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Research Article
Murraya exotica Protects Atherogenesis in Diet-induced Hypercholesterolemic Rats by Antioxidant and Antihyperlipidemic Activity

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Abstract

Background and Objective: Atherosclerosis is the major cause of death in developing country and complete management of it was not able to achieve with the available treatment options. Thus, present study evaluates the anti-atherosclerosis activity of *Murraya exotica (ME)* in hypercholesterolemic rats. **Materials and Methods:** Hypercholesterolemia was induced by feeding atherosclerosis feed to the rats for the period of 4 weeks and rats were treated with ME 100 and 200 mg kg^{-1} during the induction of hypercholesterolemia. Body weight, food and water intake was estimated every week till the end of protocol. However, at the end of protocol lipid profile in the blood and markers of liver and endothelial function and oxidative stress parameters were assessed in tissue homogenate. **Results:** Data of this study suggested that treatment with ME significantly decreases the percentage gain in the body weight of rat and food intake than negative control group. It was also observed that altered level of lipid profile get attenuated in ME treated group. In addition treatment with ME attenuates the altered level of oxidative stress parameters and markers of endothelial and liver dysfunction in hypercholesterolemic rats. Moreover, ME attenuates the markers of endothelial dysfunction in hypercholesterolemic rats. **Conclusion:** This study revealed that treatment with ME produces anti-atherosclerogenic activity on the basis of its hyperlipidemic and antioxidant effect in hypercholesterolemic rats.

Key words: *Murraya exotica*, hypercholesterolemia, atherosclerosis, antioxidant, blood lipid profile

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Atherosclerosis is one of the major cause of mortality in developing countries[1]. In atherosclerosis arteries become harden or plaque formation occurs, which is characterized deposition of cholesterol in the arteries, inflammation of vessels and dysfunction of endothelium[2]. All these event results in the obstruction of normal blood flow and thereby cause ischemia to the organs[3]. There were several factors that contribute in the development of atherosclerosis such as increased oxidative stress and hyperlipidemia. Hyperlipidemia is associated with several metabolic disorders in which cholesterol and triglyceride level enhanced[4]. Atherosclerosis causes development of several complications associated with cardiovascular disease such as heart attack, ischemic heart disease, aneurysms viz[5]. There were many factors that contribute in the development of cardiovascular events such as enhanced lipid level, blood pressure and smoking[6]. These factors were modifiable by improving life style, proper diet and avoiding smoking. Although, complete management of it was not able to achieve modification and treatment of it is still a big task for the health sector.

From last few decades medicinal plants used as alternative medicine for the management of several chronic disorders. *Murraya exotica* (Rutaceae) is traditionally used in China as herbal medicine and common name in Chinese is Chinese box[7]. The ME reported to posses anti-cancer, anti-diarrheal, anti-microbial and anti-thyroid property[8-10]. Photochemical study on ME reveals the presence of several chemical constituents such as phytosterols, flavonoids, alkaloids and coumarins[11]. An alkaloid isolated from the leaves of ME named as Yuehchukene reported to posses anti-cancer and anti-diarrheal activity[12]. Moreover, Murrangatin is a isolated coumarin from ME inhibits the epoxide hydrolase enzyme and epoxide hydrolase enzyme inhibitors effectively used in the management of diabetic complications[13]. Thus present investigation was done to evaluate the anti-atherosclerogenic effect of *Murraya exotica* in hypercholesterolemic rats.

MATERIALS AND METHODS

Animals: Sprague-Dawley rats (body weight: 200-250 g and age: 4 weeks) were used in the given investigation. All the rats were housed under a controlled condition specified as per the guidelines. All the experiments used in the given study are approved by Animal Ethical Committee of Huazhong University of Science and Technology, China (IAEC/HUST/2016/12). The study was performed in the lab of Huazhong University of Science and Technology, China during the period of August, 2016-February, 2017.

Plant extraction: Leaves of *Murraya exotica* was procured from local botanist of China and authenticated by Dr. Wang Lee, Department of Botany, Huazhong University of Science and Technology, China. Leaves of ME were dried under the shade and then power the leaves coarsely. Coarsely powder leaves of ME were placed in the glass jar with methanol for the duration of 5 days. Thereafter complete mixture was filtered and dried it with evaporator. Percentage yield of methanolic extract of of ME was found to be 8.7% w/w.

