Original Research Paper

Volume - 7 | Issue - 6 | June - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Biological Science

EVALUATION OF *XANTHIUM INDICUM* LEAF EXTRACT AND α - TOCOPHEROL ON ANTIOXIDANT DEFENCE SYSTEM AND ON SELECTED RENAL MARKERS IN STZ INDUCED DIABETIC RATS

Anila Devi Meruva — Division of Molecular Biology and Ethno pharmacology, Department of Zoology, Sri Venkateswara University, Tirupati, India.

Sathyavelu Reddy Kesireddy — Division of Molecular Biology and Ethno pharmacology, Department of Zoology, Sri Venkateswara University, Tirupati, India.

ABSTRACT

***Introduction*:** Chronic kidney disease (CKD) is a major public health problem, especially for people suffering with diabetes. Herbal formulations have attained widespread acceptability as therapeutic agents in many developing countries as anti-diabetic as well as renoprotective agents. *Xanthium indicum* (Asteraceae) has been reported with number of medicinal properties and α-tocopherol is a well known antioxidant cause nephro protection by improving the antioxidant enzyme activity and also lowering the levels of urine microalbumin and serum cystatin C in diabetic rats. The present study was to investigate the ameliorative property of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherol under hyperglycemic condition associated with kidney damage.

***Methods*:** seven groups of male albino rats six in each group, received the following treatment scheduled for 4 weeks: Normal control, *Xanthium indicum*, α-tocopherol, Diabetes control, Glibenclamaide8 treated diabetic, α-tocopherol treated diabetic and *Xanthium* treated diabetic. Evaluations were made for the activites of antioxidant enzymes like SOD, CAT, GP_x and levels of urine microalbumin and serum cystatin C by the method of nephelometry in all experimental rats.

***Results*:** A significant improvement in the activities of SOD ($P < 0.05$), CAT ($P < 0.001$) and GPx ($P<0.001$) and microalbumin in urine ($P<0.001$) and serum cystatin C levels ($P<0.001$) were significantly decreased with the treatment of plant extract and α-tocopherol in diabetic rats.

***Conclusion*:** Hydromethanolic extract of *Xanthium indicum* leaves and α-tocopherol possesses a potent capacity that attenuated the nephrotoxicity by lowering urine microalbumin and serum cystatin C levels in diabetic conditions.

KEYWORDS : *Xanthium indicum,* α-tocopherol, SOD, CAT, GPX microalbumin, cystatin C, diabetes.

Introduction

Diabetes is a metabolic disorder characterized by chronic hyperglycaemia, disturbances in carbohydrate, fat, protein metabolism results from defects in Insulin action.[1] Approximately 40% of patients with type I diabetes and 5-15% of patients with type II diabetes eventually develop End Stage Renal Disease (ESRD).[2] GFR (Glomerular filtration rate) estimation is essential for the evaluation of patients with chronic kidney disease (CKD) and is useful to scrutinize chronic kidney disease also in high-risk groups as persons with diabetes mellitus.[3]

Oxidative stress is said to be an increase in the steady-state levels of reactive oxygen species. It has been concerned in the pathogenesis of diabetic nephropathy.[4] Natural defence mechanism exists against oxidative stress through endogenous or exogenous antioxidant substances. Superoxide Dismutase (SOD) is the most important antioxidant enzyme because it is found virtually in all aerobic organisms. It immediately converts superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) which is then detoxified to water either by catalase (CAT) in the lysosomes or by glutathione peroxidise (GPx) in the mitochondria. [5] Hyperglycemia not only generates more reactive oxygen metabolites but also attenuates antioxidative mechanisms through nonenzymatic glycosylation of antioxidant enzymes.[6]

Microalbuminuria arises from the increased passage of albumin through the glomerular filtration barrier. This is due to glomerular endothelial dysfunction and in particular damage to its glycocalyx in diabetic conditions.[7] Microalbuminuria is the strong predictor of diabetic nephropathy, which is the main cause of morbidity and mortality in patients with diabetes mellitus.[8] Cystatin C is another good marker for estimating GFR, particularly in patients with mild to moderate renal impairment.[9] Serum cystatin C low-molecular-weight (13.3K) protease inhibitor, positive charge at physiological p^H levels facilitate the glomerular filtration that is freely filtered across the glomerular membrane and then reabsorbed and metabolized in the proximal tubule, was proposed as a new endogenous marker of GFR.[10,11]

Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect itself and many phytochemicals can protect humans against diseases.[12] *Xanthium indicum* belongs to the family Asteraceae, is a coarse annual plant which has been reported for a number of medicinal properties such as control blood sugar in diabetic patients, treatment of rheumatic pain ,[13] anti-bacterial and cytoxic activities in methanolic extract of the plant leaves .[14] It was also to be noted that antinociceptive activity of leaf extract of the plant has been reported before,[15] improve the performance of growth and cocoon characteristics of silkworm larvae,[16] inhibitory activity of prostaglandins[17] antioxidant and antidiarrhoeal activity[18]. The recent reports have been demonstrated that *X. indicum* stem has hypoglycemic activity.[19] This plant is reported to contain α and γ-tocopherols, polyphenols, glucoside, xanthostrumarin and xanthonolides as the main constituents.[20]

α-tocopherol is widely used as adjuvant in the treatment of diabetic patients.[21] As an antioxidant, α-tocopherol improves outcomes related to pancreas physiology in diabetes,[22] which may improve functional outcomes of diabetes in animal models. α-tocopherol supplementation inhibits the glycation of haemoglobin, a biomarker for the diagnosis of diabetes in clinical studies, by interrupting glycosylation at an early step in the Maillard reaction[23] or by partially inhibiting the formation of advanced glycolated endproducts (AGEs).[24]

The goal of the present study was to determine the effect of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherol on antioxidant enzymes like SOD, CAT, GPx and renal markers like urine microalbumin as well as serum cystatin C in STZ induced diabetic rats.

Material and methods

Collection of plant material and preparation of plant extract

The leaves of *Xanthium indicum* were collected from Tirumala hills, Chittoor district. The air-dried leaves of *Xanthium indicum* were grounded into a fine powder, and the extract was evaporated to dryness.

Procurement of chemicals

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

Experimental animal models

Wistar strain male albino rats (3 months of age, 200–230 g) were used in the present study. To induce diabetes rats were given a single

intravenous injection of 50 mg/kg streptozotocin (STZ; Sigma-Aldrich, Inc., St. Louis, Mo., USA). The urine was collected for 24 hours under a layer of toluene (to inhibit bacteria growth) and stored at 4°C for later analysis. At the end of the four-week treatment, the blood samples were collected from a tail vein.

Grouping of animals

The rats were divided into 7 groups of six in each group and treated for four weeks as follows: Group I –Normal Control (NC), were administered with 0.9% of NaCl/kg body weight via orogastric tube, Group II – *Xanthium indicum* treated (*Xi*t) rats received 200mg/kg body weight of plant extract, Group III – α-tocopherol treated rats (Tpt) received 100mg/kg body weight of α-tocopherol, Group IV – Diabetic Control (DC) rats received 50mg/kg body weight of STZ after fasting, Group V – Diabetic + Glibenclamide (Di + Glbt) Diabetic rats received 20 mg/kg body weight of glibenclamide, Groups VI - Diabetic+ α-tocopherol (Di + Tpt) Diabetic rats received 100 mg/kg body weight of α-tocopherol and Group VII – Diabetic + *Xanthium indicum treatment* (Di + *Xi*t) Diabetic rats received 200 mg/kg body weight of *Xanthium indicum* leaf extract.

Biochemical Assays

The superoxide dismutase (SOD) activity was assayed in the kidney homogenates by the method of Misra and Fridovich.[25] The catalase (CAT) activity was determined at room temperature by using the method of Aebi.[26] Se-Dependant Glutathione Peroxidase (GPx) activity was determined by a modified version of Flohe and Gunzler[27] at 37°C. Urine micoalbumin and serum cystatin C levels were estimated by particle-enhanced Immunonephelometry.

Histological analysis

The kidney tissues were fixed in neutralized formalin, dehydrated with ethanol and embedded in paraffin wax (56°C). Serial sections (5µm) were taken and stained with haematoxylin and eosin following the earlier described methods of Ross and Reith (1989).

Statistical analysis

The data are expressed as mean of 6 determinations ± standard deviation (SD).The differences among groups were analysed by one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 13.5, SPSS Inc., Chicago, Illinois, USA, was used for this analysis.

Results

Table 1and 2 shows a statistically significant increase (***P<0.001) in urine microalbumin levels and serumCystatin C levels in streptozotocin induced diabetic rats with respect to normal control and there was a significant decrease (****P*<0.001) in urine microalbumin levels in diabetic rats treated with plant extract and α-tocopherol.

A significant reduction in SOD (**P*< 0.05), CAT (****P*< 0.001) and GPx (***P<0.001) activities were observed in diabetic control rats when compared to normal control rats. The activities of these antioxidant enzymes were significantly increased in diabetic rats by treating with plant extract and α-tocopherol (Figure 1, 2 and 3).

In STZ-induced diabetic control rats, degenerative changes in Glomeruli/ Bowman's capsules and focal necrosis of Renal tubules in the kidney tissue were observed. The cytoplasm resolution of abnormal cells changed and vacuolar modifications occurred. According to the association between cell shape and cell function, these changes may correspond to an adaptation of cells to a new situation such as increased load due to congestion. Diabetic rats were treated with plant leaf extract and α-tocopherol,the kidney renal parenchyma showing regeneration changes in distal tubules (D) and bowman's capsule (BC) where necrosis takes place (Figure 4).

