Original article

Antioxidant and antidiabetic activity of *Dillenia pentagyna* Roxb. fruit extract

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Abstract

The aim of this study is to evaluate the enzymatic antioxidant activity and antihyperglycaemic activity of hydroalcoholic (25:75) extract of *Dillenia pentagyna* Roxb. fruits. Acute oral toxicity study was carried out in healthy male Wister rats (150-200 g). The dose was finally made to 200 mg/kg and 400 mg/kg b.w. for oral administration after the LD₅₀ estimation. The results showed that the enzymatic antioxidant of *D. pentagyna* were in fresh fruit extract as determined by the SOD, APX, POD, and GR assays. Oral administration of *D. pentagyna* extract in rat showed significant restoration of blood glucose to normal level. After 21st day of treatment, level of blood glucose, hepatic and renal markers (ALP, SGOT, SGPT and BILIRUBIN) were significantly decreased when compared with the diabetic control. Lowering of blood glucose and other associated markers suggest that *D. pentagyna* extract possess potent antidiabetic activity which can be attributed to antioxidative properties in fruit extract. The findings in this study confirm the antidiabetic properties of *D. pentagyna* fruits which justify its traditional usage in the management of diabetes.

Key words: *Dillenia pentagyna* Roxb., alloxan, antioxidant, antidiabetic, medicinal plant

1. Introduction

Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. *D. pentagyna* Roxb. is an herbal plant species commonly known as ‘agai’ respectively belonging to family Dilleniaceae (Dubey et al., 2009; Yadav et al., 2015). Diabetes mellitus (DM) is considered one of the main threats to human health in the 21st century. In developing countries, the prevalence of diabetes is increasing very fast. The World Health Organization (WHO) estimated that around 70 million people suffering from diabetes mellitus (David et al., 2010). Globally, DM presents enormous and increasingly important public health issues. The prevalence of DM in all age groups are estimated to be 2.8% (170 million) in 2000 and the rate is expected to rise to 4.4% (366 million) in 2030 (Fonseca, 2006). The mechanism of alloxan action has been intensively studied, predominantly in vitro and is now characterized quite well. Using isolated islets (Weaver et al., 1978a) and perfused rat pancreas (Kliber et al., 1996). It was proved that alloxan damages an emergent elevation in insulin ejection in the presence or absence of glucose. It was validated that alloxan induces a quick rise in insulin secretion in the presence or absence of glucose. This miracle seemed just after alloxan treatment and was not observed after repetitive exposure of islets to this diabetogenic agent (Weaver et al., 1978b). Alloxan-induced insulin liberation is pursued by complete straining of the islet respond to glucose even when high concentrations of this sugar were used (Kliber et al., 1996). However, many researchers recommended that the choosiness of alloxan act is not fairly suitable (Szkudelski et al., 1998). The diabetogenic representative alloxan is having a tendency to mix with water and chemically changeable compound. As an outcome, there is an urge to quest for compounds with effective antidiabetic activity when contracted orally (Trejo et al., 1996). An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Catalase, peroxidase, superoxide dismutase and the non-enzymatic antioxidant compounds such as phenols, ascorbate and glutathione have been viewed as a synergistic antioxidant defensive system, whose combined purpose is to protect cells from active oxygen damage (Awad et al., 2011). The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies (Cragg et al., 1997). But, we clearly knew that the use of oral antiabetic is limited due to their adverse side effects including hematological, hypoglycaemic coma and disturbances of liver and kidney functions (Taylor et al., 2006). Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents.

2. Materials and Methods

2.1 Fruits

The basic fruit material of *D. pentagyna* used for the investigation was obtained from Bhinga Forest Range of Uttar Pradesh, India. The Voucher specimen (EBH No.: 265233) of the plant deposited in Ethnobotanical Herbarium, National Botanical Research Institute, Lucknow, Uttar Pradesh, India.
2.2 Chemicals

All chemicals were obtained from the following sources: ethanolic alcohol (boiling range 65-80 °C) (S.D. Fine chemicals Ltd., India); Na₃HPO₄•2H₂O (Rankem), K₂HPO₄•2H₂O (Rankem), Trichloroacetic acid (Bio-Rad), EDTA (Ranbaxy), Nitroblue tetrazolium salt (Rankem), H₂O₂ (Ranbaxy), Guaiacol (Rankem), CaSO₄ (Ranbaxy), Thiobarbituric acid (Rankem), Poly Vinyl Polypyrrolidone (Rankem) whereas alloxan was purchased from the Loba chemie, Mumbai, India. Commercially available kits for biochemical analyses such as glucose, metformin were done using commercial diagnostic kits following manufacturer’s instructions. All reagents used in study were of analytical grade.

