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Research Article

PREVENTIVE EFFECT OF AEGLE MARMELOS FRUIT EXTRACT ON ISOPROTERENOL INDUCED ATHEROGENESIS IN RATS

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ABSTRACT

Aim of the Study: To evaluate the preventive role of Aegle marmelos fruit extract (AMFE) on Isoproterenol (ISO) induced atherogenesis in rats.

Materials and Methods: Atherogenesis was induced in Wister albino rats by administration of ISO (85 mg/kg B.W. for 2 consecutive days). Aqueous extract of AMFE was administered orally to the experimental animals at the dose of 150 mg/kg/day for 45 days. Preventive effect was assessed by the estimation of serum and cardiac tissue lipid profile, heart co-efficient, A.I, total proteins and A/G ratio.

Results: The presence of various active principles like alkaloids, terpenoids, tannins, flavonoids and sterols AMFE exhibited protective effect against ISO induced atherogenesis in rats. The heart co-efficient was significantly decreased in AMFE rats compared to ISO administered rats.

Conclusion: The study revealed that the AMFE possesses strong antiatherogenic property against ISO adverse effects on heart.

Keywords: *Aegle marmelos*; Atherogenesis; Isoproterenol; Lipid profile.

INTRODUCTION

Research in cardiovascular pharmacology in the past few years has been mainly focused on hypolipidemic agents including herbal drugs. Atherosclerosis is a complex disease underlying cause of heart attack, stroke, and peripheral

vascular disease, is a main cause of morbidity and mortality worldwide, characterized by an excessive inflammatory, fibro-fatty, proliferative response to damage of the artery wall involving several cell types, particularly smooth muscle cells, monocyte-derived macrophages, T-lymphocyte and platelets[1]. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of atherosclerosis and progression of atherosclerotic lesions[2]. One of the initial events in the development of atherosclerosis is the accumulation of cells containing excess lipids within the arterial wall. A lot of studies have found that risk for atherosclerosis is higher in subjects with hyperlipidemia and decrease following phytomedicine and or dietary supplementation with antioxidants. Similar observations have been reported in animal models of hyperlipidaemia[3]. However there is no data available on preventive role of AMFE to claim antiatherogenic potency in ISO model.

ISO is a potent synthetic catecholamine which causes the development of infarct-like lesions when injected into animals. These lesions are morphologically similar to those of coagulative myocytolysis or myofibrillar degeneration, which is one of the signs of acute myocardial infarction in man[4]. Therefore, isoproterenol-induced myocardial injury serves as a well standardized model to study the beneficial effects of many natural compounds and synthetic drugs.

In the recent years, there is growing interest in herbal medicine all over the world, as they have little or no side effects. Traditional medicinal plants having cardioprotective property can prove to be useful source for the development of new cardioprotective agents as pharmaceutical entities or simple dietary adjuvant to existing therapies. *Aegle marmelos* (L.) belongs to family Rutaceae. It grown throughout the India as well as in Burma, Bangladesh and Pakistan. Its medicinal properties have been described in the ancient medical treatise in Sanskrit, Charka Samhita. It is used in several indigenous systems of medicine in India[5]. The whole plant and various parts (root, root bark, stem bark, leaf and fruit) are all used in traditional medicines[6]. The extracts of A. marmelos have demonstrated anti-inflammatory[7], antioxidant[5], antihyperlipidemic[8], radioprotective[9], antimicrobial[10], antiparasitic[11], hypoglycemic and antihyperglycemic[12] activities. Since heart diseases has posed a great challenge in front of us it

has become essential to discover plant based drugs which are safe and very useful in cardiac disease. Despite its extensive medicinal use no information is available related to its use on ISO induced atherogenesis status in rats. Hence the present work investigates the therapeutic efficacy of AMFE to prevent ISO-induced atherogenesis in rats.

MATERIALS AND METHODS

Chemicals

ISO (D,L-4-(2-(isopropylamino)-1-hydroxyethyl) pyrocatechol) used to induce cardiac damage was purchased from Sigma-Aldrich (USA). All other chemicals were of analytical grade and were supplied by Sisco Research Laboratories (Mumbai, India).

