

## Chapter

# Potential Antioxidative Effects of Kolaviron on Reproductive Function in Streptozotocin-Induced Diabetic Wistar Rats

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## Abstract

The present study investigated the effects of Kolaviron (KV) on the testicular and epididymal tissue antioxidant status in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ at 50 mg/kg body weight. The antioxidant status was studied by evaluating epididymal and testicular levels of malondialdehyde (MDA), a lipid peroxidation (LPO) marker, and the activities of catalase (CAT) glutathione peroxidase (GPX) and superoxide dismutase (SOD) were also assessed using biochemical techniques. Diabetes induction resulted in testicular and epididymal LPO and adversely affected the activities of antioxidant enzymes evident by a noticeable decrease in enzyme activity in both tissues. The potential antioxidative effects of KV in the testicular and epididymal tissues of STZ-induced diabetes were revealed by its ability to mitigate against LPO and increase the activity of antioxidant defense enzymes in the reproductive tissues studied. KV might potentially be used as an antioxidant as well as antidiabetic treatment; however, further studies are needed.

**Keywords:** antioxidant, diabetes, infertility, Kolaviron, oxidative stress, streptozotocin

## 1. Introduction

Infertility is a reproductive health disease defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Male factor infertility can contribute between 30 and 50% to this condition and may arise from several factors such as physiological, systemic pathologies, genetic abnormalities, environmental pollution, and oxidative stress (OS) [2, 3].

OS is described as an imbalance between the production of reactive oxygen species (ROS) and their removal or reducing agents called antioxidants [4]. This state of OS potentially leads to the damage of biomolecules such as proteins, nucleic acids, and lipids [4]. In recent years, OS has become more prevalent and has significantly contributed to abnormal sperm morphology [5–8] and sperm quality

and quantity [6, 7, 9]. In the testes, OS is capable of disrupting the steroidogenic capacity of Leydig cells as well as the spermatogenesis process [10]. Spermatozoa contain polyunsaturated fats (PUFAs) and limited cytoplasm antioxidant enzymes [11] and are susceptible to oxidative attack. The free radical attack can induce lipid peroxidation (LPO) and DNA fragmentation, disrupting both sperm development and motility [2, 11].

Numerous disorders of the male reproductive system such as cancer, varicocele, cryptorchidism, testicular torsion of the spermatic cords, and diabetes have been associated with male infertility due to OS caused by the uncompensated hyperproduction of ROS [12]. The OS derived from diabetic mellitus (DM) may affect the male reproductive function [11]. Diabetes mellitus is a group of metabolic conditions that are characterized by high glucose levels (hyperglycemia) caused by abnormal insulin secretion/insulin deficiency, abnormal insulin action, or both. It has been demonstrated that diabetes has a direct effect on male fertility [13]. OS in diabetic patients develops from pathways including the nonenzymatic, enzymatic, and mitochondrial signaling pathways [12–14]. Although the problems arising from DM have been widely investigated, the mechanisms responsible for the male reproductive dysfunction are still poorly understood [15]. In hyperglycemic patients, glucose undergoes autoxidation and reacts with proteins leading to the development of Amadori products and advanced glycosylation end products (AGEs). In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in the enhanced production of superoxide [11, 16]. Another enzymatic generation of ROS is via the mitochondrial respiratory chain through the oxidative phosphorylative process where electrons are transferred from electron carriers NADH and FADH<sub>2</sub>, through four complexes in the inner mitochondrial membrane, to oxygen, generating ATP in the process [17]. Hyperglycemic conditions disturb endothelial cells, and ROS are produced which participate in the development of diabetic complications. There are enzymatic overproductions of ROS in diabetes through NADPH oxidase that enhance O<sub>2</sub><sup>-</sup> [18].

Diabetes-related OS, endocrine disorders, and neuropathy may contribute to the reproductive impairment by causing sexual function alterations including testicular function and epididymal sperm transit [19]. Moreover, under OS condition the protective effects of testicular and epididymal antioxidant enzymes decline [6, 20].

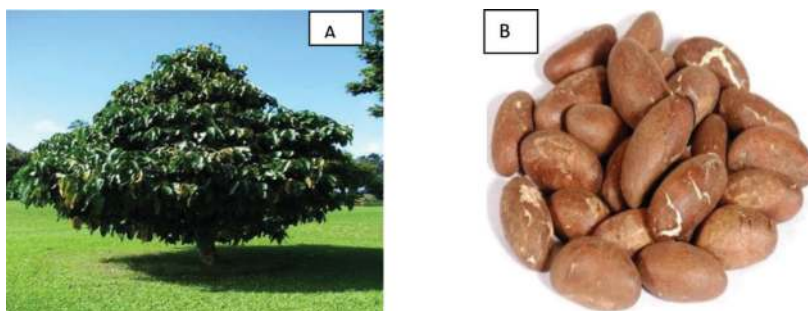
Many artificial and natural agents possessing antioxidant properties, such as dietary antioxidants, may be of great importance as additional protective measures and have been proposed to prevent and/or treat oxidative damage induced by DM [6]. The use of derivatives of plant materials might be important and effective because they are less toxic and affordable as well as minimize side effects or risks caused by other options [20]. Phytochemicals are considered strong natural antioxidants and play an important role in healthcare systems [6]. They have adaptive characteristics to respond to stress and help regulate the interconnected endocrine, immune, and nervous systems [6]. Moreover, ethnobotanical research, literature reviews, and experimental studies reported the beneficial effect of plant materials which have been used many years ago in prevention and treatment of diabetes as well as its complications [20]. More than 1200 flowering plants have been claimed to possess antidiabetic properties [22–24]. These properties have been found to be present in different parts of plants such as the aerial parts, bark, flowers, roots, seeds, leaves, bulbs, tubers, and/or the whole plant [22–24].

