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Telfairia occidentalis ATTENUATES STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY IN WISTAR RATS

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ABSTRACT
The morbidity and mortality of diabetic nephropathy (DN) have been rising rapidly in the worldwide population. *Telfairia occidentalis* (TO) is a popular plant and food item reported to have high content of antioxidant, anti-inflammatory, immunomodulatory and hypoglycemic properties. The present study investigated the effects of *T. occidentalis* against streptozotocin-induced diabetic nephropathy in Wistar rats. Thirty five rats were randomly assigned into five groups of Seven (7) rats each. The control group received distilled water for 28 days, the streptozotocin (STZ), TO1, TO2, and MET groups received 10% fructose for 14 days prior to single intraperitoneal injection of 40mg/kg body weight STZ; after which they were treated with 0.5ml distilled water, 200mg/kg body weight TO, 300mg/kg body weight TO, and 300mg/kg body weight MET respectively. Treatment with *T. occidentalis* (200mg/kg and 300mg/kg) significantly ($p<0.05$) improved the activities of the antioxidant enzymes namely SOD, CAT and GPx activities as well as GSH and Vitamin C levels with a concomitant decrease in MDA level in the kidney of diabetic nephropathy rats. In the same vein, biomarkers of inflammation, namely: TNF-α, IL-1β, NO and iNOS levels along with MPO and COX-2 activities were markedly reduced in the kidney of diabetic nephropathy rats when treated with *T. occidentalis* (200mg/kg and 300mg/kg). Also, the activity of caspase-3 was markedly reduced in the kidney of diabetic nephropathy rats when treated with *T. occidentalis* (200mg/kg and 300mg/kg). *Telfairia occidentalis* attenuated oxidative stress and inflammation as well as caspase-3 in the kidney of streptozotocin-induced diabetic nephropathy in rats.

KEYWORDS: Antioxidant enzymes, Caspase-3, Diabetic nephropathy, Inflammation, *Telfairia occidentalis.*

INTRODUCTION
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia with disturbances in carbohydrate, fat, and protein metabolism.[1] Patients with diabetes mellitus often have poor glycemic control and develop numerous microvascular complications, including nephropathy.[2,3]

Diabetic nephropathy (DN), also called diabetic kidney disease (DKD), or chronic kidney disease (CKD), is one of the most frequent and severe chronic complications of DM resulting from microvascular lesions in the renal glomeruli and tubules. It has been the leading cause of CKD and renal failure in developed countries. In the past two decades, the morbidity and mortality of DN have been rising rapidly in the worldwide population.[4,5,6,7]

Although the pathogenesis of DN is not fully understood, multifactorial interaction between lipid disorders, oxidative stress, renal hemodynamic changes, polyol activation, inflammatory pathways, and mitogen-activated protein kinase signaling pathways have been implicated.[8][9][10] Among which, excessive production of reactive oxygen species (ROS), induced by high blood glucose levels is believed to be the initiator for diabetes and its complications[11] ROS levels significantly increase during the pathogenesis of renal cells and the kidney tissues of diabetic mice.[12] ROS causes continuous oxidative stress and reduces the expression of antioxidative enzymes, such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in glomerular mesangial cells (GMCs).[13] It also promotes the generation of a large number of cytokines and extracellular matrix (ECM), which ultimately initiates and contributes to the development of diabetic renal fibrosis.[5,14,15] Therefore, strong antioxidants could potentially serve as treatments to diabetes related diseases.

Telfairia occidentalis, commonly known as fluted pumpkin, is a member of the Cucurbitaceae family. The stem, leaves and seeds are popularly used in preparing soups. The highly nutritious seeds are also eaten roasted or boiled. *T. occidentalis* has been reported to possess

antioxidant effect, antiplasmodial and antibacterial effects, antianaemic and antidiabetic effects, immunodulatory, anticancer and antiinflammatory effects, hepatoprotective effects and male fertility activity.[16] Given the widely reported properties of *T. occidentalis*, the study sought to investigate its effect on streptozotocin-induced diabetic nephropathy in diabetic rats.

MATERIALS AND METHODS

Chemicals and reagents

Streptozotocin and adenosine monophosphate (AMP) was purchased from Sigma Aldrich, St Louis, USA. Caspase-3, Tumor necrosis factor alpha (TNF-α), Interleukin-1β (IL-1β), Cyclooxygenase-2 (COX-2) and Inducible nitric oxide synthase (iNOS) ELISA kits were procured from CUSABIO Life Science Inc. (Wuhan, China). Creatine and Urea assay kit were purchased from Randox laboratories limited, USA. All other reagents were obtained from the British Drug Houses (Poole, Dorset, UK).

