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Telfairia occidentalis Ameliorates Streptozotocin-induced Testicular Oxidative stress by Restoring Endogenous Antioxidant Enzyme Activity and Inhibiting Apoptosis, and Pro-inflammatory cytokines

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Abstract

The study investigated the protective role of *Telfairia occidentalis* (TO) against Streptozotocin-induced testicular damage in male Wistar rats, by observing the levels of prooxidants, endogenous antioxidant enzymes inflammatory biomarkers, as well as apoptotic proteins. Thirty-five animals were used for this study and shared into five groups of seven animals each. Group 1 (normal control) received distilled water throughout the experiment. Groups 2-5 received 10% fructose ad libitum for 14 days followed by a single injection of 40 mg/kg body weight streptozotocin, intraperitoneally. After confirmation of diabetes mellitus, group 2 rats received 0.5ml distilled water, group 3 received TO (200 mg/kg body weight), group 4 received TO (300 mg/kg body weight) and group 5 received Metformin (300 mg/kg body weight). All treatments lasted for 28 days, followed by the sacrifice of all experimental animals and the harvest of their testes for biochemical analyses. Results revealed that streptozotocin administration decreased the activities of the antioxidant enzymes SOD, GSH, GST, GPx, CAT while elevating MDA levels in groups 2-5 animals when compared with group 1. Treatment with TO showed increased activities of antioxidant enzymes and reduced MDA levels.

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Furthermore, streptozotocin administration increased the levels of the inflammatory biomarkers MPO, IL-1β, TNF-α, iNOS, COX-2, and the apoptotic protein, caspase-3 in groups 2-5 animals when compared with group 1. However, these elevations were reversed by the administration of TO. In conclusion, *Telfairia occidentalis* demonstrates significant protective effects on the antioxidant status and inhibits oxidative stress markers, and inflammatory cytokines in the testes of diabetic rats. This indicates its potential therapeutic value in mitigating diabetes-related testicular complications through the modulation of oxidative stress and inflammatory pathways.

Keywords: Testicular damage, Streptozotocin, *Telfairia occidentalis*, Antioxidant enzymes, inflammatory biomarkers

INTRODUCTION

Millions of individuals are impacted by diabetes mellitus (DM), a dangerous metabolic disease. In actuality, it is predicted that by 2040, there would be 642 million cases of DM worldwide (Ogurtsova *et al.,* 2017). While nephropathy, retinopathy, and diabetic cardiomyopathy are the most frequently studied complications of diabetes, male sexual and reproductive dysfunction which affects both diabetic patients and animals is gaining more attention from researchers (Agbaje 2007; Kanter *et al.,* 2012). In the context of diabetes mellitus, low testosterone levels and testicular injury can result in erectile dysfunction, decreased sperm motility, and reduced seminal fluid volume (Feyli *et al.,* 2017). Even though one of the most common consequences of diabetes is testicular failure, the fundamental mechanisms in the testes that cause hypogonadism remain poorly understood. The process of removing dying cells from the population of proliferating or differentiating cells is known as apoptosis or programmed cell death; it is believed to be a key factor in the pathophysiology of hypogonadism (Hikim and Swerdloff, 1999). One of the main causes of infertility in diabetic animals is thought to be apoptotic cell death, which is markedly elevated in the seminiferous tubules of streptozotocin (STZ)-induced diabetic mice and rats (Guneli *et al.,* 2008; Sainio-Pöllänen *et al.,* 1997).

The pathophysiology of decreased spermatogenesis and germ cell loss is influenced by oxidative stress (Cai *et al.,* 2000; Tsounapi *et al.,* 2012). Excessive oxidative stress in diabetic testes can lead to depleted antioxidant defenses as well as an overproduction of reactive oxygen species (ROS). Cell damage or malfunction will eventually result from superoxide

accumulation if the equilibrium between ROS formation and ROS scavenging mechanisms is upset. Moreover, oxidative damage caused by diabetes might trigger apoptosis (Kilarkaje and Al-Bader, 2014). Antioxidants are being used more and more as potentially helpful therapeutic agents due to the negative effects of oxidative stress (Heeba and Hamza 2015).