2.3 Preparation of fruit extract

The fruits of *D. pentagyna* were collected, washed with distilled water and oven dried at 70°C for 24 h. The oven dried fruits (0.5 gm) of *D. pentagyna* were subjected to pulverization to get coarse powder. The powder, thus, obtained was extracted with hydro-alcoholic (25:75) water and 95% ethanol, using Soxhlet apparatus. The extract was concentrated using rotary evaporator under reduced pressure at 40°C to obtain (yield 9.6% w/w) a semi-solid mass. It was labeled and stored in a glass bottle for further studies. The dose was finally made to 200 mg/kg and 400 mg/kg body weight for oral administration after the LD₅₀ estimation while evaluate the enzymatic activity, we have taken fresh fruit tissues (200 mg each), were of analytical grade.

2.4 Maintenance of animals and approval of protocol

Healthy male wister rats (150-200 g) were maintained in an air-conditioned experimental room at 12 h light: dark cycles. The animals were randomized into experimental and control groups and were housed in a polypropylene cages. Standard pellets were used as a basal diet during the experimental period. The control and experimental animals were provided with purified drinking watered libitum. The animals were maintained in accordance with the “CPCSEA guidelines for laboratory animal facility” (Committee for the Purpose of Control and Supervision on Experiments on Animals) and the approval number is (CPCSEA Regd. No.1129/bc/07/ CPCSEA, dated 13/02/2008).

2.5 Acute oral toxicity studies

*D. pentagyna* fruit at the dose range of 100-2000 mg/kg b.w. oral gavage administration different group of rats, comprised of 6 rats in each group. Animals were kept under close observation for 4 h after administering the fraction for behaviour, neurological and autonomic profile and then observed for any change in the general behaviour or physical activities, mortality was recorded within 72 h. Acute toxicity was determined according to the method of Lorke (Lensen, 2008).

2.6 Experimental design

Five groups of rats, six rats in each received the following treatment schedule:

Group I : Normal control (saline)

Group II : Alloxan treated control (150 mg/kg b.w. i.p)

Group III : Alloxan (150 mg/kg b.w. i.p) + *D. pentagyna* (fruit extract at the dose of 200 mg/ kg b.w)

Group IV : Alloxan (150 mg/kg body weight i.p) + *D. pentagyna* (fruit extract at the dose of 400 mg/kg b.w)

Group V : Metformin (100 mg/kg b.w. i.p)

2.7 Collection and analyses of blood

After the last dose, animals were fasted for 12 h and sacrificed. Blood samples were collected by orbital sinus puncture method (Reitmann and Frankel, 1957). Serum was formed in brief; blood samples were emit from orbital sinus using non-heparinised capillary tubes, collected in dried centrifuge tubes and permitted to clot at room temperature for 3 h. Serum was separated from the clot by centrifuged at 3000 rpm for 15 min at room temperature. Serum was collected carefully and kept at – 20°C until analysis of glucose (Jendrassik and Grolf, 1938). Glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT) activities were measured according to the method described by Kleiner et al. (2005) while bilirubin activity was measured as mentioned by Ayesha et al. (2008).

2.8 Histopathological studies

A portion of the liver was cut into two to three pieces of approximately 6 mm³ sizes and fixed in 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 µm thickness of liver tissue were cut and stained with haematoxylin-eosin. The thin sections of liver were made into permanent slides and examined at 20X magnification using high resolution microscope with photographic facility and photomicrographs were taken.

2.9 Antioxidant enzyme activities

*D. pentagyna* Roxb. is a common, being used by tribal so we estimate the enzymatic antioxidant such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione reductase (GR) activity. SOD activity was verified by in vitro assay of the inhibition of NBT reduction. NBT reduction was measured according to the method of Beyer and Fridovich (1987). A fruit was homogenized in 1ml cold 100 mM K-phosphate buffer (pH 7.8) containing 0.1 mM ethylene diaminetetraacetic acid (EDTA), 1% (w/v) polyvinyl-pyrrolidone and 0.5% (v/v) triton X-100. One unit of SOD activity was defined as the number of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. For the determination of APX, fruit tissue was homogenized in 100 mM Na-phosphate buffer (pH 7.0) containing 5mM as A, 10% glycerol and 1mM EDTA. APX activity was determined in 1ml reaction mixture containing 50 mM K-phosphate buffer (pH 7.0), 0.1mM as A (extinction coefficient, 2.8 mM⁻¹ cm⁻¹) and 0.3 mM H₂O₂. The decrease in absorbance was recorded at 290 nm for 3 min (Chen and Asada, 1989). For GR, fruit tissue was homogenized in 100 mM Na-phosphate buffer (pH 7.0) containing 1mM EDTA. Glutathione reductase (GR) activity was assayed by following the reduction of 5, 5’-dithio-bis (2-nitrobenzoic acid) (DTNB) at 412 nm (extinction coefficient, 13.6 mM⁻¹ cm⁻¹) following Putter (1974). The activity was assayed for 5 min in a reaction mixture comprised of 50 mM K-phosphate buffer (pH 7.0), 20.1 mM guaiacol, 12.3 mM H₂O₂ and suitable amount of enzyme extract from fruits.
2.9 Statistical analysis

