Effect of *Aegle marmelos* Correa. (Bael) fruit extract on tissue antioxidants in streptozotocin diabetic rats

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A study was undertaken to evaluate the anti-lipid peroxidative activity of an aqueous extract of *A. marmelos* fruits (AMFEt) in streptozotocin diabetic rats in heart and pancreas. Oral administration of AMFEt for 30 days (125 and 250 mg kg\(^{-1}\) body weight twice daily) produced a significant decrease in the elevated levels of peroxidation products, viz. thiobarbituric acid reactive substances and hydroperoxides in the tissues of diabetic rats. The depressed activities of superoxide dismutase, catalase and glutathione peroxidase and lowered glutathione content in the heart and pancreas of diabetic rats were found to increase on treatment with AMFEt. AMFEt at a dose of 250 mg kg\(^{-1}\) was more effective than glibenclamide (300 µg kg\(^{-1}\)) and both reversed all the values significantly. Thus AMFEt exhibits anti-oxidative activity in streptozotocin diabetic rats.

**Keywords**: *Aegle marmelos*, Anti-lipid peroxidase, Diabetic, Anti-oxidative

*Aegle marmelos* Correa. (Bael), indigenous to India, is grown throughout the sub-continent as well as in Burma, Pakistan and Bangladesh. Indigenous people use both leaves and fruits of this plant to treat diabetes mellitus. Preliminary reports indicate the blood glucose-lowering activity of green leaves\(^1\) and root bark of *A. marmelos*. The crude extract of the leaves and the alkaloid prepared from it also exhibit hypoglycaemic effect in diabetic rats\(^3,4\). Available literature, showed that no experimental work has been carried out to verify the claims on the antioxidant activity of *Aegle marmelos* fruits. Therefore, it was considered worthwhile to undertake this study to evaluate the antioxidant activity of an aqueous extract of *Aegle marmelos* fruits in streptozotocin diabetic rats.

During the development of diabetes, free radicals are generated by inflammatory cells in the course of inflammatory events\(^8\). As a result, inevitable impairment of the \(\beta\)-cells occurs\(^8\). The imbalance between uncontrolled reactive oxygen species (ROS) generation and the activity of scavenging systems, resulting probably from chronic hyperglycaemia, leads to the production of excess free radicals which in turn cause damage to lipid membranes and cellular death\(^8\).

**Materials and Methods**

*Plant extract*— *Aegle marmelos* fruit extract (brown dry powder) was received as a gift from Chemiloids (Manufacturers and exporters of herbal extracts), Vijayawada, Andhra Pradesh, India. Herb to product ratio is 8:1. The extract was suspended in distilled water prior to use. Following constituents are reported to be present in *Aegle* fruit\(^5,7\):

- Aegelin, alloimperatorin, imperatorin, marmelide, marmelosin, marmesin, psoralen, rhamnose, scoparone, scopoletin, skimmian, tannic acid, umbelliferone, xanthotoxol and \(\beta\)-sitosterol.

**Drugs and chemicals**— Streptozotocin and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis Mo, USA). All the other chemicals used were of analytical grade.

**Animals**— Female albino Wistar rats, weighing 160-190 g were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and used in the present study. Diabetes was induced in rats by a single ip injection of freshly prepared streptozotocin (45 mg kg\(^{-1}\) body wt\(^8\)) in citrate buffer (0.1 \(M\), pH 4.5) in a volume of 1 ml kg\(^{-1}\). Two days after STZ-administration, blood glucose levels of each rat was determined. Rats with a fasting blood glucose range of 250-300 mg dl\(^{-1}\) were considered diabetic and included in the study.

**Experimental design**— A total of 30 rats (6 normal, 24 STZ-diabetic surviving) were used. The rats were divided into 5 groups of 6 rats each. Group 1: Normal untreated rats. Group 2: STZ-diabetic rats. Groups 3

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and 4: STZ-diabetic rats administered with AMFEt (125 mg kg\(^{-1}\) body wt and 250 mg kg\(^{-1}\) body wt respectively) in distilled water using an intragastric tube twice a day for 30 days, Group 5: STZ-diabetic rats given glibenclamide (300 \(\mu\)g kg\(^{-1}\) body wt) in distilled water using an intragastric tube twice a day for 30 days. After 30 days of treatment, all the rats were sacrificed after fasting overnight. Blood was collected in potassium oxalate and sodium fluoride containing tubes for the estimation of fasting blood glucose. Heart and pancreas were removed immediately, rinsed in ice chilled normal saline and weighed.

**Biochemical estimations**—Fasting blood glucose\(^{10}\), thiobarbituric acid reactive substances (TBARS)\(^{12}\), hydroperoxides (HP)\(^{12}\), reduced glutathione (GSH)\(^{13}\), superoxide dismutase (SOD)\(^{13}\), catalase\(^{15}\) and glutathione peroxidase (GPX)\(^{16}\) were determined.

**Statistical analysis**—Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). All the results were expressed as mean ± SD for 6 rats in each group. \(P\) values < 0.05 were considered as significant.

**Results**

Table 1 illustrates the levels of blood glucose in normal and diabetic rats with or without treatment with AMFEt and glibenclamide. The diabetic rats showed a significant increase in blood glucose over the normal. Oral administration of a high dose of AMFEt to diabetic rats exceptionally maintained the blood glucose to near normal status.

Table 2 shows the concentration of TBARS and hydroperoxides in the heart and pancreas of normal and diabetic rats with or without treatment with AMFEt and glibenclamide. The diabetic rats showed a significant increase in TBARS and hydroperoxides in heart and pancreas. Oral administration of a high dose of AMFEt only maintained the TBARS and hydroperoxides to near normal status in the heart and pancreas of diabetic rats.

