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EFFECT OF *TERMINALIA BELLERICA* FRUIT ROXB ON ALLOXAN INDUCED DIABETIC RELATED ATHEROSCLEROSIS ON WISTAR ALBINO RATS

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ABSTRACT

To investigate the effect of *Terminalia bellerica* (Family: Combretaceae)fruit extracts on diabetic related atheroclerosis. Different extracts such as Hexane (HETB), Chloroform (CETB), Ethanol (EETB), Aqueous (AETB) at the dose of 200mg/kg were administered to high fat diet associated with alloxan induced diabetic hyperlipidemic rats. It was found that the fruit extracts significantly reduce the Total cholestrol, LDL, VLDL levels and significant increase in HDL Levels. Aqueous extract of *Terminalia bellerica* fruit extracts, have more significant activity on reducing the Total cholestrol, LDL,VLDL levels and significantly increase in HDL Levels. Histopathology results also proves that there is a less accumulation of lipids in the walls of the arch of aorta in aqueous extract.

KEY WORDS: Diabetes, Atheroclerosis, High fat diet.

INTRODUCTION

Diabetes is a chronic disease characterised by high blood glucose levels (Ramzi S *et al.*, 2003)**.** In addition to hyperglycemia, hyper cholesterolemia is the most common complications of diabetes mellitus (Kumar P *et al.*, 2010)**.** Hyperglycemia, Hypercholesterolemia leads to the disease called atheroslerosis . Herbal drugs are prescribed widely even their biological active compounds are unknown ,because of their effectiveness , less side effect & relatively low cost .one such plant expected to have diabetic related athereosclerosis activity is *Terminalia bellerica*, it is a well known traditional plant which is locally known as dhandrika.it act as laxative , regenerative,beneficial for hair,throat,eyes,skin disease,cough cold,asthma,to arrest the bleeding,induce deep sleep (Latha RCR and Daisy P, 2010) it was considered worthwhile to investigate the effect of fruit of *Terminalia bellerica* on diabetic related atherosclerosis.

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MATERIALS AND METHODS

Collection Plant material

Fruit of *Terminalia bellerica* was collected from the herbal garden PRIST University,Thanjavur,Tamilnadu, India. Its identity was confirmed by the taxonomist at St Joseph College , Trichirapalli, Tamilnadu, India by comparing it with the authentificated specimen (Voucher specimen number:AD 001) deposited at the St Joseph College.

Preparation of plant extract

The dried fruits of *Terminalia bellerica* was cutted into pieces was extracted using the soxhlet apparatus with Hexane , Chloroform, Ethanol and Aqueous on the polarity basis. the extract was filtered and the filterate was evaporated by the rotary vacuum evaporator $\leq 40^0$c to get the brownish black semi solid mass and the extractive yield of the extracts was Hexane-21%, Chloroform-31%, Ethanol-47% and Aqueous -63%.

Experimental Animals

Albino rats of weighing between 150-200 gm were used for this study. The animals were housed under standard

conditions and room temperature (25 ± 2 ^{0}c). All animals fed with standard rat pellet diet (M/S Pranav agro industries Ltd., India.) under the trade name Amrut rat feed and hand free access to tap water *add libitum*. The study has got approval from Institutional Animal Ethical committee, PRIST University. Thanjavur. Animal house registration number 743/abc/CPCSEA. Proposal approval number M.Pharm 02/2010-11 and the proposal was approved by the meeting which was held on 20.12.2010.

Acute oral toxicity

Acute toxicity study was conducted as per OECD 425 guideline (OECD, 2001). Female albino wistar rats (150-200 gm) maintained under standard laboratory condition was used. A total of five animals were used for this study which received a single dose (2000mg/kg, Body weight) of the extract. Animals were kept overnight fasting prior to drug administration. Then, food was with held for further 3-4 hours. Animals were observed individually once during the first 30 minutes after dosing, periodically during of 14 days. Once daily cage side observations included changes in eyes and mucus membrane, skin and fur, respiratory rate, CNS changes and gross pathological examinations were carried out.

Preparation of High fat diet

Cholesterol (3%), Casein (10%), Fructose (40%), Olive oil (10%) or coconut oil (5%) and water (15%) was added to one kg of normal diet. The cake was cut into pieces and dried at room temperature for 3 days before feeding to rats (Bahramica S *et al.,* 2008)

Chemicals used

Ethanol, Chloroform, Hexane, Alloxan, cholesterol, casein, sodium cholate, fructose, sodium carbonate, butanol, Tween80 and kits for the estimation of serum glucose, cholesterol, HDL, triglycerides (Vital diagnostic Pvt. Ltd., Thane, Maharashtra, India) were used in this study.

