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Hypoglycemic and hypolipidemic effects of Caralluma tuberculata and its safety on liver and kidneys of diabetic rats

Diyabetik sıçanların karaciğer ve böbrekleri üzerinde Caralluma tuberculata’nın hipoglisemik ve hipolipidemik etkisi ve güvenliği

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Abstract: Objective: Caralluma tuberculata is a succulent plant that grows in some regions of Baluchestan province in Iran, and is widely used by natives as antidiabetic agent. This study evaluates the antidiabetic effects of aerial part suspension of Caralluma tuberculata (SCT) at two doses of 100 and 200 mg/kg and its safety on liver and kidneys of Streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was rendered via single dose of STZ (60 mg/kg, injected intraperitoneally). Forty eight rats were classified into 6 groups as follow; (I): Normal control, (II): Normal + SCT (200 mg/kg), (III): STZ Diabetic, (IV): STZ + vehicle, (V): STZ + SCT (100 mg/kg), (VI) STZ + SCT (200 mg/kg). The effects of 45 days of treatment with the SCT on oral glucose tolerance test (OGTT), lipid profile, hematological and biochemical parameters evaluated.

Results: SCT treated groups exhibited a significant (p<0.05) improvement in abnormalities of OGTT, biochemical and hematological parameters compared with the diabetic control group. Furthermore, SCT at both doses, returned significantly (p<0.01) diabetes-induced changes in lipid profile except HDL-C levels that only, were significantly (p<0.05) increased at dose of 200 mg/kg. There was no significant difference in hematological, liver and kidney parameters between normal control and normal animals receiving SCT.

Conclusion: The present results revealed that Caralluma tuberculata could be beneficial for amending hyperglycemia, hyperlipidemia, and hematological changes induced by diabetes. It may also protect the liver and kidneys against complications caused by diabetes without any toxic effects.

Keywords: Caralluma tuberculata, diabetes, hyperglycemia, oral glucose tolerance test, hyperlipidemia

Özet: Amaç: Caralluma tuberculata İran’ın Beluçistan bölgesinde yetişen, lezzetli bir bitkidir ve yaygın olarak yerliler tarafından anti-diabetik olarak kullanılmaktadır. Bu çalışmada, Caralluma tuberculata (SCT)’nin antenlerinden elde edilen suspansiyonun 100 ve 200 mg/kg lik dozlarla Streptozotocin (STZ) ile indüklenmiş diyabetik sıçanlarda kullanımı ve karaciğer ve böbrekler üzerindeki güvenliği incelenmiştir.

Metod: Sıçanlarda periton içine 60mg/kg STZ verilerek diyabet oluşturulmuştur. Kirk sekiz sıçan, (I): Normal control, (II): Normal + SCT (200 mg/kg), (III): STZ Diabetic, (IV): STZ + vehicle, (V): STZ + SCT (100 mg/kg), (VI) STZ + SCT (200 mg/kg) olmak üzere altı gruba ayrılmıştır. 45 günlük SCT tedavisi sürecinde glukoz toleransı (OGTT) lipid profileri, kan ve biyokşmyasal parametreleri değerlendirilmiştir.

Bulgular: SCT ile tedavi edilen grupların, diyabetik kontrol grubuna göre, biyokşmyasal ve kan parametreleri ile OGGT anomalilerinde belirgin bir (p<0.05) sağaltım

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Jafar Poodineh and Alireza Nakhaee: Hypoglycemic and hypolipidemic effects of Caralluma tuberculata

Introduction

Diabetes mellitus (DM) is an endocrine, metabolic disorder characterized by hyperglycemia and distorted lipids, carbohydrates and proteins metabolism [1]. According to World Health Organization (WHO), there are 346 million patients with DM worldwide, and most of the deaths associated to this disease occur in nations with little and mediocre income [2]. International Diabetes Federation reported that the incidence of DM among Iranian population in 2011 was 9.3% and it is predicted to reach 13.1% in 2030. Despite these high incidence rates, there is not a definite and real treatment for diabetes at present [3,4]. Currently available pharmatherapies for the treatment of DM include oral hypoglycemic agents and insulin. However, these drugs do not bring back normal glucose homeostasis, and have some side effects. In comparison with chemical agents, herbal drugs are safer, cheaper and more effective, thus conventional anti diabetic plants can be explored [5].

