Pharmacogn. Res., 2023; 15(4):813-818.
https://www.phcogres.com

Original Article

Attenuation of Oxidative Stress and Nephrotoxicity with Supplementation of *Pimpinella tirupatiensis* Tuberous Root in Streptozotocin-induced Diabetic Rats

Thopireddy Lavanya[1], Kakarla Chandra Mohan[2], Kesireddy Sathyavelu Reddy[3], Saddala Rajeswara Reddy[4,*]

[1]Department of Zoology, KVR Government College for Women (A), Cluster University, Kurnool, Andhra Pradesh, INDIA.
[2]Department of Zoology, SCNR Government Degree College, Proddatur, Andhra Pradesh, INDIA.
[3]Department of Zoology, S.V. University, Tirupati, Andhra Pradesh, INDIA.
[4]Department of Biotechnology, Division of Ethnopharmacology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, INDIA.

ABSTRACT

Background: The endemic plant of Seshachalam hills, Eastern Ghats, India, known as *Pimpinella tirupatiensis* (Pt). **Objectives:** It was examined in this study for its protective effects against oxidative damage in the kidney of Streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** After the induction of diabetes with Streptozotocin (40 mg kg-1 b.w), PTAq, an aqueous extract of Pt was directed orally at a dose of 750 mg kg-1 b.w. day-1. **Results:** Diabetic rats displayed significant decreases in Glutathione (GSH), ascorbic acid, and Vitamin E content ($p<0.01$) and significant elevations in lipid peroxidation (as MDA), uric acid content, and Xanthine Oxidase (XOD) activity ($p<0.01$). However, PTAq supplementation in the diabetic group resulted in significant ($p<0.01$) increases in Glutathione (GSH), Ascorbic Acid (AA), and Vitamin E content and declined MDA content, uric acid content, and XOD activity. **Conclusion:** The results advocate that Pt has the potential to avert diabetic complications triggered by oxidative stress in experimental diabetic rats and could have therapeutic implications for diabetes management in the future.

Keywords: *Pimpinella tirupatiensis*, Non-enzymatic antioxidants, Lipid peroxidation, Glutathione, Ascorbic acid.

Correspondence:
Dr. Rajeswara Reddy Saddala
Assistant Professor, Department of Biotechnology, Division of Ethnopharmacology, Dravidian University, Kuppam-517426, Andhra Pradesh, INDIA.
Email: drsrr2017@gmail.com

Received: 16-05-2023;
Revised: 23-06-2023;
Accepted: 02-08-2023.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic condition characterized by high Blood Glucose Levels (BGL) caused by impaired insulin action, secretion, or both on target tissues.[1] Prolonged periods of high blood sugar levels are allied with both microvascular complications, such as retinopathy, neuropathy, and nephropathy, as well as macrovascular complications, such as coronary and peripheral arterial diseases and stroke, which are prevalent in all types of diabetes mellitus. These complications can damage various organs and are often identified at a late stage or with inadequate medical supervision.[2] According to the International Diabetes Federation (IDF), in 2015, roughly 415 million individuals aged 20-79 years had diabetes mellitus, and it is anticipated that this number will increase by an additional 200 million by 2040, resulting in a significant escalation in the global burden of the disease.[3]

DOI: 10.5530/pres.15.4.086

Copyright Information :
Copyright Author (s) 2023 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

The condition of oxidative stress can occur as a result of an upsurge in the production of Reactive Oxygen Species (ROS), a decline in antioxidant defense, or both. ROS, which is produced unavoidably during metabolic processes, is a significant contributor to oxidative stress. The principal source of ROS generation is the release of electrons from the mitochondrial respiratory chain, which then transfer to molecular oxygen and produce the superoxide anion (O^{2-}). The alterations caused by ROS can result in damage to cells, tissues, or organs, and are regarded as the primary cause of various diseases.[4]

The cellular damage caused by hyperglycemia is mainly caused by oxidative stress. Increased glucose levels can prompt the production of free radicals, and when the body's natural defense mechanisms are inadequate to counteract the heightened production of Reactive Oxygen Species (ROS), an imbalance occurs between ROS and their protection, resulting in the domination of oxidative stress.[5,6]

The harmful effects of oxidative stress on cellular physiology, particularly on tissues with low intrinsic antioxidant defenses like the islet, can be observed in diabetes mellitus. The chronic hyperglycemia and oxidative stress have a detrimental impact on

the functionality of vascular, retinal, and renal tissues through various biochemical pathways and mechanisms.[7-9]

Over the last few years, there has been a growing interest in understanding how oxidative stress contributes to diabetes-related problems. Furthermore, investigators have been studying the possibility of utilizing natural agents derived from botanical sources to alleviate oxidative stress and control diabetes mellitus.