Telfairia occidentalis, a tropical vine plant native to West Africa, is valued for its nutritional and medicinal properties (Adaramoye *et al.,* 2007). It is known to contain bioactive compounds, including flavonoids and polyphenols, which exhibit antioxidant properties (Saalu *et al.,* 2010). Evidence supports the benefits of *Telfairia occidentalis* in mitigating the adverse effects associated with diabetes (Horsefal and Spiff, 2005). Therefore, this study was designed to investigate the potential of *Telfairia occidentalis* to ameliorate streptozotocin-induced testicular damage.

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

Telfairia occidentalis (TO) leaves were obtained from a nearby farm in Okuku, Cross River State, Nigeria. The botanical verification of the plant was conducted at the herbarium of the Department of Botany, University of Ibadan where a specimen was deposited in the herbarium and the voucher number: UIH 63457 was issued. The TO leaves were air-dried and then ground using an electronic blender. Following this, the leaves were immersed in absolute ethanol (1:3 ratio) for 72 hours. The resulting solution was filtered through Whatman paper size 1, and the filtrate was subsequently concentrated at 40ºC.

Animal Model

Ethical approval for the treatment and handling of experimental animals was obtained from the Faculty of Basic Medical Science Animal Ethical Committee University of Cross River State with an approval number: FBMS/UNICROSS/21/035. A total of thirty-five (35) male albino rats of the Wistar strain, weighing approximately 100-250g, were procured from the animal holding facility within the Faculty of Basic Medical Sciences at the University of Cross River State. These rats were housed in well-ventilated plastic cages with wire mesh covers, distributed across five groups, each containing seven rats. Prior to the initiation of the study, a 14-day acclimatization period was observed, during which the rats had unrestricted access to rodent chow and drinking water.

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 14 days

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

Group 3: Served as the TO1 group and received 10% fructose for 14 days prior to single administration of streptozotocin 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 testes homogenate

All rats were euthanized 24 hours following the last treatment. The testes were carefully removed, washed in 1.15% potassium chloride, and homogenized in 0.1 M phosphate buffer (pH 7.4). The resultant homogenates underwent centrifugation at 10,000g and temperature of 4°C for 10 minutes, using a cold centrifuge, to yield the post-mitochondrial fraction utilized for the biochemical assays.

Determination of Oxidative stress biomarkers in testes

Catalase (CAT) activity was estimated using hydrogen peroxide as substrate according to the method of Clairborne. (1995), Glutathione peroxidase (GPx), Glutathione (GSH) and Glutathione S-tansferase (GST) activity was determined by the method of Rotruck. (1973). Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich. (1972). Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi *et al.* (2005) and expressed as micromoles of MDA per gram tissue.

Determination of Testes Inflammatory and Apoptotic Biomarkers

Myeloperoxidase (MPO) activity was determined according to the method of Granell *et al.* (2003). Nitric oxide (NO) level was determined according to the method of Ajayi *et al.* (2018). Caspase-3, TNF-α, IL-1β, 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).

Statistical analysis

All data were expressed as mean ±SEM. Statistical analysis using Analysis of Variance and complemented with Duncan post hoc using GraphPad Prism statistics, version 8.0. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The results in Figure 1 (a-d) revealed that administration of Streptozotocin (STZ) in rats significantly ($p<0.05$) reduced the activities of SOD, GSH, GST, and GPx. Treatment with *Telfairia occidentalis* (TO) (200mg/kg and 300mg/kg) significantly ($p<0.05$) increased SOD, GSH, GST and GPx compared with rats exposed to STZ.

Results in Figure 2 (a-d) shows that STZ administration in rats significantly ($p<0.05$) reduces CAT activities with a concomitant increase in MDA, NO and MPO activities compared with control. Treatment with *Telfairia occidentalis* (TO) (200mg/kg and 300mg/kg) significantly ($p<0.05$) increased CAT with a corresponding decrease in MDA, NO, and MPO level compared with STZ exposed rats.

