
Effects of lycopene on kidney antioxidant enzyme activities and functions in streptozotocin-induced diabetic Wistar rats

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Abstract: The present study assessed the effects of lycopene on kidney antioxidant enzymes activities and functions in streptozotocin-induced diabetic Wistar rats. Diabetes was induced in animals by single *intra-peritoneal* injection of streptozotocin. Thereafter the animals were randomly assigned into the following groups: Group I and II (Normal control + olive oil and Diabetic control + olive oil) while Group III to VI were treated with (10, 20 and 40 mg/kg of lycopene and 2 mg/kg glibenclamide) respectively. All treatments were given once daily orally for four weeks. Results obtained showed that blood glucose was significantly ($P < 0.05$) reduced. MDA concentration was reduced in kidney tissue, with increased activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) in diabetic animals administered with lycopene when compared with diabetic control group. There was significant ($P < 0.05$) increase in the level of serum sodium ion and reduction in serum urea level in diabetic rats treated with lycopene when compared with the diabetic control group. Histological findings showed improved renal architecture as reflected by reduced glomerular and tubular necrosis in all treated groups when compared with control group. It can be concluded that lycopene protects against diabetes-induced kidney damage through elevation of endogenous antioxidant enzymes and improved renal dysfunction in diabetic animals.

Keywords: Diabetes Mellitus, Lycopene, Streptozotocin, Electrolytes, Antioxidants, Kidney

1. Introduction

Diabetic nephropathy (DN) has become a worldwide epidemic, accounting for approximately one third of all cases of end stage renal disease [1]. DN is classically defined as the increase in protein excretion in the urine. The early stage of DN is characterized by a small increase in urinary albumin excretion (microalbuminuria), while overt diabetic nephropathy is defined as the presence of macroalbuminuria or proteinuria [2]. Pathophysiological changes associated with diabetic nephropathy include renal and glomerular hypertrophy, mesangial cell hypertrophy and matrix accretion, glomerular basal membrane thickening and functional alterations in glomerular filtration barriers [3], leading to a progressive reduction in the filtration surface of the glomerulus, a process known as glomerulosclerosis [4]. Furthermore, there is now compelling evidence to suggest

that disruption of the tubulointerstitial architecture is as important, if not more important in contributing to kidney injury as glomerular damage [5]. An upregulation of reactive oxygen species in diabetes has been implicated in the pathogenesis of kidney injury [6]. Forbes *et al.* [6] have shown that in diabetic kidney, there is enhanced glucose uptake in many of the cell populations including glomerular epithelial cells, mesangial cells and proximal tubular epithelial cells, leading to the excessive production of intracellular reactive oxygen species, making these cells particularly susceptible to the diabetic milieu. Reports from Ceriello *et al.* [7] suggest that glucose alters antioxidant defenses in endothelial cells as well as in patients with diabetic complications such as diabetic nephropathy [8]. From example, the concentration of the antioxidant

(glutathione) has been found to decrease in a range of organs including the kidney, liver, pancreas, plasma, and red blood cells of chemically induced diabetic animals [9]. Thus, increased reactive oxygen species in diabetes is not only the result of their increased production, but also a consequence of impaired antioxidant defenses [10]. Furthermore, reports exist from both clinical and pre-clinical studies, to suggest that oxidative stress accompanies the progression of diabetic nephropathy. Hyperglycaemia has been shown to increase 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative mitochondrial DNA damage in diabetic rat kidneys [11]. Therefore, development of targeted therapeutics is needed in order to ameliorate or eliminate diabetes associated kidney damage. In both experimental and clinical models of diabetes, antioxidants have been reported to reduce markers of oxidative stress [12, 13]. Besides, some studies have showed that antioxidants are effective and cheaper than conventional therapy in management of some diseases [14]. Carotenoids have extensive applications as anti-oxidants in dietary supplements and have extensively studied because of their potential health benefits. More recently, protective effects of carotenoids against serious disorders such as cancer [15], heart disease [16] and degenerative eye disease [17] have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants. The carotenoids used as food ingredients include astaxanthin, beta-apo-carotenal, canthaxanthin, beta-carotene, lutein, zeaxanthin and lycopene [18]. Lycopene has been reported as one of the strongest antioxidants among dietary carotenoids found in foods. The higher antioxidant capacity has been linked to the fact that the β -cycle in its structure is opened [19]. Lycopene, the predominant carotenoid in tomatoes, has the highest antioxidant activity among all dietary carotenoids [20]. Tomato fruits are virtually the sole dietary source of lycopene [21]. Other sources of lycopene include: Gac (*Momordica cochinchinensis* Spreng) fruit, tomatoes and tomato products, including ketchup, tomato juice, pizza sauce, watermelon, papaya, pink grapefruit, and pink guava [22]. Several studies have been carried out into lycopene's antioxidant properties and an increasing body of evidence based on laboratory and population-based research has demonstrated the role of lycopene in health and disease. However, there are limited studies on the nephroprotective activity of lycopene in diabetes. Therefore, the present investigation was aimed at evaluating the effect of lycopene on changes in antioxidant enzymes activity and functions in streptozotocin-induced diabetic Wistar rats.

2. Materials and Methods

2.1. Materials

2.1.1. Animals

Adult Wistar rats of both sexes weighing 150 to 200 g were obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University Zaria Kaduna State. The animals were kept and maintained under

laboratory condition of temperature, humidity and light. The animals were housed five animals per cage. They were fed on standard commercial feeds with water *ad libitum*.

2.1.2. Chemicals and Lycopene

Streptozotocin was purchased from Sigma chemicals (St Louis U.S.A), while Lycopene (30 mg capsule, General Nutrition Corporation, Pittsburgh, U.S.A.). It was reconstituted in olive oil (*Goya en espana*, S.A.U., Savilla, Spain) to appropriate working dosage as earlier described by Ogundejiet *al.* [23] with modifications. All other chemicals and solvents used were of analytical grade.