Discussion

Diabetic nephropathy (DN) is the most common cause of renal damage in the World that often progresses to end-stage renal disease.[28] The balance between hyperglycemia and the different genetically determined antioxidant cellular defences, mainly enzymatic ones (superoxide dismutase, catalase, Glutathione peroxidise), account for the different effects of hyperglycemia related free radical production in the development of diabetic complications, the increased susceptibility to oxidant injury.[29] In the present investigation, it was found that activity of SOD is significantly decreased in diabetic rats. Superoxide anion is one of the initiators of free radical reactions plays an important role in the determination of SOD levels and the products of membrane lipid peroxidation and other oxidants like H_2O_2 may react with SOD, resulting in oxidative modification, thereby its activity is reduced.[30] Hydrogen peroxide (H_2O_2) is a by-product of normal cellular respiration and is also formed from superoxide anion by the action of superoxide dismutase.[31] The increased frequency of diabetes associated with catalase deficiency and the observed low catalase activity due to accumulation of free radicals, superoxide radicals and hydrogen peroxide. GPx catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of glutathione (GSH).[32] Optimum level of GSH is required in body which in turn potentiates GPx activity to stay healthy.[33] GPx activity is also reduced in diabetic condition; this may be due to inactivation of the enzyme involved in disposal of oxygen species and also insufficient availability of GSH.[34] The high flux of glucose through the activated polyol pathway in diabetes may consume NADPH, which results in decreased level of reduced GSH and consequent decrease in the activity of GPx.[35] The reduced availability of NADPH, which could be either due to reduced synthesis or increased metabolization of NADPH through some other pathway, could be also responsible for low levels of reduced glutathione[36] there by GPx activity is reduced in diabetic rats. Amassing of superoxide (O_2^-) radicals, H_2O_2 in kidney of diabetic rats demonstrate reduced activities of SOD, CAT and GP_X.

The leaves of *Xanthium indicum* was reported to have anti diabetic activity due the presence of phenolic compounds.[37] leaf extract of *Xanthium indicum* treated diabetic rats shown the improved activities of SOD, CAT and GP_X because presence of phenolic compounds such as tannins and flavonoids, and also polyphenols because of their scavenging ability with reactive oxygen species (ROS) and chelating ability with divalent cations due to hydroxyl groups.[38] The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[39] Diabetic rats were treated with α-tocopherol increases the activities of SOD, CAT and GP_X. α-tocopherol was considered to be a potent antioxidant, by preventing oxidative injury and restoring renal tissue antioxidants and glutathione redox balance.[40] Treatment with α-tocopherol has also been demonstrated to improve functional outcomes, like insulin sensitivity in type 2 diabetic subjects.[41] α-tocopherol promoted a reduction in the indicators of oxidative stress, protein glycation, lipid peroxidation thus an increase in SOD[42] CAT and GP_Xactivity. α-tocopherol is the lipid soluble, chain breaking, antioxidant in mammalian tissue[43] and in particular is capable of quenching the propagation of free radical reaction within cell membranes without altering the production of free radicals compounds through the donation of a hydrogen atom from the phenolic hydroxyl group with subsequent formation of tocopherol dimmers or quinines.[44] In diabetic nephropathy, α-tocopherol significantly reduce the lipid peroxidation there by GPx activity was increased.[45]

The elevated microalbumin in urine is indicator of renal disease, especially in diabetics.[46] We examined in the present research work the albumin levels in urine were increased to drastically 7 fold in diabetic rats (P<0.001) than the normal controlled rats. The proteinuria that develops after induction of diabetes is mainly due to an increased excretion of low molecular weight proteins.[47] Albumin is normally excreted as a mixture of intact protein and fragments that are produced during renal passage.[48] Increase in albumin excretion rate in early diabetes due to the disproportionate increase in the excretion of intact albumin.[49] The change in the ratio of intact versus degraded excreted albumin that accompanies the increase in albumin excretion rate in early diabetes is due to the inhibition of degradation of albumin at a post-glomerular site; that is, after the albumin has passed the glomerular filtration barrier.[48] Cystatin C is produced by all nucleated cells at a constant rate and its serum level is relatively unaffected by age, sex, body composition, diet, and exercise.[50] Due to the uncontrolled blood glucose levels, metabolic wastes may deposit in the vital organs such as kidneys, the toxic concentration of blood sugar damages the kidney tissue. Serum cystatin C might meet the need for detecting trends in renal function over time when glomerular filtration rate (GFR) is normal or elevated.[51] In some conditions like diabetes with ischemic nephropathy, nodular glomerulosclerosis and renal failure, serum cystatin C levels were significantly increases.[52] From the present experiment, it was observed that serum cystatin C levels were increased nearly 7 fold in diabetic rats when compared to the

normal controlled rats.

