Comparative effects of *Aegle marmelos* extract and alpha-tocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction

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ABSTRACT

Introduction: We demonstrate the effect of *Aegle marmelos* leaf extract (AMLEt) and alpha-tocopherol on plasma lipids, lipid peroxides and marker enzymes in rats with isoproterenol (ISO)-induced myocardial infarction.

Methods: Rats were pre-treated orally for 35 days with different doses of an aqueous AMLEt (50 mg/kg, 100 mg/kg and 200 mg/kg) prior to ISO-induced myocardial infarction. The effects on creatine kinase, lactate dehydrogenase, plasma thiobarbituric acid reactive substances, lipid hydroperoxides, serum lipids and lipoproteins were studied.

Results: Pre-treatment with AMLEt at doses of 100 mg/kg and 200 mg/kg bodyweight for 35 days showed a significant effect on the activities of marker enzymes, lipid peroxides, lipids, lipoproteins and antioxidant enzymes in ISO-treated rats. The effect of AMLEt 200 mg/kg was found to be equal to the effect of alpha-tocopherol 60 mg/kg.

Conclusion: *Aegle marmelos* leaves possess antihyperlipidaemic effect in rats with ISO-induced myocardial infarction.

Keywords: *Aegle marmelos*, isoproterenol, lipids, lipoproteins, myocardial infarction

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INTRODUCTION

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, causes severe oxidative stress in the myocardium, resulting in infarct-like necrosis of the heart muscle with an increase in the levels of lipids in the myocardium[1]. Free radical generation and lipid peroxidation could be involved in ISO-induced cardiac damage[2]. The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial infarction[3], due to altered lipid metabolism.

Cardiovascular disease is the leading cause of mortality in many parts of the world. Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects. *Aegle marmelos* Correa, commonly known as Bael, is indigenous to India and is also found in Myanmar, Pakistan and Bangladesh. It is one of the most useful medicinal plants in India. Its stem, bark, root, leaves and fruits have medicinal value, and it has a long tradition as a form of herbal medicine. The leaves are widely used to treat diarrhoea, dysentery, heart palpitations and eye diseases[4].

Preliminary studies have shown that the leaves of *Aegle marmelos* possess a "cardiotonic" effect on frog's heart[5]. In our previous study, we have reported that AMLEt pre-treatment to ISO-treated rats increased the activities of superoxide dismutase, catalase and glutathione peroxidase in the heart tissue[6]. α-tocopherol, a well-known antioxidant, offers protection against experimental myocardial infarction induced by ISO[7]. This study examines the ameliorative effect of the leaves of *Aegle marmelos* on cardiac enzymes, lipid peroxides, serum lipids and lipoproteins.

METHODS

Forty-two female albino Wistar rats, weighing 150g to 170g, were used. They were housed in polypropylene cages (47 x 34 x 20 cm) lined with husk, changed every 24 hours under a 12 hour light/12 hour dark cycle at around 22ºC, and had free access to tap water and food. The rats were fed a standard pellet diet (Pranav Agro Industries Ltd, Pune, Maharashtra, India) comprising 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose and 55% nitrogen free extract (carbohydrates). The diet provided metabolisable energy of 3,000 kcal/kg. This study was approved by the Ethical Committee of Annamalai University.

ISO hydrochloride and digitonin were used (Sigma Chemical Company, St. Louis, MO, USA) and all other biochemicals and chemicals were of analytical grade. *Aegle marmelos* leaf extract (AMLEt) powder was authenticated by a botanist in

Table I. Effect of AMLEt and α-tocopherol on CK, LDH, TBARS, HP and serum lipid levels in rats with ISO-induced myocardial infarction.