Experimental Design

Male wistar rats (3-4 weeks old) were fed a high fat diet *ad libitum* during 30 days followed by a single dose of Alloxan (120 mg/kg body weight, Intraperitoneally) to induce diabetes (Celine Bouvet, 2007) Diabetes was confirmed after 3 days of Alloxan monohydrate injection, the blood samples were collected through tail vein and blood glucose levels were estimated by one touch glucometer which was purchased from Johnson & Johnson (Bernard cosyns, 2008). The rats having blood glucose levels more than 200mg/dl were selected and used for the present study.

All the groups treated with various extracts for 30 days. The animals were divided into following 6 groups of 6 each,

Group I : Served as a control

Group II : High fat diet associated with Alloxan (120mg/kg/B.wt)

Group III : High fat diet associated with Alloxan and treated with HETB fruit (200mg/kg/B.wt)

Group IV : High fat diet associated with Alloxan and treated with CETB fruit (200mg/kg/B.wt)

Group V : High fat diet associated with Alloxan and treated with EETB fruit (200mg/kg/B.wt)

Group VI : High fat diet associated with Alloxan and treated with AETB fruit (200mg/kg/B.wt)

After 30 days the animals were anesthetized by an intraperitoneal injection of Pentobarbitone sodium (60 mg/kg body weight). Blood was collected from the left ventricle and Serum was estimated for the analysis of LDL, VLDL, Total cholesterol and HDL. For each rat, the Aorta was rapidly dissected out & stored in buffered formalin (10%) for later assessment of Atherosclerotic lesion area.

Statistical Analysis

The data obtained in the studies were subjected to one way analysis of variance (ANOVA) for determining the significant difference. The results from the test groups were compared with respective disease controls. All values were expressed as mean ± SEM.

RESULTS

Acute toxicity studies

Acute toxicity studies revealed that *Terminalia bellerica* fruit extracts were practically non toxic when administered orally to rats. The LD_{50} value was more than 2000mg/kg body weight.

Blood glucose level

One month high fat diet associated with an injection of Alloxan induced a significant increase in blood glucose levels (Table: 1).

Total Cholesterol

Rats treated with high fat diet associated with Alloxan only (Disease control group) showed a higher concentration of serum Total Cholesterol (252±1.23) compared to normal group rats (126±1.36), aqueous extract (124.6±0.76) and chloroform extract (124.5±0.76) shows more significant activity in reducing the cholesterol level compare to the disease control group. Ethanol (129.8±0.73) and hexane extract (134±0.74) also reduce the Total Cholesterol but it is less efficient than the Aqueous and chloroform extract.

LDL

The present study data indicated that rats treated with high fat diet associated with Alloxan only (disease control group) showed a higher concentration of serum LDL (176.83±1.85) when compared to normal group rats (124.4±1.42), Aqueous extract (115±1.35) and chloroform extract (128.1±0.47) shows more significant activity in

reducing the LDL level compare to the disease control group, Hexane extract (124.8±1.01) and ethanol extract (117±1.18) also shows significant reduction in the serum LDL level.

HDL

Rats treated with Aqueous and chloroform extracts of *Terminalia bellerica* is capable of increasing the serum level of good cholesterol (*i.e.* HDL-C) , the Chloroform extract of *Terminalia bellerica* showed significant improvement in the HDL levels but Aqueous extract was showed less significantly increasing the HDL levels compare to the chloroform extract. Aqueous extract (67.83±0.54) and chloroform extract (64.4±0.70) both are having significant improvement in the HDL level when compare to the disease control (34.2±0.54) and other groups of extracts.

Table 1. Effect of Alloxan on blood glucose level in 30 days high fat diet treated rats

Groups	Treatment	Blood Glucose Levels (mg/dl)
I	Control	112.66±0.7
II	Disease control	307±3.93
III	HETB (200mg/kg	301.88±16
IV	CETB (200mg/kg)	294.16±0.9
V	EETB (200mg/kg)	233.53±0.8
VI	AETB (200mg/kg)	304.16±1.6

All values are expressed as mean ± SEM, n=6.

