Lycopene Ameliorates Atherogenic Cardiovascular Risk in Streptozotocin-Induced Diabetic Hyperlipidaemia in Wistar Rats

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Abstract

Diabetes mellitus is a chronic metabolic disorder of the endocrine system and a risk factor for many cardiovascular diseases. Several studies have reported the beneficial roles of lycopene in the treatment of various ailments, but they were mainly epidemiological. This study was investigated the ameliorative effects of lycopene in atherogeneity of streptozotocin-induced diabetic hyperlipidaemia in Wistar rats. Diabetic rats were randomly divided into following groups: Group I: Normal control + OL (0.5 ml/kg b w), Groups II: Diabetic control + OL (0.5 ml/kg b w) respectively. Diabetic Groups III-V received 10 mg/kg b w, 20 mg/kg b w, 40 mg/kg b w of lycopene while Diabetic Groups VI + glibenclamide 2 mg/kg b w. There was a significant (p<0.05) reduction in blood glucose level. The results showed a significantly (p<0.05) decreased serum, TC, triglyceride and LDL levels with a corresponding elevated serum HDL level in diabetic-lycopene treated animals. Serum atherogenic risk predictor indices and cardiac risk ratio were significantly decreased (p<0.05) while HDL-cholesterol/TC was increased significantly (p<0.05) when compared with diabetic control group. It can be concluded that lycopene ameliorated atherogenic diabetic-induced dyslipidaemia, hence may have cardio-protective effects.

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1. Introduction

1.1 Literature Review

Diabetes mellitus is a chronic metabolic disorder of the endocrine system (Li et al., 2004) and among the most common disorders in both developed and developing countries (Makund et al., 2008; Zhou et al., 2009). It has become a global metabolic epidemic, affecting important biochemical activities in nearly every age group (Gupta, 2008; Singh et al., 2012). It has been estimated that the number of people with diabetes will rise from the present 150 to 230 million in 2025 (Iraj et al., 2009; Abu-Zaiton, 2010). 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 (Wang et al., 2012). Chronic hyperglycaemia leads to many long-term complications in the eyes, kidneys, nerves, heart, and blood vessels (Lyra et al., 2006; Laakso, 2010; Murti et al., 2012). Stratmann and Tschoepe (2009) 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 (Stratmann and Tschoepe, 2009; Shaikh and Somani, 2010). In addition, management of diabetes mellitus without any side effects is still a challenge to the medical system. Many drugs are available for use in the treatment of diabetes, but their long-term use may cause adverse side effects and hence. This leads to increasing demand for natural products with potent anti-diabetic activity and fewer side effects (Nabeel et al., 2010; Grover et al., 2002). 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 (Singh et al., 2012). Lycopene being an antioxidant has been suggested to protect critical bimolecules including lipids, protein and DNA from free radicals (Ashwani and Prachi, 2013). In both experimental and clinical models of diabetes, antioxidants have been reported to reduce markers of oxidative stress (Johansen et al., 2005; Fenercioglu et al., 2010; Neri et al., 2010). Besides, some studies have showed that antioxidants are effective and cheaper than conventional therapy in management of some diseases (Trevithick et al., 2004) 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 (Maritim et al., 2003; Johansen et al., 2005; Fenercioglu et al., 2010).

1.2 Lycopene
Lycopene belongs to a large group of pigments known as carotenoids (Ernst, 2002). It is a red pigment that occurs naturally in certain fruits, vegetables, algae, and fungi. Tomatoes and tomato-based products are the major sources of natural lycopene in the human diet (Nguyen and Schwartz, 1999). Other significant sources of lycopene include watermelon, pink grapefruit, pink guava, and apricots (Nguyen and Schwartz, 1999). Lycopene occurs in the all-trans and various cis configurations. Naturally-occurring lycopene present in red tomato fruits consists predominantly (94-96%) of all-trans-lycopene (Schierle et al., 1997). It is one of the most potent antioxidants among dietary carotenoids. It has a single-oxygen-quenching ability twice as high as that of beta-carotene (vitamin A relative) and 100 times higher than that of alpha-tocopherol (vitamin E relative), which in turn has 125 times more quenching action glutathione (water soluble) (Atessahin et al., 2005; Rafi et al., 2007). Ambreen et al. (2014) reported that lycopene has potential to prevent various chronic ailments like dyslipidemia, diabetes, oncogenesis, neurodegenerative diseases osteoporosis and so on.

