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Lycopene ameliorates atherogenic cardiovascular risk in streptozotocin-induced diabetic hyperlipidaemia in wistar rats

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ABSTRACT

The aim of the study was conducted to investigate the ameliorative effects of lycopene in atherogenecity of streptozotocin-induced diabetic hyperlipidaemia in Wistar rats. Thirty (30) Wistar rats of which twenty of them were made diabetic by single intraperitoneal injection of 60 mg/kg b w of streptozocin. After which the diabetic animals were randomly divided into the following groups: Groups (I, II, III, IV, V and VI). Group I and II (NC + 0.5 ml of olive oil asn DC + 0.5 ml of olive oil), while Group III-V (received 10, 20 and 40 mg/kg b w of lycopene) and Group VI (received 2 mg/kg b w of glibenclamide). All administration was by oral gavage and it lasted for four weeks. After the last day of treatment, blood sample was collected and serum separated and used for determination of serum lipid profile. Atherogenic indices and cardiac risk ratio were calculated. There was a significant ($P < 0.05$) dose dependent reduction blood in glucose level with an increase of insulin concentration which was not statistically significantly when compared with diabetic control rats. The results showed a significantly ($P < 0.05$) decreased serum levels of total cholesterol, triglyceride and low-density lipoprotein with a corresponding elevated serum high-density lipoprotein level in diabetic-lyccopene treated animals in comparison with the diabetic control group. Serum atherogenic risk predictor indices (LDL-cholesterol/ HDL-cholesterol, and log (TRIG/HDL-cholesterol) and cardiac risk ratio were significantly deceased ($P < 0.05$) while HDL-cholesterol/TC was increased significantly ($P < 0.05$) when compared with diabetic control group. The experimental animals also showed a significantly ($P < 0.05$) increased percentage protections especially with the highest dose of lycopene recording the highest increased protection. From the available evidence in this study it can be concluded that lycopene ameliorated atherogenic diabetic-inducd dyslipidaemia, hence may have cardio-protective effects due to their high antioxidant activity.

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KEYWORDS

Diabetes mellitus;
Atherogenesis;
Dyslipidaemia;
Lycopene;
Lipid profile.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of the endocrine system[1] and among the most common disorders in both developed and developing countries[2][3]. It has become a global metabolic epidemic, affecting important biochemical activities in nearly every age group[4][5]. It has been estimated that the number of people with diabetes will rise from the present 150 to 230 million in 2025[6][7]. Uncontrolled chronic hyperglycaemia as a result of absolute insulin deficiency (type 1 diabetes) or insulin resistance with or without insulin deficiency (type 2 diabetes) is one of the primary causes of diabetic complications in a number of organs[8]. Chronic hyperglycaemia leads to many long-term complications in the eyes, kidneys, nerves, heart, and blood vessels[9][10]. It has been reported that diabetes is a risk factor for cardiovascular disease[11] and more than 70% of type 2 diabetic patients die of cardiovascular diseases[12]. Cardiovascular diseases accounts for up to 80% of premature mortality in diabetic patients (Winer and Sowers, 2004). The major cardiovascular diseases related to diabetes include atherosclerosis which is a major risk factor for Coronary Artery Disease (CAD)[14]. Besides oxidative stress, dyslipidemia is also a common metabolic disturbance associated with diabetes, as 97% of diabetics are dyslipidemic[15][16]. Atherogenic dyslipidemia, is characterised by increased levels of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and decreased high density lipoprotein (HDL)[17][18]. It has become clear that ameliorating oxidative stress using antioxidants might be an effective strategy for the treatment of diabetes mellitus and also reducing diabetic complications[19][20].[21] reported that diabetes is known to have a multi-factorial pathogenicity and therefore, demands a multi-modal therapeutic approach. Great efforts have been made in the understanding and management of diabetes mellitus[21][22]. In addition, management of diabetes mellitus without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with potent anti-diabetic activity and fewer side effects[23]. The recognition of the potential role for nutraceuticals and dietary supplements in helping to reduce health risks and improve health quality is on the increase[24]. Lycopene being an antioxidant has been suggested to protect critical bimolecules including lipids, protein and DNA from free radicals[25]. Many drugs are available for use in the treatment of diabetes, but their long-term use may cause adverse side effects and hence, the increased search for natural remedies for the effective treatment of diabetes exists[26]. In both experimental and clinical models of diabetes, antioxidants have been reported to reduce markers of oxidative stress[27][28][29]. Besides, some studies have showed that antioxidants are effective and cheaper than conventional therapy in management of some diseases[30] including diabetes mellitus. Therefore, antioxidants or nutrients with high antioxidant capacity may offer additional health benefits with potential for limiting the progression of diabetes and its related complications[31]. Lycopene is a red pigment that occurs naturally in certain fruits, vegetables, algae, and fungi. It belongs to a large group of pigments known as carotenoids[32]. Tomatoes and tomato-based products are the major sources of natural lycopene in the human diet[33]. Other significant sources of lycopene include watermelon, pink grapefruit, pink guava, and apricots[33]. Lycopene occurs in the all-*trans* and various *cis* configurations. Naturally-occurring lycopene consists predominantly of all-*trans*-lycopene. For example, lycopene present in red tomato fruits typically contains 94-96% of all-*trans*-lycopene[34]). Lycopene is one of the most potent antioxidants among dietary carotenoids. It has strong antioxidant property which can neutralize oxygen-derived free radicals[35]. The presence of large number of double bonds is responsible for its fairly high free radical scavenging or singlet oxygen quenching ability of lycopene[36]. As an antioxidant, lycopene has a single-oxygen-quenching ability twice as high as that of beta-carotene (vitamin A relative) and 100 times higher than that of alphatocopherol (vitamin E relative), which in turn has 125 times more quenching action gluthathione (water soluble)[37].[38] reported that lycopene has potential to prevent various chronic ailments like dyslipidemia, diabetes, oncogenesis, neurodegenerative diseases osteoporosis and so on.