Data from the experiments were presented as mean ± SD. Statistical analysis was done by using the SPSS 16.0 and Graph Pad Prism Program. The level of significance was set at $p < 0.001$.

3. Results

3.1 Effect on serum glucose

Alloxan (150 mg/kg b.w.) administration resulted in significant elevation of glucose level. Administration of *D. pentagyna* at a dose of 200 and 400 mg/kg b.w. administered for 21st days were able to correct this aberration significantly ($p < 0.001$). The results of all the formulations tested are presented in (Table 1 and Figure 1). Before treatment schedule, fasting blood glucose level in all animals was within normal range. After treatment with alloxan, the fasting blood glucose level was significantly changed and it was significantly ($p < 0.001$) reduced by 21st days treatment with hydroalcoholic extract of *D. pentagyna* that is comparable to the standard metformin HCl (100 mg/kg b.w).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CN</th>
<th>DR</th>
<th>DR+R200</th>
<th>DR+R400</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>85.67±0.33</td>
<td>298.34±0.41</td>
<td>234.34±0.27</td>
<td>134.45±0.12</td>
<td>145.67±0.15</td>
</tr>
</tbody>
</table>

Table 1: Effect of *D. pentagyna* on fasting blood glucose (FBG) level in normal and alloxan induced diabetic rat (AIDR).

![Figure 1: Effect of *D. pentagyna* on fasting blood glucose (FBG) level in normal and alloxan induced diabetic rat (AIDR). CN=Normal; DR=Diabetic rat; R200=Plant extract at 200 dose; R400 = Plant extract at 400 dose; MET (Metformin) =100 mg/kg Body weight. Results are presented as mean ± SD, (n=6), Dunnet test showed $p<0.001$ for R400 vs normal control (***) and $p<0.001$ for R400 vs diabetic control group (##).](image)

3.2 Serum marker enzymes activity

The results of statistical analysis showed a significant difference in the mean values of SGOT, SGPT, ALP and bilirubin levels in *D. pentagyna* extract treated and diabetic control groups ($p < 0.001$). This difference indicates the effectiveness of *D. pentagyna* in reducing serum liver marker enzyme levels. As it is seen in (Table 2 and Figure 2), the effectiveness of shallot extract significantly changes based on its dosage ($p < 0.001$) and the high dose of the extract (400 mg/kg b.w) has even been more effective than metformin in decreasing liver marker enzyme levels ($p < 0.001$).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>DR</th>
<th>R200</th>
<th>R400</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (IU/ml)</td>
<td>113.3±0.22</td>
<td>211.6±0.38</td>
<td>180.5±0.31</td>
<td>165.3±0.25</td>
<td>167.3±0.51</td>
</tr>
<tr>
<td>SGOT (IU/ml)</td>
<td>35 ± 0.12</td>
<td>76.56±0.21</td>
<td>65.98±0.30</td>
<td>45.98±0.29</td>
<td>49.58±0.15</td>
</tr>
<tr>
<td>SGPT (IU/ml)</td>
<td>80.23 ±0.12</td>
<td>58.67±0.23</td>
<td>45.45±0.12</td>
<td>39.67±0.27</td>
<td>48.45±0.21</td>
</tr>
<tr>
<td>BILIRUBIN (mg/dl)</td>
<td>0.68 ± 0.06</td>
<td>1.23±0.01</td>
<td>1.10±0.13</td>
<td>0.87±0.015</td>
<td>0.73±0.014</td>
</tr>
</tbody>
</table>

Table 2: The effects of hydroalcoholic extract of *D. pentagyna* on liver marker enzymes level
3.3 Amelioration of liver pathology in diabetic rat by effect of *D. pentagyna* extract

