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Research Article

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Antiobesity activity of ethanolic extract of fruits of *Terminalia bellirica* on atherogenic diet induced obesity in experimental rats

Mary Shoba Das C.* and Gayathri Devi S.**

**Research Scholar, Department of Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore*
***Associate Professor, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore*

ABSTRACT

Obesity is a common metabolic disorder and rapidly becoming a global public health problem in the 21st century. Millions of adults are diagnosed as obese each year. Therefore, the present study was designed to investigate the effect of ethanolic extract of fruits of Terminalia bellirica (EFTB) on Atherogenic Diet (AD) induced obesity in rats. Obesity was induced by oral feeding of AD for a period of 45 days. Experimental rats were divided into 4 groups (n=6): Group I: control rats, Group II: obesity control, Group III: obesity+500mg/kg body weight of ethanolic extract of fruits of Terminalia bellirica (EFTB) and Group IV: obesity+10mg/kg body weight of Sibutramine (standard drug). Body weight gain, feed and water intake, body temperature, BMI, atherogenic index (AI), organ weight and lipid profile were measured. Continuous administration of ethanolic extract of fruits of Terminalia bellirica at the dose of 500mg/kg had significant ($p<0.05$) effect on the parameters assessed similar to that of standard drug Sibutramine (10mg/kg). The results of the present study revealed that the ethanolic extract of fruits of Terminalia bellirica could be a useful intervention in the treatment of obesity. Further investigation is needed to identify the therapeutic potential and the exact mechanism of Terminalia bellirica for the treatment of obesity.

Keywords: Ethanolic extract, obesity, atherogenic diet, *Terminalia bellirica*

INTRODUCTION

Obesity is associated with an array of health problems in adult and pediatric population including metabolic syndrome, hypertension, diabetes and liver diseases. In human, adipose tissue represents an active endocrine organ that by releasing the large number of bioactive mediators plays an important role in modulating hemostasis, blood pressure, lipid and glucose metabolism and inflammation. It has been shown that weight loss as modest as 10 % could significantly improve the risk of several chronic diseases [1]. Overweight and obesity are defined as an abnormal or excessive fat accumulation that may impair health. The most common method used to assess obesity and overweight is the body mass index (BMI). BMI is a simple, rapid and inexpensive method that can be applied generally to adults and is significantly correlated with total body fat as well as morbidity and mortality.

Now a day the prevalence of obesity is increasing worldwide. Numerous diseases are caused due to obesity. These include type 2 diabetes, hypertension, dyslipidemia, ischemic heart disease, stroke, obstructive sleep apnea, asthma, nonalcoholic steatohepatitis, gastroesophageal reflux disease, degenerative joint diseases, infertility and polycystic ovary syndrome; various malignancies and depression [2]. The primary treatment for obesity is dieting and physical

exercise. If this fails, anti-obesity drugs or surgery is performed to reduce appetite or inhibit fat absorption. Considerable efforts have been devoted to the discovery of antiobesity drugs worldwide. Despite the remarkable progress in the management of obesity by synthetic drugs, there has been a renewed interest in medicinal plants because of the side effects of synthetic drugs. The discovery of new drugs from traditional medicine is not a new phenomenon. Many Indian medicinal plants are reported to be useful in obesity. However, search for new antiobesity drugs continues [3].

Terminalia bellirica commonly known as dhandrika in Tamil and it belongs to the family Combrataceae. It is reported to promote digestive power, wound healing, curative of ulcers, local swelling, anemia and chronic recurrent fever. The fruits are purgative, laxative, gastroprotective and are used to alleviate asthma, piles and cough. *Terminalia bellirica* has been reported to exhibit a variety of biological activities such as anticancer, antimutagenic and antiviral activity [3]. With this back ground, the present study was designed to evaluate the antiobesity activity of the ethanolic extract of fruits of *Terminalia bellirica* on Atherogenic Diet (AD) induced obesity in experimental rats.

EXPERIMENTAL SECTION

Plant material and extract preparation

The fruits of *Terminalia bellirica* were collected from Velliangiri hills, Coimbatore and duly authenticated by Botanical Survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/2014/Tech 510. The collected fruits were washed, dried and powdered. The powdered fruits were then subjected to ethanolic extraction by using Soxhlet apparatus.

