Jurnal Biologi Volume: 2, Nomor 1, 2024, *Page*: 1-11

Effect of *Artemisia annua* Extract on Kidney Functions and Histology in Experimental Diabetic Rats

Marwa Azeez Akoul

Biotechnology Department, College of Applied Science, Fallujah University, Anbar, Iraq

DOI: https://doi.org/10.47134/biology.v2i1.3316
*Correspondensi: Marwa Azeez Akoul
Email: Marwa.azeez@uofallujah.edu.iq

Received: 18-09-2024
Accepted: 20-10-2024
Published: 22-11-2024

Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Abstract: *This study sought to understand the impact of an alcoholic extract of Artemisia annua on kidney function in experimental diabetic rats. For this study, fifty adult male albino rats were randomly assigned to one of five groups: G1 received unrestricted access to food and water. G2: To induce diabetes, a single intravenous injection of STZ (60 mg/kg) is administered. The third group undergoes insulin subcutaneous injections for four weeks after each STZ injection. The fourth group takes Artemisia annua extract orally (by gavage) at a dose of 75 mg/kg for four weeks following each STZ injection. The fifth group takes insulin subcutaneously (by gavage) and Artemisia annua extracts orally (70 mg/kg) for four weeks following each STZ injection. We took blood samples from the orbital sinus to estimate creatinine, urea, uric acid, SOD, GPX, and CAT in the serum. We extracted the kidneys for histopathological investigation after slaughtering the animals. The current results demonstrated a significant reduction in all kidney functions, including creatinine and urea, as well as uric acid, following treatment with insulin, Artemisia annua extract, or a combination of insulin and extract. According to these results, the amount of SOD in G3, G4, and G5 is significantly higher than in G1 and G2. However, there were no significant differences between the groups in the amounts of GPX and CAT. The histopathological results of the kidney in groups G1 and G4 revealed a normal histological appearance. Kidneys of G2 exhibit glomerular atrophy and interstitial hemorrhage. G3 displayed tubular epithelial degeneration, necrosis, mesangial cell hyperplasia, glomerular atrophy, and inflammatory cell infiltration. The kidney of G5 displays blood vessel dilatation, congestion, glomerular atrophy, and acute swelling of epithelial cells. In conclusion, the alcoholic extract of Artemisia annua reduces the kidney function in experimental diabetic rats and can alleviate the histological damage of diabetes on renal tissue.*

Keywords : *Artemisia annua, Diabetes, Kidney, ROS, Histopathology.*

Introduction

According to Reutens & Atkins, 2011, diabetic nephropathy is a major consequence of diabetes mellitus that leads to a significant amount of death and disability globally. The anatomical and functional anomalies that define diabetic renal failure, which is primarily caused by hemodynamic dysfunctions, are outlined by (Cheng & Harris, 2014). According to (Pourghasem & Shafi, 2015), diabetes individuals might have several glomerular and tubulointerstitial problems, such as podocyte loss, thickening of the glomerular basement membranes, expansion of the tubular basement membranes, interstitial fibrosis, and tubular atrophy. The importance of oxidative stress in the progression of diabetic kidney failure has long been recognized. According to (Forbes *et al.*, 2008), diabetic nephropathy is thought to

be mainly caused by free radicals produced by mechanisms such as glycolysis, xanthine oxidase, and advanced glycation. The accumulation of evidence also points to chronic hyperglycemia-induced reactive oxygen species (ROS) in mitochondria as a potential starting point for these harmful pathways (Forbes *et al.,* 2008). Many cell types in the kidney, such as podocytes, mesangial cells, and proximal tubular epithelial cells, increase glucose absorption in people with diabetes. Thus, diabetic complications may affect not only the kidneys but also the retina and peripheral nerve neuronal and glial cells. A further complication of chronic hyperglycemia is the quickening of the AGE production process. In diabetes, a cycle of reactive oxygen species (ROS) production and advanced glycation end products (AGEs) develops during the synthesis of these products. As a result of their interaction with various populations of renal cells, the kidneys eliminate most AGEs (Kashihara *et al.,* 2010). In reaction to an excess of reactive oxygen species (ROS) production, many antioxidant mechanisms are activated in the cells of mammals. Superoxide dismutase (SOD) is the most crucial enzyme in the antioxidant system; it converts superoxide radicals to hydrogen peroxide. Once catalase has broken down hydrogen peroxide, it leaves behind water and oxygen. One of the most successful medications for treating diabetic renal illness to date are renin-angiotensin system (RAS) blockers (Chawla *et al.,* 2010). Herbal remedies with strong anti-inflammatory and antioxidant capabilities have emerged as a new tool in the fight against hyperglycemia and other metabolic complications in diabetes in recent years. One major problem with lipid metabolism in diabetes is hypertriglyceridemia, which occurs when chylomicrons, very low-density lipoprotein, and other triglyceride-carrying lipoproteins rise (Biesenbach, 1989).