Xanthium indicum possess diuretic properties.[53] The *Xanthium indicum* extract exerted its diuretic activity possibly by inhibiting tubular reabsorption of water and accompanying anions, as such action has been hypothesized for some other plant species.[54] Therefore *Xanthium indicum* extract significantly increased the GFR due to (a) A detergent like interaction with structural components of glomeruluar membranes. (b) A decrease in renal perfusion pressure, attributable to decrease in the resistance of the afferent arteriole and/or an increase in the resistance of the efferent arteriole and/or. (c) The direct effect on the arteriole wall affecting glomerular blood flow.[55] *X. indicum* treated diabetic rats shown 68% decrease in microalbuminuria when compared to the diabetic controlled rats. Shravani *et al*.,[56] studied the diuretic activity of *Xanthium indicum* in albino rats. The petroleum ether extract in normal saline showed significant increase in diuresis, natriuresis, kaliuresis, glomerular filtration rate. All extract causes increase in urine elimination and increase in Na^+, K^+, Cl^- excretion compared to normal saline. They reported that the diuretic activity of the extract may be due to the presence of flavanoides, saponins, and Organic acids. Treatment of hydromethanolic leaf extract of *Xanthium indicum* (200 mg/kg/day) for four weeks causes 42% reduction of serum cystatin C levels when compared to STZ rats. α-tocopherol reduce the serum levels of C-reactive protein (CRP) and advanced glycation end products, expression of cell adhesion molecules and inflammatory mediators.[57] Koya *et al*.,[58] reported that hemodynamic abnormalities in diabetic rats were normalized by treatment with α-tocopherol. The hypertrophic response usually found in diabetes was prevented by α-tocopherol administration; tubular acidification, which was significantly changed by diabetes. α-tocopherol is able to protect the harmful effects of diabetes on renal function.[59] Treatment with α-tocopherol has all been shown to significantly reduce urinary albumin excretion ratio among diabetics.[60] The present work reveals that α-tocopherol treated diabetic rats shown nearly 71% decrease in microalbumin levels were observed than the diabetic controlled rats. Chronic use of α-tocopherol can enhance antioxidant enzymes, resulting in vasodilatation of afferent and/or efferent arterioles, decreased pre and/or post-glomerular resistances, and reduced changes observed in renal morphology of glomerular and vascular hypertrophy. It is known that glomerular hypertrophy is related to the development of glomerulosclerosis[61] where the prevention of this alteration can contribute toward preventing the development of kidney disease. Increased renal vascular resistance is also related to the reduced levels of renal plasma flow (RPF) and Glomerular filtration rate (GFR). From the study, it was observed that α-tocopherol treated diabetic rats exhibited nearly 50% decreased levels when compared to the diabetic rats.

Conclusion

Accumulation of free radicals and increased formation of advanced glycation end-products (AGE) in the renal system leads to decline the activities of antioxidant enzymes like SOD, CAT, GPx and also GFR. The present investigation reveals that *X. indicum* extract andα-tocopherol may improve the antioxidant status in diabetes and decrease the levels of serum cystatin C, in this manner both plant extract and α-tocopherol might improve GFR in diabetic condition.

Acknowledgments

The authors convey their gratitude to Head of Department Zoology, S.V.University for providing the lab facilities.

Ethical issues

The usage of animals was approved by the Institutional Animal Ethics Committee in its resolution No: 09/ (i)/a/CPCSCA/ IAEC/ SVU/ KSR-TL/dated 26-06-2008.

Tables

Table 1.

Effect of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherol on Urine micro albumin levels in Normal control (NC), *Xanthium indicum* (*Xi*t), α-tocopherol (Tpt), Diabetes control (DC), Glibenclamaide treated diabetic (Di+ Glbt), α-tocopherol treated diabetic (Di+ Tpt) and *Xanthium indicum* treated diabetic (Di+*Xi*t) male albino rats.

Urine Microalbumin levels	Experimental Groups (Treatment)						
	NC	Xit	Tpt	DC	Di+Glbt	Di+Tpt	Di+Xit
Mean ±SD	2.466 ±0.156	2.760 ±0.145	3.157*** ±0.211	18.821*** ±1.852	9.498*** ±0.760	5.485*** ±0.534	6.048*** ±0.660
Percent Change (%)		(+11.922)	(+28.021)	(+663.219)	(+285.158)	(+122.429)	(+145.255)

All values are expressed as mean ± SD values with six replicates. ***P<0.001 compared with normal control. The values are expressed micro albumin levels in mg/l.

Table 2.