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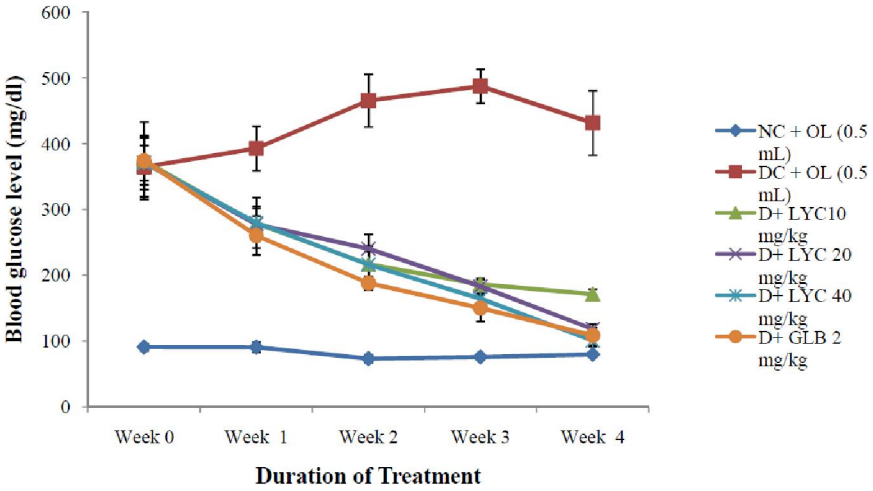

DC+OL = Diabetic rats + olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats + olive oil (0.5 ml), D+ LYC10 mg/kg = Diabetic rats + 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats + 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Daibetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg

Figure 1 : Effects of lycopene on blood glucose level in streptozotocin-induced diabetic wistar rats

Until now, the possible beneficial effect of lycopene for the control of progression of diabetes has not been given much serious attention. Several studies have reported the beneficial roles of lycopene as antioxidant in the prevention and amelioration various ailments, but most of investigations are mainly epidemiological in nature and coupled with the fact that there is limited information on the effects of lycopene on blood glucose level and it cardiovascular complications in animals as model. This study was designed to investigate the ameliorative effects of lycopene on atherogenic cardiovascular risk in streptozotocin-induced diabetic hyperlipidaemia in Wistar rats.

MATERIALS AND METHODS

Materials

Experimental animals and management

Adult Wistar rats of both sexes weighing 150 to 200 g were be obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria, Kaduna State. The animals kept and maintained under laboratory condition of temperature, humidity and light. The animals were housed five animals per cage. The animals were fed on standard commercial feeds with water *ad libitum.*

Chemicals and lycopene

Streptozotocin and DPPH (1-1- diphenyl 2-picryl hydrazyl) was purchased from Sigma chemicals (St Louis U.S.A), while Lycopene (30 mg capsule, General Nutrition Corporation, Pittsburgh, U.S.A.) was procured from Live well Pharmacy, Ceddi Plaza Central Area, Abuja, Federal Capital Territory, Nigeria. It was reconstituted in olive oil (*Goya en espana*, S.A.U., Savilla, Spain) to appropriate working dosage. All chemicals and solvents used were of analytical grade.

Induction of experimental diabetes mellitus

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozocin (STZ) dissolved in fresh 0.1M cold citrate buffer of pH 4.5 into 18 h-fasted rats[39]. 72 hours after STZ injection, blood was taken from tail artery of the rats. Animals having blood glucose levels greater than 200mg/dl were considered diabetic and included in the study. The diabetic animals were randomly divided into different groups.

Experimental protocol and treatment

In the experiment, a total of 30 Wistar rats were used; the animals were randomly divided into six groups of five rats each as follows:

Group I: Normal control (NC) and was administered 0.5 ml/kg body weight of olive oil used in dissolving lycopene

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Groups II: (DC) Diabetic control and was treated with 0.5 ml/kg body weight of olive oil used in dissolving lycopene

Group III: Diabetic and treated with 10 mg/kg b w of lycopene

Group IV: Diabetic and treated with 20 mg/kg b w of lycopene

Group V: Diabetic and received 40 mg/kg b w of lycopene

Group VI: Diabetic and received Glibenclamide 2 mg/kg b w

30 mg lycopene in a gelatinous capsule (General Nutrition Corporation, Pittsburgh, U.S.A.) was reconstituted in olive oil (*Goya en espana*, S.A.U., Sevilla, Spain) to appropriate working concentration as described by[40] and[41] with little modifications. All administration was given orally once daily by gavage for four (4) weeks.

Determination of blood glucose level

Blood glucose level was determined by collection of blood sample from the tail artery of the rats at interval of 0 Week, 1st Week, 2nd Week, 3rd Week and 4th Week of the treatment period respectively by glucose-oxidase principle described by[42] using digital glucometer (Accu-chek Advantage) and was expressed in the unit of mg/dl.

Blood sample collection and serum preparation

After the last day of treatment (28 days) all animals from each group was sacrificed using light chloroform after 24 hours and blood was collected through cardiac puncture into a specimen bottles and was allowed to clot and separated by centrifugation at 2,000 × *g* for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany) and the supernant obtained was used for the determination of physiological and biochemical parameters respectively.

Determination of serum insulin level

The estimation of serum insulin levels was done by radio-immunoassay (RIA) using Mercodia Ultrasensitive Rat Insulin ELISA kits (10-1251-01).

Determination of lipid profile

Serum total cholesterol was determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited kits, Unite kingdom) as described by method of[43], while serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of[44]. The serum level of HDL-C was measured by the method of[45] and the serum level of (LDL-C) was measured according to protocol of[46] using the relationship the following formula:

$$\text{LDL cholesterol} = \frac{[\text{Total cholesterol}] - [\text{HDL cholesterol}] - \text{Triglyceride}}{2.2}$$

All the values obtained were expressed in the unit of (mmol/L).

Determination of Atherogenic Risk Predictor Indices and cardiac risk ratio

Atherogenic Risk Predictor Indices were calculated as earlier reported by Ikewuchi and Ikewuchi[47][48][49][50]. While the percentage protection was calculated based on the method of[51] using the following formulae:

$$\text{Cardiac Risk Ratio} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL cholesterol}}{\text{HDLcholesterol)}} \quad \text{and} \quad \log \frac{\text{Triglyceride}}{\text{HDL cholesterol}}$$

$$\text{Percentage Protection} = \frac{\text{AI of Diabetic Control} - \text{AI of Diabetic Treated}}{\text{AI of Diabetic Control}} \text{ X } 100$$

Statistical analysis

Data obtained from each group was expressed as mean ± 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.

