Effect of *Termenalia catappa* Leaf Extract on Cafeteria Induced Obesity in Rats

Sridatha. P*, Subbareddy.P, Sowjanya.K, Sudheer. A
Department of Pharmacology
Raghavendra Institute of Pharmaceutical Education & Research (RIPER),
Anantapuramu, Andhra Pradesh, India.
puranamdatha@gmail.com

ABSTRACT

Obesity is a leading cause of death worldwide, with increasing rates in adults and children. To investigate anti-obesity effect of *Termenalia catappa* leaf extract on cafeteria induced obesity in rats. For anti-obesity experiment, animals were divided into five groups of six animals and housed in cages. Normal control group continued to be fed a laboratory pellet chow ad libitum. Cafeteria diet-control group received cafeteria diet in addition to the normal pellet diet. The remaining three groups were fed with cafeteria diet and NPD along with *T. catappa* leaf extract (200 mg/kg, p.o.), *Termenalia catappa* leaf extract (400 mg/kg, p.o.) and orlistat (30 mg/kg, p.o.). Treatment was continued for 4 weeks. Animals and various adipose pads were weighed and serum total cholesterol, triglyceride, LDL, VLDL and HDL-C were measured after 4 weeks of treatment. At the end various adipose tissues, aorta were removed and processed for Histopathological study. The *Termenalia catappa* leaf extract -treated groups showed a significant decrease in body weight, and various adipose pad weight and serum TC, TG, LDL, VLDL and increase in HDL levels after 4 weeks treatment and decrease in the adipose tissue size and adipocyte number. At present study, *Termenalia catappa* leaf extract can inhibit the development of obesity and hyperlipidemia on cafeteria induced obesity in rat. It is because of various phytoconstituents of leaf extract. But further studies are still waiting for establishing mechanism and isolation of phytoconstituents. By observing above results *Termenalia catappa* leaf extract can act as adjuvant in obesity treatment.

Key Words: Antiobesity**,** Termenalia catappa, Cafeteria diet, hyperlipidemia

INTRODUCTION

Obesity, a nutritional disorder, is defined as nonstandard or unwarranted fat accumulation and growth of adipose tissue leading to obesity (A.E. Decle, 2011). Because of its rising prevalence and its association with chronic health disorders such as insulin resistance, dyslipidemia, hypertension, cardio vascular diseases, nonalcoholic fatty liver and osteoarthritis, obesity has become a major health concern in developed and developing countries (D.B.F. Carla, 2011). Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems. People are considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, exceeds 30 kg/m^2 (D.T. Villareal, 2011). Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis Obesity is most commonly caused by a combination of excessive food energy intake, lack of physical activity, and genetic susceptibility. Dieting and physical exercise are the mainstays of treatment for obesity. Diet quality can be improved by reducing the consumption of energy-dense foods such as those high in fat and sugars, and by increasing the intake of dietary fiber. Anti-obesity drugs may be taken to reduce appetite or decrease fat absorption when used together with a suitable diet. If diet, exercise and medication are not effective, a gastric surgery may be performed to reduce stomach volume and/or bowel length, leading to feeling full earlier and a reduced ability to absorb nutrients from food (Kuller LH, 1997). Obesity is a leading cause of death worldwide, with increasing rates in adults and children. Authorities view it as one of the most

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serious public health problems of the 21st century. Obesity is stigmatized in much of the modern world (particularly in the Western world), though it was widely seen as a symbol of wealth and fertility at other times in history, and still is in some parts of the world In 2013, the American Medical Association classified obesity as a disease (Birari, R.B., Bhutani, K.K., 2007).

The present study is aimed at investigating the Phytochemical, anti-obesity studies on Aqueous extract of *Termenalia catappa* (AETC) leaves with a view to justify the folklore use of this plant. This kind of detailed scientific study has not been documented so far, the *Termenalia catappa* leaves are used traditionally in treatment of wide range of disorders including the anti-obesity. *Termenalia catappa* is a large tropical tree in the leadwood tree family, Combretaceae, that grows mainly in the tropical regions of Asia, Africa, and Australia. It is known by the common names Bengal almond, country almond,]Indian almond, Malabar almond, sea almond tropical almond. The leaves contain several flavonoids (such as kaempferol or quercetin), several tannins (such as punicalin, punicalagin or tercatin), saponines and phytosterols. Due to this chemical richness, the leaves (and the bark) are used in different herbal medicines for various purposes. For instance in Taiwan, fallen leaves are used as an herb to treat liver diseases. In Suriname, an herbal tea made from the leaves is prescribed against dysentery and diarrhea. The leaves may contain agents for prevention of cancers (although they have no demonstrated anti-carcinogenic properties) and antioxidants, as well as anti-clastogenic characteristics. Extracts of *T. catappa* have shown activity against *Plasmodium falciparum* chloroquine (CQ)-resistant (FcB1) and CQ-sensitive (HB3) strains (Pankaj Oudhia et.al, 2008)