Preparation of *Aegle Marmelos* Unripe Fruit Extract

Fresh Aegle marmelos unripe fruits were collected around Sri Krishnadevaraya University premises and verified with specimens available at the Botanical Herbarium, Dept of Botany, Sri Krishnadevaraya University, Anantapur, A.P, India, with the help of Prof. T. Pullaiah. Fruits were air dried and powdered in an electric blender. The powder was boiled in distilled water. After filtration through whatmann No.40 filter paper the extract was evaporated to dryness by slow heating and continuous stirring in a water bath. The dark brown residue left behind was collected and was used as the drug. The extract was tested for total phenolic compounds (26%) including its flavonoid and tannoid content. The extract was dissolved in distilled water prior to administration.

Experimental Design

Adult male albino Wistar rats weighing 150-180g were divided in to four groups of eight animals in each, procured from National Institute of Nutrition, Hyderabad, India. Atherogenesis was induced by s.c., injection of IPL 85 mg/kg b.wt, dissolved in physiological saline, twice at an interval of 24h for two days[13]. AMFE was pretreated to rats orally at a dose of 150 mg/kg b.wt/day for a period of 45 days, and then administered IPL at the dose of 85 mg/kg b.wt. All the groups were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The extract dose used in this study was fixed based on the acute oral toxicity study as well as mentioned in the literature on the pharmacological effects. The present study was approved by Sri Krishnadevaraya University institutional

animal ethical committee. At the end of the experimental period, the rats in each group were fated overnight and then sacrificed by cervical dislocation. Blood was collected with and without anticoagulant by cardiac puncture, plasma and serum was separated for various biochemical estimations. The heart was excised immediately from the animals, washed off blood with ice-chilled saline. A known weight of the heart tissue was homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical estimations.

Biochemical analysis

Body weight of control and experimental groups were recorded prior to treatment and then post treatment. Heart weight is recorded at the end of experimental period immediately after dissection of rats. Plasma total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C)[14], triglycerides (TG)[15], glucose[16], carried using commercial diagnostic kits (Qualigens Diagnostics, Mumbai, India). The low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C)[17], atherosclerotic index (AI) were calculated as described previously[18]. The plasma total phospholipids[19], free fatty acids[20], cardiac tissue TC[21], TG[22], PL[19], were analyzed.

Statistical analysis

All the grouped data were statistically analyzed and significance of changes caused by the various treatments was determined using one-way analysis of variance followed by DMR test. The level of statistical significance was set at p 0.05.

RESULTS

Table 1 depicts the levels of plasma TC, HDL-C, LDL-C, VLDL-C, TG, PL, FFA, AI, total proteins and A/G ratio in control and different experimental groups. ISO administration is associated with pronounced metabolic abnormalities in lipid profile. In ISO administered rats the levels of TC, LDL-C, VLDL-C, TG, PL, FFA and AI were increased significantly (p 0.05) and those of HDL-C decreased significantly (p 0.05), compared with control rats. The rats pretreated with AMFE showed significant (p 0.05) decrease in TC, LDL-C, VLDL-C, TG, PL, FFA,

glucose and AI those of HDL-C were increased significantly (p□0.05) when compared to ISO administered rats and showed no significant change when compared to controls.

The cardiac tissue lipid profile of control and different experimental groups is presented in Table 2. In ISO administered group, the cardiac tissue TC, TG, FFA and TC to PL ratio were significantly (p□0.05) increased and a significant (p□0.05) decrease in PL compared to control. Rats pretreated with AMFE showed significant (p□0.05) decrease in TC, TG, FFA, TC to PL ratio with concomitant increase in PL, compared to ISO administered rats.

Fig. 1 (a) and (b) shows the initial and final body weight of control and experimental animals. The final body weight of ISO administered rats was significantly (p□0.05) decreased, compared to control where as in AMFE pretreated rats the same was increased significantly (p□0.05), compared to ISO administered rats.

Fig. 2 (a) and (b) shows the heart weight and heart coefficient of control and experimental animals. The heart weight and heart coefficient values of ISO administered rats were significantly (p□0.05) increased, compared to control and the same values were decreased significantly (p□0.05) in AMFE pretreated rats, compared to ISO administered rats.

