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Original Research Article

Evaluation of antiatherogenic effect of *Alternanthera brasiliana* Linn kuntz in rats on atherogenic diet

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ABSTRACT

Background: Hypercholesterolaemia is a major risk factor for systemic atherosclerosis and a well-known etiological factor for cardiovascular diseases and its complications which is a leading cause of mortality worldwide. In a recent study, the antihyperlipidemic activity of dried leaves extract of *Alternanthera brasiliana* has been evaluated. Hence, the present study was undertaken to investigate the anti-atherosclerotic potential of the methanolic extract of the leaves of *Alternanthera brasiliana* L. Kuntz (MEAB) in high fat diet induced hypercholesterolemic rat model.

Methods: Thirty (30) wistar albino rats of either sex were randomly divided into five groups: first two groups received normal diet and high fat diet respectively and the remaining three groups received high fat diet supplemented with methanolic extract of *Alternanthera brasiliana* (MEAB) administered orally daily at two different doses: 200 mg/kg and 400 mg/kg and Atorvastatin 10 mg/kg/day orally as standard respectively. Serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) was estimated after 12 weeks. Atherogenic index was calculated from the results of lipid profile. At the end, the aorta was removed for assessment of atherosclerotic plagues.

Results: Our results showed that MEAB possessed significant cholesterol lowering potency as indicated by decrease in serum total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL-C) accompanied by an increase in serum high density lipoprotein (HDL-C) and reduces the atherosclerotic lesion of aorta ($p < 0.05$).

Conclusions: These results strongly suggests that MEAB can prevent the progress of atherosclerosis likely due to the effect of *A. brasiliana* on serum lipoproteins and its antioxidant and anti-inflammatory properties. It could be a potential therapy for the prevention and treatment of atherosclerosis.

Keywords: *Alternanthera brasiliana*, Antiatherogenic, Atorvastatinflavonoids, Methanolic extract

INTRODUCTION

Atherosclerosis remains the major cause of mortality and morbidity around the world. The prevalence of atherosclerosis is increasing steadily due to ageing population, rapid urbanization and economic development promoting habits of diet rich in saturated fat and diminished physical activity. It is predicted that by 2020 it will become the leading cause of total disease burden.

Many researchers have defined atherosclerosis as an oxidative, inflammatory and thrombotic disease of the arterial wall that is precipitated by elevated levels of low density lipoprotein (LDL) cholesterol in the blood.[1] It is the thickening and loss of elasticity of the walls of the arteries that is associated with the formation of atherosclerotic plagues within the arterial intima. Atherosclerosis is a complex multifactorial disease which develops in the arterial wall in response to

various stimuli like hyperlipidaemia, hypertension, hyperhomocystenemia, oxidative stress etc. Vascular injury in the form of endothelial injury is a critical initiating event in the pathogenesis of atherosclerosis. There is chronic damage to arterial endothelium by turbulence of blood flow or other injuries, which leads to non-denuding functional alteration of endothelial cells. There is lipid accumulation, adhesion of monocytes and platelets. Along with altered endothelium, these cells release various growth factors leading to migration and proliferation of smooth muscle cells and ultimately forms a typical atherosclerotic plague.[2]

Alternanthera brasiliana Linn Kuntz belongs to the family Amaranthaceae, popularly known as Brazilian joy weed, is an evergreen, perennial herb, native to tropical and subtropical regions of Australia, South America and India.

It is a herb indigenous to Brazil, prostrate and branchy, presenting a circular to polygonal stem, long internodes and swollen nodes where opposite leaves attach, The inflorescence is cymes, composed of hermaphrodite, actinomorphous and monocyclic flowers.[3]

Traditional uses

It is an ornamental as well as medicinal plant, traditionally used against inflammation, cough, diarrhoea in Brazilian medicine. Also used as galactogogue, cholagogue, abortificient and febrifuge. In some places it is used for night blindness and hazy vision. The herb is said to possess diuretic properties and its decoction is taken in gonorrhoea.[4]

Phytochemical constituents

Previous phytochemical studies of *Alternanthera brasiliana* reported the presence the presence of flavonoids: 3-O-robinobioside derivatives of kaempferol and quercetin, vitamins (riboflavin and niacin), beta cianin and steroids such as beta sitosterol and stigmasterol.[4]

Pharmacological activity

Various bioactivities like wound healing, anti-inflammatory and analgesic, anti-ulcer, anti-tumour, lymphocyte proliferation inhibition, antioxidant, antimicrobial and antiviral properties of the plant were studied.[4-7]

In a recent study, anti-hyperlipidaemic activity of dried leaves extract of *Alternanthera brasiliana* in fructose induced hyperlipidaemic rats has been evaluated.[8] However, to the best of authors knowledge the anti-atherogenic activity of *Alternanthera brasiliana* has not been evaluated. Hence, the present study is aimed to evaluate the anti-atherogenic activity of methanolic extract of *Alternanthera brasiliana* in high fat diet induced hypercholesterolemic rat model.

