

**NEUROPROTECTIVE EFFECT OF *PIMPINELLA TIRUPATIENSIS*  
TUBEROUS ROOT AQUEOUS EXTRACT ON BRAIN ANTIOXIDANT  
STATUS IN STZ-INDUCED DIABETIC RATS**

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**ABSTRACT**

The aim of the present study was to evaluate the protective effect of *Pimpinella tirupatiensis* tuberous root aqueous extract (Pt.Aq.e) against diabetes induced neuropathy in rats. Diabetes was induced in Wistar rats with a single intraperitoneal injection of STZ (40 mg/kg). Pt.Aq.e (750 mg/kg/b.w./day) and glibenclamide (GLB) (20 mg/kg/b.w./day) were administered orally for 30 days. After 30 days the antioxidant enzymes and MDA content was measured in diabetic and control groups. A marked decrease in antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione (GSH) content and increase in Malondialdehyde (MDA) was observed in the diabetic rats. After treatment with *Pimpinella tirupatiensis* tuberous root aqueous extract increased activities of antioxidant enzymes in diabetic rats.

Moreover, *Pimpinella tirupatiensis* tuberous root aqueous extract administration decreased the MDA content, which was earlier increased in the diabetic rats. These results suggest that *Pimpinella tirupatiensis* exhibit a neuroprotective effect by accelerating brain antioxidant defense mechanisms and down regulating the MDA content to the normal levels in the diabetic rats. Thus, *Pimpinella tirupatiensis* eliminated the free radical toxicity in diabetic rats.

**KEY WORDS:** Diabetes, *Pimpinella tirupatiensis*, Antioxidant enzymes, glibenclamide.

## INTRODUCTION

Diabetes mellitus is the world's largest endocrine disorder. The World Health Organisation (WHO) reported that 300 million people suffer diabetes mellitus by the year 2025. India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes will affect approximately 57 million people by the year 2025 in India.<sup>[1]</sup> Diabetes is characterized by hyperglycemia and metabolic abnormalities due to decreased insulin levels, causing metabolic and physiological changes in various organs including brain.<sup>[2]</sup> Hyperglycemia associated with diabetes increases the glucose autooxidation and protein glycation and the subsequent oxidative degradation of glycated proteins leads to enhanced production of reactive oxygen species (ROS).

The over production of free radicals and ROS results in enhanced lipid peroxidation, damages to DNA and protein degradation and exhaustion of the antioxidative defense systems.<sup>[3]</sup> Many functional and structural disorders, related to diabetes, were observed in the central and peripheral nervous systems.<sup>[4]</sup> Oxidative stress induced by chronic hyperglycemia has been associated with dysfunction and apoptosis of several cell types, including pancreatic  $\beta$  cells, endothelial cells.<sup>[5]</sup> neurons and glial cells.<sup>[6]</sup>

The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies.<sup>[7]</sup> Many plants have been reported effective for treating diabetes, though their mechanism of action is not known.

*Pimpinella tirupatiensis* (*Pt*) is an herbaceous medicinal plant, distributed on Tirumala hills of chittoor district, Andhra Pradesh.<sup>[8]</sup> It is endemic species of Umbellifereae and seasonal occurrence with underground tubers root system.<sup>[9]</sup> It acts as antifertility, anti ulcer and aphrodisiac agent.<sup>[10]</sup> Though there is no scientific evidence to support the antidiabetic property of *Pimpinella tirupatiensis* in the management of diabetes.

The present study was planned to know the effect of *Pimpinella tirupatiensis* in STZ diabetic rats. This is the first investigation to study the effect of *Pimpinella tirupatiensis* in diabetic rats and also with reference to brain antioxidant enzymes there was no reported data.

## MATERIAL METHODS

*Pimpinella tirupatiensis* plant 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. *Pimpinella tirupatiensis* tuberous roots were dried and powdered. The powder was stored in airtight containers and was used for the extraction of the bioactive compounds in different solvents.

### Preparation of extract

The *Pimpinella tirupatiensis* tuberous root was air dried in the shade, powdered and the powder was used for the extraction of potential antidiabetic principles into water solvent. *Pimpinella tirupatiensis* tuberous root powder was soaked in water 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 aqueous extract is 8.25% (w/w in terms of dried starting material).