Table 2. Effect of *Terminalia bellerica fruit* extracts on serum levels of Total Cholesterol, HDL Cholesterol, LDL Cholesterol and VLDL Cholesterol in Normal and High Fat diet with Alloxan treated wistar rats

Groups	Treatment	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
I	Control	126±1.36	64.66±1.33	124.4±1.42	30.86±0.32
II	Disease control	$252±1.23^{A\#}$	$34.2±0.54^{A\#}$	$176.83±1.85^{A\#}$	$71.06±0.35^{A\#}$
III	HETB (200mg/kg)	$134±0.74^{B\#}$	$59.16±0.4^{B**}$	$124.8±1.01^{B**}$	$31.13±0.01^{B\#}$
IV	CETB (200mg/kg)	$124.6±0.76^{B\#}$	$64.4±0.70^{B\#}$	$128.1±0.47^{B**}$	$28.96±0.03^{B\#}$
V	EETB (200mg/kg)	$129.8±0.73^{B\#}$	$57.33±1.02^{B**}$	$117±1.18^{B**}$	$30.46±0.20^{B\#}$
VI	AETB (200mg/kg)	$124.5±0.7^{B\#}$	$67.83±0.54^{B\#}$	$115±1.35^{B**}$	$26.93±0.17^{B\#}$

*All values are expressed as mean ± SEM, n-6. A-Group I vs Group II, B-Group II vs Group III, Group IV, Group V, Group VI; **P<0.01; #P<0.001*

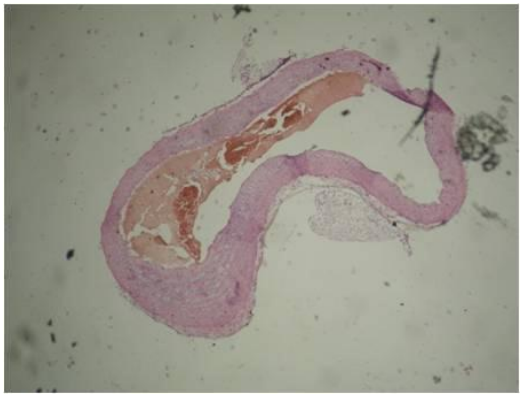

Figure 1. Disease control shows more Plaque area in the walls of arch of aorta (40X bmp)

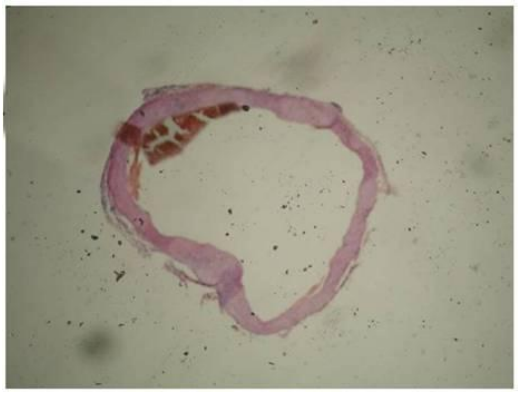

Figure 2. HETB 200mg/kg shows less Plaque area in the walls of arch of aorta (40X bmp)

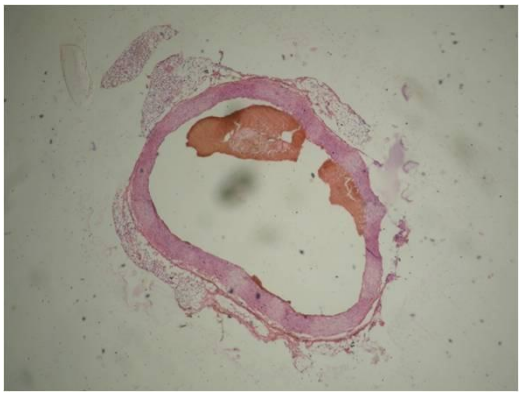

Figure 3. CETB 200mg/kg shows less
Plaque area in the walls of arch of aorta (40X bmp)

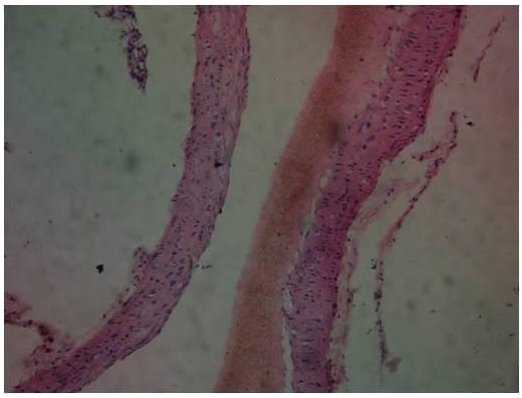

Figure 4. EETB 200mg/kg shows
Plaque area in the walls of arch of aorta (40X bmp)

