ANTI-OXIDATIVE AND ANTI-DIABETIC EFFECT OF POD EXTRACT OF PITHECELLOBIUM DULCE (ROXB.) BENTH, ON LIVER, KIDNEY AND PANCREAS IN ALLOXAN INDUCED DIABETIC MALE SWISS ALBINO MICE

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ABSTRACT
Pithecellobium dulce has previously been used for the treatment of various diseases. In this study the pod extract of P. dulce is being used to investigate the anti-oxidative and anti-diabetic activity in alloxan induced diabetic male Swiss albino mice and the tissues were also studied for histo-pathological changes induced after induction of diabetes and subsequent extract treatment. 24 male Swiss albino mice each of 25-32g of body weight were divided in 4 groups (each group was maintained with 6 mice), I Normal Control (NC), II Diabetic Control (DC), III Glibenclamide Treated (GT) and IV P. dulce pod extract treated group (PDPE). Diabetes was induced to DC, GT and PDPE by a single intraperitoneal injection (IP) of alloxan monohydrate of 150mg/kg B.w. Group IV was orally administered by a single dose of 300mg/kg B.w./d of PDPE extract, whereas diabetic group III was treated with glibenclamide (10mg/kg of B.w.) for 45 days. Fasting Blood Glucose levels of all the four groups were measured using blood glucose test strips of Dr. Morpen’s glucometer at a regular interval of 0, 15, 30 and 45th days and various tissue homogenates viz liver, kidney, pancreas was used for the estimation of enzymatic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx). Hepatic, Renal and Pancreatic tissues from the four groups were studied for histological changes, induced after Alloxan injection and subsequent extract treatment. Data obtained from the study shows significant (P<0.05) reduction in FBG levels by 38.1% after the 45 days treatment of PDPE and significantly (P<0.05) normalized enzymatic antioxidants to such an extent that helped in the reduction of oxidative damage in the tissues of...
diabetic animals. Histopathological examination shows regeneration of tissues in their normal state. It can be concluded from this study that the pods of *P. dulce* shows significant favorable effects in various physiological and histopathological parameters distorted during diabetic demonstration, and these effects correspond to the standard drug treated glibenclamide group.

**KEYWORDS:** Diabetes mellitus, Antioxidants, Antidiabetic, Histopathology, Alloxan and Glibenclamide.

**INTRODUCTION**

Diabetes Mellitus (DM) is a multi - factorial disorder characterized by hyperglycemia, high blood glucose level. Presently, DM affects a number of people in the world and so is considered as a major public health problem in developing as well as in developing countries. The prevalence of DM in 2011 is nearly 366 million people worldwide (2.8% of the population). Its incidence is increasing swiftly, and expected to double by the year 2030.

The largest number of generation of free radicals and oxidative stress is assumed to play a major function in the pathogenesis of DM and its late complications. In DM, major possible cause of oxidative stress and damage caused to proteins includes production of free radicals by auto-oxidation of sugars and sugar adducts of proteins as well as by auto-oxidation of unsaturated lipids in plasma and membrane proteins. It can be assumed that the oxidative stress in DM is amplified by a constant cycle of metabolic stress, damage of tissues and cell death which may lead to the increased production of free radicals and compromised scavenging system of free radicals, which aggravate the oxidative stress. Certainly, there is a prevalent believe of a potential role of reactive oxygen species (ROS) generated as a product of hyperglycemia, which may cause various secondary complications of DM such as, retinopathy, cardiomyopathy, etc. Moreover, the existence of higher glucose or glycated protein concentration in DM increases lipid peroxidation and consequently, lipid peroxides may lead to increases the extent of advanced glycation end products (AGEs).

Several allopathic drugs like sulfonylureas, biguanides, thiazolidinedions, alpha-glucosidase and meglitinides, available in the market are used for the treatment of diabetes and associated complications. However, these drugs play a valuable role in the management of DM, but have limitations due to undesirable adverse effects such as hypoglycemia, weight gain, and inability arrest pancreas degeneration or diabetic implications which have been linked to diabetes-induced oxidative stress. According to ethnobotanical medicinal practice
approximately 800 plants are used to treat and manage DM. Though, numerous medicinal plants traditionally reported to have hypoglycemic properties with their efficacy, low incidence of side effects, low cost, safety, effectiveness and availability when compared to synthetic drugs.\(^9,10\) Because of this reason, there is an increasing interest in the natural product remedies with a basic approach towards the nature.

The aim of the present study is to investigate in-vivo anti-diabetic effect of *P. Dulce* pods in alloxan induced diabetic male Swiss albino mice and also investigated in-vivo anti-oxidants levels and the histology of liver, kidney and pancreas brought about by PDPE administration to alloxan induced diabetic mice.