### Animals and treatment

Total numbers of 30 Male albino wistar strain rats, aged 3-4 months ( $200\pm 250$  g) were used for the present study. The rats were maintained on standard pellet diet ((M/s Hindustan Lever Ltd., Mumbai) 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 experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee (Regd No. 438/01/a/CPCSEA/dt.17.07.2001) in its resolution number 09 (iii)/a/CPSCA/IAEC/07-08/SVU/Zool/KSR-DVNBK/dated 26/6/08.

### Induction of diabetes

The animals fasted over night and diabetes was induced a single intra peritoneal injected with a freshly prepared STZ (40 mg/kg b.w) dissolved in ice cold 0.1M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12-15 hr as per the method followed by Rakieten et al., (1963). 8 hr after STZ administration the rats were kept for next 24 hr on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of producing fatal

hypoglycemia due to destruction of  $\beta$  cells which in turn results in to massive pancreatic insulin release. Diabetes was assessed by determining the fasting blood glucose after 48 hr of injection of STZ. The blood glucose levels in STZ rats were increased to markedly higher levels than normal. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (blood glucose level  $\geq 250$  g/dl) were selected.

### Experimental design

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

#### 1. Group I- Normal control (NC)

Six rats were received the 0.9%Nacl / kg bodyweight 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 (50 mg / kg b.w.) intraperitoneally to the fasted rats.

#### 3. Group III – Diabetic + *Pimpinella tirupatiensis* (D+Pt.e)

Diabetic animals were treated with *Pimpinella* orally with 750 mg/kg b.w/day of Pt aqueous extract for 30 days.

#### 4. Group IV - *Pimpinella tirupatiensis* (Pt.e)

Normal animals were treated with *Pimpinella* orally with 750 mg/kg b.w/day of Pt aqueous extract for 30 days.

#### 5. Group V Diabetic + Glibenclamide (D+Glb)

Diabetic animals were treated with 20 mg/kg/day of glibenclamide for 30 days.

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

### Analytical procedures

Superoxide dismutase (SOD) activity was assayed in the tissue homogenates by the method of Misra and Fridovich (1972) at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein. Catalase (CAT) activity was determined

at room temperature by using the method of Aebi (1984) and absorbance of the sample was measured at 240 nm for 1 min in a spectrophotometer. Activity of glutathione peroxidase (GPx) was determined by the method of Flohe and Gunzler (1984), in the presence of NADPH and absorbance was measured at 340 nm, using cumene hydrogen peroxide. Glutathione reductase (GR) activity was determined according to the method of Carlberg and Mannervik (1985). The concentration of reduced glutathione (GSH) in brain homogenates was measured, as described by Akerboom and Sies (1981). All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry *et al.*, (1951), using bovine serum albumin (BSA) as a standard. The blood glucose levels were measured by using an Accucheck glucometer (Roche – Germany).

### Procurement of Chemicals

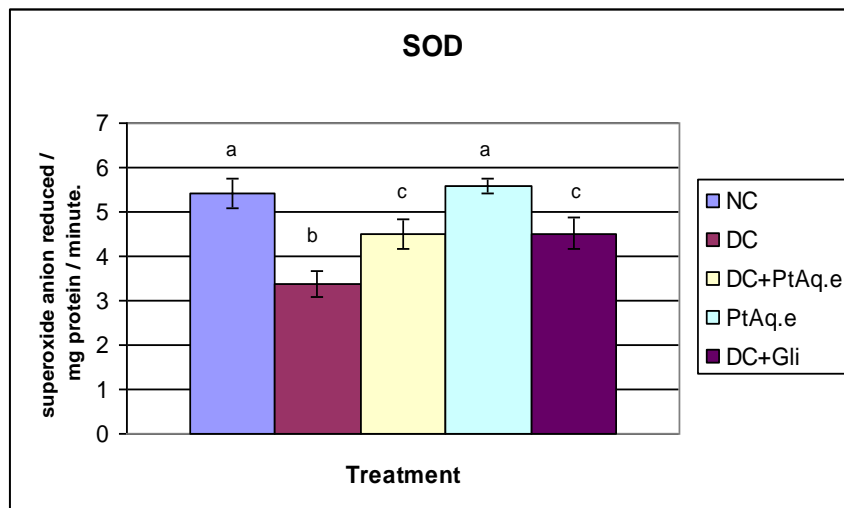
All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

