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Original Article

'Adavikothimeera' (*Pimpinella tirupatiensis*) Ameliorates Oxidative Stress in Streptozotocin-Induced Diabetic Rats

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ABSTRACT
Pimpinella tirupatiensis (Apiaceae) widely used as traditional medicine for various diseases including diabetes in India. However, its use has not been scientifically validated. The aim of this study was to examine the antidiabetic potential of Pimpinella tirupatiensis extract on the oxidative stress markers in streptozotocin-induced diabetic rats. Diabetes was induced by a single dose of streptozotocin (40 mg/kg) administered by intraperitoneal (i.p) way. Plant extract (750 mg/kg body weight) was administered to diabetic rats for 4 weeks. Activity of antioxidant enzymes (SOD, Catalase, and GPx), level of lipid peroxidation were estimated in diabetic rats. Treatment plant extract to diabetic rats showed significant reversal of disturbed antioxidant status and peroxidative damage. These results indicate that Pimpinella tirupatiensis extract possesses protective effect against streptozotocin-induced oxidative stress.
Key words: *Antioxidant enzymes; Pimpinella tirupatiensis; Streptozotocin.*

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INTRODUCTION

Reactive oxygen species (ROS) are constantly generated in vivo for physiological purposes. Their productions are often balanced by antioxidant defense system. However, excess Reactive oxygen species (ROS) production beyond the ability of antioxidant defence system can cause oxidative damage to protein, lipid and nucleic acid[1]. Antioxidant defense include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), in addition to low molecular agents and dietary antioxidants. Disturbing of oxidantantioxidant balance system is involved in development of many chronic diseases such as atherosclerosis, cancer and diabetes[2]. Diabetes is chronic metabolic disorder that continues to present a major worldwide health problem. Despite the efficiency of insulin treatment and other chemical therapies to control many features of diabetes, there are common incidents of diabetic complications such as vascular dysfunctions, nephropathy, neuropathy and retinopathy [3]. The role of oxidative damage in the etiology of diabetes and progression of diabetic complications is now well established [4]. Several studies have shown that antioxidant treatments improve markers of oxidative stress and lipid peroxidation in diabetic patients and animals [5, 6] found that plant extracts possessed antioxidant properties in streptozotocin-induced diabetic rats.

Pimpinella tirupatiensis is an endemic species with seasonal occurrence with underground tubers, distributed on Tirumala Hills (1000meters above the sea level of Chitoor district, Andhra Pradesh, India) [7, 8] is one such plant Indian traditional medicine and the plant has been a popular remedy for various diseases such as antitumor genic, antimicrobial, purgative, abortificient, analgesic, antiseptic, antipyretic and anti-inflammator [9]. The present study was conducted in order to

demonstrate the effects of *Pimpinella tirupatiensis* extract against oxidative stress in streptozotocin-induced diabetic rats, and the efficacy was compared with an oral antidiabetic agent, glibenclamide.

MATERIALS AND METHODS

Plant collection and preparation of extracts

Pimpinella tirupatiensis tuberous roots were collected from Tirumala Hills of Seshachalam range of Eastern Ghats (Chitoor district, Andhra Pradesh, India) during the raining season and identified by the taxonomist of herbarium Dept. of Botany, S.V. University, Tirupati Andhra Pradesh, India. The tubers were dried at room temperature for 2 days, crushed by an electrical grinder and then powdered. Extraction was performed by taking 50 g powder in 500 ml of distilled water for 18 h in the Soxhlet apparatus (Optic tech Delhi). The obtained deep brown aqueous extract was dried at reduced pressure and finally lyophilized (lyo 1580 Optic tech Delhi).This extract was used for further studies. The yield of the aqueous extract is 8.5% (w.w in term of dried starting material).

Selection of animals

Wistar strain albino rats of male sex, (n = 30), weighing 180 ± 10 g and six months age obtained from the Indian Institute of Science, Bangalore were used in this study. All the rats were maintained in the polypropylene cages, at an ambient temperature of 27 ± 2 0C 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. The experiments were carried out 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

Chemicals used in the present study were Analar Grade (AR) and obtained 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 40mg/kg body weight was injected intraperitoneal in a volume of 1ml/kg body weight (Sezik *et al.,* 2005). Because streptozotocin is capable of producing lethal hypoglycemia as a result of enormous pancreatic insulin release, rats were treated with 20% glucose (5-10ml) orally after 6 h of injection for the next 72 hours to prevent hypoglycemia. Neither death nor any other adverse effect was observed at the tested concentration throughout the experiment. After one week, rats with diabetes (i.e., high blood glucose levels, 200-300 mg/dL) that exhibited glycosuria and hyperglycemia were selected for the experiment.