Pimpinella tirupatiensis Bal. and Subr. is a plant that belongs to the Apiaceae family and is known locally as Konda Kothimeera. It is exclusively found in the Tirumala hills situated in the Seshachalam range in the Eastern Ghats of Andhra Pradesh, India.[10] The tuberous root of *Pimpinella tirupatiensis* (Pt) has hexane and ethyl acetate fractions that have shown a diverse range of anti-microbial activity and contain significant quantities of alkaloids, flavonols, flavones, and volatile oils. Among the primary compounds identified are β-bisabolene, Δ-3-carene, cis-carveol, elemol, Δ-cadinol, methyl geranate, and γ-nonalactone.[11] In addition, the root extract of Pt is used for the treatment of skin diseases.[12] To the best of our knowledge, this is a significant study that investigates the effect of Pt on the kidney's non-enzymatic antioxidant status in experimental diabetic rats. This study was conducted to analyze the impact of *Pimpinella tirupatiensis* on kidney lipid peroxidation and non-enzymatic antioxidants in STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

The chemicals utilized for these experiments were of Analar Grade (AR) and were attained from reputable technical companies, including Sigma, Fisher, Merck, Ranbaxy and Qualigens.

Extraction of Plant Material

The roots (tuberous) of Pt were attained at Tirumala hills in the Seshachalam range of Eastern Ghats, Andhra Pradesh, India. It was acknowledged by the taxonomist of the department of botany at S.V. University, Tirupati, Andhra Pradesh, India. The roots were dehydrated and milled into a fine powder. 500 g of Pt root powder was water-logged in 1500 mL of water in distinct glass vessels for two days at 25°C. After that, the solvent was filtered, and the procedure was done three or four more times until the extract was colorless. A rotary evaporator (Model no. HS-2005V) was used to concentrate the extract, distil it under decreased pressure, and then freeze-dry it using a lyophilizer (Lyodel). In terms of the dried starting material, the yield of the aqueous extract was 8.5% (w:w). Just before giving it to the animals during the experiment, the crude extract was diluted with distilled water.

Experimental Animals

Male Wistar strain albino rats weighing 230 ±20 g (*n*=30) was attained from the Indian Institute of Science (IISc), Bangalore. The rats were kept in hygienic polypropylene cages, with six rats per cage, and were maintained in a room with a controlled temperature of 27 ± 2°C and a 12 hr light-dark cycle. Throughout the experimental period, the rats had access to a standard pellet diet (Lipton Rat Feed Ltd., Pune) and water *ad libitum*. The Institutional Animal Ethics Committee (Regd. No. 438/01/a/CPCSEA/dt.17.07.2001) approved the guidelines and protocol for the experiments, and the committee's resolution number 09 (iii)/a/CPCSCA/IAEC/07-08/SVU/Zool/KSR-TL/dated 26/6/08 was followed.

Induction of Diabetes

To induce experimental diabetes, the rats were fasted overnight for 12-15 hr and then given a single intraperitoneal injection of STZ (40 mg/kg b.w) liquified in ice-cold 0.1M citrate buffer (pH 4.5). To counteract drug-induced hypoglycemia, the rats were given a 15% glucose solution to drink for 24 hr after STZ direction. Diabetes was confirmed 48 hr later by measuring fasting blood glucose levels. Rats with fasting blood glucose levels above 250 mg/dL after one week of STZ injection were included in the study.[13]

Experimental design

30 rats were separated into five groups of six animals each.

Group I (NC): Untreated normal rats were administered normal saline (0.9%) daily for 30 days by orogastric tube.

Group II (DC): Untreated diabetic rats were directed with normal saline (0.9%) daily for 30 days by orogastric tube.

Group III (D+PTAq): Diabetic rats-750 mg/ kg b. wt./day of PTAq for one month by orogastric tube.

Group IV (C+PTAq): Normal rats-750 mg/kg b. wt./day of PTAq for one month by orogastric tube.

Group V (D+GLB): Diabetic rats-20 mg/kg b. wt./day of glibenclamide for one month by orogastric tube.

After 24 hr, all of the animals were sacrificed, and kidney tissue was removed. After washing with ice-cold saline, the tissue was immediately submerged in liquid nitrogen and preserved at -80°C for subsequent biochemical examinations.

