# Methanolic extract of *Pueraria tuberosa* Linn ameliorates renal injury and oxidative stress in rats with alloxan-induced diabetes

Short Title: *Pueraria tuberosa* ameliorates renal injury

Durgavati Yadav[*], Vivek Pandey[*], Shivani Srivastava[*], Mohan Kumar[§], Yamini Bhusan Tripathi[*] *Department of Medicinal Chemistry, Institute of Medical Science, Banaras Hindu University, Varanasi-221005, India.
[§]Department of Pathology, Institute of Medical Science, Banaras Hindu University, Varanasi-221005, India.

Corresponding Address:
Prof. Yamini Bhusan Tripathi
Department of Medicinal Chemistry, Institute of Medical Sciences,
Banaras Hindu University, Varanasi-221005, India.



**Abstract:**

**Aim of the study:** Excessive free radicals generated by chronic hyperglycaemia contribute to the development of diabetic nephropathy (DN). Here, the effect of *Pueraria tuberosa* in alloxan induced DN has been explored.

**Methodology:** DN was induced by administering alloxan (120 mg/kg in chilled normal saline, ip) in albino rats (CF strain, both sexes, 7-8 weeks old) at regular intervals to maintain higher blood glucose. Tubers of *Pueraria tuberosa* (Bidarikand, PT) has been taken for the study as it is reported in the treatment of kidney disorders. Renal damage was assessed by renal functional tests, histopathology and oxidative stress. Antioxidant parameters were measured; serum urea and creatinine levels were assessed in four groups with PTME treated group (40 mg/100g BW for seven days and 20 mg/100g BW for 14 days to diabetic rats of alloxan treated and two groups each with PTME to normal treated rats), diabetic control and normal group.

**Results:** PTME showed significant protection ($P<0.05$) by decreasing urea and creatinine as compared to the diabetic control. It improves kidney physiology by increasing its decreased antioxidant level. Histopathology reports supported the experimental findings.

**Conclusion:** These results suggested that PT might act as beneficial against renal impairment through antioxidant mechanisms.

**Keywords:** Antioxidants, Bidarikand, Glomerulosclerosis, Nephropathy, Tubular damages.

**Running title:** Amelioration of alloxan induced DN by methanolic fraction of *Pueraria tuberosae*.

## 1. Introduction:

Diabetes is a group of metabolic diseases with chronic hyperglycemia resulting from defects in insulin metabolism. There is complex imbalance among carbohydrate, lipid and protein with long term micro & macro vascular complications (Of and Mellitus, 2014). There is steep rise in the prevalence of metabolic syndrome and Type 2 diabetes worldwide; extremely pronounced in Asian countries and highly dramatic in India.

In urban Indian population, there is occurrence of nephropathy and microalbuminuria, and it was found to be 2.20 and 26.90% respectively (Unnikrishnan et al., 2007). DN accounts for no less than 46% of the chronic kidney diseases & frequent cause of the chronic renal failure and end-stage kidney diseases in the United States (Collins et al., 2015). India is declared as the diabetic capital of the world. Chronic hyperglycemia is the main culprit responsible for the development of oxidative stress and free radicals (Yan, 2014). As a clinical syndrome, DN has distinctive feature with decline in GFR (glomerular filtration rate), persistent albuminuria and arterial hypertension in absence of any other kidney diseases (Tripathi and Yadav, 2013). Kidneys are easily prone to damaging effects caused by oxidative stress which in turn cause vascular complications. These changes results in high secretion of profibrotic cytokines, such as transforming growth factor β (TGF-β), TNF-α (Tumour necrosis factor-α) resulting to haemodynamic changes (Wang et al., 2012).

The earliest clinical evidence of nephropathy is an increase in microalbuminuria (defined as >30 mg/day) into the macroalbuminuria range (>300 mg/day) (Roshan and Stanton, 2013). Renal function is compromised even prior to the onset of nephropathy. There are several redox changes that occur early and changes as nephropathy progresses. Several studies have proven that prevention of diabetes related complications has been nearly correlated with ingestion of various fruits, medicinal plant parts and vegetables (Kooti et al., 2016).

Tubers are used for the treatment of dysuria, cough, rheumatism, erysipelas and malarial fever. Roots are used in medicine as a demulcent, chyawanprash- a health tonic, as cataplasam for swellings of joints, as lactogogue and cardiotonic. It is also used as emetic, galactogogue and tonic (Maji et al., 2014). Tuberous roots of PT showed significant response on kidney. The mechanism of action of the effective plants could be due to its antioxidant potential since plants are rich in polyphenolic compounds. Antioxidants play major role in scavenging free radicals and detoxification (Ighodaro and Akinloye, 2018). Reactive oxygen species (ROS) caused from oxidative stress reacts with polyunsaturated fatty acids fatty acids leading to the peroxidation of lipid and irreversible cellular macromolecule modifications (Ayala et al., 2014). Typical markers of oxidative stress already been reported in both humans and experimental animals including increased ROS, MDA (Malondialdehyde) and reduced levels of antioxidants. An imbalance in the production of ROS and antioxidants is involved in renal failure (Ratliff et al., 2016). DN causes progressive renal alterations with maximum chances of irreversibility. In the present study effect of *Pueraria tuberosa* has been seen in alloxan induced DN by assessing the antioxidant levels and kidney damage markers.

## 2. Material and Methods:

Protocol for animal experimentation and the animal handling studies were approved by Animal ethical committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi (Letter No. Dean/2012-2013/192). Both male and female albino rats of Charles Foster strain (7-8 weeks old) weighing 120-160 g were purchased from the Central animal house of our Institute. All the rats were fed standard pellet laboratory diet and were provided with water *ad libitum*. Glucose, urea and creatinine were determined from commercial kits available (Accurex Biomedical Private Limited, New Delhi, India). For histological analysis, one kidney was taken from the dissected rats, washed with normal saline then fixed in Bouin's fluid for 24 hours, finally embedded in paraffin wax to prepare 3-4 μm tissue slices. For histological details, tissue slices were stained with Periodic acid-Schiff staining procedures for specific visibility through microscope.

