British Journal of Medicine & Medical Research
7(9): 762-770, 2015, Article no.BJMMR.2015.386
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Hypoglycaemic Effect of Lycopene in Streptozotocin-Induced Diabetic Wistar Rats

Ejike Daniel Eze[1*], Aliyu Mohammed[2], Yusuf Tanko[2], Abubakar Ahmed[3] and Karimah Mohammed Rabiu[4]

[1]Department of Physiology, Faculty of Basic Medical Sciences, Bingham University, Karu, Nasarawa State, Nigeria.
[2]Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.
[4]Department of Biochemistry, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors EDE and AM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author YT managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors AA and KMR managed the experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/15908
Editor(s):
(1) Kate S. Collison, Department of Cell Biology, King Faisal Specialist Hospital & Research Centre, Saudi Arabia.
Reviewers:
(1) Anonymous, India.
(2) Ds sheriff, Faculty of Medicine, Benghazi University, Benghazi, Libya.
(3) Anonymous, Malaysia.
(4) Araceli Loste, Animal Pathology, Veterinary Faculty, University of Zaragoza, Spain.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=949&id=12&aid=8634

Original Research Article

Received 24th December 2014
Accepted 12th March 2015
Published 31st March 2015

ABSTRACT

Aim: The study was designed to investigate the hypoglycaemic potential of lycopene in streptozotocin (STZ)-induced diabetic Wistar rats.
Methodology: To achieve this, a total of thirty (30) adult Wistar rats of both sexes were used. The animals were made diabetic by single intraperitoneal injection of freshly prepared (60 mg/kg body weight) of STZ. Diabetes was confirmed by the presence of high blood glucose ≥ 200 after 72 hr. Diabetic animals were divided into six (6) groups (1, 2, 3, 4, 5 and 6) comprising five animals each. Animals in Group 1 (Diabetic control) and Group 2 (Normal control) received 0.5 ml of olive oil, while those in groups 2, 3, 4, 5 and 6 were administered 10, 20, 40 and 2 mg/kg b w of lycopene

**Corresponding author: Email: daneze4@gmail.com;*

and glibenclamide respectively orally once daily for a period of four weeks. After the last day of treatments, all animals were sacrificed and blood samples collected and the serum separated for determination of serum insulin concentration. The liver tissue was excised and homogenized in equivalent volumes of phosphate buffer for the determination of hepatic glucokinase enzyme activity.

Results: The results obtained showed that lycopene at all doses significantly ($P < 0.05$) decreased the blood glucose concentration steadily from (431.4±48.84 mg/dL) to (171.1±7.65, 118.4±1.97 and 100.8±6.89 mg/dL) after four weeks of treatment. The Serum insulin level was increased from (3.02±0.24 µIU/mL) to (4.02±0.70, 3.96±1.41 and 5.06±0.96 µIU/mL), but was not significant ($P>0.05$), when compared with diabetic control animals. The activity of hepatic glucokinase was significantly ($P<0.05$) increased from (8.78±1.11 ng/mL) to (11.96±0.54, 14.23±0.88 and 15.78±0.27 ng/mL), when compared with diabetic control group.

Conclusion: It is therefore, suggested that antidiabetic-activity may be linked to enhanced glucokinase enzyme activity and not due to increased serum insulin level as the elevation was not statistically significant ($P > 0.05$) when compared with the diabetic control group. It is recommended that, lycopene may be used as a dietary component in controlling sustained hyperglycaemia in diabetes.

Keywords: Diabetes mellitus; lycopene; hypoglycaemia; insulin; rats; glucokinase.