Several modern ethnobotany studies have documented the numerous indications of Artemisia annua (SCOPA) in Afghanistan, Saudi Arabia, Pakistan, Iran, as well as China for ailments such as ear pain, cardiovascular conditions, hyperglycemia, diabetes, gallbladder, liver, as well as digestive disorders; a host of infectious and inflammatory diseases; and a host of other conditions (Youssef, 2013; Sher *et al.,* 2016). This study aimed to know the effect of alcoholic extract of *Artemisia annua* on kidney function in experimental diabetic rats.

Methodology

This research included fifty mature male albino rats that weighed about 150-200 g. They were provided with water at will and kept in transparent plastic cages with a regular pellet rodent food. The rats were kept in a typical laboratory setting with a temperature of 25±2oC, a relative humidity of 50±15%, and a 12-hour light-dark cycle.

Induction of diabetes

A single intravenous administration of Streptozotocin (60 mg/kg body weight) one time made the animals diabetic after they had fasted overnight. After Streptozotocin administration, the animals' diabetic status was tracked for seven days in a row to ensure stability. Only rats with fasting blood glucose levels of 300 mg/dl or above were chosen as diabetic rats for the present investigation on the eighth day after alloxan injection. A single injection of physiological saline served as a placebo for the control animals.

Collecting Plants for Extracting Their Essential Oils

Aerial parts were sourced from the Iraqi market. After washing, drying, and powdering the plant, it was macerated in 70% ethanol while being sometimes stirred and shaken. A blackish-brown concentration was obtained by allowing the filtered mixture to evaporate the ethanol at 45°C. Before being used, the produced extract was stored at 4°C.

Animal groups

For this study, fifty adult male albino rats were randomly assigned to one of five groups: G1 (unrestricted access to food and water) G2: to induce diabetes, a single intravenous injection of STZ (60 mg/kg), The third group undergoes insulin subcutaneous injections for four weeks after each STZ injection. The fourth group takes Artemisia annua extract orally (by gavage) at a dose of 70 mg/kg for four weeks following each STZ injection. The fifth group takes insulin subcutaneously (by gavage) and Artemisia annua extract orally (75 mg/kg) for four weeks following each STZ injection.

To evaluate blood glucose and kidney functions, blood samples were taken from the orbital sinus and stored at -20°C after the experiment ended. The animals were then gently slaughtered and their kidneys were promptly extracted and preserved for the purpose of oxidative stress testing.

The animals were all killed and their kidney were taken out. For the purpose of histopathology, kidney samples were collected and processed using a tissue processor (Leica manufactured). For kidney section staining, haematoxylin and eosin Y (H/E) dyes were used. Histopathological examination of the pancreatic sections was carried out using an Olympus light microscope.

Assessment of serum biochemical parameters

Following the directions on the packaging, the commercial kits were able to detect serum glucose levels, Urea, creatinine and uric acid.

SOD activity

The SOD activity was assessed at 570 nm using a colorimetric technique. The quantity of enzyme needed to inhibit the rate of MTT decrease by 50% was determined as one unit of SOD. Units per milligram of protein were used to display the findings. Hydrogen peroxide (30 mM) was used as the substrate in the Aebi technique to test catalase activity. The amount of hydrogen peroxide consumed per milligram of protein sample was used to estimate one unit of catalase activity.

Catalase activity

The catalase activities were carried out according to the protocol developed by (Shah & Khan, 2014). The findings were recorded with some modest adjustments in units/mg protein. A CAT test was conducted using H2O2, and the absorbance was measured at 240 nm.

GPX activity

A commercially available kit was used to measure the GPX level. GPX is responsible for reducing 5,5-dithiobis (2-nitrobenzoic acid), which produces a product that is yellow in color. You may measure the absorbance of the yellow product at 405 nm69, which is directly proportional to the quantity of GPX.

Statistical analysis

The mean ± SEM was used to represent the data. After doing a one-way ANOVA, we compared the means using a post hoc LSD test. We regarded a p-value less than 0.05 to be statistically significant.

Result and Discussion

The current results showed that all kidney functions including creatinine, urea as well as uric acid were reduced significantly after treatment with insulin, *Artemisia annua* extract, or in combination insulin and extract (Table 1).