Genus Caralluma is a member of Asclepiadaceae family, which is also recognized as the milkweed family because many of its members have milky latex [6]. Several species of this genus are suitable for human consumption and used in numerous countries as medicinal plants [7]. Species of Caralluma tuberculata (C. tuberculata) is a succulent plant which lacks leaves, and grows in some regions of Baluchestan province in Iran. In these areas, the plant is known as “Marmootk” and the aerial part powder suspension of Caralluma tuberculata (SCT) is widely used by the natives to treat diabetes. Despite the high prevalence of diabetes in the country and the extensive use of C. tuberculata by natives, to date, no studies have been conducted to investigate the possible antidiabetic effect and safety of C. tuberculata in this area. Therefore, the present study was planned to assess the antidiabetic effects of Iranian species of Caralluma tuberculata on Streptozotocin (STZ)-induced diabetic rats.

Material and Methods

Plant material

The aerial part of C. tuberculata was collected in April 2012 from Irandegan District that is a district in Khash County, Sistan and Baluchestan Province, Iran. The plant was identified by Dr. Valizadeh, at the Biology department of Sistan and Baluchestan University, Zahedan, Iran, and voucher specimen was deposited in the Herbarium of this department. The collected plant materials were washed carefully with water and shade dried for 21 days. The dried material pulverized using an electrical grinder. The powder materials were kept at refrigerator (2–8°C) until future use.

Animals and the experimental protocol

The 48 male Wistar rats (230±20 g) were kept under standard environmental conditions (12 h light and 12 h dark cycles at 25°C±2 and 60–80% relative humidity) and nourished with a standard diet and water ad libitum. They were randomly divided into six groups with eight rats in each group, and treated as follow; Group I, Normal healthy control rats; Group II, Normal rats administered with the 200 mg/Kg/day of SCT; Group III, STZ-induced diabetic control rats; Group IV, Diabetic rats administered with the distilled water; Group V, Diabetic rats administered with the 100 mg/Kg/day of SCT and Group VI, Diabetic rats administered with the 200 mg/Kg/day of SCT. The administrations of SCT in groups of II, IV, V and VI were performed in a final volume of 0.5 mL by intra-gastric gavage. The experiment was continued for 45 days. All the studies were directed in accordance with “Guide for the Care and Use of Laboratory Animals”.

Induction of diabetes

After accommodation for one week, diabetes was induced in the overnight fasted animals with a single intraperitoneal injection of streptozotocin (Sigma, S0130) prepared
in fresh 100 mM citrate buffer (pH=4.5) at dose of 60 mg/kg of body weight [8]. The glucose level was estimated 3 days after STZ injection in tail vein using an Accu-Check Active glucometer (Roche Company, Germany). Rats with fasting blood glucose higher than 200 mg/dl were considered as diabetic and used in the experimental design.

**Oral glucose tolerance test (OGTT)**

OGTT was carried out two days before the end of the experiment and after overnight fasting [9]. To perform OGTT, a fasting blood samples (FBS) were taken and blood glucose determined. Thereafter, treated rats were administered with a single oral dose of the allocated management (200 mg/kg of SCT for groups of II and VI, distilled water for group of IV, 100 mg/kg of SCT for group of V). Thirty minutes later, all the animals were fed with glucose solution (2 g/Kg) via intragastric tube and blood glucose was estimated at 30, 60, 90, and 120 minutes following the administration. During OGTT, Accu-Check Active glucometer was used to measure glucose levels in blood sample taken from tail vein.

**Blood sampling**

On the last day of the study, after an overnight fasting, two blood samples were collected from the heart of the animals into EDTA - tubes for hematological experiments and fresh centrifuge tubes to separate the serum for biochemical assays. Sera were stored at -20°C until analyzed.

**Measurement of lipid parameters**

The serum lipid parameters include: Total cholesterol (TC), Triglycerides (TG) and High Density Lipoprotein cholesterol (HDL-C) which were measured using commercial available kits (Pars Azmoon Company, Iran) in an auto-analyzer (Biotecnica BT-3000, Italy). Low Density Lipoprotein Cholesterol (LDL-C) was calculated by using the Friedewald equation.

**Measurement of liver and kidney function parameters**

The levels of the serum creatinine, urea, uric acid, total bilirubin, as well as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities were determined by auto-analyzer (Biotecnica BT-3000, Italy), using relevant kits (Pars Azmoon Company, Iran).

**Results**

In analyzing all parameters no significant difference were observed between normal rats and normal rats administrated with SCT. Also, the difference between diabetic control rats and diabetic rats administrated with distilled water was not significant.

**Effects of SCT on OGTT**

Diabetic control rats demonstrated a highly significant elevation (p<0.05) at the FBS and blood glucose concentration at 30, 60, 90 and 120 minutes after glucose loading compared with normal healthy rats. Compared with diabetic control group, in SCT treated groups (V and VI), the levels of FBS and blood glucose at 30, 60, 90 and 120 min after glucose loading, significantly decreased (p<0.05); however, it did not place inside the normal range (Table 1).