Determination of the effective dose

To find out the effective dose, three groups comprising of five animals each were used. Various doses (250, 500, and 750 mg/kg body weight) of the root extract of *Pimpinella tirupatiensis* were administered to the diabetic rats (1-3). Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, and 5 h after the administration of different dosage. Blood glucose was measured by using dextrostix (Glucose oxidase method) with Basic One Touch Accuchec Glucometer. Extract of *Pimpinella tirupatiensis* at a dose of 750 mg/kg body weight showed a highly significant effect than 250 and 500 mg/kg body weight. Based on these data, the effective dose was fixed at 750 mg/kg body weight and used for further analysis.

Experimental design

Thirty rats were divided into five equal groups (each six) and treated as follows;

I. Group 1: Normal control rats given only normal saline
II. Group 2: Diabetic control (streptozotocin 40 mg/kg body weight) rats
III. Group 3: Normal rats treated with extract of *Pimpinella tirupatiensis* at the dose of 50 mg/kg bodyweight, daily for one month by an orogastric tube.
IV. Group 4: Another set of diabetic rats received *Pimpinella tirupatiensis* extracts at the dose of 750 mg/kg body weight, daily for one month by an orogastric tube.
V. Group 5: Diabetic rats were orally administered with a standard anti-diabetic drug glibenclamide at the dose of 20 mg/kg body weight daily for one month by an orogastric tube.

Collection of sample

The animals were anaesthetized with ether, and the fasting blood samples were collected through retro orbital plexus puncture at end of the treatment. This was put into serum tubes (BD Vacutainer® Plus plastic serum tube). The Blood samples were centrifuged at 1000 X g for 10 min and the supernatant obtained was used for assays of various biochemical examinations.

Biochemical analysis of serum

In the current study individual body weight was recorded weekly during the treatment period, serum glucose levels were estimated by oxidase method [10]. The extent of serum lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (MDA) by the method of Ohkawa *et al.,[11]* as a marker of oxidative stress, superoxide dismutase (SOD) estimated in the serum by the method [12] serum catalase (CAT) was estimated by the method [13]. Serum glutathione peroxidase (GPx) activity was assayed by the method [14].

Statistical analysis

The data was expressed as mean ±SD of six rats per group and the statistical significance 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 *Pimpinella tirupatiensis* on body weight

Body weights of diabetic rats were significantly reduced when compared to control rats. This was reversed by treatment with *Pimpinella tirupatiensis* extract or by glibenclamide.

Table: 1 Effect of treatment *P. tirupatiensis* extract for one month on body weight of control and experimental rats

Groups	Body weight (g)				
	0 week	1 week	2 week	3 week	4 week
Normal control (NC)	187±3.5	189±4.5	199±3.7	210±2.9	221±3.3
Diabetic control (DC)	163±4.97	147±9.8[a]	139.5±2.7[a]	125±2.2[a]	109±3.97[a]
P. tirupatiensis (Pt)	188±5.19	192±10.5	198±6.8	210±4.2	223±5.19
Diabetic plus P. tirupatiensis (D+Pt)	164±4.72	175±8.1[b]	182±3.5[b]	189±2.8[b]	196±5.92[b]
Diabetic plus *glibenclamide*(*D+Gl)*	168±5.55	182±4.7[b]	193±1.3[b]	202±3.5[b]	213±3.35[b]

Each value is mean± SD for six rats in each group. [a]$p < 0.01$ by comparison with normal rats.
[b]$p < 0.01$ by comparison with diabetic rats.

Effect of *Pimpinella tirupatiensis* on glucose levels: Fasting serum glucose levels of diabetic groups was significantly higher than that of control. Treatment with *Pimpinella tirupatiensis* or glibenclamide normalized blood glucose levels of diabetic groups.

Table: 2 Effect of treatment *P. tirupatiensis* extract for one month on serum glucose and body weight of control and experimental rats.

