Effect of Glibenclamide and Tomato lycopene extract on some biochemical parameters in serum of alloxan Induced diabetic rabbits

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both, and occurs in almost all populations of the world a variable prevalence. In the present study we evaluated the effect of glibenclamide, and tomato lycopene extract on blood glucose, enzymatic antioxidants, glycated hemoglobin (HbA1c), and malondialdehyde (MDA) in 50 alloxan induced diabetic rabbits. The results showed a significant (P < 0.001) increase in the level of glucose, HbA1c, MDA in alloxan diabetic rabbits in comparison to the control rabbits. A Significant decrease in the level of serum glucose, HbA1c, and MDA in alloxan diabetic rabbits when received glibenclamide daily as single dose 0.5 mg/kg body weight for 20 weeks, and 2 and 4 mg/kg of tomato lycopene extract for 20 weeks respectively. The result of this study showed a significant (P < 0.05) decrease in the mean of serum catalase in alloxan diabetic rabbits in comparison with control group after treatment with 0.5 mg/kg glibenclamide, and 2, 4 mg/kg of lycopene extract a significant (P < 0.05) increase in serum catalase was observed in alloxan diabetic rabbit.

The aim of the study was to evaluate the effect of glibenclamide and tomato products on antioxidative status in alloxan induced diabetic rabbits.

Keyword: diabetes, glibenclamide, lycopene, antioxidants.
تأثير مستخلص لأيوكوبين الطماطة والكلابينكلاميد على بعض المعايير الكيميائية في مصل الدم الآرانب المحدث بها السكري بواسطة الألوكسان

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الملخص

يعد داء السكري أحد الاضطرابات الإيذائية الذي يتميز باختفاء مستوى الكلوتز في الدم نتيجة خلل أنسوبي، فعمل الإنزيم أو كلاهما، وبدأت دراسة هذا السكري على مستوى مختلف في معظم دول العالم. في الدراسة الحالية تم تقييم تأثير مستخلص لأيوكوبين الطماطة على كلوتز الدم والإنزيمات مضادة للأكسدة بالألدهايد والهيموكموبين المسكر في الأرانب السكري في الطبيعة. أظهرت النتائج انخفاضاً معنويًا في مستوى الكلوتز و الالدهايد والهيموكموبين المسكر عندما استخدم جلابينكلاميد بتركيز 0.5 ملغم / كغم من وزن الجسم يومياً لمدة 20 أسبوع. وعند استخدام خلصة لأيوكوبين للطماطة بتركيز 2 و 4 ملغم/كغم من وزن الجسم ولعدة 20 أسبوع مقارنة مع مجموعة السيطرة. كما نوّجت نتائج انخفاضاً معنويًا (P < 0.05) في مصل الأرانب السكري بخلاف الكتاليز في مصل الأرانب المحدث بها السكري بالإضافة مراقبة مع علامة السكري. و بعد العلاج بجلابينكلاميد أزمنة الخالص بتركيز 0.5 ملغم / كغم و خلاصة لأيوكوبين للطماطة بتركيز 2 و 4 ملغم/كغم من وزن الجسم ونحوت تمريح ارتفاعاً معنويًا (P < 0.05) في مستوى الكتاليز في صلب الأرانب المحدث بها السكري بالإضافة مراقبة مع نتائج السيطرة. لذلك، علامة تأثير جلابينكلاميد واستهلاك منتجات الطماطة على الانزيمات مضادة للأكسدة في مصل الأرانب المحدث بها السكري بواسطة الألوكسان.

الكلمات الدالة: داء السكري، جلابينكلاميد، لأيوكوبين، مضادة الأكسدة.
1. INTRODUCTION

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The World Health Organization estimates that over 170 million people worldwide are afflicted with this chronic condition and projects that numbers will rise to over 360 million by the year 2030 [1].

Reports suggest that patients with diabetes mellitus are susceptible to increased levels of oxidative stress [2,3]. Poor intravascular substrate control is largely regarded as a factor contributing to the increase in superoxide anions (O$_2^-$) that has previously been observed in diabetic serum [4]. Other potential mechanisms relating to enhanced oxidative stress in diabetes include a compromised antioxidant defense system, glucose autoxidation, the formation of advanced glycated end-products and a change in the glutathione redox status [5].