*Pithecellobium dulce* (Roxb.) Benth

*P. dulce*, commonly known as “jungle jalebi” is an evergreen flowering plant from the family Fabaceae, cultivated throughout the plains of India and also in Andamans.\(^11\) This plant is reported to possess various medicinal properties such as antimicrobial, dermatitis\(^12\), anti-inflammation\(^13\), emollient, abortifacient and antidiabetic\(^14\) and antioxidative properties.\(^15\)

**MATERIAL AND METHODS**

**Chemicals**

Alloxan monohydrate was purchased from SD Fine chemical (Mumbai, India). Other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), SRL (India), CDH (India), Qualigens (India/ Germany).

**Collection and Preparation of plant extract**

Taxonomically identified plant *P. dulce* Pods were collected from the Khejari nursery, Jaipur, Rajasthan, India. Shade dried pods were subjected to size reduced to a coarse powder which was then soxhlet extracted with 50% hydro-ethanol. This extract was then concentrated to dryness under reduced pressure at 60±1°C in a rotator vacuum evaporator. The extract was then dried at 40-45°C in hot air oven till solid to semisolid mass was obtained. During the experimental period, the suspension of pods extract was prepared in 20% tween 20 in normal saline.

**Animal Care and monitoring:** The healthy Swiss albino male mice (*Mus Musculus*) of 4-6 months old and of 25-32g in weight were procured from the C.C.S. Haryana Agricultural University, Hissar, India. All the animals were housed under standard laboratory environment
12:12 h L: D cycle light, 23±2°C temperature and 55±5% relative humidity. For feeding standard rat pellet diet and tap water ad libitum were provided to the experimental animals. All these animals were maintained and treated as per the directions of the Institutional Animal Ethical Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (574/02/ab/CPCSEA).

**Diabetes Induction and Treatments**

In this study, all the animals were divided in four groups (I) Normal Control (NC), (II) Diabetic Control (DC), (III) Glibenclamide Treated (GT) and (IV) *P. dulce* pod extract treated (PDPE). After 18 hour fasting period, the mice from all the groups were made diabetic by single intra-peritoneal injection (IP) of alloxan monohydrate. For single mice the alloxan was used 150mg/kg body weight, which was freshly dissolved in normal saline.\[16\] Subsequent of alloxan administration, the food and water were freely accessible to the treated animals and to control the drug induced hypoglycemic shock they were provided with 5% glucose solution to drink overnight. After one week of alloxan injection, the Fasting Blood Glucose (FBG) levels were determined by means of one touch ultra glucometer (Dr. Morpen’s Glucometer) and with compatible blood glucose strips.\[17\] Animals with 140mg/dl or above FBG levels were considered as diabetic\[18,19\] and were selected for treatment with Standard drug Glibenclamide (10mg/kg body weight) or pod extract (300mg/kg body weight). The drug and the pod extract were administered orally, once in a day for 45 days.

**Estimation of FBG levels of anti-diabetic activity**

FBG levels of all the four experimental groups were noted by glucometer (Dr. Morpen One Touch Glucometer) at the time interval (Before inducing, zero day, 15, 30 and 45th day) of the experiment by collecting a drop of blood from the tail vein of each animal. FBG levels were expressed in mg/dl.

**Tissue homogenate preparation and estimation of antioxidants**

After 45 days of experimentation, the animals were sacrificed by cervical dislocation and were dissected under aseptic conditions. Then, Liver, Pancreas and Kidney were removed from each animal, freed from adhering tissues, washed with ice-cold normal saline solution (0.9%) and blotted dry and weighted. Thereafter, each tissue was separately homogenized in ten times of its volume of 0.2M Tris HCl with the help of Homogenizer. The homogenate was filtered through cheese cloth to remove any lumps that may be present. The filtrate was centrifuged at 10,000 RPM for 20 minutes at 4°C. The supernatant so obtained was used for
estimation of enzymatic antioxidants viz., Superoxide dismutase (SOD)\textsuperscript{[20]}, Catalase (CAT)\textsuperscript{[21]}, glutathione peroxides (GPx).\textsuperscript{[22]}

**Histo-pathological Examination**

Selected tissues from all the four groups were studied for histological changes, induced after Alloxan injection and subsequent extract treatment. For this, about 2mm thick piece of tissue was fixed in Bouin’s fluid for 24 hours, followed by thorough washing in water and then dehydrating them through alcoholic series. The tissues were then being kept in xylene for ½ to 1 hour. After cleaning in Xylene, tissues were embedded in paraffin wax at 53°C, blocks were prepared and cut at 6 microns. The ribbons were shaded on clean slides using Meyer’s albumin. After 24 hours of drying, the sections were stained with hematoxylin and counter stained with eosin and then mounted in D.P.X. The permanent mounts were then being photo-micrographed.\textsuperscript{[23]}

**Statistical Analysis**

Results are expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey’s post hoc multiple comparison test using SPSS (version 16.0) and students’ t - test using Sigma Plot (version 8.0). The values of P<0.05 were considered as statistically significant.

