INTRODUCTION

Diabetes is common metabolic disorder characterized by hyperglycemia resulting from insufficient insulin secretion, or action or both. The World Health Organisation (WHO) reported that 300 million people would suffer from diabetes mellitus by the year 2025. India is one of the leading countries for the number of people with diabetes and it is estimated that diabetes will affect approximately 57 million people by the year 2025 in India. Hyperglycemia is connected with antioxidative defense system which is accepted to be important in the rise of reactive oxygen species (ROS) and free radicals that cause development and progress of diabetic complications. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins. Several scientific reports indicate that diabetic complications are associated with overproduction of (ROS) and accumulation of lipid peroxidation by-products. Free radicals generated in diabetes may lead to several kinds of diabetic complications including nephropathy, neuropathy, cardiopathy etc. Presently available synthetic antidiabetic drugs including sulfonylureas, biguanide, thiazolidinedione and a-glycosidase display undesirable side effects, such as hypoglycaemic coma and hepatorenal disturbances. Hence, the search for safer and more effective antidiabetic drugs has continued. Following the World Health Organisation (WHO) recommendation for research on the herbal medicinal plants in the management of diabetes mellitus, investigations on antidiabetic agents derived from herbal medicinal plants have also gained energy. Medicinal plants have the benefit of having lower side effects. Some of them have been used in traditional and Ayurveda medicine from the ancient time in many countries. In the course of our research project on herbal medicinal plants, we investigated P. tirupatiensis is an endemic species. It is restricted to Seshachalam hills, Eastern Ghats of India. The Vernacular Name of P. tirupatiensis is Adavikothimeera and it belongs to Apiaceae (Umbelliferae) Family. In the present study, attempts have been made to decrease oxidative stress of diabetes by P. tirupatiensis, without compromising its antidiabetic effects, and also to discover whether this treatment can repair the altered antioxidant defence system in the liver and pancreas of STZ-induced diabetic rats.

MATERIALS AND METHODS

Collected the plant materials

The P. tirupatiensis was collected from Tirumala Hills of Chittoor district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by the concerned herbarium officer, Dept. of Botany, S.V. University, Tirupati Andhra Pradesh. Voucher specimen (1533) was deposited in the campus.

Preparation of extract

The P. tirupatiensis tuberous root was dried in the shade, powdered and the powder was used for the extraction of potential antidiabetic principles into organic solvent (ethyl acetate). P. tirupatiensis tuberous root powder was soaked in ethyl acetate in different glass jars for 2 days at room temperature and the solvent was filtered. This was repeated three to four times until the extract give no coloration. The extract was distilled and concentrated under reduced pressure in the Rotary Evaporator (Model no-HS-2005V) and finally freeze dried by lyophilizer (Lyodel). The yield of the ethyl acetate extract was 3.298% (w/w) in terms of dried starting material.

Selection of animals

Wistar strain male albino rats (n = 30), weighing 130 ± 10 g and six months age were used in this study. All the rats were
maintained in the polypropylene cages (six rats per cage), at an ambient temperature of 25 ± 2°C with 12-h-light/12-h-dark cycle. Rats allowed free access to standard chow (Hindustan Lever Ltd., Bangalore, India) and water ad libitum during the study. All the experiments in this study were performed according to the regulations for the care and use of laboratory animals and this study was approved by the Institutional Animal Ethical Committee and its resolution number; 09 (ii)/a/CPCSCA/IAEC/07-08/SVU/Zool/ dated 26/6/08.

Chemicals and reagents

All the chemicals used in the current study were Analara Grade (AR) and brought from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburgh, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and Qualigens (Mumbai, India).

Induction of diabetes

Streptozotocin (STZ) solution was freshly prepared in 0.1M citrate buffer (pH 4.5) and 40 mg/kg body weight (b.w.) was injected intraperitoneally in a volume of 1ml/kg b.w.11. Because STZ is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose (5-10ml) orally after 6 h of injection for the next 48 hours to prevent hypoglycaemia. Neither death nor any other adverse effect was observed at the tested concentration throughout the study. After one week, rats with diabetes (i.e., high blood glucose levels, 200-300 mg/dL) that exhibited glycosuria and hyperglycaemia were selected for the experiment.