Objective: To investigate the anti-obesity effect of *Termenalia catappa* leaf extract on cafeteria induced obesity in rats

MATERIALS AND METHODOLOGY

Materials: Orlistat was a generous gift from Intas Pharmaceuticals Ltd, Ahmedabad. All other chemicals used were of analytical grade.

Authentication and Extraction of Termenalia catappa

The leaves of *Termenalia catappa* were collected from in and around Anantapuramu and authenticated by Dr.J.Ravendra Reddy Department of Phamacognosy RIPER, anantapuramu. The specimen voucher (RIPER/COG/04) was retained in the Department of Pharmacology, RIPER Anantapuramu. Freshly collected leaves of *Termenalia catappa* dried for 14 days under shade condition.

Extraction of Termenalia catappa leaves

For the present study, 500 gm of the powdered leaves of *Termenalia catappa* were extracted by cold maceration method with water as solvent. The maceration was continued for 72 hours after which, the contents were filtered and boiled by using water bath. A resinous greenish extract was obtained which was calculated for the yield and stored in desiccators till further study (Khandelwal KR, 2006).

Preliminary Phytochemical study

The freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents. The extract was screened for various chemical constituents employing standard screening tests such as alkaloids with Mayer and Dragendorff's reagent, saponins (frothing test), tannins ($FeCl_3$), glycosides (NaCl and Fehling's solution A and B), flavonoids (NaCl and HCl), phenols ($FeCl_3$ and $K_3Fe(CN)_6$) . Phytochemical screening of the AETC showed the presence of alkaloids, flavonoids, tannins, saponins (C.K.Kokate, 1994).

Experimental Animals

Female albino rats (150-200 g body weight) were used for this study. They were housed at ambient temperature (22 ±10C), relative humidity (55±5%) and 12h/12h light dark cycle. Animals had free access to Amrut brand rat pellet diet supplied by The Institutional Animal Ethical Committee (878/ac/05/CPCSEA/008/2013) has approved the experimental protocol at Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapuramu, Andhra Pradesh, India.

Induction of obesity

Obesity was induced by oral feeding of freshly prepared cafeteria fat diet for a period of 28 days. Cafeteria fat diet was prepared with a composition of condensed milk-48gm, bread-48gm, chocolate-18gm, biscuits-36gm, dried coconut-36gm, cheese-48gm, boiled potatoes-60gm prepared as per nutrition guidelines. The prepared diet was not stored and whenever required we prepared freshly (Harris RB, 1993).

Experimental Protocol

After acclimatization for 10 days, the animals were randomly divided into five groups of six animals each and treated as follows:

- Group I (Normal healthy control): fed with standard Pellet diet *ad libitum* daily for 28 days.
- Group II (Cafeteria diet control): fed with Cafeteria diet for 28 days.
- Group III (Standard): fed with Cafeteria diet + Orlistat 30 mg /kg orally suspended in 0.5% cmc (Aniket Karmase et.al, 2013)
- Group IV (Test-1): fed with Cafeteria diet + AETCleaves 200mg/kg orally for 28 days (Saurabh Arjariya, 2013)
- Group V (Test-2): fed with Cafeteria diet+ AETC leaves 400mg /kg orally for 28 days

Measurement of Body Weight Gain

The body weight was determined every weekly before the treatment with standard and test drugs.

Measurement of Biochemical Parameters in Serum

The animals were kept overnight fasting before biochemical estimation. Twenty-four hours after the last dose treatment (day 29), Blood samples were collected under slight ether anesthesia by retro orbital puncture in appendroff tubes and allow to clot. The clotted blood samples are centrifuged at 3000Xg for 15 min. Separated serum carefully withdrawn with help of micro pipettes into another appendroff tubes and kept at -70oC up to further usage for biochemical analyses. The serum is utilized for estimation of CH, TG, LDL, VLDL, HDL, SGOT and SGPT determined according to Standard methods using diagnostic kits from Erba diagnostics, by using Autoanylser ERBA CHEM7 (Shiv Kumar, 2011).