Biochemical assays

Estimation of MDA content

In order to determine the level of MDA, 0.1 mM EDTA (5% w/v) was added to 50 mM phosphate buffer (pH 7.0), which was used to homogenize the kidney tissue. The homogenates were centrifuged for 10 min at 4°C at 10,000 rpm, and the supernatant collected was used for analysis. More specifically, 50 µL of 8.1% SDS was added to 200 µL of tissue extract before being vortexed and left to sit at room temperature for 10 min. The mixture was then cooked in a boiling water bath for 60 min before the addition of 375 µL of 0.6% thiobarbituric acid and 375 µL of 20%

acetic acid (pH 3.5). After that, room temperature was reached by the samples. Then, 12.5 mL of a 15:1 mixture of butanol and pyridine was added, vortexed, and centrifuged for 5 min at 10,000 rpm. Using 1,1,3,3-tetraethoxypropane as a reference, the coloured layer (500 µL) was measured at 532 nm. µ moles of malondialdehyde produced per gram wet weight of the tissue was used to express.[14]

Glutathione (GSH) content estimation

The method outlined by Theodorus *et al*.[15] was used to calculate glutathione content. 0.1 M phosphate buffer (pH 7.0) with 0.001 M EDTA was used to homogenise kidney tissue. Protein was then precipitated using 1 mL of 5% sulfosalicylic acid (w/v) solution. The supernatant obtained after centrifuging at 5000 g for 15 min at 4°C was utilised as the enzyme source. The reaction mixture was composed of 2.5 mL of 0.1 M potassium phosphate buffer, 2.0 mL of DTNB (1.5 mg/mL), 0.02 mL of NADPH (4 mg/mL of 0.5% $NaHCO_3$), 0.02 mL of glutathione reductase (6 units/mL), and the required volume of tissue source. The reaction's absorbance was measured at 425 nm in comparison to the reagent blank after 0.41 mL of the enzyme source was added. The glutathione content of the tissue was calculated as µ moles of glutathione oxidized/ gm wet weight of the tissue.

Ascorbic acid content estimation

The technique developed by Omaye *et al.*[16] was used to measure the amount of ascorbic acid in the kidney tissue. The kidney tissue was homogenized with 10% TCA (w/v) and centrifuged at 3500 g for 20 min at 40°C. The DTC solution (diphenylhydrazine-thiourea-copper sulphate solution) was then combined with 1 mL of the supernatant and incubated for 3 hr at 37°C. After that, 1.5 mL of ice-cold, 65% H_2SO_4 was added, thoroughly mixed, and the solution was let to stand for a further 30 min at room temperature. At 520 nm, the produced colour was compared to the reagent blank. Ascorbic acid was measured in mg per gramme of tissue weight under moist conditions.

Assessment of α-tocopherol (Vitamin E)

0.1 mL of lipid extract, after being combined, 1.5 mL of ethanol and 2 mL of petroleum ether were added, centrifuged for 10 min at 3000 g. At 80°C, the supernatant was dried off before 0.2 mL of a 2,21-dipyridyl solution and 0.2 mL of a ferric chloride solution were added and thoroughly mixed. This had 2 mL of butanol added and was held in the dark for 5 min. At 520 nm, the absorbance was then measured. A blank containing simply the reagent and standards of α-tocopherol in the range of 10-100 µg were used in the same manner. µ moles of Vitamin E/mg of tissue.[17]

Estimation of Uric acid

Renal tissue was homogenised in a 15% lithium carbonate (w/v) solution, and then 0.66 N H_2SO_4 and 0.50 mL of 10% tungstate (w/v) were added. The mixture was centrifuged at 5000 g for 5 min. at 4°C. 1 mL of sodium hydroxide solution and 1 mL of uric acid reagent were added to the supernatant, and the produced colour was quantified at 680 nm in a spectrophotometer against the reagent blank. The results were reported as mg of Uric acid/ gm wet weight of the tissue.[18]

Estimation of Xanthine Oxidase activity (XOD)

The enzyme source, 50 µm of xanthine, 0.1 µm of NAD, 0.4 µm of INT, and 100 µm of sodium phosphate buffer (pH 7.4) were all present in the test mixture. The reaction was allowed to start after adding 20 mg of the enzyme source, and it was kept at 37°C for 30 min. The reaction was ceased by adding 5 mL of glacial acetic acid, and the formazan that resulted was take out into 5 mL of toluene and measured at 495 nm in contrast to a toluene blank. The enzyme activity was calculated as the quantity of formazan generated per milligrams of protein per hour.[19]

Statistical analysis

The data were presented as mean values with their Standard Errors (SE). SPSS software (Version 13.5; SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel were used for the statistical analysis. We utilized one-way Analysis of Variance (ANOVA) to compare the groups, and we found that there were significant differences between them using Duncan's multiple range test as a post-hoc test. The significance level used for all statistical analyses was $p < 0.01$.

