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Efficacy of *Pimpinella tirupatiensis* extract on brain oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats

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Abstract
The present study was designed to investigate the protective role of *Pimpinella tirupatiensis* in streptozotocin (STZ)-induced diabetic rats by administering oral dose 750 mg/kg body weight. Glibenclamide was taken as the standard drug (600 μg/kg b. w. body weight). Diabetes was induced in adult male albino rats, weighing 180-200 g, by administration of STZ (40 mg/kg of body weight) intraperitoneally. Diabetic rats showed an increase in MDA, uric acid content and XOD activity in their brain homogenates. They showed a decrease in levels of ascorbic acid, GSH and GST activity, in brain homogenates. Oral administration of *Pimpinella tirupatiensis* extracts and glibenclamide decreased MDA, uric acid content, XOD activity and elevations in the activity of GST, ascorbic acid and GSH in brain homogenates were observed in diabetic animals. These findings suggest that *Pimpinella tirupatiensis* aqueous extract treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and brain damage.

Keywords: Diabetes, *Pimpinella tirupatiensis,* brain, rats

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to either insulin resistance or insufficient insulin secretion by β-cells of the pancreas [1]. Recent estimates indicate that more than 171 million people in the world suffer with diabetes and this is projected to increase 366 million by 2030 [2]. Diabetes is strongly associated with both micro vascular and macro vascular complications, including retinopathy, nephropathy, and neuropathy (micro vascular) and ischemic heart disease, peripheral vascular disease and cerebrovascular disease (macro vascular), resulting in organ and tissue damage in approximately one third to one half of people with diabetes [3]. STZ-induced diabetes mellitus is associated with the generation of reactive oxygen species (ROS) causing oxidative damage [4]. Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes impaired GSH metabolism [5] and decreased ascorbic Acid [6]. In diabetes mellitus, increased ROS activity initiates peroxidation of lipids and MDA accumulation, which in turn can stimulate glycation of proteins in diabetes [7].

Herbs and phytochemicals play a major role in the discovery of new therapeutic agents and have received attention as sources of antioxidants, hypoglycemic, and antihyperlipidemic agents. There are numerous traditional plants mentioned in Siddha and Ayurvedic system of medicine which are used as antidiabetic agents. *Pimpinella tirupatiensis* Bal. and Subr. (Family Apiaceae; local name, konda kothimera) is a rare and endemic medicinal plant and restricted to the Seshachalam hills of the Eastern Ghats, India [8]. This medicinal herb is considered to be an excellent candidate for oral therapy as it is effective, non-toxic and without serious side effects the whole plant of *Pimpinella tirupatiensis* is used to treat cough, stomach, liver problems, asthma, ulcer and tooth ache [9]. This plant root extract is also used to treat skin disease and is used as an antimicrobial agent [10] it is even given in the treatment of venereal disease and peptic ulcers [11]. In the present study, we have examined the protective role of *Pimpinella tirupatiensis* aqueous extract treatment on brain oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats.

2. Materiel and Methods

2.1. Animals

Wistar strain male albino rats, aged 3 months (180-200 g) were used for the present study. The total number of animals used for this study is 30. The rats were maintained on standard pellet diet and provided access to water *ad libitum*. They were housed in clean, dry polypropylene cages and maintained in a well-ventilated animal house with 12 h light-12 h dark cycle. All the experiments were carried out between 8 am to 10 am in order to avoid circadian rhythm induced changes. The study was approved by the University Animal Ethical Committee and experiments were performed according to the regulations for the care and use of laboratory animals and its resolution number; 09 (ii)/a/CPCSCA/IAEC/07-08/SVU/KSR-DVNK/Zool/ dated 26/6/08.

2.2. Plant material collection and extraction

Tuberous roots of *Pimpinella tirupatiensis* were collected from Shesachalam hills, (Chittoor district, Andhra Pradesh, India) during the raining season. Pimpinella tirupatiensis tubers were dried at room temperature and tubers were powdered in an electrical grinder then stored at 5 °C until further use. Tubers powder 500g was extracted with distilled water 1L for a period of over 24 hours. After filtration, the residue obtained was given resuspended in equal volume of distilled water for 48 hours and filtered again. The above two filtrates were mixed and the solvent was evaporated in a Rota Vapor (Model No-HS-2005V) at 50-65 °C under reduced pressure and then

lyophilized to get a powder and the same was used for the study.