RESULTS

Effect of lycopene on blood glucose level in streptozotocin-induced diabetic wistar rats

Figure 1 shows that the comparison between the normal control (NC) and diabetic control (DC) showed a significantly increased ($P < 0.05$) blood glucose concentration from (91.0 ± 5.74) to (364.4 ± 44.50) on week 0. That is before the commencement of treatment. However, treatment of diabetic animals with the graded doses of lycopene (10, 20

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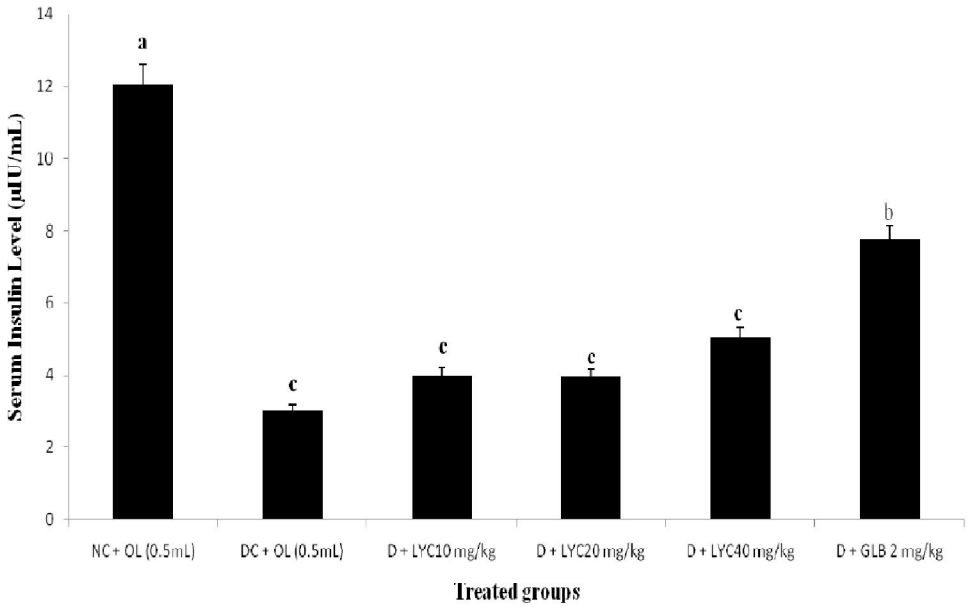

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Figure 2 : Effects of lycopene on serum insulin level in streptozotocin-induced diabetic wistar rats

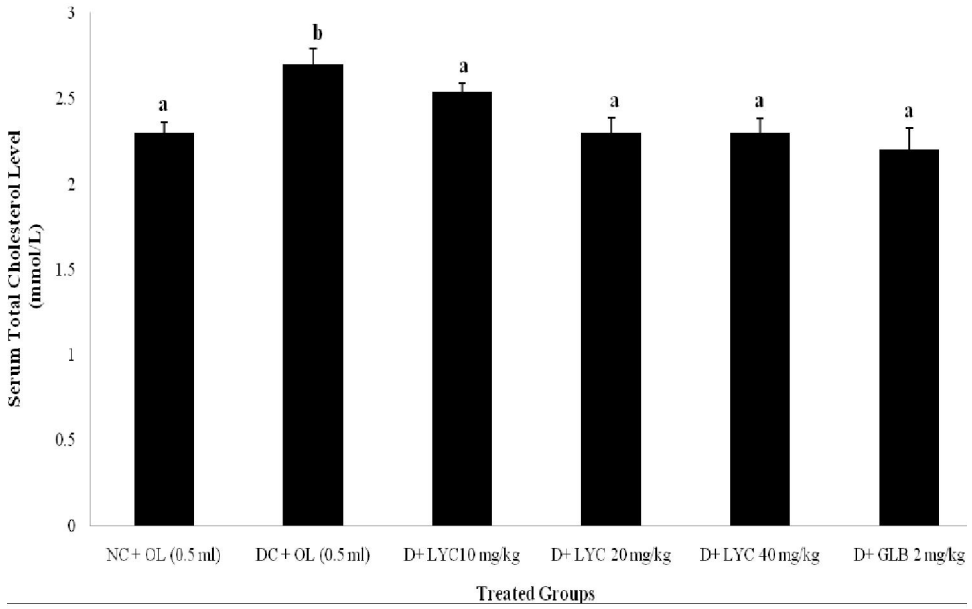

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Figure 3 : Effects of lycopene on serum total cholesterol level in streptozotocin-induced diabetic wistar rats

and 40 mg/kg) and standard drug Glibenclamide (2mg/kg) significantly ($P < 0.05$) decreased the blood glucose concentration from (392.6 ± 33.52) to (278.2 ± 26.40, 277.43 ± 24.33, 279.8 ± 38.47 260.3 ± 29.74) after week 1, (465.2 ± 39.81) to (216.4 ±19.55, 240.2 ±21.60 216.0 ±28.51 and 188.0 ± 10.06) after week 2, (487.0 ± 25.64) to (186.2 ±9.20, 183.0 ± 10.57, 164.4 ± 21.19 and 150.2 ± 20.28) after week 3 and (431.4 ± 48.84) to 171.1 ± 7.65, 118.4 ± 1.97 100.8 ± 6.89 and 108.8 ± 16.74) after

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week 4 when compared with corresponding diabetic untreated group.

Effect of lycopene on serum insulin level in streptozotocin-induced diabetic wistar rats

Results obtained indicated that the serum insulin levels decreased significantly ($P < 0.05$) to (3.02 ± 0.24) in the diabetic untreated animals (DC) following streptozotocin (STZ) treatment from (12.04 ± 0.93) in normal control (NC) when compared. However, administration of various doses (10, 20 and 40 mg/kg) of lycopene to diabetic rats elevated serum insulin level to 4.02 ± 0.70, 3.96 ± 1.41 and 5.06 ± 0.96 but was not statistically significant when compared with diabetic control group. Conversely, treatment of diabetic animals with the standard drug (Glibenclamide) 2 mg/kg produced a significantly ($P < 0.05$) increased the serum insulin level to (7.76 ± 0.42) when compared with the diabetic control group that recorded (3.02 ± 0.24) Figure 2.

