

## Telfairia occidentalis ATTENUATES LIVER DAMAGE IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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### ABSTRACT

**Background:** The onset of diabetes is associated with the development of biochemical and functional abnormalities, including oxidative stress and apoptosis in the liver. *Telfairia occidentalis* (TO) is a popular plant and food item reported to have high of antioxidant, anti-inflammatory, immunomodulatory and hypoglycemic properties. The present study investigated the potential antihepatotoxic properties of *T. occidentalis* in animal model of diabetes- induced liver damage.

### Methods:

Thirty-five rats were assigned into five groups of Seven (7) animals each. Group 1 (Normal control) received 0.5ml distilled water for 28 days, Group 2 (Diabetic control) received 10% fructose (w/v) for 14 days prior to single intra-peritoneal injection of 40mg/kg body weight streptozotocin (STZ), Group 3 (TO1 group), received 10% fructose (w/v) for 14 days prior to single intra-peritoneal injection of 40mg/kg body weight STZ, followed by treatment with 200mg/kg body weight TO for 28 days, Group 4 (TO2 group) received 10% fructose (w/v) for 14 days prior to single intra-peritoneal injection of 40mg/kg body weight STZ, followed by treatment with 300mg/kg body weight TO for 28 days, while Group 5 (MET group) received 10% fructose (w/v) for 14 days prior to single intra-peritoneal injection of 40mg/kg body weight STZ, followed by treatment with 300mg/kg body weight Metformin for 28 days.

**Results:** Treatment with TO at 200mg/kg and 300mg/kg significantly ( $p < 0.05$ ) augmented the activities of superoxide dismutase, catalase and glutathione peroxidase as well as reduced glutathione level with a concomitant decrease in malondialdehyde level in the liver of diabetic rats. Also, Inflammatory biomarkers namely TNF- $\alpha$ , IL-1 $\beta$ , NO and iNOS levels along with MPO and COX-2 activities were significantly decreased in the liver of diabetic rats when treated with *T. occidentalis* (200mg/kg and 300mg/kg). Furthermore, the activity of caspase-3 was markedly reduced in the liver of diabetic rats when treated with *T. occidentalis* (200mg/kg and 300mg/kg).

**Conclusion:** *Telfairia occidentalis* attenuated oxidative stress, inflammation and activation of caspase-3 in the liver of streptozotocin-induced diabetic rats via antioxidant and anti-inflammatory mechanisms.

**KEYWORDS:** Inflammation; Apoptosis; Oxidative stress; Liver; Streptozotocin

## INTRODUCTION

Diabetes is a lifelong chronic disease, which is one of the most severe endocrine metabolic disorders worldwide.<sup>[1]</sup> This common metabolic disease can result in severe structural and functional complications in the liver.<sup>[2][3]</sup> In addition to unfavourable effects on many tissues and organs, it has been suggested that diabetes mellitus also adversely affects hepatobiliary functions in diabetic patients and animals. Further, it is reported that hepatobiliary disorders such as hepatic inflammation, non-alcoholic fatty liver disease, nonalcoholic steatohepatitis, hemochromatosis, autoimmune hepatitis, cirrhosis, hepatocellular carcinoma, acute liver failure, and cholelithiasis can ensue under diabetes.<sup>[2][3][4]</sup> Although the liver is a central organ in metabolism and has been recognized to be injured in this disease,<sup>[5]</sup> the hepatic damage and dysfunction in diabetes have not been well addressed.<sup>[6]</sup>

The onset of diabetes in the liver is associated with the development of biochemical and functional abnormalities, including oxidative stress and apoptosis.<sup>[7][8]</sup> In addition, inflammation and necrosis or fibrosis of non-alcoholic fatty liver disease has been reported to follow diabetes.<sup>[9]</sup> Madar *et al.* have also found that STZ-induced diabetes increased iNOS activity in the rat liver and in the isolated hepatocytes.<sup>[10]</sup> Taurino *et al.* have found that the diabetic liver presents abnormalities in the 14-3-3 proteins, which constitute a family of conserved molecular chaperones with roles in the regulation of metabolism, signal transduction, cell cycle control, protein trafficking and apoptosis.<sup>[11]</sup>

Nowadays, medicinal plants have received attention in the management of diabetes because they offer some hope and have less side-effects than the conventional medications.<sup>[12]</sup> A recent study has shown that higher habitual flavonoid intake from fruits and vegetables during adolescence is relevant for the prevention of risk factors of type 2 diabetes in early adulthood.<sup>[13]</sup>

