An investigation of the anti-diabetic effects of an extract from *Cladonia humilis*

Yibing Zhang[1], Jinli Shi[2], Yong Zhao[2], Haifeng Cui[2], Chunyu Cao[2] and Sha Liu[1*]

[1]School of Traditional Chinese Medicine, Chongqing Medical University, Jieqing Road, Huxi University City, Chongqing, PR China
[2]School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, PR China
[3]Institute of Chinese Materia Medica, China Academy of TCM, Beijing, PR China

Abstract: The effect of *Cladonia humilis* on glycaemic metabolism was researched in this studied. The blood glucose, insulin secretion and glycogen synthesis of the hyperglycemic mice induced by alloxan were analyzed respectively. The gluconeogenesis and the sugar tolerance of the normal mice were also analyzed in this paper. After the hyperglycemic mice were orally administrated with *Cladonia humilis* extract, the blood glucose was decreased ($p<0.05$), the level of insulin secretion and glycogen synthesis were elevated ($p<0.05$, $p<0.01$, respectively). In addition, *Cladonia humilis* extract could inhibite the gluconeogenesis ($p<0.01$) and improve the sugar tolerance in normal control mice. These results maybe account for the causes of *Cladonia humilis* extract-induced significant decreases of the blood glucose in hyperglycemic mice.

Keywords: Lichens; blood glucose; diabetes; insulin.

INTRODUCTION

Lichens are symbiotic organisms composed of fungi and algae. The fungus forms a three-dimensional thallus, within which the photosynthetic partners are located (Collins and Farrar 1978). Lichens have demonstrated a capacity to survive the more challenging extremes of condition (Sancho *et al*., 2007). Many lichen extracts have been used in folk medicine. Screening tests with lichens have demonstrated the frequent occurrence of metabolites with antibiotic, antimycobacterial, antiviral, anti-infammatory, analgesic, antiproliferative, antipyretic, and cytotoxic properties (Bucar *et al*., 2004; Guo *et al*., 2010; Kumar and Müller 1999; Liu *et al.,* 2010. Müller 2001;Omarsdottir *et al*., 2007). However, its anti-diabetic propertie has not yet been discussed in terms of modern pharmacological concepts. The hypoglycemic activity of Cladonia humilis is reported in this study.

Ahti reported that Cladonia humilis contains atranorin and fumarprocetraric acid (Ahti *et al*. 1996). However, there is no information and research on activities of *Cladonia humilis*. For the first time, the pharmacological activity of Cladonia humilis is reported.

MATERIALS AND METHODS

Lichen

Cladonia humilis was collected from Xijiang, China in August of 2009. The material was identified by Dr. Zheng, a staff member of the Botany Department at Shandong University.

Chemicals

Alloxan was purchased from Sigma Co., Ltd. L-alanine was purchased from Betapharma Co., Ltd., Shanghai, China.

Lichen Extract (LE)

The air-dried and powdered lichen material (300g) was extracted with methanol at 40°C. The first extraction was completed for 4h. After the extract was filtered, it was concentrated in a Rotavapor at 40°C. The methanol extract obtained at the end of this process was then solved in water. This watered extract was consumed using petrol ether. The remaining extract was lyophilized and used in this study.

Animals

Kunming strain mice (20-22g), were purchased from the Experimental Animal Center, Chongqing Medical University, China. The mice, maintained at room temperature under alternating natural light/dark photoperiod, had access to standard laboratory food and fresh water *ad libitum.* It was performed in accordance with the Guide for the Care and Use of Laboratory Animals. Care was taken to minimize discomfort, distress, and pain to the mice.

Induction of diabetes

Mice, fasted for 12 h, were injected intravenously with alloxan (75mg/kg) solution made with saline. Forty-eight hours later, blood was collected from the tail veins of the animals. The blood glucose level of mice greater than 11.1mmol/L was selected as hyperglycemic mice.