*Garcinia kola* (*G. kola*), commonly referred to as bitter kola, is one of such plants that has been widely used in ethnomedicine [25]. The therapeutic and medicinal values of *Garcinia kola* are the subject of many studies, and several researchers have described their functional health benefits [5, 26–30]. *G. kola* is an angiosperm plant that belongs to the family of Guttiferae (Clusiaceae) [5, 28–30]. It adapts and grows

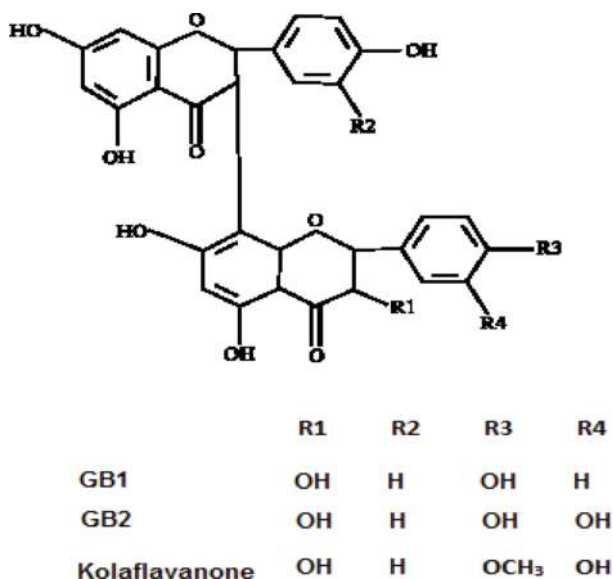
up in moist lowland forest and subtropical or tropical region. *Garcinia kola* tree is up to 14 m high and produces brown nut-like seeds (**Figure 1**). *G. kola* is highly valued in African countries such as Nigeria, Benin, Cameroon, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Senegal, and Sierra Leone. These countries used *G. kola* seeds as source of food and medication [5].

Moreover, phytochemical screening of *Garcinia kola* seed showed the presence of polyphenol compound, which is a bioflavonoid Kolaviron (KV). This extract was found in a 2:2:1 ratio of bioflavonoid GB1, GB2, and kola flavanone [21] (**Figure 2**). Polyphenolic compounds are composed of three benzene rings with hydroxyl (OH) groups which preserve antioxidant activity [31, 32] (**Figure 2**).

KV, extract from *G. kola* nut, has shown great potential for use in therapeutic medicine against many health-threatening chronic diseases of the liver and reproductive system and diabetes [5, 25–30]. It is widely used in traditional medicine in southern Nigeria for the treatment of different conditions associated with increased OS [5]. KV is known to possess antihyperglycemic effects in normal and alloxan- and streptozotocin (STZ)-induced diabetic animals [21, 25]. Moreover, KV has elicited strong antioxidant activity in in vivo and in vitro models [33]. This property is due to the high flavonoid (bioflavonoids) contents which are able to terminate the free radical chain reactions in response to OS [33].



**Figure 1.**  
*Garcinia kola* tree (A) and its brown seeds (B).



**Figure 2.**  
 Chemical structure of KV isolated from *Garcinia kola* seed.

This study was therefore designed to evaluate any potential effects of KV in boosting testicular and epididymal antioxidant status in STZ-induced diabetic Wistar rats.

## 2. Materials and methods

### 2.1 Plant materials and KV extraction

Fresh seeds of *G. kola* were purchased from the Bodija market in Ibadan, Oyo State, Nigeria, and authenticated by Professor E. Ayodele at the Department of Botany, University of Ibadan. A voucher specimen (FHI-109777) is available at the University of Ibadan, at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan.

KV was extracted and isolated according to the method of [34]. The seeds were peeled, sliced, and air-dried (25–28°C). Briefly, the powdered seeds were extracted with light petroleum ether (boiling point, 40–60°C) in a soxhlet for 24 h. The defatted dried product was repacked into the soxhlet and extracted with acetone. The extract was concentrated and diluted twice its volume with water and extracted with ethylacetate (6 × 300 mL). The concentrated ethylacetate yielded a golden yellow solid termed KV.

Liquid chromatography-mass spectrometry (LC-MS) analysis of the *Garcinia kola* seed extract was performed on a Dionex HPLC system (Dionex Softron, Germering, Germany) equipped with a binary solvent manager and autosampler coupled to a Bruker ESI Q-TOF mass spectrometer (Bruker Daltonik GmbH, Germany) as previously described [35]. KV was separated by reversed-phase chromatography on a Thermo Fischer Scientific C18 column 5 µm, 4.6 × 150 mm (Bellefonte, USA), using gradient elution with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as solvent at a flow rate of 1.0 mL min<sup>-1</sup>, an injection volume of 10 µL, and an oven temperature of 30°C. MS spectra were acquired in negative mode using the full scan and auto MS/MS (collision energy 25 eV) scan modes with dual spray for reference mass solution. Electrospray voltage was set to +3500 V. Dry gas flow was set to 9 L min<sup>-1</sup> with a temperature of 300°C, and nebulizer gas pressure was set to 35 psi.

### 2.2 Treatment of animals and ethical clearance

Sixty adult male Wistar rats, weighing between 240 and 290 g, were purchased from the animal facility of the Medical Research Council, South Africa. The animals were accommodated individually in plastic cages. They were supplied with water and standard rat feed ad libitum. Animals were maintained under standard laboratory conditions at 22 ± 2°C with a 12-h light/dark cycles and humidity at 55 ± 5%. Body weights were measured from the onset of the study and monitored throughout the feeding period until sacrifice. All animals received care according to the principles of Laboratory Animal Care of the National Society of Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health publication no. 80-23, revised 1978). The study was approved by the Ethical Committee of the Faculty of Health and Wellness Sciences, University of Cape Peninsula Technology, South Africa (Cape Town, South Africa) (NHREC: REC-230408-014).