Effect of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherol on Serum cystatin C levels in Normal control (NC), *Xanthium indicum* (*Xi*t), α-tocopherol (Tpt), Diabetes control (DC), Glibenclamaide treated diabetic (Di+ Glbt), α-tocopherol treated diabetic (Di+ Tpt) and *Xanthium indicum* treated diabetic (Di+*Xi*t) male albino rats.

Serum Cystatin C levels	Experimental Groups (Treatment)						
	NC	Xit	Tpt	DC	Di+Glbt	Di+Tpt	Di+Xit
Mean ±SD	0.072 ±0.031	0.141 ±0.032	0.182*** ±0.010	0.514*** ±0.142	0.374*** ±0.026	0.261*** ±0.025	0.299*** ±0.014
Percent change (%)		(+95.833)	(+152.77)	(+651.388)	(+419.44)	(+262.5)	(+315.27)

All values are expressed as mean ± SD values with six replicates. ***P<0.001 compared with normal control. The values are expressed micro albumin levels in mg/l.

Figures

Figure 1.

Effect of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherolon Superoxide dismutase (SOD) activity in Normal control (NC), *Xanthium indicum* (*Xi*t), α-tocopherol (Tpt), Diabetes control (DC), Glibenclamaide treated diabetic (Di+ Glbt), α-tocopherol treated diabetic (Di+ Tpt) and *Xanthium indicum* treated diabetic (Di+*Xi*t) male albino rats. All values are expressed as mean ± SD values with six replicates. *P<0.05 compared with normal control. The values are expressed superoxide ion reduced /mg protein/min.

Figure 2.

Effect of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherolon Catalase (CAT) activity in Normal control (NC), *Xanthium indicum* (*Xi*t), α-tocopherol (Tpt), Diabetes control (DC), Glibenclamaide treated diabetic (Di+ Glbt), α-tocopherol treated diabetic (Di+ Tpt) and *Xanthium indicum* treated diabetic (Di+*Xi*t) male albino rats. All values are expressed as mean ± SD values with six replicates. ***P<0.001 compared with normal control. The values are expressed µmoles of H_2O_2 degraded /mg protein/min.

Figure 3.

Effect of hydromethanolic leaf extract of *Xanthium indicum* and α-tocopherolon Glutathione Peroxidase (GPx) activity in Normal control (NC), *Xanthium indicum* (*Xi*t), α-tocopherol (Tpt), Diabetes control (DC), Glibenclamaide treated diabetic (Di+ Glbt), α-tocopherol treated diabetic (Di+ Tpt) and *Xanthium indicum* treated diabetic (Di+*Xi*t) male albino rats. All values are expressed as mean ± SD values with six replicates. ***P<0.001 compared with normal control. The values are expressed µmoles of NADPH oxidised /mg protein/min.

Figure 4.

Histopathology of kidney tissue of Normal control (NC), *Xanthium indicum* (*Xi*t), α-tocopherol (Tpt), Diabetes control (DC), Glibenclamaide treated diabetic (Di+ Glbt), α-tocopherol treated diabetic (Di+ Tpt) and *Xanthium indicum* treated diabetic (Di+*Xi*t) male albino rats. The changes were observed as follows

A. The normal architecture of the kidney tissue with lower magnification (10X).

B. The normal architecture of the kidney tissue with lower magnification (10X).

C. The normal architecture of the kidney tissue with lower magnification (10X).

D. Degenerative changes in Glomeruli/ Bowman's capsules and Renal

tubules along with necrotic changes in kidney tissue with lower magnification (10X).

E. Regeneration changes in Glomeruli and necrosis takes place and tissue shows similar to normal cyto-architecture with lower magnification (10X).

F. Regeneration of necrosis and congestion takes place and tissue shows similar to normal cyto-architecture with lower magnification (10X).

G. Regenerative changes shows similar to normal cyto-architecture of kidney tissue with lower magnification (10X).

Figure 1.

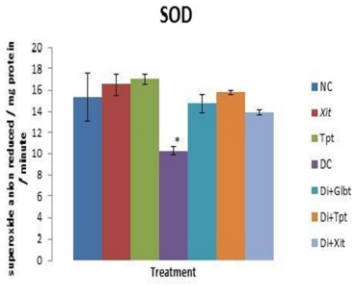


Figure 2.

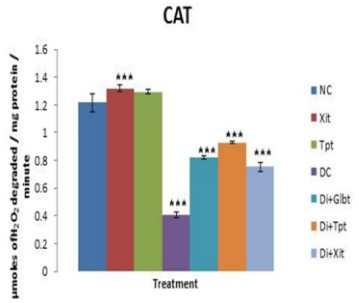


Figure 3.

