Antioxidative Effects of Allium Cepa Essential Oil in Streptozotocin Induced Diabetic Rats

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Abstract

Aim. This study was undertaken to investigate the antioxidative effect of allium cepa essential oil in streptozotocin induced diabetic albino rats through assessment of the lipid peroxidation, serum lipid profile, blood glucose and serum insulin, in addition to histological and histochemical study of liver and kidney.

Material and Methods. Diabetes mellitus was induced in 20 out of 30 adult male albino rats, using subcutaneous injection of 60 mg/kg b.w. streptozotocin. Diabetic rats were divided into two groups, one was taken onion essential oil orally (100 mg/kg b.w.) for 21 days (treated group). The other group received no onion oil. The remaining non-diabetic rats (negative control group) received neither streptozotocin nor the onion essential oil.

Results. It was found that treatment with onion essential oil, resulted in a significant decrease in serum lipids, lipid peroxide formation, blood glucose and increase in serum insulin. All the histological differences caused by diabetes were converted to normal in the treated group.

Conclusion. We suggest here that the mode of action of allium cepa (onion) as antidiabetic may be caused by the antioxidant properties of its essential oil components thereby preventing hyperglycemia and reducing lipid profile together with converting liver and kidney pathology caused by diabetes to normal pattern.

Introduction

Many traditional treatments have been recommended in the complementary medicine for treatment of Diabetes Mellitus; however, the mechanism of most of the herbs used has not been defined [1]. Allium is a genus of some 500 species belonging to the family Liliaceae [2]. Allium cepa or common onion, is a member of this family, it has a long history of medicinal use [3]. Such plants have been used for many centuries for their pungency and flavouring value, for their medicinal properties, and in some parts of the world, their use also has religious connotations [4]. Its usefulness has been discovered independently by many cultures on several continents [5]. Onions are mentioned in ancient Egyptian writings. Egyptians numbered over 8000 onion-alleviated ailments. They were fed together with garlic to workers building pyramids and were found in the tomb of King Tut [6].

Onions are rich in two chemical groups that have perceived benefits to human health. These are the flavonoids and the alk(en)y cysteine sulfoxides (ACSOs), both being strong antioxidants. Two flavonoid
subgroups are found in onion, the anthocyanins, which impart a red/purple colour to some varieties and flavanols such as quercetin and its derivatives responsible for the yellow and brown skins of many other varieties [7]. The constituents of onion contain only traces (0.01%) of essential oil, which mostly consists of sulfur compounds mainly in the form of cysteine derivatives, viz. S-alkyl cysteine sulfoxides which decompose into a variety of thiosulfimates and polysulfides by the action of an enzyme allinase on extraction. The decomposed products are volatile and possess multiple effects as antidiabetic, antibiotic, hypcholesterolaemic, fibrinolytic, anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic and antibiotic effects [8]. In addition to free sulfoxides in alliums, there are nonvolatile sulfur-containing peptides and proteins which possess various activities and thus make allium cepa an important source of therapeutic agents [9].

Diabetes is the most common metabolic disorder worldwide and is a major public health problem. Its frequency increases every day in all countries. However, in developing African countries, few people have access to drugs. In addition, in Africa, traditional beliefs induce people to use medicinal plants whenever they have health problems. Thus, many people in these developing countries use plants for the treatment of diabetes [10]. Yet, few studies are focused on proving their efficacy especially for their products.

Different researches on onion were done using dietary onion. Park et al. [11] investigated the effects of dietary onion flesh or onion peel on lipid peroxides and DNA damage in aged rats. They found that Onion flesh or onion peel enhanced antioxidant status in aged rats and may be beneficial for the elderly as a means of lowering lipid peroxide levels. Some others use onion juice; El-Demerdash et al. [12] concluded that onion and garlic juices exerted antioxidant and antihyperglycemic effects and consequently may alleviate liver and renal damage caused by alloxan-induced diabetes. But few researches investigated the effect of onion essential oil in diabetic rats. So, the aim of this study is to investigate the antioxidative effect of allium cepa essential oil in streptozotocin induced diabetic albino rats.

Materials and Methods

Materials

- Essential oil of red onion (0.05%) prepared and isolated according to Harborne [13].
- Streptozotocin (STZ) was purchased from sigma chemical company, St Louis, Missouri. USA.
- Chloroform, Methyl alcohol, ether were purchased from BHD, England.

Animals tested

Thirty male albino rats weighing 150-200g were supplied by the Animal House of National Research Center, Cairo-Egypt. Rats were caged under controlled temperature 20-24°C and 12 h light/dark cycle. They were fed with standard laboratory chow and water ad libitum.

Induction of Diabetes

Rats were kept on fasting prior to streptozotocin injection. On the day of administration, STZ was freshly dissolved in 50 Mm sodium citrate (pH 4.5) solution containing 150 mM NaCl and subcutaneous injection was given at the dosage of 60 mg/kg b.w. Blood glucose concentration was checked by the glucose oxidase method [14] after 3 days of STZ injection. The animals with glucose concentration exceeding 200 mg /dl were considered diabetic.