The pharmacological agents currently employed, such as sulfonylureas (e.g., glibenclamide), biguanides (e.g., metformin), thiazolidinediones (e.g., pioglitazone) and α-glycosidase inhibitors (e.g., acarbose) act to selectively modulate a specific pathological pathway [6, 7].

Antioxidants are protective agents that inactivate reactive oxygen species (ROS) and thereby significantly delay or prevent oxidative damage. The role of dietary antioxidants including Vitamin-C, Vitamin-E and B-carotenes in disease prevention has received much attention in recent years [8] and much work has been done on them. Very recently a new carotenoid compound called Lycopene, a red pigment which is rich in tomatoes and tomato based food products which is thought to play an important role in defense against chronic diseases like cancer, coronary heart diseases has been discovered [9-12]. Lycopene is a carotenoid compound, an acyclic isomer of B-carotene and does not show any pro vitamin A activity. It is a highly unsaturated hydrocarbon containing eleven conjugated and two unconjugated double bonds. It is the most predominant carotenoid in human plasma present naturally in greater amount than B-carotene and other dietary carotenoids. This perhaps indicates its greater biological significance in human antioxidant defense system [13].

Tomatoes are a valuable source of several micronutrients and phytochemicals including carotenoids, polyphenols, potassium, folate, ascorbic acid and α-tocopherol. An extensive literature survey from all scientific sources revealed that lycopene has antioxidant and anti-
diabetic activity. But the influence of lycopene on diabetic patients who are under the treatment is not clear[14,15].

This study aimed to evaluate the hypoglycaemic and antioxidant activities of glibenclamide, and tomato lycopene extract in alloxan induced diabetic rabbits.

2. MATERIAL AND METHODS

Sample Preparation

- **Drugs solution:** Glibenclamide 0.5 mg was dissolved in 1 ml distilled water[16].

- **Extraction of Lycopene:** The Tomatofruits *Lycopersicum esculentum* were used in this study as lycopene source. 500 gm of tomato fruit were chopped to small pieces and mixed with distilled water (1 weight: 3 volumes). This mixture was immediately shaken vigorously in a blender for 10 minutes. The mixture then left for two hours in mixer under cooled condition, and then the thick suspension was filtered through several layers of quiz and the extract centrifuged to remove residual materials. The extract volume reduced in incubator at 25 C and crude powder extract obtained.

  The lycopene content ranged from 11.47 to 53.90 mg/100 g in tomato paste, 20 mg lycopene powder was weighed and dissolved in 10 ml of distilled water to give 2 mg/ml solution. This solution was administered at a dose of 2 mg/kg and 4 mg/kg body weight using clean and dry oral feeding needle for 21 days.

**Experimental animals**

The experimental study was carried out on 125 adult rabbits (about 1.5-2.1 kg), during the period from June 2011 to May 2012. The animals were divided into 5 groups each group consists of 25 animals:

**Group 1 (G1):** diabetic rabbits received an alloxan (180 mg/kg intravenous injection)[17].

**Group 2 (G2):** diabetic rabbits received Glibenclamide 0.5 mg/kg.

**Group 3 (G3):** diabetic rabbits received lycopene 2 mg/kg[15].

**Group 4 (G4):** diabetic rabbits received lycopene 4 mg/kg[15].

**Group 5 (G5):** healthy control rabbits.
Induction of diabetes mellitus

The animals were Diabetes induced by injection of alloxan tetra hydrate (Koch-light laboratories, Coin brook, England -0158 Alloxan puriss) at a dose of 180 mg body weight intravenous in marginal ear vein [17] then after 6 hours the animals were injected with 10 ml of 10% glucose solution subcutaneously. The control group received an equivalent amount of normal saline.

Glucose solution 10% was given for 24 hours instead of the tap water in order to reduce alloxan hypoglycemic shock. The animals with high blood glucose concentration more than 300 mg/dl were considered as diabetic and employed in this study. Tests were performed on diabetic rabbits a week after the onset of diabetes.

