Invitro Studies on Antidiabetic Effect of Aegle Marmelos Plant Aqueous Extract

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Abstract: Diabetes has become a major metabolic disorder disease that is increasingly becoming a killer disease worldwide. The aim of this study was to evaluate (bael tree) Aegle marmelos plant extract has the potential efficiency to manage diabetes mellitus type 2, that affects kidney and liver, in streptozotocin induced rats. Wistar strain male rats were used grouped into three groups. The first group was used as control, the second group was induced diabetes by inducting streptozotocin 70mg/kg, the third group was induced diabetes and treated with A.marmelos aqueous plant leaves extract in the increasing dosage of 250mg/kg, 350mg/kg and 450mg/kg body weight from the 3rd to 21st day. Induction of streptozotocin affected the liver, kidney and their histopathology. Biochemical tests and histopathological studies were conducted. After administration of Aegle marmelos extract in increasing level, glucose level was significantly p<0.05 reduced. The kidney and liver parameters creatinine, urea, cholesterol protein, alanine transaminase (ALT), aspartate transaminase (ALT), alanine phosphate (ALP), albumin were also significantly p<0.05 reduced from 16th day of study. Histopathological results showed reduced inflammatory cellular lining in liver for the rats that were treated with the aqueous plant extract after inducing diabetes, while the histopathological test for pancreas showed reduced focal damage for the animals that were treated with the plant extract after inducing diabetes. From this results we found that Aegles Marmelos plant has therapeutic effect as renoprotective, hepatoprotective, and hypoglycaemic.

Keywords:

1. Introduction

About 347 million people have diabetes in adult population 20-79 years old. USA and Caribbean region have a higher prevalence of 11% while Middle East and North Africa are next with 9.2% reported by World Health Organization(25). India is the top country with the highest number of diabetic patients in the world followed by China. This number is expected to rise. Although Africa still faces other challenges and not many people have done diabetes test, diabetes could also become a major challenge(11). South Africa, Nigeria, Kenya and Mozambique that have better economies could face this problem as a major challenge in future.

2. Management

Metformin is a commonly used as an oral anti-diabetic drug currently. Overweight patients mainly prefer it since it is considered weight neutral. It is under biguanide class and works by reducing hepatitis glucose. The most adverse effect is gastrointestinal irritation (9). Metformin is used in setting of mild-moderate renal insufficiency. In traditional days and today also, some herbs have been used to control and manage diabetes such as: Ginseng has shown to fight diabetes (a 2000 study at Toronto University) revealed that 3 gram dose of ginseng extract significantly reduce the blood sugar spike, which occurs after taking a high carbohydrate meal. Holy basil also known as tulsi has shown to reduce both blood sugar and lipid levels. (7,14,18). Gymnema Sylvester a Hindi name translates as sugar destroyer and the plant is said to reduce the ability to detect sweetness. It is regarded the most powerful herbs it may work as boosting the activity of enzymes that help use glucose or stimulates production of enzymes. (22).Aegles Marmelos a plant of Indian origin, with phytopharmacological properties has shown to reduce metabolic disorder such as cholesterol level and blood sugar levels. (7,14,18).

3. About Aegle Marmelos

Aegle Marmelos is a plant that belongs to a family of rutaceae. It is 6-8 metres of height with aromatic leaves; flowers are nearly 2cm wide boom in clusters, sweet scented and greenish white, the fruits are oblong pyriform in shape.(8) This is a plant of Indian origin that plays an important part in the Hindu religion (20). It is also present in South East Asia regarded as sacred tree. It grows in dry forests and hills. The plant has been used for so many pharmacological activities such us; antidyspepsia, antidiarrheal, antioxidants, as a laxative, antulcer and cardio protective (10). It has also been used to inhibit antifungal activity and antibacterial activity (15).It is also used as pesticide and has shown to have nutritional properties. The parts of the plant were taken and all the structures were characterized by extensive 1D and 2D Nuclear Magnetic Resonance (NMR). From the roots, twigs and leaves consist of alkaloids and coumarins which include; oxazalin & 2-phenyl-5-(4-methoxyphenyl)-6-oxazoline (12). Skimmianine (Aurapten. Xanthotoxin) Marmin (20).