Table 1. Comparison between different groups in Kidney functions

Group	Mean ±SE		
	Creatinine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)
G1	0.263 ±0.02 ab	1.66 ±0.04 b	20.93 ±0.73 d
G2	0.31 ±0.001 a	1.78 ±0.09 b	61.03 ±2.13 a
G3	0.24 ±0.02 b	1.33 ±0.07 c	48.01 ±0.17 b
G4	0.239 ±0.02 b	2.02 ±0.04 a	38.14 ±2.07 c
G5	0.24 ±0.02 b	1.270 ±0.07 c	44.51 ±3.08 bc
LSD	0.045 *	0.230 **	7.48 **
P-value	0.0242	0.0001	0.0001

The kidneys keep the chemical composition of bodily fluids optimal by filtering out waste metabolites including urea, uric acid, and creatinine. Kidney failure was shown by elevated creatinine, urea, and uric acid levels in the present investigation including diabetic rats. Prior research has shown that diabetic rats have renal impairment as evidenced by an increase in renal markers (Jarald *et al.*, 2008; Chandramohan *et al.*, 2009). Renal marker elevations in diabetic rats may be attributable to metabolic disturbances as shown by elevated xanthine oxidase activities, lipid peroxidation, triacylglycerol, and cholesterol levels (Madinov *et al.*, 2000). The normalization of these indices in diabetic rats treated with *Artemisia annua* extract suggests that metabolic disruptions of other pathways, including protein and nucleic acid metabolism, were reduced, leading to better glycaemic management. Results from treating diabetic rats with an ethanolic extract of the Artemisia turanica were similar to those found here (Yazdi *et al.*, 2020). Research has also shown that the Artemisia compound cirsimaritin may regulate digestive, immunological, and neurological processes and reduce kidney damage (González-Trujano *et al.*, 2017; Abdelhalim *et al.*, 2015; Amakura *et al.*, 2014).

The present results a significant increase in SOD concentration in G3,G4 and G5 as compared with G1 and G2, while the GPX and CAT activities showed non-significant differences between all groups (Table 2).

Table 2. Comparison between difference groups in Anti-Oxidants

Groups	SOD (U/ml)	GPX (U/ml)	CAT (U/ml)
G1	38.61 ±1.18 b	60.39 ±3.53	158.07 ±37.83
G2	37.09 ±2.62 b	62.84 ±3.08	168.37 ±21.79
G3	48.03 ±0.26 a	64.87 ±1.98	141.04 ±8.64
G4	46.03 ±1.95 a	59.16 ±2.38	147.96 ±29.44
G5	51.29 ±1.59 a	61.99±2.31	108.51 ±22.76
LSD	6.68 **	10.49 NS	87.20 NS

Histopathological results of kidney in G1 showed normal histology appearance (Figure 1). Kidney of G2 exhibit glomerular atrophy and interstitial hemorrhage (Figure 2). A tubular epithelial degeneration with necrosis and Mesangial cell hyperplasia with glomerular atrophy and inflammatory cell infiltration were showed in G3 (Figure 3). Figure 4 shows a normal histology architecture in G4. Kidney of G5 showing blood vessels dilations with congestion, glomerular atrophy and acute epithelial cell swelling (Figure 5).