Induction of hypercholesterolemia: Hypercholesterolemia was produced by high fat diet as per the previously reported method. All the animals were divided into 4 different groups like control group which receives standard diet, negative control group which receives high fat diet (diet with 0.5% thiouracil, 1% cholic acid and 4% cholesterol), ME (100 and 200 mg kg^{-1}) which receives high fat diet with ME 100 and 200 mg kg^{-1} orally for the duration of 4 weeks.

Consumption of water, intake of water and body weight: Consumption of water and food intake was estimated on the first day of protocol and thereafter at the end of every week. Body weight of all the animals was estimated initially before the treatment and also estimated the same after the interval of each week.

Biochemical estimation: All the animals were anesthetized by anesthetic ether and blood was collected from the retro orbital plexus of each animal for the estimation of biochemical parameters. Thereafter, all the animals were sacrificed by cervical dislocation and aorta and liver was isolated.

Preparation of tissue homogenate: Tissue homogenate of isolated organ was prepared as per the previously described method. Homogenate of aorta was prepared in 100 mM KH_2PO_4 buffer and supernatant was used for biochemical estimation by centrifuging it at 12000 rpm for the duration of 30 min. However, liver tissue was homogenate in 1.17% of KCl and centrifuges the homogenate at 800 rpm for the duration of 5 min to get the supernatant.

Estimation of biochemical parameters: Biochemical parameters such as lipid profile and total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and

serum alkaline phosphatase (ALP) were determined by using autoanalyzer. Moreover, production of NO was estimated in the tissue homogenate of aorta. Here in, 100 µL of supernatant tissue homogenate 100 µL of Griess reagent was added and kept it for incubation at room temperature for the duration of 10 min micro-plate reader was used to estimate the optical density at 550 nm.

Activity of tissue enzyme was also estimated in the tissue homogenate of liver and aorta. Activity of superoxide dismutase and catalase was estimated by determining the change in the colour at 560 and 620 nm using UV spectroscopy. However, level of malonyldialdehyde (MDA) was estimated by determining the absorbance at 532 nm.

Statistical analysis: Statistical analysis reported in the form of Mean±SD. One way analysis of variance (ANOVA) was performed for the comparison of results and $p<0.05$ was significant value. GraphPad Prism version 5.0 for Windows (San Diego, CA, USA) was used to analyze the results.

RESULTS

Effect of ME on consumption of water, intake of water and body weight: Effect of ME on consumption of water, intake of water and body weight in hypercholesterolemic rats was shown in Fig. 1. Observation of the study suggested that percentage of weight gain by the rat of negative control group significantly enhanced compared to control group. However treatment with ME significantly reduces the percentage of weight gain and food intake by rats than negative control group during the treatment protocol. There was significant increase in the consumption of water in ME treated group compared to negative control group with the treatment protocol i.e., up to 4th week of treatment protocol.

Effect of ME on lipid profile: Data of the study suggest that there was significant increase in the total cholesterol (145.6±5.22) and triglyceride level (102.4±3.82) in negative control group of rats than control group. However, treatment