Biochemical analysis

-Blood samples
At the end of experiment, 5 ml of blood samples were collected by ear vein of the rabbits from(125rabbits) and 1 ml of the sera prepared through centrifuging at 2500×g for 15 minutes at 30°C.

-Determination of Blood Glucose
The samples collected from fasting rabbits were used to determination of the fasting blood glucose that measured by enzymatic method [18].

-Determination of glycated hemoglobin
A standard colorimetric method was used to determine the glycated hemoglobin [19].

-Erythrocyte Malondialdehyde assay
Malondialdehyde (MDA) was assayed according to the method of Ohkawa, et al.[20]. The reactions to form thiobarbituric acid reactive substances (TBARs) depend on condensation of two molecules of MDA to generate a reddish chromogen that absorbs light at 532 nm wave length.

-Determination of erythrocyte catalase activity (CAT)
Serum Catalase activity (CAT) was determined according to the method used by Ohkawa, etal.[20].
-Statistical Analysis

The data for various biochemical parameters were expressed as mean ± SD and compared using one way analysis of variance (ANOVA) test. Values were considered statistically significant when p < 0.05, (Graph Pad Software, Inc. San Diego, CA).

3. RESULTS

In present study, serum glucose levels increased high significantly (P <0.01) in rabbits after alloxan administration (355.82±8.4 mg/dl) as compared with control group (130.14±4.3 mg/dl). Table(1) showed a high significant decrease in the level of glucose (P <0.01) in alloxan diabetic rabbits (170.9 ±1.87 mg/dl) when received glibenclamide daily as single dose 0.5 mg/kg body weight for 20 weeks as compared with group 1. The intake of 2 mg/kg of lycopene extract for 20 weeks produced high significant (P < 0.001) decrease (199.68±2.9 mg/dl) in comparison with group1, while after 20 weeks of treatment with 4 mg/kg of lycopene extract, the mean of serum glucose level decreased significantly (P < 0.01) (182.00±2.1 mg/dl) when compared with group1, as shown in. The results also showed no significant differences in glucose level in group 3 and 4 in comparison to that treated with glibenclamide 0.5 mg/kg.

The level of glycated hemoglobin increased significantly (P <0.05) in alloxan diabetic rabbits (7.02±0.49) in comparison to the control group (3.91±0.18). While Glycated hemoglobin levels increased in alloxan diabetic rabbits (4.3±0.2) %Hb received glibenclamide 0.5 mg/kg body weight for 20 weeks in comparison to group1. Treatment with 2 mg/kg of lycopene extract for 20 weeks caused a significant decrease(P <0.05) in the level of glycated hemoglobin in alloxan diabetic rabbits (5.87±0.32)%Hb when compared with control group, while after 20 weeks of treatment with 4 mg/kg of lycopene extract caused a significant decrease(P <0.05) in the level of glycated hemoglobin in alloxan diabetic rabbits (3.82±0.16)%Hb in comparison with control group. The results of this study showed significant decreases (P <0.05) in Glycated hemoglobin in alloxan diabetic rabbits treated with lycopene extract (2, and 4 mg/kg) in comparison to that treated with glibenclamide 0.5 mg/kg %Hb. The mean of serum catalase decreased significantly (P <0.05) in alloxan diabetic rabbits (7.5±1.1 U/ml) in comparison with control healthy group (12.75±1.12 U/ml). After treatment with glibenclamide 0.5 mg/kg body weight for 20 weeks a significant (P <0.05) increase in serum catalase was observed in alloxan diabetic rabbits (8.46±1.4 U/ml) when compared with group 1 as shown in table1. The results also showed that level of catalase...
elevated significantly (P <0.05) in alloxan diabetic rabbits when treated with 2 mg/kg of lycopene extract for 20 weeks, while the serum catalase increased significantly to (11.8±0.87 U/ml) when alloxan diabetic rabbits intake 4 mg/kg for 20 weeks of lycopene extract when compared with the serum catalase level in group 1, as shown in table (1). No significant differences (P > 0.05) found in serum catalase level in alloxan diabetic rabbits treated with lycopene extract (2mg/kg) in comparison to that treated with glibenclamide 0.5 mg/kg.