Experimental design and treatment

Thirty rats were divided into five equal groups and treated as follows;

**Group I**: Normal control rats, given normal saline (1ml/kg)

**Group II**: Diabetic control rats, received STZ- in single dose (40 mg/kg, i.p.)

**Group III**: Diabetic rats treated with *P. tirupatiensis* extract (750 mg/kg)

**Group IV**: Normal rats treated with *P. tirupatiensis* extract (750 mg/kg)

**Group V**: Diabetic rats treated with glibenclamide (20 mg/kg).

Animals were treated by oral gavage twice a day for a period of 30 days.

After above treatments, animals were fasted for 12 h, anaesthetized between 8.30 a.m. and 9.30 a.m. using ketamine (24 mg/kg body weight, intramuscular injection). Livers and pancreas were immediately dissected out, washed in ice-cold saline solution to remove the blood. The sample was sliced into pieces and homogenized in cold phosphatic buffer solution (pH 7.0) to given a 10% homogenate (w/v). The homogenates were centrifuged at 5000 g for 10 min at 0°C and the supernatants were taken for biochemical evaluation.

Biochemical examination

The blood glucose levels were measured with an Accu Check Glucometer with a small drop of blood from tail of each rat on the glucometer strip.12, 13 Superoxide dismutase (SOD) activity was assayed by the method of Sun, et al.14. Catalase (CAT) activity was estimated by the method of Aebi,15. Activity of glutathione peroxidase (GPx) was determined by the method of Paglia and Valentine,16. Reduced glutathione (GSH) level was estimated by using the method of Dringen and Hamprecht.17, Vitamin C was determined by the method of Omaye et al.,18 and Vitamin E was estimated by the method of Aebi19. Lipid peroxidation level was estimated by using the method of Jamall and Smith.20. All enzymes activities were as per mg of protein and protein content was determined by the method of Bradford using assay kit (Sigma Diagnostics, St Louis, MO).

Statistical analysis

The results were expressed as mean ± SEM of six rats per group and the statistical significant was evaluated by one-way analysis of variance (ANOVA) using the SPSS (version 15.0) program followed by LSD. Values were considered statistically significant when (p < 0.01).

RESULTS

Effect of *P. tirupatiensis* on blood glucose and body weight levels in STZ- induced animals

Physiological parameters including fasting blood glucose levels and body weights of rats in the control and experimental groups are presented in Table 1 and 2.

Fasting blood glucose levels of diabetic rats were significantly amplified as compared with that of the control ones (P < 0.01). The enhanced of fasting blood glucose levels in diabetic rats were significantly reduced with treatment *P. tirupatiensis* (P < 0.01).

Reduction in body weight was observed in STZ-induce diabetic rats when compared to control group (P < 0.01). Rise in body weight was observed in extract of *P. tirupatiensis* treated diabetic rats when compared to untreated diabetic groups (P < 0.01). No change was observed in treated with extract groups and normal control rats.

Effect of *P. tirupatiensis* on antioxidant enzymes activity in STZ- induced animals

As evident from Fig. 1, 2 and 3, SOD, CAT and GPx activities were significantly lower in liver and pancreas of STZ-induced diabetic group as compared to normal control group (P < 0.001) SOD, CAT and GPx activity in extract treated diabetic group was found to be significantly increased than that in untreated diabetic group (P < 0.01). No change was observed in rats treated with extract and control rats.

Effect of *P. tirupatiensis* on non enzymatic antioxidant levels in STZ- induced animals

Reduced glutathione (GSH), Vitamin C and Vitamin E levels were depleted in STZ- induced diabetic rats as compared to normal control group (P < 0.01). Administration of extract of *P. Tirupatiensis* significantly (P < 0.01) increased levels of GSH, Vitamin E and Vitamin C were observed in liver and pancreas in STZ induced diabetic groups. No change was observed in rats treated with extract and normal control rats (Figs. 4, 5 and 6).

Effect of *P. tirupatiensis* on malondialdehyde level in STZ- induced animals

In diabetic condition, significant enhanced in lipid peroxide was observed in liver and pancreas when compared to normal control rats (P < 0.01), as evident from Fig. 7. On management with extract of *P. tirupatiensis*, significant decreased in the level of malondialdehyde was observed when compared with untreated diabetic groups (P < 0.01), no significance was found in levels of malondialdehyde formation in normal control group and extract treated control group rats.