### Statistical analysis

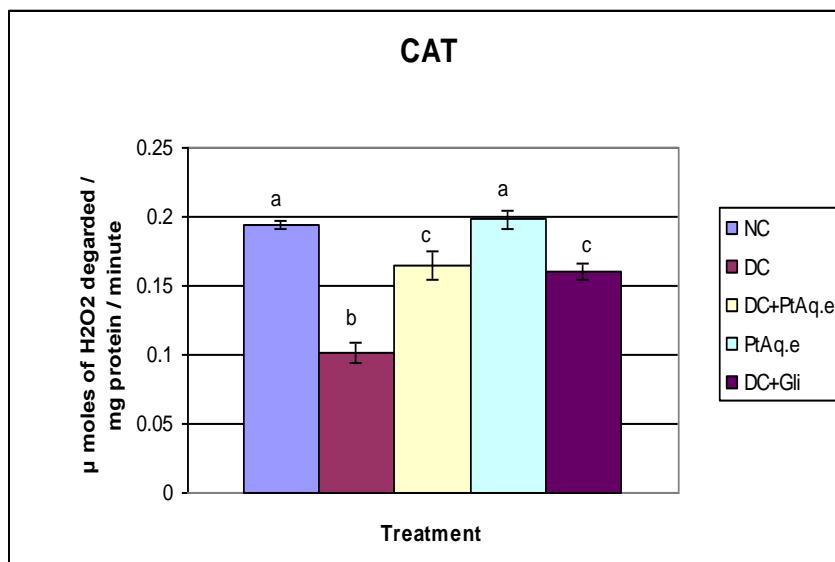
Results are expressed as means  $\pm$  standard deviation (SD). Variance analysis was done with Duncan's multiple comparison tests among data were carried out using the SPSS (Version 15; SPSS Inc., Chicago, IL, USA) and M.S. Office, excel software for the significance of the main effects (factors), and treatments along with their interactions. Statistical significance was set at  $P < 0.01$ . The p-values are presented with obtained data.

## RESULTS

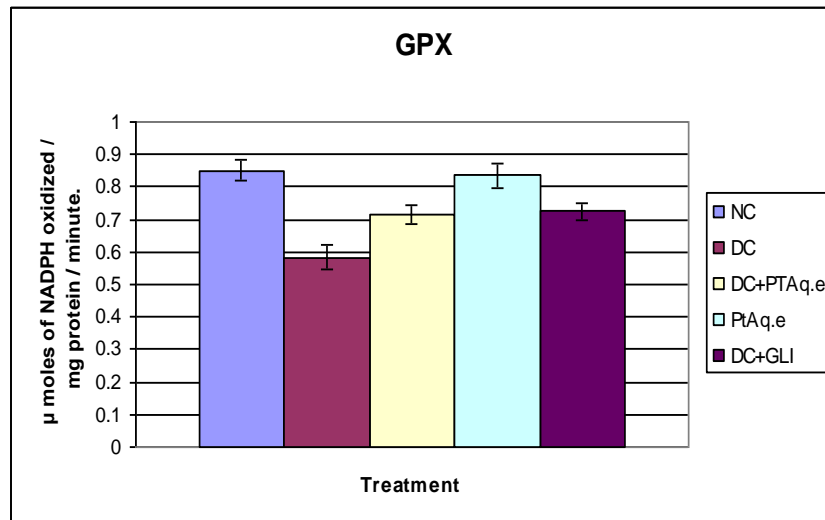
**Figs. 1–5** depict activities of SOD, CAT, GPx, GR, GSH content and MDA content in brain tissue of normal control, diabetic control, Pt treated diabetic rats, Pt treated normal rats and GLB treated diabetic rats. SOD, CAT, GPx, GR, activities, GSH content was significantly decreased and MDA content were significantly ( $p < 0.01$ ) increased in diabetic rats when compared to normal rats. After treating with Pt the activities of SOD, CAT, GPx, GR, GSH content was increased and MDA content were significantly decreased ( $p < 0.01$ ), and the when compared to the diabetic control rats. No significant changes were observed in the SOD, CAT, GPx, GR, activities and GSH content of control rats treated with Pt.e when compared to the control rats.