Experimental design

Thirty-six hyperglycemic animals were allocated equally

**Corresponding author:* e-mail: shaliuchongqing@yeah.net

into 4 groups: hyperglycemic mice, LE (50mg/kg) treated mice, LE (100mg/kg) treated mice. Twelve normal mice were injected intravenously with the normal saline and used as the control group. The four groups of mice were treated orally by saline, LE (50mg/kg), LE (100mg/kg) and saline respectively. Fifteen days later, the animals were fasted overnight and sacrificed by decapitation. The liver was dissected out for the measurement of hepatic glycogen. The blood samples were collected to be centrifuged at 3000 rpm for 20 min. The serum separated for the measurement of insulin.

Estimation of serum insulin

Serum insulin level was analyzed with an enzyme-linked immunosorbant assay kit (Biosource, Europe).

Estimation of hepatic glycogen

The liver, homogenized in ice-cold 0.6 M $HClO_4$, was centrifuged at 3000g and subjected to determination of free glucose in liver by the glucose oxidase method (Arayne *et al*., 2007).

Estimation of Gluconeogenesis

Thirty-six healthy mice were selected and allocated equally into 3 groups: LE (50mg/kg)-treated group, LE (100mg/kg)-treated group, saline-treated group (control group). The 3 groups of animals treated orally with LE (50mg/kg), LE (100mg/kg) and saline respectively. Fifteen days later, animals were fasted 12h and were injected (s.c.) with L-alanine after the last administration. The blood samples were collected from the tail vein of the mice at the 0^{th} min and 60^{th} min respectively. The blood glucose level was analyzed.

Estimation of sugar tolerance

Thirty-six normal mice were selected and allocated equally into 3 groups: LE (100mg/kg)-treated group, saline-treated groups (control group). Seven days later, after the last administration, the first and second groups were injected intravenously with glucose (2 g/kg) and the third group with saline (control group). The blood samples were collected from the tail vein of the mice at 0, 30, 60 and 120 min respectively. The blood glucose level was analyzed.

STATISTICAL ANALYSIS

All data were analyzed by Duncan's multiple-range test. The data are shown as the mean±SEM. The significant level of 5% was used as the minimum acceptable probability for the difference between the means.

RESULTS

Effect of LE on blood glucose level

The results of blood glucose in hyperglycemic mice induced by alloxan are presented in table 1. The levels of blood glucose decreased after administration of LE (100mg/kg) and LE (50mg/kg) *($p<0.05$)*.

Table 1: Effect of LE on blood glucose levels in hyperglycemic mice

Different groups	Blood glucose (mmol/L)
Alloxan-treated	21.2 ± 2.1^{a}
Alloxan and LE (50mg/kg)-treated	13.1 ± 3.2^{b}
Alloxan and LE (100mg/kg)-treated	10.5 ± 2.0^{b}
Control group	5.9±1.2

The different letters (a and b) indicate a statistical difference ($p<0.05$)

Effect of LE on serum insulin level

As shown in table 2, the serum insulin levels were elevated after administration of LE (50 mg/kg) (8.6 ± 0.6 μU/mL, $p<0.05$) and LE (100 mg/kg) (9.7 ± 0.4 μU/mL, $p<0.05$*)*. However, the same results did not occur in the hyperglycemic group (3.7 ± 1.3 μU/mL).

Table 2: Effect of LE on serum insulin level in hyperglycemic mice

Different groups	Serum insulin (μU/mL)
Alloxan- treated	3.7 ± 1.3^{a}
Alloxan and LE (50mg/kg)-treated	8.6 ± 0.6^{b}
Alloxan and LE (100mg/kg)-treated	9.7 ± 0.4^{b}
Control group	5.5±1.2

LE-treated groups were compared with diabetic group. The different letters (a and b) indicate a statistical difference, $p<0.05$.

Effect of LE on hepatic glycogen level

LE produced the increase in the level of hepatic glycogen. The glycogen levels were 21.2 ± 3.2 mg/g tissue in LE (100 mg/kg) treated mice ($p<0.05$). Treatment of diabetic mice with LE (50mg/kg) also produced increase in the levels of hepatic glycogen to 15.8 ± 0.3mg/g tissue. Concentrations of hepatic glycogen were lower in diabetic mice (14.1 ± 3.8 mg/g) than those in normal mice (22.1 ± 4.3mg/g) and LE (100mg/kg) treated mice (table 3).