*Telfairia occidentalis* (TO), routinely referred to as fluted pumpkin, fluted gourd and “Ugu,” is a popular plant and food item amongst the populace in Nigeria due to its consumable seeds and edible leaf. It is an integral part of folkloric medicine in Nigeria<sup>[14][15]</sup> The seeds are rich in phytosterols, phytochemicals, sterols, polyunsaturated fatty acids, tocopherol, carotenoids and proteins.<sup>[16]</sup> Flavonoids, alkaloids, saponins, phenolics, anthraquinones and tannins were also found in methanolic seed extract of TO.<sup>[17]</sup>

*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.<sup>[18]</sup>

More so, it has been opined that bioactive constituents of TO can trigger varied physiological, biochemical and morphological effects,<sup>[19]</sup> and this may account for the divergent influence of TO in experimental models. For instance, Nwidu and Oboma recently reported that *T. occidentalis* (Cucurbitaceae) pulp extract mitigated rifampicin-isoniazid-induced hepatotoxicity in an *in vivo* rat model of oxidative stress.<sup>[20]</sup> Also, Oladele *et al* demonstrated that *T. occidentalis* mitigated dextran sodium sulfate- induced ulcerative colitis in rats via suppression of oxidative stress, lipid peroxidation, and inflammation.<sup>[21]</sup> Hence this study investigated the potential antihepatotoxic properties of *T. occidentalis* in animal model of streptozotocin diabetes- induced liver damage.

## MATERIALS AND METHODS

### Chemicals

Streptozotocin was procured from Sigma Aldrich, St Louis, USA. Interleukin-1 $\beta$  (IL-1 $\beta$ ), Cyclooxygenase-2 (COX-2), Caspase-3 and Inducible nitric oxide synthase (iNOS) kits were purchase from CUSABIO Life Science Inc. (Wuhan, China). All other reagents and kits were obtained from the British Drug Houses (Poole, Dorset, UK) and Randox USA.

### Preparation of Plant Extract

*Telfairia occidentalis* (TO) leaves were harvested from a local farm in Okuku, Cross River State, Nigeria. The botanical identification of the plant was carried out at the herbarium of the Department of Botany, University of Ibadan. The TO leaves were air-dried and grinded using an electronic blender. TO leaves were soaked in absolute ethanol (1:3) for 72 hours and filtered with Whatman paper size 1 and the filtrate was then concentrated at 40°C.

### Animal Model

Thirty-five (35) male albino rats Wistar strain weighing about 100-250g were obtained from the animal holding facility of the Faculty of Basic Medical Sciences, University of Cross River state. They were placed in well ventilated wire netted plastic cages of five groups containing seven rats each. The rats were acclimatized for 14 days before the commencement of study and were allowed access to rodent chow and drinking water *ad libitum*. All the rats 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. The experiment was performed according to the US NAS guidelines and approval of institutional animal ethics committee.

### Experimental Design

The rats were randomly assigned into 5 groups of 7 rats each. The treatment regimen is as described below

Group 1: Served as control and received distilled water for 42 days

Group 2: Served as the diabetes group and received 10% fructose for 14 days prior to single administration streptozotocin (STZ) 40mg/kg

Group 3: Served as the TO1 group and received 10% fructose for 14 days prior to single administration of streptozotocin (STZ) 40mg/kg followed by treatment with 200mg/kg body weight TO for 28 days

Group 4: Served as the TO2 group and received 10% fructose for 14 days prior to single administration of streptozotocin (STZ) 40mg/kg followed by treatment with 300mg/kg body weight TO for 28 days

Group 5: Served as the MET group and received 10% fructose for 14 days prior to single administration of streptozotocin (STZ) 40mg/kg, followed by treatment with 300mg/kg body weight Metformin for 28 days.

### Animal Sacrifice and Preparation of Post Mitochondrial Fraction of Liver Homogenate

All rats 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 liver 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°C for 10 minutes, to obtain the post mitochondria fraction that was used for the biochemical assay.

### Determination of Alanine aminotransferase, Aspartate aminotransferase and Alkaline Phosphatase

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) were determined in the plasma as described in the manufacturer's protocol Randox laboratories limited, USA.

