The protective role of onion oil (Allium cepa L.) extract on some physiological parameters on Streptozotocin induced diabetes in male mice

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Abstract:

This study was carried out to investigate the most effective compound extracted from approved selected anti diabetic plants on blood glucose, Serum insulin, lipid profile level and protective effect against oxidative stress in Streptozotocin induced diabetic male mice. 30 albino male mice were divided into 3 groups 10 mice each one. Group 1 normal control, group2 diabetic control, Group3 received essential onion oil (50mg/kg B.w. orally),at the end of experiment Blood glucose, insulin levels, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol were estimated. Oxidative stress biomarkers represented in the Amount of thiobarbituric acid reactive substances (TBARS) and nitric oxide were Determined. Liver and kidney removed for histopathological examination. the Results is isolated compound(onion oil) improved diabetes status but the most potent was observed as anti diabetic & antioxidant effect.

These results suggest that administration of the onion oil used as antidiabetic agent And improved diabetic status.

dur القائً المستخلص زٌت البصل على بعض المعابٌ الفسلجٌة في ذكور الفئران المستحدث فيها السكري بمركب الستربتوزوتوكٌن

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خلاصة:

أُجريت هذه الدراسة لمعرفة فاعلية المركب المستخلص (زيت البصل) المعروف بتأثير مضاد للسكر على جملة من المعابير الفسلجية في الفئران مثل قٌاس مستوى السكر والأنسولين في المصل والتحايل الدهنية مثل نسبة الكولسترول ا كلي والدهون النافعة والضارة ودور المستخلص ضد الإجهاد التاكسدي الناتج من الإصابة بالسكر المستحدث من الستربتوزوتوكٌن في الفئران الذكور إذ تضمنت الطريقة استخدام ثلاثون فارا ذكرًا مختيرًا قسمت إلى ثلاث مجموعات السبعة ملغرام والبيطرة ثانية مجموعة السكري والمجموعة الثالثة المجموعة المعالمة يستخلص زيت البصل بجرعة 50 ملغرام/كم فصيًا. وفي نهاية التجربة حسب المعابير أعلاه إضافة إلى قٌاس الإجهاد التاكسدي والعامل المرتبط بكمية حامض الثاي باربيتالوك وأوكسيد النتريك، أيضاً تم إزالة الكبد والكليتين لغرض عمل المقاطع النسيجية.
سجلت النتائج وجود فرق معنوي بين المجاميع الثلاث عند مستوى معنوي 5% حدوث انخفاض في نسبة السكر والدهون الضارة والكولسترول في حال استخدام المستخلص نستنتج من ذلك بأن للمستخلص تأثير مضاد للسكري والإجهاد التاكسيدي

**Introduction:**

Regardless of the type of diabetes, patients are required to control their blood glucose level with medication and /or by adhering to an exercise program and a dietary plan. Oral antidiabetic agents exert their effects by various mechanisms

1. stimulating beta cells in the pancreas to produce more insulin (sulfonylurea and meglitinides),
2. increasing the sensitivity of muscles and other tissues to insulin (thiazolidinediones),
3. decreasing gluconeogenesis by the liver (biguanides), and delaying the absorption of carbohydrates from gastrointestinal tract (alphaglucosidase).

These treatments are associated with adverse effects, and some may produce toxic effects and unfortunately, none of the currently used antidiabetic agents provide all the required advantages necessary for successful management including adequate hypoglycemic activity, modification of insulin secretion, peripheral insulin resistance and sufficient safety. Until now the search for new antidiabetic agents represents a challenge to medical professions.

For many years, many herbs and plant products have been shown to have hypoglycemic action, one of them is essential oil of onion (Allium cepa L.) containing compounds such as daily disulfides and their oxidized thiols, these constituents may contribute to the protective effects of onion against oxidative stress in STZ-induced diabetic mice. Hence, the present study investigation was undertaken to assess the antihyperglycemic, antilipidemic and antioxidant role of the essential oil of onion in streptozotocin diabetic male mice

**Materials And Methods:**

Streptozotocin was purchased from sigma chemical company, St Louis, Missouri, USA. Chloroform, Methyl alcohol, ether were purchased. Glucose was estimated using kit (glucose PAP enzymatic oxidize method purchased from Stanbio Laboratory, Inc.). Serum insulin levels were determined by Biosource-INS-ELISA.

Serum cholesterol and Triglycerides HDL and LDL were estimated by quantitative enzymatic colorimetric method using kits purchased from Stanbio Laboratory, Inc., Texas, USA. Serum nitric oxide (NO) was evaluated by measuring levels of nitrite by Griess reaction. Lipid peroxidation as TBARS was estimated from serum level of malondialdehyde (MDA) which is allowed to react with thiobarbituric
acid (TBA) in acidic medium. The color produced was measured\(^{11}\).