Table 3 shows the changes in the concentrations of reduced glutathione and the activity of glutathione peroxidase in the heart and pancreas of normal and diabetic rats with and without treatment with AMFEt and glibenclamide. There was a significant reduction in glutathione and glutathione peroxidase in heart and pancreas in diabetic rats. Oral administration of AMFEt at a high dose to diabetic rats significantly increased glutathione and glutathione peroxidase to near normal values in heart and pancreas. Glibenclamide at the applied dose is not that effective.

Table 4 depicts the changes in the activities of superoxide dismutase and catalase in heart and

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**Table 1** — Effect of aqueous AMFEt on blood glucose in diabetic rats as compared to glibenclamide

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg dl(^{-1}))</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Normal</td>
<td>81.65 ± 4.81</td>
<td>85.80 ± 4.40*</td>
</tr>
<tr>
<td>STZ-treated</td>
<td>286.21 ± 13.20</td>
<td>336.83 ± 22.51(^{1})</td>
</tr>
<tr>
<td>STZ-treated + AMFEt 125 mg / kg twice a day</td>
<td>270.60 ± 16.51</td>
<td>162.20 ± 10.90*</td>
</tr>
<tr>
<td>STZ-treated + AMFEt 250 mg / kg twice a day</td>
<td>280.43 ± 19.22</td>
<td>96.21 ± 5.33*</td>
</tr>
<tr>
<td>STZ-treated + glibenclamide (300 (\mu)g / kg) twice a day</td>
<td>285.41 ± 18.40</td>
<td>130.00 ± 8.01*</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript differ significantly at \(P < 0.05\) (DMRT).

**Table 2** — Effect of aqueous AMFEt as compared to glibenclamide on thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in heart and pancreas of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart (mM 100 g(^{-1}) wet tissue)</th>
<th>Pancreas (mM 100 g(^{-1}) wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBARS</td>
<td>HP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TBARS</td>
</tr>
<tr>
<td>Normal</td>
<td>0.48 ± 0.01(^{a})</td>
<td>17.28 ± 1.21(^{a})</td>
</tr>
<tr>
<td>STZ- treated</td>
<td>0.62 ± 0.02(^{b})</td>
<td>26.38 ± 1.88(^{b})</td>
</tr>
<tr>
<td>STZ- treated + AMFEt 125 mg / kg twice a day</td>
<td>0.56 ± 0.01(^{c})</td>
<td>22.06 ± 1.22(^{c})</td>
</tr>
<tr>
<td>STZ- treated + AMFEt 250 mg / kg twice a day</td>
<td>0.51 ± 0.02(^{d})</td>
<td>18.13 ± 0.86(^{d})</td>
</tr>
<tr>
<td>STZ- treated + glibenclamide (300 (\mu)g / kg) twice a day</td>
<td>0.54 ± 0.02(^{d})</td>
<td>19.65 ± 1.01(^{e})</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript differ significantly at \(P < 0.05\) (DMRT).
pancreas of normal and various groups of the diabetic rats described above. There was a significant decrease in the activities of superoxide dismutase and catalase in the heart and pancreas of diabetic rats. Oral administration of a higher dose of AMF Et to diabetic rats showed a significant effect and restored the activities of these enzymes to near normal status.

For all the parameters studied, AMF Et at doses of 125 and 250 mg kg⁻¹ showed significant effects with the higher dose giving a far better effect. Glibenclamide also showed a significant effect in all the parameters studied in diabetic rats. However, the effect exerted by AMF Et (250 mg kg⁻¹) was more effective than glibenclamide.

Discussion

Administration of aqueous AMF Et twice a day for 30 days showed a significant effect on lipid peroxidation and antioxidants in STZ-diabetic rats. An observed increase in the levels of TBARS in heart and pancreas may be due to increased susceptibility of the tissues of diabetic rats to lipid peroxidation. Increase in lipid peroxides in the heart and pancreas of STZ-diabetic animals have also been observed.

Oral administration of AMF Et decreases the concentration of TBARS in the heart and pancreas of STZ-diabetic rats.

Hydroperoxides are molecules with high toxicity potential for destroying enzymes and cell membranes. An increase in hydroperoxides in heart and pancreas is related to induced diabetes also. This may be due to a decrease in the activities of antioxidant enzymes, which is a favourable factor for uncontrolled generation of free radicals and subsequent generation of lipid hydroperoxides. AMF Et administration lowers hydroperoxides in heart and pancreas of STZ-diabetic rats.

Increased lipid peroxidation under diabetic conditions may be due to increased oxidative stress in the cells as a result of the depletion of antioxidant scavenger systems. Diminished levels of both non-enzymic and enzymic antioxidants are noted in the present study. Under in vitro conditions, GSH acts as an antioxidant and its decrease is reported in the heart and pancreas of STZ-induced diabetic condition. Decreased glutathione levels in diabetes have been considered to be an index of increased oxidative stress. The increased GSH content in the heart and
pancreas of rats treated with AMFET may be one of the factors responsible for the inhibition of lipid peroxidation.

Superoxide dismutase catalyses the dismutation of the highly reactive superoxide anion to oxygen and hydrogen peroxide. Catalase and glutathione peroxidase are considered biologically essential in the reduction of hydrogen peroxide. Reports have shown that the activities of SOD, catalase and glutathione peroxidase were lowered in STZ-diabetic rats. The AMFET-treated diabetic rats showed decreased lipid peroxidation associated with increased activities of these antioxidant enzymes. Studies have shown that alkaloids, coumarins and tannins have antioxidant effect which may be due to the presence of these chemical constituents in the fruits.

The above findings show that the water extract of *A. marmelos* fruits protects the antioxidant systems directly or by stimulating the antioxidant enzymes in diabetic rats. Further work is needed to find out the exact mechanism of action of the fruit extract.

References


