ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)：239 – 242
http：//www.chinaphar.com

Antidiabetic effect of *Oenanthe javanica* flavone

YANG Xin-Bo[1]，HUANG Zheng-Ming，CAO Wen-Bin，ZHENG Ming，CHEN Hong-Yan，ZHANG Jing-Zhen
(*Department of Pharmacology，Beijing Medical College of PLA，8 Dongdajie Road，Beijing 100071，China*)

KEY WORDS *Oenanthe javanica*；flavones；blood glucose；insulin；experimental diabetes mellitus；hypoglycemic agents；lipids and antilipidemic agents；amylases；islets of Langerhans

ABSTRACT

AIM：To study the antidiabetic effect of *Oenanthe javanica* flavone (*Oj*F). 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 Analysor. Serum insulin was determined by radioimmunoassay. The pancreas and islets were examined under microscope. RESULTS：*Oj*F 200 mg·kg^{-1} reduced the blood glucose in normal mice from 0.5 to 6 h after a single administration ig. *Oj*F 200 and 400 mg·kg^{-1} ig daily for 10 d decreased the blood glucose in alloxan-induced hyperglycemic mice ($P < 0.05$，$P < 0.01$). *Oj*F promoted the release of insulin both in normal and in diabetic mice. *Oj*F decreased serum triglyceride and raised the lowered pancreatic amylases in diabetic mice ($P < 0.01$，$P < 0.01$). The islet-injured changes of *Oj*F-treated group were similar to those of control in histology examination，but to a lesser degree. CONCLUSION：*Oj*F possessed the hypoglycemic and hypotriglyceride actions，mainly concerned with promoting release of insulin from B-cells in islets of langerhans .

INTRODUCTION

Oenanthe javanica (Oj)，Umbelliferate，is used to treat sudden attack of high fever，polydipsia and hypertension in folk remedy. Oj shows liver-protective[1]，hypotensive，anti-arrhythmic，and anti-anaphylactic effects[2]. In our previous study，Oj reduced blood glucose (Glu) 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[3]. *Oenanthe javanica* flavone (*Oj*F) 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 *Oj*F.

MATERIALS AND METHODS

Preparation of *Oj*F *Oj* were collected from Yanbian Autonomus Region in autumn and identification was done by Prof XIAO Hui-Zhong，Department of Phytochemistry，Yanbian Medical University. *Oj*F 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 *Oj*F was 51.67 % in whole extacts. The latter was determined according to aluminium nitrate reagent method. The components of *Oj*F were analyzed by reverse HPLC. *Oj*F contained 7.4 % quercetin.

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

Normal mice experiment Kunming mice，♂，weighing 20.1 g ± 1.4 g，were provided by Laboratorial Animal Center，Academy of Military Medical Sciences (Grade Ⅱ，№ 013023). Mice were divided into 4 groups：control (NS，20 mL·kg^{-1})；positive drug control (Gli，100 mg·kg^{-1})；*Oj*F 100 mg·kg^{-1}，and *Oj*F 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 *Oj*F on Glu Mice were divided into 2 groups：NS (20 mL·kg^{-1}) and *Oj*F 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，and 6 h after administration.

Preparation and treatment of hyperglycemic mice Kunming mice，♂，weighing 22.6 g ± 1.6 g，were

[1] Correspondence to Prof YANG Xin-Bo. Phn 86-10-6694-7723. E-mail Simbalee@Publica.bj.cninfo.net
Received 1999-01-11 Accepted 1999-08-19

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Mar；21 (3)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

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} blood and the variation of $\bar{x}$ of Glu in 4 groups was < 0.9 mmol·L^{-1} blood. The normal control (Normal) 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) Retrobulbar blood was spun at 22.4 × *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 Analysor, while Glu in time-response relation examination was determined by One Touch Basic Glucometes (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 *Oj*F on Glu and Ins in normal mice *Oj*F 200 mg·kg^{-1} decreased the Glu ($P < 0.01$) and increased serum Ins ($P < 0.01$; Tab 1).