METHODS

The study was conducted from February 2015- February 2016.

Collection and authentication of plant material

Leaves of *Alternanthera brasiliana* were collected from medicinal garden of College of Veterinary science, Guwahati during the month of February to June and identified by Taxonomist Dr I.C Barua, Principal scientist, Department of Agronomy, Assam Agricultural University, Jorhat, Assam. Voucher specimen number AAU/CVSC/PHT/02. A voucher specimen was deposited in the herbarium of the institute.

Preparation of the plant extract

Fresh leaves of *Alternanthera brasiliana* were washed thoroughly with distilled water, shade dried and powdered. About 250 grams of powdered leaves was soaked in 1000 ml of methanol for 72 hours in a beaker and the mixture is stirred every 18 hours using sterile glass rod. The filtrate was obtained after passing through Whatman filter paper no. 1 for three times and solvent was evaporated at 50-60° C under reduced pressure using a rotary vacuum evaporator.

A dark brown methanol extract of *Alternanthera brasiliana* was obtained which was stored in airtight container at 4°C for further use.

Phytochemical analysis

MEAB was subjected to quantitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, terpenoids and others as per standard methods.[9]

Drugs and chemicals used

- Atorvastatin powder was obtained from Sun Pharma Lab. Ltd, East Sikkim
- The kits for estimation of Total Cholesterol, Triglycerides and HDL- cholesterol were obtained from Coral Clinical system, Goa, India.
- Methanol was obtained from Merck, India
- Potassium Phosphate Buffer, Hydrogen Peroxide solution and Tricarboxylic acid were obtained from Sigma private Ltd, Bangalore, India
- Thiobarbituric acid was obtained from HiMedia Laboratories Private Ltd, Mumbai, India.

Diet used in the study

Normal diet

Standard animal diet consisting of Bengal gram, wheat, maize, soya bean with water *ad libitum*.

High fat diet for induction of atherosclerosis

Mixture of coconut oil and Vanaspati ghee in a ratio of 2:3 (v/v) at a dose of 10 ml/kg body weight per day.[10]

Experimental animals

Healthy wistar albino male rats (*Rattus norvegicus*) 7-8 weeks old weighing 150-200 grams were taken from the Central Animal House, Assam Medical College, Dibrugarh (Registration number 634/02/a/CPCSEA) dated 19/05/02. The animals were housed in polypropylene cages under standard condition of 12 hours light and dark cycle and normal room temperature. Animals were fed with normal diet and water *ad libitum*. Before starting the study permission from the Institutional Animal Ethics Committee was taken vide approval number IAEC/AMC/001 dtd 12/06/2014. The study was conducted according to CPCSEA guidelines.

Acute oral toxicity test

Acute oral toxicity test was done following OECD guidelines 425 (up and down method). MEAB was found safe at 2000 mg/kg dose.[11]

Experimental Design

Animals were randomly assigned into five groups with six animals in each group (n=6).

Group A

Normal control (given only normal saline 10 ml/kg/day and normal diet)

Group B

Experimental control (received high fat diet at a dose of 10 ml/kg body weight /day along with normal diet)

Group C

Test drug (received high fat diet with normal diet and methanolic extract of *Alternanthera brasiliana* [MEAB] at a dose of 200 mg/kg/day)

Group D

Test drug (received high fat diet with normal diet and MEAB at a dose of 400 mg/kg/day)

Group E

Standard drug (received high fat diet with normal diet and Atorvastatin at a dose of 10 mg/kg/day).[12]

Standard drug Atorvastatin (10 mg/kg) and Methanolic extract of *Alternanthera brasiliana* were given orally with the help of feeding cannula for 12 weeks. Weight of each animal was taken at the beginning of the experiment and at the end of 12 weeks.

Collection of blood sample and estimation of lipid profile

Under all aseptic conditions and general anaesthesia, blood samples were collected by retro orbital puncture.[13] About 4 ml of blood was collected from each animal in separate sterile vials and kept for some time. The serum is collected and further centrifuged for 5 minutes at 3000 rpm. The serum thus obtained was used for biochemical analysis. Biochemical analysis of the sample was done in Biochemistry Department, Assam Medical College and Hospital.