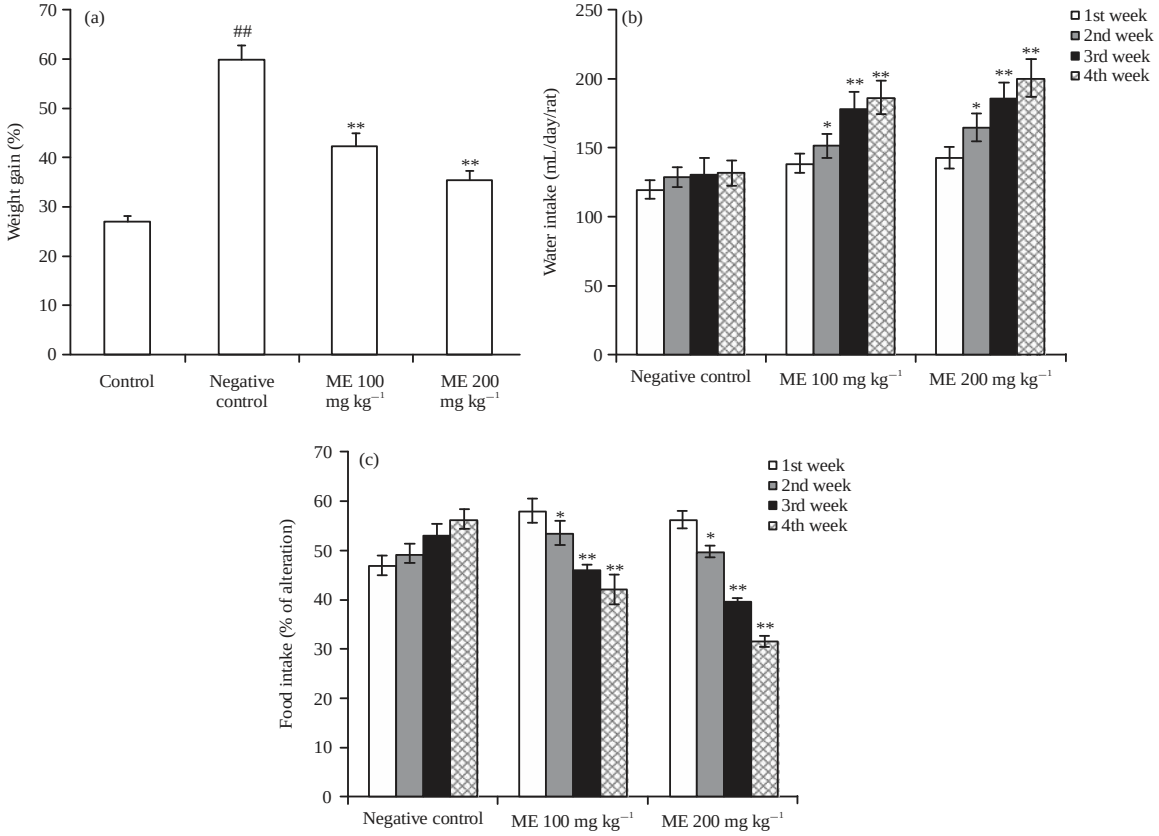

Fig. 1(a-c): Effect of ME on the different parameters in hypercholesterolemic rats, (a) Weight gain, (b) Consumption of water and (c) Food intake

Mean±SD (n = 10), ##p<0.01 compared to control, *p<0.05, **p<0.01 compared to negative control

Table 1: Effect of ME on lipid profile in hypercholesterolemic rats

Groups	Triglyceride (mg dL^{-1})	Total cholesterol (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})	Atherogenic index
Control	65.76±1.47	72.39±2.83	32.48±0.62	8.36±0.28	14.27±0.42	1.08±0.08
Negative control	102.40±3.82##	145.60±5.22##	11.91±0.21##	28.25±1.13##	39.82±2.12##	12.46±0.42##
ME 100 mg kg^{-1}	82.40±2.15**	91.82±2.40**	20.63±1.07**	18.28±0.82**	21.73±0.93**	4.25±0.15**
ME 200 mg kg^{-1}	70.90±3.69**	76.41±1.37**	29.42±1.64**	10.04±0.32**	18.26±0.56**	2.18±0.12**

Mean±SD (n = 10), ##p<0.01 compared to control, *p<0.05, **p<0.01 compared to negative control

Table 2: Effect of ME on the oxidative stress parameters in tissue homogenate in hypercholesterolemic rats

	Liver			Aorta		
Groups	SOD (unit/mg^{-1}/ protein)	CAT (unit/mg^{-1}/ protein)	MDA (μM/100 g of tissue)	SOD (unit/mg^{-1}/ protein)	CAT (unit/mg^{-1}/ protein)	MDA (μM/100 g of tissue)
Control	2.15±0.14	3.98±0.40	3.25±0.30	2.98±0.40	1.82±0.20	1.91±0.13
Negative control	7.93±0.91##	0.19±0.03##	12.82±1.40##	10.82±1.12##	0.36±0.04##	10.42±1.60##
ME 100 mg kg^{-1}	4.32±0.28**	1.07±0.11**	8.37±1.10**	6.23±1.07**	0.89±0.07**	8.82±1.20*
ME 200 mg kg^{-1}	3.61±0.11**	2.79±0.14**	6.51±0.73**	4.72±0.26**	1.12±0.11**	7.19±0.49**

