



Aqueous extract of *Inocutis levis* improves insulin resistance and glucose tolerance in high sucrose-fed Wistar rats

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ABSTRACT

Introduction: *Inocutis levis* is a polypore fungus belonging to Hymenochaetales of basidiomycetes. The specie is mainly distributed in Asia and grows on living angiosperm trees. This study investigates the effect of aqueous extract of *I. levis* on insulin resistance and glucose tolerance in high sucrose fed (HSF) rats.

Methods: Male rats were given 30% sucrose solution for 12 weeks. The extract of *I. levis* at doses of 50 and 150 mg/kg body weight was intraperitoneally administered for 2 weeks in HSF rats. Blood glucose, serum insulin, insulin resistance index (HOMA-IR) and histological changes of pancreas were evaluated.

Results: Sucrose solution significantly increased the insulin, glucose and HOMA-IR levels, compared to the control. At the dose of 50 mg/kg the extract decreased glucose and insulin, and improved insulin resistance relative to that of HSF rats which did not take the extract. Histological studies on pancreas tissue indicated no significant difference in the size of Langerhans islets of HSF rats and HSF rats treated with the extract at dose of 50 mg/kg compared to the control group. But, treatment with the dose of 150 mg/kg of *I. levis* increased islets diameter and number of cells in the islets of Langerhans in HSF group.

Conclusion: *Inocutis levis* extract may have hypoglycemic effects, by regulating insulin secretion and improving glucose tolerance, and seems to be able to ameliorate pancreatic islets in HSF rats. Our findings indicate that *I. levis* can be considered as a potential treatment with medicinal properties to alleviate insulin resistance and to prevent risk of type 2 diabetes in a dose dependent manner.

Implication for health policy/practice/research/medical education:

The findings indicate that *I. levis* may improve glucose intolerance and might be effective in blood glucose regulation. On the other hands, *I. levis* can regulate insulin secretion in a dose dependent manner. The results also point out that *I. levis* at the dose of 50 mg/kg is able to delay onset of type 2 diabetes. Thus, the administration of *I. levis* may be considered as a potential source of new natural therapeutic agent that can control insulin resistance and reduce the risk of diabetes. Further studies are necessary to discover the active ingredients of *I. levis* extract that can control insulin resistance.

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Introduction

Insulin resistance is associated with lowering insulin sensitivity in liver tissue, adipose tissue and skeletal muscles (1). It has been shown that lack of insulin secretion and insulin resistance can play a critical role in diabetes (2). Pancreatic β -cells produce insulin and maintain blood

glucose concentration (3). Insulin links to the surface of insulin sensitive tissues such as skeletal muscles and liver, and regulates the glucose uptake (2). High glucose serum levels increase toxic reactive oxygen species (ROS) which leads to oxidative stress and diabetes (3). On the other hand, the destruction of pancreatic beta cells is induced

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by oxidative stress (4).

Sucrose, glucose and fructose solutions are normally consumed as sugar in human diets. Yet, it has been shown that diets with high sucrose and high fructose can reduce insulin sensitivity in animals (5), and cause skeletal muscle insulin resistance (6). Moreover, a number of studies have indicated that high fat and high sucrose feed lower the uptake of glucose in muscles (6,7). Likewise, consumption of foods containing high levels of simple sugars can reduce insulin sensitivity (8) and can induce diabetes (5).

Herbs have been used as alternative medicines in various diseases (9). Several mushrooms are regarded as natural health food and have been used as remedies for diseases therapy for thousands of years (10). Many mushrooms are source of metabolites such as polysaccharides, polyphenols, lectins, terpenes, alkaloids, and antibiotics that are considered as natural medicine (11). It has been demonstrated that some mushrooms have anti-tumor, anti-inflammatory, antimutagenic, antihepatotoxic, antidiabetic and antioxidant properties (11,12). Antioxidant compounds can potentially promote β -cells survival in type 1 diabetes (4). Some fungal polysaccharides are assumed to repair the function of pancreatic tissue, increase insulin secretion and improve insulin sensitivity in peripheral cells (10).

Hymenochaetales (belonging to the order Hymenochaetales) is a large family of macrobasidiomycetous fungi with worldwide distribution (11). Several members of this family, especially those belonging to the genera *Phellinus* Quél and *Inonotus* P. Karst., have been intensively studied for their significant medicinal properties (13), and a number of species are consumed in Japan, Korea, China and India (11). *Inocutis levis* (P. Karst.) Y.C. Dai (= *Inonotus levis* P. Karst., *Inonotus pseudohispidus* Kravtzev) is a member of Hymenochaetales family with poroid (polypore) fruiting bodies, the species is mainly distributed in Asia, and grows on living angiosperm trees (14). *I. levis* is more or less widely distributed in Iran, commonly found in urban areas on trunk of plants and elm trees (15). To our knowledge, no studies have yet been done about the effects of *I. levis* on insulin resistance and anti-diabetic properties of *I. levis*. In this study we examined the effect of aqueous extract of *I. levis* on blood glucose level, serum insulin and insulin resistance in sucrose feeding rats.

