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Effect of *Pimpinella tirupatiensis* Extract on Brain Free Radical Toxicity in STZ Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to investigate the effects of *Pimpinella tirupatiensis* on oxidative stress markers in different regions of the brain (cerebral cortex, cerebellum, hippocampus, and Pons medulla) in rats with diabetes. Diabetes was induced in wistar rats by injecting them with STZ (40 mg/kg). The rats were then administered *Pimpinella tirupatiensis* aqueous extract (750 mg/kg /

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b.w. /day) and glibenclamide (Glb) (20 mg/ kg / b.w. /day) orally for 30 days. After 4 weeks of high blood sugar, the activity of antioxidant enzymes was measured in both the diabetic and control groups. Diabetes can worsen nerve damage and cause oxidative damage due to high blood sugar levels. The diabetic rats displayed a significant decrease in levels of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and reduced glutathione. However, when ginger was administered orally, it enhanced the activity of antioxidant enzymes in the diabetic rats. This suggests that *Pimpinella tirupatiensis* has a protective effect on the brain by enhancing antioxidant defense mechanisms in diabetic rats. Furthermore, it has a neuroprotective effect by enhancing the brain's antioxidant defense mechanisms and thereby attenuating the progression of hyperglycemia and some complications caused by diabetes in the rat brain.

Keywords: Diabetes; Pimpinella tirupatiensis; SOD; GPx; glutathione; glibenclamide.

1. INTRODUCTION

“Chronic hyperglycemia, which is a hallmark symptom of diabetes, leads to serious metabolic imbalances and pathological changes in various tissues. The number of people with diabetes was 366 million in 2011 and is expected to reach 552 million by 2030 [1]. Diabetes is a group of metabolic diseases characterized by high blood sugar levels due to defects in insulin secretion, action, or both” [2].

“The neurological consequences of diabetes mellitus in the Central Nervous System (CNS) are now receiving greater attention. Cognitive deficits, along with morphological and neurochemical alterations illustrate that the neurological complications of diabetes are not limited to peripheral neuropathies. Diabetes is also associated with microvascular-lar complications including diabetic encephalopathy with progressive tissue damage in the central nervous system” [3]. “This complication is characterized by impairment of cognitive functions and electrophysiological changes” [4]. “Oxidative stress plays an important role in the enhancement of diabetes complications, including learning and memory impairments, as a result of the increased generation of free radicals and diminished antioxidant defenses” [5]. “These free radicals lead to increased neuronal death in several brain areas, including the hippocampus, and DNA damage, through protein oxidation and peroxidation of membrane lipids” [6]. The CNS has a high demand for oxygen and contains unsaturated lipids, which increases its susceptibility to the production of oxygen radicals and lipid peroxidation [7]. “Researchers have suggested that oxidative stress in diabetes may be caused by increased production of reactive oxygen species (ROS) through glucose auto-oxidation, decreased levels of tissue glutathione, and impaired antioxidant enzyme function” [8].

“The current antidiabetic medications have drawbacks in terms of their side effects, thus suggesting a need for a safe drug discovery system with a focus on natural plant sources” [9]. “Such a system would be able to effectively manage oxidative stress and diabetes mellitus without causing any additional adverse effects. Taking into account these findings, it is essential to consider the potential benefits of antioxidants as a promising pharmacological approach in diabetes management” [10].

Pimpinella tirupatiensis (Pt) is an herbaceous medicinal plant found on the Tirumala hills in the Chittoor district of Andhra Pradesh. It is a species of umbellifereae and grows seasonally with underground tubers as its root system. Some traditional uses of *Pimpinella tirupatiensis* include being an antifertility [11], anti-ulcer [12], and aphrodisiac agent [13]. Few studies have been achieved with this plant, and there is presently little or no information regarding the effect of *Pimpinella tirupatiensis* on the brain of rats with STZ-induced diabetes. This study aims to evaluate the neuroprotective effects of *Pimpinella tirupatiensis* on brain damage caused by STZ-induced diabetes.