**Effect of SCT on lipid parameters**

Changes in the lipid parameters are presented in Table 2. The serum levels of TC, TG and LDL-C in STZ-induced dia
Effect of SCT on liver function parameters

Table 3 shows the changes in the liver function parameters of rats in different experimental groups. STZ-Diabetic rats demonstrated a significant increase in the levels of total bilirubin, serum ALP, ALT, and AST activities compared to those in normal rats. Treatment with 100 and 200 mg/kg SCT significantly decreased (p<0.01) all these parameters compared to diabetic untreated rats.

Effect of SCT on kidney function parameters

The serum levels of creatinine, urea, and uric acid of different experimental groups are shown in Table 4. Each of the three parameters, in diabetic control group, significantly increased (p<0.05) compared to normal control group. Diabetic rats administered with the SCT, at both doses, showed a significant (p<0.05) decrease in these three parameters when compared to diabetic untreated rats.

Effect of SCT on hematological parameters

In diabetic untreated rats, compared to normal control rats, all the hematological parameters significantly decreased (p<0.01), whereas WBCs significantly increased (p<0.01). Diabetic rats treated with both doses of SCT, after 45 days, showed significant improvement (p<0.05) in entire hematological abnormalities compared to diabetic control group (Table 5).
The present study was designed to investigate the hypoglycemic, hypolipidemic and hematoprotective effects of *C. tuberculata* and associated toxicity on STZ-induced diabetic rats. The WHO has recommended investigation of herbal medicines because herbal medicines have fewer side effects than synthetic agents [10]. In the present study the powder of aerial part of the *C. tuberculata* was used, because to be similar to the way in which this herb is used.

### Discussion

Table 3: Effect of *Caralluma tuberculata* treatment on liver function parameters of rats in different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>143.57±28.25</td>
<td>47.71±05.74</td>
<td>265.75±15.35</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Normal+200 SCT</td>
<td>145.75±22.17</td>
<td>59.63±06.51</td>
<td>262.18±17.21</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>STZ diabetic</td>
<td>276.86±20.24*</td>
<td>154.57±10.02*</td>
<td>779.07±52.21*</td>
<td>0.31±0.03*</td>
</tr>
<tr>
<td>STZ Veh</td>
<td>256.25±22.43*</td>
<td>162.25±12.77*</td>
<td>756.75±56.38*</td>
<td>0.27±0.03*</td>
</tr>
<tr>
<td>STZ+100 SCT</td>
<td>180.38±25.45**</td>
<td>100.5±06.30**</td>
<td>393.66±26.35**</td>
<td>0.22±0.03**</td>
</tr>
<tr>
<td>STZ+200 SCT</td>
<td>150.50±23.76**</td>
<td>81.88±05.92**</td>
<td>333.55±31.81**</td>
<td>0.20±0.02**</td>
</tr>
</tbody>
</table>

Normal+200 SCT; normal rats receiving 200 mg/kg/day of aerial part suspension of *C. tuberculata*, STZ-Veh; diabetic rats gavaged with distilled water, STZ+100 SCT; diabetic rats receiving 100 mg/kg/day of aerial part suspension of *C. tuberculata*, STZ+200 SCT; diabetic rats receiving 200 mg/kg/day of aerial part suspension of *C. tuberculata*. *P<0.01 compared with control rats (group I). **P<0.01 compared with diabetic rats. Data are mean±SD for eight animals in each group.

Table 4: Effect of *Caralluma tuberculata* treatment on kidney function parameters of rats in different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinin (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.50±0.06</td>
<td>41.13±6.1</td>
<td>1.58±0.08</td>
</tr>
<tr>
<td>Normal+200 SCT</td>
<td>0.51±0.06</td>
<td>44.50±5.6</td>
<td>1.61±0.06</td>
</tr>
<tr>
<td>STZ diabetic</td>
<td>0.84±0.07*</td>
<td>79.00±7.6*</td>
<td>2.57±0.11*</td>
</tr>
<tr>
<td>STZ Veh</td>
<td>0.81±0.08*</td>
<td>80.38±5.1*</td>
<td>2.45±0.11*</td>
</tr>
<tr>
<td>STZ+100 SCT</td>
<td>0.67±0.07***</td>
<td>58.56±6.0**</td>
<td>1.97±0.10**</td>
</tr>
<tr>
<td>STZ+200 SCT</td>
<td>0.62±0.06**</td>
<td>54.78±6.0**</td>
<td>1.77±0.09**</td>
</tr>
</tbody>
</table>

Normal+200 SCT; normal rats receiving 200 mg/kg/day of aerial part suspension of *C. tuberculata*, STZ-Veh; diabetic rats gavaged with distilled water, STZ+100 SCT; diabetic rats receiving 100 mg/kg/day of aerial part suspension of *C. tuberculata*, STZ+200 SCT; diabetic rats receiving 200 mg/kg/day of aerial part suspension of *C. tuberculata*. *P<0.01 compared with control rats (group I). **P<0.01 compared with diabetic rats. *P<0.05 compared with diabetic rats. Data are mean±SD for eight animals in each group.