Groups	Glucose (mg/dL)				
	0 week	1 week	2 week	3 week	4 week
Normal control (NC)	87±0.9	89±11.5	89±1.66	87±1.9	88±2.33
Diabetic control (DC)	353±1.07	367±8.8[a]	375±2.89[a]	385±2.2[a]	390±3.97[a]
P. tirupatiensis treatment (Pt)	87±0.12	88±11.5	87±2.29	88±11.2	85±2.99
Diabetic plus *P. tirupatiensis* (D+Pt)	375±1.92	275±8.1[b]	175±3.62[b]	189±2.8[b]	184±2.28[b]
Diabetic plus *glibenclamide*(*D+Gl)*	347±1.65	262±2.7[b]	166±2.65[b]	180±3.5[b]	171±0.55[b]

Each value is mean± SD for six rats in each group. [a]$p < 0.01$ by comparison with normal rats. [b]$p < 0.01$ by comparison with diabetic rats.

Effect of *Pimpinella tirupatiensis* on antioxidant enzymes

A significant reduced in superoxide dismutase (SOD) and catalase (CAT) were observed in diabetic groups as compared to control groups (Figs 1 & 2). Treatment with *Pimpinella tirupatiensis* or

glibenclamide resulted in significant increased in superoxide dismutase (SOD) and catalase (CAT) activities to approach control values. Similarly, glutathione peroxidase (GPx) activity of diabetic rats was significantly reduced compared to control (Fig. 3). Treatment with *Pimpinella tirupatiensis* or glibenclamide normalized glutathione peroxidase (GPx) of diabetic rats.

Fig: 1 Effect of treatment *P. tirupatiensis* extract for one month on serum superoxide dismutase of control and experimental rats. Each value is mean± SD for six rats in each group. [a]$p < 0.01$ by comparison with normal rats. [b]$p < 0.01$ by comparison with diabetic rats.

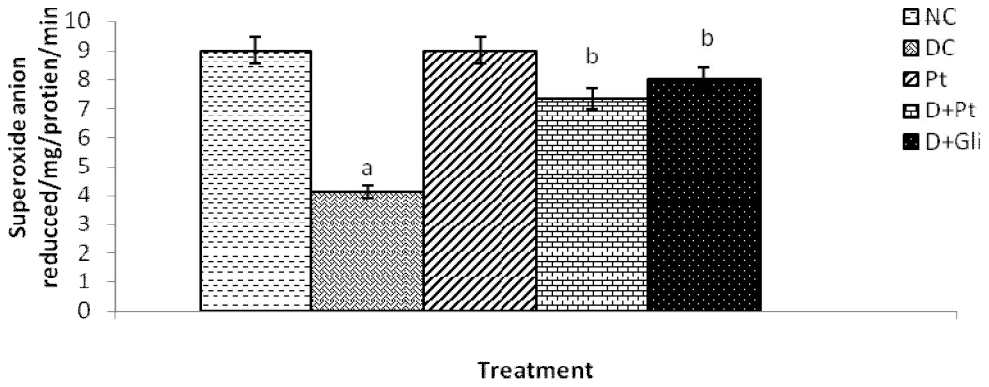


Fig: 2 Effect of treatment *P. tirupatiensis* extract for one month on serum catalase of control and experimental rats. Each value is mean± SD for six rats in each group. [a]$p < 0.01$ by comparison with normal rats. [b]$p < 0.01$ by comparison with diabetic rats.