RESULTS

Figure 1 shows the amounts of lipid peroxidation in diabetic and normal rats. Malondialdehyde (MDA) concentrations were greater in the diabetic rats than in the normal control rats. MDA levels were lowered after a month of treatment with PTAq and GLB. Similar to how GLB reduced MDA levels, PTAq did the same. When normal rats were given PTAq, there were no observable significant changes in MDA levels. The levels of Glutathione (GSH), Vitamin C, and Vitamin E in the kidneys of the normal and diabetic groups are shown in Figures 2, 3, and 4. The diabetic group's renal GSH, ascorbic acid, and Vitamin E content significantly decreased ($p < 0.01$) according to the study. However, after treating the diabetic rats with PTAq and GLB, the content of GSH, Vitamin C, and Vitamin E significantly increased ($p<0.01$), and the contents in both groups were restored compared to the diabetic group. The elevated levels of GSH, ascorbic acid, and Vitamin E were comparable to those of the rats treated with GLB. In normal rats treated with PTAq, no discernible changes in GSH, ascorbic acid, or Vitamin E levels were found. Figure 5 demonstrates that the estimated uric acid level in the diabetic group was significantly ($p < 0.001$) higher, and that treatment with PTAq reduced the elevated content. The PTAq-treated control group showed no discernible alterations. Finally, as shown in Figure 6, diabetic rats had significantly higher Xanthine Oxidase

(XOD) activity than control rats, and the elevated XOD activity was reduced by PTAq administration

DISCUSSION

Complications of DM can be expressed through four primary mechanisms triggered by hyperglycemia, including the making of ROS, stimulation of protein kinase C, polyol pathway, and the formation of progressive glycosylation end products (AGE) and hexamine path.[20]

Persistent and chronic hyperglycemia causes severe oxidative stress in diabetic experimental animal models, diminishing the antioxidative defence system's activity and raising the quantities of oxygen free radicals.[21] The generation of extremely reactive oxygen radicals, which are hazardous to cells, particularly the cell membrane, results in lipid peroxidation and damage to cellular organelles, affecting pancreatic -cell function.[22]

Lipid peroxidation levels can be determined by measuring MDA, a convenient indicator of lipid peroxidation.[23] MDA is produced through lipid peroxidation and can crosslink collagen molecules, stabilizing them and enabling further glycation, which increases the potential for glycated collagen to initiate more lipid peroxidation and release additional MDA. Thus, MDA may serve as a connection between glycation and subsequent lipid peroxidation.[24,25]

To halt the lipid peroxidation chain reaction, antioxidants that scavenge free radicals are commonly used. Researchers have turned to plant-derived antioxidants to address this issue. In this study, *Pimpinella tirupatiensis* tuberous root aqueous extract of was utilized, and the results exhibited a increase in MDA levels in the renal tissue of diabetic rats. The oral treatment of PTAq to diabetic rats, on the other hand, effectively lowered the increased levels of lipid peroxidation to near-normal levels. These findings suggest that PTAq may have antioxidant properties that protect tissues from lipid peroxidation.

GSH is a non-enzymatic antioxidant that can directly and indirectly scavenge free radicals and ROS via enzymatic processes.[26] The lessening of GSH in tissues is a critical factor that allows lipid peroxidation to occur.[27] Antioxidants that aid in the maintenance of GSH concentrations can help to restore cellular defence mechanisms, inhibit lipid peroxidation, and protect tissues from oxidative damage.[28] The GSH concentration was found to be low in the diabetic group in this investigation, which is consistent with the findings of Eliza *et al.* 2010,[29] which showed a decrease in GSH concentration in diabetic rats. However, the oral administration of PTAq significantly increased GSH levels in this study, indicating that PTAq's effect could be attributed to the reduction of oxidative stress and inhibition of GSH utilization.

By interacting with essential antioxidants such as Vitamins E and C, glutathione can improve antioxidant defence. To terminate the radical chain reaction and prevent PUFA peroxidation in cellular and subcellular membrane phospholipids, Vitamin E can transfer its phenolic hydrogen to a peroxyl free radical of peroxidized polyunsaturated fatty acids (PUFA). Vitamin C is a powerful antioxidant that binds to oxygen free radicals and Vitamin E.[30] When it combines with a Vitamin E radical, it produces a Vitamin C radical while also replenishing Vitamin E. Then, GSH can convert a Vitamin C radical back to Vitamin C.[31]

Diabetic rats were found to have significantly lower levels of Vitamin C in their kidneys. However, our study found that diabetic rats had more levels of Vitamin C, which is consistent with previous studies in the diabetic kidney[32] and liver.[33]

