STREPTOZOTOCIN-INDUCED OXIDATIVE STRESS IN DIABETIC RATS - A DEFENSIVE EFFECT OF PSYDRAX DICOCOS

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ABSTRACT

Objective: The present study was aimed to evaluate the antihyperglycemic activity and in vivo antioxidant effect of methanolic extract of whole plant of Psydrax dicoccos (MEPD) belonging to the family Rubiaceae.

Methods: MEPD was prepared by Soxlet extraction. Wistar rats weighing (180–200 g) were divided into six groups (n=6), with three doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of extract. Metformin was used as a standard drug. Diabetes was induced by streptozotocin (STZ) (40–50 mg/kg,i.p) in control group. The animals were treated with different doses of extracts for 21 days, and on the 22nd day, the blood glucose levels along with antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and lipid peroxidase (LPO) were determined.

Results: The phytochemical screening of the extract showed the presence of carbohydrates, phenolics, flavonoids, glycosides, and tannins. The methanolic extract of MEPD at the dose of 200 mg/kg body weight showed a significant reduction in blood glucose levels (**p<0.001) with the value of 151.2 mg/dl on the 22nd day at 8 h. A promising antioxidant effect was also evident from the determination of antioxidant enzymes such as SOD, CAT, and LPO.

Conclusion: The P. dicoccos extract revealed a potential effect of antihyperglycemic activity and combating nature on oxidative stress induced by STZ.

Keywords: Psydrax dicoccos, Streptozotocin, Blood glucose, Catalase, Lipid peroxidase.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia with hampered metabolism of vital biological components such as carbohydrate, protein, and lipids. The effects are mainly due to the defective insulin secretion, loss of insulin receptors, or both [1]. Earlier century in India, two physicians "Charaka and Susruta" reported the disease. However, in the 18th-19th century, hyperglycemia is less visualized clinically, identified by uncontrolled glycosuria, and usually diagnosed in later stage of life; currently, it is recognized as type 2 diabetes mellitus. Persistent higher blood glucose levels generate free radicals that may lead to the development of oxidative stress in distinct parts of the body which further culminate to various complications such as cardiovascular, neurodegenerative, chronic kidney failure, ocular damage, and vascular diseases [2]. Free radicals are highly unstable reactive oxygen species (ROS) produced regularly in various normal biological reactions in the human system. Whenever these free radicals are produced, their effects are monitored by defense system of the body which includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glutathione reductase (GR), and lipid peroxidase (LPO) [3]. This “oxidative shielding” acts as a protective mechanism to counter the attack of toxic pathogens or noxious chemicals or to destruct the cell by apoptosis and thus preventing its advancement to neighboring cells [4]. The term “oxidative stress” is biological state in which ROS and RNS level attain a maximum peak, either by excess production or insufficient removal. Since ROS and RNS have been a highly reactive molecule, it will cause some alteration to vital biological proteins, carbohydrates, DNA, lipids, and other molecules, further prelude to physiological dysfunction of cells and pathologic condition such as diabetes, heart diseases, and cancer [5].

Antioxidants are the compounds that can prevent (or) inhibit oxidation chain reaction process in living cells [6]. The use of synthetic agents is frequently associated with several undesirable side effects and fails to correct the fundamental biochemical lesion and diabetic complications. From the researcher’s point of view, investigation on medicinal plants has gained importance because of their natural origin and fewer side effects [6]. New therapies are needed which control the hyperglycemic condition and prevent long-term complications. Although there are drugs which were reported to control blood glucose levels of diabetic subjects/animal models, these failed to control complications of diabetes because of side/toxic effects such as hepatotoxicity or cardiac failure. Therefore, authentic evaluation is needed for herbal drug with an expectation of product efficacy, safety, and therapeutic risk/benefit. Hence, the current research aims to scientifically investigate the traditionally used medicinal plants in the treatment of diabetes mellitus, their insulin secretagogue action, and potent antioxidant effect.