Mean±SD (n = 10), ##p<0.01 compared to control, *p<0.05, **p<0.01 compared to negative control

Table 3: Effect of ME on the biochemical parameter in hypercholesterolemic rats

Groups	Bilirubin (mg dL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})
Control	0.34±0.02	24.39±2.17	27.11±1.21	43.77±2.17
Negative control	6.14±0.32##	48.92±3.98##	54.92±3.48##	94.52±6.42##
ME 100 (mg kg^{-1})	3.27±0.21**	32.45±1.35**	42.37±3.91**	61.09±5.81**
ME 200 (mg kg^{-1})	2.04±0.12**	27.33±1.33**	34.68±2.12**	54.76±3.82**

Mean±SD (n = 10), ##p<0.01 compared to control, *p<0.05, **p<0.01 compared to negative control

with ME significantly reduces the level of total cholesterol and triglyceride in the blood of hypercholesterolemic rats than negative control group. In addition to it, level of HDL was significantly enhanced and LDL, VLDL and atherogenic index was significantly decreased in ME treated group of rats than negative control group. This attenuation of altered lipid profile of hypercholesterolemic rats was achieved by treatment with ME in a dose dependent manner (Table 1).

Effect of ME on the parameters of oxidative stress: Effect of ME on the oxidative stress parameters in tissue homogenate of liver and aorta of hypercholesterolemic rats was given in Table 2. It was observed that treatment with ME significantly decreases the activity of SOD and enhances the activity of CAT in the tissue homogenate of liver and aorta of hypercholesterolemic rats than negative control group of rats. However, level of MDA was significantly decreases in the ME treated groups than negative control group of rats.

Effect of ME on biochemical parameters: Effect of ME on the biochemical parameter of liver function in hypercholesterolemic rats was shown in Table 3. Biochemical parameters such as bilirubin, ALP, AST and ALT was found to be significantly increases in ME treated group than control group of rats. However treatment with ME significantly decreases the level of bilirubin, ALP, AST and ALT than negative control group.

Effect of ME on the endothelial dysfunction markers: It was observed that markers of endothelial dysfunction such as NO, fibrinogen and PLT get altered in the negative control group (Fig. 2a-c). There was significant decrease in the level of fibrinogen and PLT in ME treated group than negative control group. Level of NO was found to be significantly enhanced in ME treated group than negative control group in a dose dependent manner (Fig. 2).

DISCUSSION

Present study evaluates the anti-atherosclerotic activity of ME in diet induced hypercholesterolemic rats. In this report atherogenesis was produced by diet induced hypercholesterolemia. Effect of ME was assessed by estimating the lipid level in the blood and parameters of oxidative stress in the liver and aorta tissue homogenate. In addition markers of endothelial and liver dysfunction were also estimated.