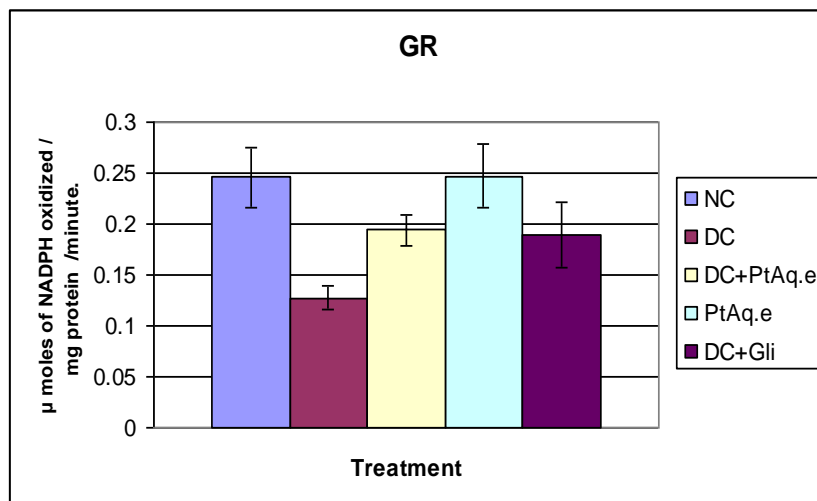
**Fig.1:** Changes in SOD activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



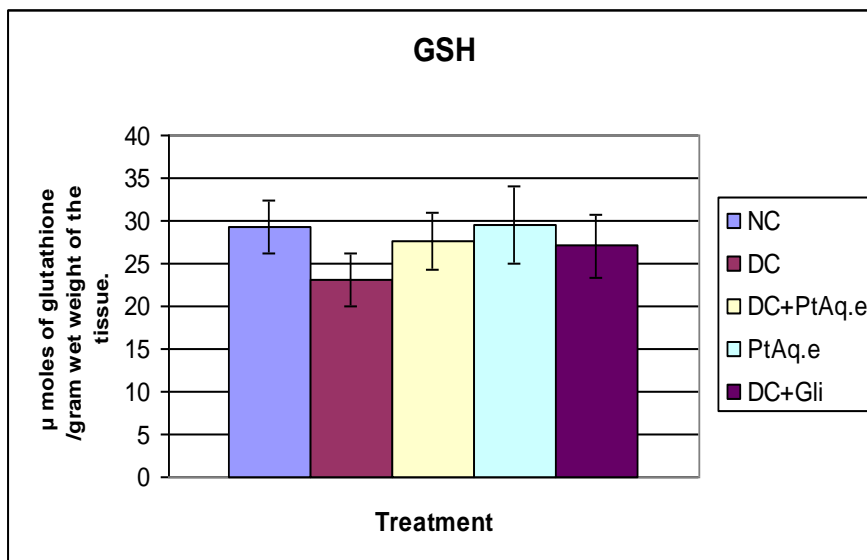
**Fig.2:** Changes in CAT activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



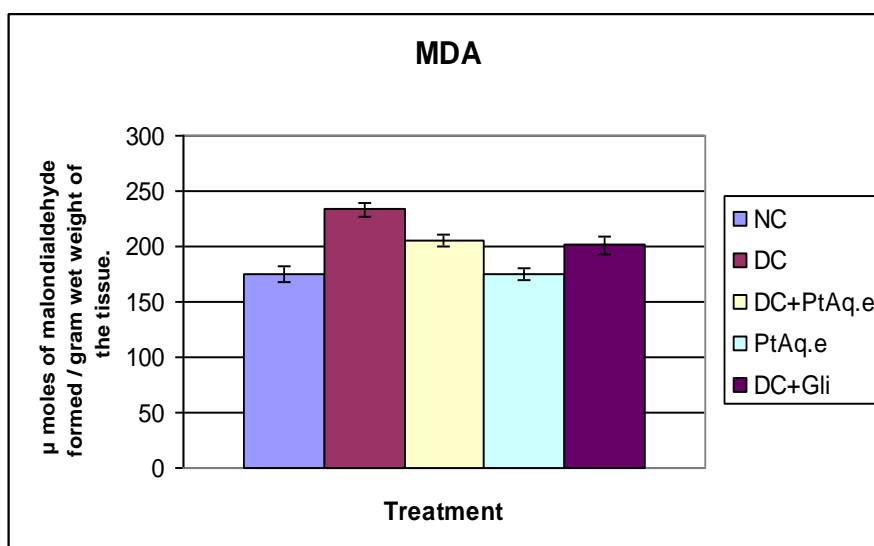
**Fig.3:** Changes in GPx activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), *Pimpinella tirupatiensis* aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



**Fig.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 *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



**Fig.5:** GSH content in the brain of Sedentary Control (SC), 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  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



**Fig.6:** MDA content in the brain of Sedentary Control (SC), 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  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



