Antidyslipidaemic effect of Aegle marmelos Linn. fruit on Isoproterenol induced myocardial injury in rats

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Citation

Abstract
The present study was designed to verify the antidyslipidemic effect of Aegle marmelos Linn. unripe fruit aqueous extract (AMUFAEt) against isoproterenol (IPL) induced cardiac stressed rats. Rats were divided into four groups (of six each): group I of healthy controls, group II of AMUFAEt treated (150 mg/kg body weight, for 45 days), group III of IPL treated rats (85 mg/kg body weight, once a day for 2 days) and group IV of AMUFAEt treated (150 mg/kg body weight, for 45 days) and then IPL administered. The levels of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and phospholipids (PL) was significantly increased, while the levels of high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) decreased in serum of IPL treated rats. In AMUFAEt pretreated rats the dyslipidaemic effects of IPL were compensated to near normal levels. Blood glucose, protein levels were not significantly altered. The study shows that the extract has significant antidyslipidaemic effect.

INTRODUCTION
The Indian system of medicines, Viz Ayurveda, Siddha, Unani and Homeopathic system predominantly use plant based raw materials and most of their preparations and formulations. Herbal medicines are becoming more and more popular nowadays. Among the entire flora, 35,000 to 70,000 species have been used for medicinal purposes \([1]\). In India, of the 17,000 species of higher plants, 7500 are known for medicinal uses. This is the highest proportion of medicinal plants known for their medical purposes in any country of the world for the existing flora of that respective country \([2]\). Demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at desirable price and sometime the only source of health care available to the poor.

Many herbal secondary metabolites, chemical compounds and herbal formulations have been studied for their biological actions related to prevent human diseases by using models such as IPL-induced myocardial infarctions \([3]\). Since IPL induced myocardial infarction serves as a well standardized model to study the beneficial effects of many drugs. IPL, a non-selective \(\beta\)-adrenergic agonist, has been reported to cause oxidative stress in the myocardium resulting in infarct like necrosis of the heart muscle and increase in the levels of lipids in the myocardium \([4]\). Free radical generation and lipid peroxidation could be involved in IPL-induced cardiac damage \([5]\). The pathophysiological changes during IPL induction are comparable to those taking place in human myocardial infarction \([6]\), due to alter lipid metabolism.

In many countries, herbal therapies are among the most popular of all “alternative treatments” \([7]\). Aegle marmelos has been used for centuries as an herbal medicine. It is commonly known as Bael, is indigenous to India and is one of the most useful medicinal plants in India. Its stem, bark, root, leaves and fruits have medicinal value. The ancient systems of medicine, including Roman, Ayurveda, Greek, Siddha and Unani, have mentioned its therapeutic applications in cardiovascular disorders, diabetes, diarrhea and dysentery \([8]\). Other actions like antifungal \([9]\), antibacterial \([10]\), antipROTOzoal \([11]\), hypoglycemic \([12]\), antioxidant \([13]\), antiviral \([14]\) and cardioprotective effects have been studied using various parts of the plant \([15,16]\). Besides its antioxidant properties, AMUFAEt interacts by various other mechanisms in a complex way to elicit its therapeutic effects. Several phytochemical constituents like aegelin, alloimperatorin, marmelide, marmeline, marmelosin, marmesin, psoralen, skimming, tannic acid, xanthotoxol and \(\beta\)-sitosterol are reported to be present in
Aegle marmelos fruit \[23,24,25\]. However, AMUFAE potential as a antidyslipidemic related to cardioprotective agent has not been extensively studied.

**MATERIALS AND METHODS**

**PLANT EXTRACT AND CHEMICALS**

The AMUFAE (brown dry powder) was received as a gift from Laila Impex (Manufacturers and exporters of herbal extracts), Vijayawada, Andhra Pradesh, India. The extract was suspended in distilled water prior to use. IPL was procured from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Adult male albino rats of Wister strain weighing 150-250 g were used for the study. They were acclimatized to animal house conditions, fed with commercial pelletized rat chow (Hindustan Lever Ltd., Bangalore) and had free access to water. The rats were divided into 4 groups each consisting of 6 animals.

**EXPERIMENTAL DESIGN**

Group I served as normal control, Group II, AMUFAEt treated (150 mg/kg body weight, for 45 days), Group III, IPL treated rats (85 mg/kg body weight, once a day for 2 days), Group IV, AMUFAEt treated (150 mg/kg body weight, for 45 days) and then IPL administered once a day for 2 days.

**BIOCHEMICAL ASSAYS**

After the experimental period the rats were sacrificed by cervical decapitation. Blood was collected and the serum separated was used for the assay of Lipid peroxide in serum was determined by the method of \[26\].

Protein was estimated by the method of \[27\] et al. Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were assayed by enzymatic kit methods using an autoanalyzer (AU-5232, Olympus Corporation, Tokyo, Japan). Serum phospholipids was determined as inorganic phosphorus according to the method of \[28\]. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) fractions were calculated as LDL-C = TC-(HDL-C + VLDL-C) and VLDL= TG/5, respectively. Atherogenic Index (A.I) was calculated according to the formula = TC – HDL-C/HDL-C.