Experimental animals

Male Wistar rats of 8 – 10 weeks old, weighing 100 – 200 grams were used for this study were procured from KMCH college of Pharmacy, Coimbatore, India. They were acclimatized to animal house conditions and it was housed in polypropylene cages at room temperature (25° - 30° C) and at 45 - 55% relative humidity for 12h, each of dark and light cycle and had free access to drinking water and fed with standard pellet diet. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The animal ethical committee of the Institute gave its approval to conduct the animal experiments (KU/IAEC/Ph.D/128).

Acute toxicity studies

Acute oral toxicity study was performed as per Organization for Economical Co-operation and Development (OECD) guidelines No.423. The rats (n=6) selected by random sampling technique were used for the study. The animals were kept fasting overnight prior to drug administration providing only water. A group of 6 animals was administered the plant extract in graded doses of 0.25g/kg, 0.5g/kg, 1.0g/kg and 2.0g/kg rat. Animals were observed individually once during the first 30 minutes after dosing, periodically for 14 days. Daily once cage side observations were made which included changes in eyes and mucus membrane, skin and fur, respiratory tract, CNS changes and gross pathological examinations. The dose selection was performed by taking $1/10^{th}$ of the lethal dose (LD_{50}).

Preparation of Atherogenic diet (AD)

Atherogenic diet (AD) is a hypercaloric diet. It was prepared by mixing the following the following ingredients in fixed percentage. AD contains (100g): Cholesterol – 2 %, Cholic acid – 1 %, Dalda – 20 %, Coconut oil – 6 %. The feed was prepared, dried and administered every day morning to 45 days to the experimental animals with water *ad libitum*.

Experimental design for antiobesity activities

The male Wistar rat was divided into four groups with six rats each for each treatment period. The groupings are as follows:

Group I: Control
Group II: Rats received atherogenic diet and served as obese control
Group III: Rats received atherogenic diet + EFTB (500mg/kg)
Group IV: Rats received atherogenic diet + Sibutramine (10mg/kg)

The experiments were carried for 45 days. At the end of the study period the animals were sacrificed after an overnight fasting. The blood samples were collected from the left ventricle and used for the biochemical analysis.

Body weight of experimental rats
The body weight (gm) of the experimental rats was recorded on the day one and then every week for 45 days using digital weighing balance.

Feed and water intake of experimental rats
The daily feed and water intake of experimental rats were measured daily morning for 45 days of the study.

Body temperature of experimental rats
The body temperature of the experimental rats was recorded on the day 45 using rectal telethermometer before and after drug administration at 30, 60, 90, 120 and 180 min with a contact time of 1 minute.

Organ weight of experimental rats
On the final day of the study the animals were sacrificed and then the organs like liver, heart, kidney, spleen, spleen, pancreas and intestines were removed, washed with saline, blotted in a filter paper and weighed.

BMI of experimental rats
BMI of the experimental rats were measured before and after of the treatment period by the formula:

BMI = body weight (g)/ length^2 (cm^2)

Atherogenic index (AI) and protection of experimental rats
Atherogenic index and percentage protection [4] of the experimental rats were calculated by the using the following formula:

$$\text{Artherogenic Index (AI)} = \frac{\text{Serum cholesterol}}{\text{Serum HDL – cholesterol}}$$

$$\% \text{ Protection} = \frac{\text{AI (control)} - \text{AI (treated)}}{\text{AI (control)}} \times 100$$

Lipid profile
The lipid profile parameters like total cholesterol [5], triglyceride [6], HDL [7], LDL and VLDL were evaluated in the serum of the experimental rats.

Statistical analysis
All the results were expressed as mean of 6 replicates ± standard error of mean and were analyzed by Analysis of Variance (ANOVA). Differences between groups were considered significant at $p<0.05$ level.

RESULTS AND DISCUSSION

Acute toxicity studies
The animals fed with the ethanolic extract of fruits of *Terminallia bellirica* were found to be healthy and safe in the doses used and there was no mortality upto a dose of 2000mg. There was no significant change in the behaviour of animals like alertness, anxiety, motor activity, restlessness, tremors and appearance of animals was observed. The oral administration of the extracts did not cause mortality in all the groups of the study till 14 days of experimental periods. The plant extract had no adverse effect indicating that the medium lethal dose (LD_{50}) could be greater than 2000 mg.