Preparation of *Telfaria occidentalis* leaf Extract

Telfaria occidentalis (TO) leaves were harvested from a local garden in Okuku. Yala Local Government Area of Cross River State, Nigeria. The botanical identification of the plant was done at the herbarium of the Department of Botany, University of Ibadan. The TO leaves were air-dried in the Biochemistry laboratory and grinded using an electronic blender. 700g of the pulverized TO was weighed and soaked in 2100ml of ethanol for 72 hours and sieved using a muslin cloth before filtering with Whatman filter paper (size 1) and the filtrate was concentrated at 40ºC.

Animal Model

Thirty-five (35) male Albino rats weighing about 150-200g were obtained from the animal house of the Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku campus. They were placed in well ventilated wire netted plastic cages of five groups containing seven rats per group. The Animals were acclimatized for a period of 14 days before the commencement of experiment and were allowed access to standard laboratory chow and drinking water *ad libitum*. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health.

Experimental Design

The rats were randomly assigned into five groups of seven (7) rats per group. And the treatment was done as explained below.

Control: served as control and received distilled water for 28 days.

STZ group: received 10% fructose for 14 days prior to single administration of 40mg/kg body weight streptozotocin (STZ)

TO1 group: received 10% fructose for 14 days prior to single administration of 40mg/kg body weight STZ, followed by 200mg/kg body weight TO for 28 days

TO2 group: Received 10% fructose for 14 days prior to single administration of 40mg/kg body weight STZ, followed by 300mg/kg body weight TO for 28 days

MET group: Received 10% fructose for 14 days prior to single administration of 40mg/kg body weight STZ, followed by 300mg/kg body weight Metformin for 28 days

Animal Sacrifice and Preparation of Post Mitochondrial Fraction Collection

All animals were sacrificed 24 hours after the last treatment. The blood was collected via cardio-puncture into EDTA bottles and centrifuged at 3000 RPM for 15mins to obtain the plasma. The Kidney was excised, rinsed in 1.15% potassium chloride and homogenized in 0.1 M phosphate buffer (pH 7.4). The resulting homogenates were then centrifuged at 10,000g at 4^{o}C for10 min. The supernatants were used for the biochemical assay.

Determination of Creatinine and Urea

Creatine and Urea were determined in the plasma as described in the manufacturers protocol Randox laboratories limited, USA.

Determination of Renal Oxidative stress

The Supernatant fraction of the Kidney homogenates were collected for the estimation of catalase (CAT) activity using hydrogen peroxide as substrate according to the method of Clairborne [17] Hydrogen peroxide generation was assessed by the method of Wolff.[18] Glutathione peroxidase (GPx) activity was determined by the method of Rotruck.[19] Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich.[20] Myeloperoxidase (MPO) activity was determined according to the method of Granell *et al.*,[21] MPO activity was expressed as umoleH_2O_2/min/mg protein. Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi *et al.*,[22] and expressed as micromoles of MDA per gram tissue. Nitric oxide (NO) level was evaluated by measuring the colonic nitrites content, the stable end products of nitric oxide (NO). Colonic nitrites content was obtained using a sodium nitrite curve as standard and expressed as µ m of nitrites/mg protein.[23]

Determination of Renal Inflammatory and Apoptotic Biomarkers

Caspase-3, TNF-α, IL-1β, iNOS and COX-2 concentrations in the supernatant of the kidney

homogenate were measured by ELISA kits according to the manufacturers' instruction. (CUSABIO Life Science Inc., Wuhan, China).

Histological Assessment

The kidney was excised washed in phosphate-buffered saline, and fixed with 10% formalin overnight. Evaluation of kidney histological architecture was done by staining with hematoxylin and eosin. Mounted slides were examined under a light microscope

RESULTS

***T. occidentalis* improved 5' Nucleotidase activity, antioxidant response and reduced oxidative stress indices in the kidney of streptozotocin-induced diabetic nephropathy in rats**

As shown in Fig. 1 and in Table 1, the results revealed that treatment of rats to streptozotocin caused a significant decrease (P< 0.05) in SOD, CAT and GPx activities as well as GSH and Vit C levels with a concomitant increase in MDA level in the kidney of diabetic rats when compared to control. It also triggered increases in urea and creatinine levels when compared with the normal control. However, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) significantly reduced urea and creatinine levels and also improved the activities of the antioxidant enzymes as well as decreased the levels of MDA when compared with rats exposed to STZ alone. Also, treatment with metformin (300mg/kg) also showed similar trend with *T. occidentalis* group when compared with rats exposed to STZ alone.