2.2. Methods

2.2.1. Induction of Diabetes Mellitus

Experimental diabetes mellitus was induced by single *intra-peritoneal* injection of 60 mg/kg body weight dose of streptozotocin (STZ) dissolved in fresh 0.1M cold citrate buffer of pH 4.5 into 18 h-fasted Wistar rats. Seventy two (72) hours after STZ injection, blood was taken from tail vein of the rats. Animals having blood glucose levels ≥ 200 mg/dl were considered diabetic and included in the study. The diabetic animals were randomly divided into different groups [24].

2.2.2. Experimental Design

In the present study, a total of 30 Wistar rats were used. They comprised of twenty five (25) diabetic and five (5) normal animals and were randomly divided into six groups of five rats each as follows:

Group 1: Normal control (NC) and administered (0.5 ml/kg body weight) olive oil

Group 2: (DC) Diabetic control and administered (0.5 ml/kg body weight) olive oil

Group 3: (D + LYC 10 mg/kg) Diabetic and treated with 10 mg/kg b w of lycopene

Group 4: (D + 20 mg/kg) Diabetic and treated with 20 mg/kg b w of lycopene

Group 5: (D + 40 mg/kg) Diabetic and received 40 mg/kg b w of lycopene

Group 6: (D + GLB 2 mg/kg) Diabetic and received Glibenclamide 2 mg/kg b w

All administration was given orally once daily for four weeks. The study was carried out according to the specification of the Ahmadu Bello University Animal Research Committee.

2.2.3. Determination of Fasting Blood Glucose Level

Fasting blood glucose level was estimated at interval of 0 week, 1st week, 2nd week, 3rd week and 4th week of the treatment period respectively by glucose-oxidase principle as described by Beach and Turner [25] using digital glucometer (Accu-chek Advantage) and was expressed as mg/dL.

2.2.4. Blood Sample Collection and Serum Preparation

Twenty four hours after last administration animals from each group were euthanized by exposure to light chloroform soaked in cotton wool placed in anesthetic box. Blood was

withdrawn by cardiac puncture into specimen bottles and was allowed to clot. The serum was separated by centrifugation at 2,000 g for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany), after which the supernatant obtained and used for the determination of serum electrolytes.

2.2.5. Tissue Sample Collection and Homogenates Preparation

Kidney tissue (1.0 g) was dissected out and immediately washed with ice-cold normal saline, weighed and homogenized immediately in equivalent volume of ice-cold phosphate buffer of 0.1 M of pH 7.4. The homogenates were centrifuged at $2,000 \times g$ for 5 min to remove debris and supernatant collected for evaluation of some of the kidney tissue antioxidant enzyme activities and malondialdehyde (MDA) concentration.

2.3. Assessment of Some Kidney Tissue Oxidative Stress Bio-makers

2.3.1. Estimation of Kidney Tissue Lipid Peroxidative Changes (MDA) Concentration

Kidney tissue Malondialdehyde Concentration (MDA) levels were measured by the double heating method as described by Placer *et al* [26]. The principle of the method was based on the spectrophotometric measurement of the colour during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substance was calculated by the absorbance coefficient of malondialdehydethiobarbituric acid complex and expressed in nmol/mg protein.

2.3.2. Determination of Kidney Tissue Superoxide Dismutase Activity (SOD)

Total superoxide dismutase activity was measured spectrophotometrically using xanthine/xanthine oxidase as an O_2^- generating system and nitro-blue tetrazolium (NBT) as a detector as described by Suzuki [27]. Briefly, each sample was diluted 1:10 with phosphate buffer (50 mM, pH 7.5, EDTA 1 mM). Sodium-carbonate working solution (50 mM, xanthine 0.1 mM, NBT 0.025 mM, EDTA 0.1 mM), xanthine oxidase (0.1 U/mL in ammonium sulfate 2 M) and sample or blank (phosphate buffer 50 mM, pH 7.5; EDTA 1 mM) were mixed in a cuvette. The change in absorbance per minute at 560 nm (DA560) was calculated. Enzymatic activity was expressed in units of SOD activity per mg of protein (U/mg protein). One unit of SOD activity is defined as the amount of enzyme needed to inhibit the reaction of O_2^- with NBT by 50%.

2.3.3. Determination of Kidney Tissue Catalase Activity (CAT)

Catalase activity in the kidney tissue was evaluated by measuring the decrease in H_2O_2 concentration at 240 nm as described by Aebi [28]. Briefly, working solution (phosphate buffer 100 mM; H_2O_2 10 mM) and sample were mixed in a cuvette. The change in absorbance per minute at 240 nm (DA240) was calculated. Enzyme activity was expressed in units of CAT activity per milligram of protein (U/mg protein).

One unit of CAT activity is defined as the amount of enzyme needed to reduce 1 μ mol H_2O_2 /min.

2.3.4. Determination of Kidney Tissue Glutathione Peroxidase Activity (GPx)

Glutathione Peroxidase activity in the kidney tissue was measured by monitoring the continuous decrease in NADPH concentration using H_2O_2 as a substrate as described by Flohe and Günzler [29]. Briefly, in a cuvette, potassium phosphate buffer (500 mM), EDTA (50 mM), sodium azide (20 mM), glutathione reductase (15 U/mL), NADPH (1.5 mM), reduced glutathione (250 mM), sample and H_2O_2 (10 mM) were mixed; the absorbance was followed at 340 nm, and the change in absorbance per minute (DA340) was calculated. Two blanks, one without H_2O_2 and another without sample, were run simultaneously. Enzyme activity was expressed in units of GPx activity per mg of protein (U/mg protein). One unit of GPx activity is defined as the amount of enzyme that oxidizes 1 μ mol of NADPH per min.