DISCUSSION

Diabetes is probably one of the fastest growing metabolic diseases in the world. Hyperglycaemia, the primary clinical manifestation of diabetes mellitus, is associated with the development of micro- and macro-vascular diabetic complications. Herbal remedies have been in use for
centuries in the management of diabetes\textsuperscript{23}, but only a few have been scientifically evaluated. Therefore, we have investigated the effect of extract from \textit{P. tirupatiensis} on lipid profiles and biomarkers of oxidative stress, in tissues of STZ-induced diabetic rats. Streptozotocin \textit{(2-deoxy-2-(3-(methyl-3-nitrosoureido)-d-glucopyranose)} is an antibiotic obtained from \textit{Streptomyces achromogenes}, has been widely used for inducing diabetes in the experimental animals\textsuperscript{24}. Streptozotocin selectively destroys pancreatic insulin secreting \(\beta\)-cells, due to excess production of reactive oxygen species (ROS)\textsuperscript{25-28} interact with biological macromolecules such as DNA damage, protein degradation, lipid peroxidation and finally culminating in the damage of various organs such as liver, kidney, brain, eyes and haemopoietic system\textsuperscript{29,30}. In the current study, we observed streptozotocin treatment resulted in rise in blood glucose level along with significant reduction in body weight of animals, which could be due to poor glycemic control. The excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin absence results in muscle wasting and weight loss in diabetic animals\textsuperscript{31-33}. Increase in insulin levels upon management with \textit{P. tirupatiensis} in diabetic rats resulted in enhanced glycemic control, which prevented the loss of body weight. SOD protects tissues against oxygen free radicals by catalyzing the deletion of superoxide radical \((O_2^-)\), which harms the membrane and biological structures\textsuperscript{34}. Catalase has been shown to be responsible for the detoxification of significant amounts of H\textsubscript{2}O\textsubscript{2}\textsuperscript{35}. SOD and CAT are the two major key scavenging enzymes that eliminate the toxic free radicals in vivo\textsuperscript{36}. The antioxidative defence system enzymes, e.g. SOD and CAT, showed lower activities in liver, kidney heart and brain, during diabetes and the results agree well with the previous reported data\textsuperscript{7-30}. The reduced activities of SOD and CAT may be a response to raised production of H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2}\textsuperscript{-} by the auto-oxidation of the excess of glucose and non-enzymatic glycation of proteins\textsuperscript{37,38}. Pigeot et al.\textsuperscript{39} have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The reduced activity of SOD and CAT could also be due to their diminished protein expression levels in the diabetic condition\textsuperscript{40}, as recently observed in liver\textsuperscript{41}. Glutathione peroxidase (GPX) a key redox regulator was significantly decreased during diabetic condition, which could be due to the increased endogenous production of superoxide anions. H\textsubscript{2}O\textsubscript{2}, the substrate of GPX is itself toxic and can react with O\textsubscript{2} in the presence of metal ions to form OH and result in higher levels of MDA formation\textsuperscript{42,43}. Increased peroxidative damage reported in diabetic animals disturbs the membrane function by affecting the membrane fluidity and changing the activity of membrane bound enzymes and receptors\textsuperscript{44}. These results are matching with the finding of Friesen et al.\textsuperscript{45}. In the present study, treatment with \textit{P. tirupatiensis} showed significant reduction in lipid peroxidation associated with augmented activity of antioxidant enzymes such as SOD, CAT and GPXs. The decreased level of ascorbic acid or vitamin C during diabetic condition may be due to either augmented utilization as an antioxidant defence against excessive ROS or to a diminish in GSH level, since glutathione is required for the recycling of ascorbic acid and vitamin E is alipophic antioxidant and inhibits lipid peroxidation, scavenging lipidperoxyl radical stydiold lipid hydro peroxides and the \(\alpha\)-tocopherol radical\textsuperscript{46-55}. Treatment with extract of \textit{P. tirupatiensis} brought ascorbic acid and Vitamin E to near control levels. Glutathione an important inhibitor of free radical mediated lipid peroxidation\textsuperscript{4}. It is involved in several reactions in the body and is one of the most prominent non- enzymatic antioxidant. Deficiency of GSH may lead to various complications such as neuropathy, myopathy and cataract during diabetic condition\textsuperscript{55,56}. The declined level of GSH in diabetes can also be due to NADPH depletion or its increased utilization in removal of peroxides produced due to oxidative stress\textsuperscript{48,57,58}. In context, several scientists have also reported diminished levels of glutathione during diabetic condition\textsuperscript{59}. The reduction of GSH may be responsible for low GPx activity in diabetic tissues, as GSH is a cofactor and also a substrate of this enzyme.