Group	CK (IU/L)	LDH (IU/L)	Plasma TBARS (nM/ml)	Plasma HP (valuesx 10^{-5} mM/dL)	Cholesterol (mg/dL)						Lipids (mg/dL)		
					Total	Ester	Free	HDL	LDL	VLDL	Trigly-cerides	Free fatty acids	Phos-pholipids
I	231.4±22.5*	72.61±6.2*	2.11±0.18*	8.21±0.78*	95.2±8.3*	60.1±5.8*	35.2±2.3*	22.2±1.4*	65.3±4.4*	7.72±0.48*	38.6±3.4*	23.1±2.0*	80.5±6.4*
II	220.6±20.2*	71.8±6.0*	2.07±0.17*	8.15±0.72*	93.8±8.6*	59.4±5.4*	34.6±2.8*	22.4±1.3*	64.2±3.9*	7.21±0.42*	37.0±3.1*	22.6±1.7*	79.2±5.1*
III	384.0±33.1**	133.2±12.2**	4.89±0.43**	14.94±1.36**	124.0±12.1**	76.5±7.1**	48.7±4.3**	16.8±1.3**	94.6±8.0**	12.26±0.80**	61.3±5.4**	39.5±3.7**	96.6±8.6**
IV	362.1±35.8**	116.0±10.8**	4.68±0.41**	14.53±1.13**	120.5±10.9**	74.8±6.2**	46.8±4.1**	17.3±1.2**	91.3±8.1**	11.74±0.94**	58.7±5.2**	37.1±3.3**	94.5±8.3**
V	301.7±25.5#	109.7±8.5#	3.93±0.37#	12.86±1.03#	112.0±11.0#	70.4±6.0#	42.4±4.0#	18.6±1.6#	82.7±7.4#	10.72±0.88#	53.6±4.9#	33.7±2.6#	90.3±7.2#
VI	270.4±19.5##	88.5±6.9##	2.80±0.25##	10.13±1.07##	106.3±10.8##	67.3±6.3##	40.8±3.7##	19.3±1.7##	77.6±6.3##	9.30±0.86##	46.5±4.3##	29.1±2.2##	86.4±6.5##
VII	274.3±24.6##	92.0±7.5##	2.91±0.26##	10.20±0.81##	104.6±9.3##	66.8±5.9##	39.2±2.9##	19.9±1.3##	74.8±5.9##	9.16±0.76##	45.8±4.0##	28.3±2.1##	86.1±6.8##

Group I: control rats; Group II: rats treated with AMLEt (200 mg/kg); Group III: rats subcutaneously injected with ISO; Group IV: rats treated with AMLEt (50 mg/kg) + ISO; Group V: rats treated with AMLEt (100 mg/kg) + ISO; Group VI: rats treated with AMLEt (200 mg/kg) + ISO; Group VII: rats treated with α-tocopherol (60 mg/kg) + ISO. Values not sharing a common superscript (*, **, #, ##) differ significantly at $p<0.05$ (DMRT).

the Department of Botany, Annamalai University. It was suspended in distilled water prior to use. The rats were pre-treated orally with AMLEt for 35 days. After the treatment period, ISO hydrochloride (200 mg/kg) dissolved in normal saline was administered subcutaneously once a day for two days[7].

The rats were divided into seven groups of six rats each: control rats; rats orally treated with AMLEt (200 mg/kg) for 35 days[6]; rats subcutaneously injected with ISO (200 mg/kg dissolved in saline) once a day for two days; rats orally pre-treated with AMLEt (50 mg/kg) for 35 days and then subcutaneously injected with ISO (200 mg/kg) once a day for two days; rats orally pre-treated with AMLEt (100 mg/kg) for 35 days and then subcutaneously injected with ISO (200 mg/kg) once a day for two days; rats orally pre-treated with AMLEt (200 mg/kg) for 35 days and then subcutaneously injected with ISO (200 mg/kg) once a day for two days[6] and rats orally pre-treated with α-tocopherol (60 mg/kg) for 35 days and then subcutaneously injected with ISO (200 mg/kg) once a day for two days[7].

At the end of the study, all the rats were sacrificed on the morning of the third day after injection of ISO, by cervical decapitation after an overnight fast. Blood was collected and the plasma and serum were separated for various biochemical estimations. The activities of serum creatine kinase (CK) and lactate dehydrogenase (LDH) were assayed by the methods of Okinaka et al[8] and King[9], respectively. The levels of thiobarbituric acid reactive substances and lipid hydroperoxides were determined by the methods of Yagi[10] and Jiang et al[11], respectively. The levels of serum total, free and ester cholesterol, triglycerides, free fatty acids and phospholipids were estimated according to the methods of Zlatkis et al[12], Varley et al[13], Foster and Dunn[14], Falholt et al[15] and Zilversmit and Davis[16], respectively. Serum high density lipoprotein (HDL) was determined according to the technique of Wilson and Spiger[17]. LDL and very low density lipoprotein fractions were calculated as VLDL = triglycerides/5 and LDL = total cholesterol – (HDL cholesterol + VLDL cholesterol), respectively.

Statistical analysis was performed using one way analysis of variance followed by Duncan's Multiple Range test. Results were expressed as mean ± standard deviation for six rats in each group. A value of $p<0.05$ was considered statistically significant.

RESULTS

The CK and LDH levels, TBARS and HP, total, free and ester cholesterol levels, HDL, LDL and VLDL levels and also the levels of triglycerides, free fatty acids and phospholipids in serum in normal and experimental rats are shown in Table I. Rats treated with ISO showed a significant increase in these levels, but there was a significant decrease in HDL levels. Rats pre-treated with AMLEt (100 mg/kg, 200 mg/kg) for 35 days significantly decreased the levels of CK, LDH, TBARS, HP and other lipids and lipoproteins levels with subsequent increase in the level of HDL cholesterol to ISO-treated rats.