Materials and methods

Fungal specimens

Fresh fruiting bodies of *I. levis* were collected from trunk of living *Ulmus glabra* in Tehran city during 2015. Specimens were dried using an electric mushroom dryer. Identification was followed by Ghobad-Nejhad and Kotiranta (14). Voucher specimens were deposited in the herbarium unit of Iranian Research Organization for Science and Technology, Tehran (ICH herbarium) under numbers Ghobad-Nejhad 4083, 4084, and 4085.

Animals

Male Wistar rats (150-250 g) were purchased from the Pasteur Institute in Tehran, Iran. The rats were maintained at $24\pm 2^\circ\text{C}$, 50-55% humidity and 12-hour light/dark cycles. Thirty-two rats were divided into 4 groups ($n=8$) including control group, sucrose group receiving 30% sucrose solution (Suc) as drinking water for 12 weeks, sucrose fed groups were intraperitoneally given aqueous extract of *I. levis* at doses of 50 and 150 mg/kg (Suc+Ext) for 2 weeks.

Preparation of aqueous extract of *Inocutis levis*

Dried samples of *I. levis* were powdered. Fine powder (240 g) of *I. levis* was macerated with 200 mL distilled water at room temperature for 3 days and was filtrated with a filter paper. The extraction was evaporated in water bath at $40-50^\circ\text{C}$. The extract was maintained in refrigerator at 4°C .

Biological measurements

Blood glucose level was determined by a glucose test Lifan kit (Lifen, Taiwan,) after overnight fasting. Glucose solution was given to the rats via gavage (1 g/kg body weight). Blood glucose level was determined at 0, 30, 60 and 120 minutes from tail vein. The serum insulin was measured by rat insulin ELISA kit. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated by the following formula: $[\text{Fasting serum insulin } (\mu\text{g/L}) \times \text{Fasting blood glucose (mg/dL)}] / 22.5$.

Histological studies

Pancreas tissues of the rats were fixed in 10% buffered formaldehyde solution. Tissues were embedded in paraffin, sectioned at $5\ \mu\text{m}$ and stained with hematoxylin and eosin (H&E). Slides of all groups were studied with light microscope. The islets diameters were determined using micrometer and the number of islet cells were counted.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc analysis. Statistical significant was accepted at $P < 0.05$. The measurements were presented as the mean \pm standard deviation (SD).

Results

Effect of aqueous extract of *Inocutis levis* on blood glucose

Effect of aqueous extract of *I. levis* on blood glucose in all rats were summarized in Table 1. Sucrose gradually increased glucose levels during 12 weeks. The levels of fasting glucose in serum significantly elevated in high sucrose fed (HSF) groups compared to the control. Also, the levels of fasting glucose significantly reduced in HSF groups that treated with doses of 50 and 150 mg/kg aqueous extract of *I. levis* intraperitoneally for 2 weeks. The results showed that extract of *I. levis* has a preventive effect on increasing of blood glucose levels in HSF rats.

Table 1. Effect of aqueous extract *Inocutis levis* on blood glucose, insulin levels and HOMA-IR in sucrose drinking rats

	Control	Sucrose	Suc+Ext (150 mg/kg)	Suc+Ext (50 mg/kg)
Glucose (mg/dL)	98.75±3.54	115.87±5.19*	97.25±6.22#	93.50±8.84#
Insulin (µg/L)	0.232±0.177	1.356±0.801**	2.450±1.298#	0.399±0.129##
HOMA-IR	1.108±0.85	6.983±2.760**	7.55±0.98#	2.244±0.632###

The data were analyzed using one-way ANOVA and Tukey's post hoc tests. The data are mean ± SD (n = 8).

* Compared to control, # Compared to sucrose drinking rats. *P < 0.05, **P < 0.01, #P < 0.05, ##P < 0.01.