**2.1 Preparation of methanolic extract by Soxhlet method:**

Coarse root powder obtained from the Department of Ayurvedic Pharmacy, Faculty of Ayurveda, Banaras Hindu University, was extracted in a soxhlet apparatus at 60-80˚C for 18 h to get the methanolic extract. Obtained filterate was concentrated in vacuum distillation unit. It was transferred to previously weighed empty porcelain dish and was subjected to evaporation in a vacuum dessicator till constant weight was attained without any solvent left in the extract. The obtained solvent free semisolid matter was termed as methanolic fraction of *Pueraria tuberosae* (PTME).

**2.2 Standardisation of the DN model:**

Diabetes was induced in total 64 rats by a single intraperitoneal injection of Alloxan (Sigma, USA) i.e. 120 mg/kg of body weight, were freshly dissolved in normal saline at chilled condition. Alloxan is a toxic glucose analogue that preferentially accumulates in pancreatic beta cells via GLUT-2. One group was taken as normal control group and received the same volume of normal saline as the drug treated groups. Four days after injection, rats with high fasting blood glucose levels in the range of 13.90-22.20 mmol/L were considered as diabetes mellitus (DM) rats.

Rats were divided into six groups each containing eight numbers:

Group one- Normal control

Group two- Diabetic control

Group three- Dose treatment with 20 mg/100g BW to diabetic rats

Group four- Dose treatment with 40 mg/100g BW to diabetic rats

Group five- Dose treatment with 20 mg/100g BW to normal rats

Group six- Dose treatment with 40 mg/100g BW to normal rats

In alloxan induced diabetes rat model, nephropathy developed slowly after 45 days of post-injection, although hyperglycemia occurs within 72 h of intraperitoneal injection. Mortality index was high in animals who had received alloxan. Ten animals (15.7%) initially died within the first 30 days after diabetes

induction due to metabolic disorders and the drug's toxic actions. While remaining six diabetic animals were (9.30%) died upon late follow-up mainly due to starvation. Few rats were excluded due to blood glucose levels lower than 200 mg/dl. Blood and urine samples were collected on seventh day. Blood was collected from retro-orbital vein from the eyes. Plasma was isolated from the blood for further estimation of glucose, urea and creatinine. Every week, after drug administration, individual 24 h urine sample collections were collected by using metabolic cages. Blood glucose levels of DN rats were monitored every week. They were given supportive regular insulin treatment at a dose of 1 IU/kg of body weight (three times every week) when necessary to maintain the constant fasting blood glucose in the range of 13.90-22.90 mmol/L for preventing apparent exhaustion or ketosis during the experiment. Three rats were given insulin treatment due to very high blood glucose levels. These were considered in the diabetic control group. Rats with blood glucose in the range of 13.90-22.90 mmol/L did not receive insulin treatment to avoid the possible synergistic effect or interaction of insulin and drug. Finally rats were sacrificed and their kidney was saved for assessment of activity of antioxidant enzymes. Blood urea and creatinine levels were monitored regularly (Mackay and Mackay, 1927; McPherson and Pincus, Elsevier, 2017)

Dose was prepared in 20% Tween 20 and normal group has received the Tween 20 as drug vector. Drug was administered orally by using catheter at the same time on daily routine basis and drug dosage in Tween 20 (Polyoxyethylene 20) does not exceed more than 1ml (Ravindran et al., 2011). A weighed amount of PTME was dissolved in Tween 20 and taken as stock and working solution of dose was prepared. 20mg/10g BW and 40mg/100g BW was calculated by considering body weight of each individual rat. Rats were sacrificed and dissected by using anesthesia pentobarbital (10 mg/Kg BW, ip) after seven days of PTME (Methanolic fraction of *Pueraria tuberosa*) treatment which were receiving dose of 40 mg/100g BW. Fresh kidney slices were excised and rinsed with cold normal saline; dried on filter paper and stored at -80°C until for further analysis. Another group was treated with the PTME dose for 14 days at 20 mg/100g BW. 10 % kidney homogenate was made in ice cold PBS buffer (w/v). Homogenization procedure was carried out for 2 min at 5000 g. Samples were centrifuged (×8000 g for 30 min at 4°C) and kept at -80°C. Determinations of the various parameters were made on the serum and kidney homogenates using commercially available kits. In

diabetes, urinary protein excretion has been found to be responsible for glomerular dysfunction. Creatinine clearance is an indicator of glomerular hyperfiltration and dysfunction (Kagoma et al., 2012).

Creatinine clearance (CrCl) was calculated by using the formula:

$$\frac{\text{Urinary creatinine (mg/ml)} \times \text{urine volume (ml/kg)}}{\text{Creatinine in serum (mg/ml)}}$$

Kidney index (KI) was calculated for all groups. KI (kidney weight/body weight) is an important indicator of renal morphology and marker for body weight loss and renal size. With advancement in renal dysfunction, there was reduction in body weight and increase in kidney weight. Renal function is compromised with hypertrophy of renal structures (Alicic et al., 2017).

**2.3 Superoxide dismutase activity determination:**

The principle of superoxide dismutase (SOD) activity determination method was based on the inhibition of nitrobluetetrazolium (NBT) reduction by xanthine oxidase system as a superoxide radical generator (Kakkar et al., 1984). One unit of SOD was defined as the enzyme activity causing 50% inhibition of xanthine in the nitrobluetetrazolium reduction rate. The SOD activity was expressed as units per enzymatic tissue protein (U/mg protein).