### 2.3 Experimental induction of diabetes

The animals were fasted overnight, and diabetes was induced by a single intraperitoneal injection (50 mg/kg body weight) of freshly prepared STZ

solution (Sigma, USA) dissolved in 0.1 M cold citrate buffer at pH 4.5 [36]. Five days after the STZ injection, blood glucose levels were measured using a portable glucometer (Accu-Chek, Roche, Germany) in blood collected from the tail, and diabetes status was confirmed when glucose level was above 18 mmol/L.

## 2.4 Experimental design

The overall time period for the current study was 6 weeks. Rats were randomly divided into five groups (n = 12 per group) as follows:

- Group 1 (N): control animals (healthy, nondiabetic animals); received dimethylsulfoxide (DMSO) orally.
- Group 2 (N + KV): control animals received KV dissolved in DMSO orally.
- Group 3 (D): untreated diabetic group; injected with a single dose of STZ (50 mg/kg) intraperitoneally.
- Group 4 (D + KV): received KV (100 mg/kg) orally five times weekly starting 5 days post STZ injection; this served as the KV-treated diabetic group.
- Group 5 (D + INS): received subcutaneous insulin (INS) injection (2 u/kg) every other day starting 5 days post STZ injection; this served as the insulin-treated diabetic group.

## 2.5 Sample collection and preparation

At completion of the treatment periods, rats were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). Fasting blood glucose levels were measured after 4 h of fasting (usually between 10 am and 2 pm). Blood samples were collected from the abdominal aorta into glucose tubes (containing sodium fluoride/potassium oxalate) and EDTA-containing tubes. The epididymis and testes were also excised and weighed. The tissue samples were snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

Briefly, 250  $\mu\text{L}$  of phosphate buffer (50 mM  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 0.5% (v/v) TritonX-100, pH 7.5) was added to 50 mg of testicular or epididymal tissue. The homogenates were transferred into tubes and centrifuged at 10,000 rpm for 10 min at  $4^{\circ}\text{C}$ . The supernatants were subsequently transferred to new tubes and kept at  $-80^{\circ}\text{C}$  until used.

## 3. Biochemical assays

### 3.1 Determination of protein concentration

Testicular and epididymal protein levels were determined using the bicinchoninic acid (BCA) method as describe by [37]. Briefly, BCA working reagents, samples, and standards were prepared referring to the manufacturer's instructions for the assay kit supplied by Sigma Aldrich.

### 3.2 Lipid peroxidation

Malondialdehyde (MDA) levels were determined in the samples through a modern HPLC-based thiobarbituric acid (TBA) assay method. This method is highly



specific because it quantifies the genuine MDA-(TBA)<sub>2</sub> adduct formed [38]. The quantitative analysis of MDA was performed using a modified method of Cuny et al. [39] on a Spectra SYSTEM™ HPLC (Agilent Technology, 1200 series, Germany).

Briefly, 50 µL of sample was mixed with 375 µL orthophosphoric acid 0.44 M, 125 µL thiobarbituric acid, and 225 µL distilled water. This mixture was heated at 100°C for 60 min and cooled on ice. Thereafter, 775 µL of alkaline methanol was added, and the sample was subsequently vortexed and centrifuged at 3500 rpm for 3 min at 4°C. The supernatant (1 mL) was collected; 500 µL of n-hexane was added and centrifuged at 14,000 rpm for 2 min. The supernatant (500 µL) was collected in chromatographic tubes and injected into the HPLC system. The readings were performed after 10 min, and sample concentration MDA levels were expressed in µmol/g of tissue.

### **3.3 Superoxide dismutase activity**

Superoxide dismutase (SOD) activity was determined by a modified method from Ellerby and Bredesen [40]. Briefly, samples were run in duplicate, in a 96-well plate; 15 µL of 6-HD was added to 6 µL of supernatant. An amount of 170 µL of diethylenetriaminepentaacetic acid (DETAPAC) solution (0.1 mM) in SOD assay buffer and readings were taken immediately at 490 nm for 4 min at 1 min intervals. The activity of SOD was calculated from a linear calibration curve and expressed as µmol/mg protein.

### **3.4 Catalase activity**

The catalase (CAT) activity was assessed according to Aebi [41]. The CAT induced decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into water and oxygen. The rate of disintegration is proportional to the concentration of CAT activity. The CAT activity was determined by measuring the change in absorbance of H<sub>2</sub>O<sub>2</sub> and sample mixture. Briefly, the CAT assay was performed in duplicate; 150 µL H<sub>2</sub>O<sub>2</sub> was added to 20 µL of sample. Readings were determined by using a spectrophotometer (Thermo Electron Corporation, Multiskan Spectrum, USA) at 240 nm wavelength. The CAT activity was expressed as µmol/mg of protein.

### **3.5 Glutathione peroxidase activity**

The activity of glutathione peroxidase (GPx) is derived from the oxidation of reduced β-NADPH in a conjugated glutathione reductase (GR) system using H<sub>2</sub>O<sub>2</sub> (12 mM) as a substrate. Glutathione peroxidase reacts with H<sub>2</sub>O<sub>2</sub> oxidizing reduced glutathione (GSH) to oxidized glutathione (GSSG). In brief, the GPx assay was performed in duplicate in a 96-well UV Costar plate. Each well contained 215 µL assay buffer (AB: 50 mM potassium phosphate, 1 mM EDTA, pH 7.0), 5 µL GSH (30.7 mg/mL in water), 5 µL GR (0.1 U/mL in AB), and 20 µL of sample, and 5 µL NAD(P)H was added to the mixture. Two readings were recorded [38]. The first was the background of oxidation at 340 nm for 3 min in 30 s intervals for samples (A<sub>1</sub>) and blank (A<sub>1b</sub>). The second reading was performed after adding 50 µL H<sub>2</sub>O<sub>2</sub>. This reading monitored the decrease of H<sub>2</sub>O<sub>2</sub> due to NAD(P)H oxidation at 340 nm for 2 min. The GPX activity was expressed in µmol/mg of protein.