2. MATERIALS AND METHODS

Pimpinella tirupatiensis plant was obtained from Tirumala Hills in Chittoor District, Andhra Pradesh, India. It was taxonomically identified and verified by the herbarium officer at the Department of Botany, S.V. University, Tirupati, Andhra Pradesh. A voucher specimen (1533) was provided and kept on the campus. The tuberous roots of *Pimpinella tirupatiensis* were dried and ground into a powder. This powder

was stored in airtight containers for further use. Bioactive compounds were then extracted from the powder using different solvents.

2.1 Preparation of Extract

The tuberous root of *Pimpinella tirupatiensis* was dried in the shade before being ground into a powder. This powder was utilized to extract potential antidiabetic compounds by employing water as the solvent. To begin, the powdered root was immersed in water in separate glass jars and left to soak for two days at room temperature. Afterwards, the resulting mixture was filtered. This process was repeated three to four times until the extract no longer exhibited any color. Subsequently, the extract was distilled and concentrated under reduced pressure using a rotary evaporator (model no-hs-2005v). Finally, the concentrated extract was freeze-dried using a lyophilizer (lyodel). The yield of the aqueous extract was determined to be 8.25% (w/w based on the dried starting material).

2.2 Animals and Treatment

The present study utilized a total number of 30 male albino Wistar strain rats, aged 3-4 months and weighing between 200-250 g. These rats were fed a standard pellet diet from M/S Hindustan Lever Ltd., Mumbai, and were given unrestricted access to water. They were housed in clean and dry polypropylene cages within a well-ventilated animal house that operated on a 12-glhour light-12-hour dark cycle. All experiments were conducted between 8 am and 10 am to mitigate any potential circadian rhythm-related changes. The experimental procedures adhered to the guidelines and protocol approved by the Institutional Animal Ethics Committee.

2.3 Induction of Diabetes

The animals were fasted overnight and diabetes was induced by injecting a single dose of freshly prepared STZ (40 mg/kg b.w) into the peritoneum, dissolved in ice-cold 0.1M citrate buffer (pH 4.5). This injection was given after the rats had been fasted for 12-15 hours, following the method described by Rakieten et al., 1963. To prevent hypoglycemia, the rats were given a 15% glucose solution for 24 hours, starting 8 hours after the STZ administration. STZ has the potential to cause fatal hypoglycemia by destroying β cells in the pancreas, leading to excessive release of insulin. Diabetes was assessed by measuring the fasting blood glucose levels 48 hours after the STZ injection. The blood glucose levels in the STZ rats were significantly higher than those in the normal rats. After one week, when the diabetes condition had stabilized, rats with severe hyperglycemia (blood glucose level ≥250 g/dl) were selected. Blood samples were collected from the tail vein of the rats.

2.4 Experimental Design

The rats were divided into 5 groups, six rats in each group and treated as follows:

Group I- Normal control (NC). Six rats were received the 0.9%Nacl / kg bodyweight via orogastric tube for a period of one month.
Group II -diabetic control (DC). Six rats were used as diabetic control rats by the injection of STZ (50 mg / kg b.w.) intraperitonially to the fasted rats.
Group III - (DC+Pt. Aq. e) Diabetic animals were treated orally with 750 mg/kg b.w/day of Pt aqueous extract for 30 days,
Group IV - (NC+Pt. Aq. e) Normal animals were treated orally with 750 mg/kg b.w/day of Pt aqueous extract for 30 days
Group V (D+Glb) Diabetic animals were treated with glibenclamide µg/kg body weight in aqueous solution orally for a period of 30 days.

After completion of 30 days of treatment, the animals were sacrificed by cervical dislocation and the brain tissues were excised at 4^0C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80^0C for further biochemical analysis.

