Effect of Lycopene on Thyroid Profile in Streptozotocin-Induced Diabetic Wistar Rats

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Abstract

The present study evaluated the effect of lycopene on thyroid profile in streptozotocin (STZ)-induced diabetic rats. A total of thirty (30) Wistar rats of both sexes weighing between 150-200 g were completely randomized in six groups (1-6) comprising five rats each. Normal control animals in group 1 received 0.5 ml of olive oil used in dissolving lycopene. Animals in group 2-5 which were induced with diabetes by intraperitoneal administration of STZ (60 mg/kg body weight) were respectively administered 0.5 ml olive oil, 10, 20 and 40 mg/kg body weight of lycopene. The diabetic animals in group 6 received glibenclamide. Administration of lycopene was done once daily for 28 days. The results showed that lycopene administration at all doses significantly (P<0.05) decreased the fasting 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) especially after four weeks when compared with the diabetic control group. 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) after lycopene treatment to diabetic rats, but this increase was not significant (P > 0.05) when compared with diabetic control group. The level of triiodothyronine (T₃) was significantly (P < 0.05) reduced to (1.00 ± 0.09 ng/mL) in the diabetic control animals in comparison with those recorded in rats of normal animals (1.44 ± 0.12 ng/mL). Following oral administration of lycopene and glibenclamide, the levels of T₃ was significantly (P < 0.05) elevated to (1.06 ± 0.08, 1.34 ± 0.09, 1.52 ± 0.12 ng/mL) and (1.36 ± 0.08 ng/mL) in diabetic animals when compared to diabetic control group. The total thyroxine (T₄) level was significantly (P < 0.05) lowered in STZ-induced diabetic animals that were not treated to (67.20 ± 1.28 ng/mL) when compared with normal control rats that recorded (71.60 ± 3.14 ng/mL). On treatment with lycopene and glibenclamide, there was a significant (P < 0.05) increase in T₄ level to (77.00 ± 2.07 ng/mL) only with the highest dose (40 mg/kg body weight) of lycopene when compared with diabetic control animals while no significant (P > 0.05) change was observed with 10 and 20 mg/kg body weight of lycopene when compared with diabetic control rats. Overall, these findings established the fact that lycopene through its antioxidant effect exhibited antidiabetic activity and thus can be recommended for use in the management of diabetes mellitus. It may also contribute to protection against thyroid disorders.

Keywords: Lycopene, Thyrosine, Triiodothyronine, Diabetes Mellitus, Blood Glucose, Glibenclamide, Thyroid Profile.

1. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and defective metabolism of glucose and lipids [1]. Diabetes is not a single disease rather it is a heterogeneous group of syndromes characterized by an elevation of blood glucose caused by relative or absolute deficiency of insulin. The metabolic disorder is associated with complications such as blindness, stroke, heart and blood vessel disease, kidney failure, retinopathy, amputation, neuropathy, ulceration and gangrene of extremities [2-3]. Diabetes can be divided into two main groups based on their requirements of insulin:
Insulin dependent diabetes mellitus (Type 1) and non-insulin dependent diabetes mellitus (Type 2). Type 1, known as juvenile onset or Insulin Dependent Diabetes Mellitus (IDDM); it manifests before 20 years of age [4] and its origin is usually ascribed to autoimmune disorder that destroys the beta cells of islet of Langerhans that are responsible for insulin production in the body. Individuals with this form of Type 1 diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis [5]. Markers of immune destruction, including islet cell autoantibodies and/or autoantibodies to insulin are present in 85-90% of individual with Type 1 diabetes mellitus [6]. Type 2 diabetes mellitus is a chronic progressive disease typified by a loss of glycaemic control over time as the insulin secreting pancreatic beta cells lose their ability to compensate for the prevailing levels of insulin sensitivity. The hyperglycemia of type 2 diabetes is associated with an increased risk of micro vascular (retinopathy, neuropathy, nephropathy) and macro vascular (myocardiac infarction, stroke) events.

Diabetes mellitus is a global disease found in all nations of the world [7]. A recent study by the World Health Organization (WHO) estimated that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million or more by 2030 [8]. In Nigeria, the incidence of diabetes has been put at 2,819,000 for year 2010 and was projected to rise to 5,316,000 in 2030 [9].

Lycopene is one of the most powerful antioxidants among dietary carotenoids found in foods especially tomato [10]. It has gained increased attention for its health giving properties [11-13]. It is a bright red carotene and a member of the carotenoid family of phytochemicals that gives plants its characteristics red colour [14, 15]. Lycopene is also found in watermelon, papaya, pink grapefruit and pink guava [15].