### **3.6 Statistical analysis**

Data are expressed as mean ± standard deviation (mean ± SEM). One-way analysis of variance (ANOVA) was used to test for significance between the groups.

The Bonferroni multiple comparison analysis was used to compare the differences between the groups. Differences were considered significant at  $P < 0.05$ . The GraphPad PRISM 5 software package was used for all statistical evaluations and graphical representations.

## 4. Results

### 4.1 Plasma glucose levels in diabetic and nondiabetic groups before initiation of treatments

**Figure 3** shows the non-fasted plasma glucose levels in both nondiabetic (N) and diabetic (STZ) groups before the start of KV and insulin (IN) treatments. The average fasting glucose level was significantly higher in the D group than the N group ( $28.19 \pm 2.25$  mmol/L versus  $9.93 \pm 0.51$  mmol/L,  $p < 0.05$ ).

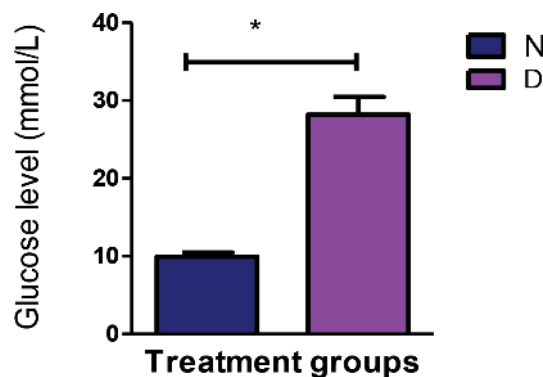
### 4.2 Evaluation of body weights after subjecting the rats to various treatments

The induction of diabetes with STZ resulted in a significant loss of body weight (**Figure 4**). KV administration to normal rats did not affect body weight compared to untreated nondiabetic rats. On the other hand, the body weights of KV-treated diabetic rats significantly increased compared to diabetic control. Injection of the standard antidiabetic drug, insulin, also improved body weight gains in diabetic rats compared to the untreated diabetic rats.

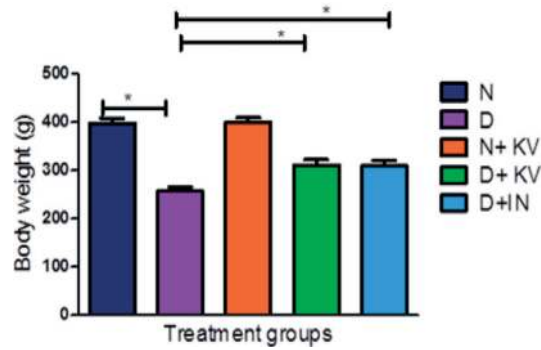
### 4.3 Evaluation of testicular and epididymal weights of rats subjected to various treatments

**Figure 5** presents the testicular weights of rats treated with STZ, KV, and/or IN. Diabetes induction decreased testicular weight in rats, whereas KV treatment of diabetic rats reversed this alteration. This is evident by a significant increase in testicular weight in KV-treated diabetic rats in comparison to untreated diabetic rats. However, insulin treatment did affect testicular weight in diabetic rats when compared to untreated diabetic rats.

**Figure 6** presents epididymal weights of rats subjected to different treatments. Diabetic rats had a lower epididymal weight compared to the nondiabetic rats ( $0.431 \text{ g} \pm 0.062 \text{ g}$  versus  $0.529 \pm 0.058 \text{ g}$ ,  $p < 0.05$ ). Treatment of diabetic rats with KV significantly increased epididymal weight in comparison to

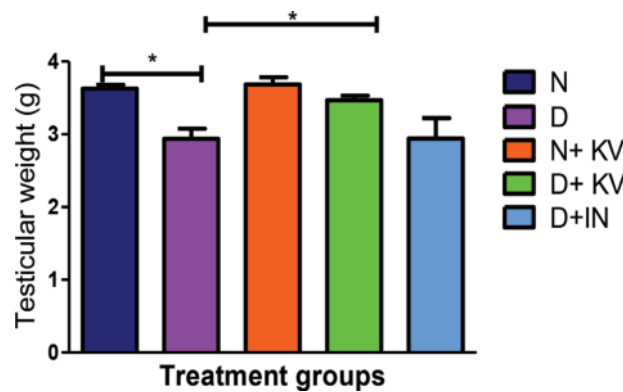


**Figure 3.** Glucose levels in diabetic and nondiabetic rats before KV and IN treatments. Data are presented as mean  $\pm$  SEM. (\*) indicates significant difference with  $p < 0.05$ . N, nondiabetic control group; D, diabetic group.



**Figure 4.**

Effect of KV and insulin on body weight of rats. Data are presented as mean  $\pm$  SEM. (\*) indicates significant difference with  $p < 0.05$ . N, nondiabetic control group; D, diabetic group; N + KV, nondiabetic group treated with KV; D + KV, diabetic group treated with KV; D + IN, diabetic group treated with insulin (standard drug).