Table 3: Effect of LE on serum insulin level in hyperglycemic mice

Different groups	Hepatic glycogen (mgg tissue)
Alloxan- treated	14.1 ± 3.8
Alloxan and LE (50mg/kg)-treated	15.8 ± 0.3
Alloxan and LE (100mg/kg)-treated	$21.2 \pm 3.2^{*}$
Control group	$22.1 \pm 4.3^{*}$

The marks (*) indicate a statistical difference,*$p<0.05$ vs. Alloxan-treated group.

Effect of LE on Gluconeogenesis

At the 60th min, the level of blood glucose in the LE (100mg/kg) group was not increased significantly (from 92.2±16.7 mg/mL to 96.8±8.2 mg/mL) after the mice were injected (s.c.) with L-alanine. On the contrary, the level of blood glucose of the mice in the control group was increased significantly after the mice were injected (s.c.) with L-alanine ($p<0.01$, from 91.4±12.7mg/mL to 110.0±11.2mg/mL). The result was shown in Table.4.

Table 4: Effect of LE on Gluconeogenesis

Different groups	Blood glucose (mg/mL) at 0th min	Blood glucose (m/mL) at 60th min
Alloxan and LE (50mg/kg)-treated	91.5±14.5	97.8±10.9
Alloxan and LE (100mg/kg)-treated	92.2±16.7	96.8±8.2[a]
Saline-treated	91.4±12.7	110.0±11.2[b]

LE-treated groups were compared with diabetic group. The different letters (a and b) indicate a statistical difference, $p<0.01$.

Effect of LE on sugar tolerance

The ascension of blood sugar induced by glucose was inhibited 30 mins later in LE (100mg/kg)-glucose group and saline-glucose group (Fig.1). The level of blood sugar in LE (100mg/kg)-glucose group was very close to that in control group 120mins later. However, the level of blood sugar in the saline-glucose group was not decreased compared with control group. As shown in fig.1, the sugar tolerance of normal mice was improved after administration (ig) with LE (100mg/kg).

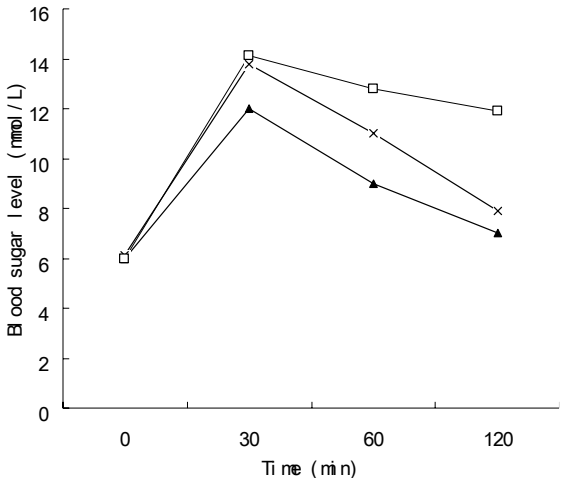

Fig. 1: Effects of LE on sugar tolerance of normal mice. (▲Control group, □Saline-glucose group, × LE -glucose group)

DISCUSSION

For the first time, the hypoglycemic activity of *Cladonia humilis* is reported in the present study. Our results showed that blood glucose in alloxan-induced hyperglycemic mice was significant decreased after LE treatment. Alloxan also produces reactive oxygen species resulting in reduced synthesis and release of insulin (El-Alfy *et al*., 2005). In our study, we found LE treatment increased the level of insulin secretion in the hyperglycemic mice throughout the total duration of the study. It is possible that LE could aid in releasing of insulin from the surviving β-cells.

Induction of diabetes with alloxan was associated with decrease in hepatic glycogen (LV *et al*., 2009). It could be attributed to the decrease in the active form of enzyme glycogen synthetase probably because of low levels of insulin (Gad *et al*., 2010). Glycogen storage in the liver is another way to maintain blood glucose concentration in mammals. In the present study, LE showed normalization of the depressed hepatic glycogen levels.