Effect on serum total cholesterol concentration

The study showed that levels of serum total cholesterol was significantly ($P < 0.05$) increased to 2.70 ± 0.09 in the diabetic untreated group (DC) when compared to the normal control group that recorded (2.30 ± 0.06). Oral administration of lycopene, especially at doses 10 and 40 mg/kg and Glibenclamide (2 mg/kg) significantly ($P <0.05$) decreased the serum total cholesterol level to (2.30 ± 0.09 and 2.30 ± 0.08) when compared with diabetic untreated animals that recorded (2.70 ± 0.09) Figure 3.

Effect on serum triglyceride level

The result of serum triglyceride level shown in Figure 4 showed significant ($P < 0.05$) increase in the lavel of serum triglyceride to (1.36 ± 0.09) in diabetic control animals (DC), when compared with the normal control group (NC) that recorded (0.58 ± 0.08). Administration of graded doses of lycopene to diabetic rats produced a significant ($P < 0.05$) reduction of serum triglyceride level to (1.00 ± 0.07, 0.72 ± 0.06 and 0.52 ± 0.04) in a dose dependent manner when compared with the diabetic control group. The effect of lycopene on serum triglyceride reduction was to similar to glibenclamide (2 mg/kg) which recorded (0.54 ± 0.07) when compared with the diabetic conrol group.

Effect on serum high density lipoprotein-cholesterol level

Result obtained showed that the mean serum high density lipoprotein level of diabetic control group (DC) was significantly ($P < 0.05$) decreased to (0.86 ± 0.06) when compared with the normal control animals (NC) that showed (1.26 ± 0.05). However, treatment of diabetic animals with 10, 20 and 40 mg/kg of lycopene resulted to a significantly ($P < 0.05$) elevated level of serum HDL-c to (1.18 ± 0.05, 1.26 ± 0.16 and 1.44 ± 0.17). However, diabetic group treated with glibenclamide (2 mg/kg) produced a non significant ($P > 0.05$) change on serum HDL-c level when compared with the diabetic untreated animals Figure 5.

Effect on serum low density lipoprotein-cholesterol level

Figure 6 shows that the mean serum LDL-c level was significantly ($P < 0.05$) increased in the diabetic untreated animals to (1.57 ± 0.10) when compared with normal control animals (NC) that had (0.92 ± 0.07). Treatment of diabetic animals with various doses of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) significantly ($P < 0.05$) reduced the serum level of LDL-c to (1.16 ± 0.06, 0.89 ± 0.20 0.53 ± 0.22, 1.11 ± 0.05) and (1.11 ± 0.05) in a dose dependent manner when compared with the diabetic control group.

Effect on Serum Atherogenic Risk Predictor indices (LDL-cholesterol/HDL-cholesterol)

The result of the study obtained showed that the mean values of atherogenic risk predictor indices (LDL-cholesterol/HDL-cholesterol) was significantly ($P < 0.05$) increased in the diabetic control group (DC) to (1.88 ± 0.25), when compared with the normal control group (NC), which recorded (0.74 ± 0.82). However, there was a significant ($P < 0.05$) reduction in the serum level of (LDL-cholesterol/HDL-cholesterol) to (1.00 ± 0.10, 0.81 ± 0.22, 0.45 ± 0.21) and (1.15 ± 0.08) in the diabetic groups that received (10, 20 and 40 mg/kg) of lycopene and glibenclamide (2 mg/kg) when compared with the

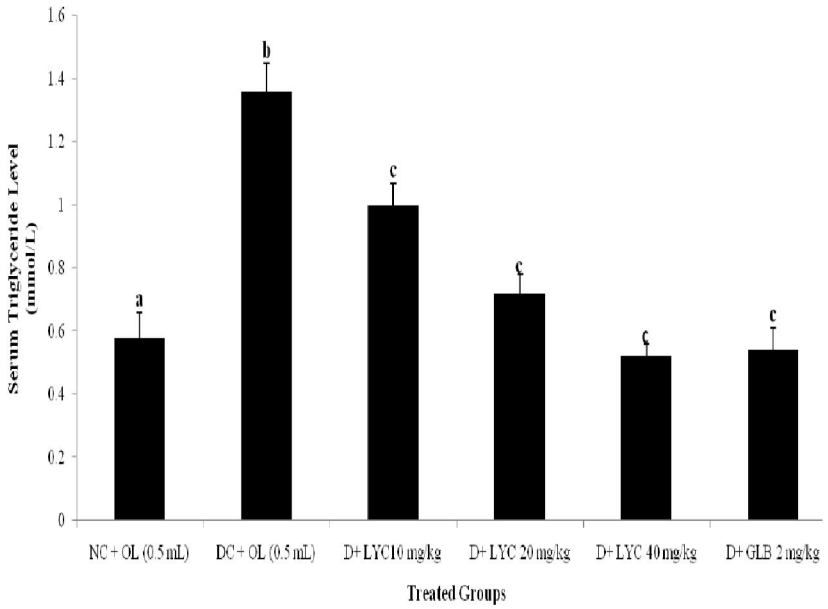

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Figure 4 : Effects of lycopene on serum triglyceride level in streptozotocin-induced diabetic wistar rats

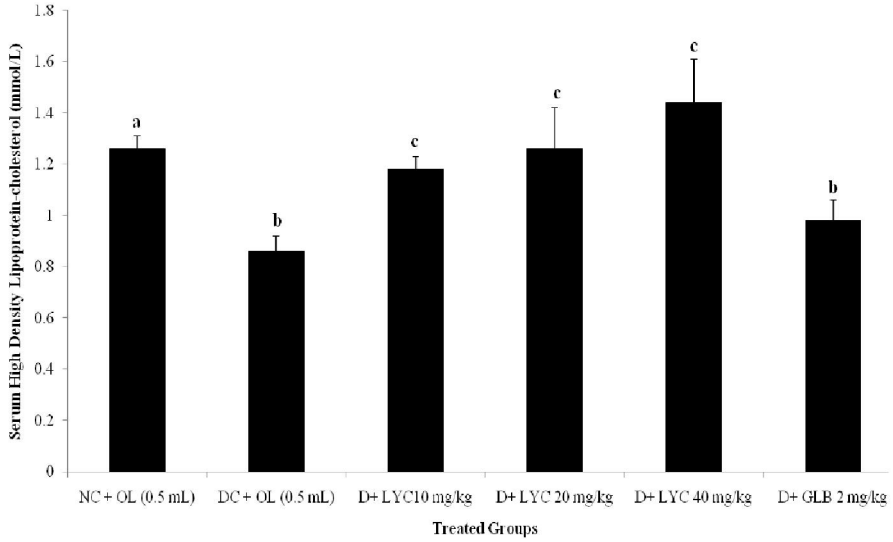

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Figure 5 : Effects of lycopene on serum high-density lipoprotein cholesterol level in streptozotocin-induced diabetic wistar rats

diabetic untreated animals Figure 7.