Effect of aqueous extract of *Inocutis levis* on serum insulin level and HOMA-IR

After 12 weeks, fasting serum insulin levels were measured in all groups and the results were summarized in Table 1. In HSF groups, serum insulin levels were higher than the control group. Serum insulin levels in the rats that received 50 mg/kg aqueous extract of *I. levis* for 2 weeks following high sucrose diet decreased compared to sucrose feeding rats. Also, 150 mg/kg aqueous extract of *I. levis* increased serum insulin level in the rats feeding sucrose for 12 weeks compared to the control group and the group that received 50 mg/kg aqueous extract of *I. levis*. Our results show that HOMA-IR was significantly elevated in HSF group and HSF group treated with a dose of 150 mg/kg extract of *I. levis* compared to the control group, but it was lowered in HSF group treated with a dose of 50 mg/kg aqueous extract of *I. levis* for 2 weeks.

Effect of aqueous extract of *Inocutis levis* on oral glucose tolerance

The blood glucose levels were elevated after 30 minutes and lowered at 60 and 120 minutes in the control group. In the rats fed with sucrose solution, the level of blood glucose increased after 30 minutes but at 60 and 120 minutes was higher compared to the control group. Whereas, in HSF rats that were treated with doses of 50 and 150 mg/kg aqueous extract of *I. levis* for 2 weeks, blood glucose level increased after 30 minutes. At 60 and 120 minutes the blood glucose was lowered compared to HSF group (Table 2).

Effect of aqueous extract of *Inocutis levis* on Langerhans islets

Histological study of pancreas showed that islets diameter of pancreas was not changed in HSF rats compared to the

control group and in HSF rats treated with a dose of 50 mg/kg aqueous extract of *I. levis* no alteration in size of islets was observed compared to HSF rats. However, in HSF rats that treated intraperitoneally with 150 mg/kg aqueous extract of *I. levis* for 2 weeks, the islets diameter and number of cells in islets increased compared to HSF rats (Table 3; Figure 1).

Discussion

It is well-known that high sucrose diet induces metabolic disorders such as glucose intolerance, hypertension, hyperlipidemia, insulin resistance and atherosclerosis (7). In this research, high sucrose feeding for 12 weeks induced insulin resistance in the Wistar rats as an animal experimental model (8). In the present study, high sucrose feeding increased fasting blood glucose, serum insulin and HOMA-IR values, glucose intolerance and insulin resistance compared to the control group. The previous studies showed that high fat and/or high fructose diet increased blood glucose, serum insulin and HOMA-IR in rodents after 8 weeks and caused insulin resistance (16,17). In the present study, we showed that the administration of the aqueous extract of *I. levis* at a dose of 50 mg/kg for 2 weeks in HSF rats could significantly lower fasting blood glucose levels, serum insulin and HOMA-IR compared to HSF rats. It seems that *I. levis* may enhance glucose intake in tissues. In a study, Hong et al showed that MT- α glucan extracted from the polypore fungus *Grifola frondosa* (Dicks.) decreased blood glucose level and insulin concentration in a dose dependent manner in KK-A γ mice as a diabetic animal model (18). Our results indicated that the extract of *I. levis* at a dose of 50 mg/kg improved insulin resistance. Treatment with the extract of *I. levis* at a dose of 150 mg/kg led to glucose hemostasis but, increased serum insulin with elevated insulin secretion from β -cells.

Table 2. Effect of aqueous extract *Inocutis levis* on glucose tolerance test in sucrose drinking rats

	Blood glucose (mg/100 mL)			
	Fast	30 min	60 min	120 min
Control	88.85±13.20	103±5.93	73.12±6.08	76.28±14.63
Sucrose	118.28±10.06*	128.5±15.85*	117.16±10.18***	110.42±5.82***
Suc+Ext (150 mg/kg)	100.5±8.14	109.33±7.25	90±6.08##	91.33±12.24##
Suc+Ext (50 mg/kg)	92±13.66	91.12±12.24###	90.75±10.91##	89±7.03##

The data were analyzed using one-way ANOVA and Tukey's post hoc tests. The data are mean ± SD (n = 8).

* Compared to control, # Compared to sucrose drinking rats. *P < 0.05, ***P < 0.001, ##P < 0.01, ###P < 0.001.

Table 3. Effect of aqueous extract *Inocutis levis* on the number of islet cells and the diameter of islets (µm) in pancreatic tissues in the rats

Treatment Groups	Islets diameter (µm)	No. of cells
Control	13.14±6.06	36.05±19.42
Sucrose	15.4±6.72	41.17±21.53
Suc+Ext (150 mg/kg)	19.89±8.54 [#]	90.69±36.73 ^{###}
Suc+Ext(50mg/kg)	12.53±5.48	60.66±17.58

The data were analyzed using one-way ANOVA and Tukey's post hoc tests. The data are mean ± SD (n = 8).

[#] Compared to sucrose drinking rats, *P* < 0.05, ^{###}*P* < 0.001.