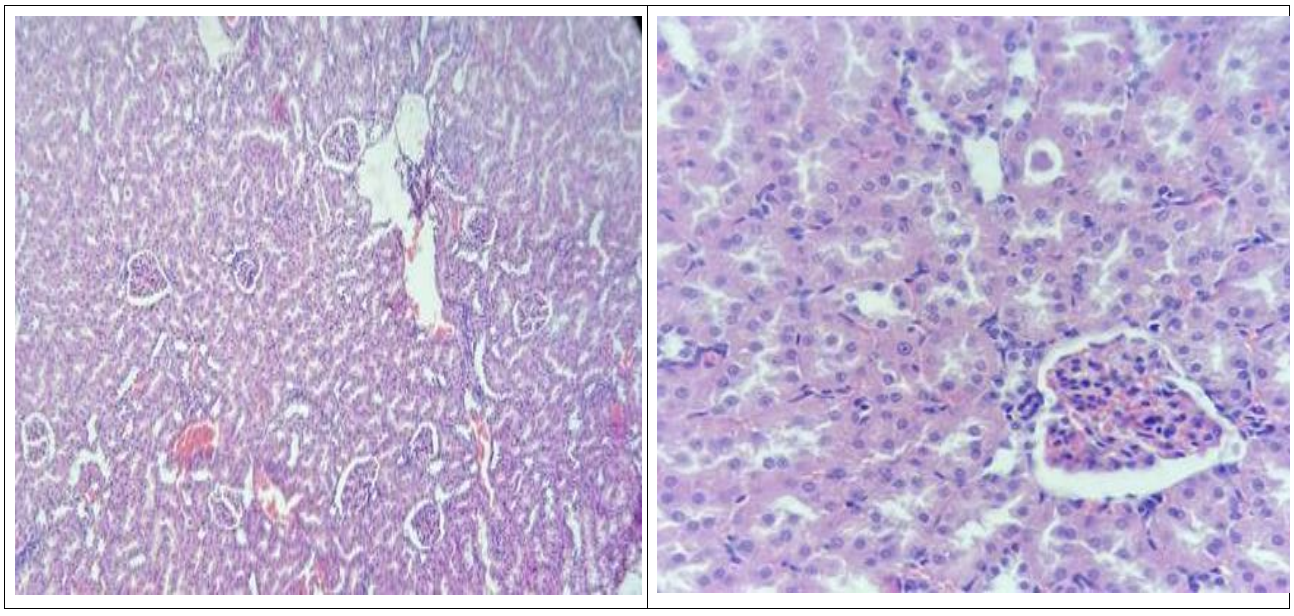

Fig. 1. The kidneys of the control group seem histologically normal in this photomicrograph. H&E stain AX100 and BX400.

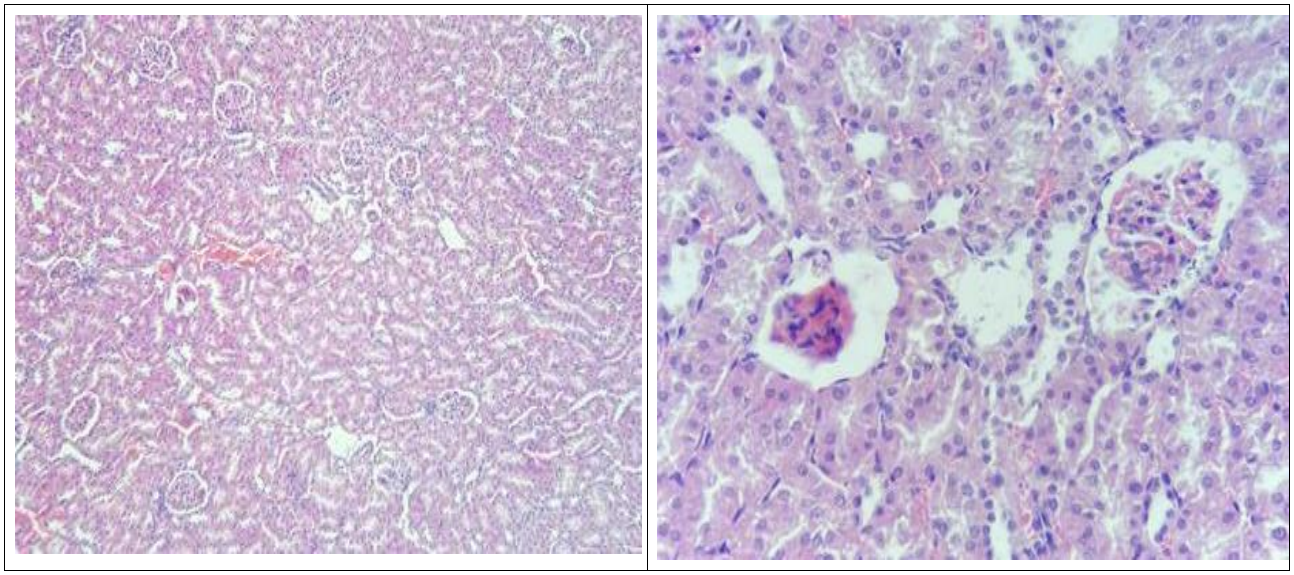

Fig. 2. Interstitial haemorrhage and glomerular shrinkage are shown in this photomicrograph of a diabetic kidney slide. Both the AX100 and the BX400 from H&E.

