Antidiabetic effect of *Oenanthe javanica* flavone

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**KEY WORDS** *Oenanthe javanica* flavone, blood glucose, insulin, experimental diabetes mellitus, hypoglycemic agent, lipids and antilipidemic agent, amylases, islets of Langerhans

**ABSTRACT**

AIM To study the antidiabetic effect of *Oenanthe javanica* flavone. **METHODS** Mice were injected iv with alloxan 90 mg kg⁻¹ to induce diabetes. Blood glucose, serum lipid and pancreatic amylase were determined with Automatic Biochemistry Analyser. Serum insulin was determined by radioimmunoassay. The pancreas and islets were examined under microscope. **RESULTS** OjF 200 mg kg⁻¹ reduced the blood glucose in normal mice from 0.5 to 6 h after a single administration ig. OjF 200 and 400 mg kg⁻¹ ig daily for 10 d decreased the blood glucose in alloxan-induced hyperglycemic mice: P < 0.05. OjF promoted the release of insulin both in normal and in diabetic mice. OjF decreased serum triglyceride and raised the lowered pancreatic amylases in diabetic mice: P < 0.01. The islet-injured changes of OjF-treated group were similar to those of control in histology examination but to a lesser degree. **CONCLUSION** OjF possessed the hypoglycemic and hypotriglyceride actions mainly concerned with promoting release of insulin from B-cells in islets of langerhans.

**INTRODUCTION**

*Oenanthe javanica* Umbelliferate is used to treat sudden attack of high fever, polydipsia and hypertension in folk remedy. Oj shows liver-protective, hypotensive, anti-arrhythmic, and anti-anaphylactic effects. In our previous study, Oj reduced blood glucose in normal and diabetic mice. The constituents of Oj are stated to consist of volatile oils 0.066 % phthalic acid ester, amino acids and flavonoids. *Oenanthe javanica* flavone OjF is considered to be one of main components and its content is about 1.2 % in whole plant. The aim of this study was to investigate the antidiabetic action of OjF.

**MATERIALS AND METHODS**

Preparation of OjF Oj were collected from Yanbian Autonomous Region in autumn and identification was done by Prof XIAO Hui-Zhong Department of Phytochemistry Yanbian Medical University. OjF was extracted in the same department with acid-base extraction method. The rate of recovery was 1.2 % from dried plant and the content of OjF was 51.67 % in whole extracts. The latter was determined according to aluminium nitrate reagent method. The components of OjF were analyzed by reverse HPLC. OjF contained 7.4 % quercetin.

Reagents Alloxan was from Sigma No 910505. Gliclazide Glu was produced by Yadong Medicinal Industry Co Shanghai No 940503. Phenformin was a product of Jiangsu Jintan Pharmaceutical Factory No 949081. Insulin kit was a product of DPC Co USA No TIN2-0012.

Normal mice experiment Kunning mice weighing 20.1 g ± 1.4 g were provided by Laboratory Animal Center of Academy of Medical Sciences Grade II No 013023. Mice were divided into 4 groups: contro NS 20 mL kg⁻¹, positive drug control Glu 100 mg kg⁻¹ OjF 100 mg kg⁻¹ all OjF 200 mg kg⁻¹. All mice were fasted 2 h before administration. Retrobulbar blood was taken 5 h after treatment.

Determination of the time-response relationship of OjF on Glu Mice were divided into 2 groups: NS 20 mL kg⁻¹ and OjF 200 mg kg⁻¹. Animals were fasted 2 h before treatment and blood samples were taken from caudal vein at 0, 0.5, 1, 2, 4, 6 h after administration.

Preparation and treatment of hyperglycemic mice Kunning mice weighing 22.6 g ± 1.6 g were
injected with alloxan 90 mg kg\(^{-1}\) through caudal veins. After 72 h the blood samples were taken for determining Glu. The mice were divided into 4 groups according to their Glu\(^+\) but the positive drug control was substituted by phenformin\(^-\) Phe\(^+\). The level of Glu was > 14.5 mmol L\(^{-1}\) and the variation of Glu in 4 groups was < 0.9 mmol L\(^{-1}\) blood. The normal control\(\) mice were injected with NS. The drugs were given ig for 10 d. After the last treatment\(\) the mice were fasted for 5 h\(\) then blood samples were collected.

**Determination of Glu** total cholesterol Chol\(\) triglyceride Trig\(\) and pancreatic amylase Amy\(\) Retrolbulbar blood was spun at 22.4 \(\times\) g for 10 min. Glu was analyzed by Glucose-oxidase method Chol and Trig by Enzyme end-point method and Amy by Rate method. The above serum values were determined with Automatic Biochemistry Analyzer while Glu in time-response relation examination was determined by One Touch Basic Glucometer LIFESCAN USA.