Tab 1. Effect of *Oj*F on Glu and Ins in normal mice. $n = 8$ mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	Dose/mg·kg^{-1}	Glu/mmol·L^{-1}	Ins/mU·L^{-1}
Control		5.6 ± 0.7	17.2 ± 3.3
Gli	100	4.3 ± 2.0^b	26.0 ± 5.6^c
*Oj*F	100	4.7 ± 0.5^b	20.7 ± 2.8^b
	200	4.0 ± 1.0^c	26.3 ± 7.2^c

Effects of *Oj*F 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 *Oj*F 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).

Tab 2. Effect of OiF on Glu and Ins in diabetic mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* normal. $^eP < 0.05$, $^fP < 0.01$ *vs* control.

	Dose/mg·kg^{-1}	Mice	Glu/mmol·L^1	Ins/mU·L^{-1}
Normal		9	7.0 ± 0.5	18.0 ± 2.8
Control		9	20.1 ± 2.5^c	12.2 ± 3.0^b
Phen	100	9	12.8 ± 3.3^f	14.3 ± 2.1
*Oj*F	200	9	16.3 ± 6.5^e	16.4 ± 6.2
	400	8	7.8 ± 3.6^f	24.9 ± 8.4^f

Time-response relationship of *Oj*F 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).

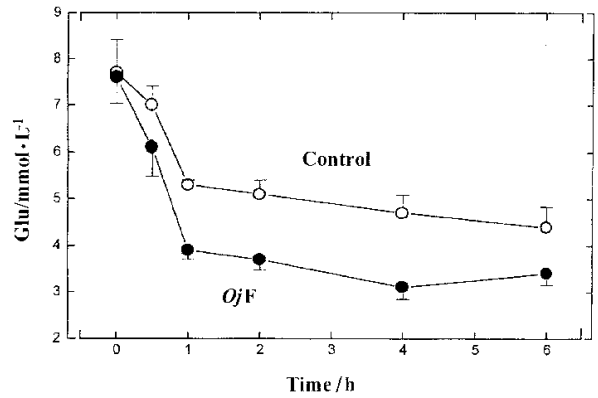

Fig 1. Time-response relationship of *Oj*F (200 mg·kg^{-1}) on Glu in normal mice. $n = 10$ mice. $\bar{x} \pm s$. The blood samples were taken for Glu determination at 0.5, 1, 2, 4, and 6 h after a single ig administration.

Effects of *Oj*F 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 *Oj*F-treated groups.

Effects of *Oj*F on Chol, Trig, and Amy in alloxan diabetic mice *Oj*F 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 *Oj*F on Chol, Trig, and Amy in diabetic mice. $\bar{x} \pm s$. $^{b}P < 0.05$ *vs* normal. $^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* control.

	Dose /mg·kg^{-1}	Mice	Chol /mmol·L^{-1}	Trig /mmol·L^{-1}	Amy /IU·L^{-1}
Normal		9	2.5 ± 0.3	1.20 ± 0.18	2601 ± 203
Control		8	2.8 ± 0.6	1.54 ± 0.26^{b}	1796 ± 309^{b}
Phen	100	9	3.1 ± 0.6	1.61 ± 0.34^{bd}	2209 ± 245^{e}
*Oj*F	200	7	2.7 ± 0.5	1.11 ± 0.20^{e}	2081 ± 218^{e}
	400	8	2.7 ± 0.8	0.80 ± 0.23^{f}	2271 ± 274^{f}

follows. In NS-treated group (control), 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 *Oj*F -treated group, pancrea leaflets structure showed normal appearance. The change in islets was similar to that of control: some islets presented shrunken-size and were mononuclear-infiltrated; but there also existed a few normal sized islets.

DISCUSSION

The effect of *Oj*F on Glu has not been so far reported. The present experiments suggest that *Oj*F was able to decrease Glu in normal animals, as well as in diabetic animals, and also cause blood lipid lowering, mainly Trig. These results demonstrate that *Oj*F 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 *Oj*F, the variations of serum Ins and pancreatic histology were observed, and synchronized with the determination of Glu. The results manifested an effective promoting release of Ins; which was likely to be the major cause of lowering the Glu by *Oj*F. As for histopathology examination, there was slight difference in islet tissues between *Oj*F-treated group and untreated group in alloxan animals observed under light microscopy. However, in our previous research, we found that pancreatic Amy was decreased greatly in alloxan diabetic animals[4]. This observation explained that alloxan was capable of damaging pancreatic exocrine gland besides B-cells, and the level of pancreatic Amy might be an index of pancreas injury. *Oj*F was in a position to recover the decrease in Amy induced by alloxan, thus indicating that *Oj*F, in a certain degree, exerted an anti-injury action, protecting pancreas from chemical damage and thus may be favourable to treatment of diabetes.