Psyrax dicoccos Gaertn. (Syn. Canthium dicoccum [Gaertn.]) belongs to the family Rubiaceae and the plant is found in all parts of tropical regions of India. It is an unarmed shrub, grows up to 3 m tall. All the plant parts have been recognized to have medicinal properties such as anti-inflammatory, digestive, anti-necrotic, hepatoprotective, neuroprotective, and antioxidant property. It is also used for fever and also applied as plasters; decoction of the root is used in diarrhoea. Bark powder with sesame oil is used in rheumatic pains. Used in inflammation, during night boiled leaf extract is taken for 2 months [7]. A number of flavonoids have been shown to suppress carcinogenesis in various animal models. Flavonoids are also responsible as effective scavengers, which participate in antioxidant mechanism. The present study was taken up to evaluate the antihyperglycemic property and antioxidant effect of whole plant extract (methanolic) of P. dicoccos streptozotocin (STZ)-induced diabetic rats [8].

METHODS

Collection of plant material and preparation of extract [9]

The whole plant P. dicoccos was procured from Tirumala Hills, Tirupati, and was authenticated by Dr. Madhava Chetty, Botanist, Sri
Venkateshwara University, Tirupati, Andhra Pradesh. The plant material was powdered and extracted with methanol in a Soxhlet apparatus at a temperature of 60°C for 12 h. The resultant extract was filtered, and the filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure and stored in a desiccator.

**Preparation of extract**

Suspension of methanolic extract of *Psydrax dicoccos* (MEPD) was prepared in 0.1% dimethyl sulfoxide as a suspending agent. The extract was administered at three doses of 100, 200, and 400 mg/kg, respectively.

**Preliminary phytochemical analysis**

The MEPD was screened for the presence of various phytoconstituents such as alkaloids, carbohydrates, phenolics, flavonoids, glycosides, and tannins.

**Acute toxicity studies** [10]

Albino rats weighing 150–250 g were selected by random sampling technique and used for the study. Acute oral toxicity was performed as per OECD-423 guidelines (acute class method). The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation, and the groups were observed for 14 days. If mortality was observed in two or three animals, among six animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose.

If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions, and mortality for 72 h. It was observed that the test extract was not mortal even at a dose of 2000 mg/kg body weight. Hence, 250 mg/kg and 500 mg/kg doses were selected for the study.

**Animals**

Adult male rats of Wistar albino strain weighing between 150 and 200 g were obtained from our college animal house. They were kept in polypropylene cages with not more than five animals per cage and allowed to get acclimatized to a standard laboratory diet. The animals were adapted to laboratory condition before the experiments at constant room temperature of 22±1°C temperature with 12 h light and dark cycle. Feed (standard pellets) and drinking water were provided ad libitum. The experimental protocol was duly approved by the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision on Experiments on animals through its reference no: IAEC/SYCP/2016/007, Dated: 27/2/16.

**Induction of experimental diabetes** [11]

Induction of experimental diabetes: Diabetes was induced by a single intraperitoneal injection of a freshly prepared STZ solution (Sisco Research Laboratories Pvt., Ltd., Mumbai-93, India) (Batch No: T-835796) (Dose: 30–50mg/kg) in citrate buffer 0.1 M, pH 4.5 to overnight fasted rats. Diabetes was identified by polydipsia, polyuria, and by measuring blood glucose levels 48 h after injection of STZ.

**RESULTS AND DISCUSSION**

From ancient times, diabetic patients have used medicinal plants to maintain blood glucose level. In this regard, the present study was extended to show the influence of extracts on the blood glucose levels and oxidative stress.

The preliminary phytochemical analysis showed that MEPD was found to possess various phytoconstituents such as carbohydrates, phenolics, flavonoids, glycosides, and tannins, whereas alkaloids were absent.

MEPD (200 mg/kg) and metformin (50 mg/kg) showed significant (p<0.001) fall in blood glucose levels at 1 h, 2 h, 4 h, 6 h, and 8 h, respectively, when compared with DC group (Table 1).

Table 2 shows the effect of administration of MEPD on MDA, CAT, and SOD in liver tissue of different groups of rats. There was a significant (p<0.001) elevation in tissue MDA in diabetic rats as compared to normal rats, whereas significant (p<0.05) decrease in tissue CAT and SOD in diabetic rats as compared to normal rats.