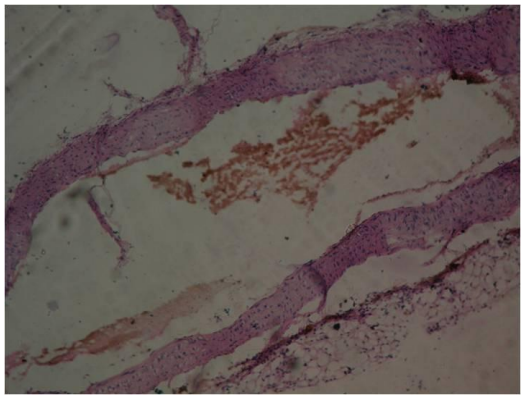

Figure 5. WETB 200mg/kg shows less
Plaque area in the walls of arch of aorta (40X bmp)

VLDL

Rats treated with high fat diet associated with Alloxan (disease control) shows high concentration of VLDL (71.06±0.35) compare to normal rats .VLDL was significantly reduced by oral administration of Aqueous extract (26.93±0.17) and Chloroform extract (28.96±0.03) of *Terminalia bellerica* when compared to the ethanol extract (30.46±0.20) and Hexane extract (31.13±0.01) treated groups.

Histopathology

Induction of diabetes was associated with a 5-fold increase in plaque area in the arch of aorta in disease control rats. Ethanol, Hexane, Chloroform extracts reduced the formation of plaque when compare to the disease control group. In Aqueous extract treated group most plaques were fatty streaks only indicate that it has more significant activity than other extracts (Figure 5).

DISCUSSION

Alloxan is well known for its selective islet β- Cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals after treatment with 120mg/kg.30 days administration of various extract of *Terminalia bellerica* fruit decreases the serum levels of Total Cholesterol, LDL Cholesterol and VLDL Cholesterol and at the same time increased HDL Cholesterol and significant maximum in reduction in Total Cholesterol, LDL Cholesterol and VLDL Cholesterol was found in aqueous extract.

The anti diabetic related atherosclerosis effect of *Terminalia bellerica* fruits may be due to the presence of more than one active principle and their synergistic properties. Preliminary phytochemical analysis of *Terminalia bellerica* fruits extracts revealed phenolic compounds and tannins as major constituents. Fruit contains 23.60 to 37.36% tannins such as chebulinic acid, chebulagicacid, 1, 3, 6-trigalloylglucose and 1, 2, 3, 4, 6-pentagalloyl glucose, glucogallin, ellagic acid, Gallic acid etc., (Row LR and Murty PS, 1970).

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus. High levels of total cholesterol and more importantly LDL cholesterol in blood are major coronary risk factors (Tchobroutsky G, 1978) insulin deficiencies causes an increase in free fatty acid mobilization from adipose tissue which results in increased production of cholesterol rich LDL particle and dislipidemia. In the present study, treatment with

Terminalia bellerica fruit extracts improved improved the lipid profile by reducing the serum levels of Total Cholesterol, LDL Cholesterol and VLDL Cholesterol and at the same time increased HDL Cholesterol. The Hypolipidemic and Cardio protective activity of *Terminalia bellerica* fruit extracts in hypercholesterolemia rats was reported (Tariq M *et al.,* 1977; Shaila HP *et al* ; 1955) and which might be due to the presence of beta sitosterol, as plant sterols are well known for its cardio protective property (Jones PJH *et al.,* 1997) Further, c-peptides were found to effectively prevent and even reverse cardiovascular disease in diabetic rats (Ido Y *et al* ., 1997) and improve blood flow in the heart of diabetic patients (Hansen A *et al* ., 2002). Therefore, the normal lipid profile in extract treated diabetic rats might be due to the significant increase in their c-peptide levels.

CONCLUSION

The study conclusively state that the aqueous extract of *Terminalia bellerica* have significant activity on reducing the Total cholesterol, LDL and VLDL levels, and also significantly increased HDL levels and histopathology results shows that , aqueous extract treated group there is less accumulation of lipids in the walls of the arch of aorta. So it can be concluded from the results obtained in the present investigation that aqueous extract of *Terminalia bellerica* possess significant diabetic related atherosclerosis activity.

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