2.4. Evaluation of Renal Function

The level of serum urea was determined using the method of Tietz *et al.* [30]. Serum sodium and potassium ions were measured by the flame photometry method of Vogel [31] and bicarbonate ion was determined using the titration method of Segal [32]. Chloride ion was analyzed using the method of Schales and Schales [33].

2.5. Histological Preparation of Kidney Tissues

At the end of four weeks of lycopene and drug treatment, all animals from each group were euthanized and kidney tissues dissected out and fixed immediately in 10% neutral formal-saline fixative solution for histological studies with Haematoxylin and Eosin. The slides were viewed at the magnification of X 250 and photomicrographs taken.

2.6. Statistical Analysis

Data obtained from each group were expressed as mean \pm SEM. The data was statistically analyzed using ANOVA with Tukey's *Post hoc test* to compare the levels of significant between the control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software and Microsoft Excel (2007). The values of $p \leq 0.05$ were considered as significant.

3. Results

Results obtained showed that after STZ injection there was significant ($P < 0.05$) increase in the fasting blood glucose level in diabetic control animals when compared with the normal control group. These values represent the fasting blood glucose levels at week 0 that is, before treatment commenced. Following treatment with lycopene and glibenclamide, there was significant ($P < 0.05$) steady decrease on blood glucose levels especially after week 4 when compared with corresponding diabetic control group (Table

1).

The concentration of MDA, a maker of lipid per oxidation was significantly ($P < 0.05$) higher in the kidney of diabetic control rats (2.45 ± 0.10) when compared with those obtained in normal control animals (1.60 ± 0.15). However, lycopene treatment at all doses and glibenclamide lowered the level of kidney MDA to (1.80 ± 0.19 , 1.36 ± 0.18 , 1.42 ± 0.12) and (1.44 ± 0.11) when compared to diabetic control animals (Figure 1).

The activity of SOD was significantly ($P < 0.05$) depleted in the kidney of diabetic untreated rats (1.66 ± 0.05) as compared to the animals in the normal control group. Decreased activity of SOD was elevated to (1.80 ± 0.08 , 2.08 ± 0.21 , 2.32 ± 0.12) and (2.22 ± 0.06) when the diabetic animals was treated with (10, 20 and 40 mg/kg) of lycopene and glibenclamide (2 mg/kg) in comparison with diabetic control group (Figure 2).

Result obtained also showed a significantly ($P < 0.05$) decreased catalase activity in kidney of diabetic untreated group (43.20 ± 1.16) when compared with that obtained in the normal control group (52.20 ± 1.39). Administration of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) caused a significant ($P < 0.05$) increase on the level of CAT to (44.80 ± 0.90 , 47.80 ± 0.90 , 52.60 ± 1.86) and (52.00 ± 0.95) when compared with the diabetic control group (Figure 3).

The results obtained showed that kidney GPx activity was significantly ($P < 0.05$) lowered in diabetic control animals (36.00 ± 0.84) when compared with those obtained from the normal control animals (46.62 ± 0.66). Lycopene and glibenclamide administration at dose concentrations of (10, 20 40 mg/kg) and (2 mg/kg) to diabetic animals, significantly

($P < 0.05$) increase the activity of GPx to (41.20 ± 0.50 , 46.00 ± 1.79 , 49.20 ± 1.80) and 46.80 ± 0.97 when compared with the diabetic control group (Figure 4).

Table 2: showed the results of changes in some kidney serum electrolytes of both control and experimental groups. There was a significant ($P < 0.05$) depleted serum sodium ion concentration with a concomitant elevated serum urea level in diabetic untreated group (128.60 ± 0.60 and 5.04 ± 0.18), when compared with animals in the normal control animals (139.00 ± 0.84 and 2.68 ± 0.16). However, oral administration of (10, 20 and 40 mg/kg) of lycopene and (2 mg/kg) glibenclamide produced a significant ($P < 0.05$) increase of (138.80 ± 0.97 , 135.20 ± 3.93 , 130.40 ± 4.69) and (139.80 ± 1.20) in the serum sodium ion level and reduction of (3.14 ± 0.12 , 2.20 ± 0.31 , 2.40 ± 0.18) and (2.84 ± 0.24) in the serum urea level when compared with the diabetic control group. No significant ($P > 0.05$) difference was recorded in the serum potassium, chloride and bicarbonate ions concentrations of all diabetic animals treated various doses of lycopene when compared with the diabetic control group.

The histological changes of kidney tissues of control and experimental groups treated with graded doses of lycopene and glibenclamide are shown in (Plates I to VI). The microscopic findings revealed intact and normal architecture of glomeruli, renal tubules and epithelium in kidney tissue of normal control rats treated with olive oil (Plate I). However, the histological finding of kidney tissue of streptozotocin-induced diabetic untreated group showed severe glomerular necrosis with lymphocyte hyperplasia when compared (Plate II). Administration of lycopene and glibenclamide to diabetic animals showed improved renal damage as reflected by reduce glomerular and tubular necrosis (Plate III to VI).

Table 1. Effects of Lycopene Administration on Blood Glucose Levels in Streptozotocin-induced Diabetic Wistar Rats.

Treatment Groups(n=5)	Fasting blood glucose level (mg/dL)				
	Week 0	Week1	Week 2	Week 3	Week4
NC + OL (0.5 ml)	91.0 \pm 5.74 ^a	90.8 \pm 7.50 ^a	73.4 \pm 5.87 ^a	76.0 \pm 3.86 ^a	79.8 \pm 2.94 ^a
DC + OL (0.5 ml)	364.4 \pm 44.50 ^b	392.6 \pm 33.52 ^b	465.2 \pm 39.81 ^b	487.0 \pm 25.64 ^b	431.4 \pm 48.84 ^b
D+ LYC10 mg/kg	373.8 \pm 36.45 ^b	278.2 \pm 26.40 ^c	216.4 \pm 19.55 ^c	186.2 \pm 9.20 ^c	171.1 \pm 7.65 ^c
D+ LYC 20 mg/kg	371.2 \pm 40.82 ^b	277.43 \pm 24.33 ^c	240.2 \pm 21.60 ^c	183.0 \pm 10.57 ^c	118.4 \pm 1.97 ^c
D+ LYC 40 mg/kg	370.6 \pm 26.54 ^b	279.8 \pm 38.47 ^c	216.0 \pm 28.51 ^c	164.4 \pm 21.19 ^c	100.8 \pm 6.89 ^c
D+ GLB 2 mg/kg	374.2 \pm 58.65 ^b	260.3 \pm 29.744 ^c	188.0 \pm 10.06 ^d	150.2 \pm 20.28 ^c	108.8 \pm 16.74 ^c