**2.4 Catalase activity determination:**

Catalase (CAT) activity was determined according to Aebi's method (Aebi, 1984). The essentials of CAT activity determination method were based on the determination of the rate constant of the $H_2O_2$ decomposition rate at 240 nm. The CAT activity results were expressed as U/mg protein.

**2.5 Malondialdehyde level determination:**

Malondialdehyde (MDA) level was determined using by Wasowicz's method which was based on the reaction of MDA with thiobarbituric acid (TBA) at 95-100°C (Hong et al., 2000). MDA or MDA-like substances and TBA together to produce a pink pigment with an absorption maxima of 532 nm. Results

were expressed as nanomoles per gram wet tissue protein (nmol/g wet tissue) according to standard graphics which was prepared with serial dilutions of standard 1,1,3,3-tetraethoxypropane.

**2.6 Protein carbonyl level determination:**

Protein carbonyl (PC) contents were determined spectrophotometrically (SSL 210, India) with the reaction of the carbonyl group with 2, 4-Dinitrophenylhydrazine to form 2, 4-Dintrophenylhydrazone (Makni et al., 2010). The results were given as nmoles of protein carbonyl/ mg of protein.

**2.7 Determination of protein content**

Protein content of the experimental samples were measured by Bradford assay using crystalline BSA as standard.

**2.8 Glomerulosclerosis index:**

PAS stained sections were examined using Nikon eclipse 50i microscope. One hundred glomeruli per section were randomly selected and the degree of damage assessed by using semi-quantitative scoring method: grade 0, normal glomeruli; grade 1, sclerotic area upto 25% (minimal sclerosis); grade 2, sclerotic area 25 to 50% (moderate sclerosis); grade 3, sclerotic area 50 to 75% (moderate to severe sclerosis); grade 4, sclerotic area 75 to 100% (severe sclerosis). Glomerulosclerosis index (GSI):

$$\frac{(1 \times n_1) + (1 \times n_2) + (1 \times n_3) + (1 \times n_4)}{n_0 + n_1 + n_2 + n_3 + n_4}$$

where $n_x$ is the number of glomeruli per section (Kim et al., 2017). This analysis was performed with the observer unknown to the treatment group.

**2.9 Assessment of tubulointerstitial damage:**

PAS stained sections were examined using Nikon microscope. The degree of tubulointerstitial damage was defined as tubular atrophy or dilatation and interstitial proliferation. The degree of tubulointerstitial damage was graded on a scale; grade 1, affected area less than 10%; grade 2, affected area 10 to 25%; grade 3, affected area 25 to 75%; grade 4, affected area greater than 75% (Piecha et al., 2008).

### 3.0 Statistical Analysis:

The data has been subjected to statistical analysis by one way Analysis of Variance (ANOVA) followed by Dunnet's t- test and $P<0.05$, 0.001 were considered as significant. IBM SPSS 20 software was used for the analysis of data.

## 3. Results and Discussion:

### 3.1 Standardisation of the DN conditions in alloxan induced diabetes (AID)

Symptoms of DN appeared after 50 days of giving alloxan. It was confirmed by performing renal function tests as shown in the Table 1. Creatinine level increases with advancement of chronic hyperglycemia. Urea level also increases on the $50^{th}$ day. The alloxan destroys pancreatic-β cell population via formation of reactive oxygen species like nitric oxide (Szkudelski, 2001).

### 3.2 Effect of PT on body weight and survivality curve in AID rats

Effect of PTME on body weight was shown in Fig. 1. Body weight was rapidly reduced in alloxan treated; reduction was higher on $50^{th}$ day. In normal rats, weight increases optimally. Loss in body weight started to recover in PTME treated rats, almost reached similar to healthy weight animals. During experiment few animals were died because of severe diabetic condition as given by survival chart (Fig 2). Rats with severely damaged kidneys (which have developed irreversible ketosis) did not able to survive, hence graph was little zigzag. Kaplan-Meir survival curve is a non-parametric statistic curve which is used to estimate the survival at particular period of time. It is generally applied to calculate the length of time upto which particular number of animals survived after the treatment.

### 3.3 Effect of PTME on renal function

Serum creatinine decreased after PTME treatment (32.72% after fourteen days of PTME (20 mg/100g BW) and 26.54% after seven days of PTME (40 mg/100g BW). Renal function tests showed positive effect of the PTME treatment. Urea decreases 34.38% after fourteen days of PT (20 mg) and 22.65% after seven days of PTME (40 mg). Glucose level also decreases 43.66% after seven days of PTME and 21.34% after fourteen days. This may be attributed to the presence of phenols, flavonoids, alkaloids, saponins, tannins and phylobatanins that have been associated with hypoglycemic activity (Wintola and Afolayan, 2011).

Decrease in the parameters was depicting dose wise response in the treated group (Table 2). Lower dose for longer duration will be better than higher dose since the rats started to die at higher dose as reported in our earlier studies done in the laboratory conditions. The PTME showed ameliorating effect in this model by free radical scavenging when administered orally. Furthermore we demonstrated that PTME accelerates the recovery of renal damage and function by restoring renal architecture after tubular damage. PTME exerts this beneficial effect through the inhibition of excessive free radical formation. This fraction had shown nephroprotection in STZ (streptozotocin) induced diabetic nephropathy model by MMP-9 expression as reported in our laboratory findings (Tripathi et al., 2016).

**3.4 Effect of PTME on liver function**

PTME showed hepatotoxicity as shown by earlier reports conducted in our laboratory conditions. Here we have assessed liver function tests to reduce the combined effect of hepatoxicity and drug treatment. There was no rise in liver function enzymes (Table 3) in experimental control and the drug treated groups. The research has revealed an important antioxidant effect, with the increase of SOD, CAT activity in the treated group and the decreased lipid peroxides and protein carbonyl after the treatment.