**Animals:** The experiments were done using thirty male albino mice of strain, weighing 27.4-33.0 g. The animals were maintained in controlled temperature (20±1°C), humidity (65%) and a 12 h dark-light cycle, with balanced food and free access to water. The protocol for these experiments was approved by veterinary medicine- alqadisiya. They divided into three groups 10 mice each.

**Induction of diabetes:** Experimental diabetes was induced in overnight fasted mice by intraperitoneal injection of STZ (Sigma-Aldrich Corp, St. Louis, MO, USA), 60 mg kg\(^{-1}\) body weight, dissolved in 0.9% NaCl solution. After 5 days, mice with glycemia above 17 mmol L\(^{-1}\) (at fasting state) were included in the study. Control mice were injected with saline solution, blood glucose level was measured in male mice to check for diabetes.

**Treatment:** treatment was started on the sixth day after STZ injection and this was considered as the first day of treatment. The treatment was continued for 30 days. The mice have been divided into three groups comprising 10 animals in each group as follows Group I was injected with sterilized buffer alone as normal control group. Group 2 received saline, Group 3 received essential oil of onion (50 mg/kg b.w. orally). The dose chosen according to evaluation of the acute toxicity of all different

The medium 50 lethal doses (LD\(_50\) ) were determined\(^{12,13}\).

**Serum analysis:** After 30 days of treatment, food was withdrawn from the mice and they were fasted overnight but had free access to water. The experimental animals were anaesthetized with diethyl ether. Whole blood samples were collected from orbital venous plexus and emptied into plain tubes and allowed to clot. The clotted blood samples were there after centrifuged to recover serum from clotted cells. Serum was carefully separated and stored frozen until used for glucose, triglycerides, cholesterol, HDL, LDL, nitric oxide and TBARS in essential oil of red onion (0.05%) Na, K and Ca determinations. Serum glucose, triglycerides and cholesterol were estimated using an automatic analyzer (Reflotron® Plus System, Roche, Germany). Serum Na, K and Ca were measured using Automated Clinical Chemistry Analysis System, Dimension® type RXL Max (Dade Behring Delaware, DE 19714, USA)\(^{14}\).

**Statistical analysis:** The data were expressed as mean± Standard Deviation (SD). Statistical comparisons were performed by one way Analysis Of Variance (ANOVA) followed by Duncan’s Multiple Range Tests (DMRT). The results were considered statistically significant if the P-values were less than 0.05.

**The Histological and Histochemical Studies:**
After blood sampling for the
biochemical analysis, the animals were sacrificed, quickly dissected, and small slices of the liver and kidney were taken and fixed in 10% formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 6 μm in thickness were prepared and stained with Haematoxylin and Eosin to examine under microscopy. Periodic acid-Schiff method was applied for visualization of the polysaccharide material.

**Results:**

The characteristic abnormalities observed in the diabetic mice were shown in Table (1). In diabetic mice, the blood glucose significant increases in the levels of serum triglycerides, cholesterol and Na, The level of serum K was statistically decreased in diabetic mice (group 2) compared with other groups. The level of serum Ca was significantly increased in group 2 after 30 days. observed in diabetic mice when compared to control, while serum insulin is decreased significantly when compared to control animals. A reduction was observed in blood glucose and increase in serum plasma in diabetic mice treated with essential oil of onion, Table (1) The maximum increases of serum glucose after 30 (390.2%) days were observed in STZ-diabetic mice, while the treatment of diabetic mice with essential onion extract (group 3) reduced the elevations of percentage change after 30 (270.2%), The maximum increases of serum triglycerides (154.3%), cholesterol (140.20%), Na (16.2%) and Ca (9.8%) and the maximum decrease of K (11.9%) were noted in STZ-diabetic mice Table(3,5).