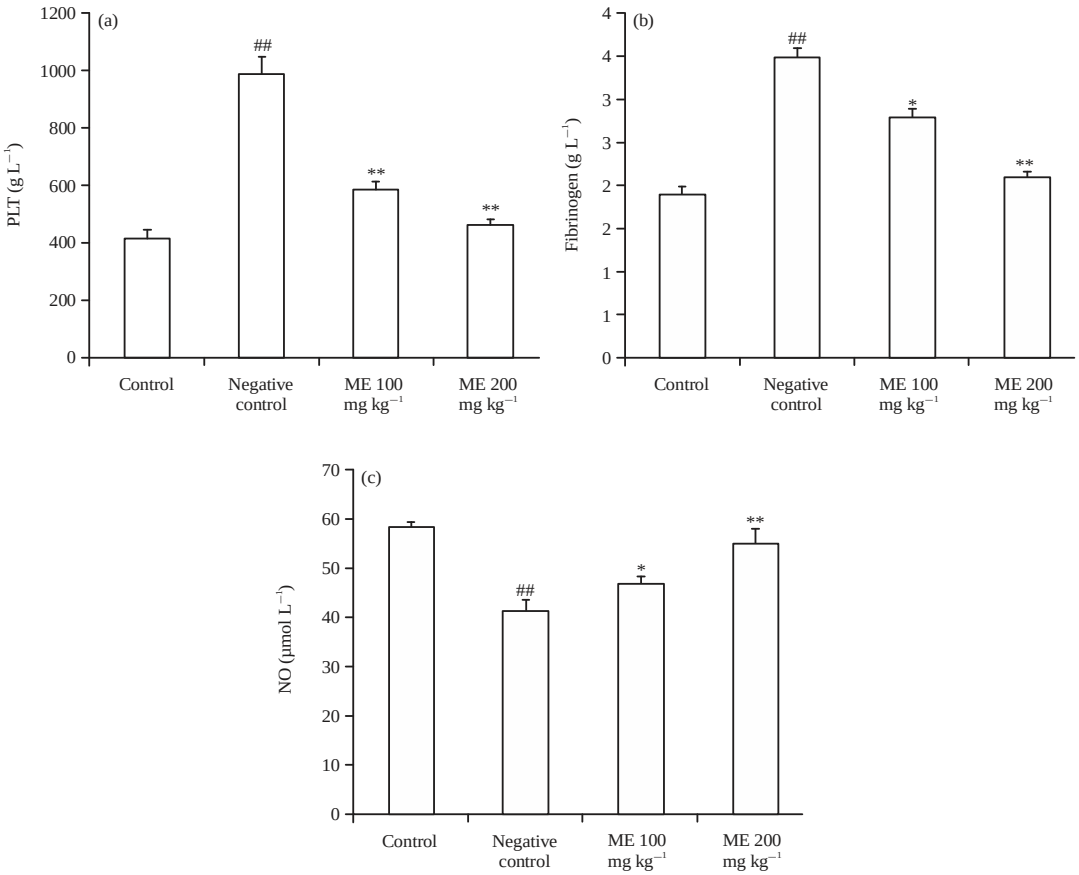

Fig. 2(a-c): Effect on the markers of endothelial dysfunction in hypercholesterolemic rats, (a) PLT, (b) Fibrinogen and (c) NO
Mean±SD (n = 10), ##p<0.01 compared to control, *p<0.05, **p<0.01 compared to negative control

Atherosclerosis can be prevented by protecting endothelial injury through decrease in the accumulation of cholesterol to the wall of the vessels[14]. Several factors that were contributed in the development of atherosclerosis such as oxidative stress and hyperlipidemia by altering the endothelial function. Reported study suggested that intake of high fat diet i.e., atherosclerosis diet improves the weight gain in the rats[15]. Present investigation reported that treatment of ME decreases the diet and percentage of weight gain and increases the consumption of water in hypercholesteromic rats than negative control group.

Data of present study supports the previously reported reports. Altered lipid profile is one of the major causes of cardiovascular diseases as increased level of lipid plays important role for the development of chronic disorders such as cardiovascular disorders[16]. In addition, it also contributes in the endothelial injury by enhancing the level of MDA and free radicals[17]. Result of this study suggested that treatment with ME attenuates the altered level of lipid in the hypercholesterolemic rats. Here activity of oxidative enzymes and level of MDA in the tissue homogenate of liver and aorta was attenuated with the ME in hypercholesterolemic rats.

Moreover, markers of endothelial function such as NO, fibrinogen and serum platelet get altered in atherosclerosis[18]. In the development of atherosclerosis activation of platelet played a vital role as it releases some of the substances that results in the accumulation of platelet and help in the formation of thrombi in the blood vessel[19]. Thus reduction in the concentration of platelet helps in the prevention of atherosclerosis. ME contain several chemical constituents such as coumarin and coumarin is reported to posses strong anti-inflammatory, anticoagulant and antioxidant property. Coumarin shows anticoagulant activity based on its antiplatelet activity[20]. Present study also revealed that treatment with ME significantly attenuates the markers of endothelial function in hypercholesterolemic rats.

CONCLUSION

Present study concludes that treatment with ME attenuates the atherosclerosis in diet-induced hypercholesterolemic rats by reducing the oxidative stress and hyperlipidemia.

SIGNIFICANT STATEMENT

This study discovers the protective effect of *Murraya exotica* against atherogenesis in diet-induced hypercholesterolemic rats. This investigation provides the alternative treatment for the management of atherosclerosis on the basis of its antihyperlipidemic and antioxidant activity.

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