The level of erythrocyte malondialdehyde was significantly(P <0.001) increased in their alloxan diabetic rabbits(15.17±3.83 μmol/L) when compared to the group 1 (8.45±1.97μmol/L) as shown in table (1), while the level of malondialdehyde in alloxan diabetic rabbits decreased(13.61±3.16μmol/L) significantly (P <0.05) when treated with glibenclamide 0.5 mg/kg body weight for 20 weeks compared with group 1. while there is no significant differences (P >0.05) demonstrated in malondialdehyde level in alloxan diabetic rabbits (15±3.8 µmol/L) treated with 2mg/kg of lycopene extract for 20 weeks in comparison with group 1, also the results showed that there is no significant differences (P > 0.05) in serum malondialdehyde level in alloxan diabetic rabbits (14.41±3.61µmol/L) treated with 4 mg/kg of lycopene extract for 20 weeks in comparison with group 1, as shown in Table (1). Significant differences(P <0.05) observed in serum malondialdehyde level in alloxan diabetic rabbitstreated with lycopene extract (2mg/kg) when compared to that treated with glibenclamide 0.5 mg/kg, but there was no significant differences (P > 0.05) in the level of serum malondialdehyde in alloxan diabetic rabbits intake 4 mg/kg.

**Table (1):** Effect of Lycopene and of glibenclamide on fasting blood glucose, HbA1c, MDA and Catalase levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dl)</th>
<th>HbA1c (% Hb)</th>
<th>Catalase (U/ml)</th>
<th>MDA (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>355.82±8.4</td>
<td>7.02±0.49</td>
<td>7.5±1.1</td>
<td>15.17±3.83 ***</td>
</tr>
<tr>
<td>G2</td>
<td>170.9±1.87</td>
<td>4.3±0.2</td>
<td>8.46±1.4</td>
<td>13.61±3.16</td>
</tr>
<tr>
<td>G3</td>
<td>199.68±2.9</td>
<td>5.87±0.32</td>
<td>9.94±0.96</td>
<td>15±3.8</td>
</tr>
<tr>
<td>G4</td>
<td>182.00±2.1</td>
<td>3.82±0.16</td>
<td>11.8±0.87</td>
<td>14.41±3</td>
</tr>
<tr>
<td>G5</td>
<td>130.14±4.3</td>
<td>3.91±0.18</td>
<td>12.75±1.12</td>
<td>8.45±1.97</td>
</tr>
</tbody>
</table>

*(P < 0.05), **(P < 0.01), ****(P < 0.001)
4. DISCUSSION

Diabetes is a metabolic disorder characterized by chronic hyperglycaemia leading to various dysfunctions in the body. Free radical generation is currently suggested to play an important role in the causation and complications of the disease [21].

In present study, the mean serum glucose level increased in rabbits after alloxan administration as compared with control group (table 1). The immediate effect of alloxan is the elevation of the blood glucose [22, 23]. Alloxan is the most prominent diabetogenic chemicals in diabetes research, following its administration; alloxan is concentrated in the islets of Langerhans in pancreas and damages the β cells of the islets in pancreas, by the liberation of oxygen free radicals, and in the liver where it is reduced to dialuric acid with a reduction in antioxidant status [24, 25]. Dialuric acid is unstable in aqueous solutions and undergoes oxidation back to alloxan, accompanied by generation of O₂, H₂O₂ and OH radicals by Fenton type reaction. Hydroxy radicals generated causes single stranded breaks in the islets cell DNA [25-28].

A significant decrease in serum glucose level observed in alloxan diabetic rabbits after treatment with glibenclamid and lycopene extract for 20 weeks as compared before treatment (Table 1). The results of this study agree with results of other studies that found tomato products including powder, paste and tomato catchup sauce have significantly beneficial therapeutic effects in rats [29-31]. Ali and Agha [31] showed that lycopene extracted from tomatoes is able to reduce concentrations of glucose. In recent years, various plant extracts have been claimed to be useful for the treatment of diabetes mellitus. According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of beta cells or by protecting the pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action or its effect on beta cells to release insulin and activate the insulin receptors to absorb the blood sugar [32-35].