**Determination of serum insulin** Ins\(\) The Ins was determined by radioimmunoassay using insulin reagent kits and Gamma counter.

**Pathological examination** On the last day of treatment\(\) the mice were exsanguinated. Pancreas were put into 15 \% formaldehyde solution. HE stain and paraffin-sections were made.

**Statistics** Data were expressed as \(\bar{x} \pm s\) and analyzed by the one-way ANOVA test.

## RESULTS

**Effects of OJF on Glu and Ins in normal mice**

OJF 200 mg kg\(^{-1}\) decreased the Glu\(\) \(P < 0.01\) and increased serum Ins\(\) \(P < 0.01\) Tab 1\(\)\(\).  

<table>
<thead>
<tr>
<th>Dose/mg kg(^{-1})</th>
<th>Glu/nmol L(^{-1})</th>
<th>Ins/mU L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.6 ± 0.7</td>
<td>17.2 ± 3.3</td>
</tr>
<tr>
<td>Glu</td>
<td>4.3 ± 2.0(^b)</td>
<td>26.0 ± 5.6(^c)</td>
</tr>
<tr>
<td>OJF</td>
<td>4.7 ± 0.5(^b)</td>
<td>20.7 ± 2.8(^a)</td>
</tr>
<tr>
<td>200</td>
<td>4.0 ± 1.0(^a)</td>
<td>26.3 ± 7.2(^c)</td>
</tr>
</tbody>
</table>

**Effects of OJF on Glu and Ins in alloxan diabetic mice** The levels of blood Glu in diabetic mice were much higher\(\) while the Ins levels were lower\(\) \(P < 0.05\) when compared with normal mice. The mice treated with OJF 200 or 400 mg kg\(^{-1}\) for 10 d showed decreased Glu levels \(P < 0.05\) \(P < 0.01\) and increased Ins levels \(P < 0.05\) \(P < 0.01\) Tab 2\(\).

<table>
<thead>
<tr>
<th>Dose/mg kg(^{-1})</th>
<th>Glu/nmol L(^{-1})</th>
<th>Ins/mU L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.0 ± 0.5</td>
<td>18.0 ± 2.8</td>
</tr>
<tr>
<td>Control</td>
<td>20.1 ± 2.5(^a)</td>
<td>12.2 ± 3.0(^b)</td>
</tr>
<tr>
<td>Phen</td>
<td>12.8 ± 3.3(^c)</td>
<td>14.3 ± 2.1</td>
</tr>
<tr>
<td>OJF</td>
<td>16.3 ± 6.5(^b)</td>
<td>16.4 ± 6.2</td>
</tr>
<tr>
<td>400</td>
<td>7.8 ± 3.6(^b)</td>
<td>24.9 ± 8.4(^c)</td>
</tr>
</tbody>
</table>

**Time-response relationship of OJF on Glu**

OJF 200 mg kg\(^{-1}\) decreased the Glu levels remarkably from 0.5 to 6 h after a single ig administration\(\) Fig 1\(\).

![Fig 1. Time-response relationship of OJF 200 mg kg\(^{-1}\) on Glu in normal mice. \(n = 10\) mice. \(x \pm s\). The blood samples were taken for Glu determination at 0.5 \(1\) \(2\) \(4\) and 6 h after a single ig administration.](attachment:image)

**Effects of OJF on Chol** Trig\(\) and Amy in normal mice There was not much difference in the concentrations of serum Chol\(\) Trig\(\) and Amy in normal mice between control and OJF-treated groups.

**Effects of OJF on Chol** Trig\(\) and Amy in alloxan diabetic mice OJF 200 and 400 mg kg\(^{-1}\) decreased serum Trig in alloxan diabetic mice \(P < 0.05\) \(P < 0.01\) and increased serum Amy \(P < 0.05\) \(P < 0.01\) Tab 3\(\).

**Pathology features** The histopathologic findings observed under light microscopy were described as
Tab 3. Effects of OjF on Chol$ï$ Trig$ï$ and Amy in diabetic mice. $x \pm s$. $^aP<0.05$ vs normal. $^bP>0.05$ $^cP<0.05$ $^dP<0.01$ vs control.

<table>
<thead>
<tr>
<th>Dose /mg kg$^{-1}$ Mice</th>
<th>Chol /mmol L$^{-1}$</th>
<th>Trig /mmol L$^{-1}$</th>
<th>Amy /IU L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9</td>
<td>2.5 $\pm$ 0.3</td>
<td>1.20 $\pm$ 0.18</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>2.8 $\pm$ 0.6</td>
<td>1.54 $\pm$ 0.26b</td>
</tr>
<tr>
<td>Phen</td>
<td>100</td>
<td>3.1 $\pm$ 0.6</td>
<td>1.61 $\pm$ 0.34d</td>
</tr>
<tr>
<td>OjF</td>
<td>200</td>
<td>2.7 $\pm$ 0.5</td>
<td>1.11 $\pm$ 0.20a</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>2.7 $\pm$ 0.8</td>
<td>0.80 $\pm$ 0.23f</td>
</tr>
</tbody>
</table>

follows. In NS-treated group$ï$ control$ï$ pancreas leaflets presented normal structure while the size and cell number of islets were reduced in total animals $n = 8$. Furthermore, mononuclei infiltrated around islets and small blood vessels. In OjF -treated group$ï$ pancreas leaflets structure showed normal appearance. The change in islets was similar to that of control$ï$ some islets presented shrunken-size and were mononuclear-infiltrated but there also existed a few normal sized islets.