**Table 1: Effect of MEPD on blood glucose levels in diabetic rats**

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>Serum glucose levels (mg/dl)</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>94.4±3.8</td>
<td>86.5±3.21</td>
<td>91.3±2.03</td>
<td>82.9±0.35</td>
<td>85±0.36</td>
<td>82.6±0.95</td>
</tr>
<tr>
<td>DC</td>
<td></td>
<td>268.5±5.5**</td>
<td>272.2±3.95**</td>
<td>280.9±3.26**</td>
<td>281.4±7.8**</td>
<td>275.8±1.14**</td>
<td>271.3±1.14**</td>
</tr>
<tr>
<td>DC+Metformin (50 mg/kg)</td>
<td></td>
<td>280.7±3.3</td>
<td>262.6±3.15</td>
<td>175.3±3.98</td>
<td>138.3±1.82</td>
<td>275.8±1.14**</td>
<td>271.3±1.14**</td>
</tr>
<tr>
<td>DC+MEPD (100 mg/kg)</td>
<td></td>
<td>270.4±9.6</td>
<td>255.6±3.08</td>
<td>250.1±2.2</td>
<td>261.2±2.62</td>
<td>251.6±1.22</td>
<td>259±2.62</td>
</tr>
<tr>
<td>DC+MEPD (200 mg/kg)</td>
<td></td>
<td>275.6±3.48</td>
<td>255.6±3.67</td>
<td>185.5±3.6</td>
<td>165.9±3.01</td>
<td>160.02±2.11**</td>
<td>150.6±3.27**</td>
</tr>
<tr>
<td>DC+MEPD (400 mg/kg)</td>
<td></td>
<td>270.6±3.66</td>
<td>255.8±3.66</td>
<td>250.78±2.67</td>
<td>245.07±1.68</td>
<td>240.8±1.12</td>
<td>240.67±1.67</td>
</tr>
</tbody>
</table>

The data are expressed in mean±SEM, n=6 in each group, **p<0.001, significant, compared to DC. MEPD: Methanolic extract of *Psydrax dicoccos*, DC: Diabetic control, SEM: Standard error of the mean
Table 2: Effect of MEPD on antioxidant enzymes in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment and dose (mg/kg)</th>
<th>SOD</th>
<th>CAT</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>68.7±0.98</td>
<td>22.05±0.56**</td>
<td>15.57±0.48**</td>
</tr>
<tr>
<td>II</td>
<td>DC</td>
<td>17.87±0.89**</td>
<td>8.19±1.19**</td>
<td>56.33±1.72**</td>
</tr>
<tr>
<td>III</td>
<td>DC+Metformin (50 mg/kg)</td>
<td>48±0.77**</td>
<td>18.01±0.66**</td>
<td>18.06±1.08**</td>
</tr>
<tr>
<td>IV</td>
<td>DC+MEPD (100 mg/kg)</td>
<td>18.67±2.98**</td>
<td>9.98±2.16**</td>
<td>56.66±2.06**</td>
</tr>
<tr>
<td>V</td>
<td>DC+MEPD (200 mg/kg)</td>
<td>38.67±3.01**</td>
<td>42.06±2.26**</td>
<td>18.06±1.89**</td>
</tr>
<tr>
<td>VI</td>
<td>DC+MEPD (400 mg/kg)</td>
<td>19.67±0.6**</td>
<td>9.12±1.16**</td>
<td>52.66±1.16**</td>
</tr>
</tbody>
</table>

The data are expressed in mean±S.E.M; n=6 in each group; **p<0.001, significant, compared to DC. MEPD: Methanolic extract of *psydrax dicoccos*, DC: Diabetic control, SEM: Standard error of the mean. SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde. NS: Non-significant

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AUTHORS’ CONTRIBUTION

Both the authors have equal contribution with regard to the work and write up.

CONFLICTS OF INTEREST

The authors have no conflicts of interest absolutely with anyone.

REFERENCES