

## Effects of Lycopene on Liver Markers and Glucokinase Activity in Experimentally-induced Diabetes Mellitus Rat Model

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*Diabetes mellitus,  
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**Abstract:** The study examined the effects of lycopene on markers of liver function and glucokinase activity in experimentally-induced diabetes mellitus rat model. To accomplish this, experimental diabetes were induced by single intra-peritoneal administration of streptozotocin (60 mg/kg b w) into animals. Diabetic and normal animals were randomized into the following groups: Group I: Normal control rats that received (0.5 mL) of olive oil; Group II: Diabetic control animals that received (0.5 mL) of olive oil); while Group III- VI received (10, 20 and 40 mg/kg of lycopene and 2 mg/kg b w glibenclamide) respectively. All treatments were given orally once daily for four weeks. There was a significant ( $P < 0.05$ ) and progressive reduction on blood glucose concentration, with non- significant ( $P > 0.05$ ) increase in serum insulin level when compared with diabetic control group. There was a significant ( $P < 0.05$ ) increase in the activity of liver glucokinase enzyme as well as a significant ( $P < 0.05$ ) decrease on the activities of serum liver enzymes (AST, ALT and ALP) in diabetic animals administered with lycopene when compared with those of diabetic control animals. Following available evidence from our findings, lycopene may be suggested as a promising dietary agent in the management of diabetes and hepato-cellular damage that usually occurs in diabetes mellitus.

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## INTRODUCTION

Diabetes mellitus is defined as a metabolic disorder usually characterized by hyperglycemia and deficient secretion or action of endogenous insulin (ADA, 2009). Diabetes is reported to be one of the most important diseases worldwide and is beginning to reach epidemic proportions. It has been estimated globally that the proportion of adult population with diabetes will increase to 69% in the year 2030 (Shaw *et al.*, 2010). Increased oxidative stress has been widely accepted to be implicated in the development and progression of diabetes and its complications (Ceriello, 2000). Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses (Baynes, 1999). The main sources of oxidative stress in diabetes mellitus comprise various enzymatic pathways, non-enzymatic pathways and mitochondrial pathways (Patel and Sharma, 2014). Enzymatic pathways includes, auto-oxidation of glucose, cellular oxidation, decreased cellular antioxidants, like glutathione and as well as impaired actions of antioxidant defense enzymes like superoxide dismutase (SOD) and catalase (CAT), elevated levels of some pro-oxidants such as ferritin a protein containing 20% iron that is found in the intestines and homocysteine are the

probable sources of oxidative stress in diabetes mellitus (Patel and Sharma, 2014). In the oxidative stress initiated by non-enzymatic sources, hyperglycemia which directly causes increased free radicals or reactive oxygen species generation has been implicated. Glucose undergoes auto-oxidation and generates hydroxyl radicals. Besides these glucose also reacts with proteins in a non-enzymatic way leading to the improvement of advanced glycation end products which alters protein and cellular/immune function, and binding of advanced glycation end products to their receptors may lead to modification in cell signaling pathways and additional production of reactive oxygen species at multiple steps during this biological process (Davies, 1997). Hepatobiliary disorders such as inflammation, necrosis or fibrosis of non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, hepatitis C, acute liver failure, and cholelithiasis are some of reported complications of diabetes mellitus. Thus, treatment of this disease goes beyond mere glycaemic control. Streptozotocin (STZ) is frequently used to induce experimental type1 diabetes in animal models (Yamagishi *et al.*, 2001). The cyto-toxic action of streptozotocin is shown to be mediated through free radicals generation and streptozotocin

has been demonstrated to have toxic and carcinogenic effects on organs such as pancreas, kidneys and including liver (Mohammed *et al.*, 2012).