Glibenclamid reduces serum glucose level in alloxan diabetic rabbits by stimulating insulin release from beta-cells of the pancreas. Glibenclamid is one of the sulphonylureases that are useful in the treatment of diabetes mellitus, which is produced its effect via stimulating of endogenous insulin release from pancreas, enhancing peripheral tissue utilization of glucose, or by decreasing the absorption of glucose by intestine [22, 36, 37].

Lycopene, a red carotenoid pigment in tomatoes and tomato-based products, is an acyclic form of beta-carotene with a potent antioxidant effect. It has attracted substantial interest.
during recent times for its beneficial in reducing oxidative stressing coronary heart diseases and other chronic diseases by increasing serum total antioxidant level[14, 38].

There were a significant elevation in glycosylated hemoglobin, (P <0.05) in alloxan diabetic rabbits when compared with corresponding control group. During diabetic mellitus, the excessglucose present in blood reacts with hemoglobin to form glycosylated haemoglobin. It has been reported that various proteins, including hemoglobin, albumin, collagen, low density lipoprotein, or crystalline proteins undergo non-enzymatic glycation in diabetes. The rate of glycation is proportional to the concentration of blood glucose [39-41]. Glycosylated haemoglobin has been found to be increased over a long period time in diabetes[42].There is an evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition [43]. Therefore, the measurement of glycosylated hemoglobin is supposed to be a very sensitive index for glycemic control [44].

Administration of lycopene extract to alloxan diabetic rabbits caused a significant decrease(P <0.05) in the level of glycated hemoglobin when compared with alloxan diabetic rabbits as shown in figure 2. These results are in agreement with the results of Bose&Agrawal, [44] who improved that long-term administration of tomato that existed lycopene for type II diabetic patients reduced Glycosylated Hemoglobin [HbA1c]. The free radicals generated in this group, in addition to protein glycation as a result of hyperglycemia could inactivate Glycosylated Hemoglobin [42]. In this study serum catalase decreased significantly (P <0.05) in alloxan diabetic rabbits in comparison with control healthy group. These results are in agreement with the results of other studies had observed that superoxide dismutase, catalase and glutathione peroxidase decrease in liver, kidney and heart in tissues of patients with diabetes mellitus while the level of reactive oxygen species such as superoxide anion radicals increase[29, 32]. Where the effect of supplementation with tomato lycopene extract was observed a significant(P <0.05) increased in serum catalase in alloxan diabetic rabbits compared to the control group. The results of this study conform to results of Ibrahim et al.[30], and Ali et al.,[31] showed that lycopene extracted from tomatoes is able to reduce concentrations of glucose, hydrogen peroxide, serum lipids and increase insulin concentrations, catalase, superoxide dismutase and glutathione peroxidase in diabetic rats. Also Lycopene has a high antioxidative activity and exerts a protective effect in various diseases [45].
Lipid peroxidation indices measured in plasma-included malondialdehyde, lipid hydroperoxides, and lipoperoxides, which were significantly elevated in diabetic patients regardless of the presence of complications [46]. Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes such as malondialdehydethat will damage cell membranes[47]. In this study thereis a significant increase in the mean of serum Malondialdehyde level in alloxan diabetic rabbits when compared to the control group, and no significant differences demonstrated in Malondialdehyde level in alloxan diabetic rabbits treated with lycopene extract when compared to group 2. These results agreement with [49] that indicate the significantly higher levels of lipid peroxidation ratein diabetes when compared with control, and no significant improvement in the lipid peroxidation rate in diabetic patients of tomato supplementation (200g/day), and also several studies have reported have significant increase in lipid peroxides in diabetes mellitus [45, 47, 48]. Generally, the effect of the treatment with lycopene extract compared with the effect of glibenclamide, is known as standard drug for diabetes.

We concluded from this study that administration of tomato product produced significant decrease in serum glucose, Malondialdehyde and Glycosylated Hemoglobin [HbA1c] level, and significant increase in serum catalase level in alloxan diabetic rabbits.

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