**3.5 Effect of PTME on urinanalysis in AID rats**

Urinary protein was decreased as dose dependent concentration in the treated group (Table 4). The first parameter, bilirubin was negative in all the groups; this showed that liver functioning was in the normal condition. Urobilinogen was in normal range in treated and experimental control. There was secretion of glucose in the urine of experimental control (abnormal functioning of glucose metabolism) rats while there was no secretion of glucose in the urine of normal rats (glucose was absorbed in renal tubules hence no abnormal functioning of glucose metabolism). As the different doses of PTME was administered to treated groups, there was improvement in the glucose metabolism, lesser amount of glucose was secreted in 20 mg/100 g BW rats as compared to the 40 mg/100g BW rats. Proteinuria occurred in the experimental control rats but slowly it was diminished in drug treated rats with same trend as followed in case of glucose secretion. Presence of nitrites in the urine sample shows bacterial infection, since it is negative in all the samples hence there was no bacterial infection occurred in the studied urine samples. When there was abnormal secretion of glucose, protein, specific gravity of the urine also increases but with treatment with PTME, specific gravity comes in the normal range.

**3.6 Effect of PTME on antioxidant enzymes in liver and kidney homogenates**

Antioxidant enzymes have lower values in the experimental control, but the level of antioxidants significantly raises in PTME treated group (40 mg/100g BW) for seven days treated rats in kidney. PTME group with 20 mg for fourteen days had also shown protective response. This shows the normal function of the liver enzymes.

Liver antioxidant enzyme levels were shown in Table 5 and kidney antioxidant enzyme levels were shown in Table 6.

### 3.7 Effect of PTME on kidney histopathology

There was deposition of eosinophilic materials in the intermediate substantiate of medulla in the kidney indicating the early sign of renal fibrosis. There was increase of blood flow with accumulation of lipofuscin granule as reason of wear and tear. The average weight of kidneys has been increased in comparison to non diabetic rats. There was hypertrophy of the kidney which leads to the increased kidney index with polyuria altering GFR. There was extension in mesangial matrix of AID rats as compared to the normal rats (Figure 4A). Tubulointerstitial damage was assessed to see changes in the tubules. Tubulointerstitial damage in the kidney is an important manifestation of the alloxan induced diabetic nephropathy. Drug treatment significantly prevented glomerular hypertrophy and glomerulosclerosis. The AID diabetic rats showed mesangial matrix expansion compared to normal control rats (Fig. 4A). Treatment with PTME for two weeks markedly ameliorated the regeneration of epithelial lining and tubular damages when compared with the untreated AID diabetic rats (Fig. 4B).

### Conclusion:

Induction of diabetes in the present study caused a significant decline in MDA and a rise in SOD and CAT in kidney as compared with the normal. Treatment with plant extract significantly reduces the elevated urea and creatinine levels and reverses the histological changes. Therefore our present study aimed at to determine the ameliorating effect of extract on secondary diabetic complications. DN therapy in today's world needs multi-targeted drug action because of its various complexities relating to hyperglycemia. Polyherbal formulations and their preparations can be very promising since it has lesser adverse effects.

Thus it could be suggested that methanolic fraction of PT has significant role in protection against DN. The mechanism of action could be through its antioxidant and anti-inflammatory property. The polyphenols, alkaloids, flavonoids present in the PT might be responsible for this activity.

**Acknowledgments:**

We thank Banaras Hindu University for the financial support; Prof. Yamini B. Tripathi conceived and designed the experiments. Durgavati Yadav performed the experiments and analyzed the whole data with grading score of the glomerulus. Vivek Pandey and Shivani Srivastava prepared the histological slides and Prof. Mohan Kumar analyzed the these slides.

**Conflict of interest:**

The authors declare that they have no conflict of interest to disclose.

**References**

Aebi, H., 1984. Catalase in *vitro*, in: Methods in Enzymology. https://doi.org/10.1016/S0076-6879(84)05016-3

Alicic, R.Z., Rooney, M.T., Tuttle, K.R., 2017. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin. J. Am. Soc. Nephrol. 12, 2032–2045. https://doi.org/10.2215/CJN.11491116

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 2014, 360438. https://doi.org/10.1155/2014/360438

Collins, A.J., Foley, R.N., Gilbertson, D.T., Chen, S.C., 2015. United States Renal Data System public health surveillance of chronic kidney disease and end-stage renal disease. Kidney Int. Suppl. https://doi.org/10.1038/kisup.2015.2

Hong, Y.L., Yeh, S.L., Chang, C.Y., Hu, M.L., 2000. Total plasma malondialdehyde levels in 16 Taiwanese college students determined by various thiobarbituric acid tests and an improved high-performance liquid chromatography-based method. Clin. Biochem. https://doi.org/10.1016/S0009-9120(00)00177-6

Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 54, 287–293. https://doi.org/10.1016/J.AJME.2017.09.001

Kagoma, Y.K., Garg, A.X., Li, L., Jain, A.K., 2012. Reporting of the estimated glomerular filtration rate decreased creatinine clearance testing. Kidney Int. 81, 1245–1247. https://doi.org/10.1038/ki.2011.483

Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys. https://doi.org/10.1097/YCO.0b013e3280117733

Kim, D.R., Lee, S.Y., Kim, J.S., Kim, Y.G., Moon, J.-Y., Lee, S.H., Lee, T.W., Ihm, C.G., Jeong, K.H., 2017. Ameliorating Effect of Gemigliptin on Renal Injury in Murine Adriamycin-Induced Nephropathy. Biomed Res. Int. 2017, 1–10. https://doi.org/10.1155/2017/7275109

Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., Asadi-Samani, M., 2016. The role of medicinal plants in the treatment of diabetes: a systematic review. Electron. physician 8, 1832–42. https://doi.org/10.19082/1832