Carotenoids are a class of more than 600 natural pigments that are present in fruits and vegetables (Khachik *et al.*, 1991). Epidemiological data have demonstrated a clear inverse association between diets high in carotenoid-rich fruits and vegetables and reduced incidence of variety of diseases (Van't Veer *et al.*, 2000). Lycopene is a potent antioxidant of carotenoid family, and naturally occurring compound that gives characteristic red color to tomato, watermelon, pink grapefruit, orange, and apricot (Shi *et al.*, 2004). A number of studies have also indicated the health benefits of lycopene consumption and have demonstrated its preventive and ameliorative role in the occurrence of a good number of chronic diseases including various types of cancers (Giovannucci, 2002). Although used as food colourant for many years, the investigation into the health benefits of lycopene did not begin until the last part of the 20th century (Betty *et al.*, 2004). Within a relatively short period of time, an increasing body of evidence based on laboratory, animal, and population-based research supports the role of lycopene in health and disease, and as a potential natural food antioxidant (Yang *et al.*, 2006). Over the years the use of antioxidant based formulations for management of various diseases such as atherosclerosis, stroke, diabetes mellitus, Parkinson's disease and liver disease, cancer and so on has been augmented globally. In addition, antioxidant mechanisms are diminished in diabetic patients, which may further augment oxidative stress (Rains and Jain, 2011; Maritim *et al.*, 2003). Several investigations have suggested the possible participation of dietary antioxidants, such as vitamins, in ameliorating the diabetic state and retarding the development of diabetes complications (Sheikh-Ali *et al.*, 2011).

This has led to significantly increased awareness about the role of nutritional antioxidants in preventing numerous human ailments and disorders. Thus, dietary antioxidants may therefore be the promising therapeutic approach in impeding the onset as well as in reducing diabetic related complications (Singh and Sankar, 2012). Despite enormous scientific success recorded in the treatment of liver diseases by oral hepatoprotective agents, the search for newer drugs continues because the currently synthetic drugs in use have several setbacks. Therefore, functional foods or natural food diets which have antioxidant or free radical mopping properties has become a main focus for research designed in order to prevent or ameliorate tissue injury/damage and may have a significant role in maintaining health. This study

was aimed at investigating the ameliorative effect of lycopene on liver glucokinase and enzymes activity in streptozotocin-induced diabetic Wistar rats.

## MATERIALS AND METHODS

### MATERIALS

#### Animals

Adult albino rats of both sexes that weighed between 150 and 200 g were procured from the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals kept and maintained under laboratory condition of temperature, humidity and light. The animals kept in clean aluminium cages. They were fed on standard commercial rat pellets (Vital Feeds) with free access to water.

### METHODS

#### Chemicals and lycopene

Streptozotocin was purchased from Sigma Chemicals (St Louis, U.S.A.), while lycopene was procured from (General Nutrition Corporation, Pittsburgh, U.S.A.). Lycopene was reconstituted in olive oil (*Goya En espana*, S.A.U., Sevilla, Spain), to appropriate working concentration as described by Ogundeji *et al.*, (2013) and Haribabu *et al.* (2013) with modifications to obtain the desired doses used in the study. All chemicals and solvents used were of analytical grade.

#### Induction of diabetes

Experimental diabetes was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin (STZ) dissolved in fresh 0.1M cold citrate buffer of pH 4.5 into animals deprived of feeds 18 hrs, but had access to water. Three days after streptozotocin administration, blood was taken from tail vein of the rats. Animals having blood glucose levels  $\geq 200$ mg/dl were taken to be diabetic and included in the study. Thereafter, diabetic animals were randomly assigned into different groups.

#### Experimental protocol

A total of thirty (30) Wistar rats were used in the study which included twenty five (25) diabetic animals and five (5) normal control animals. The animals were randomly divided into six (6) groups of five (5) rats each

Group I: Comprised of normal control rats that was administered with 0.5 mL of olive oil

Groups II: Consisted of diabetic control group that received 0.5 mL of olive oil

Group III: Diabetic animals that were treated with 10 mg/kg b w of lycopene

Group IV: Diabetic rats that were administered with 20 mg/kg b w of lycopene

Group V: Diabetic rats that were treated with 40 mg/kg b w of lycopene

Group VI: Diabetic animals that received glibenclamide 2 mg/kg b w

All treatments were given orally once daily for four weeks.