DISCUSSION

The effect of OjF on Glu has not been so far reported. The present experiments suggest that OjF was able to decrease Glu in normal animals$ï$ as well as in diabetic animals$ï$ and also cause blood lipid lowering$ï$ mainly Trig. These results demonstrate that OjF might not only affect blood Glu level but also prevent the complications of diabetes such as hyperlipemia.

In order to analyze the hypoglycemic mechanism of OjF$ï$ the variations of serum Ins and pancreatic histology were observed$ï$ and synchronized with the determination of Glu. The results manifested an effective promoting release of Ins$ï$ which was likely to be the major cause of lowering the Glu by OjF. As for histopathology examination$ï$ there was slight difference in islet tissues between OjF-treated group and untreated group in alloxan animals observed under light microscopy. However$ï$ in our previous research$ï$ we found that pancreatic Amy was decreased greatly in alloxan diabetic animals$ï$. This observation explained that alloxan was capable of damaging pancreatic exocrine gland besides B-cells$ï$ and the level of pancreatic Amy might be an index of pancreas injury. OjF was in a position to recover the decrease in Amy induced by alloxan$ï$ thus indicating that OjF$ï$ in a certain degree$ï$ exerted an anti-injury action$ï$ protecting pancreas from chemical damage and thus may be favourable to treatment of diabetes.

It is uncertain whether the effect of OjF on lowering Trig was only due to its hypoglycemic action or some other factor. Nevertheless$ï$ according to the dosage we used it seemed to be the latter. After administration of low dose of OjF$ï$ the content of Trig was obviously decreased$ï$ while Glu was lowered slightly. But in high dose group$ï$ the blood glucose was reduced almost to the normal level$ï$ but serum Trig was decreased below the normal level. This phenomenon implied that OjF possesses a powerful action on lipid metabolism possibly not depending only on hypoglycemic mechanism$ï$ but also on some other path.

Although this is the first time the effect of OjF on Glu is reported$ï$ a few investigations about antihyperglycemic effects of other plant flavones has been reported$ï$ such as flavones of Radix puerariae$ï$ Herba epimedii and Morus alba etc. The target organ for some hypoglycemic flavones was found to be predominantly located in pancrea islet$ï$ including B-cells$ï$ and Ins was released as a result$ï$. The above reports were consistent with our results that OjF influenced the release of Ins.

In addition$ï$ when the compositions of flavonoids which enabled the Glu decrease were identified$ï$ quercetin emerged as a common component. Whether quercetin reduces Glu is not entirely clear$ï$ the inhibitory effects of quercetin on albumin nonenzymatic glycosylation$ï$ ANG$ï$ have been demonstrated$ï$. It is considered that ANG is responsible for some diabetic complications$ï$ therefore$ï$ the medicine which inhibits such activity$ï$ for example quercetin$ï$ may exert protective actions against diabetic complications. OjF contains quercetin and the average quantity was found to be 7.4% in our previous study detailed role by which quercetin functions is essentially unknown as yet. Besides$ï$ other flavonoids such as persicarin$ï$ isorhamnetin and hyperoside have been separated by other laboratories from OjF$ï$. The explicit activity of these flavonoids needs further investigation.

REFERENCES

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目的: 研究水芹黄酮 (Z) 的抗糖尿病作用

方法: 给小鼠尾静脉注射四氧嘧啶 (Z), 造成高血糖动物模型

用自动生化分析仪测定血糖、血脂和胰淀粉酶

放免法测定血清胰岛素

光镜下观察胰腺和胰岛的组织学变化

结果: 一次给药后, 可使正常动物血糖降低

重复给药 3 天, 各组均使四氧嘧啶糖尿病动物血糖明显降低 (P < 0.01)

并促进正常动物及高血糖动物胰岛素释放

还能明显降低血清甘油三酯 (P < 0.01) 及升高糖尿病动物降低的胰淀粉酶水平 (P < 0.01)

组织学观察: 治疗组胰岛损伤的变化与对照组相似, 但程度较轻

结论: 水芹黄酮具有降低血糖和甘油三酯作用, 并对胰腺损伤有一定的拮抗作用

降血糖作用主要是由于促进了胰岛细胞释放胰岛素

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