Rats were divided into 3 groups 10 rats in each group: group I: normal control rats; group II: diabetic control rats; group III: diabetic rats received onion essential oil (100 mg/kg b.w. orally).

The dose was chosen according to its LD50 (the medium 50 lethal doses after acute toxicity).

Samples Collection

After 21 days from the beginning of the experiment, rats were fasted for 12 hours then blood samples were collected, Blood was collected retro-orbitally from the inner canthus of the eye under ether anaesthesia using capillary tubes containing sodium fluoride [15], serum and plasma were separated and centrifuged at 3000 rpm for 5 minutes. The serum and plasma were separated for measurement of glucose, insulin, cholesterol, triglycerides, HDL, LDL, nitric oxide and TBARS in different studied groups.

Biochemical Measurements

- Glucose was estimated using kit (glucose PAP enzymatic oxidase method purchased from Stanbio Laboratory, Inc. [14].
- Serum insulin levels were determined by...
Biosource- INS- ELISA according to Temple et al., 1992 [16].

- Serum cholesterol and Triglycerides HDL and LDL were estimated by quantitative enzymatic colorimetric method using kits purchased from Stanbio Laboratory, Inc., Texas, USA according to Allian et al. [17] and Friedewald et al. [18].

- Serum thiobarbituric acid reactive substances was measured indirectly by measuring serum Malondialdehyde (MDA), an end product of unsaturated fatty acid peroxidation, which can react with thiobarbituric acid (TBA) to form coloured complex thiobarbituric acid reactive substances (TBARS). Lipid peroxidation (LPO) was measured by the method of Yagi et al. [19] and expressed as μmol of MDA conjugate formed.

- Serum nitric oxide level was assayed by the method of Griess reaction according to Corats and Wakeid [20].

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 [21]. Periodic acid-Schiff method was applied for visualization of the polysaccharide material [22].

The protocol of the study was reviewed and approved by Ethical Committee of National Research Center.

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. Statistics were done using SPSS for windows, version 10.

Results

Chemical results

The characteristic abnormalities observed in the diabetic rats were shown in Table 1. In diabetic rats, the blood glucose was increased significantly while serum insulin is decreased significantly when compared to control animals. A reduction was observed in blood glucose and increase in serum insulin in diabetic rats treated with essential oil of onion.

Table 1: Effect of onion essential oil on fasting blood glucose, serum insulin levels in different groups studied of experimental animals 21 days after induction of diabetes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Serum insulin (μU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>76 ± 2.69</td>
<td>10.53 ± 0.68</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>280 ± 8.33*</td>
<td>4.17 ± 0.17*</td>
</tr>
<tr>
<td>Diabetic treated with onion essential oil</td>
<td>124 ± 5.13*</td>
<td>6.61 ± 0.33*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E. (n=10); # Significantly different from normal control (P<0.05); * Significantly different from diabetic control (P<0.05).

Table 2 showed dyslipidemia in rats 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.

Meanwhile in Table 3, serum lipid peroxides levels measured as (TBARS) were significantly increased in diabetic group in comparison to control group. Essential oil of onion is showed to significantly decrease in TBARS. Serum nitric oxide was significantly increased.

Table 3: Effect of onion essential oil on TBARS and Nitric oxide levels in different groups studied of experimental animals 21 days after induction of diabetes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (μmol/L)</th>
<th>Nitric oxide (μmol/L)</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 control</td>
<td>0.506 ± 0.07*</td>
<td>45.00 ± 7.67*</td>
</tr>
<tr>
<td>Diabetic treated with onion essential oil</td>
<td>0.302 ± 0.03*</td>
<td>16.25 ± 4.7*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E. (n=10); #Significantly different from normal control (P<0.05); * Significantly different from diabetic control (P<0.05).
in diabetic rats compared to control group, while it significantly decreased in diabetic rats treated with essential oil of onion.

Light microscopy of the kidney sections from diabetic rats showed an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles (Fig. 2-B).

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

**Histopathological Results**

**Liver:** The liver of control rats 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 rats showed periportal necrosis of the hepatocytes near the portal areas. The liver also, showed dilated and congested portal vessels as well as areas of inflammatory cell infiltration (Fig. 1-B).

In diabetic rats treated with onion oil, the liver architecture appears more or less like control with the exception of some hemorrhagic areas in the blood sinusoid (Fig. 1-C).

**Kidney:** Examination of the kidney of the control rats revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space surrounding proximal and distal tubules (Fig. 2-A).

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

In the liver of diabetic rats treated with onion oil, the polysaccharides appeared more or less like control (Fig. 3-C).

**Kidney:** Kidneys of control rats 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 rats showed an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles (Fig. 2-B).

In diabetic rats treated with onion oil, the kidney architecture appears more or less like control with the exception of some inflammatory infiltration that appeared in the interstitium (Fig. 2-C).
diabetic rats 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 rats treated with onion oil indicated that the polysaccharides of kidneys appeared more or less as control (Fig. 4-C).