Estimation of Liver, kidney, heart and adipose tissue weight

After completion of biochemical estimation, the animals were sacrificed with an overdose of diethyl ether. The abdomen was open and dissects out the liver, kidney, heart and washed with ice cold saline and blotted with tissue paper and immediately weighed with help of electronic weighing balance and parametrial adipose tissues were quickly removed and weighed.

Histopathological study

The isolated organs (adipose tissue and aorta) fixed in 10% buffered formalin, embedded in paraffin, cut to 5µm section for slides, and stained with hematoxylin and eosin. The slides were examined with a Magnus Microscope. The sections were observed under 10X and 40 X magnifications. The person who performs the histopathology does not know the treatment.

Statistical analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Bonfferoni test using computer based fitting program (Prism graph pad version 5.0). Statistical significance was set accordingly.

RESULTS

Table1: ***Phytochemical evaluation of AETC leaves***

Identification test for	Observation
Alkaloids	Positive
Flavonoid	Positive
Glycosides	Positive
Saponins	Positive
Steroids	Negative
Tannins	Positive

Table2: Effect of AETC leaves on body weight (gm) in cafeteria diet animals (n=6)

Weak	Group-I	Group-II	Group-III	Group-IV	Group-V
0	180.5±1.780	184.6±6.118 ns	183.7±4.031ns	179.7±0.9632ns	181.7±4.667ns
1	188.5±0.4625	201.5±1.454***	193.6±0.5995***	198.8±0.6636ns	196.9±0.6914*
2	191.7±1.308	212.7±3.820***	194.6±0.4083**	208.1±2.295ns	200.2±1.110*
3	193.4±0.7058	227.1±3.099***	196.8±0.9353***	220.3±2.679ns	209.2±2.556**

Values are mean ± SEM *P < 0.05 considered statistically significant compared to Cafeteria diet control.
Group I — Normal healthy control;
Group II — Cafeteria diet control;
Group III — Cafeteria diet control + Orlistat (30 mg /kg);
Group IV — Cafeteria diet control + AETC (200 mg/kg/day);
Group V — Cafeteria diet control + AETC (400 mg/kg/day).

Fall off time & locomotor activity evaluation

Rota rod apparatus

Fall of time was significantly decreased in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant increase in fall of time as compared to cafeteria diet control rats (p<0.01) [Table 3].

Photoactometer

Loco motor activity was significantly decreased in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant increase in loco motor activity as compared to cafeteria diet control rats (P<0.01) [Table 3

Table3: Effect of AETC leaves on locomotor activity & fall off time in cafeteria diet animals (n=6)

Parameter	*Group-I*	*Group-II*	*Group-III*	*Group-I V*	*Group-V*
Fall off time (min)	8.980±0.2503	2.983±0.2804***	6.950±0.3912***	4.127±0.3014 ns	5.573±0.3037**
Locomotor activity (counts)	636.3±6.692	452.0±6.083***	605.7±6.766***	475.0±7.211ns	494.7±4.096**

Values are mean ± SEM
*P < 0.05 considered statistically significant compared to Cafeteria diet control.
Group I — Normal healthy control;
Group II — Cafeteria diet control;
Group III — Cafeteria diet control + Orlistat (30 mg /kg);
Group IV — Cafeteria diet control + AETC (200 mg/kg/day);
Group V — Cafeteria diet control + AETC (400 mg/kg/day).

Effect of AETC leaves on Serum biochemical parameters:

Total serum cholesterol was increased significantly in cafeteria diet control group rats. Rats treatment with AETC leaves for a period of 4 weeks showed significant (p<0.01) decrease in serum cholesterol level compared to cafeteria diet control group rats. Serum triglyceride levels were significantly increased in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant (P<0.05) decrease in TG as compared to cafeteria diet control rats. Serum LDL-C levels were increased significantly in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant (P<0.01) decrease in LDL-C levels compared to cafeteria diet control rats. Serum HDL-C levels showed significant decrease in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant (P<0.01) increase in HDL-C levels compared to cafeteria diet control rats. Serum VLDL-C levels showed significant increase in

cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant (P<0.01) decrease in VLDL-C level compared to cafeteria diet control rats. Serum SGOT levels showed significant increase in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant (P<0.01) decrease in SGOT level compared to cafeteria diet control rats. Serum SGPT levels showed significant increase in cafeteria diet control group rats compared to normal. Treatment with AETC leaves showed significant (P<0.05) decrease in SGPT level compared to cafeteria diet control rats *[Table4].*