1. INTRODUCTION

Diabetes mellitus is a multi-systemic disorder that affects almost every cell in the body and considered one of the most important health problems worldwide [1]. It is a common metabolic disorder resulting from defects in insulin secretion or action or both, is characterized by hyperglycemia and often accompanied by glycosuria, polydipsia, and polyuria [2]. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species as a result of glucose auto-oxidation and protein glycosylation that occurs [3,4]. This leads to secondary complications affecting eyes, kidneys, nerves and arteries [5]. Diabetes mellitus is classified into two types namely: type 1 and type 2. Type 1, also known as insulin-dependent diabetes mellitus (IDDM), in which the body does not produce any insulin, most frequently occurs in children and young adults. This type of diabetes accounts for 5–10% of diabetes. Type 2, otherwise called non-insulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough, or properly uses insulin. It accounts for 90–95% of diabetes [6]. Poor and inadequate glycemic control constitutes a major public health problem and thus research on new substances with hypoglycemic properties is required. So there has been an increasing interest in the search of hypoglycemic agents from natural products, especially those derived from plants. This is because they are usually considered to be non-toxic, safe and with fewer side effects than synthetic sources. Several reports have shown that antioxidants could be useful in preventing or attenuating the adverse effects of chronic hyperglycemia [7]. Lycopene is a natural constituent of red fruits and vegetables and of certain algae and fungi [8]. It is one of the most powerful antioxidants among dietary carotenoids found in foods. It has gained increased attention for its health giving properties Lycopene is synthesized by plants and microorganisms, but not by animals. It is a red open-chain unsaturated carotenoid, acyclic isomer of beta-carotene and longer than any other carotenoid. Several studies have been conducted on lycopene's antioxidant properties and health benefits [9]. Gac fruits, tomatoes and tomato products, including ketchup, tomato juice, and pizza sauce, are the more bioavailable sources of lycopene [10]. It is also found in watermelon, papaya, pink grapefruit, and pink guava [11]. However, lycopene is more bioavailable in processed and cooked tomato products than in fresh tomatoes [11]. *In vitro,* animal and clinical studies suggest that lycopene may attenuate liver injury and possibly prevent the development of hepatocellular carcinoma [12]. Some evidence suggests that cancers of the pancreas, colon and rectum, esophagus, oral cavity, breast and cervix could be reduced with increased lycopene intake [13]. Studies [13] demonstrated that oral administration of lycopene to men with infertility significantly improved the sperm concentration in 66% of cases and motility in 73% of cases. Korucuoglu et al. [14] in their studies showed that lycopene can be used to prevent osteoporosis by its ability to reduce carbonyl levels. Carbonyl a product of

protein oxidation, leads to oxidative stress and osteoporosis. Lycopene also was shown to have an effect on proliferation and differentiation of osteoblasts, the cells responsible for bone formation [15]. Liu et al. [16] also demonstrated that lycopene exhibited anti-atherogenic effects by inhibiting the expression of inflammatory mediators in hyperhomocysteinemic rats. In addition, some studies have demonstrated the anti-diabetic effect of lycopene, however, most investigations carried out were mainly epidemiological in nature with little or no experimental validation. Also coupled with the fact that there is limited information on hypoglycaemic activity of lycopene using animals as models. Therefore, the current study aimed at evaluating the hypoglycaemic effect of lycopene in STZ-induced diabetic Wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animals and care

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

2.1.2 Chemicals and lycopene

Streptozotocin was purchased from Sigma chemicals (St Louis U.S.A), while Lycopene (30 mg) capsule was procured from General Nutrition Corporation, Pittsburgh, U.S.A.). It was reconstituted in olive oil (*Goya en España*, S.A.U., Sevilla, Spain) to appropriate working dosage. All other chemicals and solvents used were of analytical grade.

2.2 Methods

2.2.1 Induction of experimental diabetes

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

2.2.2 Experimental design and treatments

In the present study, a total of thirty (30) Wistar rats (twenty five diabetic and five normal animals) were used. The animals were randomized into six groups of five rats each:

Group I: Comprised of normal control rats that were administered with 0.5 ml/kg body weight of olive oil
Group II: Consisted of diabetic control animals that were treated with 0.5 ml/kg body weight of olive oil
Group III: Diabetic animals that received 10 mg/kg body weight of lycopene
Group IV: Diabetic rats that were administered with 20 mg/kg body weight of lycopene
Group V: Diabetic rats that were treated with 40 mg/kg body weight of lycopene
Group VI: Diabetic animals that received 2 mg/kg body weight of glibenclamide [17]. All treatments were given orally once daily for four weeks. The study was conducted according to the Ahmadu Bello University Animal Committee guideline in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Pub No.85-23 revised 1985).

2.2.3 Assessment of fasting blood glucose level

Fasting 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 method described by Beach and Turner [18] using digital glucometer (Accu-chek Advantage) and expressed as mg/dl.