Effect on Cardiac Risk Ratio (CRR) (TC/HDL-cholesterol)

Result obtained shows a significantly ($P < 0.05$) increased CRR (3.21 ± 0.28) in the diabetic control animals (DC) when compared with those in the normal group (NC) ($1.8\,3 \pm 0.08$). Oral administration of 10, 20 and 40 mg/kg of lycopene and glibenclamide 2 mg/kg produced a dose dependent decreased in CRR (2.17 ± 0.11, 1.94 ± 0.24 and 1.70 ± 0.23) and (2.26 ± 0.09) when compared with

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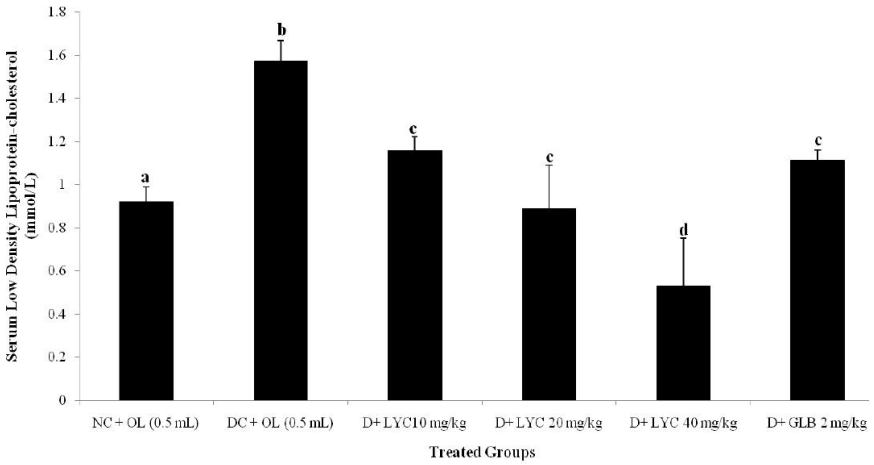

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Figure 6 : Effects of lycopene on serum low-density lipoprotein cholesterol level in streptozotocin-induced diabetic wistar rats

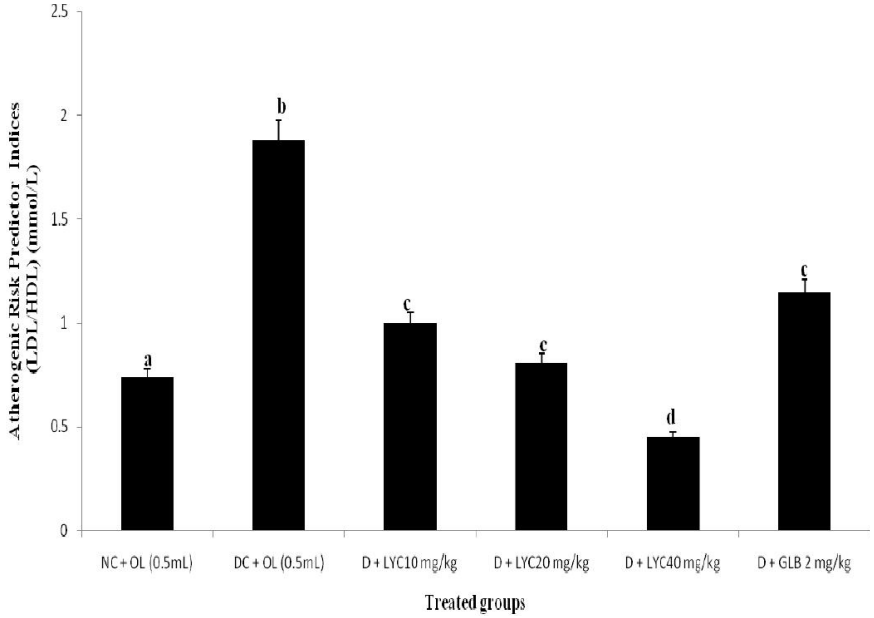

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Figure 7 : Effects of lycopene on serum atherogenic risk predictor indices (LDL-C/HDL-C) in streptozotocin-induced diabetic wistar rats

animals in diabetic control group Figure 8.

Effect on Serum Atherogenic Risk Predictor indices (HDL-cholesterol/TC)

Figure 9 shows that there was a statistically significant ($P < 0.05$) decrease in the serum level of HDL-cholesterol/TC to (0.32 ± 0.03) in the diabetic untreated animals (DC), when compared with the normal control (NC) rats that had a mean serum level of HDL-cholesterol/TC of (0.56 ± 0.02). However, the mean serum level of HDL-cholesterol/TC was increased significantly ($P < 0.05$) to (0.46 ± 0.02, 0.56 ± 0.08, 0.63 ± 0.09) and 0.45 ± 0.02 in all lycopene and glibenclamide (2 mg/kg) treated group