Similarly, STZ induction significantly ($p<0.05$) increased the levels of Interleukin-1 beta (IL-1β), Tumor Necrosis Factor alpha (TNF-α), inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase-2 (COX-2) and caspase-3 compared with control. Administration of *Telfairia occidentalis* (TO) (200mg/kg and 300mg/kg) significantly ($p<0.05$) decreased the levels of Interleukin-1 beta (IL-1β), Tumor Necrosis Factor alpha (TNF-α), inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase-2 (COX-2) and caspase-3 in the testes of diabetic rats compared with STZ induced rats as shown in Figures 3 (a-d)-4.

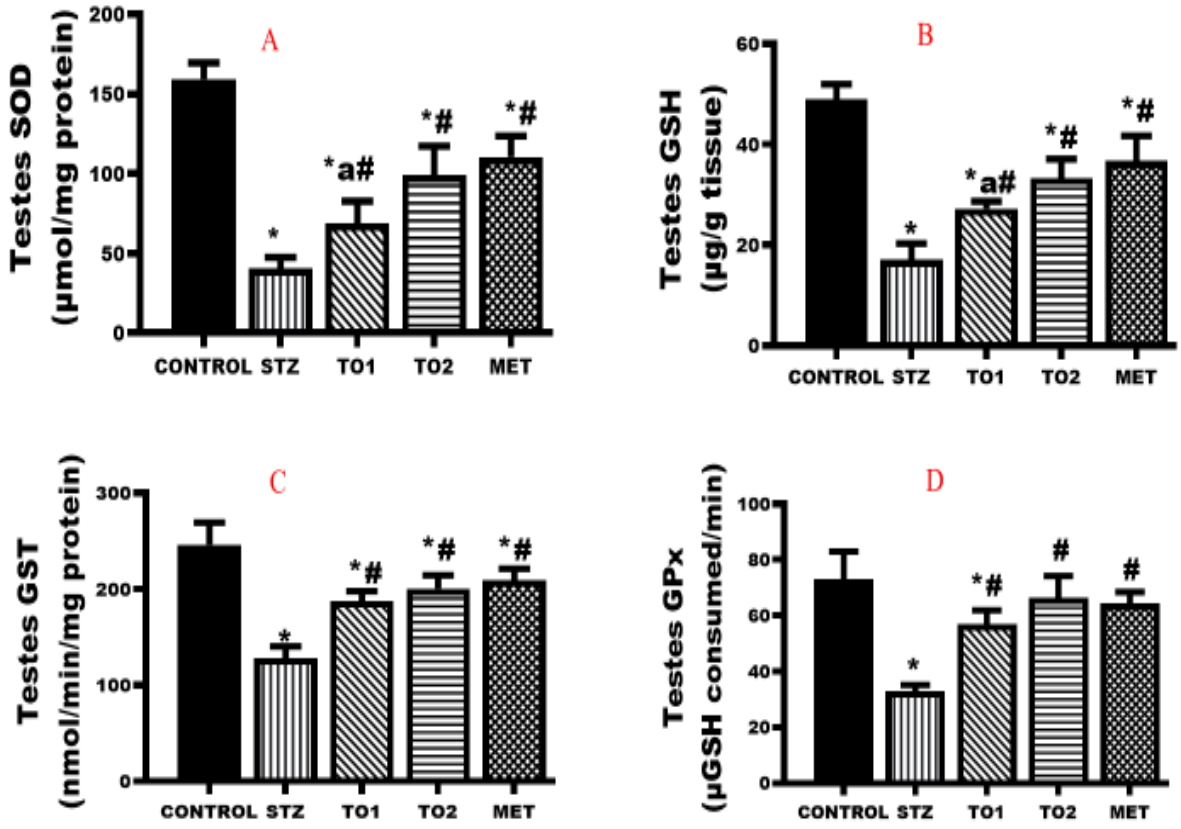


Figure 1: Effect of *T. occidentalis* (TO) on SOD, GSH, GST, and GPx activities in the testes of streptozotocin-induced diabetic rats.