Mackay, E.M., Mackay, L.L., 1927. THE CONCENTRATION OF UREA IN THE BLOOD OF NORMAL INDIVIDUALS. J. Clin. Invest. 4, 295–306. https://doi.org/10.1172/JCI100124

Maji, A.K., Pandit, S., Banerji, P., Banerjee, D., 2014. *Pueraria tuberosa* : a review on its phytochemical and therapeutic potential. Nat. Prod. Res. 28, 2111–2127. https://doi.org/10.1080/14786419.2014.928291

Makni, M., Sefi, M., Fetoui, H., Garoui, E.M., Gargouri, N.K., Boudawara, T., Zeghal, N., 2010. Flax and Pumpkin seeds mixture ameliorates diabetic nephropathy in rats. Food Chem. Toxicol. https://doi.org/10.1016/j.fct.2010.05.079

McPherson, R.A., Pincus, M.R., n.d. Henry's clinical diagnosis and management by laboratory methods.

Of, D., Mellitus, D., 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care.

https://doi.org/10.2337/dc14-S081

Piecha, G., Koleganova, N., Gross, M.-L., Geldyyev, A., Adamczak, M., Ritz, E., 2008. Regression of glomerulosclerosis in subtotally nephrectomized rats: effects of monotherapy with losartan, spironolactone, and their combination. Am. J. Physiol. Physiol. 295, F137–F144. https://doi.org/10.1152/ajprenal.00065.2008

Ratliff, B.B., Abdulmahdi, W., Pawar, R., Wolin, M.S., 2016. Oxidant Mechanisms in Renal Injury and Disease. Antioxid. Redox Signal. 25, 119–46. https://doi.org/10.1089/ars.2016.6665

Ravindran, R., Juliet, S., Gopalan, A.K.K., Kavalimakkil, A.K., Ramankutty, S.A., Nair, S.N., Narayanan, P.M., Ghosh, S., 2011. Toxicity of DMSO, Triton X 100 and Tween 20 against Rhipicephalus (Boophilus) annulatus. J. Parasit. Dis. 35, 237–239. https://doi.org/10.1007/s12639-011-0054-3

Roshan, B., Stanton, R.C., 2013. A story of microalbuminuria and diabetic nephropathy. J. Nephropathol. 2, 234–40. https://doi.org/10.12860/JNP.2013.37

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. 50, 537–46.

Tripathi, Y.B., Shukla, R., Pandey, N., Pandey, V., Kumar, M., 2016. Pueraria tuberose (PTY-2) attenuates diabetic nephropathy by up-regulating the MMP-9 expression in the kidney of diabetic rats. J. Diabetes. https://doi.org/10.1111/1753-0407.12393

Tripathi, Y.B., Yadav, D., 2013. Diabetic Nephropathy: Causes and Managements. Recent Pat. Endocr. Metab. Immune Drug Discov. 7, 57–64.

Unnikrishnan, R., Mohan, R., Rajendra, P., Mohan, D., Subramaniam, S., Raj, D., Mohan, V., 2007. Prevalence and risk factors of diabetic nephropathy in an urban south Indian population. Diabetes Care. https://doi.org/10.2337/dc06-2554.Abbreviations

Wang, J., Li, G., Wang, Z., Zhang, X., Yao, L., Wang, F., Liu, S., Yin, J., Ling, E.A., Wang, L., Hao, A.,

2012. High glucose-induced expression of inflammatory cytokines and reactive oxygen species in cultured astrocytes. Neuroscience. https://doi.org/10.1016/j.neuroscience.2011.11.062

Wintola, O.A., Afolayan, A.J., 2011. Phytochemical constituents and antioxidant activities of the whole leaf extract of Aloe ferox Mill. Pharmacogn. Mag. 7, 325–33. https://doi.org/10.4103/0973-1296.90414

Yan, L.-J., 2014. Pathogenesis of chronic hyperglycemia: from reductive stress to oxidative stress. J. Diabetes Res. 2014, 137919. https://doi.org/10.1155/2014/137919

## List of figures

### Effect PTME on alloxan induced diabetes

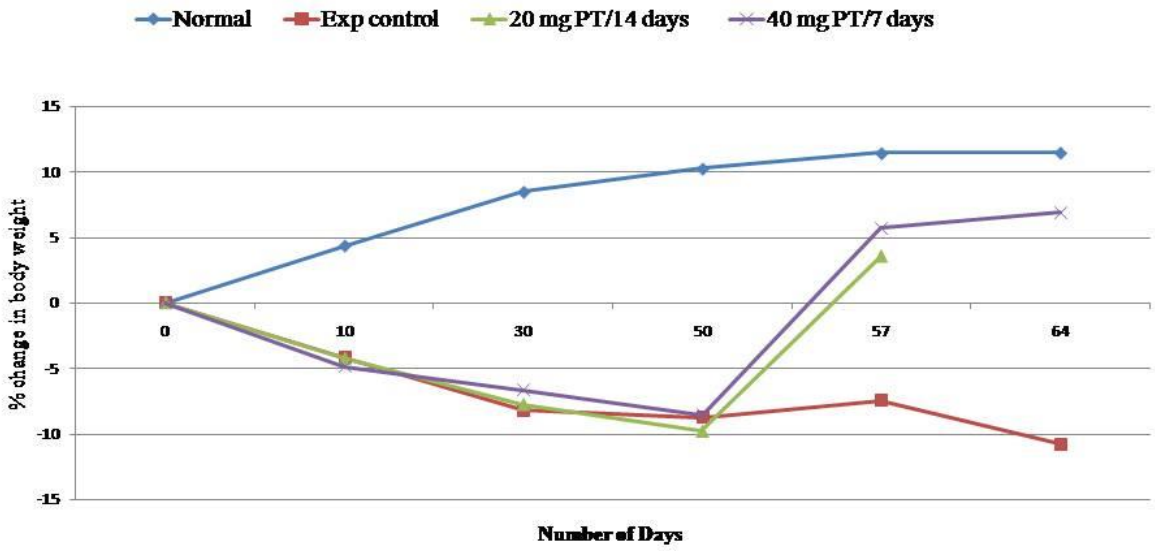


**Fig. 1 Effect of PT on alloxan diabetic rats. Normal group received normal saline intraperitoneally (ip). Experimental group received alloxan at 130 mg/kg BW, hyperglycemic condition is maintained throughout the period. 20 mg/100g BW given for 14 days while 40 mg/100g BW was given for 7 days after 50 days of diabetic condition.**