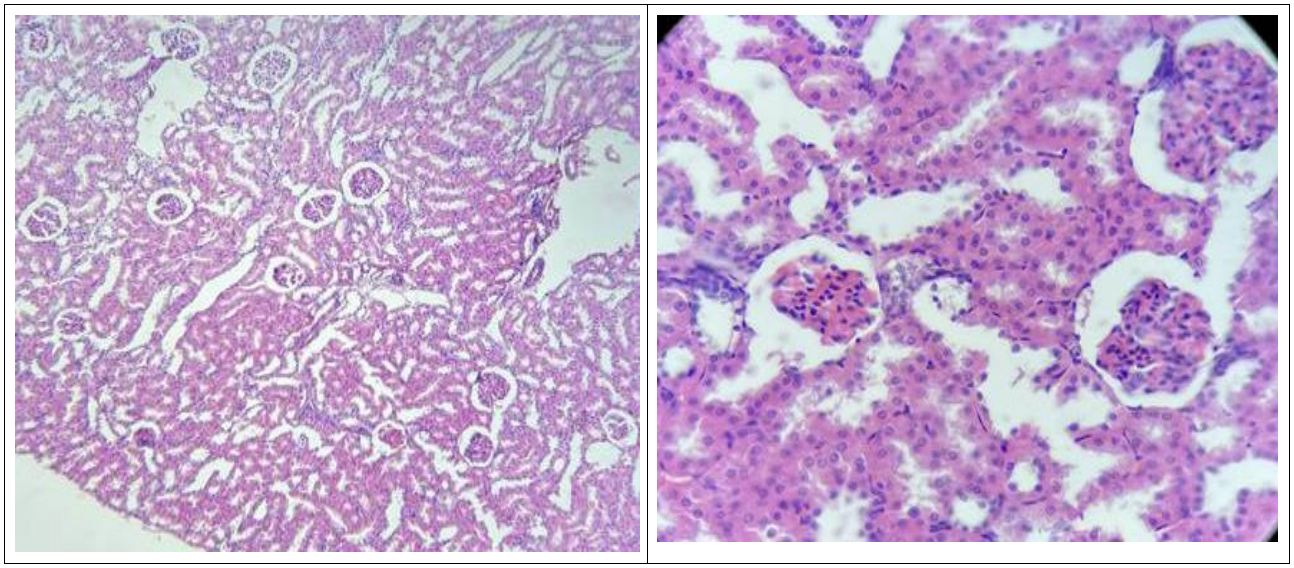

Fig. 3. The insulin group's kidneys are seen in this photomicrograph, which shows areas of tubular epithelial degradation, necrosis, mesangial cell hyperplasia, glomerular atrophy, and inflammatory cell infiltration. H&E AX100 and BX400.

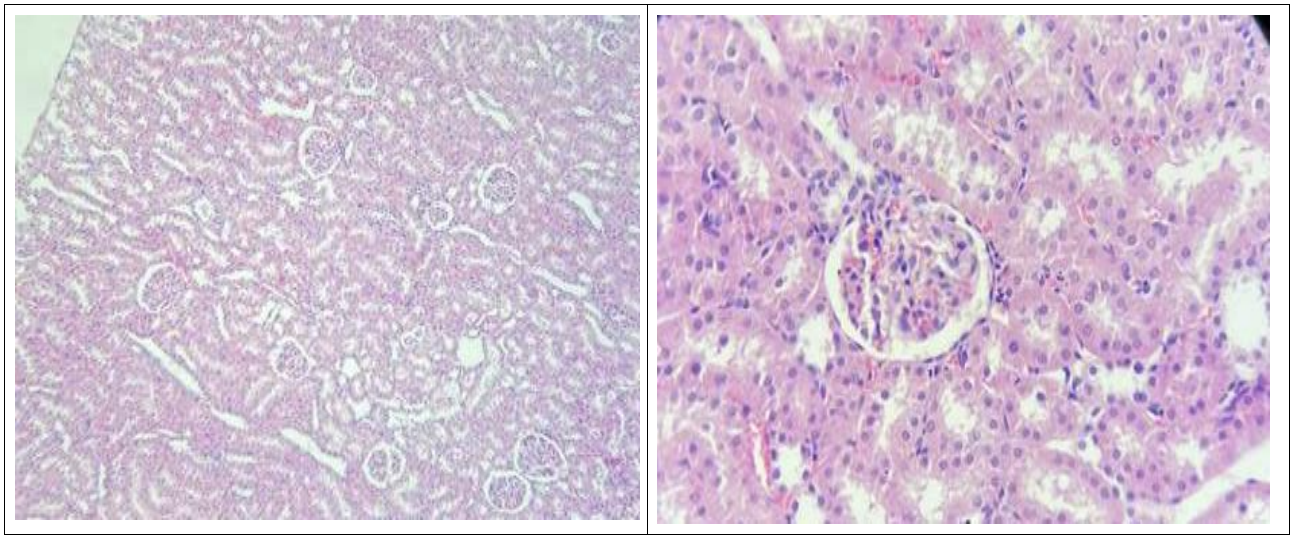

Fig. 4. The kidneys of the extract group demonstrate normal histological architecture in this photomicrograph. Both the AX100 and the BX400 from H&E.