Data is expressed as mean ± SEM; n = 7

*= significant difference from control group at $p< 0.05$

*,#= significant difference from control and BPH group at $p< 0.05$

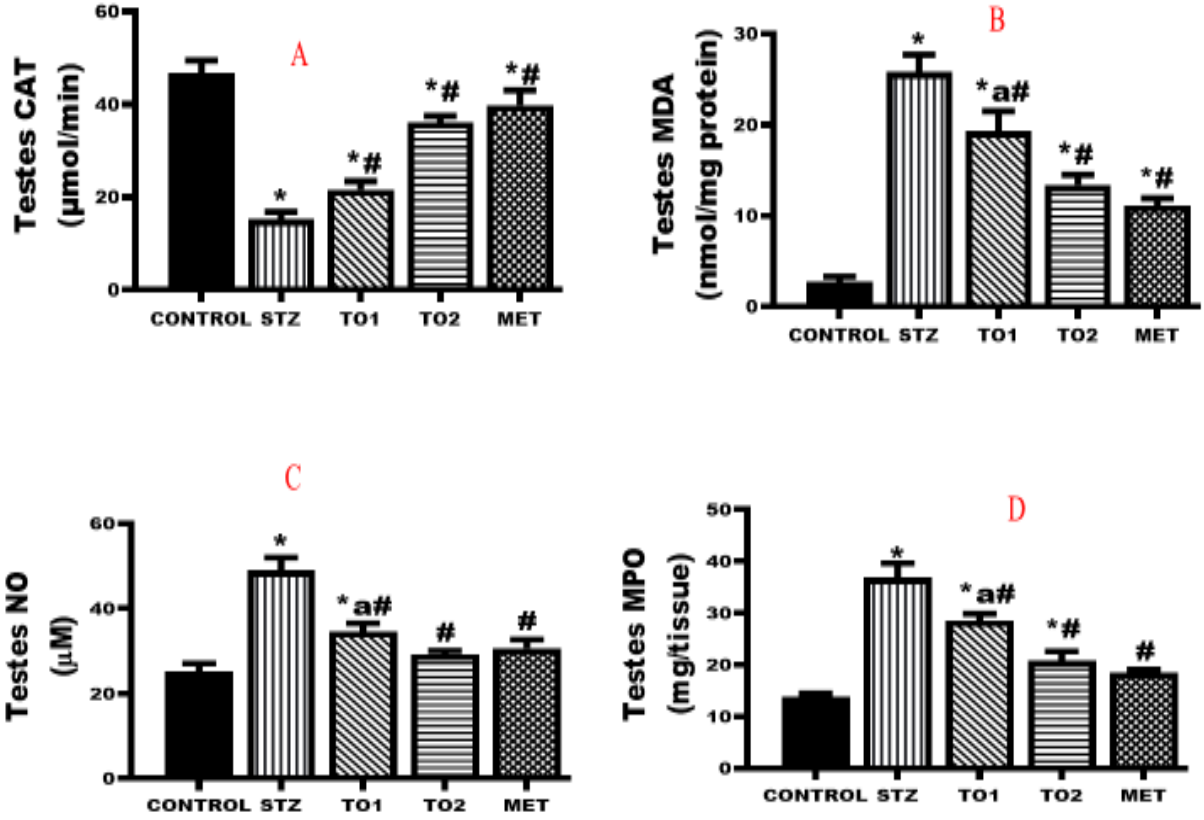


Figure 2: Effect of *T. occidentalis* (TO) on CAT, MDA, NO, and MPO activities in the testes of streptozotocin-induced diabetic rats.