### Determination of Biomarkers of Hepatic Oxidative stress

The post mitochondrial fraction of the liver was collected for the estimation of catalase (CAT) activity using hydrogen peroxide as substrate according to the method of Clairborne.<sup>[22]</sup> Hydrogen peroxide generation was assessed by the method of Wolff.<sup>[23]</sup> Glutathione peroxidase (GPx) activity was determined by the method of Rotruck.<sup>[24]</sup> Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich.<sup>[25]</sup> Myeloperoxidase (MPO) activity was determined according to the method of Granell *et al.*<sup>[26]</sup> MPO activity was expressed as  $\mu\text{mole H}_2\text{O}_2/\text{min}/\text{mg}$  protein. Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi *et al.*<sup>[27]</sup> 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  $\mu\text{m}$  of nitrites/mg protein.<sup>[28]</sup>

### Determination of Renal Inflammatory and Apoptotic Biomarkers

Caspase-3, TNF- $\alpha$ , IL-1 $\beta$ , iNOS and COX-2 concentrations in the supernatant of the liver homogenate were measured by ELISA kits according to the manufacturers' instruction. (CUSABIO Life Science Inc., Wuhan, China).

### Histological Assessment

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

**RESULTS**

**Effects of *T. occidentalis* on AST, ALT and ALP in the plasma of STZ-induced diabetes in Rats**

As shown in Table 1: There was an increase in the activities of AST, ALT and ALP in the plasma of diabetic rats as compared to control ( $P < 0.05$ ). However, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) significantly decreased the activities of AST, ALT and ALP when compared with diabetic rats. Also, a similar trend was observed with metformin (300mg/kg) when compared with diabetic rats.

Table 1: Effects of *T. occidentalis* on AST, ALT and ALP in the plasma of STZ-induced diabetes in Rats

Biomarker	Control	STZ	TO1	TO2	MET
ALT (U/L)	74.53 ± 11.2	120.2 ± 15.4*	90.81 ± 10.3	88.25 ± 6.9#	84.69 ± 14.5#
AST (U/L)	108.5 ± 12.6	250.7 ± 11.3*	164.32 ± 14.1*# <sup>a</sup>	152.83 ± 10.3*#	136.74 ± 9*#
ALP (U/L)	134.43 ± 17.5	280.78 ± 11.3*	233.44 ± 8.9*#	209.66 ± 10.2*#	219.76 ± 13.1*#

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.

**Effects of *T. occidentalis* on liver antioxidant status and oxidative stress in STZ-induced diabetes**

The result in figure 1 revealed that treatment of rats to streptozotocin caused a significant decrease ( $P < 0.05$ ) in SOD, CAT, GST and GPx activities as well as GSH level with a concomitant increase in MDA level in the liver of diabetic rats when compared to control. However, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) significantly improved the activities of these enzymes as well as decreased the level 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.