**RESULT AND DISCUSSION**

**Antidiabetic study**

In this study of anti-diabetic treatment of PDPE was evaluated in alloxan induced diabetic male mice in regular interval of 45 days. Alloxan used was reported to cause a significant decrease of insulin producing beta cells of islets of Langerhans and hence, causes hyperglycemia, a major class of diabetes mellitus. According to the previous reports the dose of 150mg/kg body weight of alloxan was selected to induce diabetes in experimental mice.\textsuperscript{[24]}

In this condition the insulin is secreted but not sufficient to regulate blood glucose level of the body and therefore, leading to the significant increase of fasting blood glucose level in alloxan induced diabetic mice. Moreover, treatment of diabetic mice with PDPE (300mg/kg body weight) for 45 days progressively reduced FBG levels (Table 1) from 208.28±2.7 mg/dl to 129±9.54mg/dl (38.1%) on the 45th day. Reduced levels of FBG by treatment of PDPE can be compared by lowering effect of glibenclamide (GT). Significant (P<0.05) reduction of FBG levels by PDPE was might be due to the amplified use of peripheral glucose or potentiating of the insulin effects.
Table 1: FBG levels (mg/dl) during 45 days treatments

<table>
<thead>
<tr>
<th>Days</th>
<th>NC</th>
<th>DC</th>
<th>GT</th>
<th>PDPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Induction</td>
<td>106.14±13.18</td>
<td>102±3.36</td>
<td>99±17.54</td>
<td>102.7±3.8</td>
</tr>
<tr>
<td>After Induction</td>
<td>106.14±13.18 a*</td>
<td>185.57±13.91 a</td>
<td>206±35.37 a</td>
<td>208.28±2.7 a</td>
</tr>
<tr>
<td>15</td>
<td>106.71±14.4</td>
<td>184.8±15.3</td>
<td>172.5±8.2</td>
<td>172.5±10.5</td>
</tr>
<tr>
<td>30</td>
<td>112±12.2</td>
<td>177.43±14.5</td>
<td>123.4±12.7</td>
<td>128.1±6.4</td>
</tr>
<tr>
<td>45</td>
<td>103.71±9.8 a</td>
<td>163.71±16.96 a b</td>
<td>95.7±18.1 a* b</td>
<td>129±9.54 a b</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 7 observations
*Before induction (Basal values); Student’s ‘t’ test is significant at P<0.05. a: significant (P<0.05) difference, a*: insignificant difference in basal values, b: significant difference (P>0.05) compared to after induction; b*: an insignificant (P<0.05) difference compared to values obtained after alloxan injection.

Anti-oxidative study
ROS are the causative factor of diabetic complications.[25] Moreover, it has also been reported that during diabetes induced oxidative stress, the antioxidant parameters were altered. In this study, alloxan was used to induce diabetes and it is being first absorbed by beta cells of the pancreas and liver and then results in the formation of ROS and besides, this the consequential oxidative stress is primarily responsible for the pathogenesis of diabetes and its complications. Decrease in the antioxidant enzymes such as SOD, CAT, GSH-Px in liver, kidney and pancreas in alloxan induced diabetic control group (Table 2). These observations were in support to the earlier findings.[26, 27] Antioxidant enzymes viz, SOD, CAT, GSH-Px levels were elevated after 45 days treatment of PDPE. The 45 days treatment of PDPE restored the level of SOD in hepatic and renal tissue to 139.80 ± 4.5 and 121.65 ± 5.16 as compared to diabetic control group and reduced in pancreatic tissue to 89.2 ±12. 46 (Table 2). Level of CAT and GSH-Px in 45 days treated PDPE group were significantly (P<0.05) standardized in hepatic, pancreatic and renal tissues. Furthermore, 45 days treated glibenclamide group could not restore enzymatic antioxidant content, as significantly (P<0.05) lower values of enzyme activities can be seen under GT. The antioxidant activity of PDPE might be due to the inhibition of glycation antioxidant enzyme.[28]