Data is expressed as mean ± SEM; n = 7

*= significant difference from control group at $p< 0.05$

*,#= significant difference from control and BPH group at $p< 0.05$

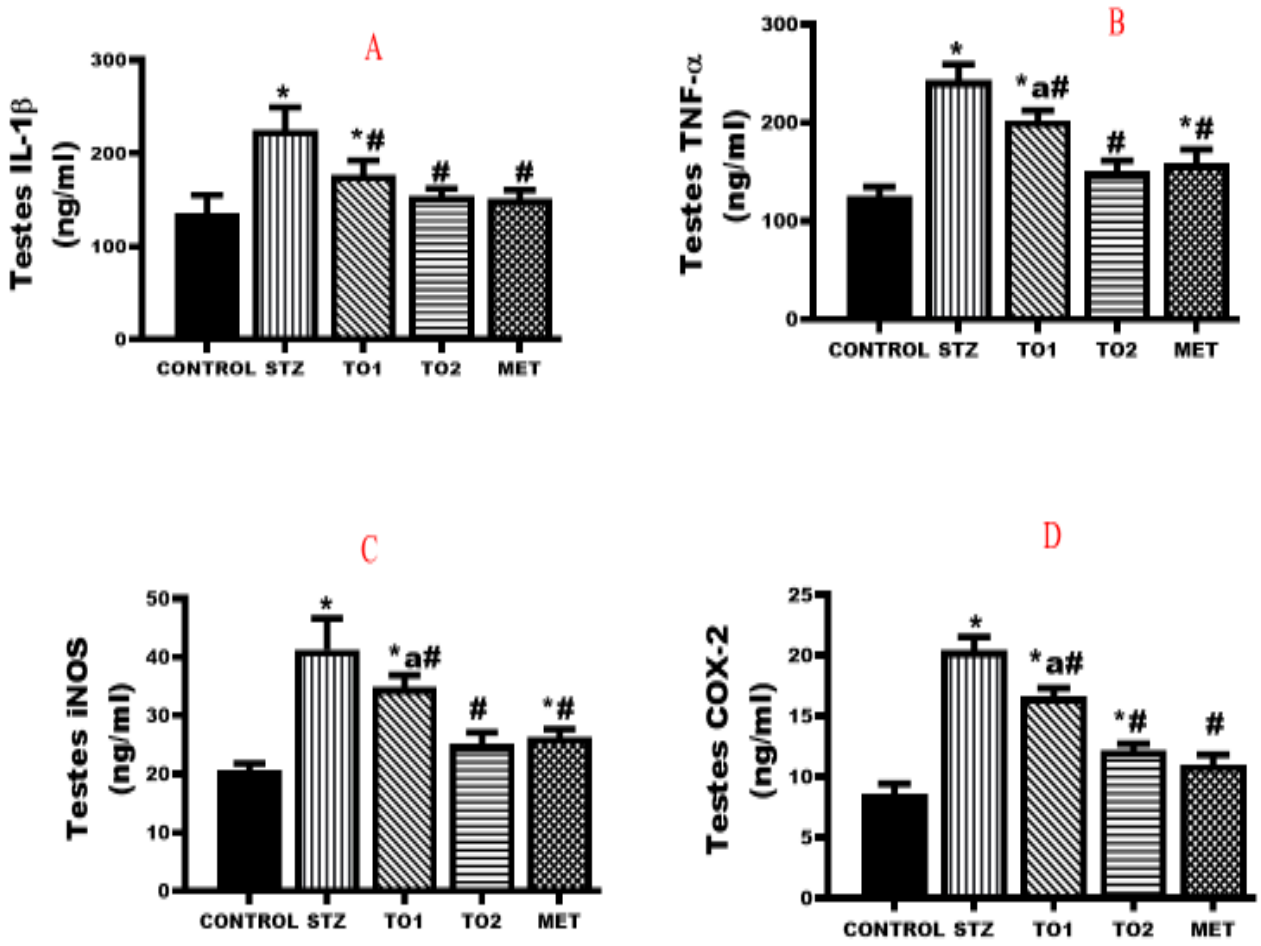


Figure 3: Effect of *T. occidentalis* (TO) on IL-1β, TNF-α, iNOS and COX-2 activities in the testes of streptozotocin-induced diabetic rats.