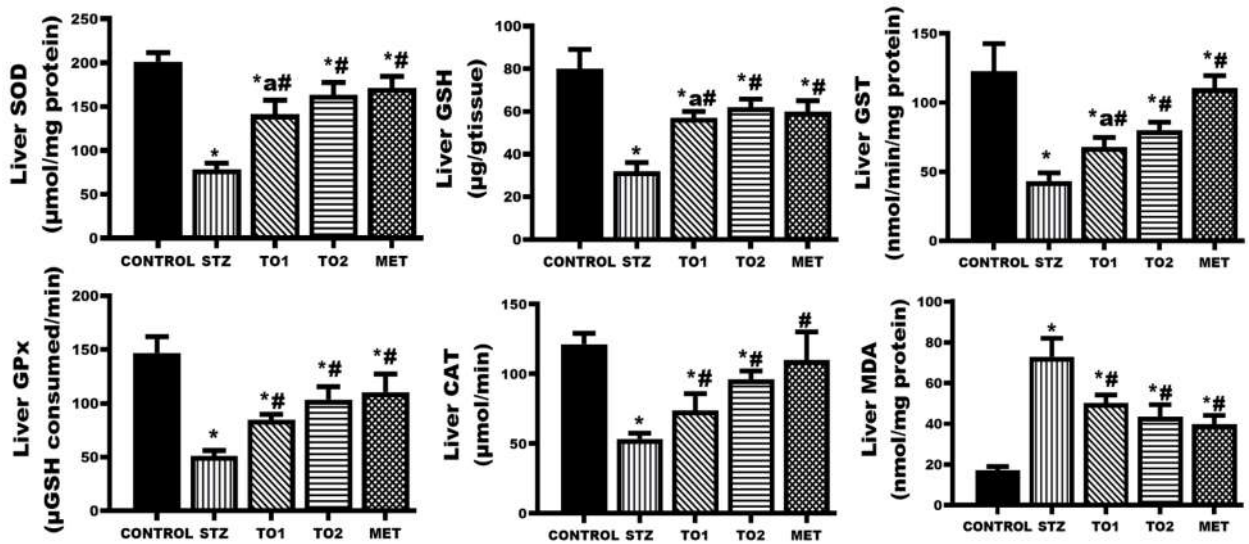


Figure 1: Effect of *T. occidentalis* (TO) on activities of SOD, GSH, GST, GPx, CAT and MDA in the liver of streptozotocin-induced diabetic rats. Data is expressed as mean ± SEM; n = 7 rats, \*  $p < 0.05$  against control, #  $p < 0.05$  against STZ, a  $p < 0.05$  against MET.

**Effects of *T. occidentalis* on liver inflammation and COX-2 activation in STZ-induced diabetes**

When compared with control (Figure 2), STZ caused a significant increase ( $P < 0.05$ ) in TNF- $\alpha$ , IL-1 $\beta$ , NO and iNOS levels along with MPO and COX-2 activities in the liver of diabetic rats. However, these inflammation biomarkers as well as COX-2 activation were markedly reduced in the liver of rats 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.

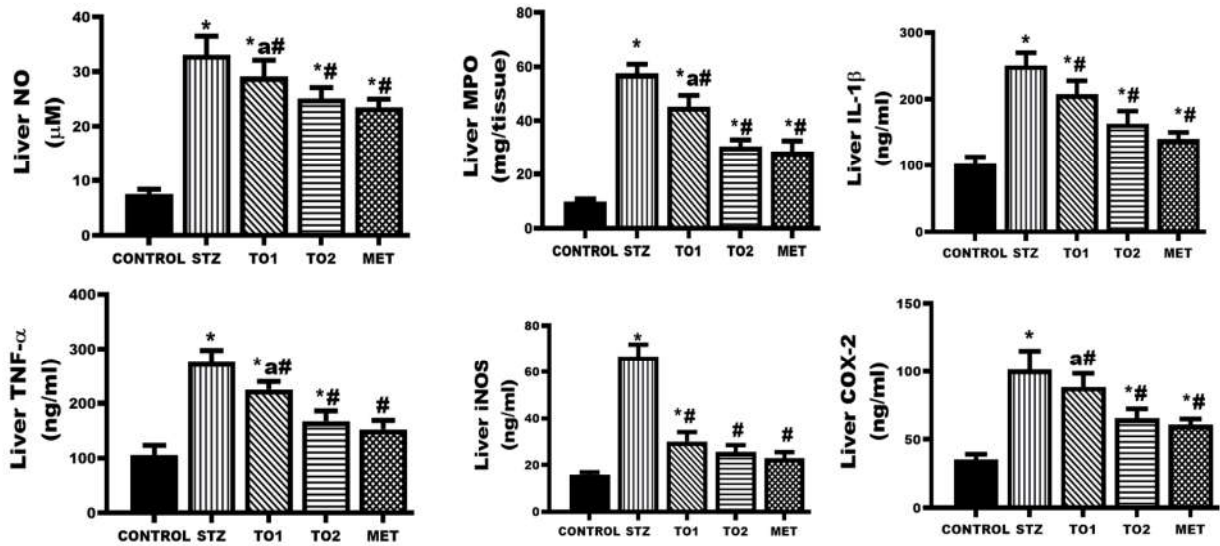


Figure 2: Effect of *T. occidentalis* (TO) on NO, MPO, IL-1β, TNF-α, iNOS and COX-2 in the liver of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, \*  $p < 0.05$  against control, #  $p < 0.05$  against STZ, a  $p < 0.05$  against MET.