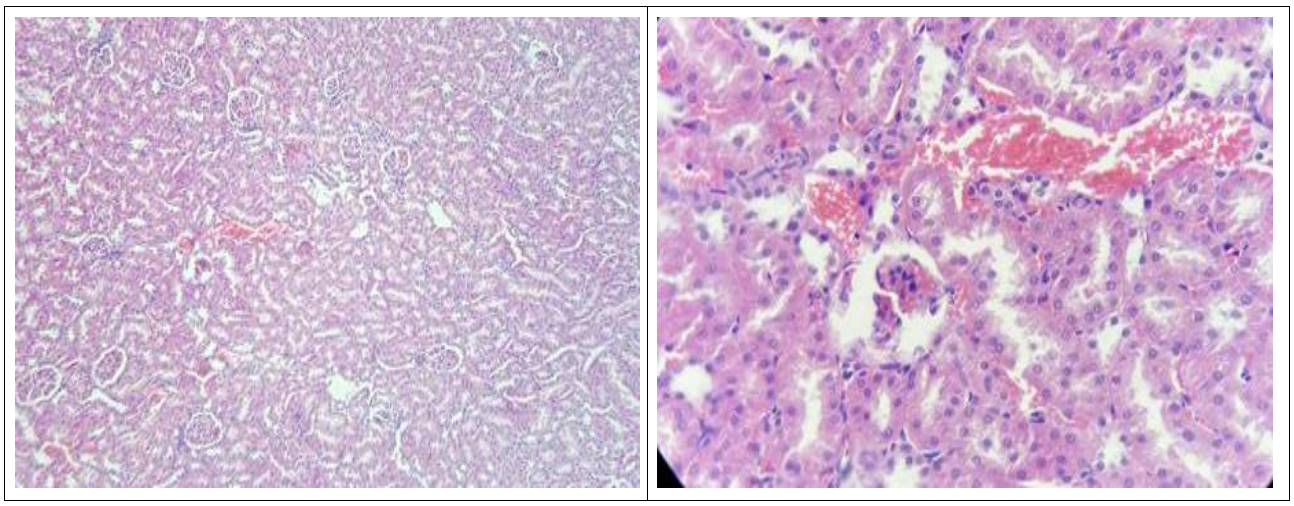

Fig. 5. The kidneys of the extract + insulin group were examined by a photomicrograph, which revealed narrowing of blood vessels with congestion, shrinkage of the glomeruli, and swelling of the epithelial cells (blue arrow) (H&E AX100, BX400).

Studies have shown that reactive oxygen species (ROS) are important in the development and occurrence of diabetes mellitus (Rehman & Akash, 2017; Haidara, 2010), and that oxidative stress is a crucial pathophysiology of both acute and chronic disease complications of diabetes. According to (Liu *et al.*, 2015), oxidative stress may harm cells in the kidneys of people with diabetes, disrupt signaling pathways, and increase the likelihood of developing DN. The unsaturated fatty acids in the biofilm might be attacked by ROS in excess, which could cause lipid peroxidation and the generation of lipid peroxides like MDA. According to (Moazamian *et al.*, 2015), the amount of malondialdehyde (MDA) in the blood may be a measure of the extent to which cells have been damaged by oxidative stress.

Superoxide dismutase (SOD) is a cellular antioxidant, an enzyme that catalyses the reaction to decompose free radicals (O2 -) and protects cells from the harmful effects of oxidative stress; GSH-Px may prevent the peroxide metabolites from accumulating in the cell because it neutralizes them. T-SOD, GSH-Px activities and the serum MDA

concentration in model group were remarkably lower than the control group; while T-SOD and GSH-Px activities and MDA concentration in ATZ group (50, 75 mg/kg) were considerably higher than the positive control group. Therefore, it suggested that ATZ may alleviate the renal injury in diabetic rats by improving the antioxidant capacity of serum to fight free radicals increases.

TGF-β is a polypeptide factor and composed of three main isomer and is involved in regulating cell haemostasis and differentiation, which interacts with a number of cell types and affect multiple steps of renal injury especially in ECM build up and gglomerular basement thickening ultimately progress into tubulointerstitial fibrosis. TGF–β1 is a major cytokine and is found abundantly dominate in kidney as described by Lo et al. (Lo *et al.,* 2012). It is considered as the major index of the phenodynamic in the both experimental and clinical fashions, which estimated the severity of proliferation, tubulointerstitial fibrosis, extracellular matrix formation, glomerular basement thickening. It showed ability to enhance the extracellular matrix formation via autocrine and paracrine ways by inhibiting the generate and activation of matrix metalloproteinase (MMP) and enhancing the expression of plasminogen activator inhibitor (PAI), which exert restraint of the breakdown of ECM consequently, allows basement membrane thickening along with proteinuria and glomeruloscelrosis alterations and swollen mesangial area as illustrated by (Lee *et al.,* 2007). Early studies have revealed a revelation that overproduction of ROS and oxidative stress caused by hyperglycaemia where considered as two critical intergrative factor which caused the upregullyion and activation of TGF-β1.Diaffeoylquinic acids have been shown to have an antihyperglycemic impact and to maintain the function and structure of pancreatic cells in a number of diabetic models (Tong *et al.,* 2015; Vitcheva *et al.,* 2018). Curiously, the mixture of A. annua's polar portion and three mains physiologically active dicaffeoylquinic acid derivatives may provide protection against diabetic complications via effective inhibition of the aldose reductase enzyme. Kuroda *et al.* (2016) found that dicaffeoylquinic acid derivatives extracted from Tussilago farfara leaves had a strong inhibitory effect on aldose reductase, which is consistent with our results. Furthermore, the same research showed that the aldose reductase enzyme was potently inhibited by the methanolic extract of three distinct Artemisia species. Aldose reductase inhibitory activity was also shown by 4,5-dicaffeoylquinic acid, which was derived from an antidiabetic methanolic extract of A. dracunculus (Logendra *et al.,* 2006).