RESULTS AND DISCUSSION

Various rats of obesity have been used to emulate obesity-like condition in humans, in order to develop effective anti-obesity treatments. Among the animal models of obesity, rats that are fed a cafeteria diet are considered useful; a high percentage of fat in their diet is considered to be an important factor in the development of obesity, leading to the accumulation of body fat (Kusunoki M, 2000).The present study showed that the administration of a cafeteria diet for four weeks, in rats, produced obesity-like conditions, with increase in body weight, parametrial adipose tissue weight, and serum lipid levels. Treatment with AETC leave at a dose of 200mg/kg/day & 400 mg/kg/day, significantly reduced the increase in body weight induced by a cafeteria diet — a clear sign of an anti-obesity effect. Obese rats because of overweight loss the ability to stand on the rotated rod in the Rota rod apparatus and exhibit the low locomotor activity in Actophotometer. Weight reduction by the AETC leave treatment normalizes the locomotor activity as well as improves the capacity to stand on rotated rod. Significant increase in serum lipids, such as total cholesterol (TC), LDL-C, and triglycerides (TG) is observed in obese animals. Treatment with AETC leave caused significant changes in the blood parameters, including decreased levels of TC, LDL-C, and TG, but increased HDL-C. Various preclinical studies stated that herbal extract containing Flavonoid; tannins have good anti-obesity effect (Gamal A. Mohamed, Sabrin R.M, 2014). Lipid lowering effect of AETC leave may be due to presence of above said active principles, which was confirmed by photochemical investigation. The extract produced a significant decrease in the liver and parametrial adipose tissue weight in comparison with the CD control group. Our extract prevents the accumulation of fat in liver and adipose tissue that's why it reduced the weight of adipose as well as liver weight.

Table 4:Effect of AETC leaves on body weight (gm) in cafeteria diet animals (n=6)

Weak	Group-I	Group-II	Group-III	Group-IV	Group-V
0	180.5±1.780	184.6±6.118 ns	183.7±4.031ns	179.7±0.9632ns	181.7±4.667ns
1	188.5±0.4625	201.5±1.454***	193.6±0.5995***	198.8±0.6636ns	196.9±0.6914*
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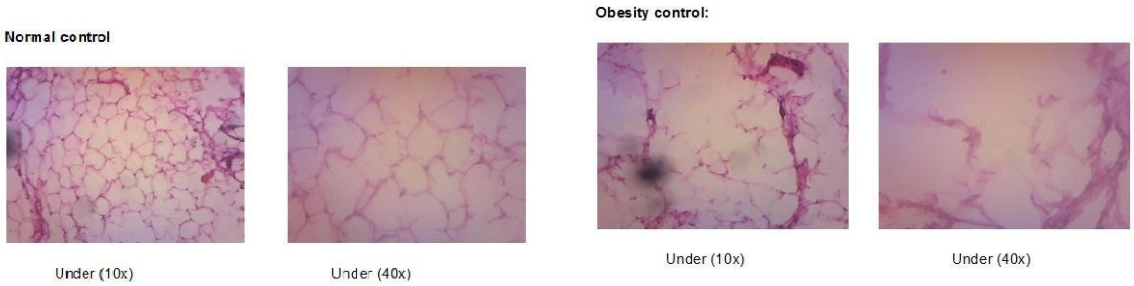

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of 200mg/kg/day & 400 mg/kg/day, significantly reduced the increase in body weight induced by a cafeteria diet — a clear sign of an anti-obesity effect.

CONCLUSION

After examination of bio-chemical, behavioral and adipose tissue parameters in normal and treated groups indicates that *Termenalia catappa* aqueous extract of leaves have anti-obesity activity. This anti-obesity is may be due to presence of Phytochemicals like alkaloids, glycosides, Flavonoid, tannins and phenolic compounds in aqueous *Termenalia catappa* leaves extracts. However, Further studies are required to isolate and characterize the phytoconstituents responsible for the anti-obese activity and confirm the anti-obesity mechanism.

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