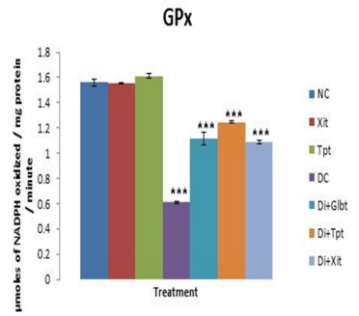
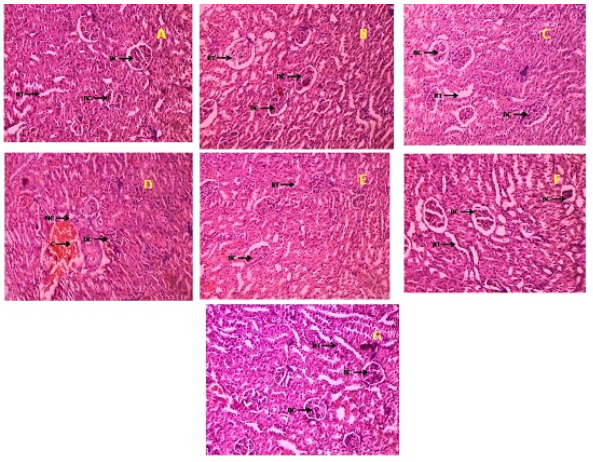


Figure 4.