#### Effects of *T. occidentalis* on liver caspase-3 activation and Histological in STZ-induced diabetes

Treatment with STZ alone caused a significant increase ( $P < 0.05$ ) in caspase-3 activity in the liver of diabetic rats when compared with control (figure 4). In contrast, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) markedly reduced the activity of caspase-3 in the liver of diabetic rats when compared with group exposed to STZ alone. Similar trend was observed in the group treated with metformin (300mg/kg) when compared with group exposed to STZ alone. The Histological examination of the liver of the diabetes rats revealed hyperplasia and severe periportal infiltration of inflammatory cells. Administration of *T. occidentalis* (200mg/kg and 300mg/kg) preserved the liver histological architecture and reduced periportal infiltration by inflammatory cells

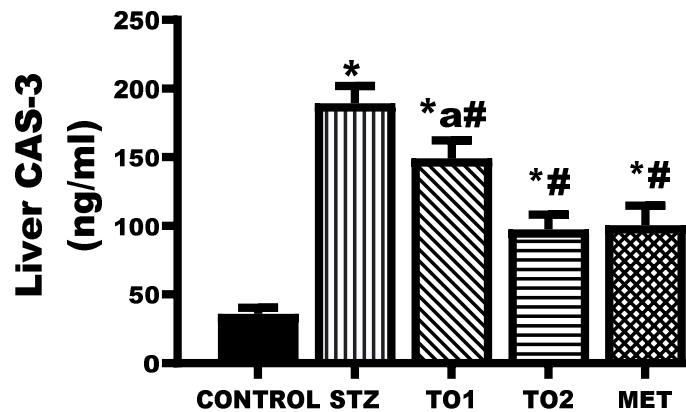


Figure 3: Effect of *T. occidentalis* (TO) on activities caspase-3 in the liver of streptozotocin-induced diabetic rats. Data are expressed as mean ± SEM; n = 7 rats, \*  $p < 0.05$  against control, #  $p < 0.05$  against STZ, a  $p < 0.05$  against MET.

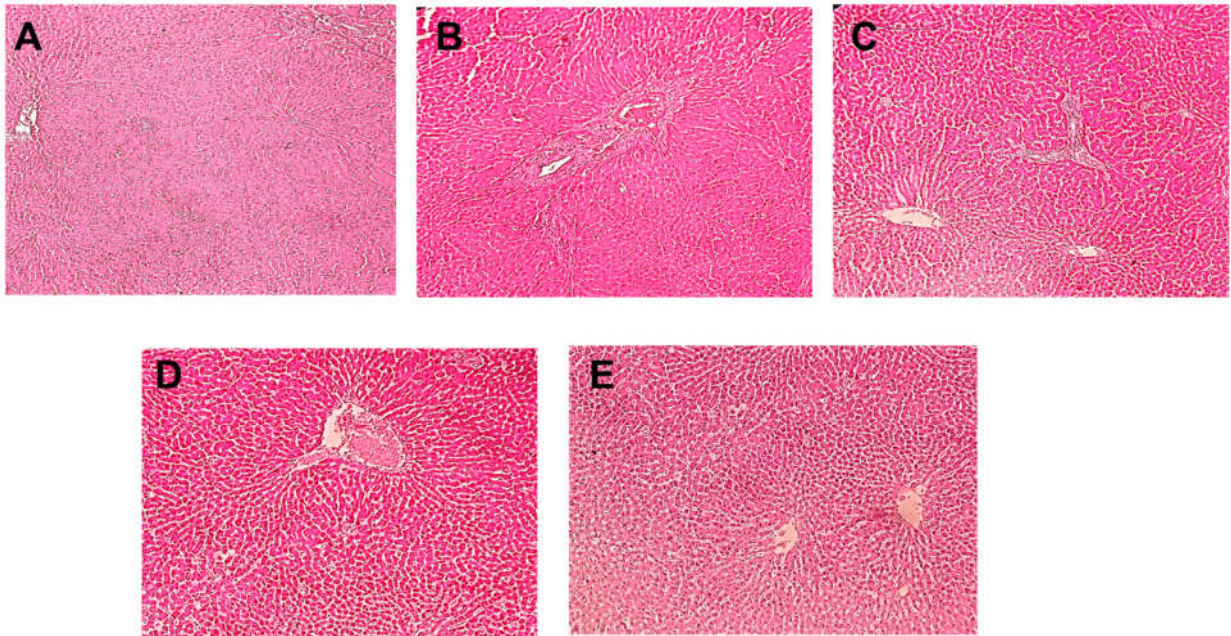


Figure 4: Hematoxylin and Eosin (H&E) stained section of the liver showing the effect *T. occidentalis* (TO) on streptozotocin-induced diabetic rats. Control (A): no visible lesion; STZ (B): hyperplasia and severe periportal infiltration of inflammatory cells. TO1 (C): Moderate periportal infiltration of inflammatory cells TO2 (D): mild periportal infiltration of inflammatory cells. MET (E): no visible lesion. Mag x 40

## DISCUSSION

Diabetes is a disease associated with associated metabolic dysfunction and pathophysiological changes in the liver.<sup>[29][30]</sup> The present study evaluated the antihepatotoxic properties of *T. occidentalis* in animal model of diabetes- induced liver damage.

