Antihyperlipidemic and renoprotective activities of methanolic extract of *Canscora decussata* extract in alloxan-induced diabetic rabbits
Antihyperlipidemic and renoprotective activities of methanolic extract of *Canscora decussata* extract in alloxan-induced diabetic rabbits

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The current study was designed to evaluate the hypolipidemic and renoprotective effects of methanolic extract of powdered *Canscora decussata* whole plant in the diabetic rabbits. Thirty rabbits were divided into five groups having 6 animals each including normal and diabetic controls groups, the remaining groups received methanolic extract in 400 and 600 mg/kg doses and another group got pioglitazone (3 mg/kg) for 30 days. Serum levels of triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, albumin, globulin and total proteins were estimated by using commercially available kits. The results showed that extract significantly (p<0.01) decreased the raised parameters including triglyceride, total cholesterol and LDL-cholesterol, atherogenic index, Coronary risk index up to normal values compared to diabetic rabbits. However, it significantly increased HDL-cholesterol, albumin, globulin and total protein levels. Therefore, it is suggested that methanolic extract of *C. decussata* exerts hypolipidemic and renoprotective effects in the alloxan-induced diabetic rabbits.
The *Canscora decussata* plant (Family: Gentianaceae), locally known as Sankhahulee, has been used in the traditional medicine for the treatment of insanity, epilepsy and nervous debility. It has been found to contain triterpines, alkaloids, and xanthones (Kokate et al., 2000). Recently, Akhtar et al., 2012 have reported its hypoglycemic effects in normal as well as in diabetic rabbits. In folklore, it is reputed as a nerve tonic, alternative, and laxative (Shah et al., 2000). Diabetes affects both glucose and lipid metabolism (Sperling et al., 2000) while renal damage is its serious complication (Alberti, 1993).

*Ipomoea staphylina* leaves (Bag and Mumtaz, 2013), *Sechium eduleon* (Mumtaz et al., 2013), *Nigella sativuson* (Begum et al., 2006), *Cinnamomum zeylanicum* (Ullah et al., 2013), and grape seed and skin (Hamlouei et al., 2012) extract shows renoprotective effect. However, as far as ascertained, no scientific study was reported to assess the antihyperlipidemic and renoprotective activities of *C. decussata* in diabetes. Therefore, the current study was conducted to determine the antihyperlipidemic and renoprotective effects of methanolic extract of *C. decussata* in the diabetic rabbits.

### Material and Methods

#### Chemicals and plant material

The plant *C. decussata* was collected from a village of Lahore, Pakistan in the month of August 2010 and got identified by the taxonomist of University of Sargodha, Sargodha. A voucher specimen (No. UOS/CD/333) was deposited in Department of Pharmacy, University of Sargodha. The plant was completely shade dried and powdered with herbal grinder. The powdered material was stored in well closed cellophane bags at 4°C in the refrigerator till further use. Chemicals used in the study include triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol kits by Fluitest, Germany; alloxan monohydrate by Research Organics, USA; Methanol by Merck Chemical Co., Germany; and Grape seed and skin by Uni-Chem, Germany. Pioglitazone was a generous gift from Maan Gee distributors, Sargodha.

#### Experimental animals

Healthy adult rabbits weighing 1,000-1,500 g were kept at animal house of the Department of Pharmay, Hamdard University Islamabad Campus, Islamabad. The animals were housed in stainless cages under standard laboratory condition (light period: 8:00 am to 8:00 pm, 21 ± 2°C, relative humidity 55%, green fodder and water were available *ad libitum*).

#### Preparation of *C. decussata* extract

Methanolic extract of *C. decussata* whole plant was prepared by cold maceration. Then extract was dried with rotary evaporator.

#### Induction of diabetes

After overnight fasting, rabbits were made diabetic by intravenous injection of fresh solution of 150 mg per kg of alloxan monohydrate in jugular vein. Rabbits received alloxan monohydrate was provided with a free access to water with 5%dextrose solution in order to protect them from hypoglycemic shock (Akhtar et al., 2002). Three days (72 hours) after injecting the alloxan blood glucose level of surviving rabbits were measured with glucometer and rabbits having blood glucose level between 250-300 mg/dL were considered diabetic and used for further study (Olajide et al., 1999; Shani et al., 1974).

#### Administration of drug suspensions

The quantity of extract was calculated on body weight and triturated with about 10 mL of 2%aqueous gum solution and the final volume was always made up to 20 mL. Then suspension was administered (p.o) to each animal by using a stomach tube and disposable syringe (Akhtar and Iqbal, 1991; Sivajothi et al., 2008).

#### Experimental design

The rabbits were divided into five groups of six animals each. Group 1 and 2 considered as normal and diabetic control and were administered orally 20 mL of 2% aqueous gum acacia solution daily for 30 days. The groups 3 and 4 were administered extract (400 and 600 mg/kg OD), while group 5 received pioglitazone continuously for 30 days respectively. After 30 days blood samples were collected for the study.