#### Evaluation of fasting blood glucose level

Fasting blood glucose level was determined by collection of blood sample from the tail vein of the rats at interval of 0 week, 1<sup>st</sup> week, 2<sup>nd</sup> week, 3<sup>rd</sup> week and 4<sup>th</sup> week of the treatment period respectively by glucose-oxidase method described by Beach and Turner (1958) using digital glucometer (Accu-chek Advantage) and was expressed in the unit of mg/dL.

#### Preparation of serum

Twenty four hours after the last treatment was administered all animals from each group were euthanized blood was collected through cardiac puncture into sample tubes. The blood in the sample tubes were allowed to stand undisturbed. The serum was thereafter separated by centrifugation at 1,986 g for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany) and transferred into sample bottles for the determination of serum insulin concentration and liver enzyme activities such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

#### Preparation liver tissue homogenate

Approximately 1.0 g of liver was dissected out, washed with ice-cold 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 1,986 g for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany) to remove debris and supernatant obtained was used for evaluation of activity of liver glucokinase.

#### 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). Briefly, all reagents and samples were brought to room temperature before use. The required amount of enzyme conjugates 1X and wash buffer solution was prepared. The samples, insulin control solutions, and calibrators were also prepared as well as sufficient microplate wells to accommodate calibrators and samples in duplicate. A recommended plate plan which includes: Cal 0-5: calibrator solutions (standards); insulin control low (ICL), Insulin control high (ICH) and sample (S) were prepared. 25  $\mu$ L each of Calibrators was pipetted into appropriate wells and 100  $\mu$ L of enzyme conjugate 1X solution added into each well

and incubated on a plate shaker 2000 g for 2 hours at room temperature. Each well was washed 6 times with wash buffer 1X solution and reaction volume discarded by inverting the micro plate over a sink. Wash solution of 350  $\mu$ L was added into each well and the wash solution discarded and tapped firmly several times against absorbent paper to remove excess liquid. This was repeated five times and to avoid prolonged soaking during washing procedure. 200  $\mu$ L substrate TMB was then added into each well. This was incubated for 15 minutes at room temperature (18 – 25 °C) and 50  $\mu$ L Stop solutions were added to each well. The plate was placed on the shaker for approximately 5 seconds to ensure mixing. The absorbance was measured at 450 nm within 30 minutes.

#### Determination of liver glucokinase enzyme activity

The activity of hepatic glucokinase enzyme activity was determined by the method of Goward (1986) by continuous spectrophotometric rate determination as follows:

##### Principle:



ATP= Adenosine 5'-Triphosphate

ADP= Adenosine 5'-Diphosphate

$\beta$ -NADP=  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

G-6PDH=Glucose-6-Phosphate Dehydrogenase

Conditions: T=30 °C, pH=9.0, A<sub>340nm</sub>, Light path=1cm

Reagents:

- 75 mM Tris HCl buffer, 100ml was prepared in deionized water using Trizma Base, Sigma Prod. No. T-1503 and adjusted to pH 9.0 at 30 °C with 1 M HCl.
- 10 ml of 600 mM Magnesium Chloride Solution (MgCl<sub>2</sub>), was also prepared in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250).
- 10 ml of 120 mM Adenosine Triphosphate Solution (ATP) was prepared in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.
- 10 ml of 360mM  $\beta$ -D(+) Glucose solution (Glucose) was prepared in deionized water using  $\beta$ -D(+) Glucose, Sigma prod.No.G-5250.
- 27 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized form solution ( $\beta$ -NADP), 30 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, sodium salt, Sigma stock No.240-330 in the appropriate volume of deionized water.
- A solution of 100 units/mL of Glucose-6-Phosphate Dehydrogenase (G-6PDH) of

Sigma product No.G-6378 in cold deionized water was prepared before use.

- 50 mM Tris HCl buffer, pH 8.5 at 30 °C (enzyme diluents), was prepared in Trizma base using deionized water, Sigma product No.T-1503, and adjust to pH 8.5 at 30 °C with 1M HCl.
- Glucokinase Enzyme Solution (GLCK); a solution containing 0.25-0.50 unit/mL of this enzyme was prepared immediately before use in cold enzyme diluents.