2.3. Induction of diabetes

Streptozotocin (STZ) solution (40mg/ml) was freshly prepared in 0.1M citrate buffer (pH 4.5) and 1ml/kg b. w of STZ solution was injected by intraperitoneally. STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, so rats were treated with 20% glucose (5-10 ml) orally after 6 h of injection for the next 48 hours to prevent hypoglycemia. 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 hyperglycemia were selected for the experiment.

2.4. Experimental design

Rats of the same age group (3 months) were divided into 5 groups, six rats in each group, and were treated as follows:

1. Group I- Normal control (NC): Six rats were received the 0.9%Nacl / kg body weight via Orogastric tube for a period of 30 days.
2. Group II -Diabetic control (DC): Six rats were used as diabetic control rats by the injection of STZ (40 mg / kg b.w.) intraperitonially to the fasted rats.
3. Group III - (DC+Pt.Aq.e): diabetic rats treated with *Pimpinella tirupatiensis* (750 mg/kg)
4. Group IV - (Pt.Aq.e): non-diabetic rats given *Pimpinella tirupatiensis* (750 mg/kg)
5. Group V (DC+Glb): Diabetic animals treated with 600 *µ*g/kg b. w. day of glibenclamide for 30 days.

Glibenclamide is a sulfonylurea antidiabetic agent, a class of drugs used to treat diabetes mellitus. This disease is a chronic metabolic illness characterized by a deficiency of insulin, a hormone produced by the pancreas which controls the sugar in the blood. For that, in this study we are using glibenclamide as a standard drug for the comparison of efficacy with *Pimpinella tirupatiensis* treated diabetic rats.

2.5. Tissue collection and Analytical procedures

After completion of 30 days treatment the animals were sacrificed by cervical dislocation and the brain tissue was excised at 4°C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at −80°C for further biochemical analysis. The selected parameters such as MDA levels, Uric acid content and XOD activity, Ascorbic acid, GSH and GST activity were monitored by the methods of Ohkawa *et al*., [12], Martinek [13], Srikanthan and Krishnamurthy [14], Omaye *et al*., [15], Theodorus *et al*., [16], Habig *et al*.,[17] respectively.

2.6. Chemicals

All the 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).

2.7. Statistical Analyses

The data were analyzed by using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office Excel software for the significance of the main effects and along with their interactions. One way analysis of variance (ANOVA) was carried out with Dunnett's multiple comparison test and differences were considered significant at $P < 0.001$

3. Results

Significant ($p< 0.001$) increase in MDA, uric acid content and XOD activity, a significant ($P<0.001$) decrease in ascorbic acid, GSH content and GST activity was observed in the diabetic treated rats when compared with normal control rats. Diabetic rats with *Pimpinella tirupatiensis* aqueous treatment showed significant ($P<0.001$) decrease in MDA, uric acid content and XOD activity and ascorbic acid, GSH content and GST activity ($P<0.001$), which reflects restoration of antioxidant enzyme system to near-normal values (Figs1-6)

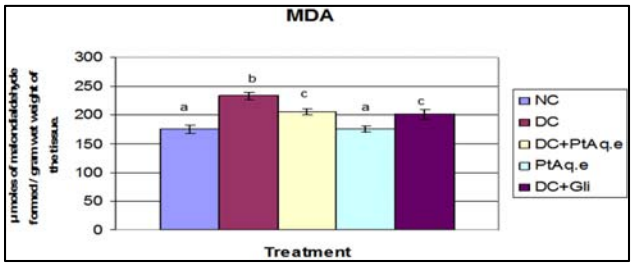

Fig 1: MDA content in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.001.

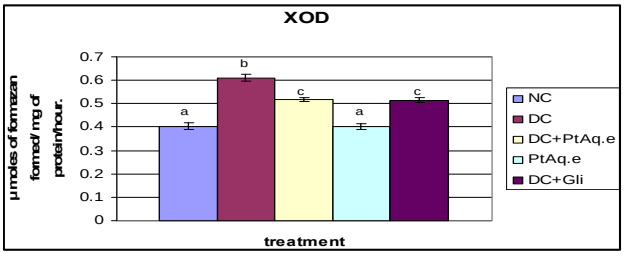

Fig 2: Changes in XOD activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at *p*<0.001.