a,b,c,d = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

a b c $P < 0.05$ Vs NC ; a b c $P < 0.05$ Vs DC

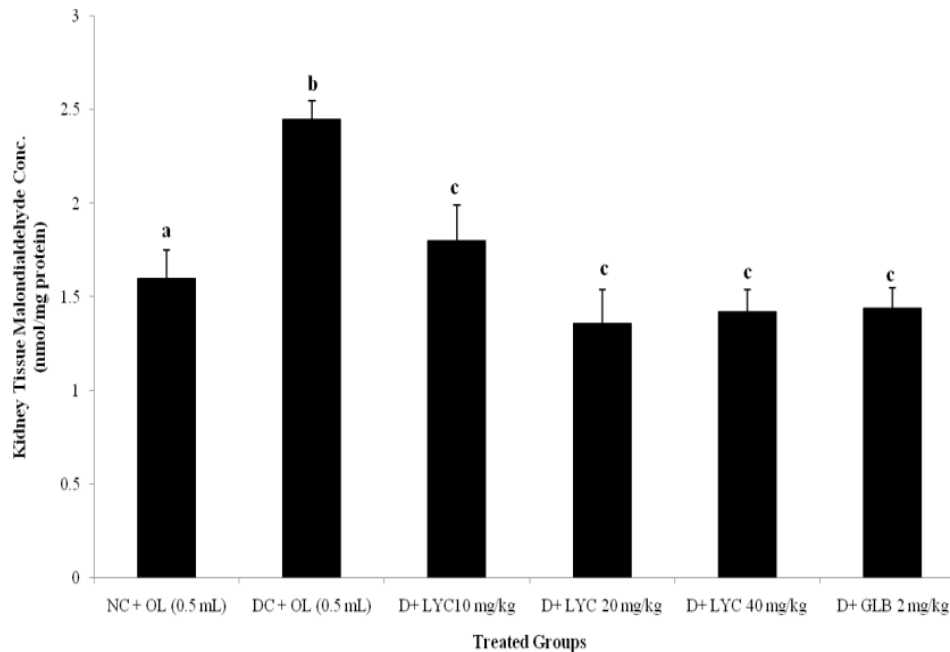
Table 2. Effects of Lycopene Administration on Some Serum Electrolytes in Streptozotocin- induced Diabetic Wistar Rats.

Treatment Groups	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO ₃ ⁻ (mmol/L)	Urea(mmol/L)
NC+OL (0.5 ml)	139.00 \pm 0.84 ^a	4.14 \pm 0.14 ^a	97.60 \pm 1.47 ^a	22.80 \pm 1.36 ^a	2.68 \pm 0.16 ^a
DC+OL (0.5 ml)	128.60 \pm 0.60 ^b	4.26 \pm 0.13 ^a	98.40 \pm 0.93 ^a	24.60 \pm 0.87 ^a	5.04 \pm 0.18 ^b
D+LYC10 mg/kg	138.80 \pm 0.97 ^c	4.08 \pm 0.17 ^a	96.40 \pm 1.17 ^a	20.80 \pm 1.02 ^a	3.14 \pm 0.12 ^c
D+ LYC 20 mg/kg	135.20 \pm 3.93 ^c	4.06 \pm 0.51 ^a	97.40 \pm 3.40 ^a	22.80 \pm 1.02 ^a	2.20 \pm 0.31 ^c
D+ LYC 40 mg/kg	130.40 \pm 4.69 ^c	3.28 \pm 0.49 ^a	92.00 \pm 3.74 ^a	21.40 \pm 2.44 ^a	2.40 \pm 0.18 ^c
D+ GLB 2 mg/kg	139.80 \pm 1.20 ^c	4.08 \pm 0.13 ^a	98.80 \pm 1.24 ^a	24.00 \pm 0.63 ^a	2.84 \pm 0.24 ^c

a, b, c = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups ; ^{a b c} $P < 0.05$ Vs NC ; ^{a b c} $P < 0.05$ Vs DC

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 mL) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated

with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg



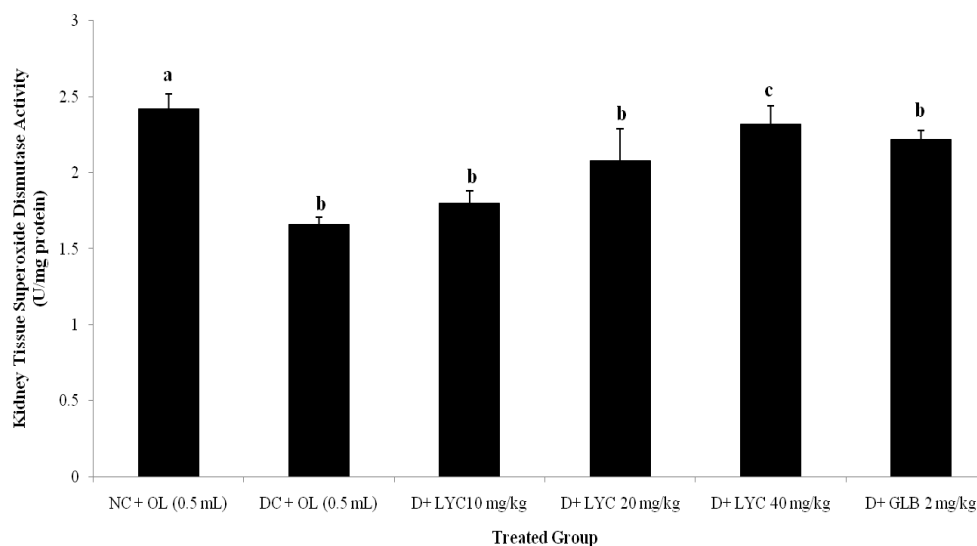
Each bar represent mean of five animals

Figure 1. Effects of lycopene on kidney tissue malondialdehyde concentration in streptozotocin-induced diabetic Wistar rats; ^{a b c} $P < 0.05$ Vs NC; ^{a b c} $P < 0.05$ Vs DC.