Gluconeogenesis is an important mechanism for maintaining blood glucose within a normal range (Xu and Guo, 2009). As shown in Table 4, the level of blood glucose of the mice in the LE group was not increased significantly ($p<0.01$). The results suggest that the effect of LE on blood glucose in animal models is due in part to the reduction in gluconeogenesis. At the same time, we can see the sugar tolerance of normal mice was improved after administration (ig) of LE.

In conclusion**,** our results showed that LE treatment markedly reduced hyperglycemia in alloxan-induced hyperglycemic mice by increasing glycogen and insulin level as well as inhibiting gluconeogenesis. The results suggest that *Cladonia humilis* extract may be used as a hypoglycemic food or medicine for hyperglycemic people in terms of this modern pharmacological study. The potential application of LE needs to be further studied.

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REFERENCES

Ahti T, Stenroos S, Chen, Guo JB and Shou Y (1996). The status of *Cladonia humilis* in east Asia. *Mycosystema*., **8**: 153-158.

Arayne MS, Sultana N, Mirza AZ, Zuberi MH and Siddiqui FA (2007). *In vitro* hypoglycemic activity of methanolic extract of some indigenous plants. *Pak. J. Pharm. Sci.*, **20**: 268-273.

Bucar F, Schneider I, Ogmundsdóttir H and Ingólfsdóttir K (2004). Anti-proliferative lichen compounds with

inhibitory activity on 12(S)-HETE production in human platelets. *Phytomedicine,* **11**: 602-606.

Collins CR and Farrar JF (1978). Structural resistance to mass transfer in the lichen *Xanthoria parietina*. *New Phytologist.,* **81**: 71-83.

El-Alfy AT, Ahmed AA and Fatani AJ (2005). Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats. *Pharmacol. Res.*, **52**: 264-270.

Gad MZ, Ehssan NA, Ghiet MH and Wahman LF (2010). Pioglitazone versus metformin in two rat models of glucose intolerance and diabetes. *Pak. J. Pharm. Sci.,* **23**: 305-312.

Guo JY, Liu TJ, Han LN and Liu YM. (2009). The effects of corn silk on glycaemic metabolism. *Nutr. Metab*., **6**: 47.

Guo JY, Han CC and Liu YM (2010). A contemporary treatment approach to both diabetes and depression by cordyceps sinensis, rich in Vanadium. *Evid-Based Compl. Alt.*, **7**: 387-389.

Kumar KC and Müller K (1999). Lichen metabolites.1. Inhibitory action against leukotriene B4 biosynthesis by a non-redox mechanism. *J. Nat. Prod.,* **62**: 817-820.

Liu Z, Li P, Zhao D, Tang H and Guo J (2010). Protective effect of extract of Cordyceps sinensis in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Behav. Brain. Funct.,* **6**: 61.

Lv Y, Han L, Yuan C and Guo J (2009). Comparison of hypoglycemic activity of trace elements absorbed in fermented mushroom of Coprinus comatus. *Biol. Trace Elem. Res.*, **131**: 177-185.

Müller K (2001). Pharmaceutically relevant metabolites from lichens. *Appl. Microbiol. Biotechnol.,* **56**: 9-16.

Omarsdottir S, Freysdottir J and Olafsdottir ES (2007). Immunomodulating polysaccharides from the lichen *Tamnolia vermicularis* var. subuliformis. *Phyto-medicine,* **14**: 179-184.

Sancho L, de la Torre R, Horneck G, Ascaso C, de Los Rios A, Pintado A, Wierzchos J and Schuster M (2007). Lichens survive in space: Results from the LICHENS experiment. *Astrobiology,* **7**: 443-454.

Xu Q and Guo J (2009). Activity and toxicity of Cr (III)-enriched Grifola frondosa in insulin-resistant mice. *Biol. Trace Elem. Res.,* **131**: 271-277.