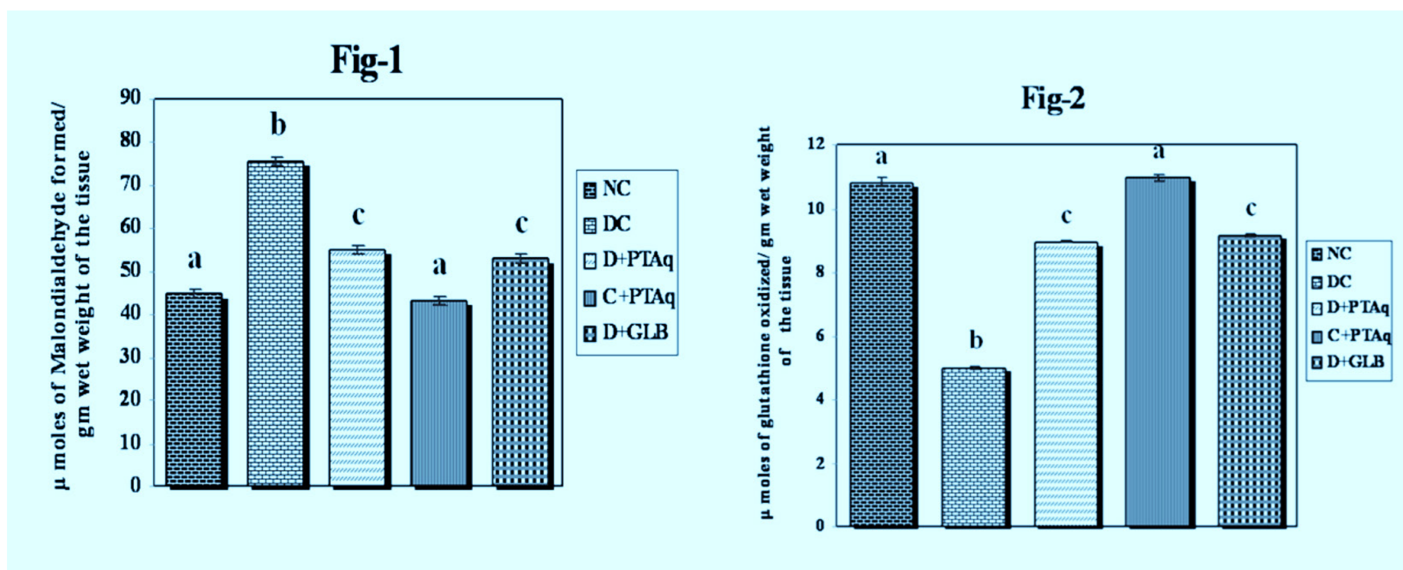

Figure 1 and Figure 2: MDA and Glutathione content in the kidney of Normal Control rats (NC), Diabetic Control rats (DC), diabetic rats treated with Pt aqueous extract (D+PTAq), Control rats treated with Pt Aqueous extract (C+ PTAq), diabetic rats treated with Glibenclamide (D+GLB). Each vertical bar represents the mean±SE. (n=6). Top of the vertical bars having the same alphabetic do not differ significantly at $p < 0.01$.

