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Original Article

Effect of *Terminalia bellerica* against high fat diet induced hyperlipidemia and obesity

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ABSTRACT

Present study was focused to investigate the anti-hyperlipidemic activity of *Terminalia bellerica* against high fat diet induced hyperlipidemia and obesity. *Terminalia bellerica* commonly known as Baheda is one of the most common plants being used in India since early times in many disorders one of the ingredients in many polyherbal formulations like Triphala etc used for cardiac disorders. The ethanolic extract of the fruits of *Terminalia bellerica* 250mg/kg & 500mg/kg body weight was administered p.o. for 20 days to test anti-hyperlipidemic activity. The parameters for evaluation of anti-hyperlipidemic activity are the physical parameters and the biochemical estimations. The physical parameters were gross examination of heart, heart weight: body weight ratio, liver weight, atherogenic index and basal metabolic index. In biochemical estimations various cardiac enzymes like lactate dehydrogenase, and the lipid profile were measured. The results of present study show that alcoholic extract of *Terminalia bellerica* (500 mg/Kg) has significant reduction in various lipid levels as well as the elevated physical parameters like heart weight: body weight ratio, body weight gain and BMI against high fat diet induced hyperlipidemia and obesity compared to clinically used drugs, Atorvastatin (10mg/kg) and Orlistat (pure drug 10mg/kg).

Keywords: *Terminalia bellerica*, Hyperlipidemia, Obesity, High fat diet, Atorvastatin, Baheda

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases [1]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary causes of death [2]. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease **[**3**].** The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing coronary heart disease or the occurrence of further cardiovascular disease like atherosclerosis or cerebrovascular disease [4]. Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide, resulting into a significant increase in mortality and morbidity related to coronary heart diseases, type 2diabetes, metabolic syndrome, stroke and cancers [5-7]. Medicinal plants have been investigated and reported to be useful in treatment of obesity, diabetes and other chronic diseases [8-9]. *Terminalia bellerica* (Family- Combretaceae) is a large, deciduous, tree with a straight steam and ash grey bark, attaining a height of 20-35 m and girth of 1.7-3m. It grows wild throughout Indian plains and on hills up to 1000m, except in arid and marshy places. It contains 20-30% Tannins, Gallic Acid, Egallic Acid, Ethyl gallate, Chebulagic acid.And also it contains Bellericanin, Phyllembin, Termilignan, Thaninilignan, 7-hydroxy-3',4'-methylenedioxy flavan and anolignan[10].It is used as remedy for Gastrointestinal disorders, and in general as health-protective. According to ayurveda system of medicine Baheda is used as a Cardiotonic, Anti-tussive, Febrifuge, Laxative and Blood Purifier. It has also been recognized as Galactogogue, Antiseptic, Anti-inflammatory, Analgesic, Antacid, has various medicinal applications [11].

MATERIALS AND METHODS

Collection and authentication of crude drug: Dried fruits of *Terminalia bellerica* were purchased from the local market of Lucknow. The drug authenticated by a botanist of National Botanical Research Institute Lucknow (UP). Ref

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No.NBRI/CIF/367/2013, Specification- NBRI-SOP-202. A specimen sample of the same was preserved in the section of the Faculty of Pharmacy, Integral University, Lucknow, with the voucher No. NBRI- SOP- 202, for future reference.

Chemicals and reagents: Atorvastatin were purchased from Sigma Chemical Co., St. Louis, USA and Orlistat (Marketed brand) from Intas Pharmaceutical. All other chemicals are of analytical grade, purchased from Merck, SD Fine chemicals, Qualigens and Hi media Pharmaceuticals. The enzymatic kits purchased from Span Diagnostics, Surat and Reckon Diagnostics, Mumbai.

Extraction of *Terminalia bellerica:* The dried fruits of *Terminalia bellerica* were crushed into small pieces and macerated with 90% ethanol for 6 days. Then extract was filtered and concentrated by evaporation to yield ethanolic extract of fruits. The concentrated extract was weighed and according to the body weight, the animals dose was calculated. It was accurately dissolved in normal saline for daily administration.

