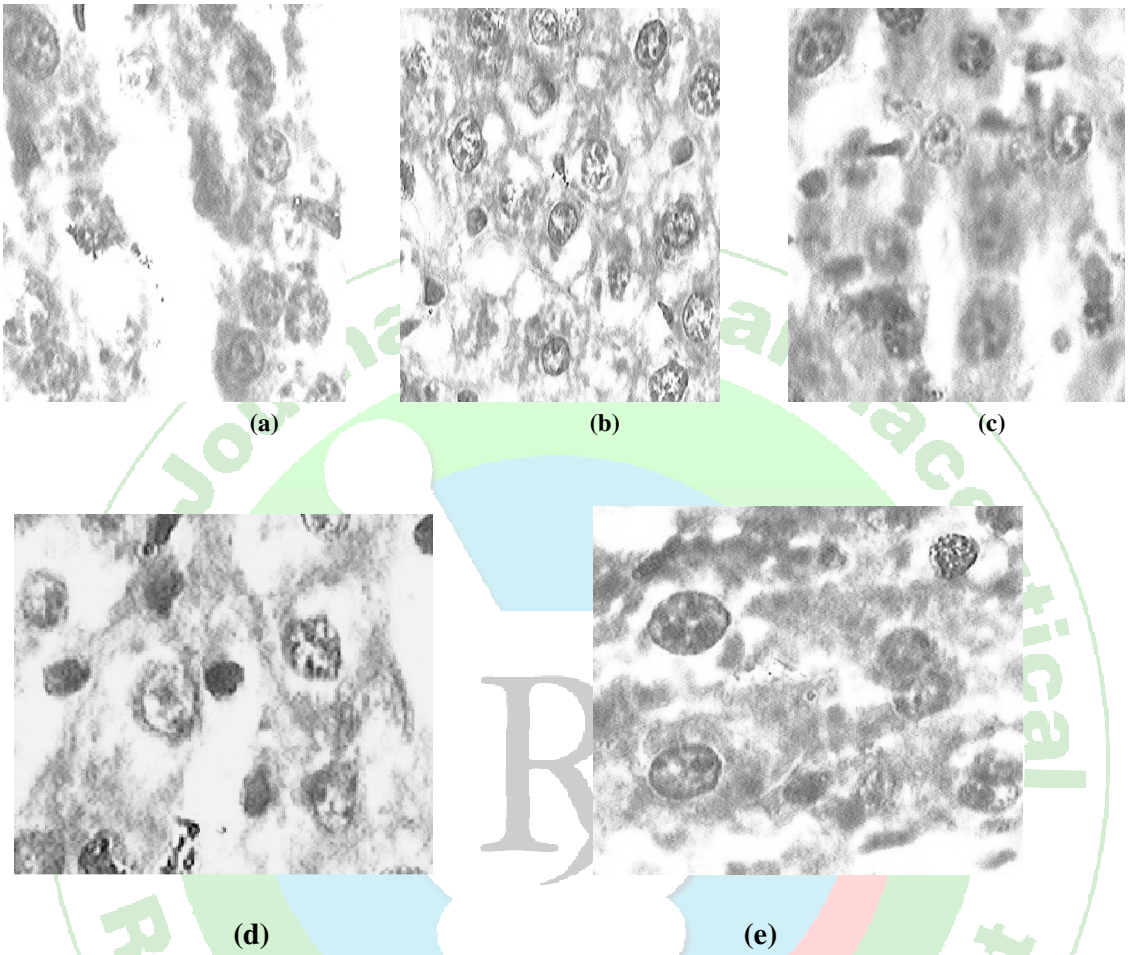

Figure 1. Histopathology of rat liver (100X); **(a)** Group I (Normal control); **(b)** Group II (Positive control HFD); **(c)** Group III [Standard drug- Atorvastatin + HFD]; **(d)** Group IV (*Terminalia catappa* Linn 200/kg body weight + HFD); **(e)** Group V (*Terminalia catappa* Linn. extract 400mg/kg body weight + HFD)

DISCUSSION

Many medicinal plants provide relief of symptoms comparable to that of conventional medicinal agents. There has been a proliferation of high-cost, anti-obesity products in the market. However, they exhibit side effects, such as gastrointestinal and kidney problems. In traditional medicine the healers use this plant against dermatitis, antipyretic and homeostatic purposes. *Terminalia catappa* leaf possess anti-obesity activity, which has not been reported previously.

The preliminary phytochemical analysis showed presence of alkaloids, tannins, flavanoids, anthraquinones and saponins, whereas steroids gave negative test for methanolic and aqueous leaf extract of *Terminalia catappa.* The methanolic leaf extract of *Terminalia catappa* (200 mg/kg and 400 mg/kg body weight) and standard drug Atorvastatin (Group III) supplementation significantly reduced body weight gain by 8% and 11% and 16 %, respectively when compared to positive control HFD (Group II) treated animals. The results of experimental

data is further supported by histopathological analysis. Histology of the liver sections of control animals showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus. The liver section of positive control HFD treated animals showed increase fat cells and most of the hepatocytes showing steatosis. Liver histology of animals treated with plant extracts (400 mg/kg body weight) and standard drug showed reduction in fat cells compared to positive control HFD (Group II). Plant extract at lower dose (200 mg/kg body weight) also showed reduction in fat cells but activity was less compared to higher dose.

Terminalia catappa leaf extract at higher dose (400mg/kg body weight) showed significant decreased in lipid profile compared to extract at lower dose (200mg/kg body weight). Thus, standard drug and *Terminalia catappa* leaf extract (400 mg/kg body weight) treated animals showed significantly decreased body weight and serum lipid level and reduction in fatty cells in all experimental models compared to positive control HFD (Group II). The results showed that treatment with standard drug Atorvastatin reduced the triglycerides of obese rats by 19% while 200 mg/kg and 400 mg/kg body weight of methanol leaf extract of *Terminalia catappa* caused a reduction of 9% and 17% respectively in treated animals.

CONCLUSION

The dietary intake of the single medicinal plants may provide a higher degree of safety and efficacy than mixed medicinal plant preparations. Improving knowledge on the use of anti-obesity medicinal preparations, and encouraging obese patients to consume them along with an enhanced exercise regimen and a healthy diet should be continued. Additional chemical, biological, and clinical studies are needed on the effectiveness of medicinal plants in ameliorating and treating obesity in humans. Such anti-obesity data would be useful for food and drug manufacturers as new products are developed, and to governments in the regulation of food products as a way to promote and enhance public health.

The results obtained from the pharmacological screening have led to the conclusions that, methanolic leaf extract of *Terminalia catappa* has significant anti-obesity and lipid lowering activities in HFD induced obese rats. Even though Atorvastatin, a synthetic anti obesity drug gave better results with significant decrease in body weight and triglycerides it may have side effects if used for long term Owing to the adverse side effects associated with synthetic anti-obesity drugs, this plant may be exploited as an weight reducing and anti-hyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. The beneficial effect of *Terminalia catappa* in serum lipid level is time and dose dependent. Polyphenolic and alkaloid compounds abundant in methanolic leaf extract of *Terminalia catappa* may be responsible for the property of anti-obesity which further needs to be evaluated.

Thus can be concluded from the present study that, *Terminalia catappa leaves* may prevent obesity by reducing excess accumulation of body fat and changing in serum lipid profile. Thus, it can be used for preparation of anti-obesity medication instead of synthetic drugs to avoid the undesirable adverse reactions.

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