From the present results, it is obviously that the percentage changes of serum parameters in group 2 were increased with the increases of experimental duration so that dyslipidemia in mice with STZ induced diabetes when compared to control group. There is a significant increase in cholesterol, triglycerides and LDL and significant decrease in HDL cholesterol when compared to control group, Administration of essential oil of onion decreased significantly the serum cholesterol, triglyceride, and LDL significantly and increases significantly HDL, Mean while in Table (4), serum lipid peroxides levels measured as (TBARS) were significantly increased in diabetic group (0.506 ± 0.07) comparison to control group (0.296±0.05), while Essential oil of onion compound showed significant decrease in TBARS(0.302 ± 0.03), Serum nitric oxide was significantly increased in diabetic mice compared (45.00 ±7.07) to control group(9.00 ± 5.22) while it significantly decreased in diabetic mice treated with essential oil of onion (16.25 ± 4.7).
### Table 1: Change in fasting blood glucose and serum insulin levels in all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Serum insulin (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>96 ± 2.59</td>
<td>10.53±0.66</td>
</tr>
<tr>
<td>Diabetic</td>
<td>390.2 ± 8.33A</td>
<td>4.17 ± 0.8</td>
</tr>
<tr>
<td>Diabetic + essential oil of onion</td>
<td>270.2± 5.13B</td>
<td>6.61±0.3B</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E. (n=10)
A significantly different from control (P<0.05).
B significantly different from diabetic control (P<0.05).

### Table 2: Change in lipid profile in different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum total cholesterol (mg/dl)</th>
<th>Serum triglycerides (mg/dl)</th>
<th>Serum HDL (mg/dl)</th>
<th>Serum LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>98.5 ±2.1</td>
<td>97.5 ±2.5</td>
<td>42.1 ±0.87</td>
<td>35.5±0.9</td>
</tr>
<tr>
<td>Diabetic</td>
<td>140.20 ±3.2A</td>
<td>154.33±6.7A</td>
<td>3 2.7 ±1.38</td>
<td>54.0±2.7A</td>
</tr>
<tr>
<td>Diabetic + essential oil of onion</td>
<td>100.6±4.2B</td>
<td>110 ±5.4B</td>
<td>48.00±1.9B</td>
<td>39.5±1.7B</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E. (n=10)
A significantly different from controls (P<0.05)
B significantly different from diabetic control (P<0.05)

### Table 3: Change in TBARS and nitric oxide levels in different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum TBARS</th>
<th>Serum nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.296 ± 0.05</td>
<td>9.00 ± 5.22</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.506 ± 0.07A</td>
<td>45.00 ±7.07A</td>
</tr>
<tr>
<td>Diabetic + essential oil of onion</td>
<td>0.302 ±0.03B</td>
<td>16.25 ± 4.7B</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E. (n=10)
A significantly different from control (P<0.05).
B Significantly different from diabetic control (P<0.05)
Table 4: changes in K, Na, Ca levels in three different groups

<p>| | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>K</td>
<td>8.8 ±0.2</td>
<td>10.8±5.5</td>
<td>16.2±3.6</td>
</tr>
<tr>
<td>Na</td>
<td>4.4 ±2.5</td>
<td>16.2±4.3</td>
<td>11.9± 2.4</td>
</tr>
<tr>
<td>Ca</td>
<td>0.9± 0.1</td>
<td>5.3 ±3.1</td>
<td>9.8±4.3</td>
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</table>

Histopathological Results:

Liver: The liver of control mice appears to be divided into the classical hepatic lobules; each is formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The cell cords were separated by narrow blood sinusoids (Fig. 1-A).

The histopathological examination of diabetic mice showed periportal necrosis of the hepatocytes near the portal areas. The livers also, showed dilated and congested portal vessels as well as areas of inflammatory cell infiltration (Fig. 1-B). In diabetic mice treated with onion oil, the liver architecture appears more or less like control with the exception of some hemorrhagic areas in the sinusoids (C).

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**Kidney:**

Examination of the kidney of the control mice revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space surrounding by proximal and distal were normal (Fig. 2-A). Light microscopy of the kidney sections from diabetic mice showed an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles (Fig. 2-B). In diabetic mice treated with onion oil, the kidney architecture appears more or less like control with the exception of some inflammatory infiltration that appeared in the interstitum (Fig. 2-C).
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Microscopy of the kidney sections from diabetic mice showed an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles (Fig.-B)

In diabetic mice treated with onion oil, the kidney architecture appears more or less like control with the exception of some inflammatory infiltration that appeared in the interstitum (Fig. 2- C).
Histochemical Results:

Liver: Examination of liver sections of control mice stained with periodic acid Schiff ’s (PAS) technique showed the abundance of glycogen in the form of purple granules and particles at one side of the cytoplasm leaving the other one almost devoid of such material in the hepatocytes. The nuclei of the hepatocytes gave negative PAS reaction indicating the absence of glycogen. The hepatocytes at the peripheral regions appeared markedly rich with glycogen particles than pericentral ones (Fig. 3-A).

The histochemical examination of diabetic mice showed pericentral depletion of the PAS +ve materials (Fig. 3-B).