1.3 Objectives of research
The main objective of this study is to establish the ameliorative effects of lycopene on atherogenic cardiovascular risk in streptozotocin-induced diabetic hyperlipidaemia in Wistar rats.

1.4 Justifications
It has been reported that diabetes is a risk factor for cardiovascular disease (Oguntibeju et al., 2009b; Laakso, 2010) and more than 70% of type 2 diabetic patients die of cardiovascular diseases (Laakso, 2001). 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) (Smith et al., 1984). Besides oxidative stress, dyslipidemia is also a common metabolic disturbance associated with diabetes, as 97% of diabetics are dyslipidemic (Lewis et al., 2009, Parhofer, 2013). Atherogenic dyslipidemia, is characterised by increased levels of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and decreased high density lipoprotein (HDL) (Adiels et al., 2008, Beliaeva, 2013). 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 (Giugliano et al., 1996; Mohamed et al., 2009).

1.5 Research problem
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 in the prevention and amelioration of various ailments, but most investigations are mainly epidemiological in nature.

2. Experimental
2.1 Materials and chemicals
Materials that were used include: Spectrophotometer (V-16 model, United Kingdom), haematocrit, Centrifuge Hettich (Universal 32, Made in Germany), Automated Analyzer (Selectra XL-model Netherland)
haematology analyzer (Systdex model 2X-2N, USA), Aumated electrolyte Analyzer, Audicom USA (AC 9900 model), electronic weighing machine, glucometer and sips (Accu-check® Advantage, Roche USA), EDTA, dissecting kits, cages and feeders, cannula, syringes and needle, glibencamide and Rats Insulin Elisa kits. 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 described by Ali et al. (2013) and Ogundeji et al. (2013) with little modifications. All chemicals andsolvents used were of analytical grade.

2.2 Experimental animals
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, Nigeria. The animals were kept and maintained under laboratory condition of temperature, humidity, light. They were fed on standard commercial feeds with water ad libitum.

2.3 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. Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h. The rats were then be kept for 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani et al., 2002). Three days 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.

2.4 Experimental protocol and treatment
In the experiment, a total of 30 Wistar rats were randomly divided into six groups of five animals. Group I: Normal control (NC) and Groups II: Diabetic control (DC) were administered 0.5 ml/kg and 0.5 ml/kg body weight of olive oil respectively. Diabetic Groups III-V were treated with 10 mg/kg b w, 20 mg/kg b w, 40 mg/kg b w of lycopene while Diabetic Groups VI received glibencamide 2 mg/kg b w. All administration were given orally once daily by gavage for four (4) weeks.

2.5 Determination of blood glucose level
Blood glucose level was determined by collection of blood sample from the tail artery of the animals on weekly bases by glucose-oxidase principle (Beach and Turner, 1958) using digital glucometer (Accu-check Advantage) and was expressed in the unit of mg/dl.

2.6 Blood sample collection and serum preparation
After the last day of treatment (28 days) all animals from each group were sacrificed using light chloroform after 24 hours and blood was collected through cardiac puncture into a specimen bottles and wereallowed 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.

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

2.8 Determination of lipid profile
Spectrophotometric determination of serum total cholesterol was carried out using enzymatic colometric assay kits (Randox Laboratories Limited kits, Unite kingdom) as described by method of Stein (1987), while serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). The serum level of HDL-C was measured by the method of Wacnic and Albers (1978) and the serum level of (LDL-C) was measured according to protocol of Friedewald et al., (1972) using the relationship the following formula

\[
\text{LDL cholesterol} = \frac{\text{Total cholesterol} - \text{HDL cholesterol} - \text{Triglycerides}}{2}
\]

All values obtained were expressed in mmol/L.