## DISCUSSION

Diabetes mellitus is a common metabolic disorder that affects the peripheral as well as the central nervous system. Brain tissue is highly susceptible to free radical damage because of its low level of endogenous antioxidants and a high content of lipids leading to lipid peroxidation and thus damage to the neuronal cell membrane. Oxidative stress plays an important role in tissue damage in CNS. It has high oxygen request and unsaturated lipid content. These two features may make the CNS target tissue oxygen radical production and lipid peroxidation. It was suggested that the possible sources of oxidative stress in diabetes include increased generation of ROS by glucose auto-oxidation, decreased tissue glutathione concentration, and impaired antioxidant enzymes.<sup>[11]</sup>

In the current study, the SOD and CAT activities was significantly ( $P < 0.01$ ) decreased in diabetic condition over normal control. This result provides support for the previously reported diabetes-induced brain oxidative stress. Oxidative stress has emerged as a critical factor in the development of chronic diabetic complications.<sup>[12]</sup> This could be due to increased utilization for scavenging free radicals. The decrease in SOD activity could result from inactivation by  $H_2O_2$  or by glycation of the enzyme, which are known to occur during diabetes.<sup>[13]</sup> The generation of  $\alpha$ -hydroxyethyl radical may lead to inactivation of these enzymes and accumulation of highly reactive free radicals also lead to deleterious effects such as loss of cell membrane integrity and membrane function. Decreased activities of CAT and SOD may be a response to increased production of  $H_2O_2$  and superoxide by the autoxidation of glucose and nonenzymatic glycation. These enzymes have been suggested as playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the distmutation of oxygen radicals and eliminating organic peroxides and hydrogen peroxides generated from inadvertent exposure to STZ. Treatment with *pimpinella* extract and *glibenclamide* has reversed the activites of SOD and CAT. This could be due to the presence of alkaloids, flavonols, flavones and volatile oils in *pimpinella tirupatiensis*. These compounds may have antioxidant properties, with this property the extract could directly scavenges the superoxide radicals.<sup>[14]</sup>

The results of the present study, reveal that GPx and GR activities were decreased in brain tissue of diabetic rats. These results are similar to many reports. Lowered activities of GPx have been well documented in STZ induced diabetic rat brain.<sup>[15]</sup> Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme. GPx an enzyme

with selenium and GST catalyzes the reduction of hydrogen peroxide to toxic compounds.<sup>[16]</sup> Decrease in GPx and GR activities indicates production of lipid peroxides and elevated H<sub>2</sub>O<sub>2</sub> production. In diabetic rats treated with the Pt aqueous extract, a significant increase in Se-GPx activity was obtained. This might reflect the antioxidant potency of the *Pimpinella*, which by reducing blood glucose levels prevented glycation and inactivation of Se-GPx. Thus, Se-GPx activity was induced to scavenge free radicals in diabetic rats.

In the current study we have observed significant decrease in GSH levels in brain during diabete condition. The detoxification pathway of ROS involves oxidation of GSH to glutathione disulfide (GSSG), resulting in decrease of GSH level.<sup>[17]</sup> Depletion of tissue GSH content enhances cellular damage caused by oxidative stress. In accordance with previous Publications<sup>[18,19]</sup>. Ozbek et al<sup>[20]</sup> found that untreated diabetes caused generally lower levels of GSH in different region. Supplementation of *Pimpinella* tuberous roots enhanced the content of GSH in the brain of STZ-diabetic rats. The increases in the content of GSH may protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to STZ. The significant increase in GSH content and GPx activity in diabetic rats treated with *Pimpinella* indicates an adaptive mechanism in response to oxidative stress.

In the present study, the formation of TBARS, a product of lipid peroxidation reaction, was significantly increased in diabetic brain tissues. Our results were also supported by studies of TBARS and hydroperoxides showed high Lipid peroxidation<sup>[21]</sup> The elevated lipid peroxidation is responsible for the formation of lipid hydroperoxides in membrane and would result in damage of the membrane structure and inactivation of membrane bound enzymes. The accumulation of lipid peroxides adds hydrophilic moieties into the hydrophobic phase and thereby brings about changes in the membrane permeability and cell functions.<sup>[21]</sup> This increased content of MDA was triggered by *Pimpinella tirupatiensis* tuberous root aqueous extract. Similar reports were found in the brain regions of diabetic rats, the elevated level of MDA was significantly decreased in animals Fed with ginger<sup>[22,23]</sup>. Safinaz & Ibrahim<sup>[24]</sup> have reported MDA levels were decreased in brain after supplementation of hesperidin. The antioxidant compounds and other pharmacological compounds of *Pimpinella tirupatiensis* extract may inhibit the production of free radicals, and reduced the products of lipid peroxidation.

From the results, 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.

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