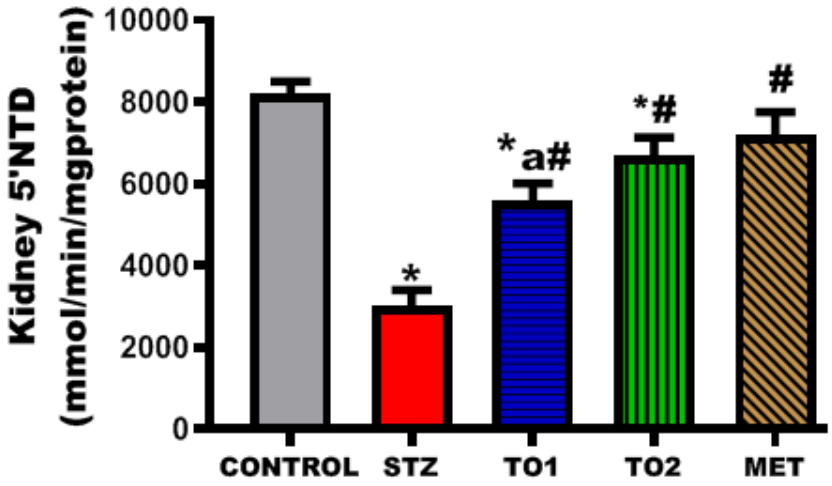

Fig. 1: Effect of *T. occidentalis* (TO) on 5'Nucleotidase (5'NTD) concentration in the kidney of streptozotocin-induced diabetic rats. Data is expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.

Table 1: Effect of *T. occidentalis* (TO) on biomarkers of kidney function and oxidative stress in the plasma and kidney of streptozotocin-induced diabetic rats.

BIOMARKERS	CONTROL	STZ	TO1	TO2	MET
SOD (µmol /mg protein)	31.7 ± 5.8	13.2 ± 0.9*	15.3 ± 1.0*# [a]	22.5 ± 2.6#	20.4 ± 3*#
CAT (µmol/min)	110.5 ± 9.2	42.4 ± 5.6*	72.7 ± 8.6*#	88.6 ± 10*#	93.7 ± 13.8*#
GSH (µg/g tissue)	53.6 ± 5.5	18.3 ± 3.2*	30.3 ± 2.7*#	41.6 ± 1.6#	46.1 ± 2.9#
GPx (µGSH consumed/min)	76.3 ± 6.0	28.8 ± 2.2*	39.5 ± 4.7*# [a]	58.4 ± 3.3#	62.3 ± 9.0#
MDA (nmol/mg protein)	2.2 ± 0.1	15.6 ± 1.3*	11.3 ± 0.9*# [a]	4.1 ± 0.05#	3.1 ± 0.1#
Vit C (µg/ml)	1.04 ± 0.01	0.36 ± 0.02*	0.59 ± 0.04*# [a]	0.74 ± 0.05#	0.85 ± 0.08#
Urea (mg/dl)	42.7 ± 3.91	85.6 ± 6.58*	75.4 ± 4.67*# [a]	61.9 ± 5.12#	48.2 ± 3.81#
Creatinine (mg/dl)	0.5 ±0.02	1.6 ± 0.04*	1.32 ± 0.09*#	1.03 ± 0.04*#	0.86 ± 0.05#

Data is expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.

***T. occidentalis* ameliorated inflammation in the kidney of streptozotocin-induced diabetic nephropathy in rats**

In comparison with control, STZ caused significant increases (P< 0.05) in TNF-α, IL-1β, NO and iNOS levels along with MPO and COX-2 activities in the kidney of streptozotocin-induced diabetic nephropathy in rats (Figs 2 to 7). Inflammation biomarkers as well as COX-2 activation were markedly reduced in the kidney of the group treated with *T. occidentalis* (200mg/kg and 300mg/kg) when compared with group exposed to STZ alone. Similar trend was also observed in the group treated with metformin (300mg/kg) when compared with group exposed to STZ alone.