Data is expressed as mean ± SEM; n = 7

*= significant difference from control group at $p < 0.05$

*,#= significant difference from control and BPH group at $p < 0.05$

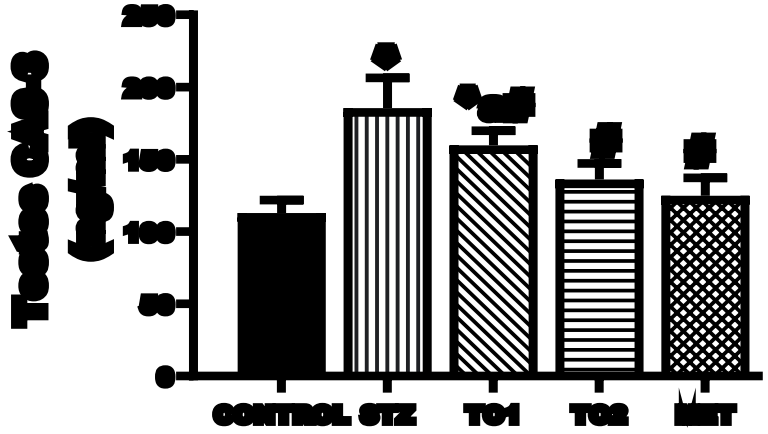


Figure 4: Effect of *T. occidentalis* (TO) on CAS-3 activities in the testes of streptozotocin-induced diabetic rats.

Data is expressed as mean ± SEM; n = 7

*= significant difference from control group at p< 0.05

#= significant difference from BPH group at p< 0.05

DISCUSSION

Cumulative evidence suggests that diabetes-induced testicular apoptosis is mostly due to elevated oxidative stress (Wang *et al.,* 2014). Oxidative stress induces testicular damage in many ways. Increased ROS production induces lipid peroxidation and mitochondrial lesions in germ cells, leading to dysfunction in testicular spermatogenesis and steroidogenesis (Aitken *et al.,* 1993; Diemer *et al.,* 2003). Moreover, increased ROS generation leads to DNA damage and germ cell abnormalities due to its genotoxic effects in the testes (Rajesh *et al*., 2001). Therefore, ameliorating oxidative stress in diabetic rats represents a method to attenuate testicular injury. In the present study, endogenous antioxidant enzymes, including SOD, GSH, GST, GPx and CAT were suppressed, while the lipid peroxidation biomarker, MDA, was significantly elevated in the testes of diabetic rats. Moreover, the present data clearly confirmed the marked increase in ROS in diabetic rat testes. These results are consistent with the increased cellular oxidative stress and lipid peroxide accumulation previously demonstrated in experimental diabetic animals (Rajesh *et al*., 2001; Ujong, 2021a; Ujong, 2021b; Ujong and Nkanu, 2021; Ujong *et al.,* 2024). Treatment with *T. occidentalis* increases endogenous antioxidant enzymes, including SOD,

GSH, GST, GPx and CAT, while decreasing lipid peroxidation biomarker, MDA. This is suggestive of the therapeutic efficacy and protective potential of *T. occidentalis*, which has been reported to contain a variety of bioactive compounds including flavonoids and phenolic acids (Aubert *et al.*, 2019).