2.2.4 Preparation of serum sample

Twenty four hours after the last day of treatment, all rats from each group were euthanized and blood was collected through cardiac puncture into sample tubes. The blood in the sample tubes was allowed to clot and the serum separated by

centrifugation at 1,964 g for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany). Serum obtained was used for the determination of serum insulin concentration.

2.2.5 Tissue homogenates preparation

Approximately 1.0 g of liver and kidney were dissected out, washed with ice-cold saline, weighed and homogenized immediately in 10 ml ice-cold phosphate buffer of 0.1 M of pH 7.4. The homogenates were centrifuged at 1,964 g for 10 minutes to remove debris and supernatant collected for evaluation of hepatic glucokinase activity.

2.2.6 Estimation 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).

2.2.7 Estimation of hepatic glucokinase enzyme activity

The activity of hepatic glucokinase enzyme activity was determined by method of Goward et al. [19] by continuous spectrophotometric rate determination.

2.2.8 Statistical analysis

Values 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 significance 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

Fig. 1 shows the comparison between the normal control and diabetic control groups as well as the diabetic rats treated with various doses of lycopene and glibenclamide. Results obtained indicated that there was a significant increase ($P < 0.05$) in the level of fasting blood glucose in the diabetic control rats three days after streptozotocin injection, when compared with those obtained in normal control animals. Treatments of diabetic animals with lycopene at all doses (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) produced a steady significant ($P < 0.05$) decrease in the fasting blood glucose concentration especially after the 3rd and the 4th weeks when compared with diabetic control group.

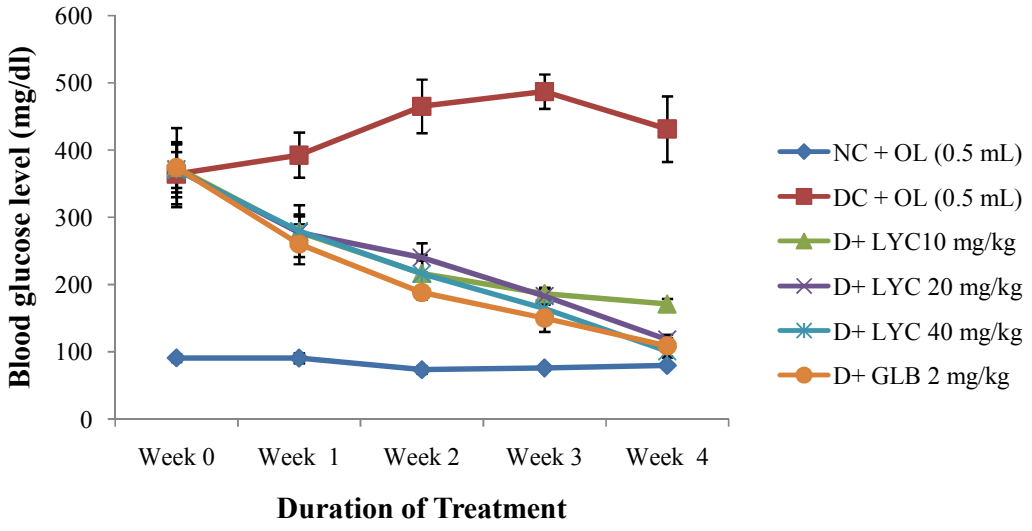

Fig. 1. Effects of lycopene on fasting blood glucose level in streptozotocin-induced diabetic Wistar rats

NC+ OL = normal (non-diabetic) rats + olive oil (0.5 ml), DC+OL = 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

There was also a significant ($P < 0.05$) reduction in serum insulin level in the diabetic control rats after STZ injection, when compared with normal control rats (Fig. 2). However, oral administration of lycopene to diabetic rats resulted in elevation of serum insulin level, but it was not statistically significant ($P > 0.05$) in comparison with the diabetic control group. Conversely, there was a significant increase ($P < 0.05$) in the level of serum insulin in diabetic animals treated with glibenclamide (2 mg/kg) when compared with diabetic control group (Fig. 2).

The activity of hepatic glucokinase was significantly reduced ($P < 0.05$) in the diabetic untreated animals when compared with normal control rats. Administration of lycopene and glibenclamide to diabetic rats significantly ($P < 0.05$) increased activity of hepatic glucokinase with the reference drug (glibenclamide) producing a better activity when compared with diabetic control group (Fig. 3).

