Restoration of Liver and Muscle Glycogen by *Eugenia jambolana* in Diabetic Rats

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Abstract: Diabetes is a debilitating disorder affecting millions today. It leads to a series of complications, crippling life. Among the several impairments, carbohydrate metabolism is also a target. The liver and skeletal muscles, important sites of carbohydrate metabolism, mediated by insulin are also affected. Diabetes has adverse effect on glucose oxidation in liver and skeletal muscles. This is probably due to inactivation of glycogen synthetase system. Plants have been used as alternate source of drugs, since traditional drugs have exhibited a lot of side effects. This study was conducted to study the reversal of this disorder by treatment with *Eugenia jambolana* extracts. This restoration of glycogen levels might be due to insulin release. Further work is in progress.

Keywords: Diabetes, glucose oxidation, glycogen, Eugenia jambolana

1. Introduction

Diabetes mellitus, a metabolic disorder is on the increase globally [1]. Hyperglycemia is the main cause of complications related to coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and premature death [2].

Assessment of tissue glycogen contents and the activities serve as markers for studying insulinomimetic activity. The glycogen content of insulin-dependent tissues is known to decrease during diabetes but the opposite happens in insulin-independent tissues like kidneys. Liver plays an important role in buffering the postprandial hyperglycemia and is involved in the synthesis of glycogen. Diabetes mellitus is known to impair the normal capacity of liver to synthesize glycogen[3-12]. Insulin plays a crucial role in lowering blood glucose level by enhancing glycogenesis in liver and muscles. Assessment of glycogen serves as a marker for studying insulinomimetic activity. Glycogen content of skeletal muscles and liver markedly decreases in diabetes [13-16]. Though biguanides, sulphonylureas and other drugs are available for treatment of type 2 diabetes and its associated complications, yet they exhibit side effects. This has led to search for newer drugs without side effects. Many experts are of the view that the complications of diabetes mellitus cannot be addressed with a unidirectional therapeutic approach. On the other hand, a holistic approach embracing several traditional systems can be of advantage in the management of diabetic pathogenesis[17]. In India, use of herbal drugs based on Ayurveda is very commonly practised from long ago and is less expensive. They are less toxic with fewer side effects compared to the synthetic drugs [18]. For these reasons, at present traditional and complementary medicine has seen an upsurge in its popularity for the treatment of several diseases.

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Since ancient times, traditional medicines all over the world have advocated the use of plants to treat diabetes. Till today more than 1200 species of plants have been screened for activity on the basis of ethnopharmacology or on random basis. Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques [19-22].

The cellular and biochemical mechanisms underlying the hyperglycemic effects are still not fully understood. The main purpose of this research was to investigate the possible pathways that may...
explain the hypoglycemic effects of the chosen plant, *Eugenia jambolana* seed extracts. *Eugenia jambolana* Lam. (Syn. *Syzygi cumunci* Skeels or *Syzygium jambolana*) belonging to the family Myrtaceae is a large evergreen tree. Flowers white 7.5-13 mm across in branched clusters at stem tips, calyx cuplike; 4 petals, fused into a cap; many stamens. Fruit variable in size up to 2.5 cm long, ellipsoid or oblong, crowned with truncate calyx-limb, black with pink juicy pulp. It is widely distributed throughout India, Ceylon-Malaya and Australia and known as Jamun, Jam, Jambul in India. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties [23]. The present review will possibly help to bridge between traditional claims and modern therapy on *E. jambolana*. Most of the plant parts of *E. jambolana* are used in traditional system of medicine in India. According to Ayurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers [23]. The fruits are acrid and sweet, cooling, dry and astringent to bowels. In order to accomplish this goal, the study was carried out to test the muscle and liver glycogen levels, which serve as indicators of studying insulinomimetic activity.

2. MATERIALS & METHODS

2.1 Plant Material

*Eugenia jambolana* Lam. (Syn. *Syzygi cumunci* Skeels or *Syzygium jambolana*) belonging to the family Myrtaceae is a large evergreen tree up to 30 m high. Bark pale brown, slightly rough on old stems. Recently the compound isolated from the seeds, already reported was targeted against diabetes and its associated urinary tract infection [24-25]. Hence, in an attempt to investigate the mode of action of *E. jambolana*, this study was undertaken to evaluate the efficiency of the plant in restoration of glucose oxidation, both in the liver and tissues in diabetes.