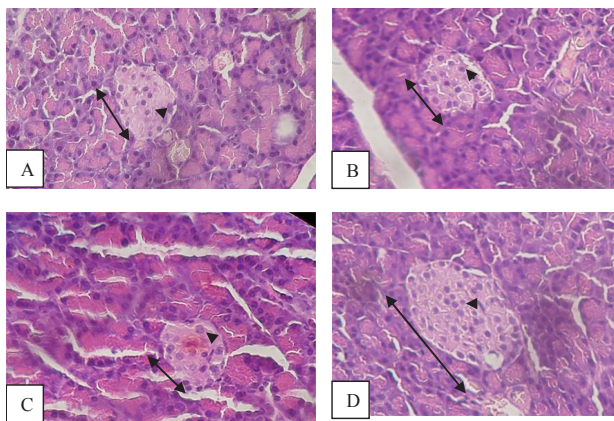


Figure 1. Histological changes in Langerhans islets of pancreas. The rats received 30% sucrose solution as drinking water for 12 weeks, sucrose fed groups were intraperitoneally given aqueous extract of *I. levis* at doses of 50 and 150 mg/kg for 2 weeks. Control (A); Sucrose drinking rats (B); Sucrose drinking rats + *I. levis* 50 mg/kg (C); sucrose drinking rats + *I. levis* 150 mg/kg (D). ↔ The figures show the diameter of Langerhans islets (µm), pancreatic islets cells (arrows). H&E (×40)

It therefore seems that treatment with the extract of *I. levis* at a dose of 50 mg/kg may ameliorate the function of β-cells in pancreatic islets. However, high dose of the extract of *I. levis* stimulates insulin secretion. According to the previous studies, some fungal polysaccharides can bind to pancreatic beta cells and stimulate the production of insulin (19). On the other hand, in another study, Soo et al. have indicated that *Phellinus linteus* (Berk. & M.A. Curtis) suppressed the secretion of insulin in β-cells (9). Also, the use of *Grifola frondosa* and *Pleurotus sajor-caju* (Fr.) extracts has been shown to improve glucose intolerance in diabetic rats (20,21). However, *Polygonatum odoratum* (Mill.), used as antidiabetic agent in Korea, can improve insulin resistance and enhance intake of glucose in skeletal muscles (22). Here, our results indicated that HSF decreased glucose tolerance in rats compared to the control, and treatment with the extract of *I. levis* at doses of 50 and 150 mg/kg in HSF rats ameliorated glucose tolerance compared to HSF rats.

It is known that hyperglycemia can decrease antioxidant enzyme activity and increase superoxide radicals that

cause insulin resistance (16). Moreover, hyperglycemia increases the production of ROS in diabetes (11). Rony et al showed that *Phellinus rimosus* (Berk.) enhanced antioxidant enzymes in tissue and could protect the cells against ROS (12). Our histological studies showed that the diameter of islets and the number of islet cells in pancreas increased in the group treated with a dose of 150 mg/kg extract of *I. levis*, but the extract of *I. levis* at the dose of 50 mg/kg did not alter the diameter of pancreatic islets in HSF rats compared to the control group and sucrose fed rats. It seems that *I. levis* can inhibit the destruction of islets against hyperglycemia. It may also increase the number of β-cells in pancreatic islets and may stimulate the secretion of insulin at the dose of 150 mg/kg. Moreover, the studies indicated that *P. rimosus* protected pancreatic islets against free radicals produced by diabetogenic agents (11). Also, the studies have shown that control of blood glucose in diabetic patients can preserve the structure and function of beta cells in pancreas tissue (23).

This study suggests that the extract of *I. levis* at the dose of 50 mg/kg can improve insulin resistance, enhance insulin sensitivity in tissues and increase glucose uptake but at high dose can be more effective in the function and the structure of the islets and increase insulin secretion and the number of cells in islets. Taken together, our findings indicate that *I. levis* can improve glucose intolerance and seems to be effective in blood glucose regulation. On the other hand, *I. levis* regulates insulin secretion in a dose dependent manner. It seems that *I. levis* enhanced peripheral glucose intake and use. Our results point out that *I. levis* at the dose of 50 mg/kg may be able to delay onset of type 2 diabetes. Thus, the administration of *I. levis* may be considered as a potential source of new natural therapeutic agent that can control insulin resistance and reduce the risk of diabetes. Further studies are necessary to discover the active ingredients of *I. levis extract* that can control insulin resistance.

Authors' contributions

FMM designed the laboratory methods, prepared paper draft and analyzed data. ZEF contributed to data collection, prepared the extract and worked in lab. AS designed animal model. MGN identified mushroom and edited paper.

Conflict of interests

None.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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