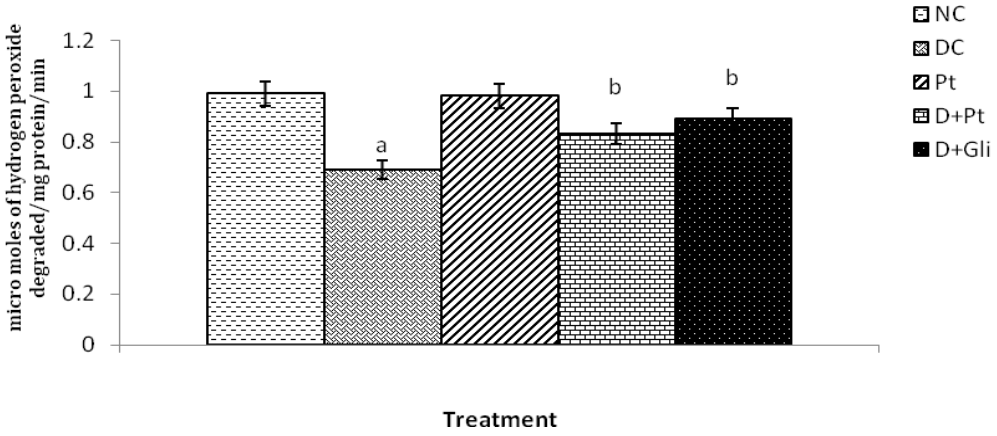
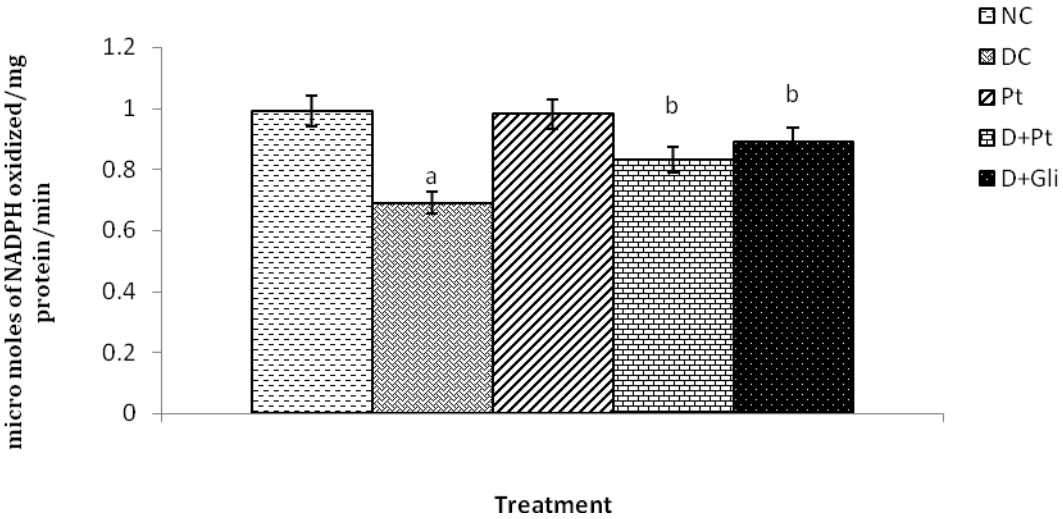


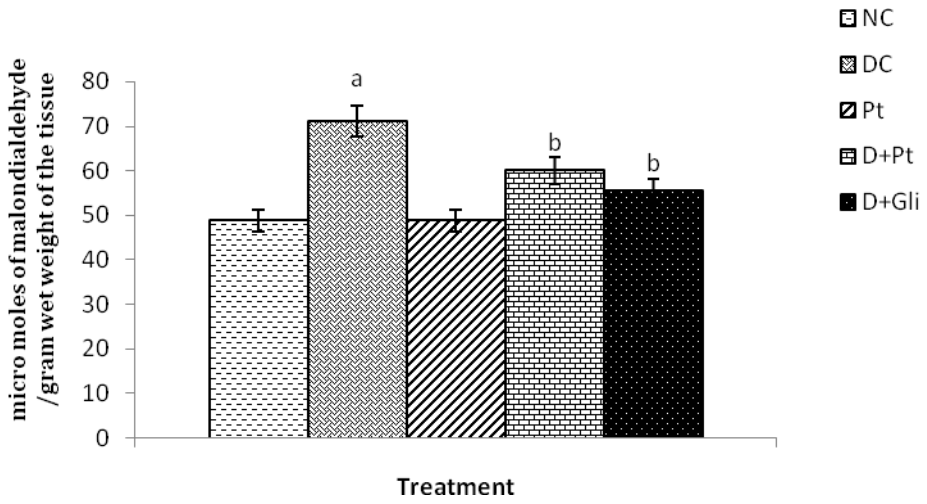
Fig: 3 Effect of treatment *P. tirupatiensis* extract for one month on serum glutathione peroxide of control and experimental rats. Each value is mean± SD for six rats in each group. [a]$p < 0.01$ by comparison with normal rats. [b]$p < 0.01$ by comparison with diabetic rats.



Effect of *Pimpinella tirupatiensis* on in lipid peroxidation

A significant increase in lipid peroxidation estimated by TBARS levels were observed in diabetic rats as compared to normal control. After treatment with extract, there were significant reductions in lipid peroxidation estimated by TBARS levels to approach normal values.

Fig: 4 Effect of treatment *P. tirupatiensis* extract for one month on serum lipid peroxidation (MDA) of control and experimental rats.Each value is mean± SD for six rats in each group. [a]$p < 0.01$ by comparison with normal rats. [b]$p < 0.01$ by comparison with diabetic rats.