Table 2: Effect of 45 days treatment of *P. Dulce* pod extracts on antioxidative status in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>DC</th>
<th>GT</th>
<th>PDPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic SOD</td>
<td>190.20±15.41</td>
<td>140.7±5.144 a</td>
<td>132.67±2.51 b*</td>
<td>139.80±4.5 b* c*</td>
</tr>
</tbody>
</table>
### Table 1: Enzyme Activity of Pancreatic and Renal Tissue

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Pancreatic</th>
<th>Renal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAT²</td>
<td>SOD¹</td>
</tr>
<tr>
<td></td>
<td>208.20±9.43</td>
<td>220.3±14.5</td>
</tr>
<tr>
<td></td>
<td>136.30±6.61 a</td>
<td>197.40±27.9 a</td>
</tr>
<tr>
<td></td>
<td>150.50±7.85 b</td>
<td>208.4±15.95 b*</td>
</tr>
<tr>
<td></td>
<td>265.30±35.56 b c</td>
<td>89.2±28.46 b c</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 7 observations.

1mg/gm tissue, 2 nm TBARS/mg Protein, 3 μ molesH₂O₂ decomposed/min/mg Protein

Students’ t–test is significant at P<0.05. a: significant (P<0.05) difference, a*: insignificant difference (P>0.05) compared to NC, b: significant difference (P<0.05) to DC, b*: insignificant difference (P>0.05) to DC, c: significant difference (P<0.05) to GT, c*: insignificant difference (P>0.05) to GT.

### Histopathological Examination

In this study, autopsies revealed significant damage in liver, kidney and pancreas of steadily diabetic animals. On the other hand, the photomicrographs of vehicle-treated mice of normal control groups (NC) demonstrate normal histological information of these tissues (fig. 1a, e & i). Liver of diabetic mice revealed impaired sinusoids, degenerative hepatocytes, infiltration of triad and some necrosis region. However, these deformities were not witnessed in PDPE treated and GT groups (fig. 1d & c). Histopathological demonstration of pancreas of alloxan administered diabetic group of animal showed damaged islets of Langerhans (fig. 1f). No perceptible islets were found in brutally and determinedly diabetic animal. On the other hand, in glibenclamide treated and PDPE treated group (fig. 1g & h) the islets were comparable to normal control ones, except only some traces of degenerative changes. Thus the histomorphological examination of pancreas revealed that the PDPE repair the action of islets of Langerhans of diabetic animals. In case of kidney, the diabetic control group (fig. 1j) indicates the shrinkage of glomeruli, which was the most remarkable characteristic observed under the renal tissue of diabetic animals. Even though, treatment with PDPE and glibenclamide Fig. L & k) subsequent to alloxan management reverted the normal composition of kidney to a certain extend. Thus, histomorphological changes in all the three tissues revealed that the alloxan administration brutally declined the histology of tissues, but PDPE and GT to assured level restored the detected distortion. In histopathological findings of pancreatic section of diabetic group, revealed that islets were less in number and damaged as compared to normal ones, which might be due to the infiltration of lymphocytes and
moreover to this, the pancreas of PDPE and GT group were comparable to those of normal ones. Similar findings were also reported earlier.\textsuperscript{[29, 30]} In case of liver of diabetic mice, impaired sinusoids, degenerative hepatocytes and some necrosis were noticed. However, these deformities in the liver of diabetic animals were restored in PDPE and GT groups. This indicated the beneficial effects of PDPE on the liver of diabetic animals. On the other hand, the kidney of the diabetic group shows glomerulosclerosis, which is the most common histological character in diabetic individuals. All these notable changes were witnessed in kidney sections of diabetic mice caused by alloxan administration were normalized by the treatment of PDPE and glibenclamide. These findings were previously reported.\textsuperscript{[30]}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{images}
\caption{Histological images of diabetic mice liver and kidney.}
\end{figure}
Fig. 1- Photomicrographs of histological details of liver, pancreas and kidney (400X) [a. Normal liver, b. Diabetic liver (showing impaired sinusoid, infiltration of portal triad and some necrosis regions); c. Glibenclamide treated liver; d. PDPE-treated liver; e. Normal pancreas; f. Diabetic pancreas (showing damaged islets); g. Glibenclamide treated pancreas; h. PDPE-treated pancreas; i. Normal kidney; j. Diabetic kidney (shrunken glomerulus); k. glibenclamide treated kidney; l. PDPE-treated kidney.

CONCLUSION

Present study confirmed that the crude hydro-ethanolic pod extract of *Pithecellobium dulce* possess significant antihyperglycemic, antioxidative potential and consequently, it gives an indication to find its use in the management of diabetes and resultant oxidative stress.

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**ABBREVIATIONS**


**REFERENCES**