In diabetic mice treated with onion oil show the distribution of polysaccharides in the liver that appear more or less like control (Fig. 3-C).
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**Kidney:** Kidneys of control mice showed the presence of polysaccharides in the form of PAS positive materials in the parietal and visceral walls of the Bowman’s capsule, capillaries of the glomeruli, the basement membrane of the proximal and distal convoluted tubules and the brush border of the proximal convoluted tubules (Fig. 4-A). Light microscopy of the kidney sections from diabetic mice showed an increase in the PAS +ve material in the mesangial cell and matrix of the glomeruli. The basement membranes of the proximal and distal convoluted tubules appear thicker as compared with the control one (Fig. 4-B). The histochemical examination of the kidney of diabetic mice treated with onion oil showed the reduction in the PAS +ve materials as compared with the normal one (Figs. 4-C)
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The histochemical examination of the kidney of diabetic mice treated with onion oil showed the reduction in the PAS +ve materials as compared with the normal one (Figs. - C ).

**Discussion:**

In the present study, diabetic mice induced by streptozotocin showed the expected elevation in plasma glucose, triglycerides, cholesterol, and LDL and decreasing HDL and insulin levels which indicating that their pancreatic B cells were irreversibly damaged and cause various metabolic disorders.[21]

These results are in agreement with. However, treatments of the diabetic mice with essential oil of onion (50 mg/ kg/day) showed a significant decrease in blood glucose and increases in insulin level and improved lipid profile as cholesterol, TG, LDL and HDL. These results are in agreement with several studies which reported that, onion intake was found to improve the diabetic status, including protection of DNA against oxidative damage, hypoglycemic and hypocholesterolemic effects.[17].

STZ diabetic mice exerted a significant elevation of lipid peroxides expressed as nmol TBARs/ml serum[18]. In this study
the production of lipid peroxides was significantly decreased by administration of onion oil group. This result may be due to the active compounds of onion oil such as daily disulphide’s and their oxidized thiols which has been reported to have an ant oxidative effect\(^{19,20}\). In agreement with our results\(^{34}\) indicated that these compounds may contribute to the protective effects of onion against oxidative stress in STZ induced diabetic mice. Nitric oxide synthesis is presenting in pancreatic B- cells and may be involved in the release of insulin under normal condition\(^{35}\). However \(^{21}\) suggested that induction of nitric oxide formation may play a role in the destruction of B-cells during the development of type I diabetes. In the present study, plasma nitrite as end product of nitric oxide activity was elevated in the untreated diabetic mice, similar results were obtained by \(^{22}\). The oral administration of essential oil of onion decreased significantly the nitric oxide level which may be due to their antioxidant ability and free radicals scavenger against oxidative damage\(^{22,23}\).

upon supplementation with certain dietary antioxidants such as vitamin E, C, and a-lipoic acid\(^{46}\). The use of other non-nutrient antioxidants such as flavonoids and polyphenols has been reported with the same advantage\(^{24,25}\). Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes. It is well known that alterations in mineral metabolism can induce disturbance in glucose metabolism \(^{26}\) and glucose intolerance which also can interfere with mineral metabolism \(^{5,6}\). There is evidence that STZ-induced diabetes in experimental animals alters trace mineral balance as a result of disturbances in pancreatic function. In the present study, the levels of serum Na and Ca were statistically elevated in diabetic mice, while the level of serum K was significantly declined. In contrast, \(^{27}\) showed that the levels of serum Na and Ca were significantly decreased in STZ-induced diabetic rats, whereas serum K levels were increased non-significantly as compared to control rats \(^{28}\). reported that the serum concentrations of Na and K of STZ-diabetic rats were decreased compared to the non-diabetic control. \(^{24}\) demonstrated that the level of serum Na was significantly decreased in alloxan-induced diabetic rats, while the level of K was statistically unchanged compared with control rats. \(^{29}\) showed that the level of serum Na was significantly unchanged in alloxan-induced diabetic rats, while the level of K was statistically increased compared with control rats. \(^{30,31}\) showed that in STZ-diabetic rats, there was a significant increase in the serum Na, K and Ca. \(^{32,33}\) demonstrated that the levels of
serum Ca were increased in STZ-diabetic rats. \(^{(34,35)}\) reported that in the STZ-diabetic rats, the blood Na and K levels were statistically increased.