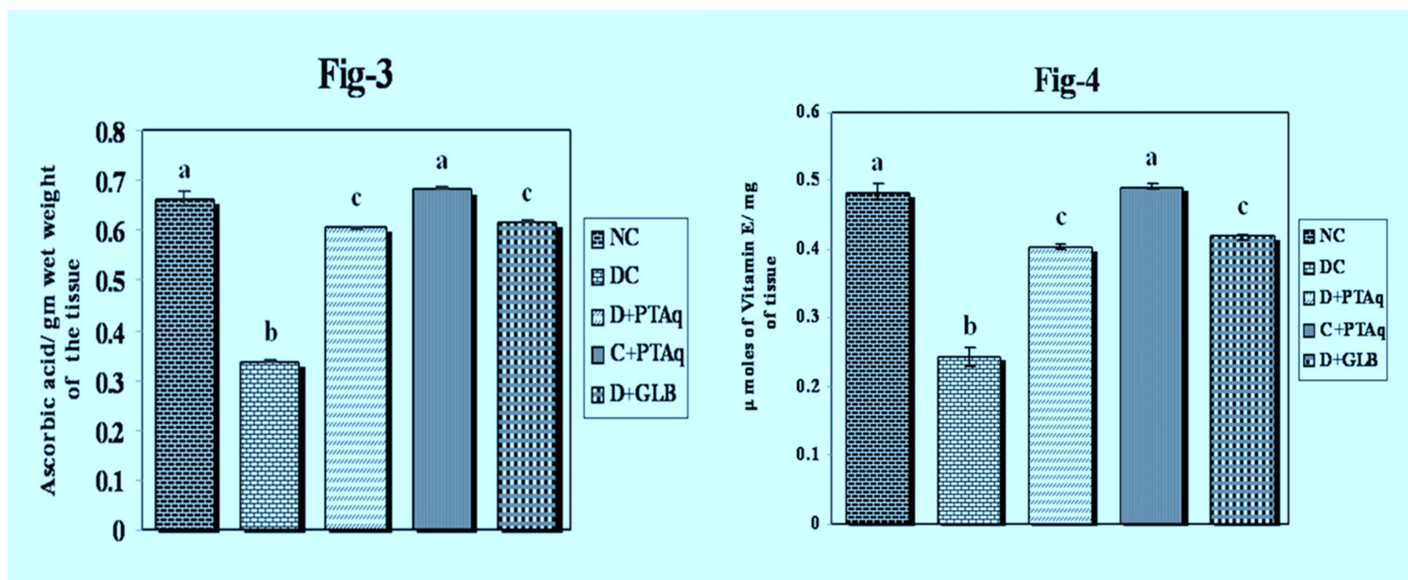

Figure 3 and Figure 4: Ascorbic acid and Vitamin E content in the kidney of Normal Control rats (NC), Diabetic Control rats (DC), diabetic rats treated with Pt aqueous extract (D+ PTAq), Control rats treated with Pt aqueous extract (C+ PTAq), diabetic rats treated with Glibenclamide (D+GLB). Each vertical bar represents the mean±SE. (n=6). Top of the vertical bars having the same alphabetic do not differ significantly at $p < 0.01$.

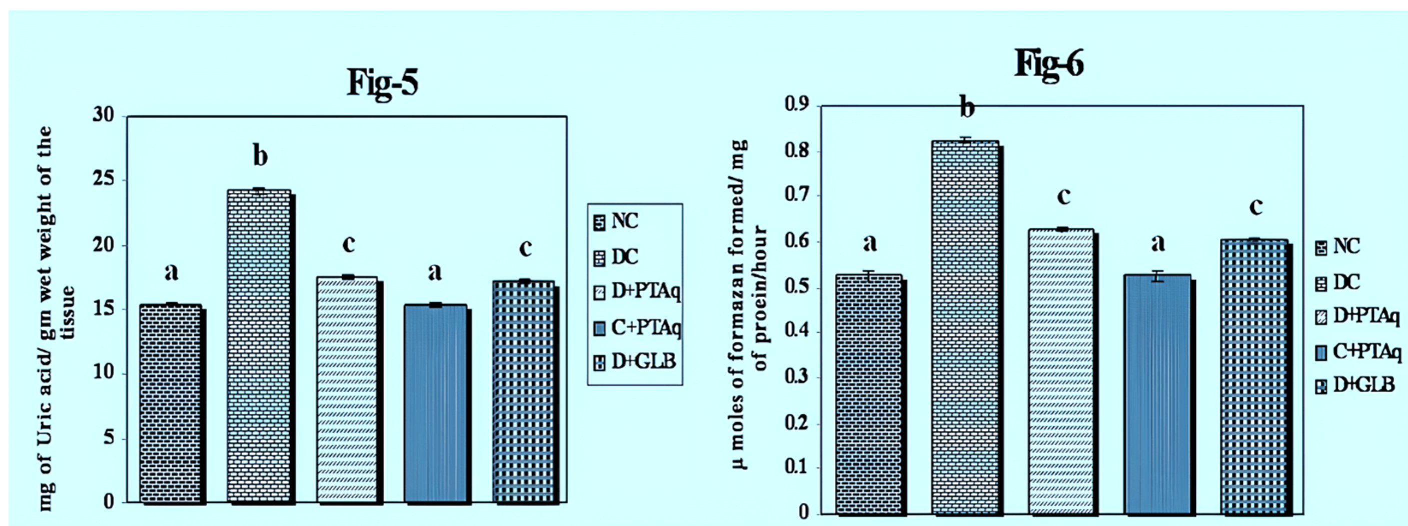

Figure 5 and Figure 6: Uric acid content and Activity of Xanthine Oxidase in the kidney of Normal Control rats (NC), Diabetic Control rats (DC), diabetic rats treated with Pt Aqueous extract (D+PTAq), Control rats treated with Pt Aqueous extract (C+PTAq), diabetic rats treated with Glibenclamide (D+GLB). Each vertical bar represents the mean±SE. (n=6). Top of the vertical bars having the same alphabetic do not differ significantly at $p < 0.01$.

Vitamin C levels may have decreased due to increased utilization of the vitamin as an antioxidant defence against ROS or a decrease in GSH, which is essential for Vitamin C recycling. Vitamin E is a physiological antioxidant and membrane stabilizer, responding with lipid peroxy radicals to break the chain reaction of lipid peroxidation and protect cell structures from damage.[34] Similarly, reduced levels of Vitamin E were also reported in diabetic liver and kidney.[32] Treatment with PTAq restored Vitamin C and E levels to almost normal levels, indicating reduced membrane damage due to the extract's antioxidant properties.