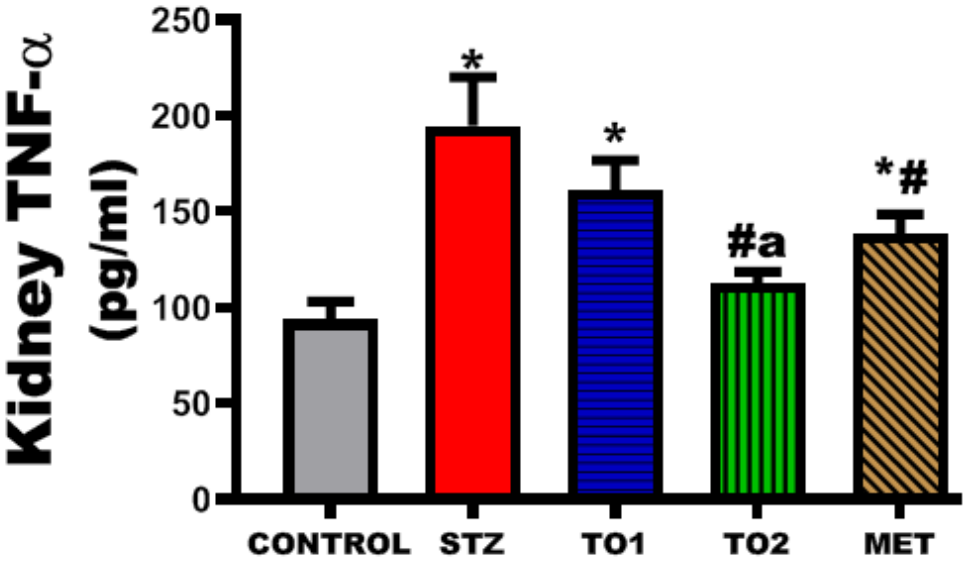

Fig. 2: Effect of *T. occidentalis* (TO) on tumor necrosis factor alpha (TNF-α) concentration in kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< 0.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.

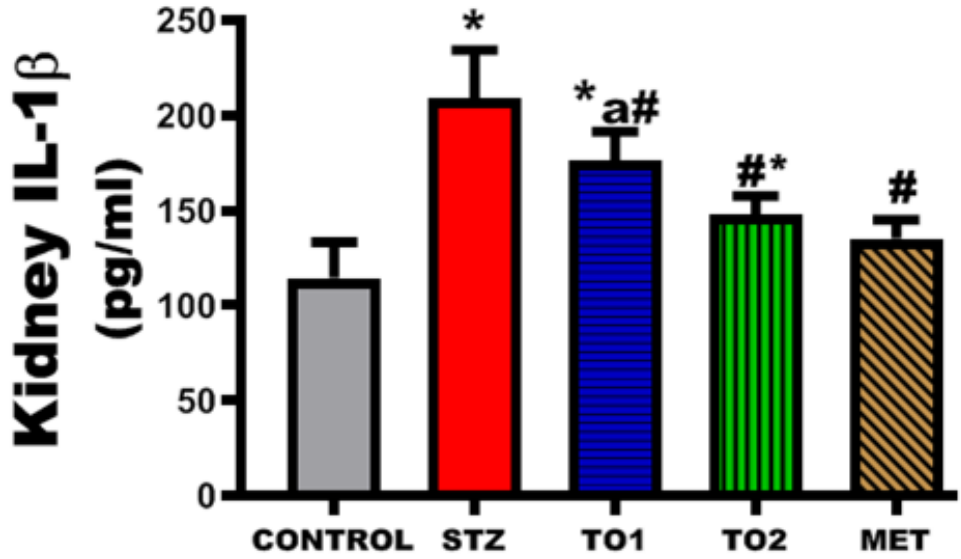

Fig. 3: Effect of *T. occidentalis* (TO) on interleukin-1β (IL-1β) concentration in the kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< 0.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.