2.2 Preparation of Plant Extract

*E. jambolana* seeds were collected from a local market in Tiruchirappalli, India and authenticated at the Department of Botany of Holy Cross College, Tiruchirappalli, India. The seeds were thoroughly washed and dried under shade. The dried seeds were powdered and extracted in a soxhlet apparatus using methanol as solvent (1 kg powder in 3 L solvent). The extract was filter-sterilized through a 0.45 μ membrane filter and evaporated to dryness in a rotary vacuum evaporator at 40°C. The yield of the methanol extract was 14.6 g %. The extracts were dried and 150 mg kg\(^{-1}\) b.w. was suspended in 5 mL of 1.0% of carboxy methyl cellulose, commonly used as a suspending solvent. It was also repeated with acetone and hexane.

2.3 Animals Feeding, Housing Conditions and Ethical Approval

All rats were fed with a standard pellet diet (Durga feeds, Bangalore, India) and tap water ad libitum and housed in a 12:12 h light: dark cycle at 22-25°C. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the US guidelines.

2.4 Induction of Diabetes and Experimental Design

Diabetes was induced in rats by intraperitoneal injection of streptozotocin (single dose of 60 mg kg\(^{-1}\) body weight) dissolved in freshly prepared 0.01 M citrate buffer (pH 4.5). Rats with fasting blood glucose more than 300 mg dL\(^{-1}\) were included in the study. The rats were randomly divided into four groups of five animals each as:

**Group 1:** Normoglycemic control. Received only vehicle (1.0% CMC) (5 mL kg\(^{-1}\))

**Group 2:** Diabetic control. Received only vehicle (1.0% CMC) (5 mL kg\(^{-1}\))

**Group 3:** Diabetic. Administered methanol extract of *E. jambolana* seeds once a day throughout 60 days with a dose of 150 mg kg\(^{-1}\) b.w.

**Group 4:** Diabetic. Glibenclamide was given once a day throughout 60 days with a dose of 0.6 mg kg\(^{-1}\) b.w.

After the 60th day treatment, the rats were sacrificed by decapitation and liver and skeletal muscles were dissected and removed to measure glucose oxidation and blood was collected for determination of blood glucose.
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**2.5 Determination of the Blood Glucose Levels**

Blood glucose was determined on the 0, 15, 30 and 60th days, using Glucose Oxidase/Peroxidase method.

**2.6 $^{14}$C-Glucose Oxidation**

$^{14}$C-glucose oxidation in liver and skeletal muscles was estimated by the method of Johnson and Turner (1971) and Kraft and Johnson (1972).

**2.7 Acute Toxicity Studies**

Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 420. Wistar albino rats of either sex (150-200gm) were used for the study and administered a limit dose of 2000 and 5000 mg/kg of the hydroalcoholic combined plant extract and animals were observed for mortality and clinical signs for the first hour, then hourly for 3 hrs and finally periodically until 48 hrs. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD50 was predicted to be above 2000 or 5000 mg/kg, if three or more rats survived. Behavioural and neurological changes such as tremors, convulsions, salivation, diarrhoea, sleep, lacrimation and feed behaviour in drug treated rats were observed for sign of acute toxicity.

**2.8 Statistical Analysis**

Statistical analysis was performed using SPSS software package, version 6.0. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). All the results were expressed as mean±SD for five rats in each group. p-values<0.05 were considered as significant.

**3. RESULTS**

Serum glucose levels in the normal untreated rats did not show any significant variation in the blood glucose throughout the experimental period. Administration of STZ (60 mg kg$^{-1}$ b.w.) led to an elevated blood glucose level, almost 5 fold increase than the untreated group, which was significant (p<0.05). Blood glucose levels measured in extracts-treated experimental rats at the end of 15, 30 and 60 days of treatment are given in Table 1. The decrease in blood glucose in the extract-treated groups could be observed from the 15th day onwards, registering a significant decrease on the 30th day, while a near total restoration comparable to the drug treated and untreated were observed on the 60th day of treatment, showing a time response pattern. Oral administration of the methanol extracts of *E. scaber* root and leaf for a period of 60 days, significantly decreased the blood glucose levels (p<0.05). On the basis of acute toxicity study there is no abnormal behavioural, neurological changes and death was observed till the end of the 14 day. Hence, the median lethal dose (LD50) of the extract was then greater than 2000 mg/kg. Hydroalcoholic combined plant extract was found to be safe up to the dose of 2000 mg/kg. Therefore, dose of 100 and 200 mg/kg b.wt. of were selected for all the experiments. Table 2 depicts the effects of the methanol extract of the root and leaf of *E. scaber* on $^{14}$C glucoso oxidation in liver and skeletal muscles of adult male diabetized rats. In control rats, liver possessed a maximum ability to oxidize glucose when compared to the skeletal muscles. STZ caused a remarkable decrease in glucose oxidation both in liver and skeletal muscles. ESR treatment restored the rate of glucose oxidation to that of control, while the increase in ESL treated samples did not match the control level.