2.9 Determination of atherogenic risk predictor indices and cardiac risk ratio
Atherogenic Risk Predictor Indices were calculated as earlier reported by Ikewuchi and Ikewuchi (2009a; 2009b; 2010) and (Owolabi et al., 2010). While the percentage protection was calculated based on the method of Dhandapani (2007) using the following formula:

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

\[
\text{Atherogenic Index (AI)} = \frac{\text{LDL cholesterol}}{\text{HDL cholesterol}}
\]

\[
\text{Rudolph Index (RI)} = \frac{\text{AI of Diabetic Control} - \text{AI of Treated Groups}}{\text{AI of Diabetic Control}}
\]

2.10 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.

3. Results

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

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 = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + glibenclamide 2 mg/kg

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

Comparison between the normal control (NC) and diabetic control (DC) (Figure 1) showed a significantly increased (\( p<0.05 \)) blood glucose concentration from (91.0 ± 5.74) to (364.4 ± 44.50) before the commencement of treatment on (week 0). However, treatment of diabetic animals with the graded doses of lycopene (10, 20 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 (182.9 ±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 week 4 when compared with corresponding diabetic untreated group.

### 3.2 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 (\( p<0.05 \)) increase of the serum insulin level to (7.36 ± 0.42) when compared with the diabetic control group that recorded (3.02 ± 0.24) (Figure 2).

### 3.3 Effect of lycopene on serum total cholesterol concentration (TC)

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).

### 3.4 Effect of lycopene on serum triglyceride level (TRIG)

The result of serum triglyceride level shown in (Figure 4) showed significant (\( p<0.05 \)) increase in the level 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 (1.0 ± 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 similar to glibenclamide (2 mg/kg) which recorded (0.54 ± 0.07) when compared with the diabetic control group.

### 3.5 Effect of lycopene on serum high density lipoprotein-cholesterol level (HDL-C)

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).

### 3.6 Effect of lycopene on serum low density lipoprotein-cholesterol level (LDL-C)

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
Figure 2: Effects of lycopene on serum insulin level in streptozotocin-induced diabetic Wistar rats. Each bar represents 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+ LYT10 mg/kg = Diabetic rats + 10 mg/kg of lycopene, D+ LYT 20 mg/kg = Diabetic rats + 20 mg/kg of lycopene, D+ LYT 40 mg/kg = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.

Figure 3: Effects of lycopene on serum total cholesterol level in streptozotocin-induced diabetic Wistar rats. Each bar represents 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+ LYT10 mg/kg = Diabetic rats + 10 mg/kg of lycopene, D+ LYT 20 mg/kg = Diabetic rats + 20 mg/kg of lycopene, D+ LYT 40 mg/kg = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.

Figure 4: Effects of lycopene on serum triglyceride level in streptozotocin-induced diabetic Wistar rats. Each bar represents 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+ LYT10 mg/kg = Diabetic rats + 10 mg/kg of lycopene, D+ LYT 20 mg/kg = Diabetic rats + 20 mg/kg of lycopene, D+ LYT 40 mg/kg = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.
Figure 5: Effects of lycopene on serum high-density lipoprotein cholesterol level in streptozotocin-induced diabetic Wistar rats. 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 = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.

![Figure 5: Effects of lycopene on serum high-density lipoprotein cholesterol level in streptozotocin-induced diabetic Wistar rats.](image1)

Figure 6: Effects of lycopene on serum low-density lipoprotein cholesterol level in streptozotocin-induced diabetic Wistar rats. Each bar represent mean of five animals. Bars with different superscripts letters (a, b, c, d) 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 = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.

![Figure 6: Effects of lycopene on serum low-density lipoprotein cholesterol level in streptozotocin-induced diabetic Wistar rats.](image2)

Figure 7: Effects of lycopene on serum atherogenic risk predictor indices (LDL-C/HDL-C) in streptozotocin-induced diabetic Wistar rats. Each bar represent mean of five animals. Bars with different superscripts letters (a, b, c, d) 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 = diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.