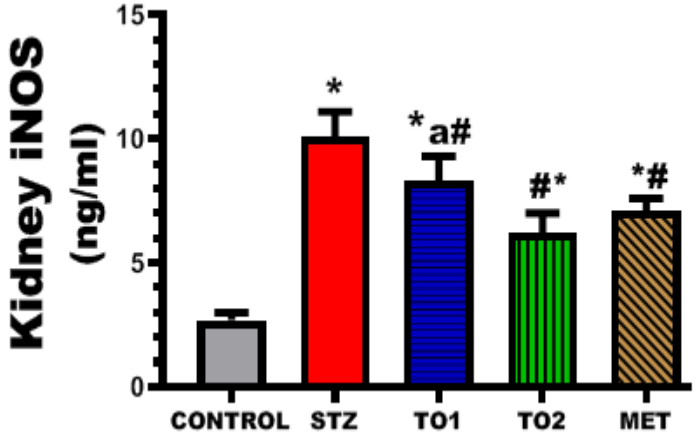

Fig. 4: **Effect of *T. occidentalis* (TO) on inducible nitric oxide (iNOS) concentration in the kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.**

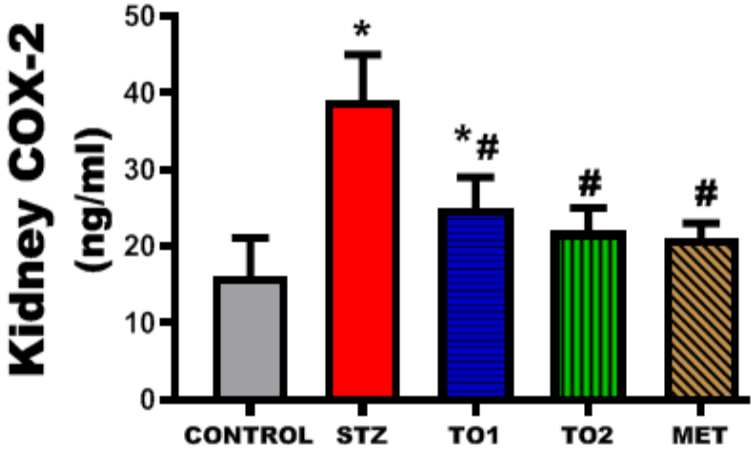

Fig. 5: **Effect of *T. occidentalis* (TO) on cyclooxygenase-2 (COX-2) concentration in the kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.**

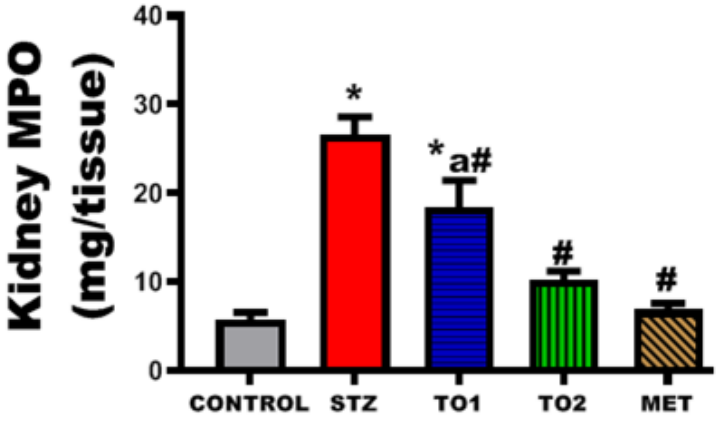

Fig. 6: **Effect of *T. occidentalis* (TO) on myeloperoxidase (MPO) activity in the kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.**

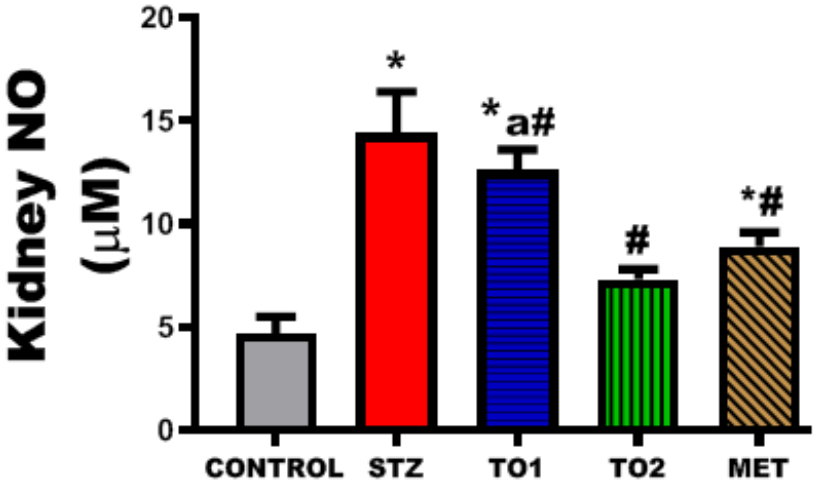

Fig. 7: Effect of *T. occidentalis* (TO) on nitric oxide (NO) concentration in the kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.