Oxidative stress (OS) is a condition defined by an imbalance between the physiological production of reactive oxygen species (ROS) and antioxidants. OS has been reported to play an important role in diabetic associated hepatic injury.<sup>[31]</sup> It has therefore, been documented that suppressing OS may alleviate diabetes-associated hepatic dysfunction. Endogenous antioxidants such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) as well as glutathione peroxidase (GPx) synergistically act as a cellular defensive mechanism against the obnoxious effect of ROS.

Superoxide dismutase (SOD) is an enzymatic antioxidant that breaks down superoxide radical to hydrogen peroxide. Catalase (CAT) decomposes hydrogen peroxide in living cells and protects the cell against oxidative damage by ROS. Glutathione is a ubiquitous non enzymatic antioxidant that facilitates the excretion of xenobiotics.<sup>[32]</sup> In the present study, it was revealed that STZ-induced diabetes caused a significant depletion of SOD, CAT and GPx activities as well as GSH levels, implying that they were highly utilized in the mop up of reactive oxygen species generated by STZ-induced diabetes in rats. An elevation of these antioxidant enzymes in the liver of diabetic rats treated with *T. occidentalis* suggests the alleviation of free radical damage. This effect most likely, is due to the antioxidant properties of *T. occidentalis*. Metformin was used as a positive control and our data showed that the protective effects of *T. occidentalis* against diabetes induced liver damage are comparable to that of metformin.

Furthermore, lipid peroxidation plays a substantial role in the development and progression of diabetic complications.<sup>[33]</sup> MDA, a product of lipid peroxidation, is an indication of the extent of lipid peroxidation in tissues. An increase in the hepatic levels of MDA has been reported in diabetic rats.<sup>[34][35][36][37]</sup> The results from the present study corroborates these observations. Treatment of diabetic rats with *T. occidentalis* significantly reduced lipid peroxidation, an indication its free radical scavenging property.

Inflammation has been reported to contribute a vast role in the onset of diabetes and diabetes complications.<sup>[38]</sup> IL-1 $\beta$  is a pro-inflammatory cytokine crucial for the activation of pro-inflammatory signaling, leading to tissue damage.<sup>[39]</sup> As an important factor in the inflammatory signaling network, TNF- $\alpha$  participates in chronic

inflammatory reactions and induces apoptosis in then liver.<sup>[40][41][42]</sup> Elevated TNF- $\alpha$  level has been shown to activate inducible nitric oxide synthase (iNOS) which subsequently increases NO generation.<sup>[43]</sup> COX-2 is an inducible enzyme that is elevated in atherosclerosis and it may contribute to initiation of atherosclerotic plaque by inducing metalloproteinases.<sup>[44]</sup> NO is a reactive nitrogen specie and can act as a potent pro-inflammatory mediator at concentrations above physiological level. In the present study, treatment with *T. occidentalis* (200mg/kg and 300mg/kg) prevented periportal infiltration of inflammatory cells in the liver and significantly reversed the increased levels of NO, iNOS, TNF- $\alpha$  and IL-1 $\beta$ , as well as MPO and COX-2 activation in the liver of diabetic rats, thus, suppressing inflammation. These findings further potentiate the anti-inflammatory properties of *T. occidentalis*.

Apoptosis is a programmed cell death that is tightly regulated to maintain tissue homeostasis, but uncontrolled apoptosis has been implicated in the pathobiology of diabetes.<sup>[45]</sup> Several studies have shown a correlation between diabetes, oxidative stress and apoptosis.<sup>[46][47][48][49]</sup> Oxidative stress and apoptosis have been reported in the liver of diabetic rats.<sup>[50]</sup> Similarly, our work corroborates these observations as caspase-3 activity was significantly increased in the liver of diabetic rats in this study. Treatment with *T. occidentalis*, however, protected hepatic cells from activation of apoptotic protein, suggesting the anti-apoptotic property of *T. occidentalis*.

In conclusion, the present study demonstrated the potentials of *T. occidentalis* in ameliorating the biochemical changes in the liver of rats following experimental induction of diabetes using STZ. The ameliorative activity of *T. occidentalis* in the liver of STZ-induced diabetic rats could partly be related to its antioxidant and anti-inflammatory properties.

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