#### Procedure:

A reaction cocktail was prepared by pipetting into a suitable container: 24 mL of Buffer, 1 mL of MgCl<sub>2</sub>, ATP, glucose and β-NADP solutions. This content was mixed, swirled and adjusted to pH 9.0 at 30 °C with 1 M HCl or NaOH, if necessary. Thereafter, 2.8 mL of the reaction cocktail was pipetted into test and blank cuvettes and then 0.1 mL of 6-GPDH solution added into test and blank cuvettes and mixed by inversion and equilibrate to 30 °C. This was monitored at A<sub>340nm</sub> using a suitably thermostatted spectrophotometer and 0.1 mL Glucokinase enzyme solution was added into the test cuvette while enzyme diluents were added into the blank cuvette. This was immediately mixed by inversion and the increase in A<sub>340nm</sub> recorded for approximately 5 minutes. Change in A<sub>340nm</sub> per minute was also obtained using the maximum linear rate for both the test and blank.

Units/ml enzyme =  $(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank}) \times 3 / (6.22)(0.1)$

3=Volume (in milliliter) of assay

6.22=Millimolar extinction coefficient of β-NADP at 340 nm

0.1=Volume (in milliliters) of enzyme used.

#### Determination of markers of liver function

Activities of serum alanine aminotransaminase was estimated by the method adopted by Tietz (1995), aspartate aminotransaminase was determined by the method of Henderson and Moss (2001) while alkaline phosphatase was determined according to the method of Scherwin (2003).

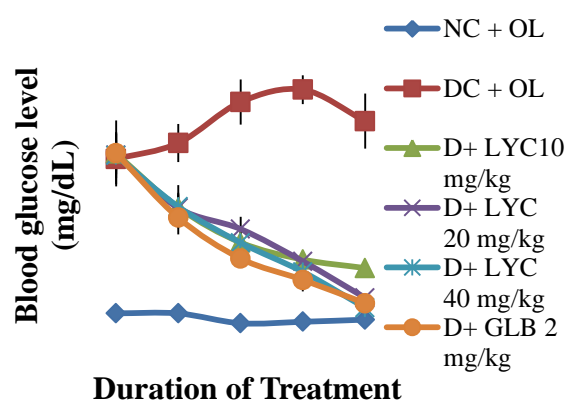
#### Statistical analysis

Data obtained from each group were expressed as mean ± SEM. The data were 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

### Changes on fasting blood glucose level in streptozotocin-induced diabetic Wistar rats

The blood glucose concentration was significantly increased ( $P < 0.05$ ) (from 91.0 ± 5.74 to 364.4 ± 44.50 mg/dl) after STZ injection on week 0. Following treatment of diabetic animals with the graded doses of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg), there was a significant ( $P < 0.05$ ) steady decrease on blood glucose levels most especially from the second week of treatment from (465.2 ± 39.81 to 216.4 ± 19.55, 240.2 ± 21.60 216.0 ± 28.51 and 188.0 ± 10.06 mg/dL) and the week 3 from (487.0 ± 25.64 to 186.2 ± 9.20, 183.0 ± 10.57, 164.4 ± 21.19 and 150.2 ± 20.28 mg/dL) and week 4 from (431.4 ± 48.84 to 171.1 ± 7.65, 118.4 ± 1.97, 100.8 ± 6.89 and 108.8 ± 16.74 mg/dL), when compared with diabetic control animals (Figure 1).