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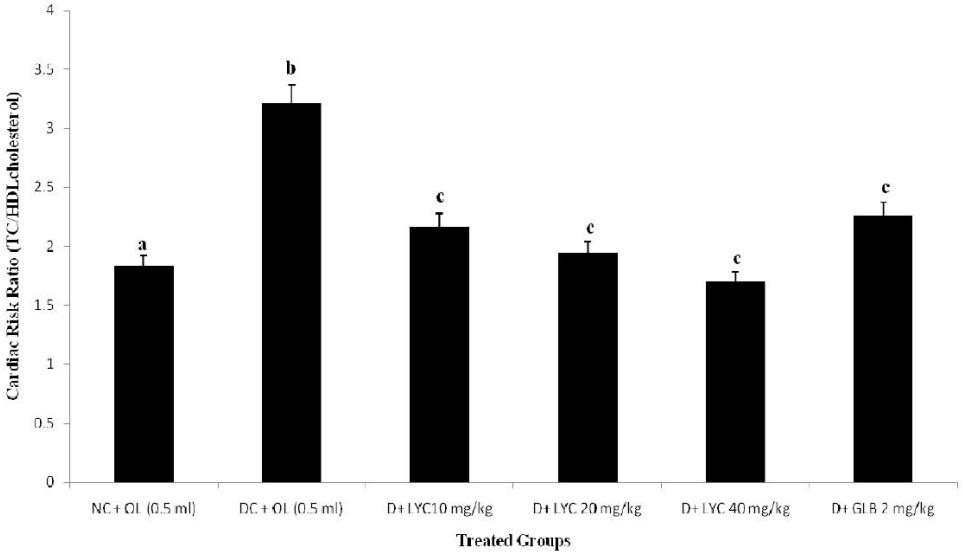

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Figure 8 : Effects of lycopene on cardiac risk ratio (TC/HDL cholesterol) in streptozotocin-induced diabetic wistar rats

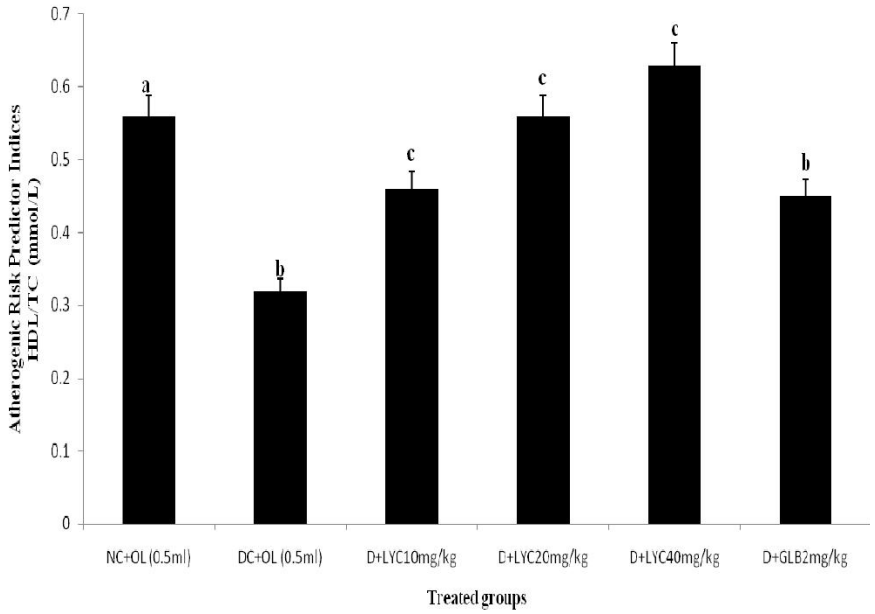

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Figure 9 : Effects of lycopene on serum atherogenic risk predictor indices (HDL-c/TC) in streptozotocin-induced diabetic wistar rats

when compared with the diabetic control group.

Effect on Serum Atherogenic Risk Predictor indices log (TRIG/HDL-cholesterol)

Result obtained revealed that the mean serum level of log (TRIG/HDL-cholesterol) in diabetic control animals (DC) significantly (P < 0.05) increased to (1.62 ± 0.17) when compared with the normal control rats (NC) (0.47 ± 0.06). Oral ad-

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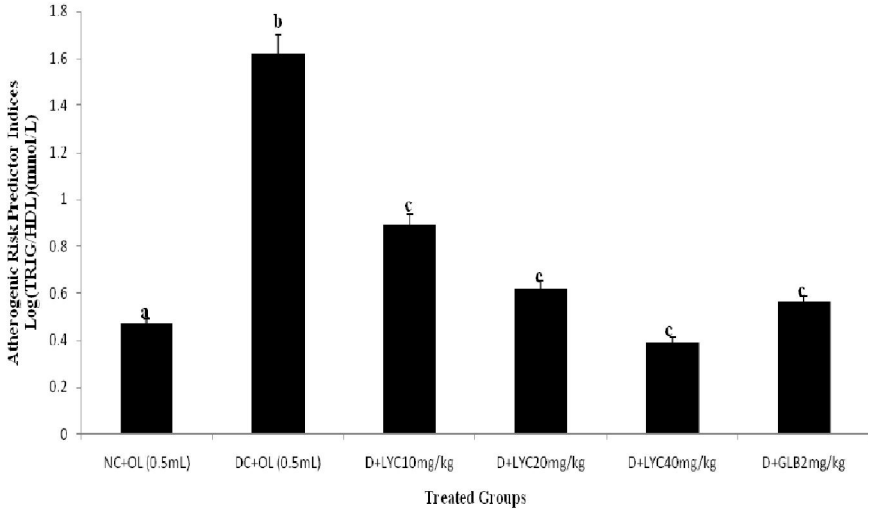
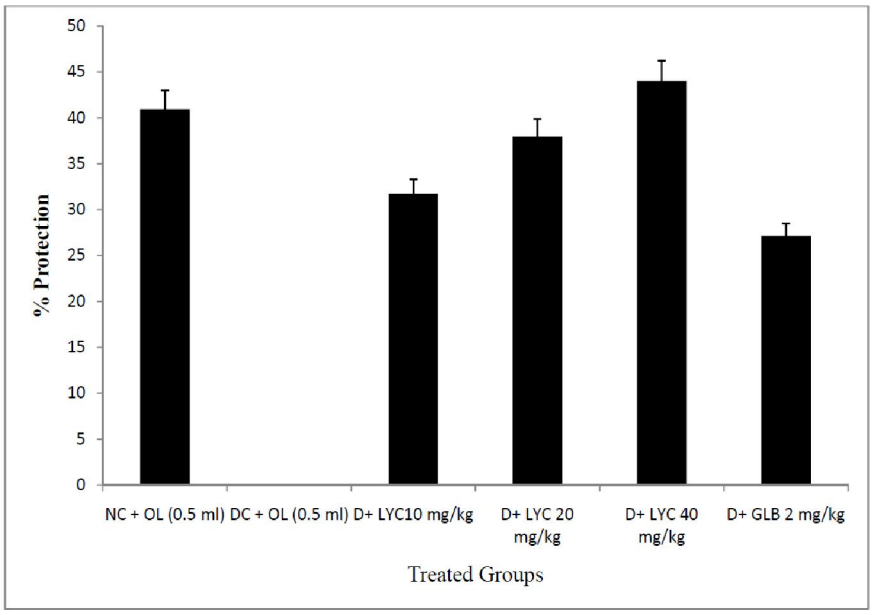