**Figure 5.**

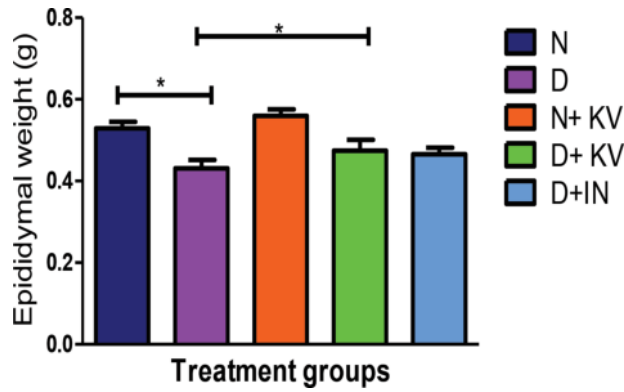
Effect of KV and insulin treatment on testicular weight of rats. Data are presented as mean  $\pm$  SEM. (\*) indicates significant difference with  $p < 0.05$ . N, nondiabetic control group; D, diabetic group; N + KV, nondiabetic group treated with KV; D + KV, diabetic group treated with KV; D + IN, diabetic group treated with insulin (standard drug).

untreated diabetic rats. KV treatment did not affect the epididymal weight of normal rats as no apparent difference was observed between N + KV group and N group ( $0.529 \pm 0.058$  g versus  $0.475 \pm 0.09$  g,  $p > 0.05$ ). On the other hand, the epididymal weight of insulin-treated diabetic rats was not significantly different compared to the untreated diabetic rats ( $0.454 \pm 0.050$  g versus  $0.431 \pm 0.062$  g,  $p > 0.05$ ).

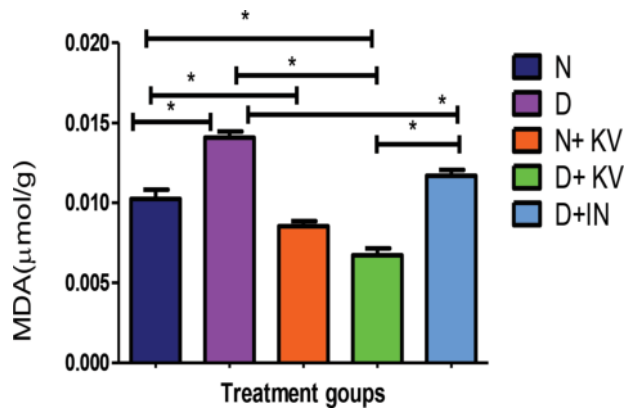
#### 4.4 Assessment of lipid peroxidation of testicular and epididymal tissues of rats subjected to various treatments

MDA levels in the testis are presented in **Figure 7** for both nondiabetic and diabetic groups treated with or without KV or insulin. The testicular MDA level was significantly higher in the D group compared to the N group ( $0.014 \pm 0.001$   $\mu\text{mol/g}$  versus  $0.010 \pm 0.002$   $\mu\text{mol/g}$ ,  $p < 0.05$ ). The MDA level in the testes of nondiabetic rats treated with KV (N + KV) was significantly lower than the N group. The testes of diabetic rats treated with KV (D + KV) showed significantly lower testicular MDA level than the diabetic control group. Also, insulin treatment significantly lowered MDA levels in diabetic rats compared to the diabetic control group. It is noteworthy that a significant reduction in MDA

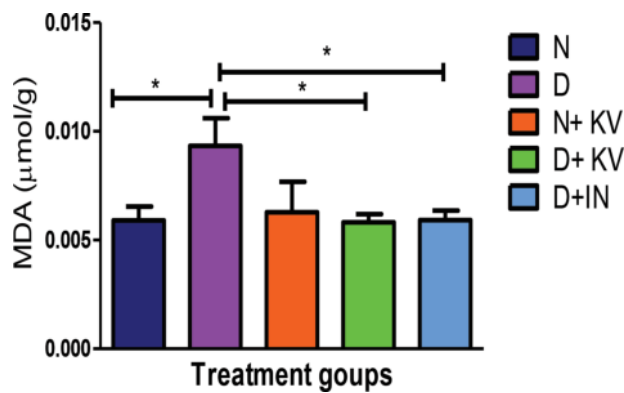




**Figure 6.** Effect of KV and insulin treatment on epididymal weight of rats. Data are presented as mean  $\pm$  SEM. (\*) indicates significant difference with  $p < 0.05$ . N, nondiabetic control group; D, diabetic group; N + KV, nondiabetic group treated with KV; D + KV, diabetic group treated with KV; D + IN, diabetic group treated with insulin (standard drug).



**Figure 7.** Effect of KV and insulin treatment on testicular tissue LPO in rats. Data are presented as mean  $\pm$  SEM. (\*) indicates significant difference with  $p < 0.05$ . N, nondiabetic control group; D, diabetic group; N + KV, nondiabetic group treated with KV; D + KV, diabetic group treated with KV; D + IN, diabetic group treated with insulin (standard drug).



**Figure 8.** Effect of KV and insulin treatment on epididymal LPO in rats. Data are presented as mean  $\pm$  SEM. (\*) indicates significant difference with  $p < 0.05$ . N, nondiabetic control group; D, diabetic group; N + KV, nondiabetic group treated with KV; D + KV, diabetic group treated with KV; D + IN, diabetic group treated with insulin (standard drug).

levels was observed in the testes of KV-treated diabetic rats when compared to the D + IN group ( $0.007 \pm 0.001 \mu\text{mol/g}$  versus  $0.012 \pm 0.001 \mu\text{mol/g}$ ,  $p < 0.05$ ).