**Effect of PTME in alloxan induced diabetic rats depicting survivality curve**

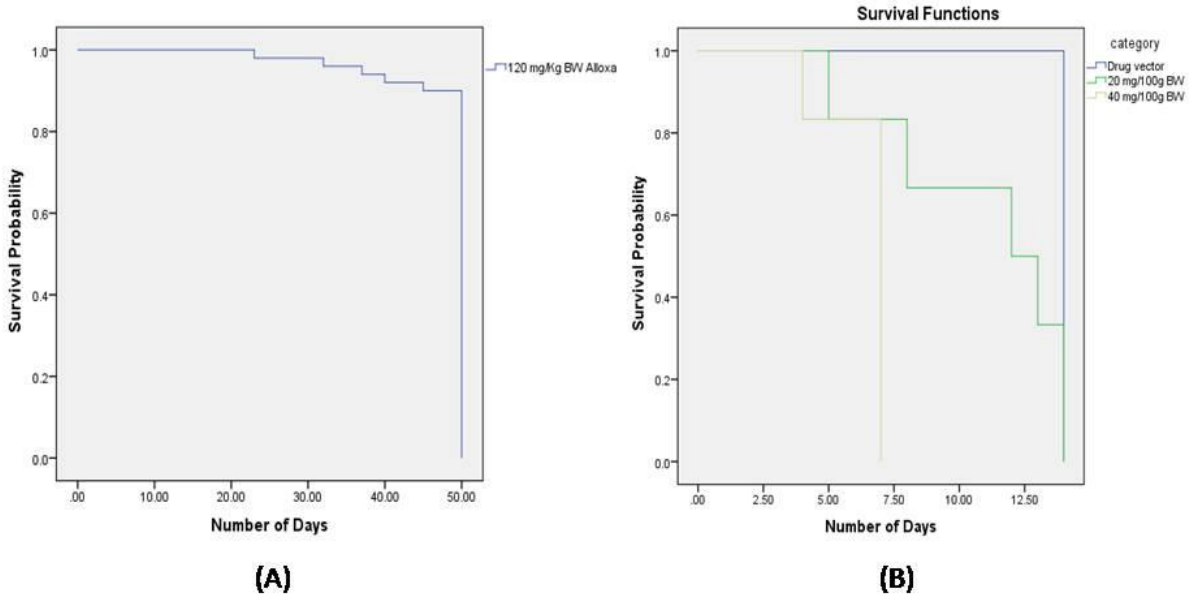


**Figure 2: (A) Probability curve is showing the rats survived upto 50 days. (B) Probability curve showing the rats survived after the dose treatment in Alloxan induced renal damage. This curve is zigzag depicting that rats were dead even at the time of treatment highlighting the severity of alloxan induced kidney damage.**