4. DISCUSSION

Experimental diabetes models have been reported to be an important tool in analyzing diabetes complications and determining treatment approaches [20]. The present study evaluated the hypoglycaemic activity of lycopene in STZ-induced diabetes mellitus. Results obtained showed that blood glucose concentration was significantly elevated in animals three days after STZ injection. This finding is in agreement with those reported by other authors [21,22]. STZ is well known for its selective pancreatic β-cell toxicity and has been extensively used for the induction of diabetes mellitus in animal models [23]. STZ induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models by selectively destroying the insulin producing β-cells by inducing necrosis and hence, causing alterations in plasma insulin and glucose concentrations [24]. Thus, the significantly reduced serum insulin level recorded in the present investigation showed that STZ administration caused destruction insulin producing β-cell in the pancreas. This finding has been reported by other investigators [25-27]. The present study showed that lycopene administration to diabetic animals increased serum insulin concentration, although this elevation was not statistically significant when compared with the diabetic control group. This observation indicated that lycopene may possess insulin secreting or releasing activity [28], even though the level of increase was not significant. Thus, this may partly account for the significantly reduced blood glucose levels recorded in the experimental animals when compared with the diabetic control group. However, glibenclamide produced a better activity than the lycopene treated groups when compared. The finding is consistent with the reports of Sevim et al. [20] and Duzguner et al. [29], who have demonstrated a significantly reduced blood glucose concentration in diabetic animals following lycopene administration. Furthermore, chronic hyperglycemia in diabetes mellitus has been shown to cause increase in the levels of free radicals generation [30]. And it has been reported that lycopene possess good free radical scavenging capacity because of its unique structure (high number of conjugated double bonds) being one of the most potent antioxidants carotenoids [31]. Therefore, anti-diabetic activity of lycopene observed in our present study may be attributed to its strong antioxidant property [31], since hyperglycaemia in diabetes mellitus has been shown to cause depletion of the cellular antioxidant defenses and increases the levels of free radicals as well. Similar study carried out by Bose and Agrawal [32] have demonstrated the ability of lycopene to quench the superoxide and other free radical anions which are released in diabetes due to abnormal glucose metabolism.

Abnormal insulin secretion is one of the characteristics of chronic hyperglycaemia in diabetes; and this often results to derangement in carbohydrate metabolism [33]. Liver has been shown to be an insulin dependent tissue, and is reportedly involved in glucose and lipid homeostasis, which is usually severely affected during diabetes [34]. Insulin influences the intracellular utilization of glucose in a number of ways. It increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase [35]. Glucokinase catalyzes the conversion of glucose to glucose - 6- phosphate and plays a central role in the maintenance of glucose homeostasis. In the liver, this enzyme is an important regulator of glucose storage and disposal [36]. In the present study, the glucokinase activity was decreased in STZ-induced diabetic untreated animals when compared to normal control group. This finding is similar with the reports of the previous studies by Iynedjian et al. [37] and Iynedjian [38] who demonstrated that hepatic glucokinase was significantly reduced in STZ-induced diabetic rats. Hence the decreased activity of hepatic

glucokinase in diabetic untreated animals recorded in the current investigation may be attributed to depleted insulin secretion. Treatment with lycopene enhanced the activity of glucokinase in liver of diabetic animals when compared with the control group. This observation suggests that increased activity may be due to stimulation of insulin secretion from pancreatic β-cells as evidenced by increased serum insulin level recorded in our current investigation [39], however this elevation was statistically significant when compared with the diabetic control group. The enhanced glucokinase activity does not appear to be related to increased insulin secretion, since there was only a modest, statistically insignificant increase in insulin levels with lycopene treatment. Thus, the increased activity of this glucose regulating enzyme may have resulted in increased glucose utilization by the cells leading to reduced levels of blood glucose [34,40].