Each bar represent mean of five animals, Bars with different superscripts letters (a, b, c) differ significantly (P < 0.05) compared with the control groups, DC+OL = Diabetic rats + olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats + olive oil (0.5 ml), D+ LYC10 mg/kg = Diabetic rats + 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats + 20 mg/kg of lycopene, D+ LYC 40 mg/ kg = Daibetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg

Figure 10 : Effects of lycopene on serum atherogenic risk predictor indices log (TRIG/HDL-C) in streptozotocin-induced diabetic wistar rats



Each bar represent mean of five animals, DC+OL = Diabetic rats + olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats + olive oil (0.5 ml), D+ LYC10 mg/kg = Diabetic rats + 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats + 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Daibetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg

Figure 11 : Effects of lycopene on percentage protection of different groups in streptozotocin-induced diabetic wistar rats

ministration of graded doses of lycopene (10, 20, 40 mgkg) and glibenclamide (2 mg/kg) resulted to significant ($P < 0.05$) decrease in the serum level of TRIG/HDL-cholesterol to (0.89 ± 0.09, 0.62 ± 0.11, 0.39 ± 0.07) and 0.56 ± 0.08, when compared with diabetic untreated Figure 10.

Effect on percentage protection of diabetic treated groups

Results obtained indicated that animals in the diabetic group were not protected (0.00 ± 0.00) when compared with animals in the normal control group (40.95 ± 6.37). However, all the concentra-

tions of lycopene and glibenclamide tested in diabetic animals produced a significantly ($P < 0.05$) increased percentage protections of (31.75 ± 7.05, 37.97 ± 9.11, 44.04 ± 9.92) and (27.14 ± 7.32) Figure 11.

DISCUSSION

It has been suggested that experimental animal models are one of the best ways to understand the pathophysiology of any disease[52][53][54]. 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, three days after STZ injection. This agrees with the reports of[55][56][57][58] who reported that blood glucose level increased significantly after three days STZ injection in rats. STZ induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models[59]. STZ selectively destroys the insulin producing β-cells which is accompanied by characteristic alterations in blood insulin and glucose concentrations[60].

Results obtained in our present study also indicated that the serum insulin levels decreased significantly in the diabetic untreated animals following streptozotocin (STZ) treatment when compared normal control rats. Streptozotocin has been reported to induce insulin-dependent dependent diabetes mellitus in animal models[61]. After administration, STZ is taken up by pancreatic β-cells *via* glucose transporter GLUT2[62]. Intracellular action of STZ results in changes of DNA in pancreatic β-cells comprising its fragmentation (Morgan *et al*. 1994). This results to impaired glucose oxidation[61] and decreases insulin biosynthesis and secretion[63][64]. However, treatment of diabetic animals with the graded doses of lycopene and glibenclamide significantly decreased the blood glucose concentration, with better effect recorded after week 3 and week 4 respectively when compared with corresponding diabetic untreated animals. This finding agrees with the report of[65] who demonstrated that five weeks of lycopene administration significantly reduced blood glucose levels in diabetic rats. Also,[66] had also demonstrated that elevated blood glucose concentration in streptozotocin-induced was significantly decreased following three weeks of lycopene treatment.[67] also reported a significantly decreased blood glucose level in diabetic rats treated with lycopene for four weeks. Similar findings were also observed in the study of[68][69] who reported that lycopene has significant, dose-dependent anti-diabetic action in streptozotocin-induced diabetic rats. The finding of this study reveals that lycopene administartion to diabetic rats did not produce any significant increase on serum insulin level when compared with diabetic control group. This finding does not corroborate the previous reports of[65][66] who showed that the depleted serum insulin level in diabetic rats was reversed following lycopene administration. Based on our present findings, it may be suggested that insulin secretion may not be part of the observed hypoglycaemic property lycopene because lycopene treatment to diabetic animals resulted to increase serum level, but the increase was not statistically significant in comparison with the diabetic control group. Contrary this findings glibenclamide produced a significantly elevated serum insulin level when compared with the diabetic control group. Glibenclamide have been reported to stimulate insulin secretion from pancreatic β-cells and also reduces hepatic glucose production resulting in reduced blood glucose level[70]. Furthermore, the improvement with glibenclamide administration in diabetic animals was evident by significant increase in the serum insulin levels as observed in the present study.[71] have demonstrated that glibenclamide is able to maintain prolonged increase in serum insulin. Glibenclamide binds to receptors on the surface of pancreatic β-cells; as a result, the cell membrane creates an influx of calcium ions and a subsequent release of insulin[72]. Oxidative stress induced by reactive oxygen species (ROS) which are generated due to hyperglycaemia has been implicated in the onset and progression of diabetes mellitus and its related complications[73][74][75]. Hyperglycemia in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increases the levels of free radicals[76][77]. Lycopene which is one of the potent antioxidants have been shown to have good free radical scavenging capacity because of its unique struc-

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ture (high number of conjugated double bonds)[78]. Therefore, hypoglycaemic effect of lycopene may also be attributed to its strong antioxidant property[79].[78] 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 was observed in the present study.