In diabetes, the xanthine oxidase enzyme system is activated, leading to increased production of uric acid and consequent free radical production, despite uric acid's known non-enzymatic antioxidant properties.[35] This study found that diabetic rats had higher uric acid levels, potentially due to metabolic disturbance in diabetes such as high activity of xanthine oxidase and elevated levels of lipid peroxidation, triglyceride and cholesterol.[36] Additionally, muscle wasting and increased release of purine due to protein glycation may contribute to elevated uric acid levels.

However, supplementation with PTAq significantly lowered uric acid levels and xanthine oxidase activity in hyperglycemic rats. This diminution in uric acid levels may be due to the reduction in xanthine oxidase activity, lipid peroxidation, and triglyceride and cholesterol levels, as high levels of these substances can lead to uric acid synthesis.

CONCLUSION

Overall, the *Pimpinella tirupatiensis* tuberous root aqueous extract can prevent and/or protect against free radical production in diabetes by significantly increasing the levels of non-enzymatic antioxidants while reducing markers of lipid peroxidation. Therefore, this study suggests that the extract has protective properties against oxidative stress in diabetic rats induced with STZ.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The outcomes of the experiment designate that Pt may be beneficial in preventing diabetic complications caused by oxidative stress in rats with diabetes. Therefore, Pt may have potential therapeutic implications for managing diabetes in the future.