Previous studies by Wang et al [16] reported that consumption of lycopene and tomato-based food products is not associated with the risk of type 2 diabetes in women while Duzguner et al [17] worked on the effect of lycopene administration on plasma glucose, oxidative stress and body weight in streptozotocin diabetic rats. Haribabu et al [18] worked on evaluation of anti-diabetic activity of lycopene and its synergistic effect with metformin hydrochloride and glipizide in alloxan induced diabetic rats in while Ali and Agha [19] worked on amelioration of streptozotocin-induced diabetes mellitus, oxidative stress and dyslipidemia in rats by tomato extract lycopene. Furthermore, Kuhad and Chopra [20] reported that lycopene ameliorates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rat while Aydin and Celik [21] reported the effects of lycopene on plasma glucose, insulin levels, oxidative stress, and body weights of streptozotocin-induced diabetic rats. Of all these studies, none has reported that the effect of the lycopene at 10, 20 and 40 mg/kg body weight. Again, studies by Karakaya and Yilmaz [22] have implicated the lycopene content of tomato for its pharmacological potentials without detailed information. Therefore, this study intends to provide information on the effects of lycopene on thyroid profile of STZ-induced diabetic rats.

2. Materials and Methods

2.1 Materials

2.1.1 Experimental Animals

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

2.1.2 Assay Kits, Drugs and Chemicals

Rats Insulin Elisa kits was procured from Bayer Diagnostics Ltd, Baroda, India. 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. Glibenclamide was obtained from Kemei Laboratories Ltd., Mumbai, India. All chemicals and solvents used were of analytical grade (BDH, UK).

2.1.3 Glucose Meter and Test Strips

Accu-check® Compact Plus Glucose Meter (Glucometer) and On-Call-Redi Blood Glucose Test Strips were products of Roche Diagnostic, Mannheim, Germany and Acon Laboratories Inc., San Diego, USA respectively.

2.2 Methods

2.2.1 Preparation of Lycopene Solution

30 (30) mg lycopene in a gelatinous capsule (General Nutrition Corporation, Pittsburgh, U.S.A.) was reconstituted in olive oil (Goya En espana, S.A.U., Sevilla, Spain) to appropriate working concentration as described by Ogundeji et al [23] with modifications to obtain the desired doses used in the study.

2.2.2 Induction of Diabetes

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight dose of STZ dissolved in fresh 0.1M cold citrate buffer of pH 4.5 into 18 h-fasted rats. Three days after STZ injection, blood was taken from tail artery of the rats. Animals having blood glucose levels ≥ 200 mg/dL were considered diabetic and included in the study. The diabetic animals were randomly divided into different groups.

2.2.3 Determination 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 week, 2 weeks, 3 weeks and 4 weeks of the treatment period by glucose-oxidase principle using digital glucometer (Accu-check® Compact Plus).

2.2.4 Experimental Protocol and Treatment

In the experiment, a total of thirty (30) Wistar rats of both sexes (twenty five diabetic and five normal control rats) were used; the animals were completely randomized into six groups (1-6) of five rats each as follows:

Group 1: Normal control animals that received (0.5 mg/kg body weight) of olive oil

Groups 2: Diabetic control rats that were administered (0.5 mg/kg body weight) of olive oil

Group 3: Diabetic animals that received 10 mg/kg b w of lycopene
Group 4: Diabetic animals that received 20 mg/kg b w of lycopene
Group 5: Diabetic animals that received 40 mg/kg b w of lycopene
Group 6: Diabetic rats that received 2 mg/kg b w of glibenclamide
All administrations were given orally once daily for four weeks.

2.2.5 Blood Sample Collection and Serum Preparation
After the last day of treatment (28th day) all animals from each group were sacrificed using light chloroform after 24 hours and 5 mL of blood was collected through cardiac puncture into specimen bottles and allowed to clot, and separated by centrifugation at 1,964 g for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany). The supernatant obtained was used for the determination of insulin and thyroid hormones levels.

2.2.6 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).

2.2.7 Determination of Serum Triiodothyronine (T3) and Thyrosine (T4)
Serum triiodothyronine (T3) was determined following the procedures outlined in the manufacture’s kits (ALPCO Diagnostics [Version 030104]). Thyrosine (T4) was determined in the serum using total thyroxine ELISA (Total T4 ELISA) method as described by Larsen et al (1973).

2.2.8 Statistical Analysis
Data obtained from each group were expressed as mean ± SEM of five determinations. 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 ≤ 0.05 were considered as significant.

