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Research Article

HYPOGLYCEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *ASTRAEUS HYGROMETRICUS* (PERS.) MORG. IN ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

Objective: All over the whole world, mushrooms have been cherished for thousands of years both as medicine and as food. In the present study, we report the hypoglycemic effects of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. (Astraeceae), in alloxan-induced diabetic mice.

Methods: *A. hygrometricus* ethanolic extract was administrated by oral route, and levels of blood glucose and body weight were measured. In a separate set of mice, an oral glucose tolerance test (OGTT) was conducted.

Results: Administration of extracts orally reduced the level of blood glucose in diabetic mice treated with alloxan, tested subsequent to acute (24 hours) and subacute (28 days) administration. The treatment with extract furthermore showed better tolerance to glucose.

Conclusion: These outcomes advocate that the extract possesses good hypoglycemic activity.

Keywords: Alloxan-induced diabetic mice, Antidiabetic activity, *Astraeus hygrometricus*, Oral glucose tolerance test.

INTRODUCTION

As the number of people with diabetes multiplies globally, the ailment takes an escalating proportion of countrywide and global health care budgets. It is anticipated to become one of the world's foremost killers and disablers within the next 25 years [1]. Regions with utmost potential are Africa and Asia, where diabetes mellitus (DM) toll may possibly augment to two- to three-folds than the current rates. Apart from presently existing therapeutic options, a lot of herbal medicines have been suggested for the management of diabetes. Conventional plant medicines are used all over the planet for an array of diabetic presentations. Plant foods have been used for the management of diabetes in developing countries where the price of the traditional medicines represents a burden to the people [2].

DM is a chronic malady caused by hereditary and/or acquired insufficiency in the making of insulin by the pancreas, or by the ineffectiveness of the insulin formed. Such a deficit results in augmented concentrations of glucose in the blood that in consequence harm several of the body's systems, specially the nerves and blood vessels [3]. Chronic hyperglycemia during diabetes causes glycation of body proteins, which consequentially leads to secondary complications distressing arteries, kidneys, nerves and eyes [4]. The curative procedures for the management of hyperglycemia consist of use of insulin and other agents such as amylin analogues, alpha glycosidase inhibitors like acarbose, miglitol and voglibiose, sulphonylureas, biguanides, etc. These drugs in addition have some undesirable effects viz., causing hypoglycemia at high doses, hepatic troubles, diarrhea and lactic acidosis [5, 6].

Mushrooms are an assemblage of fleshy macroscopic fungi that until recently, like other fungi, were integrated in the plant kingdom on account of spores and cell wall. Mushrooms have been treasured all through the globe as food and medicine both for thousands of years [7-9]. Mushrooms are exceedingly nutritive and they contain alkaloids, polyphenols, sterols and flavonoids [10]. Mushrooms are low calorie food with very minute fat content having potent antioxidants that can be valuable as a dietary supplement in favor of the patients suffering from a majority of disease conditions like Alzheimer's disease, atherosclerosis, cancer, diabetes mellitus, hypertension, inflammatory conditions, ischaemia, obesity, Parkinsonism and so on [11-15].

Astraeus hygrometricus is an ectomycorrhizal edible wild mushroom commonly known as false earthstar belonging to the Family Astraeceae. The villagers and local people eat it as a healthy food having a belief that consumption of this mushroom could prevent several age related disorders. Previous works concerning *A. hygrometricus* revealed that a water-soluble glucan, Fraction I, isolated from the aqueous extract of the fruit bodies of *A. hygrometricus*, exhibited strong activation of splenocytes [16] and the ethanolic extract showed strong *in vitro* free radical scavenging activity and *in vivo* anti-inflammatory activity [17]. In the present study, an effort was made to assess the hypoglycemic effect of an ethanolic extract of *A. hygrometricus* on blood glucose in alloxan-induced diabetic mice.

MATERIALS AND METHODS

Drugs and chemicals

Glyburide (Ranbaxy Pharma. Ltd., New Delhi, India), alloxan monohydrate (Loba Chemie Ltd., Mumbai, India), one touch horizon glucometer (Johnson & Johnson Ltd., Mumbai, India), and D-glucose (Qualigens Fine Chemicals, Mumbai, India) were purchased from respective companies.