#### Biochemical analysis

The total serum cholesterol, triglyceride and HDLs were estimated by enzymatic test kits using chemistry analyzer biolyser 100 (Merck Chemical Co., Germany). VLDL cholesterol and LDL-cholesterol were calculated using the Friedewald's et al., 1972 formula. Atherogenic index and coronary risk index was calculated by using Devaki et al., 2011 formula. Serum total protein and albumin content was determined by standard procedures in an auto analyzer using standard kits.

#### Statistical analysis

The results are expressed as mean ± SEM. The statistical analysis was carried out using paired t-test and one-way analysis (ANOVA). Statistical p value <0.05 was considered to be significant.

#### Results and Discussion

Effects of methanolic extract of *C. decussata* whole plant on serum triglyceride, cholesterol, LDL cholesterol, VLDL cholesterol, HDL-cholesterol, AI and CRI (mg/dL; mean ± SEM) levels in alloxan-induced diabetic rabbits after 30 days of extract administration are given in Table I. The serum levels of triglyceride (99.8 ± 0.31 mg/dL) and total cholesterol (129.9 ± 0.98 mg/dL) of group treated with 400 mg/kg extract were found to be significantly (p<0.001) lowered as compared to untreated alloxan-induced diabetic. Whereas, the serum level of HDL-cholesterol (34.6 ± 0.6 mg/dL) of group treated with 400 mg/kg extract was found to be...
significantly (p<0.001) increased as compared to untreated alloxan-induced diabetic. The AI and CRI also decreased significantly (p<0.001) as compared to diabetic group. The serum levels of triglyceride (83.6 ± 0.7 mg/dL), total cholesterol (123.7 ± 0.78 mg/dL), LDL-cholesterol (67.3 ± 1.4 mg/dL) and VLDL-cholesterol (16.7 ± 0.4 mg/dL) of group treated with 600 mg/kg extract were found to be significantly (p<0.001) lowered as compared to untreated alloxan-induced diabetic group. Whereas, the serum level of HDL-cholesterol (39.7 ± 1.1 mg/dL) of group treated with 600 mg/kg of methanolic extract were found to be significantly (p<0.001) increased as compared to untreated alloxan-induced diabetic group. AI and CRI also significantly lowered as compared to diabetic control group. The serum level of HDL-cholesterol (37.4 ± 0.2 mg/dL) of group treated with pioglitazone were found to be significantly (p<0.001) increased as compared to untreated alloxan-induced diabetic group (Table I).

Effects of methanolic extract of *C. decussata* whole plant on serum total protein, albumin, and globulin and A/G ratio in alloxan induced diabetic rabbits after 30 days of extract administration are given in Table II. The serum levels of total protein (7.5 ± 0.02 g/dL), albumin (4.5 ± 0.03 g/dL) of group treated with methanolic extract in dosing 400 mg/kg were found to be significantly (p<0.05) increased as compared to untreated alloxan-induced diabetic group. The serum levels of total protein (7.6 ± 0.02 g/dL), albumin (4.8 ± 0.03 g/dL), globulin (3.1 ± 0.3 g/dL) and A/G ratio (1.5 ± 0.1 g/dL) of group treated with extract in dosing 600 mg/kg were found to be significantly (p<0.05) increased as compared to untreated alloxan-induced diabetic group. Similarly, the serum levels of total protein (6.5 ± 0.2 g/dL), albumin (4.2 ± 0.1 g/dL), globulin (2.3 ± 0.1 g/dL) and A/G ratio (1.8 ± 0.01 g/dL) of group treated with pioglitazone in dosing 3 mg/kg were found to be significantly (p<0.05) increased as compared to untreated alloxan-induced diabetic group.

### Discussion

Diabetic patients have more chances to develop atheromatous complications such as ischemic heart diseases (Way et al., 2001). The decrease level of high density lipoprotein in diabetic patient is also the leading cause of atheromatous diseases (Rang et al., 2003). Oral administration of methanolic extract of *C. decussata* cause an increase in HDL and reduction in total cholesterol, triglycerides, LDL and VLDL which protect diabetic patients from atheromatous disease. The continuous administration of extract produced beneficial effect against hyperlipidemia associated with hyperglycemia in a dose dependent manner. Standard drug pioglitazone also increased serum HDL while reduces the total cholesterol, triglycerides, LDL and VLDL in dose dependent manner as reported by