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

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

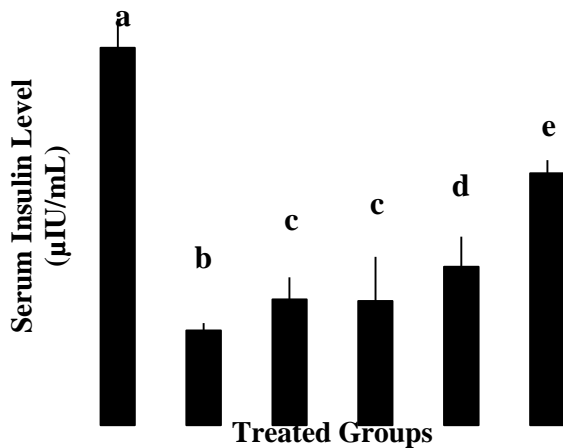
### Effect of lycopene on serum insulin level in streptozotocin-induced diabetic Wistar rats

The serum insulin levels decreased significantly ( $P < 0.05$ ) in the diabetic untreated animals to (3.02 ± 0.24 μIU/mL) following STZ treatment from (12.04 ± 0.93 μIU/mL) in normal control, when compared. Administration of various doses (10, 20 and 40 mg/kg) of lycopene did not produce any significant ( $P > 0.05$ ) increase on serum insulin levels (4.02 ± 0.70, 3.96 ± 1.41 and 5.06 ± 0.96 μIU/mL) in diabetic rats when, compared with diabetic control group. Conversely, in diabetic animals treated with glibenclamide (2 mg/kg), there was a significant ( $P < 0.05$ ) elevation on serum insulin level to (7.76 ± 0.42 μIU/mL), when compared with those of diabetic control group (3.02 ± 0.24 μIU/mL) (Figure 2).

### Effect of lycopene on liver glucokinase activity in streptozotocin-induced diabetic Wistar rats

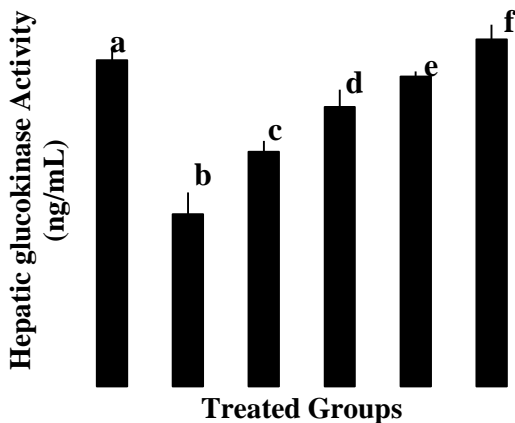
The activity of liver glucokinase was significantly ( $P < 0.05$ ) decreased from (16.62 ± 0.63 ng/mL) in

the normal control animals to  $(8.78 \pm 1.11\text{ng/mL})$  in diabetic control group, when compared. Administration of lycopene (10, 20 and 40 mg/kg) to diabetic animals resulted to a significant ( $P < 0.05$ ) increase on activity of liver glucokinase ( $11.96 \pm 0.54$ ,  $14.23 \pm 0.88$  and  $15.78 \pm 0.27$  ng/mL) respectively in a dose dependent manner with glibenclamide (2 mg/kg) producing a better activity ( $17.68 \pm 0.75$  ng/mL), when compared with the diabetic control group (Figure 3).



**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 superscript letters (a, b, c, d, e) differ significantly ( $P < 0.05$ ) compared with the control groups  
 DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, 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



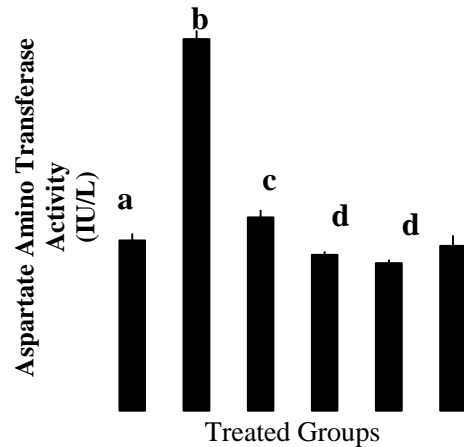
**Figure 3: Effects of lycopene on hepatic glucokinase activity in streptozotocin-induced diabetic Wistar rats.**

Each bar represent mean of five animals  
 Bars with different superscript letters (a, b, c, d, e, f) differ significantly ( $P < 0.05$ ) compared with control groups  
 DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, 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