2.5 Analytical Procedures

The activity of superoxide dismutase (SOD) was measured in tissue homogenates using the method of Misra and Fridovich [14] on a Hitachi U-2000 spectrophotometer. The absorbance was measured at 480 nm for 4 minutes. The activity of SOD was expressed as the amount of enzyme needed to inhibit the oxidation of epinephrine by 50%, which is equal to 1 unit (U) per milligram of protein. The activity of catalase (CAT) was determined at room temperature using the method of Aebi [15]. The absorbance of the sample was measured at 240 nm for 1 minute using a spectrophotometer. The glutathione peroxidase (GPx) activity was determined using the method of Flohe and Gunzler [16]. NADPH was used in the presence of cumene hydrogen

peroxide, and the absorbance was measured at 340 nm. The activity of glutathione reductase (GR) was determined according to the method of Carlberg and Mannervik [17]. The concentration of reduced glutathione (GSH) in brain regions homogenates was measured using the method described by Aker boom and Sies [18]. All enzyme activities were expressed as per milligram of protein, and the tissue protein concentration was estimated using the method of Lowry, Rosebrough, Farr, and Randall (1951) with bovine serum albumin (BSA) as a standard.

2.6 Procurement of Chemicals

The chemicals utilized in this study were of analar grade (AR) and were procured from various reputable scientific companies, including Sigma from St. Louis, MO, USA, Fisher from Pittsburgh, PA, USA, Merck from Mumbai, India, Ranbaxy from New Delhi, India, and Qualigens from Mumbai, India.

2.7 Statistical Analysis

The data in the study are presented as mean values along with their standard deviations. In order to analyze the data statistically, we utilized the statistical packages MS Excel and SPSS 11.5. To compare the different groups, we employed the one-way analysis of variance (ANOVA) technique to determine the significance between these groups. Additionally, we conducted Duncan's multiple range test as a post-hoc test to identify any significant differences among the groups. The entire statistical analysis was conducted at a significance level of 0.01.

3. RESULTS

In our study we observed SOD, CAT, GPx, GR activities, GSH content, were significantly decreased in Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM) diabetic rats compared to normal rats. After treatment with *Pimpinella tirupatiensis* and *Glibenclamide*, all measured parameters (SOD, CAT, GPx, GR activities and GSH content significantly increased compared to diabetic control rats. No significant changes were observed in Control rats treated with *Pimpinella tirupatiensis* compared to control rats (Tables 1-5).

4. DISCUSSION

Diabetes is a complex biochemical problem that affects a large number of individuals worldwide. Existing therapeutic options for diabetes often come with numerous side effects and can have limited success rates. As a result, there is a need for a promising treatment approach for diabetes. Fortunately, extensive research has shown that the plant kingdom contains a wealth of bioactive compounds that have the potential to be effective in diabetes therapy. Our study focused on evaluating the effectiveness of *Pimpinella tirupatiensis* in influencing the activities of key enzymes and other factors involved in the antioxidant defense systems of control and induced rats.

“Diabetes mellitus is associated with hyperglycaemia, the persistence of which can affect different organelles in the body system, including the brain, leading to neuropathy” [10].

Table 1. Changes in SOD activity in the brain regions of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (Pt.Aq. e), Diabetic rats treated with *Glibenclamide.* Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Brain region	SC	DC	DC+Pt.Aq. e	Pt.Aq. e	DC+Gli
CC	13.257 ±0.265	9.621* ±0.148	11.231* ±0.295	13.345* ±0.198	11.205* ±0.234
CB	17.981 ±0.387	12.497* ±0.294	15.874* ±0.124	17.987* ±0.386	15.127* ±0.364
HC	30.701 ±0.314	17.859* ±0.298	24.147* ±0.315	30.697* ±0.567	24.023* ±0.234
PM	21.756 ±0.389	14.741* ±0.254	18.754* ±0.229	21.787* ±0.141	18.147* ±0.276

All the values are mean, ± SE of six individual observations
** Values are significant at P<0.01 in Scheffe test.*
Values are expressed as µ moles of epinephrine oxidized/ mg of protein/min