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>Atherogenic index (mg/dL)</th>
<th>Coronary risk index (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>121.0 ± 0.9</td>
<td>80.6 ± 0.7</td>
<td>42.0 ± 1.2</td>
<td>58.7 ± 0.6</td>
<td>20.5 ± 0.7</td>
<td>1.3 ± 0.01</td>
<td>28.6 ± 0.02</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>266.3 ± 1.5</td>
<td>218.8 ± 1.4</td>
<td>29.7 ± 0.7</td>
<td>191.3 ± 0.7</td>
<td>45.5 ± 1.0</td>
<td>6.4 ± 0.4</td>
<td>8.9 ± 0.3</td>
</tr>
<tr>
<td>Methanolic extract 400 mg</td>
<td>129.9 ± 1.0b</td>
<td>99.8 ± 0.3b</td>
<td>34.6 ± 0.6b</td>
<td>75.3 ± 1.3b</td>
<td>19.9 ± 0.6b</td>
<td>2.1 ± 0.1b</td>
<td>3.7 ± 0.2b</td>
</tr>
<tr>
<td>Methanolic extract 600 mg</td>
<td>123.7 ± 0.8b</td>
<td>83.6 ± 0.7b</td>
<td>39.7 ± 1.1b</td>
<td>67.3 ± 1.4b</td>
<td>16.7 ± 0.4b</td>
<td>1.6 ± 0.02b</td>
<td>3.1 ± 0.3b</td>
</tr>
<tr>
<td>Pioglitazone treated</td>
<td>147.5 ± 1.0b</td>
<td>87.0 ± 0.03b</td>
<td>37.4 ± 0.2b</td>
<td>92.6 ± 1.2b</td>
<td>17.4 ± 0.4b</td>
<td>2.4 ± 0.01b</td>
<td>3.9 ± 0.1b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n= 6; *p<0.05, *p<0.001, *p>0.05 considered statistically significant as compared to diabetic control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Globulin (g/dL)</th>
<th>A/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8.0 ± 0.1</td>
<td>5.0 ± 0.02</td>
<td>3.1 ± 0.1</td>
<td>1.5 ± 0.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>4.4 ± 0.03</td>
<td>2.3 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Methanolic extract 400 mg</td>
<td>7.5 ± 0.02b</td>
<td>4.5 ± 0.03a</td>
<td>2.7 ± 0.01c</td>
<td>1.6 ± 0.04a</td>
</tr>
<tr>
<td>Methanolic extract 600 mg</td>
<td>7.6 ± 0.02b</td>
<td>4.8 ± 0.03b</td>
<td>3.1 ± 0.3a</td>
<td>1.5 ± 0.1b</td>
</tr>
<tr>
<td>Pioglitazone treated</td>
<td>6.5 ± 0.2b</td>
<td>4.2 ± 0.1b</td>
<td>2.3 ± 0.1b</td>
<td>1.8 ± 0.01b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n= 6; *p<0.05, *p<0.001, *p>0.05 considered statistically significant as compared to diabetic control group.
Kakadiya and Shah (2010). The antihyperlipidemic activity of the test extract would be due to its control of hyperglycemia which elevates the triglyceride, total cholesterol and LDL levels (Maciejewski et al., 2001). Several studies have reported that atherogenic index is an excellent predictor of HD risk and monitor for the effectiveness of lipid-lowering therapies. But, the LDL-C/HDL-C ratio has been considered more prognostic than LDL-C or HDL-C alone (Natarajan et al., 2003). In the present study the test extract significantly reduced the AI as well as TC/HDL-cholesterol ratio which show its protective effect against cardiovascular dis-eases. Similar results have reported for B. tomentosa which significantly reduced the atherogenic and cardiac risk index in diabetic rats (Devaki et al., 2011). The protein profile parameters are also disturbed during diabetes mellitus as reported by Sumana and Suryawanshi (2001) in rats. The extract of C. decussata has also shown improvement in the insulin secretion and reverses the altered protein profile in a dose dependent manner by exerting the protein sparing effect. Generally, significant increase in urinary excretion of protein, albumin and glucose levels indicates the impaired renal function in diabetes. However, the treatments with herbal remedies have been found to prevent such changes (Tedong et al., 2006). Peroxisome proliferator-activated receptor-gamma agonists have been reported to have direct beneficial effects on the diabetic renal diseases. They have shown to reduce proteinuria and improve glomerulosclerosis, both in animal and human diabetic nephropathic studies (Baylis et al., 2003), which is also shown in this study. In addition, Ohga et al., (2007) reported that pioglitazone ameliorates renal injury may be by the inhibition of NF-κB activation, ICAM-1 expression and macrophage infiltration in streptozotocin-induced diabetic rats. The present study has thus duly supported the alleged medicinal use of the plant in the traditional medicine.

It conclusion methanolic extract of C. decussata has been observed to exert significant and consistent hypolipide-mic and renoprotective effects in the diabetic rabbits which also confirm the presence of such active principle(s) which have the maximum solubility in methanol solvent.

References


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