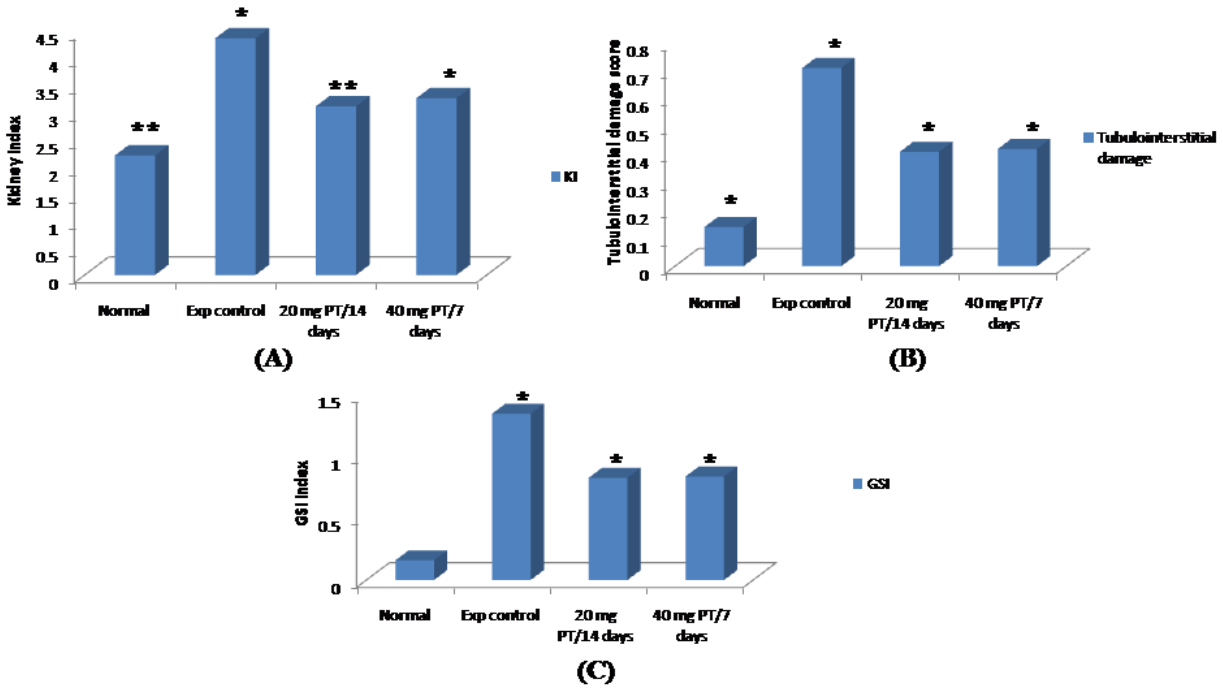


**Figure 3:** (A) Kidney index (KI) in normal, untreated and treated groups calculated by considering ratio of kidney weight and body weight. (B) It showed tubulointerstitial damage in the renal medulla in untreated and treated group. (C) This figure showed glomerulosclerosis in renal cortex in untreated and treated group. B and C were calculated by histological score with different grading. *Asterisk* mark showed that the data represent average ± SD in six separate experiments (*P < 0.05, **P < 0.001).

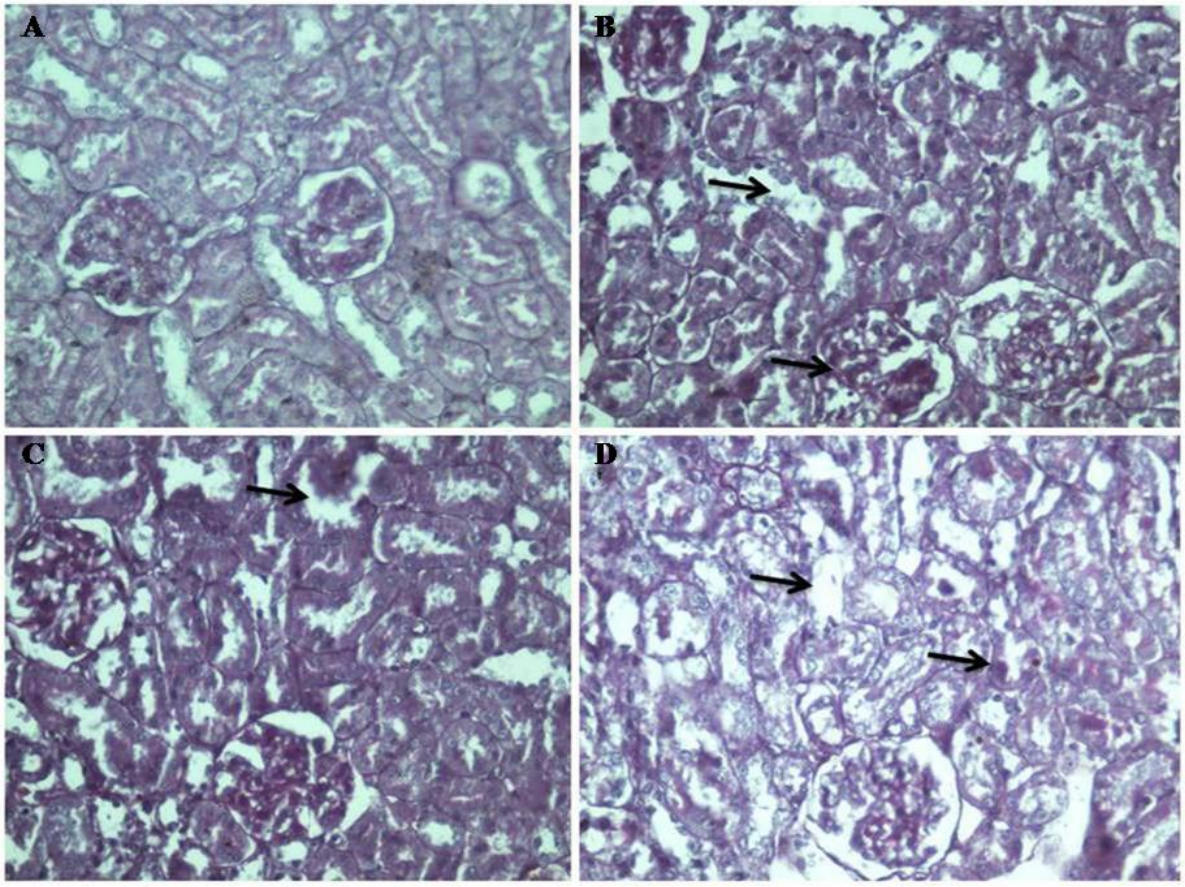

Figure 4: PAS (Periodic acid Schiff's staining) staining at 40X. (A) Normal morphology of the kidney, (B) diabetic control, arrows is showing the epithelial lining damages and accumulation of eosinophilic granules. (C) 20 mg/100 g BW PTME treated showing treatment affected areas with regeneration of epithelial lining, (D) 40 mg/100 g BW PTME treated, lesser damage to epithelial lining.

## List of Tables

Table 1: Standardisation of DN conditions in alloxan induced diabetes (AID)

| Parameters | 0th Day | | 15th Day | | 50th Day | |
|---|---|---|---|---|---|---|
| | Normal | AID | Normal | AID | Normal | AID |
| Body Weight (g) | 167 ± 2.56* | 168 ± 3.21 | 171 ± 3.51 | 157 ± 2.31 | 180 ± 3.51* | 150 ± 4.76* |
| Serum glucose (mmol/l) | 6.11 ± 0.87 | 4.98 ± 0.56 | 5.89 ± 0.56 | 13.03 ± 0.65 | 6.05 ± 0.89* | 15.98 ± 0.65 |
| Urea (mmol/l) | 1.36 ± 0.09 | 1.49 ± 0.13 | 1.59 ± 0.16 | 3.16 ± 0.18 | 1.38 ± 0.06 | 3.54 ± 0.27 |
| Creatinine (mg/dl) | 0.49 ± 0.19 | 0.53 ± 0.06 | 0.59 ± 0.09 | 3.67 ± 0.20 | 0.41 ± 0.13* | 5.41 ± 0.70 |
| Creatinine clearance (ml/Kg/min) | 12.40 ± 1.19 | 6.45 ± 0.99 | 9.69 ± 0.19 | 39.43 ± 1.89 | 12.49 ±1.79 | 56.49 ± 3.99 |