4. Materials and Methods

Plant extract preparation

Fresh leaves and twigs of the plant were collected from the green house under the guidance of a botanist. Using distilled water then cleaned them, shade dried and kept at room temperature in the department of botany. After drying,
500gms of the plant leaves and twigs was taken powdered by crashing in a blender and mixed with 500ml of distilled water magnetically stirred overnight at room temperature. The extract was filtered and the aqueous extract was administered to animals.

Animals and drugs used
Adult male albino rats Wister strain were taken, 100-120 days old. Institutional Ethics Committee approved the experiment and Care of Animals was carried out by guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Each of these group was kept in propylene cage at ambient temperature of 24 °C and 55% -65% relative humidity. A 12-hour light and dark schedule was maintained in the animal house until they are used to the laboratory condition they were then fed with commercial rat chow and had free access to water. Streptozotocin was purchased from sigma chemicals and the other chemicals used were of analytical reagent grade.

Experimental Design
The animals were divided into 3 groups and the first group (G1) was used, as control thus there was neither induction of diabetes nor treatment with A. Marmelos aqueous extract. The second group (G2) was induced diabetes by treatment with streptozotocin through intraperitonial way and diabetes was confirmed by glucose test, after this confirmation only then the treatment of aqueous plant extract was commenced. The third group (G3) was treated with streptozotocin and A. Marmelos aqueous extract through intraperitonial way, the extract was given in increasing dosage of 250mg/kg, 350mg/kg and 450mg/kg body weight. This administration of A. Marmelos aqeuas extract in group 3 was done twice daily in increasing dosage for 21 days to access the therapeutic potential of the plant extract. Pentathol sodium was used for sacrificing the groups of animals from the third day ,7th, 14th day. Histopathological studies were also conducted in the liver and pancreas to check for toxicity. This was done by taking fresh fixatives samples and stored at 3°C and passed through alcohol solutions for dehydration.

Biochemical tests
Blood glucose was determined, kidney and liver parameters that indicate effect of diabetes testwere analysed. For the blood glucose was determined by O-tulidine method. Cholesterol test was done by commercial kit method assay and Allain et al method. For the kidney test urea was tested by ChaneyMarbach method, creatinine test performed by Wit et al commercial kit assay method. Uric acid test was determined by Fossa et al method and phosphorous was estimated by Endres et al method. For the liver, Aspartate transaminase (AST) was determined by Reitman et al method, Alanine transaminase (ALT) was determined by King et al method, Alanine transaminase (ALT) was estimated by assay kit (colorimetric and fluorometric) method. Albumin was analysed by BCG Assay kit.

5. Statistical Methods
All values of the biochemical estimations were expressed as mean ± standard error mean (SEM) and were then analysed by ANOVA post hoc Dunnet t-test method using spss software. Differences were considered to show statistically significant difference using spss version 24.

6. Results
After induction of streptozotocin in increasing dosage of 250mg/kg, 350mg/kg, 450mg/kg there was increased blood sugar levelTable. On administration of Aegles marmelos extract there was significant p < 0.05 control within 14 days. Hemoglobin levels were reduced in streptozotocin treated animals, treatment with A.m. extract significantly p < 0.05 increased. This could be because of improved sugar control by the plant extract.

Table 1: Effect of A.m plant extract on serum glucose, hemoglobin, urea, creatinine, uric acid and phosphorous

<table>
<thead>
<tr>
<th>Group</th>
<th>glucose (mg%)</th>
<th>Hgbm %</th>
<th>Creatinine mg/dl</th>
<th>Urea mg/dl</th>
<th>Uric acid mg/dl</th>
<th>Phosphorous mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>93.6±6.7</td>
<td>15.65±0.104</td>
<td>0.743±0.108</td>
<td>52±0.26</td>
<td>2.06±0.252</td>
<td>7.1±0.03</td>
</tr>
<tr>
<td>diabetic</td>
<td>211.4±12.3</td>
<td>14.229±0.371</td>
<td>1.23±0.039</td>
<td>59±0.75</td>
<td>3.65±0.181</td>
<td>7.8±0.02</td>
</tr>
<tr>
<td>treated</td>
<td>134.2±6.4</td>
<td>15.679±0.123</td>
<td>1.20±0.125</td>
<td>58±0.56</td>
<td>2.52±0.25</td>
<td>6.8±0.45</td>
</tr>
</tbody>
</table>

The values are expressed in mean ± S.D followed by pos hoc dunnet t-test n=4 in each group. values that do not have a common superscript differ significantly p<0.05.