Extraction of tissue for various lipid parameters and lipid peroxidation as follows. Accurately weighed 0.5gm heart tissue was ground using mortar and pestle. An extract using chloroform methanol mixture (1:1) was made by 1:5 volumes and diluted to 20ml and centrifuged at 10000 rpm/10 min. 2ml of this supernatant was evaporated and redissolved in 1ml of acetic acid. 0.05 ml of this extract was used for the estimation of TC, TG, PL and lipid peroxides.

**STATISTICS**

All results were expressed as means ± standard error of a six individual observations. Duncan multiple range (DMR) test \[29\] was performed to know the level of significance among all experimental groups. For statistical analysis group III was compared with group 1 and group IV was compared with group III.

**RESULTS AND DISCUSSION**

The levels of TC, LDL-C, VLDL-C, TG and PL in serum were significantly increased by 46.71%, 82.00%, 65.33%, 65.23% and 29.81% in the IPL-treated rats as compared to normal control rats where as the levels of HDL-C are significantly (p <0.05) decreased (36.64%) as compared to normal control. Rats pre-treated with AMUFAEt showed significant (p <0.05) decrease in serum TC, LDL-C, VLDL-C, TG, PL and significant rise in HDL-C by 24.44%, 36.66%, 37.67%, 37.55%, 16.95% and 64.32% respectively as compared to IPL-treated rats (Table 1). These results are union with earlier findings \[23\]. Rats pre-treated with AMUFAEt showed significant (p <0.05) decrease in heart tissue TC, TG, LP and significant rise in PL by 34.40%, 27.32%, 24.59% and 65.73% respectively as compared to IPL – treated rats. The levels of TC, TG, LP in heart tissue were significantly (p <0.05) increased by 86.74%, 71.99%, 74.71% in IPL.

Each value is the mean ± S.E of six animals in each group. Group III were compared with corresponding normal control group and Group IV were compared with group III. According to DMR test p* <0.05.
Levels of TC, TG, PL and lipid peroxides in heart tissue of control and experimental animals are shown in Table 2. It is well known that abnormalities of lipid and lipoprotein metabolism are positively correlated with heart disease [33]. The abnormal high concentrations of lipids is mainly due to increase in the mobilization of free fatty acids from peripheral depots. On the other hand, catecholamine and other hormones enhance lipolysis. Thus, regulating serum lipid profile is important in prevention of cardiovascular diseases.

Figure 2

Table 2: Effect of AMUFAEt on heart tissue lipid profile.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>4.98±0.20</td>
<td>5.17±0.3</td>
<td>9.30±1.4</td>
<td>6.10±0.9</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>4.32±0.07</td>
<td>4.06±0.14</td>
<td>7.43±1.36</td>
<td>5.40±0.12</td>
</tr>
<tr>
<td>PL (mg/dL)</td>
<td>28.10±0.15</td>
<td>28.76±0.19</td>
<td>17.16±0.58</td>
<td>28.44±0.16</td>
</tr>
<tr>
<td>Lip peroxid (mg protein)</td>
<td>3.56±0.12</td>
<td>4.25±0.07</td>
<td>6.22±0.37</td>
<td>4.60±0.15</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E of six animals in each group. Group III were compared with corresponding normal control group and Group IV were compared with group III. According to DMR test p* <0.05.

Recently, there has been focus on the lipid lowering effects of dietary plants, and various plants are shown to be helpful in lowering serum lipid levels [34]. AMUFAEt pretreatment showed a significant decrease in serum total cholesterol, LDL-C, VLDL-C, TG, PL, and a significant rise in HDL-C, compared to IPL treated group. This clearly indicates the protective effect of AMUFAEt against IPL induced myocardial stress. An increased cholesterol concentration along with triglycerides in serum was considered an important risk factor for atherosclerosis [35]. In particular, many studies have found LDL-C to be the most dangerous among the serum lipids, and the oxidation of LDL leads to its increased penetration of arterial walls [36] apart that excess LDL in the blood, is deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions. According to these studies, decreased serum TC and LDL-C levels is important for reducing the risk of cardiovascular disease.

Our results showed that the AMUFAEt increased serum HDL-C concentrations when compared to IPL treated rats. HDL carries cholesterol and cholesterol esters from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids. This path way plays a major role in reducing cholesterol levels and inhibits atherosclerotic plaque formation in the aorta [37]. Our findings suggest that AMUFAEt is an effective lipid lowering agent and may protect against cardiovascular diseases that result from hyperlipidemia. The A.I. is thought to be an important factor for atherosclerosis, and was significantly lowered in the IPL induced dyslipidemic rats pre-treated with AMUFAEt. This decrease in the A.I. is however another positive change resulting from AMUFAEt treatment.

The antidyslipidemic effect of AMUFAEt could be due to different types of active principles, each with single or a diverse range of biological activities.  

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