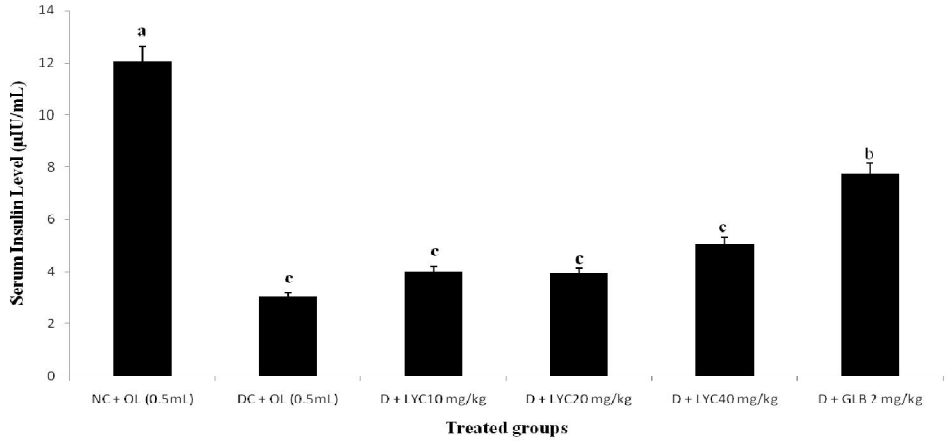

Fig. 2. Effects of lycopene on serum insulin level in streptozotocin-induced diabetic Wistar rats Bars with different superscript letters (a, b, c) differ significantly (P<0.05) when compared with the control groups

NC+ OL = normal (non-diabetic) rats + olive oil (0.5 ml), DC+OL = 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

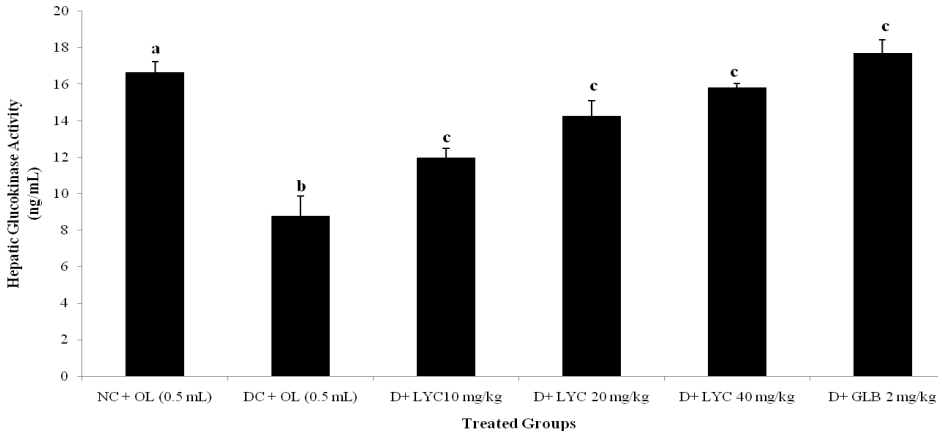

Fig. 3. Effects of lycopene on hepatic glucokinase acitivity in streptozotocin-induced diabetic Wistar rats bars with different superscript letters (a, b, c) differ significantly (P<0.05) when compared with the control groups

NC+ OL = normal (Non-diabetic) rats treated with olive oil (0.5 ml), DC+OL = diabetic rats treated with olive oil (0.5 ml), D+ LYC10 mg/kg = diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = diabetic rats treated with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = diabetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = diabetic rats treated with glibenclamide 2 mg/k

5. CONCLUSION

In conclusion, the observed significant reduction in blood glucose level in diabetic rats recorded in our present investigation suggests that lycopene may exert its hypoglycaemic activity via increased hepatic glucokinase activity and probably by stimulating insulin release from pancreatic β-cells as evidenced by elevated serum insulin level, although the increase was not significant. It is therefore suggested that lycopene may be a promising candidate as a dietary hypoglycaemic agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments were examined and approved by the Ahmadu Bello University Animal ethical committee and were therefore, performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