Bars with different superscripts letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 ml) D+ LYC10 mg/kg = Diabetic rats treated with 10

mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg



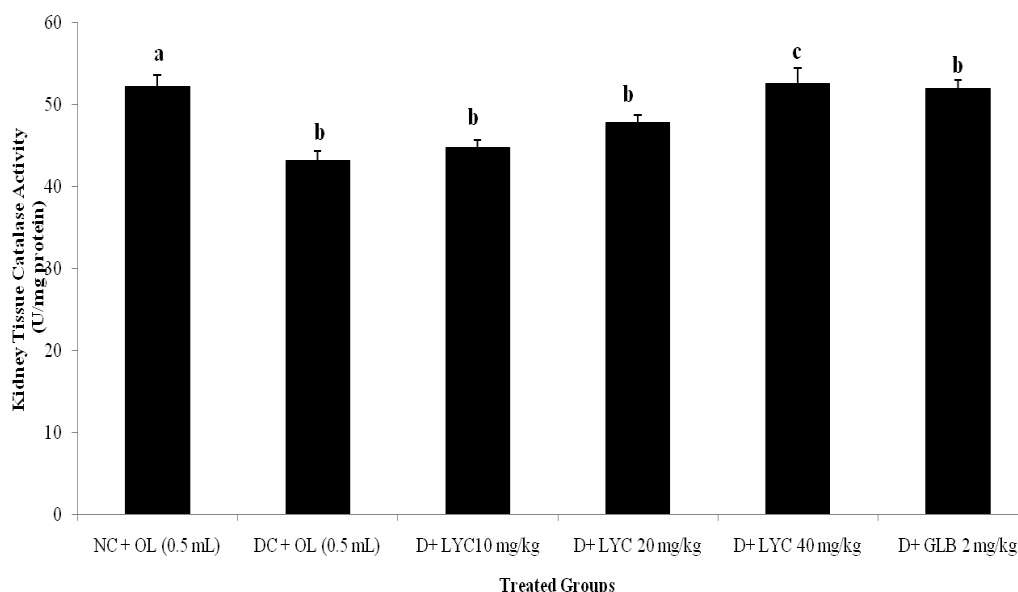
Each bar represent mean of five animals

Figure 2. Effects of lycopene on kidney tissue superoxide dismutase activity in streptozotocin-induced diabetic Wistar rats; ^{a b c} $P < 0.05$ Vs NC; ^{a b c} $P < 0.05$ Vs DC.

Bars with different superscripts letters (a, b, c) differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 ml) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated

with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg



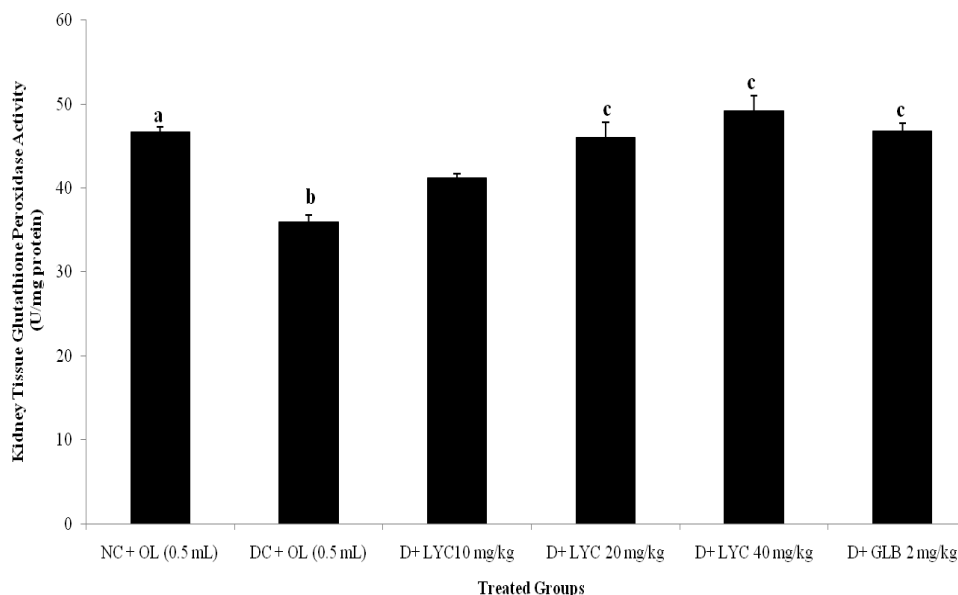
Each bar represent mean of five animals

Figure 3. Effects of lycopene on kidney tissue catalase activity in streptozotocin-induced diabetic Wistar rats; ^{a b c} $P < 0.05$ Vs NC while ^{a b c} $P < 0.05$ Vs DC.

Bars with different superscripts letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 ml) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated

with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg



Each bar represent mean of five animals

Figure 4. Effects of lycopene on kidney tissue glutathione peroxidase activity in streptozotocin-induced diabetic Wistar rats; ^{a b c} $P < 0.05$ Vs NC while ^{a b c} $P < 0.05$ Vs DC.