**Figure 8** shows the MDA level in epididymis of both nondiabetic and diabetic groups treated with KV and/or insulin. The MDA level was significantly higher in epididymal tissue of the D group compared to the N group in epididymal tissue ( $0.009 \pm 0.004 \mu\text{mol/g}$  versus  $0.006 \pm 0.002 \mu\text{mol/g}$ ,  $p < 0.05$ ). No significant differences in MDA levels were observed in the epididymal tissue of nondiabetic rats supplemented with KV (N + KV) compared to the untreated (N) rats. Furthermore, a significantly lower epididymal MDA level was observed in the D + KV group when compared to the D group. KV treatment normalized epididymal MDA level in diabetic rats, and this effect is comparable to that of insulin, the standard antidiabetic drug.

#### 4.5 Assessment of SOD activity in testicular and epididymal tissues of rats subjected to various treatments

As shown in **Table 1**, no significant differences were observed in the activity of testicular superoxide dismutase (SOD) across all treatment groups. However, in the epididymal tissue, the SOD activity was significantly reduced in the diabetic (D) group when compared to the N group ( $0.042 \pm 0.007 \mu\text{mol/mg}$ ,  $p < 0.05$  versus  $0.556 \pm 0.007 \mu\text{mol/mg}$ ,  $p < 0.05$ ). On the other hand, separate treatments of diabetic rats with KV and insulin increased SOD activity in epididymal tissue compared to untreated diabetic rats. The effects of KV on the activity of SOD in the epididymis are comparable to that of the standard drug, insulin.

#### 4.6 Assessment of the catalase activity in testicular and epididymal tissues of male Wistar rats subjected to various treatments

A significantly lower CAT activity was observed in testicular tissue of STZ-induced diabetic rats (**Table 1**) in comparison to nondiabetic rats ( $12.21 \pm 1.235 \mu\text{mol/mg}$  versus  $18.00 \pm 1.524 \mu\text{mol/mg}$ ,  $p < 0.05$ ). The supplementation of KV to nondiabetic rats (N + KV) did not significantly affect testicular CAT activity in comparison to the untreated nondiabetic group. KV treatment of diabetic rats elevated testicular CAT activity in comparison to diabetic control, and CAT activity was restored to normalcy. Likewise, a significantly higher testicular CAT activity was observed after insulin treatment in diabetic rats (D + IN) when compared to the diabetic control rats.

As shown in **Table 1**, STZ-induced diabetic rats showed significantly lower CAT activity in epididymal tissue compared to the N group ( $2.864 \pm 0.415 \mu\text{mol/mg}$  versus  $6.162 \pm 0.612 \mu\text{mol/mg}$ ,  $p < 0.05$ ). There was no significant difference in the epididymal CAT activity of rats supplemented with KV (N + KV) when compared to the N group. The separate treatment of diabetic rats with KV and insulin significantly elevated CAT activity in the epididymis compared to the diabetic controls.

#### 4.7 Assessment of the GPX activity in the testicular and epididymal tissues of male Wistar rats subjected to various treatments

The results of testicular GPX activity of rats treated with STZ, KV, and/or IN are captured in **Table 1**. A significantly lower testicular GPx activity was observed in diabetic rats compared to the N group ( $3.977 \pm 0.880 \mu\text{mol/mg}$  versus  $12.26 \pm 0.644 \mu\text{mol/mg}$ ,  $p < 0.05$ ). The nondiabetic rats treated with KV (N + KV) did not show any significant difference in testicular GPx activity compared to the N group. The testes of KV-treated diabetic rats (D + KV) showed significantly higher

Sample types and tests	Group/treatments				
	N: nondiabetic control group	D: diabetic group	N + KV: nondiabetic group treated with Kolaviron	D + KV: diabetic group treated with Kolaviron	D + IN: diabetic group treated with insulin (standard drug)
<i>Testicular</i>					
SOD (μmol/mg protein)	0.193 ± 0.010	0.198 ± 0.008	0.195 ± 0.019	0.169 ± 0.010	0.171 ± 0.020
CAT (μmol/mg protein)	18.00 ± 0.62 <sup>*</sup>	12.95 ± 0.58	15.58 ± 1.31 <sup>*#</sup>	13.60 ± 0.94 <sup>\$</sup>	11.86 ± 0.54 <sup>&amp;c</sup>
GPx (μmol/mg protein)	12.28 ± 0.227 <sup>*\$&amp;c</sup>	3.977 ± 0.280	12.06 ± 0.242 <sup>*\$</sup>	6.770 ± 0.232 <sup>*#&amp;c</sup>	8.006 ± 0.345 <sup>*&amp;c</sup>
<i>Epididymal</i>					
SOD (μmol/mg protein)	0.055 ± 0.002 <sup>*</sup>	0.042 ± 0.002	0.050 ± 0.003	0.052 ± 0.002 <sup>*</sup>	0.053 ± 0.002 <sup>*</sup>
CAT (μmol/mg protein)	2.518 ± 0.178 <sup>*#&amp;c</sup>	4.962 ± 0.807	4.433 ± 0.947 <sup>*#</sup>	4.896 ± 0.385 <sup>*</sup>	5.360 ± 0.369 <sup>&amp;c</sup>
GPx (μmol/mg protein)	12.30 ± 0.225 <sup>*</sup>	4.277 ± 0.279	11.81 ± 0.2476 <sup>*\$</sup>	7.056 ± 0.2484 <sup>*#&amp;c</sup>	8.006 ± 0.3624 <sup>*&amp;c</sup>
<i>Data are presented as mean ± SEM. N, nondiabetic control group; D, diabetic group; N + KV, nondiabetic group treated with KV; D + KV, diabetic group treated with KV; D + IN, diabetic group treated with insulin (standard drug)</i>					
<i><sup>*</sup>Represents a significant difference when compared to D</i>					
<i><sup>#</sup>Represents a significant difference when compared to N + KV</i>					
<i><sup>\$</sup>Represents significance when compared to D + KV</i>					
<i><sup>&amp;c</sup>Represents a significant difference when compared to D + IN (n = 12 per group)</i>					

**Table 1.** Antioxidant (SOD, CAT, and GPx) activities in testicular and epididymal tissues of Wistar rats after a 6-week period of treatment.