Table 5: Effect of *Caralluma tuberculata* treatment on hematological parameters of rats in different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC (x10^6/μl)</th>
<th>WBC (x10^3/μl)</th>
<th>HGB (g/dl)</th>
<th>HCT (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>PLT (x10^9/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>8.6±0.7</td>
<td>9.01±0.54</td>
<td>15.53±0.7</td>
<td>49.7±3.0</td>
<td>56.6±0.9</td>
<td>17.7±0.7</td>
<td>31.17±0.9</td>
<td>879±54.4</td>
</tr>
<tr>
<td>Normal+200 SCT</td>
<td>8.7±0.6</td>
<td>8.72±0.57</td>
<td>15.58±0.8</td>
<td>48.8±3.2</td>
<td>55.9±1.2</td>
<td>17.8±0.6</td>
<td>31.85±1.3</td>
<td>898±48.1</td>
</tr>
<tr>
<td>STZ diabetic</td>
<td>6.7±0.5*</td>
<td>11.10±0.64*</td>
<td>12.83±0.6*</td>
<td>41.7±2.8*</td>
<td>49.5±1.0*</td>
<td>14.6±0.6*</td>
<td>27.75±0.9*</td>
<td>693±37.7*</td>
</tr>
<tr>
<td>STZ Veh</td>
<td>6.9±0.5*</td>
<td>10.93±0.59*</td>
<td>13.16±0.6*</td>
<td>41.9±2.8*</td>
<td>50.3±1.6*</td>
<td>15.5±0.6*</td>
<td>27.34±1.3*</td>
<td>691±45.3*</td>
</tr>
<tr>
<td>STZ+100 SCT</td>
<td>8.1±0.6***</td>
<td>9.18±0.61**</td>
<td>14.66±0.8*</td>
<td>48.5±4.4**</td>
<td>55.1±2.2**</td>
<td>17.0±0.8**</td>
<td>30.67±1.6**</td>
<td>763±46.0***</td>
</tr>
<tr>
<td>STZ+200 SCT</td>
<td>8.2±0.7**</td>
<td>9.19±0.63**</td>
<td>14.93±0.7*</td>
<td>48.9±3.6**</td>
<td>55.7±2.3**</td>
<td>17.0±0.7**</td>
<td>30.47±1.3**</td>
<td>798±34.5**</td>
</tr>
</tbody>
</table>

Normal+200 SCT; normal rats receiving 200 mg/kg/day of aerial part suspension of *C. tuberculata*, STZ-Veh; diabetic rats gavaged with distilled water, STZ+100 SCT; diabetic rats receiving 100 mg/kg/day of aerial part suspension of *C. tuberculata*, STZ+200 SCT; diabetic rats receiving 200 mg/kg/day of aerial part suspension of *C. tuberculata*. *P<0.01 compared with control rats (group I). **P<0.01 compared with diabetic rats. ***P<0.05 compared with diabetic rats. Data are mean±SD for eight animals in each group.
as an antidiabetic agent by natives. Chemical analyses on *C. tuberculata* have shown the presence of flavonoids, sponins, phenols, flavon glycosids and several pregnane glycosides that might operate alone or synergistically to cause the antihyperglycaemic effects [6,11,12].

STZ induces diabetes through the destruction of beta cells in the pancreas and it has been reported as a good empirical model to assess the activity of anti-diabetic medicinal agents, because it is less toxic than other chemical substances causing diabetes [13]. In this study, increased blood glucose due to STZ treatment, after 45 days treatment with SCT, significantly reduced. Similar effects have been reported by the methanolic extract of *C. tuberculata* [6,7]. In addition, the hypoglycemic effect of SCT was verified by doing OGTT. The results showed that in diabetic control rats, after a glucose loading, blood glucose increased to the peak levels within 60 minutes and nearly remained constant until end of the experiment and no significant decrease in glucose levels was observed. It may be due to destruction of beta cells by STZ treatment that caused insulin deficiency and therefore exhibited abnormal glucose tolerance. The SCT treated groups, after a glucose loading, exhibited an increase in glucose levels that peaked within 30 minutes. Subsequently, it decreased by the end of the test (similar to normal control rats). This useful activity proposed that *C. tuberculata* has ingredients with anti-diabetic properties. Overall, this effect may be due to inhibition of glucose absorption, increase in the sensitivity of insulin receptors to ligands, inhibition of insulinase action, stimulation of β cells of pancreas to secrete insulin or stimulation of the peripheral tissues for glucose uptake [14].