Bars with different superscripts letters (a, b, c) differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml),

NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 ml) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated

with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil

(0.5 ml) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg.

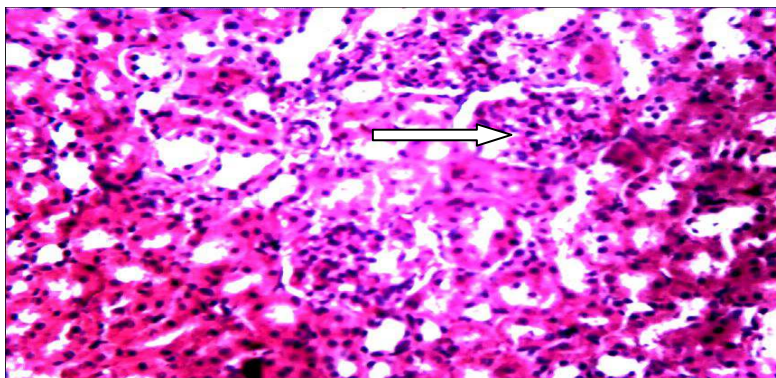


Plate I. Photomicrograph of kidney of normal control rat that received olive oil (0.5 ml) orally, showing normal glomerulus, proximal and distal convoluted tubules. Stained in H & E X 250.

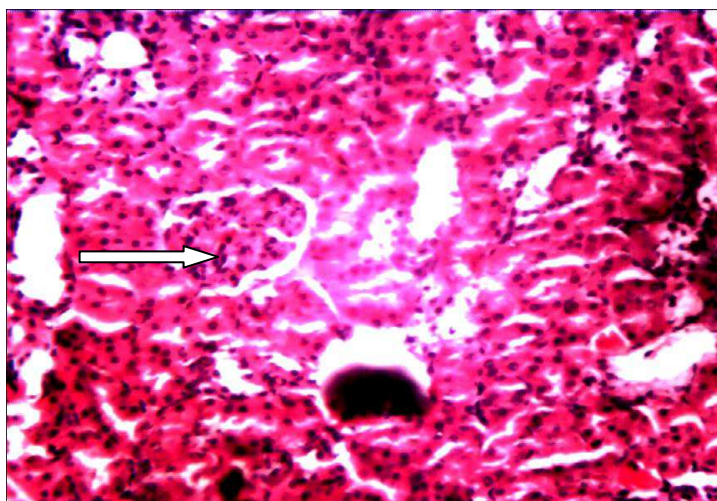


Plate II. Photomicrograph of kidney of diabetic control rat that received olive oil (0.5 ml) orally, showing severe glomerular necrosis with lymphocyte hyperplasia. Stained in H & E X 250.

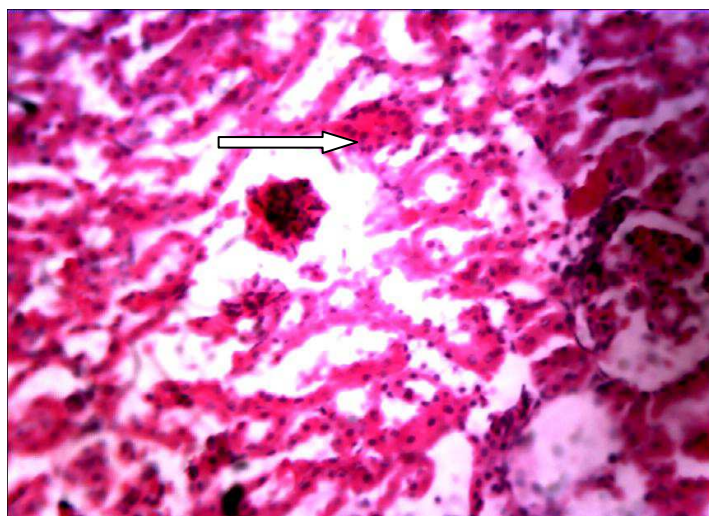


Plate III. Photomicrograph of kidney of diabetic rat administered with 10 mg/kg b w of lycopene orally, showing moderate glomerular and tubular necrosis. Stained in H & E X 250.

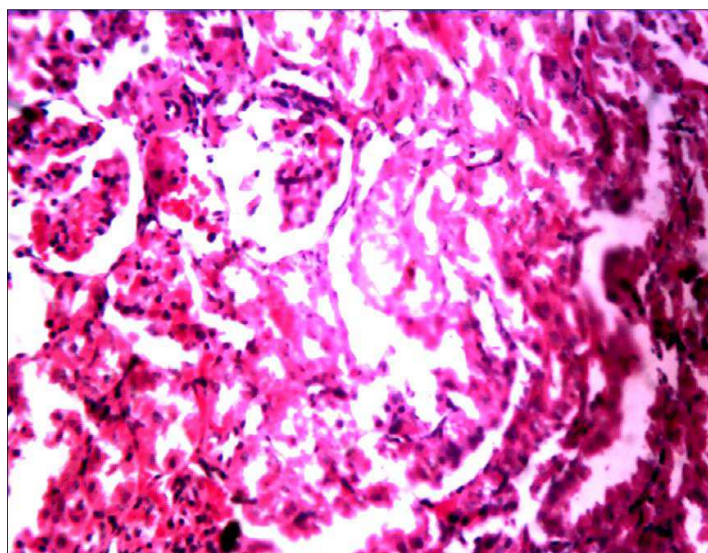


Plate IV. Photomicrograph of kidney of diabetic rat administered with 20 mg/kg b w of lycopene orally, showing moderate glomerular and tubular necrosis. Stained in H & E X 250.