REFERENCES

1. Alema NM, Periasamy G, Sibhat GG, Tekulu GH, Hiben MG. Antidiabetic activity of extracts of *Terminalia brownii* Fresen. stem bark in mice. J Exp Pharmacol. 2020;12:61-71. doi: 10.2147/JEP.S240266, PMID 32110120.
2. Meresa A, Gemechu W, Basha H. Herbal medicines for the management of diabetic mellitus in Ethiopia and Eretria including their phytochemical constituents. Am J Adv Drug Deliv. 2017;5(1):1-25.
3. Ezhilarasu H, Vishalli D, Dheen ST, Bay BH, Srinivasan DK. Nanoparticle-based therapeutic approach for diabetic wound healing. Nanomaterials (Basel). 2020;10(6):1-29. doi: 10.3390/nano10061234, PMID 32630377.
4. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5(1):194-222. doi: 10.3390/biom5010194, PMID 25786107.
5. Halliwell B, Gutteridge J. Free radicals in biology and medicine. 4th ed. New York: Oxford University Press; 2007.
6. Pandey KB, Mishra N, Rizvi SI. Protein oxidation biomarkers in plasma of type 2 diabetic patients. Clin Biochem. 2010;43(4-5):508-11. doi: 10.1016/j.clinbiochem.2009.11.011, PMID 19941844.
7. obertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem. 2004;279(41):42351-4. doi: 10.1074/jbc.R400019200, PMID 15258147.
8. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, *et al*. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: avenues for a mechanistic-based therapeutic approach. Curr Diabetes Rev. 2011;7(5):313-24. doi: 10.2174/157339911797415585, PMID 21838680.
9. Fiorentino TV, Prioletta A, Zuo P, Folli F. Hyperglycemia- induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Curr Pharm Des. 2013;19(32):5695-703. doi: 10.2174/1381612811319320005, PMID 23448484.
10. Latheef SA, Prasad B, Bavaji M, Subramanyam G. A database on endemic plants at Tirumala hills in India. Bioinformation. 2008;2(6):260-2. doi: 10.6026/97320630002260, PMID 18317578.
11. Bakshu LMD, Venkata Raju RR. Essential oil composition and antimicrobial activity of tuberous roots of *Pimpinella tirupatienesis* Bal. Flavour Fragr J. 2002;17(6):413-5. doi: 10.1002/ffj.1118.
12. Jeevan Ram A, Bhakshu LMD, Venkata Raju RR. *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J Ethnopharmacol. 2004;90(2-3):353-7. doi: 10.1016/j.jep.2003.10.013, PMID 15013201.
13. Rakieten N, Rakieten ML, Madkarni MV. Studies on the diabetogenic action of streptozotocin (NSC – 37917). Cancer Chemother Repos. 1963;29:91-8:91-8. Cancer Chemother rep.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810.
15. Akerboom TP, Sies H. Assay of glutathione, glutathione disulfide and glutathione mixed disulfide in biological samples. Methods Enzymol. 1981;77:373-82. doi: 10.1016/s0076-6879(81)77050-2, PMID 7329314.
16. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol. 1979;62:3-11. doi: 10.1016/0076-6879(79)62181-x, PMID 440112.
17. Baker H, Frank O, Angelis B, Feingold S. Plasma tocopherol in man at various times after ingesting free or acylated tocopherol. Nutr Res. 1951;21:531-6.
18. Martinek RG. Methods for the determination of uric acid. American. Clin Pathol. 1965;18(6):777-9.
19. Srikanthan TW, Krishnamurthy C. Tetrazolium test for dehydrogenases. J Sci Ind Res. 1955;14:206-7.
20. Tabatabaei-Malazy O, Khodaeian M, Bitarafan F, Larijani B, M Amoli MM. Polymorphisms of antioxidant genes as a target for diabetes management. Int J Mol Cell Med. 2017;6(3):135-47. doi: 10.22088/acadpub.BUMS.6.3.135, PMID 29682485.
21. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, *et al*. Hyperglycemia causes oxidative stress in pancreatic beta cells of GK rats a model of type 2 diabetes. Diabetes. 1999;48(4):927-32. doi: 10.2337/diabetes.48.4.927, PMID 10102716.
22. Haugaard N. Cellular mechanisms of oxygen toxicity. Physiol Rev. 1968;48(2):311-73. doi: 10.1152/physrev.1968.48.2.311, PMID 4870377.
23. Devasena T, Lalitha S, Padma K. Lipid peroxidation, osmotic fragility and antioxidant status in children with acute post-streptococcal glomerulonephritis. Clin Chim Acta. 2001;308(1-2):155-61. doi: 10.1016/s0009-8981(01)00482-x, PMID 11412828.
24. Bhatia S, Shukla R, Venkata S, Kaur J, Madhava K. Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. Clin Biochem. 2003;36:57-60.
25. Slatter DA, Bolton CH, Bailey AJ. The importance of lipid derived malondialdehyde in diabetes mellitus. Diabetologia. 2000;43(5):550-7. doi: 10.1007/s001250051342, PMID 10855528.
26. Guoyao W, Fang ZY, Yag S, Lupton RJ, Turner DN. Glutathione metabolism and implication for health. J Nutr. 2004;134:489-92.
27. Konukoğlu D, Serin O, Demiriz Kemerli G, Serin E, Hayirhoğlu A, Oner B. A study on the carotid artery intima-media thickness and its association with lipid peroxidation. Clin Chim Acta. 1998;277(1):91-8. doi: 10.1016/s0009-8981(98)00117-x, PMID 9776048.
28. Chugh SN, Kakkar R, Kalra S, Sharma A. An evaluation of oxidative stress in diabetes mellitus during uncontrolled and controlled state and after vitamin E supplementation. J Assoc Physicians India. 1999;47(4):380-3. PMID 10778519.
29. Eliza J, Daisy P, Ignacimuthu S. Antioxidant activity of costunolide and eremanthin isolated from *Costus speciosus* (Koen ex Retz) Sm. Chem Biol Interact. 2010;188(3):467-72. doi: 10.1016/j.cbi.2010.08.002, PMID 20709041.
30. Garg MC, Bansal DD. Protective antioxidant effect of vitamin C and vitamin E in streptozotocin induced diabetic rats. Indian J Exp Biol. 2000;38(2):101-4. PMID 11218824.
31. Fang YZ, Yang S, Wu G. Free radicals, antioxidants and nutrition. Nutrition. 2002;18(10):872-9. doi: 10.1016/s0899-9007(02)00916-4, PMID 12361782.
32. Murugan P, Pari L. Antioxidant effect of tetrahydrocurcumin in streptozotocin-nicotinamide induced diabetic rats. Life Sci. 2006;79(18):1720-28. doi: 10.1016/j.lfs.2006.06.001, PMID 16806281.
33. Ramesh PKV. Antioxidant role of umbelliferone in STZ-diabetic rats. Life Sci. 2006;79:1720-8.
34. Ingold KU, Webb AC, Witter D, Burton GW, Metcalfe TA, Muller DP. Vitamin E remains the major lipid soluble, chain-breaking antioxidant in human plasma even in individuals suffering from severe vitamin E deficiency. Arch Biochem Biophys. 1987;259(1):224-5. doi: 10.1016/0003-9861(87)90489-9, PMID 3688884.
35. Németh I, Tálosi G, Papp A, Boda D. Xanthine oxidase activation in mild gestational hypertension. Hypertens Pregnancy. 2002;21(1):1-11. doi: 10.1081/PRG-120002905, PMID 12044338.
36. Madianov IV, Balabolkin MI, Markov DS, Markova TN. Main causes of hyperuricemia in diabetes mellitus. Ter Arkh. 2000;72(2):55-8. PMID 10717929.

Cite this article: Lavanya T, Mohan KC, Reddy KS, Saddala RR. Attenuation of Oxidative Stress and Nephrotoxicity with Supplementation of *Pimpinella tirupatiensis* Tuberous Root in Streptozotocin-Induced Diabetic Rats. Pharmacog Res. 2023;15(4):813-8.