Experimental animals: Albino Wistar rats (l25-150g) were used for the study. They were housed five each in sanitized polypropylene cages containing paddy husk as bedding under standard laboratory conditions at room temperature (23° C± 2° C) with 12 h light / dark cycle. The animals were randomized into experimental and control groups. They had standard weighed pellets and high fat diet as basal diet and water *ad libitum.*Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) Regd. No. IU/Pharm/M.pharm/CPCSEA/12/11, Faculty of pharmacy Integral University, Dasauli, P.O. Bas-ha Kursi Road; Lucknow – 226026 (U.P).

Base line chatacterstics of obesity and hyperlipedimia induce by the high fat diet in Wister Rat: Rats were fed with normal pellet diet (NPD) for one week before the commencement of experiment. After 1 week rats were randomly divided into normal and obese groups and fed with NPD and HFD respectively for 1 week. The food intake, body weight, body mass index(BMI) and biochemical estimations (Total cholesterol, LDH, Triglycerides, and HDL) were carried out on day 8 of dietary manipulation to assess the base line characteristics of hyperlipidemia and obesity induced by HFD in rats .

Experimental scheme: Rats were fed with on normal pellet diet for 1 week before the commencement of experiment .After 1 week; Rats were randomly divided into 6 groups of 5 animals each. The dietary regimen & dosing pattern of these groups was as follows: Group 1 - Normal Control; Pellet diet for 28 days, Group 2- HFD-Control; Rats were fed with HFD along with normal pellet diet for 28 days. Group 3- Standard (Atorvastatin-10mg/kg); Rats were fed with HFD along with normal pellet diet orally for 28 days + Std Atorvastatin (10mg/kg/day) p.o. from 8thDay to 28th day, Group 4- Standard(Orlistat-10mg/kg/day); Rats were fed with HFD along with normal pellet diet orally for 28 days + Std Orlistat p.o. from 8thDay to 28th day. Group 5- Test Extract (250mg/kg); Rats were fed with HFD along with normal pellet diet for 28 days + test Extract (250mg/kg) p.o. from 8th day to 28th day, Group 6- Test Extract (500mg/kg); Rats were fed with HFD along with normal pellet diet for 28 days + test Extract (500mg/kg) p.o. from 8th day to 28th day. On 29th day, Blood was collected from the retro-orbital plexus of overnight fasted rats using micro capillary tubes and serum was separated by centrifugation at 3000rpm for 15 min. After that animals were sacrificed with high dose of ether, heart and liver were excised washed with normal saline. The Heart and then kept in formaline solution for further studies.

Assessment of lipid parameters in serum: The serum concentrations of TC, TG and HDL-C were measured with commercial kits (Lifechem), LDL-C was measured indirectly by using *Friedwald equation*, LDL-C = Total Cholesterol – HDL – (TGs /5), Atherogenic index is calculated as Total cholesterol/HDL-C [12, 13].

Assessment of lipid content in liver: Extraction of lipid content from liver was done according to [14]. Total lipids were extracted from 1gm of liver tissues, total lipids in liver tissues were homogenised with chloroform-methanol (2:1) mixture (*v/v*)to a final dilution of 20 fold the volume of tissue sample., homogenate is filtered through wattmann filter paper1into glass stopper volumetric fl ask. The crude extract is mixed thoroughly with 0.2 its volume of water and mixture is allowed to separate into 2 phases .Upper phase represents them ethenolic layer is removed and finally the lower phase collected which contains lipids [15].