In (Figure 3), panel A to D showed all the different structures of stained liver sections from normal, diabetic and diabetic rats treated with 200 mg/kg b.w. and 400 mg/kg b.w. In control, wistar rats liver section showed normal structure of central vein (CV), hepatocytes (H) well preserved and essentially normal, non-vacuolated cytoplasm, sinusoid (S) well demarcated while in diabetic rats liver section showed abnormal structure of central vein (CV), sinusoid (S) and hepatocyte (H) are not arranged in sinusoid. Cytoplasm is vacuolated. Panel C and D have showed amelioration in liver pathology upon treatment with 200 and 400 mg/kg b.w. of *D. pentagyna* extract. In panel C, central vein (CV) and sinusoid (S) are relatively well in position with arranged hepatocyte (H) in sinusoid and non-vacuolated cytoplasm. According to panel D, treated waster rats liver section showed enhanced restoration in liver structure with normal central vein (CV), sinusoids (S) and also arranged hepatocyte (H) in sinusoid with non-vacuolated cytoplasm.

**Figure 3:** H and S stained liver sections of normal, diabetic and treated rats. Panel A and Panel B showed micrograph (20X magnification) of stained liver sections from normal and diabetic rats, respectively whereas other two panels represent the liver sections from treated rats with Panel C. 200 mg/kg body weight and Panel D. 400 mg/kg body weight of *D. pentagyna* extract.

### 3.4 Antioxidant activity of *D. pentagyna* fruit at different maturation stages

The activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) were initially low at the premature stage and then increased during development at mature stage while guaiacol peroxidase (POD) was high at the premature stage and decreased during development. Whatever we have seen that the overall maximum activity detected at the mature stage in *D. pentagyna* fruits. It is seen in Table 3 and Figure 4 and values introduced as mean ± SD. It remains to be determined if fruit taken from premature stage will mature with the high quality as the process of maturation stages.

**Table 3:** The changes in the content of antioxidant enzymatic parameters during two harvesting stages. All values introduced as mean ± SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Premature fruits</th>
<th>Half-ripe fruits</th>
</tr>
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<tbody>
<tr>
<td>Superoxide dismutase (U/g FW)</td>
<td>174.10 ± 9.67</td>
<td>206.14 ± 12.80</td>
</tr>
<tr>
<td>Ascorbate peroxidase (µM/g FW)</td>
<td>406.91 ± 21.31</td>
<td>1709.52 ± 58.19</td>
</tr>
<tr>
<td>Guaiacol peroxidase (mmol g−1 FW)</td>
<td>1.06 ± 0.14</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Glutathione reductase (mmol/g FW)</td>
<td>0.12 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
</tbody>
</table>
Figure 4: The changes in the content of enzymatic antioxidants parameters during two harvesting stages. All values introduced as mean ± SD.

4. Discussion

The ethnobotanical survey can bring out many different clues for the development of drugs to treat human diseases like diabetes. Secure, dominant and inexpensive inland medications are receiving popularity uniformly among the people of both the urban and churl areas, especially in developing countries like Bhutan, Bangladesh and India (Katewa et al., 2004). In this study, D. pentagyna was selected for antidiabetic study and rejuvenating capacity of tissues. Alloxan is most commonly used to induce diabetes in animals. Alloxan is a β-cytotoxin, induces Diabetes mellitus by damaging the insulin secreting β-cells of the pancreas, resulting in decreased endogenous insulin release (Rajagopal and Sasikala, 2008). High ambient glucose can promote apoptosis, suggested by Allem et al. (2003) causing potential cellular damage as a result of hyperglycaemias in diabetes. Reactive oxygen species (ROS) are important mediators of β-cell death during the development of DM. High glucose has been postulated to generate ROS and nitrogen species in numerous cell types. In this study, significant hyperglycaemias was achieved after alloxan (100 mg/kg body weight) injection. Alloxan induced diabetic rat with more than 200 mg/dl of blood glucose level were considered to be diabetic used for the study. However, administration of the hydroalcohol extract at the dose of 200 and 400 mg/kg body weight decreased the glucose level in alloxan induced rats. The results indicated a considerable change in body and organs weight between alloxan induced and treated rats. The increase in body weight of diabetic rats as a result of D. pentagyna treatment may be ascribed to the increase in insulin release.

5. Conclusion

This study confirmed that the hydroalcoholic extract of the fruit at 400 mg/kg b.w. dose exhibited significant antihyperglycemic than at low dose (200 mg/kg b.w.) in the induced diabetic rats, various biochemical parameters like liver marker enzymes as well as regeneration of liver tissues and its beneficial metabolic effects through augmenting ALP activity. We propose that the antioxidant activity of fresh fruit tissue seems to be more influenced by genesis differences than physiological and biochemical changes. Therefore, further investigation is necessary to determine the exact phytocomponents responsible for antidiabetic effect.

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Conflict of interest

We declare that we have no conflict of interest.

References