Lipid profile is a group of blood tests which are carried out to determine the risk of coronary artery diseases (CAD). Results of lipid profile are considered as good indicators of whether someone is prone to develop stroke or heart attack, caused by atherosclerosis. Tests included in lipid profile are total cholesterol (TC), triglyceride (TRIG), high-density lipoprotein-cholesterol (HDL-c) and low-density lipoprotein-cholesterol (LDL-c)[80]. Alterations in lipid metabolism and increased mobilization of free fatty acids from muscle and fat deposition occur in tissues such as liver and heart in diabetes mellitus[81][82]. Hyperlipidaemia, a risk factor in diabetes mellitus is frequently seen among diabetic patients[83]. Serum lipid levels are commonly increased in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease[84]. The present study showed an increase in the concentration of TC, TRIG, LDL-c and a decrease in HDL-C in diabetic control animals. This is in agreement with the reports of[85][86][87][88] who have demonstrated increased serum lipids in diabetes in animals. Diabetic-induced hyperlipidemia is attributable to excess mobilization fat from the adipose due to under utilization of glucose[89][90]. The lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue[91]. The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride[92]. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots[93]. Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes[93]. The results of the present investigation showed that all doses of the lycopene administered to diabetic rats produced a significant beneficial effect on serum lipid profile in STZ-induced diabetic rats. The findings of the present study agree with studies of[94][95][96] who have reported significantly reduced serum level of TC, TRIG and LDL cholesterol in experimentally induced hypercholesterolemia in rats and rabbits supplemented with lycopene and tomato paste.[67] also showed that administration of lycopene for twenty eight days produced a significantly decreased serum TC, TRIG and LDL cholesterol with a corresponding increased serum HDL cholesterol level in rats experimentally induced diabetes. The ability of lycopene to reduce plasma cholesterol and triglycerides in diabetic animals could be explained by the insulin releasing capacity of lycopene. However, the results of present study showed that lycopene treatment to diabetic rats did not produce any significant increase on serum insulin level when compared with diabetic control group. In addition, the hypocholesterolemic effect of lycopene may also be attributable to its antioxidant property which is responsible for the decrease activity of 3-hydroxyl-3-methyl-glutaryl Co-enzyme A (HMG CoA) reductase, which is the key regulatory enzyme in cholesterol biosynthesis[97], reduction in cholesterol absorption by the intestinal wall and/or induction of LDL-receptors within the peripheral tissue[98][97]. This observed improvement in the lipid profile status of diabetic treated rats revealed the cardio-protective properties of lycopene; and may be attributable to antioxidant effects of lycopene. This beneficial effect on the lipid profile may be secondary to glycemic control. The significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals.[99] had reported that about 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease. Therefore, the observed increase in the serum HDL-C level on administration

of various doses of lycopene diabetic rats, indicates that the lycopene have HDL-C boosting effect. More so, the stabilization of serum triglyceride and cholesterol levels in rats by lycopene may be attributed to glucose utilization and hence depressed mobilization of fat[100][101].This present findings suggest that lycopene may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics.

Biomarkers of cardiovascular risk are atherogenic index (AI) and cardiac risk ratio or index (CRR), because both have been found to be the best related predictor of future cardiovascular events[102]. Atherogenic indice (AI) are the most useful index for predicting and quantifying coronary artery disease risk[103]. It is a single, independent predictor of morbidity and mortality in diabetic patients and is strongly associated with increased arteriosclerotic cardiovascular risk in diabetics as well as the general population[104][103]. The association of TRIG, TC, LDL cholesterol and HDL cholesterol in this simple ratio reflects the balance between risk and protective lipoprotein forces; and AI reflect the delicate metablic interactions within the whole lipotein complex[105]. At the end of study period, results obtained showed that the atherogenic indices (AI) (LDL-cholesterol/HDL-cholesterol), log (TRIG/HDL-cholesterol) and cardiac risk ratio (CRR) were significantly increased, while HDL-cholesterol/TC was significantly reduced in diabetic untreated animals when compared with animals in the normal control group. This observation is consistent with the reports of previous researchers[106][107][108]. Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa[109][110][111][112]. AI provides information about the atherogenicity of plasma and quantifies the response to therapeutic intervention[113]. In the present study, we observed that treatment of diabetic with graded doses of lycopene significantly reduced atherogenic indices (LDL-cholesterol/HDL-cholesterol), log (TRIG/HDL-cholesterol) and increased, HDL-cholesterol/TC in a dose dependent manner. Therefore, low atherogenic indices are protective against coronary heart disease[111]. This finding is in agreement with the reports of[114][115][116] who showed that supplementation of dietary tomato peel ultrafine powder has atheroprotective effects by significantly reducing levels of liver cholesterol and serum cholesterol STZ-induced diabetic and hyperlipidemic rats. The significant reduction in the level of log (TRIG/HDL-cholesterol) portends a decrease risk of vascular disease, since high anterogenic index of log (TRIG/HDL-cholesterol) has been positively correlated with cardiovascular disease[117]. Low total cholesterol, triglyceride and high HDL cholesterol lower the ratio and the decrease in the ratio is desirable[80]. From the present study, the values of LDL-cholesterol/HDL-cholesterol ratio is less than 2.3 and TC/ HDL cholesterol ratio is less than 3 in all diabetic lycopene treated groups while the values of HDL-cholesterol/Total cholesterol is greater than 0.3 in diabetic animals administered with lycopene, with highest increase observed with the highest dose. These values are desirable and therefore non anthogenic[113]. A total cholesterol /HDL ratio of d" 3 connotes a low risk, a ratio of around 4.5 an average risk and ratio of d" 8 a high risk of developing coronary artery disease. In the present study an increase in serum HDL with a concomitant increase in percentage of protection from atherogenesis was observed in all diabetic rats that received various doses of lycopene. This observed effect of lycopene suggests a possible protective role of lycopene against the development of atherosclerosis and coronary heart disease, as well as the dyslipidemic conditions that characterize diabetes mellitus. Low atherogenic indices are protective against coronary heart disease[111]. High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and increasing the rate of cholesterol release from the cell[47] by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues followed by esterification through lecithin: cholesterol acyltransferase and delivering it to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins and eventual elimination from the body[118][119] and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant[118][119] and anti-inflam-

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matory property.

CONCLUSION

In conclusion, the results from the present study indicate that lycopene at various doses exert an ameliorative effect on hyperglycaemia, hyperlipidemia,as well as biomarkers of cardiovascular risk i.e atherogenic index (AI) and cardiac risk index (CRR). This is evidenced by decreased elevated serum lipids TC, TRIG, LDL-c and increased the serum HDL-c levels in STZ-induced diabetic animals. However, the ability of lycopene to significantly reduce the above mentioned parameters, suggests that lycopene may be potential therapeutic agent for diabetic cardiovascular complications.

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COMPETING INTEREST

Authors declare that no competing interest exists in the publication of this manuscript.

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