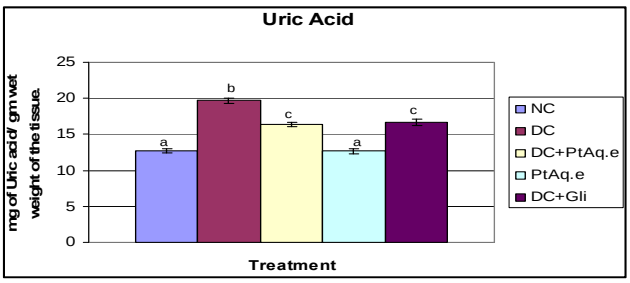

Fig 3: Uric acid content in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at *p*<0.001.

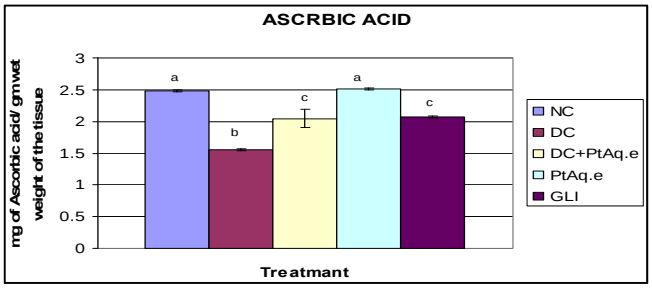

Fig 4: Content of Ascorbic acid in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.001$.

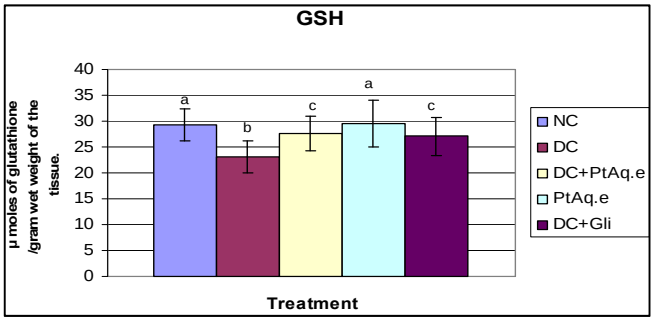

Fig 5: GSH Content in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.001$.

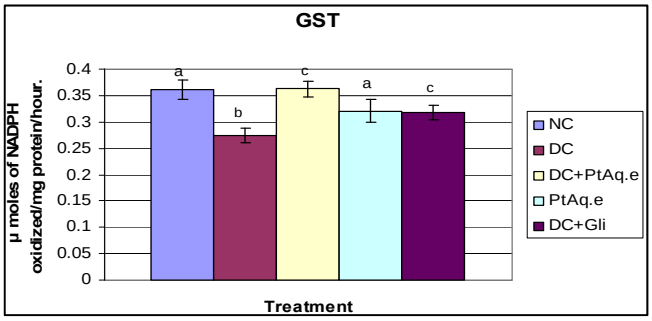

Fig 6: Changes in GST activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.001$.

4. Discussion

Diabetes is associated with a higher oxidative stress. ROS induced damage to the insulin-producing pancreatic beta-cells induces diabetes. Anti-oxidant therapy involving the use of herbs and spices has been shown to protect the tissues against such damage. It has been shown that under physiological conditions glucose may undergo auto-oxidation and contribute to ROS formation [18]. Diabetes induced oxidative damage is responsible for the changes occurring in the activities of anti-oxidant enzymes leading to impaired neuronal activity.

The present data revealed that persistent hyperglycemia through STZ generated ROS produced marked oxidant impact as evidenced by significant increase ($p<0.001$) in MDA levels in the brain tissue of diabetic rats higher than normal non diabetic animals. The increased lipid peroxidation during diabetes, as found in the present study may be due to the inefficient anti-oxidant system prevalent in diabetes [19]. This may be because; the brain contains relatively high concentration of easily peroxidizable fatty acids [20]. In addition, it is known that certain regions of the brain are highly enriched in iron, a metal that, in its free form, is catalytically involved in production of damaging oxygen free radical species [21]. In the present study *Pimpinella tirupatiensis* aqueous and glibenclamide treatments significantly reduces this enhanced lipid peroxidation and the marked activity is consistent with the previously published reports of *Pimpinella tirupatiensis* [22]. In the current study, the *Pimpinella tirupatiensis*, being an antioxidant, is thought to suppress the over generation of ROS and therefore, eliminate the intracellular ROS level of the brain tissue in the experimental rats.