Biochemical analysis

Lipid profile was estimated by colorimetric method.

- Total cholesterol was measured by CHOP/PAP method.[14]
- Triglyceride was measured by GPO/PAP method.[15]
- High-density lipoprotein (HDL-Cholesterol) was measured by PEG/CHOD-PAP method.[16]
- Low-density lipoprotein (LDL-Cholesterol) was calculated by using Friedwald's formula.[17]
- Atherogenic index was calculated by using formula of Schulpis.[18]

Serum oxidative markers

- Catalase (CAT) assay was carried out by Beers and Sizer method by continuous spectrophotometric rate determination.[19]
- Malondialdehyde (MDA) level was estimated by Satoh K method by using filter photo colorimeter.[20]

Histopathology of aorta

At the end of 12 weeks, the rats were kept in fasted state for 12 hours prior to anaesthesia and then sacrificed by cervical dislocation. The aorta was dissected out, rinsed in normal saline and kept in ice to keep the samples fresh and avoid degradation. The specimen were fixed in 10% formalin, embedded in paraffin wax and sectioned by rotary microtome at 5 µm and stained with haematoxylin and eosin (H and E) staining.

Statistical analysis

The statistical analysis was done using computerised GraphPad Prism software version 5.00. Values were expressed as mean±standard error of mean (SEM). The results of serum lipid profile of different groups were statistically analysed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests. The initial and final changes in body weights were analysed by paired t- test. For all analyses p-value <0.05 were considered statistically significant.

RESULTS

Acute toxicity study

No mortality was recorded among the rats at a maximum dose of 2000 mg/kg body weight. Hence, LD50 can be said to be above 2000 mg/kg. MEAB at doses of 200 mg/kg and 400 mg/kg which was arbitrarily selected was found to be safe.

The phytochemical analysis of MEAB showed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids and phytosterols.

Effect of MEAB on body weight

The results of body weight are shown in Table 1. The initial differences in body weights were found to be non-significant ($p>0.05$).

The final body weight after 12 weeks of treatment showed a statistically significant increase in experimental control ($p<0.05$) compared to normal group and decrease in body weight in test group (Group C and Group D) and standard group compared to Group B.

Table 1: Effect of methanolic extract of *Alternanthera brasiliana* on body weight.

Groups	Mean body weight (mg)			
	On 1st day	After 12th weeks	Change	% of increase
Normal Control	148±1.366	155.5±1.384	7	4.51
Experimental Control	152±0.774^a	262.5±1.857^a	111	42.2
Test Drug (200 mg/kg)	147.7±0.954^b	188±1.155^b	41	21.8
Test Drug (400 mg/kg)	147±1.125^b	162.7±1.994^b	15	9.25
Standard Drug	144.7±1.430^b	152.3±1.585^b	8	5.26

All values are expressed as mean±SEM (n=6). Paired t-test was done. a=$p<0.05$ when compared to normal control group, b=$p<0.05$ when compared to the experimental control group.

Table 2: Effect of methanolic extract of *Alternanthera brasiliana* on fasting serum lipid profile.

Groups	Serum Total Cholesterol (mg/dl)	Serum TG (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)	Atherogenic index (in ratio)
Normal Control	98.5±0.957	81.33±0.918	39.57±1.434	42.67±0.954	1.33
Experimental Control	170.7±1.145^a	222.5±4.815^a	103.2±0.965^a	23±0.577^a	6.39
Test Drug (200 mg/kg)	142±1.033^b	97.67±0.802^b	92.63±1.441^b	29.83±0.654^b	3.89
Test Drug (400 mg/kg)	130.7±0.760^b	85.33±0.954^b	77.77±1.485^b	35.83±1.046^b	2.71
Standard Drug	122.7±1.406^b	77±1.125^b	66.77±1.375^b	40.67±0.210^b	2.05

All values are expressed in Mean±SEM (n=6). Analysed by one way ANOVA followed by Bonferroni's multiple comparison tests. a= $p<0.05$ when compared to normal control group, b=$p<0.05$ when compared to experimental control group.

Effect of MEAB on fasting serum lipid profile

The results of fasting lipid profile are shown in Table 2. After repeated administration of the extract for 12 weeks, a significant ($p<0.05$) decrease in total cholesterol, serum LDL, serum triglycerides and significant increase in serum HDL was found in Test group (Group C and D) and standard group as compared to Experimental control group (Group B). Group B showed significant rise in total cholesterol, serum LDL and serum Triglycerides and decrease in serum HDL as compared to Normal group (Group A).