In all the parameters studied, AMLEt at a dose of 50 mg/kg showed a minor effect but it was not statistically significant. AMLEt at doses of 100 mg/kg and 200 mg/kg showed significant effects and AMLEt at a dose of 200 mg/kg was found to be more effective. Both AMLEt 200 mg/kg and α-tocopherol 60 mg/kg, were equally effective. Pre-treatment of AMLEt 200 mg/kg to normal rats did not show any significant effect.

DISCUSSION

ISO-administration in rats leads to increased lipid peroxidation and extensive necrosis of cell membranes. As a result of necrosis, the levels of diagnostic indicators

of myocardial infarction, such as CK and LDH, increase in the serum[6]. The activities of these enzymes were also increased in ISO-treated rats. This is due to leakage from the heart as a result of ISO-induced necrosis. AMLEt pre-treatment for 35 days decreased the activities of these enzyme markers. This could be due to the free radical scavenging property of the extract in the presence of antioxidative phytochemicals such as flavonoids, alkaloids, sterols, tannins and phlobatannins and flavonoid glycosides[18]. α-tocopherol pre-treatment, also decreased the activities of CK and LDH in ISO-treated rats. Since the effect of AMLEt showed a significant effect on selected marker enzymes, we investigated the anti-hyperlipidaemic effect.

Activated lipid peroxidation is an important pathogenic event in myocardial infarction, with TBARS and HP levels reflecting the major stages of the disease and its complications[19]. Excessive formation of free radicals may result in damage to the heart. A significant increase in TBARS and HP in plasma in ISO-administered rats were observed. This indicates excessive formation of free radicals and activation of lipid peroxidation. Hamberg et al[19] also reported an increase in TBARS and HP in ISO-treated rats. Pre-treatment with AMLEt in ISO-administered rats decreased the levels of TBARS and HP in plasma. α-tocopherol pre-treatment also showed a significant effect on TBARS and HP. Being a lipid-soluble chain-breaking antioxidant, α-tocopherol reacts with superoxide and lipid peroxyradicals to inhibit lipid peroxidation[7].

Lipids play an important role in cardiovascular disease, not only by way of hyperlipidaemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of cellular membranes. ISO-treated rats showed an increase in the levels of serum lipids[20]. Increased levels of total, free and ester cholesterol, triglycerides, phospholipids and free fatty acids in serum of rats treated with ISO were also observed. An increase in serum LDL and VLDL fractions, along with a decrease in HDL cholesterol, was also observed in ISO-treated rats. These changes could be due to enhanced lipid biosynthesis by cardiac cyclic adenosine mono phosphate[20]. High levels of LDL cholesterol show a positive correlation with myocardial infarction, whereas high levels of HDL cholesterol have a negative correlation[21]. An inverse relationship exists between HDL cholesterol and body cholesterol. HDL inhibits the uptake of LDL by the arterial wall and facilitates the transport of cholesterol from peripheral tissue to the liver, where it is catabolised and excreted from the body[22].

This study showed an increase in serum phospholipids in rats treated with ISO. Increased peroxidation of membrane phospholipids releases free fatty acids via phospholipase A2[23]. Free fatty acids are substances for microsomal lipid peroxidation. The higher levels of serum free fatty acids in animals treated with ISO is due to increased lipolysis[24]. Paritha and Devi have also reported an increase in serum phospholipids in ISO-treated rats[20]. Hypertriglyceridaemia and increased levels of cholesterol ester in serum might be responsible for altered cardiovascular functions[25]. Hypertriglyceridaemia observed in ISO-treated rats is due to a decrease in the activity of lipoprotein lipase in the heart, resulting in decreased uptake of triglycerides from the circulation[22].

Pre-treatment with AMLEt in ISO-treated rats showed significant effect on lipids. Hence, AMLEt offers protection against myocardial infarction induced by ISO. This might be due to effective quenching of free radicals. This prevents activation of the lipid peroxidation. α-tocopherol pre-treatment also showed a significant effect on lipids and lipoproteins. It has been reported that α-tocopherol protects against LDL oxidation, thereby inhibiting the development of atherosclerosis[20]. Administered at a dose of 200 mg/kg, AMLEt was more effective than a dose of 100 mg/kg. Both AMLEt 200 mg/kg and α-tocopherol 60 mg/kg were equally effective in ISO-treated rats. Further investigations are currently underway to find the mechanism of action and the chemical constituents responsible for the lipid-lowering property of AMLEt.

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