The body weight of the rats was measured before and after the treatment periods. The change in body weight of control and experimental rats are given in the Table 1.

Table 1. Body weight of experimental rats

Treatment groups	Body Weight	
	Before treatment	**After treatment**
Control	172± 12.86	190 ± 9.34
AD-induced	178± 9.62	223± 5.81
AD + EFTB	171± 4.76	188± 4.73
AD + Sibutramine	176± 6.28	190± 3.62

Values are expressed in mean ±S.E.M (n=6)

The rats fed with atherogenic diet increased in body weight significantly from the first week until the end of the treatment period while compared to the control rats. The rats which received ethanolic extract of fruits of *Terminalia bellirica* and standard drug (Sibutramine) underwent a loss in weight relative to the initial weight.

In a study conducted by Kameshwaran *et al*. [8], the methanolic extracts of *Tecoma stans* showed significant reduction in body weight of the Atherogenic Diet (AD) induced obese rats. In another study, the ethanolic extract of *Saccharum spontaneum* treated high fat diet induced obese rats showed a significant decrease in body weight [9].

The AD provides more calories than the normal diet, resulting in a high level of fat storage in the periepididymal region. It contains high percentage of fat and is considered to be one of the factors involved in the development of obesity, leading to the accumulation of body fat. The administration of AD for six weeks in the experimental rats produce obesity like conditions, with increase in body weight, parametrial adipose tissue weight, organ weight and serum lipid levels. The results of the present study suggests that the body weight reducing effect of fruit extract in atherogenic diet fed rats may be produced due to its hypophagic property.

Feed and water intake of experimental rats

The feed and water intake of all the rats were measured everyday at the same hour throughout the study and presented in Table 2.

Table 2. Feed and water intake of experimental rats

Treatment Groups	**Feed intake (g/100g per day)**	**Water intake (ml/100g per day)**
Control	14.40 ± 0.44	22.21 ± 1.12
AD-induced	29.80 ± 0.65	42.72 ± 0.39
AD+EFTB	17.72 ± 0.29	27.90 ± 0.62
AD+Sibutramine	18.34 ± 0.52	27.48 ± 0.51

Values are expressed in mean ±S.E.M (n=6)

The rats which received AD was found to consume more feed than the control rats which received the normal feed. The caloric content of AD was higher than the normal feed. There was a significant ($p<0.05$) decrease in feed and water intake in rats administered with ethanolic extract of fruits of *Terminalia bellirica* and Sibutramine.

Patel *et al*. [10] also reported that the oral administration of hydroalcoholic extract of bark of *Pinus sylvestris* reduce the feed intake in the HFD induced obese rats. The study done by Adikay *et al*. [11] suggested that the ethanolic extract of *Saccharum spontaneum* reduce the feed intake when compared to HFD induced obese rats.

The central controllers like NPY and peripheral controllers like gut peptides and hormonal signals are responsible for the regulation of appetite. Alteration of these controllers may results in increased appetite. In this study obese control rats showed an increased amount of feed intake when compared as the normal rats and all the treated groups. These results suggesting that the ethanolic extract of fruits of *Terminaia bellirica* playing a major role in the control of appetite signals, both centrally and peripherally.

Body temperature of experimental rats

The body temperature of the experimental rats were recorded on 45th day using rectal digital telethermometer before and after drug administration at 30, 60, 90, 120 and 180 minutes with a contact time of 1 minute and presented in Table 3 .

Table 3. Body temperature before and after drug administration of experimental rats

Treatment groups	Before Drug Administration	After Drug Administration			
		30 min	60 min	90 min	120 min
Control	**32.19 ± 0.04**	32.37 ± 0.04	32.40 ± 0.01	32.2 ± 0.02	32.41 ± 0.15
AD-induced	**30.21 ± 0.03**	**30.22 ± 0.02**	**30.10 ± 0.02**	**30.09 ± 0.04**	**30.11 ± 0.07**
AD+EFTB	**31.25 ± 0.03**	31.37 ± 0.04	31.40 ± 0.02	31.45 ± 0.04	31.53 ± 0.03
AD+Sibutramine	**30.78 ± 0.03**	32.04 ± 0.03	32.25 ± 0.03	32.45 ± 0.04	32.65 ± 0.04

Values are expressed in mean ±S.E.M (n=6)

It has been observed that the experimental rats receiving AD showed a remarkable reduction in rectal body temperature evaluated before and after drug administration at 0, 30, 60, 90 and 120 minutes. The standard drug Sibutramine significantly ($p<0.05$) increase the body temperature. Similar effect was found in the ethanolic extract of fruits of *Terminalia bellirica* which reverse the effect by increasing the body temperature significantly ($p<0.05$).