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

Although this is the first time the effect of *Oj*F on Glu is reported, a few investigations about antihyperglycemic effects of other plant flavones has been reported, such as flavones of *Radix puerariae*, *Herba epimedium*, and *Morus alba*, etc. The target organ for some hypoglycemic flavones was found to be predominantly located in pancrea islet, including B-cells, and Ins was released as a result[5]. The above reports were consistent with our results that *Oj*F influenced the release of Ins.

In addition, when the compositions of flavonoids which enabled the Glu decrease were identified, quercetin emerged as a common component. Whether quercetin reduces Glu is not entirely clear, the inhibitory effects of quercetin on albumin nonenzymatic glycosylation (ANG) have been demonstrated[6]. It is considered that ANG is responsible for some diabetic complications; therefore, the medicine which inhilsits such activity, for example quercetin, may exert protective actions against diabetic complications. *Oj*F 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, other flavonoids such as persicarin, isorhamnetin and hyperoside have been separated by other laboratories from *Oj*[7]. The explicit activity of these flavonoids, needs further investigation.

REFERENCES

1 Huang ZM, Zhang ZM, Yang XB, Cao WB. Study of Shui Qin on antihepatitis. Pharmacol Clin Chin Mat Med 1991; 7: 11-3.

2 Yang XB, Huang ZM, Cao WB, Yao XL, Zhang ZQ, Wang X, *et al*. The cardiovascular effects of the decoction of SQ. Chin Trad Herb drugs 1988; 19: 47.

3 Yin J. *Oenanthe javanica*. In: Modern study and clinic application of Chinese materia medica. Beijing: Publishing House of Traditional Chinese Medicine and Ancient Books 1997;

p 385 – 6.
4 Yang XB，Huang ZM，Zheng M，Chen HY，Cao WB，Zhang JZ. Antidiabetic effects of ethanolic extract from *Rhizoma alismatis*. Proceeding of 98 China-Japan Joint Meeting on Pharmacology；1998 Oct 17 – 20；Zhangjiajie，Hunan，China.
5 Jiang GY. Applied diabetologia. In：Zhang HF，editor. Treatment with Chinese traditional medicine. Beijing：People's Medical Publishing House 1992；p 172 – 3.
6 Ge Y，Zhang JQ，Zhou YP. The inhibitory effects of some Chinese herbs or its components on albumin nonenzymatic glycosylation. Acad J Sec Mil Univ 1995；16：333 – 6.
7 Park JC，Young HS，Yu YB，Lee JH. Isorhamnetin sulphate from the leaves and stems of *Oenanthe javanica* in Korea. Planta Med 1995；61：377 – 8.

水芹黄酮的抗糖尿病作用

杨新波[1]，黄正明，曹文斌，郑　鸣，陈鸿艳，张静珍
（北京军医学院药理室，北京 100071，中国）

关键词　水芹；黄酮类；血糖；胰岛素；实验性糖尿病；降血糖药；脂类和降血脂药；淀粉酶类；胰岛

目的：研究水芹黄酮（*Oj*F）的抗糖尿病作用. **方法**：给小鼠尾静脉注射四氧嘧啶 90 mg·kg^{-1}，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 ig *Oj*F 200 mg·kg^{-1}后 0.5 – 6 h，可使正常动物血糖降低. 重复给药 10 天，*Oj*F 200 mg·kg^{-1}和 400 mg·kg^{-1}均使四氧嘧啶糖尿病动物血糖明显降低（$P < 0.05$，$P < 0.01$）. 并促进正常动物及高血糖动物胰岛素释放. *Oj*F 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 *Oj*F 治疗组胰岛损伤的变化与对照组相似，但程度较轻. **结论**：*Oj*F 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. *Oj*F 降血糖作用主要是由于促进了胰岛 B 细胞释放胰岛素.

（责任编辑　刘俊娥）