GPx activity when compared to the D group. In addition, a significantly higher testicular GPx activity was observed in the D + IN group when compared to the D group.

Diabetes induction significantly lowered epididymal GPX activity in comparison to the nondiabetic rats (4.277 ± 0.884 μmol/mg versus 12.30 ± 0.636 μmol/mg, p < 0.05). The supplementation of KV to nondiabetic rats (N + KV) did not significantly alter GPx activity of epididymal tissue compared to the N group. However, epididymal GPX activity was significantly higher in diabetic rats treated with KV compared to the untreated diabetic group. Likewise, the treatment of diabetic rats with insulin significantly increased epididymal GPx activity compared to diabetic control.

## 5. Discussion

Diabetes associated with OS is said to impair testicular and epididymal tissue functions which can generate male infertility [8, 11, 13, 14]. Increasingly, studies demonstrate the significant impact of phytochemicals such as flavonoids in the prevention and treatment of complications related to diabetes [20, 21]. The physiological role and properties of flavonoids in the management of OS are currently being investigated in relation to male infertility. This interest is the motivation for the current study to investigate the effects of KV (KV), a known flavonoid extract of *G. kola*, on testicular- and epididymal-induced OS using a diabetic rat model.

### 5.1 Evaluation of induced diabetes with STZ before KV and insulin treatment

In the current study, the single intraperitoneal administration of STZ (50 mg/kg) in adult Wistar rats was effective in causing hyperglycemia after 5 days. This was confirmed by the significantly higher plasma glucose levels (18 mmol/l, see **Figure 3**) in the STZ group of animals which is typical of type 1 diabetes mellitus. It was therefore concluded that the diabetic animal model was successfully created and the results were similar to and supported by previous findings [42, 43] where induction of DM in Wistar rats via intravenous STZ injection (of 40 and 45 mg/kg/b.w, respectively) were confirmed by hyperglycemia after 4 days and maintained for 4 weeks.

### 5.2 Evaluation of rat body, testicular, and epididymal weights subjected to various treatments

Blood glucose levels is an indication of proper insulin function and important energy sources [44]. Insufficient insulin secretion or dysfunction of the signaling pathway results in a disturbance of glucose homeostasis. Subsequently the body will start to use other macromolecules such as lipids and proteins as sources of energy [45]. This results in shrinking of muscle tissue accompanied by a rapid weight loss in diabetic animals [45, 46]. Data from the current study showed that the body, testes, and epididymal weights were significantly lower in STZ-induced diabetic rats than the nondiabetic control group (**Figures 3–6**). These findings are in agreement with previous studies that also demonstrated a significant decrease in body, testicular, and epididymal weights in diabetic rats [5, 18, 27, 28, 33, 45, 47]. Moreover, variations in animal body and organ weights have been reported to affect spermatogenesis, sperm quality, and sperm concentration [5, 18, 27, 28, 33, 45, 47].

In the current study, diabetic animals treated with insulin improved their body and epididymal tissue weights as there was a clear regain of body weights (**Figures 3 and 4**). Synthetic insulin is a standard drug used to treat diabetes which is different from the insulin secreted naturally by the pancreas. Pancreatic insulin promotes proper metabolism, energy balance, and the maintenance of normal body weights [48]. Though the low-dose synthetic insulin used in this study had improved the weight of diabetic animals, there was still not total recovery to their normal weights.

Supplementation with KV showed similarity to the IN treatment with a significant improvement not only of the diabetic rat's body and epididymal tissue weights but also of the rat's testicular weight. Such findings are in agreement with the results of Adaramoye and Lawal [49], who reported that the treatment with KV significantly increased the weight gained by diabetic rats when compared to the untreated diabetic counterparts. Moreover, there was no significant difference in weights of nondiabetic rats supplemented with KV (N + KV) when compared to nondiabetic control group (N) (**Figures 3–6**). These results demonstrate that KV supplementation had no adverse effects on the animal weights confirming that the decrease observed in diabetic rats supplemented with KV was only due to their diabetic condition. This also implies that the body, testes, and epididymal weight improvement observed in the diabetic animals supplemented with KV might be due to its antioxidant and hypoglycemic potential to prevent OS and diabetes. It can be argued that the ability of KV to protect against weight loss might mainly be attributed to its glucose-lowering capacity [50]. Indeed, the regulation of glucose levels as the main source of energy by KV provides a platform for less

use of alternative sources of energy from body, testicular, and epididymal proteins and fats. It could therefore be postulated that KV might be considered as an antidiabetic compound in the management of weight and glucose regulation in diabetes.

### 5.3 Assessment of lipid peroxidation of testicular and epididymal tissues of rats subjected to various treatments

MDA is an end product of LPO, and the increased levels are an indication of oxidative damage [51, 52]. It has been shown that LPO induces disturbance of fine structures; alteration of integrity, fluidity, and permeability; and functional loss of biomembranes, modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms, and generates potentially toxic products [52]. Thus, LPO in vivo has been implicated as the underlying mechanisms in numerous disorders and diseases such as cardiovascular diseases, cancer, neurological disorders, and aging. The mechanism of free radical-mediated LPO reactions include [46] abstraction of bisallylic hydrogen from polyunsaturated fatty acids to give carbon-centered radicals which rearranges to more stable cis, trans-pentadienyl radicals [33], addition of oxygen to the pentadienyl radical to give lipid peroxy radicals [26], release of oxygen from the peroxy radical to give oxygen and pentadienyl radicals, which rapidly react with oxygen to give a thermochemically more stable trans, trans form preferentially than cis, trans form, and [27] intramolecular addition of the peroxy radical to the double bond to yield bicyclic prostaglandin-type products.