In obesity, there will be decrease in the diet induced thermogenisis, due to decrease in sympathetic activation of brown adipose tissue. Neuropeptide-Y (NPY) which synthesized in the brain causes increase in food intake and also inhibits thermogenisis by reducing sympathetic activation of brown adipose tissue.

BMI of experimental rats

In epidemiological studies, BMI is widely used as a measure of fatness because it is highly correlated with body fat and is nearly independent of height. The BMI of the experimental rats was taken before and after the treatment periods. The change in BMI of control and experimental rats are tabulated in the Table 4.

Table 4. BMI of experimental rats

Treatment Groups	Before Treatment	After Treatment
Control	1.49 ± 0.07	1.52 ± 0.06
AD-induced	1.43 ± 0.07	1.64 ± 0.15
AD+EFTB	1.44 ± 0.05	1.47 ± 0.05
AD+Sibutramine	1.53 ± 0.04	1.60 ± 0.02

Values are expressed in mean ±S.E.M (n=6)

A significant increase in BMI was observed in the group of rats fed with AD after 45 days as compared to normal control rats. The rats treatment with sibutramine once daily for 45 days significantly ($p<0.05$) decrease the BMI when compared with obese control rats, whereas the continuous oral administration of the ethanolic extract of fruits of *Terminalia bellirica* for 45 days results reduction in the BMI rate.

Our results were agreed with that of Suneetha *et al*. [12], who reported that the supplementation with a methanolic extract of *Sapindus emariganatus* significantly ($p<0.05$) decrease the BMI when compared with obese control rats. Garg and Singh [2], showed that the aqueous and ethanolic extracts of *Aegle marmelos* reduces the BMI in the High Fat Diet induced obese rats.

Effect on organs weight of experimental rats

On the final day of the study, the major organs like liver, heart, kidney, spleen, pancreas and intestine were removed and weighed. The change in organ weight was reported in Table 5.

Table 5: Organ weight of experimental rats

Treatment Groups	Internal Organs (g)					
	Liver	Heart	Kidney	Spleen	Pancreas	Intestine
Control	7.24 ± 0.07	1.18 ± 0.03	1.06 ± 0.03	1.12 ± 0.01	0.42 ± 0.12	5.51 ± 0.41
AD-induced	9.45 ± 0.05	1.70 ± 0.04	1.30 ± 0.02	1.19 ± 0.03	0.47 ± 0.01	7.62 ± 0.18
AD+EFTB	7.78 ± 0.04	1.24 ± 0.02	1.08 ± 0.03	1.14 ± 0.04	0.35 ± 0.01	6.35 ± 0.13
AD+Sibutramine	7.87 ± 0.07	1.27 ± 0.03	1.11 ±0.01	1.16 ± 0.05	0.34 ± 0.04	6.63 ± 0.04

Values are expressed in mean ±S.E.M (n=6)

From the table 5, it was clear that the weights of the internal organs like liver, heart, kidney, spleen, pancreas and intestine were increased significantly ($p<0.05$) in AD-induced obese rats when compared with normal rats. Ethanolic extract of fruits of *Terminalia bellirica* treated rats significantly ($p<0.05$) reduced the weight of organs when

compared with their respective control group of rats. Experimental rats which received sibutramine along with the feed significantly ($p<0.05$) reversed all the effects induced by AD.

Obesity is associated with the increased adipose tissue accumulation in the abdominal region leads to an increase in body weight, parametrial adipose tissue weight and organ weight. These results suggested that rats fed with AD supplemented daily with ethanolic extract of fruits of *Terminalia bellirica* had anti-obesity effect, which suppresses the accumulation of adipose tissue and organ weight gain. This decrease in body fat percentage accompanied by the reduction in body fat mass strongly supports the antiobesity effect of *Terminalia bellirica.*

Atherogenic Index (AI) and protection of experimental rats

The atherogenic index and protection of the experimental rats against CVD was evaluated in all the treatment groups and tabulated in Table 6.