3. Results
3.1 Effect of Lycopene on Fasting Blood Glucose Level in Streptozotocin-induced Diabetic Wistar Rats
Results obtained showed that STZ administration significantly increased (P< 0.05) fasting blood glucose concentration from (91.0 ± 5.74 mg/dL) to (364.4 ± 44.50 mg/dL) on week 0. That is before the commencement of treatment. Treatment of diabetic animals with the graded doses of lycopene (10, 20 and 40 mg/kg body weight) and standard drug, glibenclamide (2 mg/kg body weight) significantly (P < 0.05) decreased the blood glucose concentration steadily from (392.6 ± 33.52 mg/dL) to (278.2 ± 26.40, 277.43 ± 24.33, 279.8 ± 38.47 260.3 ± 29.74 mg/dL) 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 mg/dL) after week 2, (487.0 ± 25.64 mg/dL) to (186.2 ± 9.20, 183.0 ± 10.57, 164.4 ± 21.19 mg/dL and 150.2 ± 20.28 mg/dL) after week 3 and (431.4 ± 48.84 mg/dL) to 171.1 ± 7.65, 118.4 ± 1.97 100.8 ± 6.89 mg/dL and 108.8 ± 16.74 mg/dL) 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) in the diabetic control rats to (3.02 ± 0.24 µIU/mL) following streptozotocin treatment from (12.04 ± 0.93 µIU/mL) in normal control when compared. There was significantly (P > 0.05) difference between and within groups in the diabetic lycopene treated groups. However, administration of various doses (10, 20 and 40 mg/kg body weight) of lycopene to diabetic rats did not produce any significant (P > 0.05) increase on serum insulin level (4.02 ± 0.70, 3.96 ± 1.41 and 5.06 ± 0.96 µIU/mL) when compared with diabetic control group. Conversely, treatment of diabetic animals with 2 mg/kg body weight of glibenclamide produced a significantly (P < 0.05)
elevated serum insulin level (7.76 ± 0.42 µIU/mL) when compared with the diabetic (3.02 ± 0.24 µIU/mL) control group (Figure 2).

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

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

3.3 Effect of Lycopene on Triiodothyronine (T3) Level in Streptozotocin-induced Diabetic Wistar Rats

Figure 3 shows the values of effect of lycopene on total T3

concentration of control and experimental animals. The level of T3 was significantly (P < 0.05) reduced to (1.00 ± 0.09 ng/mL) in the diabetic untreated animals in comparison with those recorded in rats of normal control group that produced (1.44 ± 0.12 ng/mL) when compared. Following oral administration of lycopene and glibenclamide, the levels of T3 was significantly (P < 0.05) elevated to (1.06 ± 0.08, 1.34 ± 0.09, 1.52 ± 0.12 ng/mL) and (1.36 ± 0.08 ng/mL) in diabetic animals when compared to diabetic control group. However, the highest increase was recorded with the 40 mg/kg body weight of lycopene even better with the standard drug (glibenclamide).

Each bar represent mean of five animals

Bars with different superscript 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 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 body weight.
3.4 Effect of Lycopene on Thyrosine (T₄) Level in Streptozotocin-induced Diabetic Wistar Rats

The total thyrosine (T₄) level showed a significantly (P < 0.05) lowered in streptozotocin-induced diabetic animals that were not treated to (67.20 ± 1.28 ng/mL) when compared with normal control rats that recorded (71.60 ± 3.14 ng/mL). On treatment with lycopene and glibenclamide, there was a significant (P<0.05) increase on T₄ level to (77.00 ± 2.07ng/mL) only with the highest dose (40 mg/kg body weight) of lycopene when compared with diabetic control animals while no significant (P > 0.05) change observed with 10 and 20 mg/kg body weight of lycopene when compared with diabetic untreated rats (Figure 4).

![Fig 4: Effect of lycopene on total thyrosine (T₄) in streptozotocin-induced diabetic Wistar rats](image)

Each bar represent mean of five animals
Bars with different superscript letters (a,b,c) differ significantly (P < 0.05) compared with 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 body weight.

4. Discussion

Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity which results in hyperglycemia and abnormalities in carbohydrate, fat, and protein metabolism [24, 25]. Streptozotocin (STZ) induced diabetes is a well-documented model of experimental diabetes [26]. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used [24]. Streptozotocin-induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia [27]. STZ is a pancreatic cell toxin that induces rapid and irreversible necrosis of cells [28]. Whereas a single diabetogenic dose of STZ (70-250 mg/kg, body weight) has been demonstrated to induce complete destruction of cells in most species within twenty four hours. Multiple sub-diabetogenic doses of STZ partially damage islets, thereby triggering an inflammatory process leading to macrophage and subsequent lymphocyte infiltration, which is followed by the onset of insulin deficiency [29, 30]. Therefore, the determination of glucose concentration in the blood of animals among others is a useful, quantitative index of diabetes.