Table 2. Changes in CAT activity in the brain regions of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (Pt.Aq. 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<0.01. Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Brain region	SC	DC	DC+Pt.Aq. e	Pt.Aq. e	DC+Gli
CC	0.398 ±0.154	0.196* ±0.084	0.267* ±0.094	0.399* ±0.134	0.259* ±0 .096
CB	0.654 ±0.110	0.301* ±0.101	0.501* ±0.047	0.655* ±0.080	0.504* ±0 .097
HC	0.705 ±0.024	0.398* ±0.063	0.546* ±0.054	0.711* ±0.121	0.538* ±0 .087
PM	0.697 ±0.104	0.398* ±0.115	0.514* ±0.037	0.699* ±0.059	0.509* ±0 .146

All the values are mean, ±SE of six individual observations
** Values are significant at P<0.01 in Scheffe test.*
Values are expressed as µ moles H_2O_2 hydrolyzed/mg protein/min

Table 3. Changes in GPx activity in the brain regions of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (Pt. Aq.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<0.01. Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Brain region	SC	DC	DC+Pt.aq. e	Pt.aq. e	DC+Gli
CC	1.786 ±0.011	0.989* ±0.019	1.231 ±0.005	1.789 ±0.019	0.986* ±0 .014
CB	1.645 ±0.029	1.011* ±0.004	1.497 ±0.024	1.648 ±0.002	1.495* ±0 .019
HC	1.414 ±0.019	1.024* ±0.014	1.312 ±0.019	1.416 ±0.024	1.310* ±0 .012
PM	1.287 ±0.019	0.898* ±0.011	1.014 ±0.012	1.289 ±0.018	1.011* ±0 .014

All the values are mean, ±SE of six individual observations
** Values are significant at P<0.01 in Scheffe test.*
Values are expressed as µ moles of NADPH oxidized/ mg of protein/min

"Oxidative stress refers to the imbalance between the production and removal of reactive oxygen species (ROS). In the context of diabetic complications, increased oxidative stress plays a significant role in the development of these conditions. This imbalance can occur due to either an increase in ROS production or a decrease in the body's ability to scavenge and neutralize ROS" [19]. "Multiple studies have observed changes in the activity of antioxidant enzymes in diabetic conditions, such as superoxide dismutase (SOD) and catalase (CAT). Specifically, lower activities of SOD and CAT have been observed in the cerebral cortex, cerebellum, hippocampus, and hypothalamus during diabetes, which is consistent with previously published studies" [20].

In the current study, we found that the activities of superoxide dismutase (SOD) and catalase (CAT) were significantly decreased (p<0.01) in diabetic rats compared to normal control rats. This result supports previous findings of diabetes-induced brain oxidative stress [21], which has been recognized as a critical factor in the development of chronic diabetic complications. "This decrease in SOD activity could be attributed to inactivation by hydrogen peroxide (H_2O_2) or glycation of the enzyme, both of which are known to occur during diabetes. The inactivation of these enzymes and the accumulation of highly reactive free radicals, such as the α-hydroxyethyl radical, can have deleterious effects such as the loss of cell membrane integrity and function. The decreased

Table 4. Changes in GR 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 PtAq.e 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<0.01. Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Brain region	SC	DC	DC+Pt.Aq. e	Pt.Aq. e	DC+Gli
CC	1.897 ±0.017	0.996* ±0.125	1.231* ±0.189	1.899* ±0.212	1.230* ±0 .101
CB	4.989 ±0.017	2.019* ±0.112	3.265* ±0.197	4.998* ±0.103	3.261* ±0 .198
HC	2.885 ±0.019	1.114* ±0.148	2.010* ±0.106	2.887* ±0.198	2.005* ±0 .065
PM	4.012 ±0.040	2.986* ±0.106	3.775* ±0.073	4.019* ±0.064	3.769* ±0 .195

All the values are mean, ± SE of six individual observations
** Values are significant at P<0.01 in Scheffe test.*
Values are expressed as µ moles of NADPH oxidized/ mg of protein/min