Table 6: Atherogenic index (AI) and protection of experimental rats

Treatment Groups	AI	Protection (%)
Control	2.00 ± 0.14	-
AD-induced	12.95 ± 0.34	-
AD+EFTB	3.08 ± 0.33	76
AD+Sibutramine	3.45 ± 0.20	73

Values are expressed in mean ±S.E.M (n=6)

The table 6 clearly indicates that there was a significant ($p<0.05$) increase in the atherogenic index in rats fed with atherogenic diet when compared to control rats. Treatment with ethanolic extract of fruits of *Terminalia bellirica* and standard drug sibutramine significantly ($p<0.05$) reduced the atherogenic index. The drugs and plant extract treated groups of rats increased in percentage of protection to CVD.

The findings of Saikia and Lama [13] are in agreement with our study, who stated that the leaves of *Bougainvillea spectabilis* were able to reduce the atherogenic index in the HFD induced obese rats. The methanolic extract of leaves of *Moringa oleifera* effectively reduces the antherogenic index and increased in percentage of protection against CVD [14].

Atherogenic index is the powerful indicator of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and *vice versa*. In this study, it was observed that the ethanolic extract of fruits of *Terminalia bellirica* significantly reduced atherogenic index, indicating the protection against cardiovascular diseases.

Lipid profile of experimental rats

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus and obesity. Effect of ethanolic extracts of fruits of *Terminalia bellirica* on serum lipid profile of diabetic, obese and obesediabetic rats was determined and the results are tabulated in the Table 7.

Table 7: Lipid profile of experimental rats

Treatment groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL- C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control	91.67 ± 3.51	64.00 ± 5.57	46.00 ± 2.00	32.87 ± 3.87	12.8 ± 1.11
AD-induced	325.67 ± 5.86	121.67 ± 6.51	25.17 ± 1.04	276.17 ± 4.82	24.33 ± 1.30
AD+EFTB	132.67 ± 4.73	68.00 ± 4.58	43.33 ± 3.06	75.73 ± 8.22	13.60 ± 0.92
AD+Sibutramine	138.67 ± 8.33	63.33 ± 4.51	40.33 ± 3.51	85.67 ± 6.63	12.67 ± 0.90

Values are expressed in mean ±S.E.M (n=6)

Table 7 clearly indicates that the elevated levels of serum lipid profile except HDL-C was observed in the AD-induced obese rats when compared with control rats. Oral administration of ethanolic extract of fruits of *Terminalia bellirica* and sibutramine for 45days to obese rats showed a significant decrease in the levels of total cholesterol, triglyceride, LDL and VLDL when compared with their respective controls. Similarly the HDL-C level was found to be decreased in obese rats whereas the ethanolic extract of fruits of *Terminalia bellirica* and sibutramine treated rats showed a significant increase in the levels of HDL-C when compared with their respective controls.

Kanthlal *et al*. [15] reported that the serum cholesterol, triglyceride, LDL and VLDL cholesterol levels were significantly higher in the AD-induced rats compared to those with the normal rats, while HDL-C level was found to be decreased in the AD-induced rats. On treatment with *Tabernamontana divaricata* to the AD-induced rats, a significant reduction in serum total cholesterol, LDL-C, VLDL-C and triglyceride and a significant increase in HDL-C were observed.

In the obese condition, there will be an increase in serum lipids, such as total cholesterol (TC), triglycerides (TG), LDL-C and VLDL-C was observed in humans and also a reduction in HDL-C levels. Lipids are mostly consumed in the form of neutral fats, which are also known as triglycerides (TG). TGs form major constituents in food of animal origin and less in food of plant origin. Saturated fats increase blood cholesterol and thereby increase the risk of atherosclerosis and coronary heart diseases, abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus [8].

CONCLUSION

To conclude, the results of the present study depicted that the oral administration of the ethanolic extract (500mg/kg) of fruits of *Terminalia bellirica* is effective in reducing body weight, lipid parameters and organ weight when compared to AD induced control rats. Hence, the fruits of *Terminalia bellirica* can be potentially used in the treatment of obesity. However, further studies are required to elucidate the antiobesity effects of *Terminalia bellirica* and to identify the active components responsible for the above activities.

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