**Effects of Lycopene on markers of liver function in streptozotocin-induced diabetic Wistar rats**

Significant ( $P < 0.05$ ) increase in the activity of serum AST of  $(91.20 \pm 2.06$  IU/L) was recorded in

the diabetic control, when compared with the normal control group that had  $41.80 \pm 1.69$  IU/L. Treatment of diabetic animals with 10, 20 and 40 mg/kg of lycopene as well as (glibenclamide 2 mg/kg) significantly ( $P < 0.05$ ) reduced the activity of serum AST to  $(47.40 \pm 1.81$ ,  $38.20 \pm 0.86$  and  $36.20 \pm 0.86$  IU/L) and  $(40.40 \pm 2.58$  IU/L) for glibenclamide, when compared with the diabetic control group (Figure 4).

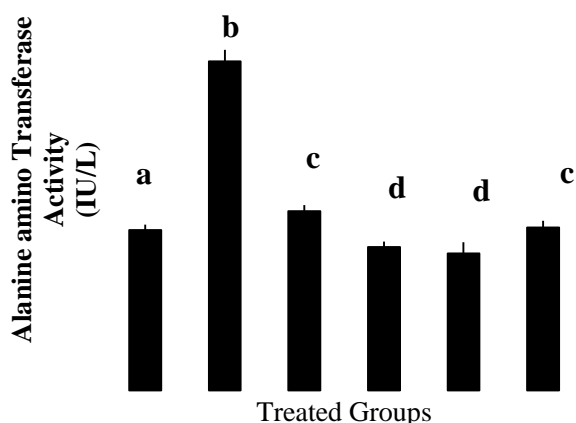


**Figure 4: Effects of lycopene on aspartate amino transferase activity in streptozotocin-induced diabetic Wistar rats**

Each bar represent mean of five animals; Bars with different superscript letters (a, b, c, d) differ significantly ( $P < 0.05$ ) compared with control groups  
 DC+OL = Diabetic rats treated with olive oil, NC+ OL = Normal rats treated with olive oil, 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

The diabetic control animals had significantly ( $P < 0.05$ ) elevated serum ALT activity of  $(93.60 \pm 3.25$  IU/L), when compared with the normal control animals group that had ALT activity of  $(45.60 \pm 1.54$  IU/L). The administration of graded doses of lycopene (10, 20 and 40 mg/kg) to diabetic resulted to significantly ( $P < 0.05$ ) decreased serum ALT activity to  $(51.00 \pm 1.73$ ,  $40.80 \pm 1.59$  and  $39.00 \pm 3.15$  IU/L) and  $(46.40 \pm 1.86$  IU/L) for the glibenclamide (2 mg/kg) diabetic treated group, when compared with diabetic control group. The effect of lycopene was found to be dose dependent (Figure 5).

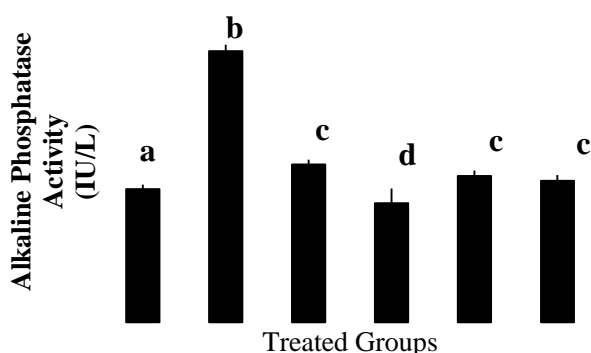
There was a significant ( $P < 0.05$ ) increase on activity of serum ALP ( $134.80 \pm 3.14$  IU/L) in diabetic untreated animals, when compared with the normal control rats that had  $(66.20 \pm 2.20$  IU/L). Administration of 10, 20 and 40 mg/kg of lycopene to diabetic animals produced a significantly ( $P < 0.05$ ) reduced serum ALP activity of  $(78.40 \pm 2.38$ ,  $59.20 \pm 7.33$  and  $72.80 \pm 2.65$  IU/L) and  $(70.40 \pm 2.87$  IU/L) for the standard drug (glibenclamide 2 mg/kg), when compared with diabetic control group (Figure 6).