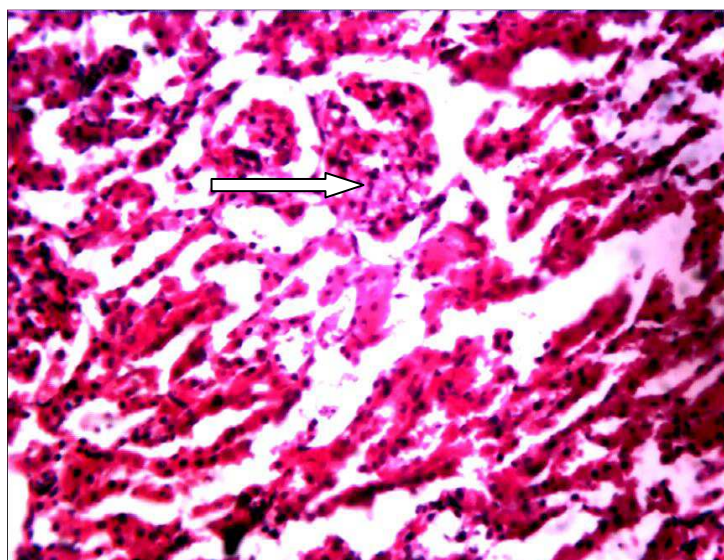


Plate V. Photomicrograph of kidney of diabetic rat administered with 40 mg/kg b w of lycopene orally, showing mild glomerular and tubular necrosis. Stained in H & E X 250.

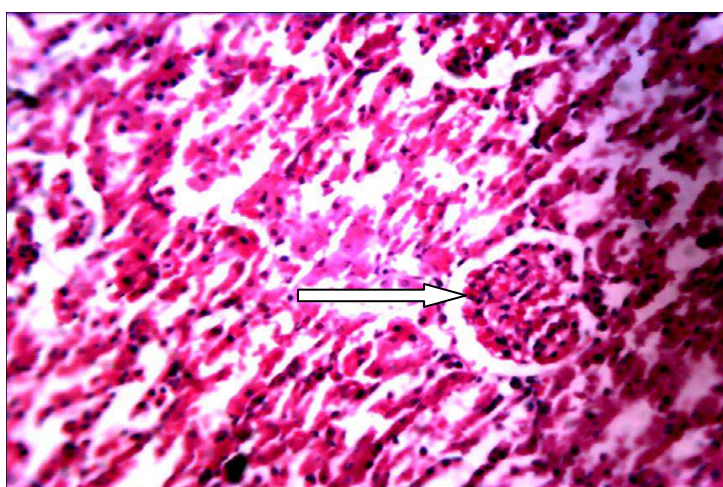


Plate VI. Photomicrograph of kidney of diabetic rat administered with 2 mg/kg b w of glibenclamide orally, showing mild glomerular and tubular necrosis. Stained in H & E X 250.

4. Discussion

In this study, the intra-peritoneal administration of streptozotocin (STZ) effectively induced diabetes mellitus in rats which was confirmed by elevated levels of fasting blood glucose, 72 hours after STZ injection. This agrees with the reports of Mohammed *et al.* [23] and Krishna *et al.* [34] who demonstrated that blood glucose level was increased significantly after 72 hours of STZ injection to Wistar rats. Streptozotocin has been shown to induce diabetes which is similar to human hyperglycaemic non-ketotic diabetes mellitus in animal models. STZ selectively destroys the insulin producing β -cells which is accompanied by characteristic alterations in blood insulin and glucose concentrations [35]. Oral administration lycopene significantly decreased the blood glucose concentration in diabetic animals with highest hypoglycaemic activity of lycopene observed after week 3 and week 4 respectively when compared with corresponding diabetic untreated animals. This finding agrees with the reports of previous investigators [36, 37, 38, 39]. Furthermore, oxidative stress induced by reactive oxygen species generated due to sustained hyperglycaemia has been implicated in the onset and progression of diabetes mellitus and its related complications [40]. Hyperglycemia in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increases the levels of free radicals [41]. Lycopene which is one of the potent antioxidants have been shown to have good free radical scavenging capacity because of its unique structure (high number of conjugated double bonds) [42]. Therefore, hypoglycaemic effect of lycopene may also be attributed to its strong antioxidant property [43]. Bose and Agrawal [42] reported that lycopene have the ability to quench the superoxide and other free radical anions which are released in diabetes due to abnormal glucose metabolism, hence resulting to decreased blood glucose concentration in diabetic animals as observed in the present study.

The biochemical indices monitored in the kidney are useful 'markers' for assessment of tissue damage. The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis [44] as well as assault on the organs/tissues and to a reasonable extent the toxicity of the drug [45]. And one of the most important among numerous diseases in which changes in antioxidant defense systems are detected is diabetes mellitus [19]. Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques [46]. The present investigation indicated that the concentration of MDA, a maker of lipid peroxidation was significantly higher in the kidney tissue of diabetic untreated rats when compared with the animals in the normal control group. This result corroborates other investigators [47, 48, 49] who have reported a significantly increased kidney MDA in experimentally induced diabetes in animals. This increase in the kidney MDA indicated enhanced lipid peroxidation