**CONCLUSION**

In summary, our study the reversal of altered antioxidant enzymes activities and peroxidative damage in tissues by \textit{P. tirupatiensis} extract suggests its antioxidant and antioxidant property and hence reveals its potential to play a crucial role in defense against free radicals. The response on treatment with the extract compared with Glibenclamide, an established oral hypoglycemic synthetic drug. Our results confirm that \textit{P. tirupatiensis} could be responsible for the restoration of metabolic activities and according protection against streptozotocin-induced diabetic rats. The mechanism could be related to scavenging activity of the \textit{P. tirupatiensis} extract. However, inclusionive chemical and pharmacological research is necessary to find out the exact mechanism of \textit{P. tirupatiensis} for its antidiabeticogenic effect.

**REFERENCES**


Argano M, Briguardello E, Tamagno O, Bocuzzi G. Dehydroepiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. J. Endocrinol, 1997;155: 233–240.


Table 1: Effect of *P. tirupatiensis* extracts on blood glucose levels in normal control and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day-1</th>
<th>Day-15</th>
<th>Day-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>87±0.9</td>
<td>89±1.66</td>
<td>89±2.43</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>363±1.07a</td>
<td>387±2.89a</td>
<td>391±3.97a</td>
</tr>
<tr>
<td><em>P. tirupatiensis</em> treatment</td>
<td>87±0.12</td>
<td>89±1.29</td>
<td>86±1.39</td>
</tr>
<tr>
<td>Diabetic + <em>P. tirupatiensis</em></td>
<td>375±1.8ab</td>
<td>176±3.62ab</td>
<td>103±3.58ab</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>347±1.75ab</td>
<td>193±2.65ab</td>
<td>101±1.15ab</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (*p* < 0.01) and diabetic control (*p* < 0.01).

Table 2: Effect of *P. tirupatiensis* extracts on changes in bodyweights in normal control and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day-1</th>
<th>Day-15</th>
<th>Day-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>187±1.5</td>
<td>198±4.1</td>
<td>214±3.3</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>163±0.97a</td>
<td>123±1.67a</td>
<td>114±2.97a</td>
</tr>
<tr>
<td><em>P. tirupatiensis</em> treatment</td>
<td>187±1.19</td>
<td>199±1.9</td>
<td>231±2.19</td>
</tr>
<tr>
<td>Diabetic + <em>P. tirupatiensis</em></td>
<td>178±0.72ab</td>
<td>188±1.65ab</td>
<td>199±3.92ab</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>181±2.55ab</td>
<td>190±2.62ab</td>
<td>201±1.35ab</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (*p* < 0.01) and diabetic control (*p* < 0.01).

Fig. 1: Effect of *P. tirupatiensis* extracts on liver and pancreas SOD activity in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, *a* *p* <0.01) and diabetic control (DC, *b* *p* <0.01).

Fig. 2: Effect of *P. tirupatiensis* extracts on liver and pancreas CAT activity in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, *a* *p* <0.01) and diabetic control (DC, *b* *p* <0.01).
Fig. 3: Effect *P. tirupatiensis* extracts on liver and pancreas GPx activity in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, $^a p < 0.01$) and diabetic control (DC, $^b p < 0.01$).

Fig. 4: Effect *P. tirupatiensis* extracts on liver and pancreas GSH levels in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, $^a p < 0.01$) and diabetic control (DC, $^b p < 0.01$).

Fig. 5: Effect *P. tirupatiensis* extracts on liver and pancreas Vitamin C levels in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, $^a p < 0.01$) and diabetic control (DC, $^b p < 0.01$).
Fig. 6: Effect *P. tirupatiensis* extracts on liver and pancreas Vitamin E level in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, *a p <0.01*) and diabetic control (DC, *b p <0.01*).

![Vitamin E Level Graph](image)

Fig. 7: Effect *P. tirupatiensis* extracts on liver and pancreas MDA level in normal control and diabetic rats. All the values are mean ± SEM of six individual observations. Values are significant compared to normal control (NC, *a p <0.01*) and diabetic control (DC, *b p <0.01*).

![MDA Level Graph](image)

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