The results clearly indicate a significantly higher and increased expression of MDA in the testicular (**Figure 7**) and epididymal (**Figure 8**) tissues of the diabetic rats when compared to the nondiabetic rats. These results are in agreement with previous study also performed on diabetic experimental animal models [49]. During diabetes, hyperglycemia causes auto-oxidation of glucose and stimulates OS through excessive free radical production. The release of free radicals causes damage to biological systems by abstracting electrons from macromolecules, thereby causing instability and disintegration [53]. For instance, peroxidation of polyunsaturated lipids on sperm membrane has been reported to cause structural alterations of the biological cell membranes as well as a change in membrane stability and function [49]. The peroxidation of sperm lipids may also disturb maturation, spermatogenesis, capacitation, acrosome reaction, and eventually membrane fusion, which results in male infertility [5, 6, 28–30].

In the present study, the protective mechanism of KV and IN has been examined in the onset of LPO related to STZ-induced diabetes. The findings from the study indicate that diabetic animals treated with IN recovered from LPO in the testes and epididymal tissues. This is observed through the decrease of MDA level to values close to baseline of nondiabetic rats (**Figures 7 and 8**).

KV showed significantly healthier responses. Not only did KV supplementation significantly reduce MDA levels in the N + KV group when compared to the N group, but it also had a better effect than IN in the restoration of testicular and epididymal MDA levels of the D + KV group when compared to D only group (**Figure 7**). These findings emphasize the potentiality of KV to better restore metabolic disorders related to OS such as diabetes and male infertility [5, 6, 28, 29].

Insulin helps to control blood glucose levels, and then this reduces the amount of free radicals released. The observed protective effects of KV in this study may be due to its antioxidant properties by scavenging the effects of hydroperoxides resulting from induced OS. This is in accordance with previous studies that have reported



the beneficial effects of KV against testicular damage induced by various chemicals [2, 5, 6, 28–30, 33, 35].

#### **5.4 Assessment of antioxidant enzymes in the testicular and epididymal tissues to various treatments**

Antioxidant enzymes such as SOD, CAT, and GPX play a crucial role in protecting the testes and epididymal tissues against OS associated damage and male reproductive disorders [43–45].

Physiological and pathophysiological conditions such as diabetes influence the level of production and activity of these antioxidant enzymes [2, 6]. The reduction in antioxidant enzymes has been previously reported in diabetic animals [20]. The observed reduction in antioxidant enzyme activities could be due to the oxidative inactivation of the enzyme by ROS or by the glycation of the enzymes [20, 45]. The reduced activity of SOD, CAT, and GPX in the epididymal and testicular tissues has been observed following STZ induction of diabetes, and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and H<sub>2</sub>O<sub>2</sub>.

In epididymal tissue the SOD activity was significantly lower in STZ-induced diabetic rats when compared to nondiabetic control rats (**Table 1**). This is in agreement with a study by Adaramoye and Lawal [49] who demonstrated that a diabetogenic agent reduced SOD activity in epididymal tissue. Both catalase and GPx activities in the testes and epididymis were significantly lower in STZ-induced diabetic rats than in the nondiabetic control rats. Glutathione peroxidase shares the substrate, H<sub>2</sub>O<sub>2</sub>, with CAT; it alone can react effectively with lipids and other organic hydroperoxides, being the major source of protection against low levels of OS. Some authors supported the idea that GPx was essential in the protection against OS under normal conditions [54]. Others believed in a protective role for these enzymes only under OS conditions [2]. Generally, in our study, the activity of testicular and epididymal antioxidant enzymes SOD, CAT, and GPx was depleted in diabetic rats. Diabetes-induced tissue OS is further supported by the elevated levels of MDA.

Numerous compounds with antioxidant activities have been shown to improve or normalize the activities of antioxidant enzymes in nondiabetic and diabetic rats, respectively [4]. In the present study, the supplementation of KV for 6 weeks to the normal rats did not change SOD, CAT, and GPx activities compared to the nondiabetic rats. This might be due to the interference between natural antioxidants produced by the body and the antioxidant effects of KV. The treatment of STZ-induced diabetic rats with KV influenced the activity of SOD, CAT, and GPx compared to the diabetic groups (**Table 1**). Similarly, to previous studies, the supplementation of dietary antioxidants to experimental animals has shown a positive correlation between natural dietary supplementation and increased antioxidant enzyme levels in induced OS models. In other studies, KV restored antioxidant enzymes in the testes of diabetes-induced rats [5, 49]. The protective effect of KV observed in the testes and epididymis of diabetic rats might either be due to the inhibition of glycation by the antioxidant enzymes or scavenging abilities of ROS, thus decreasing the formation of LPO [5, 27–30].

Insulin has been used in the management of diabetes by restoring pancreatic insulin deficiency. From our results it is clear that synthetic insulin had improved the levels of testicular and epididymal antioxidant enzymes in STZ-induced diabetic rats. These results confirm the central role of insulin in energy homeostasis and also make it an important signaling factor in the reproductive tract [2, 7]. The observed effects of KV on the testes and epididymis of STZ-induced diabetic rats in the present study could be attributed to its hypoglycemic and antioxidant properties.

## 6. Conclusion

The findings of this study emphasized the protective effects of KV against diabetes-associated OS in the testicular and epididymal tissues by enhancing antioxidant defense system in STZ-induced diabetic rats. The present study showed that that KV has the potential of being used as a treatment for diabetes-related pathologies and their complications especially testicular dysfunction. We propose further investigations to elucidate the effects of KV on male reproductive organ function in order to advance the current knowledge which could also be extended to clinical research.

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