**Figure 5: Effects of lycopene on alanine amino transferase activity in streptozotocin-induced diabetic Wistar rats**

Each bar represent mean of five animals; Bars with different superscript letters (a, b, c, d) differ significantly ( $P < 0.05$ ) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal rats treated with olive oil, D+LYC 10 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



**Figure 6: Effects of lycopene on alkaline phosphatase activity in streptozotocin-induced diabetic Wistar rats**

Each bar represent mean of five animals; Bars with different superscript letters (a, b, c, d) differ significantly ( $P < 0.05$ ) compared with control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal rats treated with olive oil, D+LYC 10 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

## DISCUSSION

Experimental animal models have been suggested as one of the best model to study patho-physiology of any disease (Chatzigeorgiou *et al.*, 2009; Ali *et al.*, 2011). Streptozotocin (STZ) induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models by selectively destroying the insulin producing  $\beta$ -cells which usually accompanied by characteristic alterations in blood insulin and glucose concentrations (Szkudelski, 2001). 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

finding has been substantiated by previous studies (Mohammed *et al.*, 2008; Krishna *et al.*, 2012).

The present investigation showed that serum insulin level was significantly decreased after streptozotocin administration when compared with normal control animals. This finding is similar to those reported by Bedoya *et al.* (1996); Mallick *et al.* (2006) and Daisy *et al.* (2012), who reported a significantly depleted insulin level in animal model administered with streptozotocin. (Elsner *et al.* (2000) reported that the mechanism by which STZ is taken up by pancreatic  $\beta$ -cells is *via* glucose transporter GLUT2. Intracellular action of STZ results in changes of DNA in pancreatic  $\beta$ -cells comprising its fragmentation (Morgan *et al.* 1994), resulting to impaired glucose oxidation and decreases insulin biosynthesis and secretion (Nukatsuka *et al.* 1990a). Oral administration of lycopene in the present study, produced a steady significant decrease on the blood glucose concentration, with better effect recorded after the third and fourth week when compared with corresponding diabetic control group. This finding agrees with the studies of other researchers (Duzguner *et al.*, 2008; Kuhad *et al.*, 2008; Ali and Agha 2009; Aydin and Celik, 2012; Sevim *et al.*, 2013). Base on the current findings, it may be suggested that the possible mechanism involved in the hypoglycaemic action of lycopene may be through stimulation of insulin secretion by the  $\beta$ -cells of pancreas as evidenced by increased serum levels. Although the elevation was not statistically significant when compared with diabetic control animals. 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. Thus, it may be suggested that insulin secretion may not be part of the observed hypoglycaemic property of lycopene. Contrary to this observation, glibenclamide administration resulted to significant elevated serum insulin level when compared with the diabetic control group. This is consistent with the reports of other investigators (Mulder *et al.*, 1991; Erejuwa *et al.*, 2011; Sayed *et al.*, 2011), who showed that glibenclamide stimulates and maintain prolonged insulin secretion from pancreatic  $\beta$ -cells as well as reduces hepatic glucose production resulting in reduced blood glucose level. The comparable effect of lycopene with glibenclamide in this study may suggest similar mechanism of action. In addition, oxidative stress induced by reactive oxygen species generated due to chronic hyperglycaemia has been implicated in the onset and progression of diabetes mellitus and its related complications (Giacco and Brownlee, 2010). Hyperglycemia in diabetes mellitus has been shown to cause depletion of

cellular antioxidant defenses and increases the levels of free radicals (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). Thus, hypoglycaemic activity of lycopene in the present study may therefore be linked to its strong antioxidant property (Atessahin *et al.*, 2005). In similar study by Bose and Agrawal (2006), showed 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 the significantly decreased blood glucose levels recorded in this study could be partly due to strong antioxidant property of lycopene.