In this study, the intra-peritoneal administration of streptozotocin effectively induced diabetes mellitus in rats which was confirmed by elevated levels of fasting blood glucose, three days after STZ injection. This finding is in agreement with the report of Mohammed et al [31] and [32] that blood glucose level increased significantly after three days of STZ injection to rats. STZ induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models [33]. STZ selectively destroys the insulin producing β-cells which is accompanied by characteristic alterations in blood insulin and glucose concentrations [30]. 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 with the normal control rats. This finding has been substantiated by other researchers [34, 35]. Streptozotocin has been reported to induce insulin-dependent diabetes mellitus in animal models [36]. After administration, STZ was taken up by pancreatic β-cells via glucose transporter GLUT2 [37]. Intracellular action of STZ results in changes of DNA in pancreatic β-cells comprising its fragmentation. This results to impaired glucose oxidation [36] and decreases insulin biosynthesis and secretion [38, 39]. 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 reports of other investigators [17, 21, 40]. The possible mechanism involved in the hypoglycaemic action of lycopene may be stimulation of insulin secretion by the pancreas or/and enhance insulin sensitivity in various organs especially the muscles by promoting glucose uptake and metabolism inhibiting hepatic gluconeogenesis. However,
the results of our present finding in this study revealed 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 [21] who showed that the depleted serum insulin level in diabetic rats was reversed following lycopene administration. Unlike glibenclamide which produced a significantly elevated serum insulin level when compared with the diabetic control group. Glibenclamide have been reported to stimulate insulin secretion from pancreatic \( \beta \)-cells and also reduces hepatic glucose production resulting in reduced blood glucose level [41]. Furthermore, the improvement with glibenclamide administration in diabetic animals was evident by significant increase in the serum insulin levels observed in the present study. This observation is consistent with the reports of previous studies [42, 43]. Furthermore, glibenclamide, a standard anti-diabetic drug, commonly used in several studies to compare the efficacy of various hypoglycemic compounds acts by various mechanisms such as binding to the ATP-sensitive potassium channel and in the process, lowers glucose levels. Others include suppressing hepatic glucose production, increasing insulin sensitivity of extra-pancreatic tissues and fatty acid oxidation, enhancing peripheral glucose uptake, decreasing hepatic glycogenolysis and gluconeogenesis as well as absorption of glucose from the gastrointestinal tract [44]. Also, the possible mechanism of action of glibenclamide for its glucose lowering activity is by potentiating insulin secretion from pancreatic beta cells or sensitizing insulin receptors [29, 41]. The comparable effect of lycopene with glibenclamide in this study may suggest similar mechanism of action. Based on our present findings, it may be suggested that insulin secretion may not be part of the observed hypoglycaemic property of lycopene as the level was not significantly increased in diabetic animals that received various doses of lycopene. Oxidative stress induced by reactive oxygen species which are generated due to hyperglycaemia has been implicated in the onset and progression of diabetes mellitus and its related complications [45-47]. Hyperglycaemia in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increases the levels of free radicals [48, 49]. 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) [50]. Therefore, the hypoglycaemic effect of lycopene may also be attributed to its strong antioxidant property [51], Bose and Agrawal [50] 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. Thyroid function test (TFTs) is a collective term for blood tests used to check the function of the thyroid [52]. A TFT panel typically includes thyroid hormones such as triiodothyronine (T\(_3\)) and thyroxine (T\(_4\). The thyroid hormones thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) are synthesized and stored in the thyroid gland and circulate in the bloodstream mostly bound to the plasma protein, thyroxine binding globulin (TBG) [53]. The thyroid gland and associated hormones are a major component of the endocrine system. They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance of the body [54, 55]. Measurement of total T\(_4\) by immunoassay is reliable and convenient methods to determine the presence of thyroid disorders in patients [56, 57]. When compared with diabetic control animals, the elevated level of T\(_3\) following oral administration of lycopene and glibenclamide at all doses have been found in hyperthyroidism due to Grave’s disease and Plummer’s disease and in acute and sub-acute thyroiditis. The elevated serum T\(_4\) levels upon administration of lycopene and glibenclamide at 40 mg/kg body weight may be attributed to hyperthyroidism due to Grave’s disease and Plummer’s disease and in acute and subacute thyroiditis. When compared with diabetic control animals, the non-significant effect in serum T\(_4\) level following administration of lycopene and glibenclamide at 10 and 20 mg/kg body weight may be adduced to maintenance of cellular integrity of the thyroid gland, associated hormones and general hormonal balance of the animals.

5. Conclusion

Available evidence from the present study indicated that the antidiabetic effect of the lycopene from tomato extract may also be attributed to its strong antioxidant property. Insulin secretion may not be part of the observed antidiabetic property of lycopene. The study also showed that lycopene reduced cellular changes induced by STZ, thus indicating that lycopene contributes to the protection against thyroid damage and the associated hormones.

6. References

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