References

1. Rajkiran E, Nusrath Y, Shashank T, Sujatha K, Keerthi P. Role of Sesamumalatum L. on nephropathy in diabetic rats. IJPSR 2011; 2(7): 1716-21.
2. Evans TC, Cappel P. Diabetic Nephropathy. Clin Diabetes 2000; 18: 7-21.
3. Sebastjan B, Radovan H, Robert E, Matej Z, Maksimiljan G, Ludvik P. Simple CystatinC Formula for Estimation of Glomerular Filtration Rate in Overweight Patients with Diabetes Mellitus Type 2 and Chronic Kidney Disease. Exp Diabetes Res 2012; 1-8: Article ID 179849.
4. MaritimAC, Sanders RA, Watkins JB. 3rd Diabetes, oxidative stress, and antioxidants: a review. J. Biochem. Mol. Toxicol 2003; 17(1): 24-38.
5. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. CardiovascDiabetol 2005; 4(1): 5.
6. Orsolic N, Basic I. Honey bee products and their polyphenolic compounds in treatment of diabetes. Phytopharmacology and Therapetutic 2008; 22(4): 455- 71.
7. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium?. Diabetologia 2008; 51(5): 714-25.
8. Ruilope LM, Segura J. Predictors of the evolution of microalbuminuria. Hypertens 2006; 48: 832–33.
9. Uzun H, OzmenKeles M, Ataman R, Aydin S, Kalender B, Uslu E, Simsek G, Halac M, Kaya S. Serum cystatin C level as a potentially good marker for impaired kidney function. ClinBiochem 2005; 38(9): 792-98.
10. Coll E, Botey A, Alvarez L, Poch E, Quinto L, Saurina A, Vera M, Piera C and Darnell A. Serum cystatin C as a new marker for non invasive estimation of glomerular filtration rate and as a marker for early renal impairment. Am J Kidney Dis 2000; 36(1): 29–34.
11. Patricia V, Manuel J, Maria-Cruz S, Jesus M, Pilar C. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. Crit Care 2005; 9(2): 139-43.
12. Farooq U, Waseem B, Muzaffar R, Tripathi J, Tharani M, Sharma MA. Comparative study of phytochemical investigation of Xanthium strumarium medicinal plant. IJRPC 2014; 4(1): 96-100.
13. Rahmatullah M, Ishika T, Rahman M, Swarna A, Khan T, Monalisa MN, Seraj S, Mou SM, Mahal MJ, Biswas KR. Plants prescribed for both preventive and therapeutic purposes by the traditional healers of the Bede community residing by the Turag River, Dhaka District. Am.-Eurasian J. Sustain. Agric 2011; 5(3): 325-31.
14. ObayedUllah M, Haque M, Urmi KF, Zulfiker AH, Anita ES, Begum M and Hamid K. Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. Asian Pac J Trop Biomed 2013; 3(1): 1-7.
15. Farhad Hossan ANM, Zaman F, Rani Barman M, Khatoon S, Zaman, M, Khatun, F, Mosaiab T, Mostafa F, Sintaha M, Jamal F, Rahmatullah M. Antinociceptive activity of Xanthium indicumJ. Koenig ex Roxb. (Asteraceae) leaves and Leucasaspera (Willd.) Link (Lamiaceae) whole plants. Adv. in Nat. Appl. Sci 2011; 5(2): 214-17.
16. Pardeshi AB and Bajad PN. Effect of Xanthium indicumlinn. plant extract on the economic parameters of silkworm, bombyxmori. IJSR 2014; 5(3): 683-86.
17. Sadhu SK, Okuyama E, Fujimoto H and Ishibashi M. Separation of Leucasaspera, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chem Pharm Bull (Tokyo) 2003; 51(5): 595-8.
18. Akter R, RaquibulHasan SM, Hossain M, Jamila M, Mazumdar EH and Rahman S. In vitro antioxidant and In vivoantidiarrhoreal activity of hydromethanolicetract of Xanthium indicumKoenig. leaves. Ejsr 2009; 33(2): 305-12.
19. Haque ME, Rahman S, Rahmatullah M, Jahan R. Evaluation of antihyperglycemic and antinociceptive activity of Xanthium indicum stem extract in Swiss albino mice. BMC Complement Altern Med 2013 13: 296.
20. Ghani A. Medicinal Plants of Bangladesh: Chemical constituents and Uses. 2nd ed. Asiatic Society of Bangladesh, Dhaka; 2003, p. 502-504.
21. Andrew R, Skyrme-Jones P, O'Brien RC, Berry KL, Meredith IT. Vitamin Supplementation Improves Endothelial Function in Type 1 Diabetes Mellitus. A Randomized Placebo-Controlled Study. J Am CollCardiol 2004; 36(1): 94- 102.
22. Sena CM, Nunes E, Gomes A, Santos MS, Proenca T, Martins MI, Seica RM. Supplementation of coenzyme Q10 and alpha-tocopherol lowers glycated hemoglobin level and lipid peroxidation in pancreas of diabetic rats. Nutr. Res 2008; 28(2):113–21.
23. Ceriello A, Morocutti A, Franceschina M, Quagliaro L, Moro M, Damante G, Viberti GC.. Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. Am. Diabetes Assoc 2000; 49(12): 2170-7.
24. Minamiyama Y, Takemura S, Bito Y, Shinkawa H, Tsukioka T, Nakahira A, Suehiro S, Okada S. Supplementation of alpha-tocopherol improves cardiovascular risk factors via the insulin signaling pathway and reduction of mitochondrial reactive oxygen species in type II diabetic rats. Free Radic. Res 2008; 42(3): 261-71.
25. Misra HP, Fridowich I. The role of superoxide anion in the autooxidationOf epinephrine and a simple assay for superoxide dismutase. K. Biol Chem 1972; 247(10): 3170-5.
26. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:125–6.
27. Flohe L, Gunzler WA. Glutathione peroxidase. Methods enzymes 1984; 105: 115-21.
28. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am SocNephrol 2008; 19(3): 433-42.
29. Subash-Babu P, Alshatwi AA, Ignacimuthu S. Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin-induced pancreatic β-cells damage in wistar rats. Biomol Ther (Seoul) 2014; 22(1): 47-54.
30. Nishal HK, Sharma MP, Goyal RK, Kaushik GG. Serum superoxide dismutase levels in diabetes mellitus with and without microangiopathic complications. JAPI 1998; 46(10): 853-5.
31. Jorns A, Tiedge M, Lenzen S, Munday R. Effect of superoxide dismutase, catalase, chelating agents, and free radical scavengers on the toxicity of alloxan to isolated pancreatic islets in vitro. Free Rad Biol Med 1999; 26(9-10): 1300-4.
32. Goyal R, Singhai M, Faizy AF. Glutathione peroxidase activity in obese and nonobese diabetic patients and role of hyperglycemia in oxidative stress. J Midlife Health 2011; 2(2): 72-6.
33. Lee YS, Kim AY, Choi JW, Kim M, Yasue S, Son HJ, Masuzaki H, Park KS, Kim JB. Dysregulation of adipose glutathione peroxidase 3 in obesity contributes to local and systemic oxidative stress. MolEndocrinol 2008; 22(9): 2176-89.
34. IIling EKB, Gray CH, Lawrence RD. Blood glutathione and non-glucose reducing substances in diabetes. Biochem J 1991; 48: 637.
35. Irshad M, Chaudhuri PS. Oxidant – antioxidant system; role and significance in human body. Indian J Exp Biol 2002; 40(11): 1233-9.
36. Madhu CG, Ojha S, Bansal DD. Antioxidant status of streptozotocin diabetic rats. Indian J Exp Biol 1996; 34(3): 264-6.
37. Agarwal A. Critical issues in Quality Control of Herbal Products. Pharma Times 2005; 37(6): 09-11.
38. Kang DG, Yun CK, Lee HS. Screening and comparision of antioxidant activity of solvent extracts of herbal medicines used in Korea. J Ethnopharmacol 2003; 87(2-3): 231-6.
39. Hasan SMR, Akter R, Hossain MM, Faruque A, Rana M.S. Antinociceptive and CNS epressant activities of Xanthium indicum Koen. in mice. Dhaka Univ J Pharm Sci 2009; 8: 99–101.