Liver glucokinase enzyme is one of the key enzymes that are involved in carbohydrate metabolism and its activity has been reported to reduce drastically during diabetes. Results obtained in our current study revealed that the activity of hepatic glucokinase enzyme was significantly decreased in diabetic untreated animals when compared with those obtained from the normal control animals. These findings have been substantiated by other investigators (Hikino *et al.*, 1989; Kalaivanan and Pugalendi, 2011) who have reported that deficiency of insulin leads to derangement in carbohydrate metabolism and reduces the activities of a number of key enzymes, including glucokinase. In addition, Zhang *et al.* (2009) had demonstrated that glucokinase enzyme activity was significantly reduced by more than 90% in the liver of diabetic rats. Oral administration of lycopene significantly increased the hepatic glucokinase activity indicating a significantly increase in glucose influx into hepatic cells, hence increase in glucose utilization and consequent significant reduction in blood glucose concentration noted in the present investigation. Nirav *et al.* (2012) demonstrated that insulin increase hepatic glucokinase activity in liver therefore, it could be inferred that the decrease in the liver glucokinase activity may be as a result of the reduced serum insulin level in the diabetic control animals observed in the present study. The findings is in consonance with the report of other investigators (Shukla *et al.*, 2007; Iynedjian, 2009; Matschinsky, 2009), who have shown that liver glucokinase is glucose rate-controlling enzyme for hepatic glucose clearance and glycogen synthesis and is the most sensitive indicator of the glycolytic pathway in diabetes and decreased levels could decrease the utilization of blood glucose for glycogen storage in the liver. The results from this study showed that administration of lycopene to diabetic rats produced a significant increased activity of hepatic glucokinase. These observations

imply that entry of glucose into the cells is facilitated by lycopene treatment, which in turn stimulates the activity of this enzyme, with a consequent decrease in the blood glucose level observed in the current study. Franz (1990) showed that liver glucokinase is sufficiently active to allow quantitative hepatic removal of alimentary glucose from the portal vein and also constitutes the first rate limiting step in the pathway that results in the storage of glucose in the form of liver glycogen.

Serum enzyme measurements are valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue (Daisy and Saipriya, 2012). Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are biomarkers of damage to the plasma membrane and endoplasmic reticulum and are often used to assess the integrity of the plasma membrane and tissues after being exposed to certain pharmacological agents (Rathod *et al.*, 2009). The study showed that the activities of serum liver enzymes AST, ALT and ALP were significantly increased in the diabetic untreated animals in comparison with those obtained in normal control animals. These findings are consistent with the studies of (Ghosh and Suryawansi, 2001; Gandhi *et al.*, 2011; Abolfathi *et al.*, 2012; Daisy and Saipriya, 2012) who have reported increased transaminase levels in streptozotocin-induced diabetes. In addition, elevation in markers of liver injury such as ALT, AST and ALP also indicate hepatocyte damage in diabetes. The increase in the levels of these enzymes in diabetes may be as a result of leaking out of these enzymes from the tissue into the blood stream (Concepción *et al.*, 1993). Aspartate transaminase and alanine transaminase are released when injury involves organelles such as the mitochondria (Kumar *et al.*, 2003). Transaminases mediate the catalysis of amino transfer actions, and are vital markers of liver injury in clinical diagnostics (Li *et al.*, 2007), alkaline phosphatase is a hydrolase enzyme located in the cytoplasm (Han *et al.*, 2006) and is responsible for removing phosphate from nucleotides and proteins released due to hepatic cellular damage. The ability of lycopene administration to diabetic animals to significantly decrease the AST serum level suggests their hepato-cellular protective function and this can be attributed to its antioxidant effects.

## CONCLUSION

The possible anti-hyperglycaemic mechanism of actions of lycopene in the present investigation is suggested to be due to increased activity of hepatic glucokinase and may not be due to stimulation of release of insulin from the pancreatic beta cells since serum insulin level was not significantly increased. Available findings from the present

investigation also showed that the elevated activities of serum liver enzymes were significantly ameliorated; therefore suggesting that lycopene may be useful in controlling hepato-cellular damage that usually occurs in diabetes mellitus.

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