DISCUSSION

Lipids play an important role in CVD, not only by way of hyperlipidaemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of cellular membranes. The increased levels of total cholesterol, LDL-C, VLDL-C and TG in ISO administered rats could be due to enhanced lipid biosynthesis by cardiac cAMP and decreased activity of lipoprotein lipase[23]. Further hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL, protein glycation, glucose-autooxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects. Besides, increased circulating cholesterol, LDL, and VLDL would have led to the uptake of cholesterol and phospholipids by platelet membranes to decrease platelet membrane fluidity, and resulted in platelet activation and aggregation, which are both primary and secondary effects on the initiation and progression of atherosclerosis and on thrombotic events[24]. Interestingly, we observed that elevated LDL-C level in ISO

administered rats. It is well known that modest increase in LDL-C show detrimental effects related to cardiovascular diseases. The increased levels of PL and free fatty acids in IPL administered rats could be due to combined effect of membrane peroxidation and phospholipase A_2[25]. Present findings were in agreement with earlier studies[26].

A decrease in the levels of plasma HDL-C observed by us in ISO administered rats could be due to increased free radical production by ISO. A decrease in serum protein is usually as a result of a fall in albumin or sometimes gamma globulin[27]. Pretreatment with AMFE showed significant increase in the levels of HDL-C, compared to ISO administered rats. This could be due free radicals scavenging property of phytochemicals. HDL may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL. In the present study increased HDL and decreased LDL levels in rats pretreated with AMFE reveals its protective effect in ISO administered rats.

Atherogenic index has been reported to be associated with atherosclerosis and to be a discriminator for presence and severity of coronary artery disease[28]. Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher is the risk of the above organs for oxidative damage[29]. It is well known that the ratio of difference between TC and HDL-C to HDL-C, greater than 4.5 is considered a powerful predictor of coronary artery disease[30]. Thus, the obtained results clearly indicates the atherogenic effect of ISO with a A.I value of 7.16, which is greater than to normal value[31]. The decrease in the A.I values of AMFE pretreated rats suggesting antiatherogenic potential of AMFE which is in agreement with the earlier report[32]. The antihyperlipidemic effect of AMFE could be inhibition of lipid absorption by saponins and tannins in the aqueous extract[33] and/or activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors. This effect also due to the presence of flavonoids which have effects on cholesterol metabolism[34].

Abnormalities in lipid profile are associated with increased risk of myocardial infarction. High level of circulating cholesterol and its accumulation in the heart tissue is usually accompanied by cardiovascular damage[35]. In the

present study ISO administered group showed increased levels of the tissue total cholesterol, TG, FFA and total cholesterol to phospholipids ratio and concomitant decrease in PL, compared to control. This could be due to enhanced lipid biosynthesis by cardiac cAMP. Intracellular calcium is an inducer of phospholipase-A2 which degrades membrane phospholipids and reported to be increased in ISO-induced MI[36]. The significant elevation noted in the levels of FFA in plasma and heart tissue of ISO-treated rats might also be due to enhanced breakdown of membrane phospholipids by the lipolytic action of phospholipase-A2[37] and transport of FFA liberated from adipose into the myocardium. Though heart can utilize FFA for its energy requirements, the excess FFA may be used for the synthesis of TG resulting in hypertriglyceridemia[38] as observed in the present study. Accelerated PL degradation in turn causes membrane dysfunction, resulting in cell injury and ultimate cell death. AMFE pretreatment significantly prevented the degradation of PL thereby increasing PL levels and decreasing the ratio of cholesterol/phospholipids (C/P). It might be due to decreased membrane lipolysis by decreasing the action of phospholipase-A2 through the depletion of intracellular calcium levels. Changes in membrane cholesterol content affect its fluidity, permeability to ions, activities of membrane bound enzymes, and increased degradation of phospholipids[39]. In this study, AMFE pretreatment restored the alterations of myocardial lipids to near normal levels, there by reducing the risk of CVD. This is an indication that the myocardial membrane is intact and not damaged. This could be due to the ability of AMFE to inhibit cAMP thereby maintaining the normal fluidity and less alteration in the structure and function of the myocardial membrane. The antihyperlipidemic effect of AMFE can be attributed to the presence of phytochemicals such as alkaloids, flavonoids, saponins and plant sterols.