40. Thamilselvan S, Menon M. Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. B. J. U.International 2005; 96(1): 117-26.
41. Jacob S, Ruus P, Hermann R, Tritschler HJ, Maerker E, Renn W, Augustin HJ, Dietze GJ, Rett K. Oral administration of RAC-alpha-lipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: a placebocontrolled pilot trial. Free Radic. Biol. Med 1999; 27(3-4): 309-14.
42. Shirpoor A, Ansari MHK, Salami S, Pakdel FG, Rasmi Y. Effect of vitamin E on oxidative stress status in small intestine of diabetic rat. World J Gastroenterol 2007; 13(32): 4340-4.
43. Sozmen EY, Sozmen B, Delen Y, Onat T. Catalase/superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poor glycemic control. Arch Med Res 2007; 32(4): 283-7.
44. Shrilatha B and Muralidhara. Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive millieu in the streptozotocin -diabetic rat. Int J Androl 2007; 30(6): 508-18.
45. Kutlu M, Nazıoglu M, Simsek H, Yılmaz T, Kukner AH. Moderate exercise combined with dietary vitamins C and E counteracts oxidative stress in the kidney and lens of streptozotocin-induced diabetic rat. Int. J. Vitam. Nutr. Res 2005; 75(1): 71-80.
46. Pazdro R, Burgess JR. The role of vitamin E and oxidative stress in diabetes complications. Mech Ageing Dev 2010; 131(4): 276–86.
47. Rigalleau V, Lasseur C, Raffaitin C, Beauvieux MC, Barthe N, Chauveau P, Combe C, Gin H. Normoalbuminuric renal-insufficient diabetic patients: a lower-risk group. Dia care 2007; 30(8): 2034-39.
48. Burne MJ, Panagiotopoulos S, Jerums G, Comper WD. Alterations in renal degradation of albumin in early experimental diabetes in the rat: a new factor in the mechanism of albuminuria. Clin Sci 1998; 95(1): 67–72.
49. Marijn MS, Reinhart S, Lien Van De V, Joris RD. Immunochemically un reactive albumin in urine: fiction or reality?. CritRev Clin lab Sci 2011; 48(2): 87-96.
50. Price CP, Finney H. Developments in the assessment of glomerular filtration rate. ClinChimActa 2000; 297(1-2): 55-66.
51. Perkins BA, Nelson RG, Ostrander BE. Blouch KL, Krolewski AS, Myers BD, Warram JH. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study. J Am SocNephrol 2005; 16(5): 1404–12.
52. Shimizu A, Horikoshi S, Rinnno H, Kobata M, Saito K, Tomino Y. Serum cystatin C may predict the early prognostic stages of patients with type 2 diabetic nephropathy. JCLA 2003; 17(5): 164-7.
53. Dra Gladys MDG, Dra. Marvadel CLPL, Eldris IH. Evaluation of thegenotoxic effect of Xhantiumstrumarium L. (Guisazo Horse). Rev Cubana Plant Med 2004; 9: 27-32.
54. Bevevino LH, Vieira FSA, Cassola AC, Sanioto SML. Effect of crude extract of roots Bredemeysra floribunda Wild I. Effect on arterial blood pressure and renal excretion in the rats. J Ethanopharmacol 1994; 43(3): 197-201.
55. Abderahim A, Jaouad E, Zafar HI, Basiaa, L. Acute diuretic effect of continuous intravenous infusion of an aqueous extact of Coriandrumsativum L. In anesthetized rats. J Ethanopharmacol 2008; 115(1): 89-95.
56. Shravani P, Laxmi M, Kumar S. Evaluation of diuretic activity of Xanthium Strumarium. IJPPR 2010; 1(1): 31-4.
57. Prasad K. Tocotrienols and cardiovascular health. Curr Pharm Des 2011; 17(21): 2147-54.
58. Koya D, Lee IK, Ishii H, Kanoh H, King GL. Prevention of glomerular dysfunction in diabetic rats by treatment with d-alphatocopherol. J Am SocNephrol 1997; 8(3): 426-35.
59. Gomes GN, Barbosa FT, Radaeli RF, Cavanal MF, Mello Aires M, Zaladek Gil F. Effect of D-a-tocopherol on tubular nephron acidification by rats with induced diabetes mellitus. Braz. J. Med. Biol. Res 2005; 38(7): 1043-51.
60. Hirnerova E, Krahulec B, Strbova L, Stecova A, Dekret J, Hajovska A. Effect of vitamin E therapy on progression of diabetic nephropathy. VnitrLek 2003; 49(7): 529–34.
61. Fogo AB. Glomerular hypertension, abnormal glomerular growth, and progression of renal diseases. Kidney Int 2000; 75: 15-21.