***T. occidentalis* attenuated Caspase-3 activation in the kidney of streptozotocin-induced diabetic nephropathy in rats**

Treatment with STZ alone caused a significant increase (P< 0.05) in Caspase-3 activity in the kidney of streptozotocin-induced diabetic nephropathy in rats when compared with control (Fig 8). In contrast, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) markedly reduced the activity of caspase-3 in the kidney of streptozotocin-induced diabetic nephropathy in rats when compared with group exposed to STZ alone. Similar trend was also observed in the group treated with metformin (300mg/kg) when compared with group exposed to STZ alone.

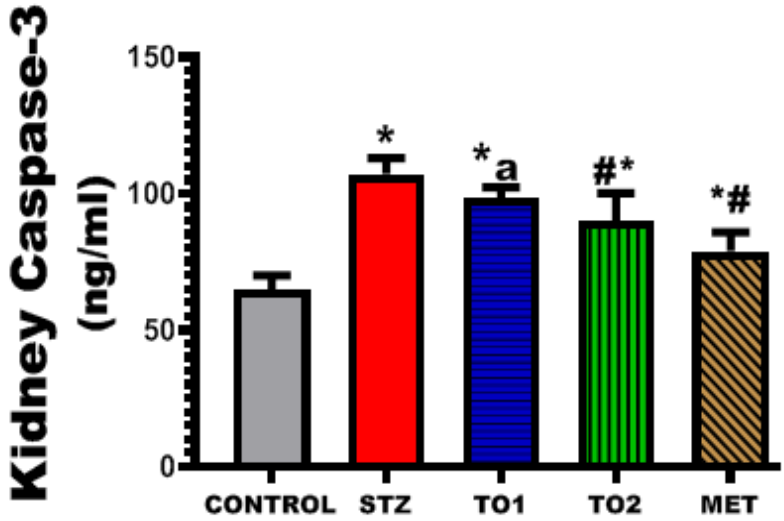

Fig. 8: Effect of *T. occidentalis* (TO) on Caspase-3 concentration in the kidney of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, * *p*< *0*.05 against control, # *p*< 0.05 against STZ, a *p*< 0.05 against MET.

***T. occidentalis* attenuated Caspase-3 activation in the kidney of streptozotocin-induced diabetic nephropathy in rats**

Treatment with STZ alone caused a significant increase (P< 0.05) in Caspase-3 activity in the kidney of streptozotocin-induced diabetic nephropathy in rats when compared with control (Fig 2). In contrast, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) markedly reduced the activity of caspase-3 in the kidney of streptozotocin-induced diabetic nephropathy in rats when compared with group exposed to STZ alone. Similar trend was also observed in the group treated with metformin (300mg/kg) when compared with group exposed to STZ alone.