The authors of this work wish to thank Mr. O. Ayegbusi of the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and Mallam Bala Mohammed, Mr Yusuf Inua, Mallam Yau Bello and Mallam Nura Mohammed of the Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria, for their research and technical assistance to ensuring that this study was successful.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta R, Bajpai KG, Johri S, Saxena A. An overview of Indian novel traditional medicinal plants with anti-diabetic potentials. African Journal of Traditional, Complementary and Alternative Medicines. 2008;5:1-17.
2. Celik IE, Yegin FO. Effect of experimental diabetes mellitus on plasma lactate dehydrogenase and glutamic oxaloacetic transaminase levels in rabbits, Turkish Journal of Biology. 2002;26:151–154.
3. Moussa SA. Oxidative stress in diabetes mellitus. Romanian Journal of Biophysics. 2008;8(3):225-236.
4. He Z, King GL. Microvascular complications of diabete. Endocrin. Metab. Clin. 2004;33:215-238.
5. Li WL, Zheng HC, Bukuru J, De KN. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. Journal of Ethnopharmacology. 2004;92: 1-21.
6. Cemek M, Kağa S, Simşek N, Büyükokuroğlu ME, Konuk M. Antihyperglycemic and antioxidative potential of *Matricaria chamomilla* L. in streptozotocin-induced diabetic rats. J. Nat. Med. 2008;62:284-293.
7. Omayma AE, Mohamed AE, Gamil EI. Lycopene and Lutein; A review for their Chemistry and Medicinal Uses. Journal of Pharmacognosy and Phytochemistry. 2013;2(1):245-254.
8. Arab L, Steck S. Lycopene and cardiovascular disease. American Journal of Clinical Nutrition. 2000;71(6):1691S-1695S.
9. Betty K, Charlotta T, Mary CH, McKeon TA. Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. Journal of Agriculture and Food Chemistry. 2004;52:274-279.
10. Gartner C, Stahl W, Sies H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. American Journal Clinical Nutrition. 1997;66:116–122.
11. Seren S, Mutchnick M, Hutchinson D, Harmanci O, Bayraktar Y, Mutchnick S, Sahin K. Kucuk O. Potential role of lycopene in the treatment of hepatitis C and prevention of hepatocellular carcinoma. Nutrition and Cancer. 2008;60(6):729735.
12. Vrieling A, Voskuil DW, Bonfrer JM, Korse CM, Van Doorn J, Cats A. Lycopene supplementation elevates circulating insulin-like growth factor binding protein-1 and -2 concentrations in persons at greater risk of colorectal cancer. American Journal Clinical Nutrition. 2007;86(5):1456-1462.
13. Gupta NP, Kumar R. Lycopene therapy in idiopathic male infertility—a preliminary