Table 5. GSH content in the brain regions of Sedentary Control (SC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (Pt Aq. 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<0.01. Cerebral Cortex (CC), Cerebellum (CB), Hippocampus (HC) and Pons Medulla (PM)

Brain region	SC	DC	DC+Pt.Aq. e	Pt.Aq. e	DC+Gli
CC	2.987 ±0.015	0.998* ±0.003	1.896* ±0.011	2.999* ±0.006	1.895* ±0 .010
CB	5.165 ±0.036	3.021* ±0.015	4.654 ±0.024	5.169* ±0.033	4.652* ±0 .019
HC	3.582 ±0.012	1.691* ±0.013	2.796* ±0.026	3.585* ±0.015	2.791* ±0 .029
PM	3.521 ±0.029	1.963* ±0.014	2.568* ±0.021	3.526* ±0.017	2.559* ±0 .019

All the values are mean, ±SE of six individual observations
** Values are significant at P<0.01 in Scheffe test.*
Values are expressed as nano moles of GSH formed/ gm wet wt. of tissue

activities of CAT and SOD may be a response to the increased production of H_2O_2 and oxygen (O2) resulting from the autoxidation of glucose and nonenzymatic glycation" [22]. "These enzymes play a vital role in maintaining physiological levels of oxygen and hydrogen peroxide by accelerating the dismutation of oxygen radicals and eliminating generated organic peroxides and hydrogen peroxides" [23]. The administration of *Pimpinella tirupatiensis* extract and glibenclamide has been shown to reverse the activities of SOD and CAT, possibly due to the presence of compounds such as alkaloids, flavanols, flavones, and volatile oils in the extract. These compounds may have antioxidant properties and directly scavenge superoxide radicals. Another study [24] has also shown that the treatment with *Gongronema latifolium* extract is able to restore the lowered levels of SOD and CAT in diabetic rat brain to normal levels.

The findings of the current study suggest that the activities of GPx and GR were reduced in the brain tissue regions of rats with diabetes. This is consistent with previous reports that have also observed decreased GPx activities in the brains of diabetic rats induced by STZ [25]. The decrease in GPx activities may be attributed to the inactivation and glycation of the enzyme [26] due to the presence of radicals [27]. GPx, an enzyme containing selenium and GST, is responsible for converting hydrogen peroxide to harmful compounds [28]. The decrease in GPX and gr activities indicates an increase in lipid peroxide production and elevated H_2O_2 levels. However, in diabetic rats treated with the *Pimpinella tirupatiensis* aqueous extract, a

significant increase in Se-GPX activity was observed. This could be due to the antioxidant properties of the aqueous extract, which help lower blood glucose levels and prevent glycation and inactivation of Se-GPX activity.

"In our current study, we have observed a significant decrease in GSH levels in the brain during diabetes. This decrease is due to the oxidation of GSH to glutathione disulfide (GSSG) as part of the detoxification pathway for reactive oxygen species (ROS)" [29]. "Depletion of GSH content in the brain regions leads to increased cellular damage caused by oxidative stress, which has been reported in previous studies" [30,31]. "In our study, supplementation with an aqueous extract of *Pimpinella tirupatiensis* tuberous roots increased the GSH content in the brain regions of streptozotocin (STZ)-induced diabetic rats. This increase in GSH content may protect cellular proteins against oxidation through the glutathione redox cycle and directly detoxify ROS generated by exposure to STZ. These findings are in accordance with previous report where treatment of diabetic rats with *Pimpinella tirupatiensis* extract was found to restore the GSH content in brain tissue" [32]. In our study, ability of *Pimpinella tirupatiensis* to reduce diabetic brain oxidative stress may be attributed to the free radical scavenging effect and the ability of this herb to preserve near normal activity levels of endogenous antioxidant enzymes.

5. 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 ethnomedical studies can yield faster hits in search of therapeutic agents from this plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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