**4. DISCUSSION**

Glucose is an important fuel for liver and muscle metabolism. Liver and skeletal muscles are major sites of carbohydrate metabolism. The decrease observed in the present study is probably due to the lack of insulin in the diabetic state, which results in the inactivation of glycogen synthetase system[26]. Muscle glycogen content was low in diabetic control animals. It increased several fold in diabetic animals treated with *Catharanthus roseus*[27], *Aegle marmelos* leaf powder[9], *Trigonella foenum*[16], D-400, a herbo-mineral formulation[28], *Momordica charantia*[29] and *Momordica cymbalaria*[30]. Treatment of alloxan induced diabetic animals with *Gymnema sylvestre* leaves resulted in an increase in glycogen content in liver and skeletal muscle. This may be due to an increase in glucose uptake by the tissues following *G. sylvestre* administration, increased glycogen synthesis or a combination of both, probably mediated through the action of insulin[31]. Treatment
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with ethanolic extract of seeds of *Eugenia jambolana* prevented the depletion in liver and skeletal muscle glycogen content in alloxan-induced diabetic rabbits[32]. Dianex, a herbal formulation containing *E. jambolana* among several other plants, when administered to STZ-diabetic mice, was found to increase liver and muscle glycogen [33]. Similar effect was reported by Babu and Prince [34], using *E.jambolana* containing Hyponidd, an herbal formulation. *Elephantopus scaber* aqueous and methanol extracts were also reported to increase the glycogen levels in STZ-diabetic rats[35]. Similar to the above findings, an increase in skeletal muscle and liver glycogen content was also found in *Eugenia jambolana* extracts treated diabetic rats. This prevention of glycogen depletion in the liver and muscles might possibly be due to stimulation of insulin release from β-cells [10].

5. CONCLUSION

Muscle and liver glycogen content was low in diabetic control animals. It increased several fold in diabetic animals treated with *E.jambolana* extracts. This may be due to an increase in glucose uptake by the tissues following administration of *E.jambolana*, increased glycogen synthesis or a combination of both, probably mediated through the action of insulin.

**Table 1. Effect of different extracts of Eugenia jambolana on blood glucose levels (mg dL-1) in normal and hyperglycemic rats at varying days**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Days</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic + EJM (150mg/kg bw)</td>
<td>523.35±3.56</td>
<td>331.7±4.6</td>
<td>207.6±5.8</td>
<td>85.74±1.94*</td>
<td></td>
</tr>
<tr>
<td>Diabetic + EJA (150mg/kg bw)</td>
<td>521.84±2.58</td>
<td>362.7±10.72</td>
<td>254.58±3.09</td>
<td>103.9±1.05</td>
<td></td>
</tr>
<tr>
<td>Diabetic + EJH (150mg/kg bw)</td>
<td>533.4±2.92</td>
<td>383.6±5.303</td>
<td>243.18±3.87</td>
<td>107.2±4.12</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SD of five rats

EJM – Eugenia jambolana methanol extract

EJA – Eugenia jambolana acetone extract

EJH – Eugenia jambolana hexane extract

**Table 2. Effect of different crude extracts of Eugenia jambolana seeds for 60 days on muscle and liver glycogen content in fasting normoglycemic and STZ induced hyperglycemic rats**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>MUSCLE GLYCOGEN (mg/g)</th>
<th>LIVER GLYCOGEN (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9.47±0.18</td>
<td>47.38±0.26</td>
</tr>
<tr>
<td>Diabetic (STZ-60mg/kg bw)</td>
<td>1.66±0.17</td>
<td>9.6±0.23</td>
</tr>
<tr>
<td>Diabetic + Humulin (0.3 IU/kg bw)</td>
<td>7.9±0.11</td>
<td>36.68±1.4</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (0.6mg/kg bw)</td>
<td>7.55±0.25</td>
<td>35.37±0.22</td>
</tr>
<tr>
<td>Diabetic + EJM (150mg/kg bw)</td>
<td>7.68±0.19*</td>
<td>36.46±0.29*</td>
</tr>
<tr>
<td>Diabetic + EJA (150mg/kg bw)</td>
<td>5.7±0.21</td>
<td>33.88±0.44</td>
</tr>
<tr>
<td>Diabetic + EJH (150mg/kg bw)</td>
<td>7.50±0.061*</td>
<td>33.29±0.42</td>
</tr>
</tbody>
</table>

Values are means ± SD of five rats. *P<0.001

EJM – Eugenia jambolana methanol extract

EJA – Eugenia jambolana acetone extract

EJH – Eugenia jambolana hexane extract

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