RESULTS

Rats were fed with on normal pellet diet (NPD) for one week before the commencement of experiment. After 1 week rats were randomly assigned into normal and obese groups and fed with NPD and HFD *ad libitum*, respectively for 1 week. The food intake, body weight, body mass index (BMI) and biochemical estimations (Total cholesterol, LDH,

Triglycerides, and HDL) were carried out on day 8 of dietary manipulation to assess the base line characteristics of hyperlipidemia and obesity induced by HFD in rats.(Table 1,2,& Fig.1)

DISCUSSION

Hyperlipidemia is a leading cause of morbidity and mortality worldwide. Prompt treatment of a hyperlipidemia is indispensable to prevent the damage and to save the life. In the traditional Indian medicinal system, a major information is reported concerned with anti-hyperlipidemic activity and *T.belerica* has been known to have potent anti- hyperlipidimic activity however it has not been extensively studied. In this context, there is a need to reveal the anti-hyperlipidemic activity of extract of *Terminalia bellerica* fruits. Atherosclerosis significantly increases the levels of serum marker enzymes such as LDH as well as increase levelsof TC, TG, HDL, which are the biochemical of hyperlipidemia. In the present study, pretreatment with alcoholic extract of *Terminalia bellerica* significantly prevents hyperlipidemia which is clearly indicated by various estimations. Present study showed that High fat diet induced significant hyperlipidemia compared to normal rats.The hyperlipidemia was also clearly assesed by various enzymes and physical parameters.*Termialia bellerica* causes control in significant Hyperlipidemia compared to normal rats as is evident from reduced levels various enzymes like TG ($p<0.001$), HDL ($p<0.001$), LDH($p<0.001$), TC.($p< 0.001$). *Terminalia bellerica* extract (250 mg/kg) doesn't show significant protective effect (p value of low dose in comparision to HFD) but *Terminalia bellerica*(500 mg/kg) showed very significant Anti-Hyperlipidemic activity against High fat diet induced Hyperlipidemia(TG,TC, HDL , LDh p-value).The heart weight body weight ratio is a very important parameter of Hyperlipidemia.*Terminalia bellerica*extract (250 mg/kg) doesn't show significant protective effect (p value of low dose in comparision to HFD) but *Terminalia bellerica* (500 mg/kg) showed very significant Anti-Hyperlipidemic activity against High fat diet induced Hyperlipidemia and Obesity {TG (p<) ,TC(p<}, HDL(p<}, LDH(p< value).All these results were compared and found similar against two clinically established standard drugs Atorvastatin and as well as Orlistat.Pretreatment with test extract, Atorvastatin, Orlistat exerted a protective effect as evident for Hyperlipidemia and Obesity.

CONCLUSION

The present study concludes that pretreatment with alcoholic extract of *Terminalia bellerica* significantly prevented from Hyperlipidemia and Obesity and decreased the levels of diagnostic marker enzymes significantly. At low dose (250mg/kg) alcoholic extract of *Terminalia bellerica* showed moderate protection against hyperlipidemia and obesity. At high dose (500mg/kg) alcoholic extract of *Terminalia bellerica* showed marked protection against High fat diet induced Hyperlipidemia and Obesity as well as by biochemical estimations of Lipid profile compare to the standard Atorvastatin (10mg/kg), Orlistat (10mg/kg). Thus the study clearly illustrates that alcoholic extract of *Terminalia bellerica* possesses protective effect against high fat diet induced hyperlipidemia and obesity.

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Table (1) Effect of HFD on body weight gain, body mass index ,Blood glucose (mg/ml) and total cholesterol (mg/dl) on 8th day after 24 h fasting (Baseline characteristics of obesity and hyperlipidemia).

Groups/ Parameters	NPD	HFD
Body weight gain (gm)	13.80 ± 2.485	30.23 ± 2.653
Body Mass Index (kg /m^2)	7.446± 1.566	3.364 ± 0.6613
Blood Glucose (mg/ml)	113.6 ± 3.832	154.3 ± 2.335
Total Cholesterol (mg/dl)	95.92±1.624	115.3 ± 1.669

Values are expressed as mean ± SEM for 5 animals in each group

Table (2)Effect of HFD on Heart weight: Body weight (X10^3), Liver weight, Atherogenic Index, Total Cholesterol, Triglycerides, HDL and LDH on 8th day after 24 hour fasting (Baseline characteristics of obesity and hyperlipidemia) of hyperlipidemia and obesity:

Groups/ parameters	NPD	HFD	*T.bellerica* (500mg/kg/)	*T.belleria* (250mg/µg)	Atorvastatin (10mg/kg)	Orlistat (10mg/kg)
Heart weight: Body weight (X10^3)	39.20±1.791	57.62±2.178	46.37±3.283	46.72±2.398	42.26±2.090	43.35±2.265
Liver weight	7.234±0.572 3.618	9.712±0.221 3.136	7.908±0.394 3.63	8.436±0.327 3.84	7.624±0.494 3.29	7.812±0.4952 3.214
Atherogenic Index	108.7±4.48	203.9±5.08	280.5±2.475	271.6±2.72	314.0±3.001	316.2±2.95
Total Cholesterol	61.92±1.550	122.4±3.346	83.52±4.378	79.61±2.333	61.91±2.892	58.21±1.682
Triglycerides	30.55±2.307	65.35±2.02	30.22±2.478	31.43±2.782	39.66±3.681	44.56±2.847
HDL	19.44±1.651	49.41±3.113	18.78±2.236	15.96±2.220	23.57±2.999	15.45±1.712
LDH						

Values are expressed as mean ± SEM for 5 animals in each group

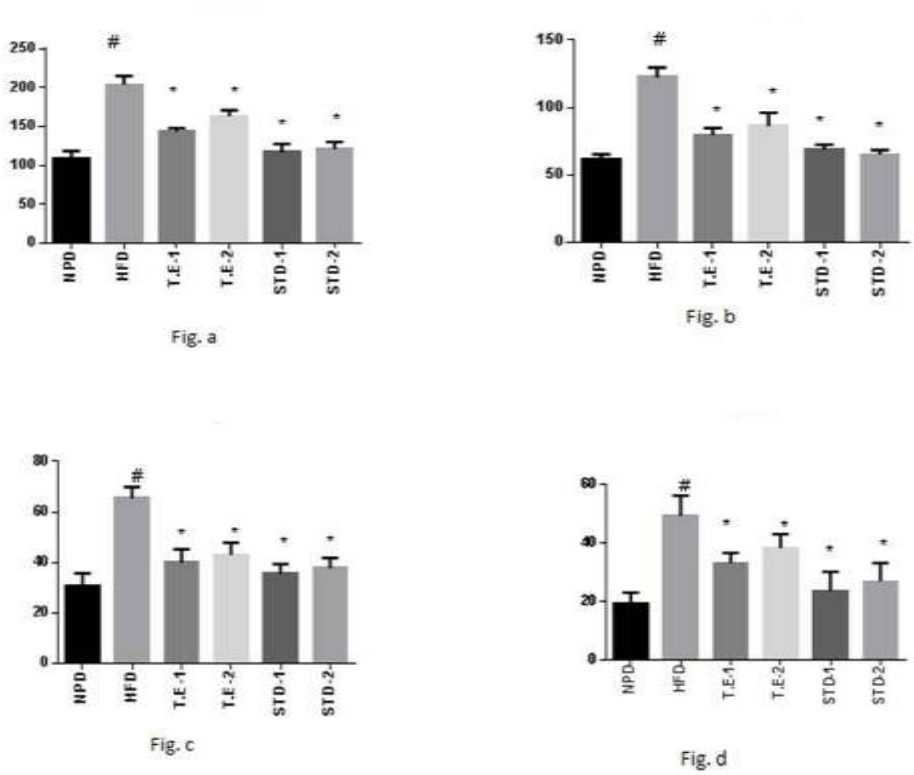

Figure (1) Values are expressed as mean ± SEM for animals in each group; where # p<0.001 when HFD compared to NPD; * p<0.001 when compared to HFD. Where; NPD - Normal Pellet Diet, HFD- High Fat Diet,T.E-1-*T.bellerica 500mg/kg*, T.E-2-*T.bellerica* 250mg/kg, STD-1-Atorvastatin(10mg/kg,b.w.), STD-2-Orlistrat(10mg/kg,b.w.);Fig. a- Total Cholesterol; Fig b- Triglycerides; Fig c- LDH; Fig d- HDL

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