DISCUSSION

Herbal therapies have been used since ancient times for the management of diabetes mellitus. About 90% of the world population in rural areas of developing countries relies solely on traditional medicines for their primary health care [15]. In the present study, the effect of *Pimpinella tirupatiensis* extract on the antioxidant status of the streptozotocin-treated diabetic rats was investigated. Streptozotocin treatment resulted in increase in serum glucose level along with significant decrease in body weight of animals. Extract treatment repaired the serum glucose level significantly and the body weight of diabetic animals to some extent. The effect observed may be due to strong antioxidant and/or improved insulin sensitivity and stimulatory action on β-cell in pancreas that could contribute to the hypoglycemic action of the extract.

We studied the effects of *Pimpinella tirupatiensis* extract on the activity of serum antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) and their effects on lipid peroxidation. We have perceived alterations in the antioxidant enzymes activities during diabetes condition. The depletion in antioxidant enzymes activities was associated with elevation of lipid peroxidation in the different tissue of diabetic rats. However, it is well known that diabetes is associated with increased oxidative stress. Free radicals, lipid peroxides and oxidation of low- density lipoproteins (LDL) have been considered as characteristic features of chronic diabetes. In diabetes, impaired glucose metabolism may lead to autooxidation of glucose and cause the nonezymatic glycation of protein through Maillard's reaction [16]. Free radicals may also be formed via the autooxidation of unsaturated lipids in plasma and membrane lipids. The produced free radicals may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation [17]. The increased susceptibility of diabetic animal's tissues to lipid peroxidation was observed by increasing the concentration of TBARS in the tissues of diabetic rats. On the other hand, antioxidant enzymes play an important role against oxidative stress. However, hyperglycemia also causes nonezymatic glycation of these enzymes [16, 18]. The present data well indicate that streptozotocin-treated diabetes disrupts actions of antioxidant enzymes [19]. These observations highlighting the critical importance of maintaining the antioxidant potential of pancreatic β-cells in order to ensure both its survival and insulin secretion capacity during times of increased oxidation stress.

The decreased activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) in diabetes condition may be due to the production of ROS such as superoxide (O_2^{-}) hydrogen peroxide (H_2O_2), and hydroxyl radical (OH.) [20, 21]. In the enzymatic antioxidant defense system,

the superoxide radical convert them to H_2O_2 and molecular oxygen [20, 22]. The observed decrease in superoxide dismutase (SOD) activity in diabetic rats could result from inactivation by hydrogen peroxide (H_2O_2), or by glycosylation of the enzymes, which have been report to occur in diabetes [23, 24]. Catalase (CAT) and glutathione peroxidase (GPx) are involved in the elimination of hydrogen peroxide (H_2O_2). Catalase (CAT) is considered as one of the main antioxidant and catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals. Decreased in catalase (CAT) activity could result from inactivation by superoxide radical and glycation of the enzyme [25].

Catalase (CAT) is known as for its involvement in detoxifying the high hydrogen peroxide (H_2O_2) concentrations, whereas glutathione peroxidase (GPx) is responsive to lower concentrations of hydrogen peroxide (H_2O_2) [19, 25]. Glutathione peroxidase (GPx) is an enzyme with selenium and plays a primary role in minimizing oxidative damage. Reduced activities of glutathione peroxidase (GPx) in diabetes state may result from radical induced inactivation and glycation of the enzyme [25]. The increase in superoxide dismutase (SOD) activity may protect catalase (CAT) and glutathione peroxidase (GPx) against inactivation by superoxide radicals as these radicals have been shown to inactivate catalase (CAT) and glutathione peroxidase (GPx) [26]. The reduced activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) may be responsible for the inadequacy of antioxidant defenses in combating reactive oxygen species (ROS) mediated damage. In this study lower levels of antioxidant enzymes and increased LPO in diabetic rats were observed with compared with normal control rats while extract of *Pimpinella tirupatiensis* partially reduced the imbalance between the generation of reactive oxygen species (ROS) and scavenging enzymes activity in diabetic rats. Therefore, treatment with extract of *Pimpinella tirupatiensis* increases the activity of antioxidant enzymes and may help to control free radicals.

CONCLUSION

In conclusion, the data of this investigation have shown that the daily administration of extract of *Pimpinella tirupatiensis* improved the alterations of antioxidant enzymes activities and lipid peroxidation level in streptozotocin-treated diabetic rats. These results suggest that *Pimpinella tirupatiensis* extract might act as a suppressor against oxidative stress through improvement of the antioxidant enzymes activities. Thus, this research provided a new approach for diabetes mellitus combination therapy in the future.

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