Effect of MEAB on serum oxidative markers

The results of Serum oxidative markers are shown in Table 3. There were a significant increase in serum catalase and significant reduction in serum Malondialdehyde (MDA) levels in MEAB treated groups and standard group compared to experimental control group.

Effect of MEAB on histopathology of aorta

Histopathological examination of abdominal aorta showed no abnormality in normal control group (Figure 1). Animals in experimental control group showed intimal thickening, internal layer is increased and cells appeared yellowish-white due to the accumulation of lipids. Separation of tunica media from intima and presence of foamy histiocytes indicated induction of atheroma (Figure 2). Animals in test and standard group showed reduced atherosclerotic injuries seen as a reduction in thickness of endothelial layer and a marked reduction in number of foam cells and inflammatory cells (Figure 3, 4) compared to experimental group.

Table 3: Effect of methanolic extract of *Alternanthera brasiliana* on serum oxidative markers.

Groups	Catalase (µmol/min/ml)	MDA (nmol/ml)
Normal Control	205.3±2.171	2.18±0.12
Experimental control	175.7±2.155[a]	9.26±0.37[a]
Test Drug (200 mg/kg)	237.3±4.492[b]	5.12±0.17[b]
Test Drug (400 mg/kg)	296.3±3.809[b]	4.38±0.14[b]
Standard Drug	244.7±2.565[b]	3.01±0.09[b]

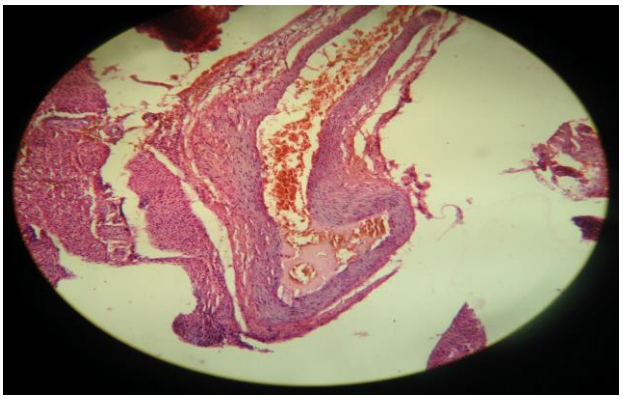

Figure 1: Normal.

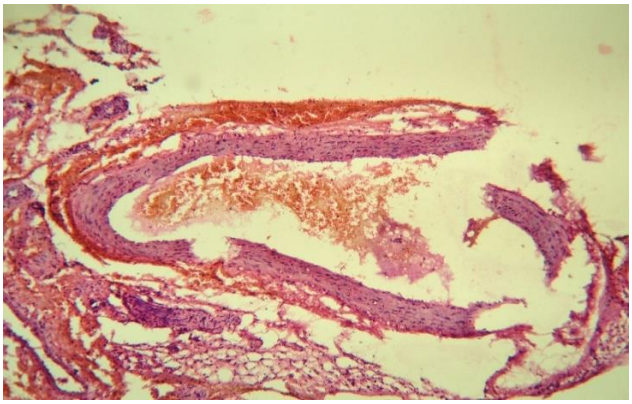

Figure 2: Experimental control.

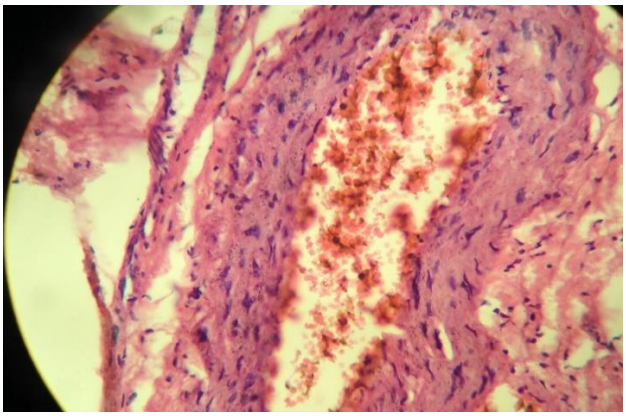

Figure 3: Test drug.

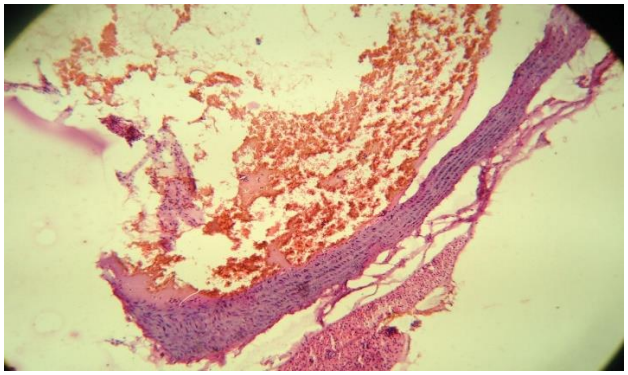

Figure 4: Standard drug.