The observed increase in the heart weight in ISO-administered rats might be due to the increased water content, oedematous intramuscular space and extensive necrosis of cardiac muscle fibers followed by the invasion of damaged tissues by the inflammatory cells. Oral pretreatment with AMFE significantly decreased the heart weight in ISO-administered rats which might be due to decreased necrosis of cardiac muscle and maintenance of normal architecture indicating the cardiotonic property of AMFE.

The heart-coefficient (heart weight/body weight x 100) is an index of cardiac hypertrophy. Cardiac hypertrophy is a general term signifying an increased workload and is characterized with an increase in cardiac mass in response to applied stimulus. Increased cardiac hypertrophy leads to CHF that appears as the final phase of most cardiac diseases[40]. The present study showed increased hypertrophy in ISO administered rats. This could be due to the increased water content, oedematous intramuscular space and extensive necrosis of cardiac muscle fibers followed by the invasion of damaged tissues by the inflammatory cells. The rats pretreated with AMFE offer protection against ISO induced cardiac hypertrophy.

CONCLUSION

In conclusion, the present study reveal that administration of AMFE for 45 days may prevent ISO induced atherogenesis due to the presence of various phytochemicals with diverse action in ISO induced atherogenesis.

Table-1: Effect of AMFE on plasma lipid profile in ISO-induced rats.

Parameter	Control	AMFE	ISO	AMFE + ISO
Total cholesterol	85.7 ± 0.20^{a}	90.3 ± 0.21^{b}	125.7 ± 0.26^{c}	95.2 ± 0.24^{a}
HDL-cholesterol	24.3 ± 0.27^{a}	26.3 ± 2.64^{a}	15.4 ± 0.28^{b}	25.3 ± 0.31^{c}
LDL- cholesterol	52.8 ± 1.47^{a}	56.2 ± 2.16^{a}	96.2 ± 0.83^{b}	60.9 ± 1.90^{c}
VLDL- cholesterol	8.5 ± 0.26^{a}	8.4 ± 0.48^{a}	14.2 ± 1.40^{b}	8.8 ± 1.26^{c}
Triglycerides	42.7 ± 0.06^{a}	42.2 ± 1.42^{a}	70.6 ± 1.29^{b}	44.1 ± 0.25^{a}
Phospholipids	75.8 ± 1.65^{a}	78.9 ± 2.50^{a}	98.5 ± 0.20^{b}	81.8 ± 0.96^{a}
Free fatty acids#	28.3 ± 1.86^{a}	25.4 ± 3.75^{a}	49.6 ± 1.62^{b}	31.1 ± 2.18^{a}
Atherogenic index	2.52 ± 0.04^{a}	2.43 ± 0.13^{a}	7.16 ± 0.16^{b}	2.75 ± 0.08^{b}

Values are mean ± SEM of eight rats in each group. ^{abc}Means in the same row not sharing a common superscript are significantly different ($p<0.05$) among groups. *values are expressed as mg/dl, # values are expressed as mEq/L.

Table-2: Effect of AMFE on cardiac tissue lipid profile in ISO-induced rats.

Parameter	Control	AMFE	ISO	AMFE + ISO
Total cholesterol	4.98 ± 0.21[a]	5.17 ± 0.03[a]	9.30 ± 0.11[b]	6.10 ± 0.09[a]
Triglycerides	4.32 ± 0.07[a]	4.40 ± 0.14[a]	7.43 ± 0.13[b]	5.40 ± 0.12[c]
Phospholipids	28.1 ± 0.25[a]	28.7 ± 0.19[a]	17.2 ± 0.58[b]	28.4 ± 0.16[a]
Free fatty acids	0.33 ± 0.06[a]	0.36 ± 0.02[a]	0.55 ± 0.02[b]	0.38 ± 0.04[a]
C/P ratio	0.17 ± 0.01[a]	0.18 ± 0.04[a]	0.53 ± 0.01[b]	0.21 ± 0.02[a]

Values are mean ± SEM for eight rats in each group. [a,b,c] Values in the same row not sharing a common superscript differ significantly (p 0.05) with each other. All the values are expressed as mg/g wet tissue.