![Figure 7: Effects of lycopene on serum atherogenic risk predictor indices (LDL-C/HDL-C) in streptozotocin-induced diabetic Wistar rats.](image3)
Figure 8: Effects of lycopene on cardiac risk ratio (TC/HDL cholesterol) in streptozotocin-induced diabetic Wistar rats. 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 = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.

Figure 9: Effects of lycopene on serum atherogenic risk predictor indices (HDL-C/TC) in streptozotocin-induced diabetic Wistar rats. 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 = Diabetic 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. 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 = Diabetic rats + 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats + Glibenclamide 2 mg/kg.
(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.

3.7 Effect of lycopene 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 diabetic untreated animals (Figure 7).

3.8 Effect of lycopene on cardiac risk ratio (crr) (td/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) (1.83 ± 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 animals in diabetic control group (Figure 8).

3.9 Effect of lycopene 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 when compared with the diabetic control group.

3.10 Effect of lycopene 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 administration of graded doses of lycopene (10, 20, 40 mg/kg) 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).

3.11 Effect of lycopene 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 concentrations 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
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 (Bedoya et al., 1996). After administration, STZ is taken up by pancreatic β-cells via glucose transporter GLUT2 (Elsner et al., 2000). 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 (Bedoya et al., 1996) and decreases insulin biosynthesis and secretion (Nukatsuka et al., 1990a; Nukatsuka et al., 1990b). 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 Aydin and Celik (2012) who demonstrated that five weeks of lycopene administration significantly reduced blood glucose levels in diabetic rats. Also, Duzguner et al. (2008) had also demonstrated that elevated blood glucose concentration in streptozotocin-induced was significantly decreased following three weeks of lycopene treatment. Sevim et al. (2013) 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 Kuhad et al. (2008) and Ali and Agha (2009) who reported that lycopene has significant, dose-dependent anti-diabetic action in streptozotocin-induced diabetic rats. The finding of this study reveals that lycopene administration 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 Aydin and Celik (2012) and Duzguner et al. (2008) 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 (Erejuwa et al., 2011). 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. Sayed et al. (2011) 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 (Martha and Karam, 2001). 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 (Jay et al., 2006; Wei et al., 2009; Giacco and Brownlee, 2010). Hyperglycemia in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increases the levels of free radicals (Sharma et al., 2010; Tsuruta et al., 2010). 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) (Bose and Agrawal, 2006). Therefore, hypoglycaemic effect of lycopene may also be attributed to its strong antioxidant property (Kumar and Kumar, 2009). Bose and Agrawal (2006) 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) (Sembulingam and Sembulingam, 2013). 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 (Bloomgarden, 2003; Shukla et al., 2012). Hyperlipidaemia, a risk factor in diabetes mellitus is frequently seen among diabetic patients (Mengesha, 2006). Serum lipid levels are commonly increased in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease (Al-Shamaony et al., 1994; Muthulingam, 2010). 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 other investigators (Mironova et al., 2000; Odetola et al., 2006, Fernandes et al., 2010, Iweala and Oludare, 2011) who have demonstrated increased serum lipids in diabetes in animals. Diabetic-induced hyperlipidaemia is attributable to excess mobilization fat from the adipose due to underutilization of glucose (Krishnakumar et al., 2000; Nimenibo-udia, 2003). 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 (Subbiah et al., 2006; Rotimi et al., 2011; Matsinkou et al., 2012). 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 (Shih et al., 1997; Suryawanshi et al., 2006). The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Ayeleso et al., 2012). 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 (Ayeleso et al., 2012). 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 previous studies (Fredrickson et al., 2007; Basunyet al., 2009; Khayat et al., 2011). Sevim et al. (2013) 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 (Olooto et al., 2014), reduction in cholesterol absorption by the intestinal wall and/or induction of LDL-receptors within the peripheral tissue (Danesh and Kanwar, 2004; Olooto et al., 2014). 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. Kwitterovich (2000) and James et al. (2010) had reported that about 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol atheroma 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 (Momo et al., 2006; Iweala and Oludare, 2011). This suggest that lycopene may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics (Sharma et al., 2003). 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 (Groveret al., 1999; Radahmadi et al., 2006; Wafà et al., 2012). Atherogenic indice (AI) are the most useful index for predicting and quantifying coronary artery disease risk (Ekpenyong et al., 2014). 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 (Steinberg et al., 2013; Ekpenyong et al., 2014). 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 metabolic interactions within the whole lipoprotein complex (Angoorbala et al., 2012). 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 (Andulla et al., 2009; Ahmed et al., 2010; Udenze et al., 2012; Ekpenyong et al., 2014; Olooto et al., 2014). 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 (Frohlich and Dobíšová, 2003; Dobíšová, 2004; Martirosyan et al., 2007; Brethm et al., 2004; Usoro et al., 2006). AI provides information about the atherogenicity of plasma and quantifies the response to therapeutic intervention (Ojiako and Nwanjo, 2005). In this 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 (Usoro et al., 2006). This finding is in agreement with the reports of (Chang and Cheong, 2007; Ogbonnia et al., 2008, Pourkabir et al., 2010; Zheng et al., 2013) 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 (Igwe et al., 2007). Low total cholesterol, triglyceride and high HDL cholesterol lower the ratio and the decrease in the ratio is desirable (Sembulingam and Sembulingam, 2013). 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 anagentic (Ojiako and Nwanjo, 2005). A total cholesterol /HDL ratio of ≤ 3 connotes a low risk, a ratio of around 4.5 an average risk and ratio of ≤ 8 a high risk of developing coronary artery disease (Saikia and Lama, 2011). 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 (Usoro et al., 2006). 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 (Ikwuchu and Ikewuchi, 2009a) 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 (Assmann and Gotto, 2004; Ademuyiwa et al., 2005) and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (Assmann and Gotto, 2004, Ademuyiwa et al., 2005; Brunzell et al., 2008) and anti-inflammatory property.