DISCUSSION

Atherosclerosis is a complex multifactorial disease which develops in the arterial wall in response to various stimuli. Hyperlipidaemia is one of the important factors associated with atherosclerosis. Also, it is one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease and stroke. While studies were going on, a strong consensus emerged and became dominant in showing that chronic inflammation was a dominant mechanism through which atherosclerosis was initiated and progressed. Oxidation and inflammation have become intimately associated in the pathophysiology of atherogenesis.[21] Statins are the drugs of choice in lowering LDL-C and non HDL-C. They act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting step in the cholesterol synthesis. Decreased intracellular cholesterol lead to upregulation of LDL receptors, thereby enhancing removal of circulating LDL-C However, long term use of Statin may cause reversible rise in hepatic aminotransferase levels, myositis and even myopathy. Rhabdomyolysis is a rare side effect and clinical trials also demonstrated that statin may increase the risk of new onset diabetes.[22] So, attention is now directed to utilize natural plant product having antiatherosclerotic property.

Renuka P. Munshi et al, also developed an experimental model of hyperlipidaemia and insulin resistance using high fat and high sugar (HFHS) diet consisting of Indian Vanaspati ghee and coconut oil with fructose in the form of sugar for 6 weeks in rats. Their study showed a significant increase in serum lipids such as total, LDL and VLDL cholesterol and serum Triglycerides along with a decrease in HDL cholesterol. Prolonged consumption of HFHS diet enhances lipid peroxidation, disturbs antioxidant defence mechanism and also leads to delayed gastric emptying. An increase in serum lipid peroxidation is evaluated in terms of malondialdehyde (MDA) levels and decrease catalase levels.[23]

In our study, methanolic extract of *Alternanthera brasiliana* demonstrated a similar effect as compared to Atorvastatin with lowering of total cholesterol, LDL, triglycerides and an increase in HDL cholesterol. Serum

catalase level was increased significantly and MDA level was reduced markedly in the rats treated with the test drug. In histopathological studies, aorta in high fat diet group animals showed increase in intimal thickening and the spaces within the tunica intima and tunica media and formation of lipoidal layer in the intima of artery. This increase in space might be attributed to the free radicals that could damage the membranes and DNA of the endothelial cells in the tunica intima and smooth muscle cells in the tunica media.[24] MEAB treated group showed reduced extent of aortic atherosclerotic involvement in hypercholesterolemic rats. The results also indicate a significant improvement in the Atherogenic index (AI). AI indicates the deposition of foam cells or plague or fatty infiltration or lipid in the heart, coronaries, aorta, liver and kidneys. The higher the AI, the higher is the risk of above organs for oxidative damage and higher the cardiovascular risk.[25] MEAB contain high concentration of polyphenols and flavonoids. Several data suggest that flavonoids like Quercetin improve dyslipidaemia, decrease oxidative stress through stimulation of lipolysis activity and upregulate the adipocyte gene expression which increases the lipid β oxidation.[26] Flavonoids inhibit LDL oxidation by several mechanisms, (a) by directly scavenging some radical species, thus acting as chain breaking antioxidants, (b) by replenishing the limited supply of endogenous chain breaking antioxidant such as α- tocopherol, by donating hydrogen atoms to the oxidised forms and (c) by chelating divalent pro-oxidant transition metals such as iron and copper, thus preventing free radical formation.[27] The mechanism underlying inhibition of aortic atherosclerotic progression of *Alternanthera brasiliana* leaves extract may be due to the free radical scavenging mechanism attributed by the presence of flavonoids and tannins. Increase in serum cholesterol and LDL-C and consequent oxidation of LDL-C are essential steps for the development of atherosclerotic plagues. It is now well established that inhibition of oxidative stress and lipid peroxidation could have beneficial effects on regression of atherogenesis.[28]

CONCLUSION

Thus, from this study it can be concluded that methanolic extract of leaves of *Alternanthera brasiliana* at a dose of 200 mg/kg and 400 mg/kg body weight orally showed significant antihyperlipidemic and antiatherogenic effect in high fat diet rat model; probable mechanism of action may be the inhibition of oxidative stress and lipid peroxidation due to the free radical scavenging and antioxidant property of flavonoids and polyphenols present in the plant extract. But further study on the exact mechanism and isolation of the active constituents are needed.

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