Discussion

During the last decade, it has become increasingly evident that many chronic diseases are accompanied by increased levels of oxidative stress exacerbated by decreased antioxidant levels [23-26].

These observations have precipitated much interest in study of the correlations between oxidative stress, antioxidant potential and development of chronic diseases in both humans and animal models. Of particular interest are the correlations between oxidative stress and development of diabetes [27-30]. This could be through the connection between oxidative stress and endothelial dysfunction leading to the development of chronic conditions such as diabetes [31]. Moreover, endothelial dysfunction is correlated with the development of complications related to diabetes mellitus [32].

Numerous herbal medicinals have been shown to affect blood glucose levels for diabetic men or diabetic induced animals including chromium [33], fenugreek [34] and garlic [35].

Studies have found that Allium cepa (onions) has blood sugar lowering effects [36, 37]. Onions are the second most important horticultural crop after tomatoes. They are used as an ingredient in many dishes and are accepted by almost all traditions and cultures. A high daily intake of onion can lead to the onion constituents and their metabolites reaching considerable concentrations in human organs. Onion oil per se is widely used as a flavour in food industry and as a raw material for many medicines and functional foods [38].

Information about the bioavailability of active ingredients in onion oil is necessary for extrapolating in vitro biological action to in vivo effects in the body [39]. However, most available reports on the bioavailability of onion constituents focused on flavonoids, which are water soluble and not present in onion oil [40]. Few studies investigated the effect of oil volatile constituents on diabetes whether through animal or human studies. This encouraged us to do this study.

In the present study, we have found that consumption of onion oil to diabetic induced rats resulted in a significant reduction of blood glucose and increase in serum insulin. This was in agreement with the human study of Augusti and Benaim [41]; Jain et al. [42] and Mathew [43]. The later found that when diabetic patients were given single oral dose of 50 g of onion juice, it significantly controlled post-prandial glucose levels.

Few studies have examined onion’s oil effects on insulin and glucose handling. Volatile oils in raw onion cloves have been shown to lower fasting glucose concentration in both diabetic animals and human subjects [44]. Some attention has been given to APDS and S-allyl-cysteine sulfoxide, a sulfur containing amino acid [37, 45]. Experiments in animal models with alloxan-induced diabetes have shown moderate reductions in blood glucose with no effect is seen in pancreatectomized animals [12].

The explanation for the hypoglycaemic action of onion oil is through the active components of onion oil which are believed to be mainly, sulfur-containing compounds – allyl propyl disulfide (APDS). Researchers have postulated that these active ingredients lower glucose levels by competing with insulin (which is also a disulfide) for insulin-inactivating sites in the liver [8] resulting in an increase of free insulin. Reported mechanisms of allium species also include increased glutathione peroxidase activity [46], and improved liver glyco-gen storage [47].

Increased synthesis of glutathione (GSH), an endogenous tripeptide thiol that directly protects cells from damage by free radicals was suggested to have...
chemopreventive effects [48]. This explained that onion, was found to reduce the incidence of cancers in several tissues in epidemiologic studies [49-51] and in experimental studies investigating the anticarcinogenic effect of the onion in multiple organs of mice, rats, and hamsters [52, 53].

Our results show that treatment of STZ-induced diabetic rats with onion oil has antioxidative effect on the TBRAS, nitric oxide and hypolipidemic activity.

Various ether soluble fractions as well as insoluble fractions of dried onion powder show antioxidant and hypolipidemic activity in diabetic rabbits. Administration of a sulfur containing amino acid from Allium cepa, S-methyl cysteine sulphoxide (SMCS) (200 mg/kg for 45 days) to alloxan induced diabetic rats significantly controlled blood glucose as well as lipids in serum and tissues and normalized the activities of liver hexokinase, glucose 6-phosphatase and HMG CoA reductase [54]. In another study diabetic rats were given yellow onion and compared with quercetin, the results suggested that onion intake suppresses diabetes-induced oxidative stress more effectively than the intake of the same amount of quercetin aglycone alone [55].

Onion contains sulfur-containing compounds such as dialkyl disulfides and their oxidized thiols, which can trap electrons from other systems [56]. Onion oil containing these compounds has been reported to have an antioxidative effect against the oxidative damage caused by nicotine in experimental animals [57]. Thus, these constituents also may contribute to the protective effects of onion against oxidative stress in STZ-induced diabetic rats.

From this study, the following conclusions can be stated. Treatment of diabetic rats with essential oil of onion, resulted in a significant decrease in serum lipids and lipid peroxide formation in addition to reduction of blood glucose and increase in serum insulin. All the pathological differences observed in liver and kidney tissues of the control positive group in comparison with the control negative group, were converted to normal in the treated group with onion oil.

We suggest here that the mode of action of allium cepa (onion) as antidiabetic may be caused by the antioxidant properties of its essential oil components through suppressing the oxidative stress thereby preventing hyperglycemia during diabetes and reducing lipid profile to almost normal together with converting liver and kidney pathology caused by diabetes to normal pattern.

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References