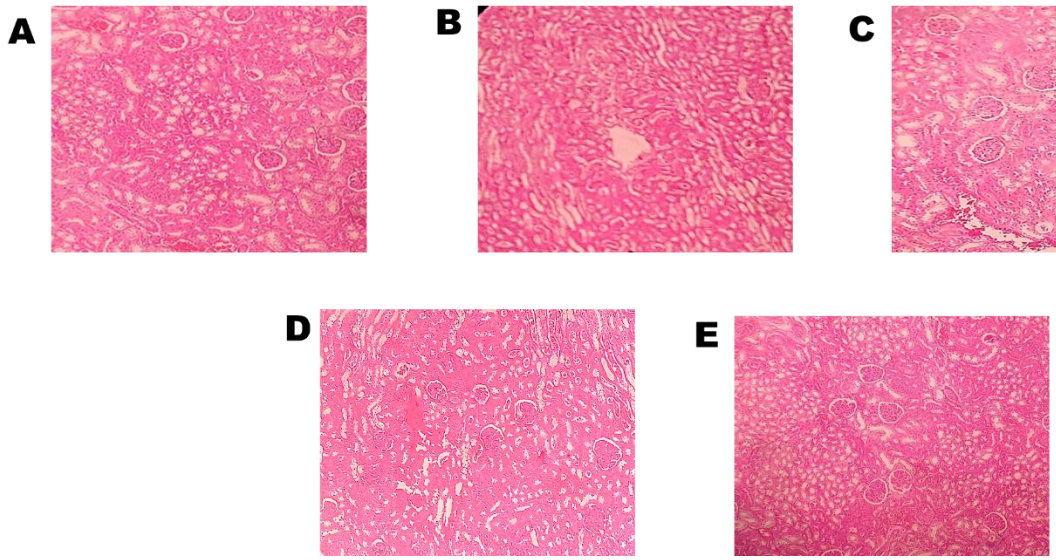

Fig. 9: **Hematoxylin and Eosin (H&E) stained section of the kidney showing the effect of *T. occidentalis* (TO) on streptozotocin-induced diabetic rats. Control (A): no visible lesion; STZ (B): severe loss of glomerulus and glomerular infiltration of inflammatory cells. TO1 (C): Mild loss of glomerulus. TO2 (D): Very mild loss of glomerulus. MET (E): The glomerulus are intact. Mag x 40.**

DISCUSSION

Several lines of evidence have convinced us that oxidative stress as well as inflammation related molecules and pathways are critically involved in the progression of diabetic nephropathy.[24][25] These molecules and pathways are candidates for the development of novel treatment modalities for diabetic nephropathy. In the present study, we evaluated the ameliorative potential of *T. occidentalis* in the kidney of streptozotocin-induced diabetic nephropathy in rats.

Oxidative stress is considered to be important in the development of diabetic nephropathy.[26][27][28] Kuo et al. suggested that the interaction between ROS production and the anti-oxidant pathways can contribute to the progression of diabetic nephropathy.[29] SOD, CAT and GPx are key enzymes that scavenge free radicals in the body. These factors can reflect the body's antioxidant capacity. Studies show that being an intracellular antioxidant, GPx protects DNA, proteins and other biomolecules from ROS. In the present study, our results showed that the administration of *T. occidentalis* (200mg/kg and 300mg/kg) significantly reversed the decrease in SOD, CAT and GPx as well as GSH and Vit C levels in the kidney of diabetic nephropathy rats. This effect is likely due to the antioxidative properties of *T. occidentalis*. We employed metformin as a positive control and our data showed that the protective effects of *T. occidentalis* against diabetic nephropathy are comparable to metformin.

MDA, a direct product of lipid peroxidation, can reflect the extent of lipid peroxidation in tissues. A study by Yılmaz *et al.*,[30] showed that MDA levels were increased in the livers of STZ-induced diabetic rats. In the present study, our results showed that administration of *T. occidentalis* (200mg/kg and 300mg/kg) markedly reversed the increase in MDA level in the kidney of diabetic nephropathy rats.

Inflammation is widely inferred as an essential aetiological factor which plays a vital role in the development of insulin resistance which significantly leads to DM. It also contributes to the foreseen diabetes complications. Hence, targeting inflammation may be a new therapy in the already expanding options for the management of diabetes mellitus and its complications. Recent reports have demonstrated that increase in inflammatory markers such as tumor necrosis factor (TNF-α) and interleukin (IL-1), C-reactive protein, interstitial cellular adhesion molecule-1, vascular cellular adhesion molecule-1, and E-selectin are associated with the development of nephropathy in patients with diabetes.[31][32] Elevated TNF-α level has been demonstrated to up-regulate the inducible nitric oxide synthase (iNOS) which subsequently increases NO production.[33]. COX-2 is an inducible enzyme that is upregulated in atherosclerotic plaques and it may contribute to initiation of atherosclerotic plaque by inducing metalloproteinases.[34] NO is well-known to be a potent pro-inflammatory mediator at high concentration. In the present study, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) significantly reversed the increased levels of NO, iNOS, TNF-α and IL-1β, as well as MPO and COX-2 activation, thus, suppressing inflammation. These findings further potentiate the anti-inflammatory properties of *T. occidentalis*.

Caspase-3 which belongs to a family of aspartate-specific cysteine proteases is the key executioner caspase regulating the apoptosis cascade.[35] The increase in caspase-3 activity indicates induction of apoptotic cell death in the untreated diabetic rats. However, treatment

with *T. occidentalis* (200mg/kg and 300mg/kg) significantly reversed the increased activity of caspase-3 in the kidney diabetic nephropathy rats.

In conclusion, the data from our study showed that *T. occidentalis* ameliorated streptozotocin-induced diabetic nephropathy rats. This effects are likely due to the antioxidant, anti-inflammatory and antidiabetic properties of *T. occidentalis.*

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