Research Highlights

Lycopene significantly (p<0.05) and dose dependently reduced blood glucose level in diabetic Wistar rats.

The drug also 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-lycopene treated animals in comparison with the diabetic control group.

This study suggests that lycopene could be a potential therapeutic agent for diabetic cardiovascular complications.

Advancement in Knowledge

Several studies have reported the beneficial roles of lycopene in the prevention and amelioration of various ailments, but most investigations are mainly epidemiological in nature. This work is the first time to the best of our knowledge that the ameliorative effect of lycopene on hyperglycaemia, hyperlipidaemia, as well as biomarkers of cardiovascular risk such as atherogenic index (AI) and cardiac risk ratio (CRR) are being carried out in laboratory animals.

Limitations and Recommendations

This study was not able to elucidate the actual mechanism of lycopene action on the reported properties. Hence we recommend that: Further investigation on the effects of lycopene should be done on inflammatory markers of diabetic animals such as TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), as elevations in inflammation maker activity has been reported to plays a critical role in several stages of microvascular and cardiovascular.
diseases that occurs in diabetes. Further studies should be carried out investigate the precise mechanism(s) involved in the protective effect of lycopene against atherogenic heart disease associated with diabetes mellitus.

**Funding and Policy Aspects**

Diabetes mellitus is a chronic metabolic disorder affecting both developing and developed countries. There is need for government and private sectors to provide fund for research in order to develop more effective treatment that can be available for all.

**Competing Interest**

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

**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 such as atherogenic index (AI) and cardiac risk ratio (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. Lycopene significantly (p<0.05) deceased serum atherogenic risk predictor indices (LDL-cholesterol/HDL-cholesterol, log (TRIG/HDL-cholesterol) and cardiac risk ratio while HDL-cholesterol/TC was increased significantly (p<0.05) when compared with diabetic control group. Lycopene significantly (p<0.05) deceased serum atherogenic risk predictor indices (LDL-cholesterol/HDL-cholesterol, log (TRIG/HDL-cholesterol) and cardiac risk ratio were significantly (p<0.05) while HDL-cholesterol/TC was increased significantly (p<0.05) when compared with diabetic control group.

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