Conclusion

Alcoholic extract of *Artemisia annua* reduce the kidney function in experimental diabetic rats and can alleviate the histological damage of diabetes on renal tissue.

Ethical Approval

Ethical approval was obtained from the Scientific Research Ethics Committee at the Fallujah University, College of Applied Sciences, Department of Biotechnology, No. 97, on 1/23/2024.

Conflict of Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdelhalim, A., Karim, N., Chebib, M., Aburjai, T., Khan, I., Johnston, G.A. and Hanrahan, J., 2015. Antidepressant, anxiolytic and antinociceptive activities of constituents from Rosmarinus officinalis. *Journal of Pharmacy & Pharmaceutical Sciences*, *18*(4), pp.448-459. DOI: https://doi.org/10.18433/J3PW38

Moazamian, R., Polhemus, A., Connaughton, H., Fraser, B., Whiting, S., Gharagozloo, P. and Aitken, R.J., 2015. Oxidative stress and human spermatozoa: diagnostic and functional significance of aldehydes generated as a result of lipid peroxidation. *MHR: Basic science of reproductive medicine*, *21*(6), pp.502-515. https://doi.org/10.1093/molehr/gav014

Rehman, K. and Akash, M.S.H., 2017. Mechanism of generation of oxidative stress and pathophysiology of type 2 diabetes mellitus: how are they interlinked?. *Journal of cellular biochemistry*, *118*(11), pp.3577-3585. https://doi.org/10.1002/jcb.26097

Amakura, Y., Yoshimura, M., Takaoka, M., Toda, H., Tsutsumi, T., Matsuda, R., Teshima, R., Nakamura, M., Handa, H. and Yoshida, T., 2014. Characterization of natural aryl hydrocarbon receptor agonists from cassia seed and rosemary. *Molecules*, *19*(4),pp.4956-4966. https://doi.org/10.3390/molecules19044956

Biesenbach, G., 1989. Disorders of lipid metabolism in diabetes mellitus. *Wiener medizinische Wochenschrift. Supplement*, *105*, pp.9-17.

Chandramohan, G., Al-Numair, K.S. and Pugalendi, K.V., 2009. Effect of 3-hydroxymethyl xylitol on hepatic and renal functional markers and protein levels in streptozotocindiabetic rats. *African Journal of Biochemistry Research*, *3*(5), pp.198-204.

Chawla, T., Sharma, D. and Singh, A., 2010. Role of the renin angiotensin system in diabetic nephropathy. *World journal of diabetes*, *1*(5), p.141. doi: 10.4239/wjd.v1.i5.141

Cheng, H. and C Harris, R., 2014. Renal endothelial dysfunction in diabetic nephropathy. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders)*, *14*(1), pp.22-33.

Forbes, J.M., Coughlan, M.T. and Cooper, M.E., 2008. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes*, *57*(6), pp.1446-1454. https://doi.org/10.2337/db08-0057

González-Trujano, M.E., Hernández-Sánchez, L.Y., Muñoz Ocotero, V., Dorazco-González, A., Guevara Fefer, P. and Aguirre-Hernández, E., 2017. Pharmacological evaluation of the anxiolytic-like effects of Lippia graveolens and bioactive compounds. *Pharmaceutical biology*, *55*(1), pp.1569-1576. https://doi.org/10.1080/13880209.2017.1310908

Lee, H.B., Seo, J.Y., Yu, M.R., Uh, S.T. and Ha, H., 2007. Radical approach to diabetic nephropathy. *Kidney International*, *72*, pp.S67-S70. https://doi.org/10.1038/sj.ki.5002389

Haidara, A.M., Z Yassin, H., Zakula, Z., P Mikhailidis, D. and R Isenovic, E., 2010. Diabetes and antioxidants: myth or reality?. *Current vascular pharmacology*, *8*(5), pp.661-672. https://doi.org/10.2174/157016110792006941

Jarald, E.E., Joshi, S.B. and Jain, D.C., 2008. Antidiabetic activity of aqueous extract and non polysaccharide fraction of Cynodon dactylon Pers.