Disorders of sodium and water balance are very common. Sodium is the principal solute in the extracellular compartment and hence the plasma osmolality largely depends on the serum sodium concentration. A decrease or increase in the serum sodium level will have an effect on the plasma osmolality and this can have deleterious effects on the whole body-in particular, the central nervous system. Severe hypo- and hypernatraemia are associated with significantly high mortality and morbidity. Moreover, inappropriate treatment may result in treatment related complications such as osmotic demyelination syndrome. Hypernatraemia is defined as serum or plasma sodium higher concentration. Hypernatraemia represents a deficit of water in relation to the body’s sodium stores. It can result from net water loss or hypertonic sodium gain. Sustained hypernatraemia can occur only when thirst or access to water is impaired \(^{(15,16)}\). K homeostasis is essential for normal myocardial function. K plays a central role in the maintenance of cellular polarization and is critical for the transmission of electrical impulses through the myocardium. Alterations in the normal balance between intracellular and extracellular K concentrations can lead to serious arrhythmias \(^{(36,5)}\). The adverse association between hypokalemia and arrhythmias in animal models appears to be more significant in the presence of acute myocardial ischemia \(^{(37)}\). The major causes of low serum K are\(^{(38)}\) decreased K intake due to intravenous feedings which do not contain K, \(^{(4,6)}\) increased loss of K in the urine due to accelerated tissue breakdown or renal lesions, \(^{(7)}\) loss from the gastrointestinal tract due to diarrhea or fistulae and \(^{(4,8)}\) shift between serum and cells due to metabolic causes, drugs or changes in pH. Serum Ca is usually measured to screen for monitor diseases of the bone or calcium regulation disorders due to hormonal disturbance (parathormone and calcitonin), vitamin D level and gastrointestinal absorption level of Ca and diseases of kidney.\(^{(38)}\) reported that the increase in serum Ca concentration in STZ-diabetic rats may result from the release of Ca from bone tissues; the femoral Ca content was found to decrease membrane lipid peroxidation and protein glycation \(^{(9,10)}\). This could be the reason for the altered flux in electrolytes balance that resulted in the elevated extracellular concentration of Na and Ca in STZ-induced diabetic mice. Although the data obtained in this study do not allow any definite conclusions to be drawn on the mechanism of action of extract on the levels of studied serum electrolytes in the experimental diabetic mice, it has been suggested
that the active natural compounds of Diabetes produces substantial changes in the intracellular metabolism in many tissue including liver and kidney \(^{(18,19)}\). The cell toxicity caused by Photomicrographs of liver show (A) Liver thrlsat exhibit the normal structure in control mice ( B) Diabetic mice show a portal tract with dilated and congested vein. Notice, the periportal necrosis of the hepatocytes that surrounded the portal area that associated with inflammatory infiltration.( C) diabetic mice treated with onion oil show the architecture of the hepatic lobule that appears more or less like control except of some hemorrhagic area in sinusoid, that appears more or less like control except of the dilation of blood sinusoid. (H & E X 150). 2005Photomicrographs of liver show A) Liver thrlsat exhibit the normal structure in control mice.B) Diabetic mice show a portal tract with dilated and congested vein. Notice, the periportal necrosis of the hepatocytes that surrounded the portal area that associated with inflammatory infiltration. C): diabetic mice treated with onion oil show the architecture of the hepatic lobule that appears more or less like control except of some hemorrhagic area in sinusoid.

**Fig. 2:**
Photomicrographs of kidney show, (A) kidney of the control mice revealed normal structure of the glomeruli and proximal and distal convoluted tubules. B): kidney of diabetic mice shows an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles. (C) kidney of diabetic mice treated with onion oil, the architecture appears more or less like control with the exception of some inflammatory infiltration in the interstitium.

**Fig. 3:**
Photographs of sections of the liver show the polysaccharides( A) control mice showing the normal distribution, the glycogen particles accumulated at one side of the cytoplasm of hepatocytes leaving the other side almost devoid of such material. (B) liver of diabetic mice showed per central depletion of the PAS +ve materials. (C) liver of diabetic mice treated with onion oil show the distribution of polysaccharides in the liver that appear more or less like control

**Fig. 4:**
Photographs of a section of the liver show the polysaccharides, (A) Kidney of control mice show PAS positive materials in the parietal and visceral walls of the Bowman’s capsule, capillaries of the glomeruli, the basement membrane of the proximal and distal convoluted tubules and the brush border of the proximal convoluted tubules. (B) Kidney of diabetic mice shows an increase in the PAS +ve material in the mesangial cell and matrix of the glomeruli. The basement membranes of the proximal and distal convoluted tubules appear thick. (C ) kidney of diabetic mice treated with
onion oil show a reduction in the PAS +ve materials. elevation of local free radicals in b-cell after increasing free radicals in other body organs [20].

References:


13. Moncada, S., R.M. Palmer, E.A.