which could cause injury to the cells. Increased levels of lipid peroxides in the plasma are usually considered to be the consequence of high production and liberation of tissue lipid peroxides into circulation due to pathological changes [50]. Oxidative stress causes a bimolecular damage as a result of the attack of reactive species on components of living organisms and is known as oxidative damage [51]. This is caused by increased production and or reduction in the removal of reactive species by the antioxidant defenses [51]. Hyperglycaemia leads to generation of free radicals due to auto-oxidation of glucose and glycosylation of proteins [52] and induces oxidative stress which becomes the chief factor that leads to diabetic complications [53]. Abnormal elevated levels of free radicals and the simultaneous reduction of antioxidant defense can result in damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance [54]. The elevated level of lipid peroxidation causes oxidative damage by increasing peroxy radicals and hydroxyl radicals [55] and is usually measured through the catabolite, malonaldehyde (MDA), in terms of TBARS as a marker of lipid peroxidation [56]. The decrease in MDA of kidney tissues upon administration of lycopene to diabetic animals in the present study, clearly demonstrated the antioxidant property of lycopene. These findings suggest that the lycopene may exert antioxidant activity and protect the tissue from lipid peroxidation. In the same manner, the kidney antioxidant enzymes (SOD, CAT and GPx) of diabetic control animals were significantly decreased in comparison with the animals in the normal control group. These observations are in consonance with the findings of Kinalskiet *al.* [47] and Bukanet *al.* [48] who demonstrated a significant decrease in kidney antioxidant enzymes in diabetes. SOD, a superoxide radical scavenging enzyme is considered the firstline of defense against the deleterious effect of oxygen radicals in the cells and it scavenges reactive oxygen radical species by catalyzing the dismutation of O_2^- radical to H_2O_2 and O_2 [57]. SOD has been reported to be involved in the conversion of superoxide anion radicals produced in the body to hydrogen peroxide, thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite. As a result, reduction in SOD activity in diabetic animals observed in the present study may be as a results of an increased influx of O_2^- radical and hence may reflects the cause of tissue injury [57]. This finding is consistent with the report of Kedziora-Kornatowska *et al.* [58] who reported decreased renal activity of SOD three to six weeks after STZ administration. In contrast, treatment of diabetic rats with lycopene and glibenclamide enhanced the activity of SOD in comparison with the diabetic control group. On the other hand, catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [59]. The present investigation showed that STZ injection caused a significant decrease in the activity of kidney catalase level. This agrees with the finding of Rauscher *et*

al.[60] who have reported similar observation. In the current study, the decreased CAT activity in kidney of diabetic animals was significantly improved following oral administration of lycopene and glibenclamide. This observation is in consonance with the reports of Kuhadet *al.*[37] and Ali and Agha [38] who showed that administration of lycopene (90 mg/kg body weight) to streptozotocin-induced hyperglycemic rats caused increased antioxidant enzyme activities (i.e., superoxide dismutase and catalase). In addition, glutathione peroxidase (GPx) is a potent endogenous antioxidant that helps to protect cells from a number of noxious stimuli including oxygen derived free radicals [61, 62]. GPx is an antioxidant enzyme involved in the detoxification of hydrogen and lipid peroxides and also acts as a peroxynitrite reductase [63]. A significant decrease in GPx activity might be accompanied by a significant increase in lipid peroxidation as evidenced by increased kidney MDA concentration observed in the present study. The results of the current finding agree with the reports of other researchers [64]. However, administration of graded doses of lycopene and glibenclamide to diabetic animals to significantly increased level of the activity of GPx. These findings have been substantiated by other investigators [37, 38]. Therefore, the increased activity of GPx might be a protective mechanism in response to increased concentrations of H₂O₂ and other lipid peroxides in kidney of diabetic animals treated with lycopene. These observations also indicate that lycopene has nephroprotective effects. Lycopene's configuration has been reported to be responsible for its ability to inactivate free radicals and to interfere with free-radical-initiated reactions, particularly lipid peroxidation, thereby preventing tissue injury [65]. The antioxidant enzymes are very good biochemical markers of stress and their elevated activity may confirm a potential for remediation [66]. In addition, it has been reported that lycopene has high efficient antioxidant and free radical scavenging capacity [43]. Lycopene has been shown to be one of the best biological suppressants of free radicals, especially those derived from oxygen. It has the highest singlet oxygen-quenching rate of all carotenoids in biological systems [67]. The findings in the present study denoted the ability of lycopene to protect the kidney tissue from oxidative damage through elevation of endogenous antioxidant enzymes (SOD, CAT and GPx). The attenuation of kidney tissue in diabetic animals treated with lycopene, which also positively correlated with the significantly reduced kidney MDA level a marker of oxidative stress, further strengthens the notion and also suggests that lycopene may have the ability to protect the kidneys from oxidative injury.

Glycosuria, which is a pertinent diagnostic feature of diabetes, imposes dehydration via glucose osmotic diuresis [68, 69, 70]. In the present study, our findings showed that there was a significant decrease in the serum sodium ion concentration when compared with the normal control animals. Kidney function has been reported to be compromised in uncontrolled diabetes mellitus. Hence the depleted serum sodium ion may be attributed to dehydration

which is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates. However, oral administration of lycopene to diabetic rats restored the serum sodium ion level almost close to normal. On other hand, the study also showed a significant increased serum urea level in diabetic control rats in comparison with the normal control animals. Plasma urea are recognized markers of glomerular filtration rate (GFR) and in nephropathy [71]. This result is an agreement with the report of Sapna *et al.*[72] that has showed increased serum urea level in diabetic patients. An elevation of serum urea usually signifies decreased renal function [72]. More so, in diabetes there is increased catabolism of amino acids resulting in high urea formation from the urea cycle [72]. Hence in this study, the elevation of serum urea with hyperglycemia can be suggested as indicator for renal dysfunction [73], and reduced filtering capacity of kidneys which leads to accumulation of waste products within the system of diabetic animals. Treatment diabetic rats with lycopene and glibenclamide produced a significantly reduced serum urea level, suggesting its ability to protect against diabetes-induced kidney damage, by preventing altered protein metabolism and/or impaired renal function that often exist in diabetes mellitus. Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes [69, 70]. Although no significant difference was recorded in the serum potassium, chloride and bicarbonate ions concentrations in diabetic control rats when compared with normal control animals. Following treatment with lycopene the serum level of potassium, chloride and bicarbonate ions did not differ significantly with those of diabetic control animals when compared.

5. Conclusion

Available evidence obtained in the present study demonstrated lycopene produced a significant improvement in blood glucose level and attenuated kidney oxidative damage by significant reduction in kidney MDA concentration and increased kidney SOD, CAT and GPx activities in diabetic animals. There was also improved renal function as indicated by restored depleted serum sodium ion and significantly reduced serum urea level in diabetic animals. Thus, this finding suggests the ability of lycopene to protect against diabetes-induced kidney injury.

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