In the current study Xanthine oxidase was increased in diabetic rat brain. This result provides support for the previously reported diabetes-induced brain oxidative stress [24]. Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid and generates O_2•− Hydrogen peroxide formed from O^2 •− could be converted into highly reactive •OH leading to oxidative stress [23]. After treatment with glibanclamide and *Pimpinella tirupatiensis* aqueous extract to the diabetic animals the activity of Xanthine oxidase was down regulated. This could be due to the decreased degeneration of ATP, down regulation of purine metabolism that leads to the low profile of xanthine, hypoxanthine levels which are necessary forhigh activity of XOD. Similar results have been obtained by the treatment with etimode in the diabetic rat CNS [24].

Uric acid is an effective antioxidant in its ability to scavenge hydroxyl radicals, hypochlorous acid, and peroxynitrite [25]. Recent anti-oxidant biochemistry studies show Uric acid is one of the most effective free radical scavengers. Uric acid has a potent defense function against a wide variety of the cellular oxidative damage of lipids, proteins, and nucleic acids [26]. Furthermore, Uric acid is an efficient iron chelator [27] which certainly can play a major role in the overall suppressive action against oxidative stress [28]. Increased XOD activity generates oxygen radicals and uric acid from xanthine, xanthine is formed from the degradation of ATP and reoxygenation [29]. In view of significant role of uric acid as free radical scavenger and singlet oxygen quencher, high uric acid levels in the brain of diabetic rats suggest that increased Xanthine oxidase activity increased the levels of uric acid. Treatment with *Pimpinella tirupatiensis* aqueous extract decreased the uric acid levels, this may be attributed by decreasing the activity of Xanthine oxidase.

Ascorbic acid is an excellent hydrophilic, dietary antioxidant and it readily scavenges ROS and peroxyl radicals [30]. It also acts as a co-antioxidant by generating vitamin A, E and GSH from radicals. In the present study we observed vitamin C was decrease in brain of diabetic rats. Anupama *et al*., [31] reported similar results in the diabetic rat brain respectively. Such a fall in level of vitamin C could be due to the increased utilization of vitamin C in the deactivation of increased level of ROS or due to decrease in GSH level, since GSH is required in

recycling of vitamin C [32]. Another possibility is that hyperglycemia inhibits ascorbic acid and its cellular transport. Since the chemical structure of vitamin C (ascorbic acid) is similar to that of glucose, it shares the membrane transport system with glucose and hence competes with it for its transport. Thus elevation in glucose concentration may depress natural antioxidants in the body like vitamin C [33]. In this study *Pimpinella tirupatiensis* administration was shown to prevent decrease in tissue GSH and ascorbic acid concentrations due to its added role in scavenging the free radicals in diabetic rats and thereby to reduce the utilization of GSH and ascorbic acid. This may be attributed to the presence of the antioxidant compounds in the *Pimpinella tirupatiensis*.

Under in vivo conditions, GSH acts an antioxidant and decreased was reported in diabetes millitus [34].We have observed significant decrease in GSH content and GST activity in brain during diabetes. The decrease level in gsh levels represents increased utilization due to oxidative stress [35]. The depletion of GSH content may also the GST activity [36]. The increased GSH content in the brain of the rats treated with *Pimpinella tirupatiensis* and glibenclamide may be a factor responsible for inhibition of lipidperoxidation. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to STZ [36]. The significant increase in GSH content and GST in diabetic rats treated with *Pimpinella tirupatiensis* indicates an adaptive mechanism in response to oxidative stress.

Conclusion

From the results obtained we conclude that *Pimpinella tirupatiensis* tuberous root aqueous extract possess potent antidiabetic and antioxidant activity. It is hoped that the activity guided isolation of the extract of this plant may yield valuable therapeutic compound (s) useful for developing powerful hypoglycemic or antioxidant drugs. The study also demonstrates that pharmacological screening based on the ethanomedical studies can yield faster hits in search of therapeutic agents from this plant.

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