Caspase-3, a pivotal executioner caspase, plays a central role in apoptosis, the programmed cell death crucial for maintaining testicular homeostasis and germ cell development. Studies have demonstrated the involvement of caspase-3 in various testicular pathologies, including testicular ischemia-reperfusion injury (IRI), testicular torsion, and testicular germ cell tumours (TGCTs) (Shalaby *et al.,* 2011). Activation of caspase-3 triggers a cascade of events leading to DNA fragmentation and cell death. In testicular tissues, dysregulation of caspase-3 activity has been linked to impaired spermatogenesis and male infertility (Wang *et al.,* 2012). Inducible nitric oxide synthase (iNOS) is an enzyme responsible for the production of nitric oxide (NO), a signalling molecule with diverse functions in the testes. NO serves as a regulator of vascular tone, immune response, and germ cell apoptosis. While moderate levels of NO are essential for physiological functions such as spermatogenesis, excessive NO production mediated by iNOS under inflammatory conditions can lead to testicular damage and dysfunction (Herrero *et al.,* 1997). iNOS-derived NO has been implicated in testicular inflammation, germ cell apoptosis, and impaired sperm motility (Aydos *et al.,* 2004). Interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are pro-inflammatory cytokines involved in orchestrating immune responses and inflammation within the testicular microenvironment. Elevated levels of IL-1β and TNF-α have been observed in various testicular pathologies, including orchitis, varicocele, and testicular injury (Mahmoudi *et al.,* 2017). These cytokines stimulate the production of other inflammatory mediators, promote leukocyte recruitment, and contribute to oxidative stress, ultimately leading to tissue damage and impaired testicular function (Zhao *et al.,* 2014). Myeloperoxidase (MPO), primarily expressed in neutrophils, is an enzyme involved in the generation of reactive oxygen species (ROS) and oxidative stress. In the testes, MPO activity has been associated with inflammatory conditions such as orchitis and epididymitis (Tsukaguchi *et al.,* 1987). Increased MPO levels contribute to oxidative damage to spermatozoa and testicular tissues, exacerbating inflammation and impairing fertility (Mostafa *et al.,* 2006). Cyclooxygenase-2 (COX-2) is an enzyme responsible for the synthesis of prostaglandins, lipid mediators involved in inflammation and pain. In the testes, COX-2 expression is induced under inflammatory stimuli,

contributing to the production of prostaglandins and amplification of inflammatory responses (Brudieux *et al.,* 2005). Dysregulated COX-2 activity has been implicated in testicular inflammation, germ cell apoptosis, and impaired spermatogenesis (Manente *et al.,* 2006).

Furthermore, the induction of streptozotocin (STZ) markedly ($P<0.05$) increased the levels of inflammatory biomarkers, including caspase-3, iNOS, IL-1β, NO, TNF-α, MPO, and COX-2 thus, signaling testicular inflammation as a result of streptozotocin induction. This result corroborates our earlier reports that *Telfairia occidnetalis* ameliorates inflammation in the plasma and hepatic tissues of streptozotocin-induced diabetic rats by inhibiting the generation of inflammatory biomarkers (Ujong, 2021a; Ujong and Nkanu, 2021). Aja *et al.* (2021) also investigated the protective effects of *Moringa oleifera* phytocompounds against cancer upsurge and thus, documented the detrimental rise in inflammatory biomarkers. Similarly, Myeloperoxidase (MPO), Interleukin-1 beta (IL-1β), Tumor Necrosis Factor alpha (TNF-α), Nitric oxide (NO), inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase-2 (COX-2), and caspase-3 were all shown to be considerably ($P<0.05$) reduced upon administration of *Telfairia occidentalis*. This may be due to the potent antioxidant and free radical-scavenging properties of the plant extract. The leaves can help reduce oxidative stress, which is a common occurrence in disorders like cancer, hepatic ailments, and damaged testicles (Osukoya *et al*., 2016; Ujong, 2021a; Ujong, 2021b; Ujong and Nkanu, 2021; Ujong *et al.,* 2024). Therefore, Testicular damage can be mitigated and spermatogenesis can be boosted by *Telfairia occidentalis*.

CONCLUSION

Telfairia occidentalis shows significant potential as a treatment for testicular complications induced by diabetes. Its ability to modulate oxidative and inflammatory pathways, positions it as a comprehensive remedy for the diverse issues arising in diabetic testes. These findings contribute to the growing body of research advocating for the utilization of *Telfairia occidentalis* in complementary or alternative approaches to managing testicular diseases linked to diabetes.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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