Table 2: Effect of A.m plant extract on liver enzymes, cholesterol,albumin

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AST(U/L)</th>
<th>ALT(U/L)</th>
<th>ALP(U/L)</th>
<th>Albumin gm%</th>
<th>Cholesterol mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>34.83±1.598</td>
<td>23.40±0.794</td>
<td>210.14±0.652</td>
<td>6.69±0.006</td>
<td>34.80±1.514</td>
</tr>
<tr>
<td>diabetic</td>
<td>67.65±2.546</td>
<td>15.95±0.624</td>
<td>211.77±1.482</td>
<td>5.63±0.228</td>
<td>34.17±0.80</td>
</tr>
<tr>
<td>treated</td>
<td>62.46±1.366</td>
<td>18.65±0.624</td>
<td>210.98±0.678</td>
<td>6.73±0.321</td>
<td>34.23±0.268</td>
</tr>
</tbody>
</table>

The liver parameters
The values are expressed in mean ± S.D followed by pos hoc dunnet t-test n=64 in each group. values that do not have a common superscript differ significantly p<0.05.

There was significant p<0.05 increase in urea, uric acid and phosphorous in diabetic animals, while A.m. extract treated animals had significant p<0.05 decrease in Table 1. Hepatic enzymes in Table 2, significantly p < 0.05 lowered after administration of A.m. extract. Cholesterol levels significantly p< 0.05 reduced after treatment with the plant extract. It is possible to predict that the A.m. plant extract may have degraded this cholesterol by inhibition of endogenous synthesis of the cholesterol. Albumin was significantly p<0.05 increased in Table 2, after treatment A.m plant extract.

7. Discussion

The latest convention methods for treatment of diabetes have shortcomings such as side effect and others have failure in management. The plants have however shown to have no side effects but slow in efficacy.

In this study streptozotocin was used to induce diabetes since it is reported not to cause cell damage of the pancreas unlike nitric oxide. (16) Streptozotocin induction elevates the level of cholesterol (5) within the liver that are likely to increase the coronary disease. From our research, cholesterol test showed tremendous p< 0.05 reduction on treatment with the plant extract. This could be because of reduction in lipolysis. During diabetes the levels of cholesterol are increased, insulin depletes level of lipoprotein lipase leading to deranged lipids (26). Diabetes care 1991 stated that reduction in lipids would be beneficial in long-term prognosis patients. This showed this plant extract could be useful in reducing hyperlipidaemia in diabetic patients (7). There was increased sugar levels in diabetic rats while those rats treated with plant extract showed lower glucose elevation thereby displaying improvement in glucose tolerance pattern (14), this means the plant utilized the blood glucose. This hypoglycaemic effect action was either by refurbishing the islet function increasing insulin output or by facilitation of metabolites generated due to insulin action.

Diabetes has become the primary cause of renal disease (4). Induction of diabetes increased levels of creatinine, urea, uric acid and protein that indicates a kidney problem as stated by the American Diabetes Association. When the rats were treated with the extract there was much significance p < 0.05 reduction of creatinine, urea uric acid and phosphorous. This displayed the plant extract as a Renoprotective (1). Glucose is usually absorbed from the intestinal tract to the portal vein then to the liver. Raised liver enzymes is characterised to insulin resistance (23). After treatment with Aegle marmelos plant extract in diabetic induced animals, there was significant p < 0.05 reduction in liver enzymes; alanine transaminase (ALT), aspartate transaminase (AST), and alanine phosphate (ALP). This could mean the plant extract reduced the excessive hepatic glycogen thus no deposit of glycogen and possibly decreased gluconeogenesis. Histopathological studies also proved the plant extract had no toxic effect to the organs but instead it displayed therapeutic effect to the kidney and pancreas (24).

8. Contradiction

When A.marmelos aqueous plant extract was induced into the animals the vital organs such as heart, liver, kidney and pancreas were taken and evaluated, there was no gross abnormalities from the histopathological result but instead displayed a therapeutically effect on the organs. (24)

9. Conclusion

The study results indicate the active compounds in the Aegle Marmelos aqueous extract may possess diverse biological action and therapeutic value based on its antidiabetic and antilipidemic.

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References