report. International Urology and Nephrology. 2002;34(3):369-372.
14. Korucuoglu U, Ciftci B, Gulbahar O, Biri A, Nas T, Gursory R, Aricioglu A. Assessment of protein oxidation in women using raloxifene. Molecular and Celular Biochemistry. 2006;290(1-2):97-101.
15. Kim L, Rao AV Rao L.G. Lycopene II—effect on osteoblasts: The carotenoid lycopene stimulates cell proliferation and alkaline phosphatase activity of SaOS-2 cells. Journal of Medicinal Food. 2003;6(2):79-86.
16. Liu X, Qu D, He F, Lu Q, Wang J, Cai D. Effect of lycopene on the vascular endothelial function and expression of inflammatory agents in hyperhomocysteinemic rats. Asian Pacific Journal of Clinical Nutrition. 2007;16(Suppl 1):244-248.
17. Ogundeji T, Ayo JO, Aluwong T, Mohammed A. Behavioral and haematological studies on effects of lycopene in Wistar rats subjected to psychological stress. Journal of Neuroscience and Behavioral Health. 2013;5(2):30-35.
18. Beach EF, Turner JJ. An enzymatic method for glucose determination uptake in body fluids. Clinical Chemistry. 1958;4:462-468.
19. Goward CR. Enzymatic assay of glucokinase. Biochemical Journal. 1986; 237:415-420.
20. Sevim CY, Fatmagül Y, Ebubekir C. Effect of lycopene application in rats with experimental diabetes using lipoprotein, paraoxonase and cytokines. Journal of Membrane Biology. 2013;246:621–626
21. Pathak S, Dorfmueller HC, Borodkin VS, Van Aalten DMF. Chemical dissection of the link between streptozotocin, O-GlcNAc, and pancreatic cell death. Chemistry and Biology. 2008;15(8):799-807.
22. Tanko Y, Yerima M, Mahdi MA, Yaro AH, Musa KY, Mohammed A. Hypoglycemic activity of methanolic stem bark of Adansonnia digitata extract on blood glucose levels of Streptozotocin-induced diabetic Wistar rats. International Journal of Applied Research in Natural Product. 2008;1:32-36.
23. Akhtar MS, Ali MP. Study of antidiabetic effect of a compound medicinal plant prediction in normal and diabetic rabbits. J Pak Med Anoc. 1984;34:329-344.
24. Szkudelski T. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiological Research. 2001;50(6):537-546.
25. Dobretsov M, Romanovsky D, Stimers JR. Early diabetic neuropathy: Triggers and mechanisms. World Journal of Gastroenterology. 2007;13:175–191.
26. Kondeti VK, Badri KR, Maddirala DR, Thur SK, Fatima SS, Kasetti RB, Rao CA. Effect of Pterocarpus santalinus bark, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin induced diabetic rats. Food Chemical and Toxicology. 2010;48:1281–1287.
27. Juárez-Rojop IE, Díaz-Zagoya JC, Ble-Castillo JL, Miranda-Osorio PH, Castell Rodríguez AE, Tovilla-Zárate CA, Rodríguez-Hernández A, Aguilar-Mariscal H, Ramón-Frías T, Bermúdez-Ocaña DY. Hypoglycemic effect of *Carica papaya* leaves in streptozotocin-induced diabetic rats. BMC Complementary and Alternative Medicine. 2012;12:236-247.
28. Duzguner V, Kucukgul, A., Erdogan, S., Celik, S. and Sahin, K. Effect of lycopene administration on plasma glucose, oxidative stress and body weight in streptozotocin diabetic rats. Journal of Applied Animal Research. 2008;33:17–20.
29. Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes and Metabolism. 2000;26:163–176.
30. Sharma R, Buras E, Terashima T, Serrano F, Massaad CA, Hu L, Bitner B, Inoue T, Chan L, Pautler RG. Hyperglycemia induces oxidative stress and impairs axonal transport rates in mice. PloS one. 2010;5(10):e13463.
31. Bose KSC, Agrawal BK. Effect of Long Term Supplementation of Tomatoes (Cooked) on Levels of Antioxidant Enzymes, Lipid Peroxidation Rate, Lipid Profile and Glycated Haemoglobin in Type 2 Diabetes Mellitus. West Indian Medical Journal. 2006;55(4):274-278.
32. Kumar P, Kumar A. Effects of lycopene and epigallocatechin-3-gallate against 3 nitropropionic acid induced cognitive dysfunction and gluthathione depletion in rats: a novel nitric oxide mechanism. Food and Chemical Toxicology. 2009;47:2522-2530.

33. Zimmet PZ, McCarty DJ, Courten MP. The global epidemiology of non-insulin dependent diabetes mellitus and the metabolic syndrome. Journal of Diabetes Complications. 1997;11:60-68.
34. Shetti AA, Sanakal RD, Kaliwal BB. Antidiabetic effect of ethanolic leaf extract of Phyllanthus amarus in alloxan induced diabetic mice. Asian Journal of Plant Science and Research. 2012;2(1):11-15.
35. Murray RL, Granner DK, Mayes PA, Rodwell VW. Harper's Biochemistry, 25th edition, Appleton and Lange Stanford Connecticut; 2000.
36. Goyal A, Chopra M, Lwaleed BA, Birch B, Cooper AJ. The effects of dietary lycopene supplementation on human seminal plasma. BJU International. 2007;99:1456-1460.
37. Goyal RK, Bhullar SS, Singh R. Comparative sensitivity to inorganic phosphate of sucrose to starch conversion in sorghum and wheat grains raised through liquid culture. Plant Physiology and Biochemistry. 1999;28:755.
38. Iynedjian P, Gjinovci A, Renold A. Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. Journal of Biological Chemistry. 1988;263(2):740-744.
39. Iynedjian PB. Molecular physiology of mammalian glucokinase. Cellular and Molecular Life Sciences. 2009;66(1):27-42.
40. Nirav D, Amit J, Gaurang S, Vipin D, Samadhan K, Rajesh B, Mukul J. Exendin-4 reduces glycemia by increasing liver glucokinase activity: An insulin independent effect Pharmacological Reports. 2012;64:140-149.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=949&id=12&aid=8634