Fig 1 (a)

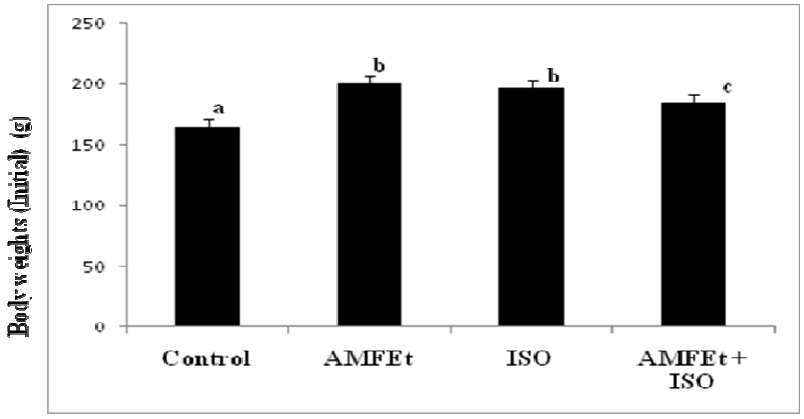


Fig 1 (b)

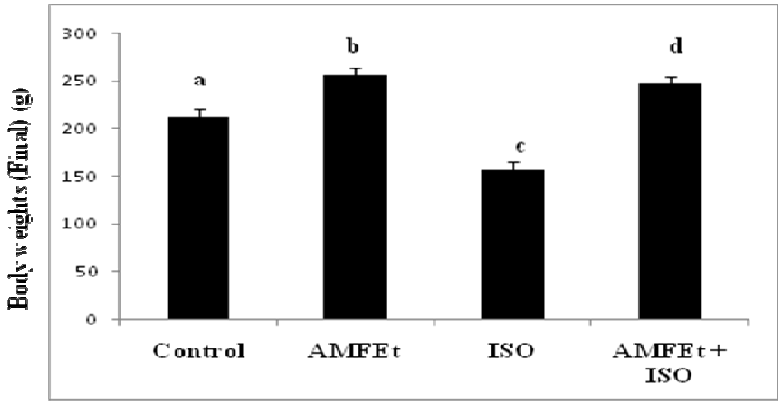


Fig 2 (a).

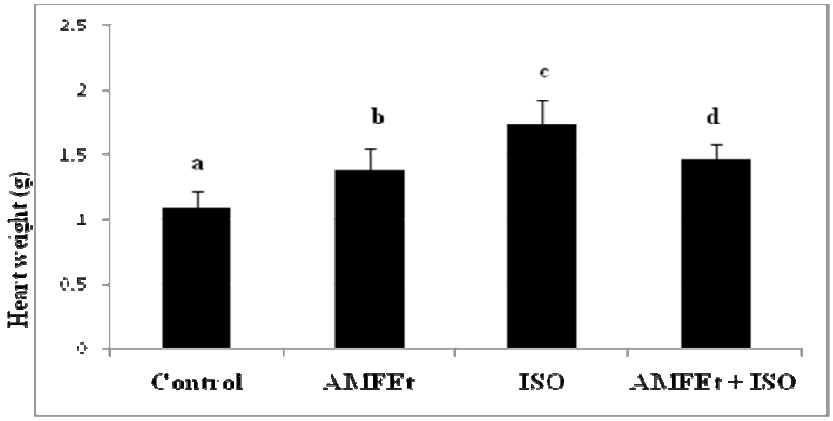
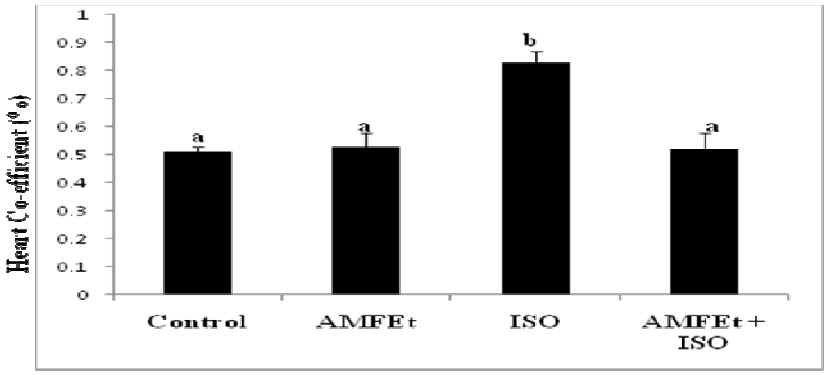


Fig 2 (b)



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