Kashihara, N., Haruna, Y., K Kondeti, V. and S Kanwar, Y., 2010. Oxidative stress in diabetic nephropathy. *Current medicinal chemistry*, *17*(34), pp.4256-4269. https://doi.org/10.2174/092986710793348581

Kuroda, M., Ohshima, T., Kan, C. and Mimaki, Y., 2016. Chemical constituents of the leaves of Tussilago farfara and their aldose reductase inhibitory activity. *Natural Product Communications*, *11*(11), p.1934578X1601101109. https://doi.org/10.1177/1934578X1601101

Liu, J., Wang, C., Liu, F., Lu, Y. and Cheng, J., 2015. Metabonomics revealed xanthine oxidase-induced oxidative stress and inflammation in the pathogenesis of diabetic nephropathy. *Analytical and bioanalytical chemistry*, *407*, pp.2569-2579. https://doi.org/10.1007/s00216-015-8481-0

Lo, C.S., Chang, S.Y., Chenier, I., Filep, J.G., Ingelfinger, J.R., Zhang, S.L. and Chan, J.S., 2012. Heterogeneous nuclear ribonucleoprotein F suppresses angiotensinogen gene expression and attenuates hypertension and kidney injury in diabetic mice. *diabetes*, *61*(10), pp.2597-2608. https://doi.org/10.2337/db11-1349

Logendra, S., Ribnicky, D.M., Yang, H., Poulev, A., Ma, J., Kennelly, E.J. and Raskin, I., 2006. Bioassay-guided isolation of aldose reductase inhibitors from Artemisia dracunculus. *Phytochemistry*, *67*(14), pp.1539-1546. https://doi.org/10.1016/j.phytochem.2006.05.015

Madianov, I.V., Balabolkin, M.I., Markov, D.S. and Markova, T.N., 2000. Main causes of hyperuricemia in diabetes mellitus. *Terapevticheskii arkhiv*, *72*(2), pp.55-58.

Pourghasem, M., Shafi, H. and Babazadeh, Z., 2015. Histological changes of kidney in diabetic nephropathy. *Caspian journal of internal medicine*, *6*(3), p.120.

Reutens, A.T. and Atkins, R.C., 2011. Epidemiology of diabetic nephropathy. *Diabetes and the Kidney*, *170*, pp.1-7. https://doi.org/10.1159/000324934

Shah, N.A. and Khan, M.R., 2014. Antidiabetic effect of Sida cordata in alloxan induced diabetic rats. *BioMed research international*, *2014*(1), p.671294. https://doi.org/10.1155/2014/671294

Sher, H., Bussmann, R.W., Hart, R. and De Boer, H.J., 2016. Traditional use of medicinal plants among Kalasha, Ismaeli and Sunni groups in Chitral District, Khyber Pakhtunkhwa province, Pakistan. *Journal of ethnopharmacology*, *188*, pp.57-69. https://doi.org/10.1016/j.jep.2016.04.059

Tong, J., Ma, B., Ge, L., Mo, Q., Zhou, G., He, J. and Wang, Y., 2015. Dicaffeoylquinic acid-enriched fraction of Cichorium glandulosum seeds attenuates experimental type 1 diabetes via multipathway protection. *Journal of agricultural and food chemistry*, *63*(50), pp.10791-10802. https://doi.org/10.1021/acs.jafc.5b04552

Vitcheva, V.B., Simeonova, R.L., Kondeva-Burdina, M.S., Zheleva-Dimitrova, D.Z., Balabanova, V.I., Yagi, S. and Gevrenova, R.S., 2018. In vivo evaluation of the antioxidant potential of dicaffeoylquinic acid isolated from geigeria alata. *Bulg. Chem. Commun*, *50*, pp.98-104.

Yazdi, H.B., Hojati, V., Shiravi, A., Hosseinian, S. and Vaezi, G., 2020. The role of Artemisia turanica extract on renal oxidative and biochemical markers in STZ-induced diabetes in rat. *Avicenna Journal of Phytomedicine*, *10*(5), p.504.

Youssef, R.S., 2013. Medicinal and non-medicinal uses of some plants found in the middle region of Saudi Arabia. *J Med Plants Res*, *7*(34), pp.2501-2513.