*P-value of all the parameters has P < 0.05 significance level

**Table 2: Effect of PT on renal function test and glucose:**

| Groups | Blood glucose (mmol/l) | Blood urea (mmol/l) | Blood creatinine (mg/dl) |
|---|---|---|---|
| Normal | 4.09 ± 0.26* | 1.32 ± 0.12 | 0.56 ± 0.07 |
| Exp control | 12.46 ± 0.45* | 2.88 ± 0.26 | 1.62 ± 0.08* |
| 20 mg PT/14 days | 7.02 ± 0.45* | 1.89 ± 0.16* | 1.09 ± 0.05 |
| 40 mg PT/7 days | 9.80 ± 0.23* | 2.17 ± 0.39* | 1.19 ± 0.06 |

*P-value of all the parameters has P < 0.05 significance level. Exp control group refers to the diabetic control group.

**Table 3: Effect of PT on liver function test:**

| Groups | SGOT | SGPT | Alkaline Phosphatase |
|---|---|---|---|
| Normal | 20.01 ± 0.17 | 26.17 ± 0.18 | 156.78 ± 11.72 |
| Exp control | 22.14 ± 1.09 | 24.36 ± 1.09 | 155.23 ± 13.09 |
| 20 mg PT/14 days | 20.73 ± 0.55 | 25.25 ± 0.79 | 154.51 ± 9.39 |
| 40 mg PT/7 days | 20.89 ± 0.34 | 26.71 ± 0.66 | 155.25 ± 9.75 |

**Table 4: Effect of PT on Urinanalysis in AID rats:**

| Groups | Bilirubin | Urobilinogen | Ketone bodies | Protein | Glucose | pH | Nitrites | Leucocytes | Specific gravity |
|---|---|---|---|---|---|---|---|---|---|
| Normal | -ve | 0.2 mg/dl | 0 | -ve | -ve | 6.5 | -ve | -ve | 1.005 |
| Exp Control | -ve | 0.2 mg/dl | 5 mg/dl | 3+,>300 mg/dl | 4+,>1000 mg/dl | 8.0 | -ve | -ve | 1.030 |
| 20 mg PT/100g BW for 14 days | -ve | 0.2 mg/dl | 0 | 1+,>30 mg/dl | 2+,100 mg/dl | 7.5 | -ve | -ve | 1.015 |
| 40 mg PT/100g BW for 7 days | -ve | 0.2 mg/dl | 0 | 2+,>100 mg/dl | 3+,>500 mg/dl | 7.5 | -ve | -ve | 1.020 |

Experiment was repeated in N=6. –ve indicates the absence of protein or glucose.

**Table 5: Effect of PT on antioxidant enzymes in liver homogenates:**

| SNo | | LPO (nMol/mg protein) | | SOD (U/mg protein) | | Cat (U/mg protein) | | Protein carbonyl (nMol/mg protein) | |
|---|---|---|---|---|---|---|---|---|---|
| | Doses | *20 mg | 40 mg | 20 mg | 40 mg | 20 mg | 40 mg | 20 mg | 40 mg |
| 1. | Normal | 0.15 ± 0.02 | 0.16 ± 0.01* | 4.99± 0.11* | 4.74 ± 0.19 | 40.17± 2.12 | 41.23 ± 2.39 | 3.25± 0.84 | 3.53 ± 0.76** |
| 2. | Exp control | 0.24 ± 0.03 | 0.35± 0.05* | 3.01± 0.38 | 3.09 ± 0.54 | 24.45± 2.37 | 25.32 ± 3.33 | 14.67±0.93 | 18.88 ± 1.20 |
| 3. | PT treated | 0.16 ± 0.02 | 0.17 ± 0.01 | 3.54± 0.05** | 3.44 ± 0.04* | 40.56± 1.07 | 42.13 ± 1.45** | 3.63± 0.23* | 3.22 ± 0.54** |

Data are expressed as mean ± standard error. P value of the parameters is $P < 0.05$ has statistical significance (*) and $P< 0.001$ has significance (**)

**Table 6: Effect of PT on antioxidant enzymes in kidney homogenates:**

| S No | | LPO (nMol/mg protein) | | SOD (U/mg protein) | | CAT (U/mg protein) | | Protein carbonyl (nMol/mg protein) | |
|---|---|---|---|---|---|---|---|---|---|
| | Doses | 20 mg* | 40 mg | 20 mg* | 40 mg | 20 mg | 40 mg* | 20 mg | 40 mg |
| 1. | Normal | 0.16 ± 0.04 | 0.17 ± 0.05* | 4.56 ± 0.12 | 4.47 ± 0.78 | 58.98 ± 2.75* | 56.01 ± 3.87 | 2.78 ± 0.99* | 2.64 ± 0.89 |
| 2. | Exp control | 0.54 ± 0.05 | 0.32 ± 0.07* | 3.73 ± 0.45 | 3.13 ± 0.87* | 29.87 ± 1.76 | 28.06 ± 3.45 | 13.67 ± 0.93* | 14.88 ± 1.20 |
| 3. | PT treated | 0.26 ± 0.04 | 0.14 ± 0.03 | 4.68 ± 0.07 | 4.93 ± 0.04 | 55.54 ± 3.65* | 56.67 ± 3.76 | 6.87 ± 1.23* | 7.63 ± 0.79* |

Data are expressed as mean ± standard error. P value of the parameters is $P < 0.05$ has statistical significance (*).