Abnormal features in the lipid profile are one of the most common disturbances in diabetes, which are found in about 40% of diabetic patients [15]. The increase mobilization of fatty acids from adipose tissue is one of the major causes of lipid metabolism disorders in diabetes. A secondary elevation of free fatty acids in the blood ultimately causes an increase in blood cholesterol during diabetes [16,17]. DM is, also, nearly constantly connected with the alterations of lipoproteins metabolism that may influence the development of the cardiovascular disease in all forms of diabetes [18]. In this study, diabetic untreated animals showed increase in the levels of TC, TG and LDL-C whereas HDL-C decreased. The SCT treated groups, after 45 days, showed an improvement in all the abnormalities. Similar results were obtained by the methanolic extracts of *C. tuberculata* in STZ-induced diabetic rats [7]. In health condition, insulin stimulates lipoprotein lipase (LPL) and consequently TG breaks down, so insulin deficiency in diabetes causes hypertriglyceridemia. The possible explanation for the effect in the SCT treated animals is that *C. tuberculata* may regenerate the beta cells in pancreas and boost insulin secretion that leads to a decrease in TG level. Insulin also hinders mobilization of fatty acid in adipose tissues. One of the side effects of some synthetic drugs which are used to treat hyperlipidemia is that while lowering TC, they reduce HDL-C levels [19]. However, in the present study, high dose of SCT, in addition to lowering TC, increased HDL-C.

The important issue that should be taken into consideration in the use of herbal remedies to treat diseases is to decide the appropriate dosage to avoid harmful effects. Therefore, to prevent toxic effects, herbal medicinal substances are strongly recommended to be used at appropriate approved doses. The serum levels of bilirubin, ALT, AST and ALP are indicators of liver function. Liver disorders affect bilirubin excretion and elevated values are applied as a marker for liver damage [20,21]. Several studies have reported increase in the serum activities of AST and ALT after 1–3 weeks of STZ administration. In this study, the same results were obtained and it will possibly be due to the liver cellular damage caused by STZ administration [22,23]. Many studies have reported that the herbal medicines could bring back the changes in the liver function parameters. Our observation, too, showed that in the groups which were administrated with the SCT for 45 days, these changes returned to near normal range. These findings may explain the safe and protective effects of *C. tuberculata*. Probably, flavonoids compounds in *C. tuberculata*, which have a membrane stabilizer property, cause these useful effects [24].

In this study, levels of urea, uric acid and creatinine in diabetic control rats increased. The high glucose levels during diabetes may lead to a decrease in glomerular filtration rate (GFR) and induce the increase in serum level of urea, uric acid and creatinine [25,26]. Also, the stimulation of the renin-angiotensin system and hyperglycemia in diabetes induces oxidative stress which is incriminated as the major cause of diabetic nephropathy [27]. In this study, SCT treated rats exhibited a significant improvement in kidney function parameters. It may be due to antihyperglycemic effect of *C. tuberculata*. These beneficial effects, Also, may be due to the existence of active compounds in plant that have antioxidant and, or radical scavenging properties [28,29].

People who suffer from diabetes show some unusual changes in different hematological parameters [30]. In this study, diabetic untreated rats showed decrease in all hematological parameters except the WBC that increased. Kidney produce 80–90% of erythropoietin and so kidney damage caused by diabetes can cause anemia [31]. It was
also reported that the increase of WBCs in diabetes could be a consequence of stress response [32]. In the SCT treated groups, levels of RBCs and related indexes significantly increased. This beneficial effects may be due to efficacy of flavonoids compounds in *C. tuberculata* which can stimulate the production or secretion of erythropoietin [33]. In the SCT treated groups, also, levels of WBCs reduced. This effect may be due to anti-inflammatory property of *C. tuberculata*.

This study showed that although oral administration of *Caralluma tuberculata* cannot normalize blood glucose in STZ-induced diabetic rats, it has remarkable hypolipidemic and hematoprotective activity. It also has protective effects against liver and kidney abnormalities which occur during diabetes.

**Ethical Considerations:** All the studies were directed in accordance with “Guide for the Care and Use of Laboratory Animals” (Number permission: 92–6160, date: 2014).

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**Conflict of Interest:** The authors have no conflict of interest.

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