Experimental animals

Swiss albino mice (25–30 g) of roughly identical age were used for the study. They were maintained at a temperature of 25°±3°C and relative humidity of 45% to 55% under 12-h light:12-h dark cycle. Water and feed were provided *ad libitum*. The animals were maintained in line with the guidelines recommended by the Animal Welfare Board and approved by our Institutional Animal Ethical Committee (IAEC) constituted following the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment, Government of India, New Delhi. All the procedures complied with the Declaration of Helsinki, as revised in 1996. It has been established beforehand that incorporation of mushroom into the drinking water and diet has no effect on glucose homeostasis of mice [18].

Acute oral toxicity studies

Healthy adult albino mice of either gender, starved overnight, were subjected to acute toxicity studies as per the guidelines (AOT no. 425) recommended by the Organization for Economic Cooperation and Development [19]. The animals were observed constantly for 2 h for behavioral, neurological and autonomic profiles for any lethality or fatality for the next 48 h.

Extraction

Basidiocarp of *A. hygrometricus* was collected from the local market and from the sal (*Shorea robusta* G.f.) forests of Bankura and West

Midnapore, West Bengal, India. Fresh mushrooms were chosen at random into three samples of 150 g each and air-dried in an oven at 40°C for 48 h. Dried powdered mushroom sample was extracted by stirring with 200 ml of ethanol at 30°C for 24 h at 150 rpm and filtering through Whatman No. 4 filter paper. The residue was subsequently extracted two times by means of additional 200 ml of ethanol as described above. The entire extract was next rotary evaporated to dryness at 40°C, redissolved in ethanol to a concentration of 10 mg/ml, and stored at -20°C for subsequent use [17].

Induction of experimental diabetes

Diabetes was induced by a solitary intraperitoneal injection of freshly prepared solution of alloxan monohydrate at a concentration of 150 mg/kg body weight dissolved in sterile 0.85% saline [20]. After 48 h, the animals showing levels of blood glucose greater than 200 mg/dl (diabetic) were chosen for further experiments. All the animals were permitted open access to tap water and pellet diet.

Collection of blood and determination of glucose content

For blood glucose determination, the blood was obtained by snipping the tail by means of a sharp razor. Blood glucose level (BGL) was determined by means of a one touch horizon glucometer. Levels of glucose were expressed in mg/dl.

Effect of ethanolic extract of *A. hygrometricus* on oral glucose tolerance test (OGTT) in normal mice

Normal mice were separated into five groups (n = 6), viz.: group I, glucose (2.5 g/kg); group II, glyburide (10 mg/kg); group III, extract (250 mg/kg); group IV, extract (500 mg/kg); group V, extract (1000 mg/kg). The animals were fasted overnight previous to initiation of the test. All the animals in different groups were loaded with D-glucose (2.5 g/kg) solution subsequent to half an hour of drug administration. Blood glucose levels were estimated preceding drug administration and at 0, 30, 60, and 120 min following loading of glucose.

Effect of ethanolic extract of *A. hygrometricus* on blood glucose in alloxan-induced diabetic mice

The scheme described by Dunn and McLetchie [21] was adopted. Diabetic Swiss albino mice of either gender were separated into three groups (n = 6), viz.: group I, vehicle (distilled water, 10 ml/kg); group II, glyburide (10 mg/kg); group III, extract (500 mg/kg). All the drugs were given via oral route.

The acute study concerned assessment of blood glucose at 0, 2, 4, 6, and 24 h following administration of drug. The subacute study involved recurring administration of drug for 28 days at prefixed times, and blood glucose levels were estimated on the 7th, 14th, 21st, and 28th days. The data were represented as mean blood glucose level and standard deviation (SD) of the mean. The mice were weighed every day throughout the study period of 28 days, and their body weights were noted. From this data, mean change in body weight ± SD was calculated. The demise of mice was noted in addition for the period of the study, and mortality percentage was calculated.

Statistical analysis

Statistical analyses was performed by student's 't' test and in all the cases results were expressed as mean ± SD (standard deviation) of no less than six individual experimental data.

RESULTS

Acute toxicity studies revealed that the extract was harmless up to a dose level of 5000 mg/kg of body weight. No lethality or any toxic reactions were found up to the conclusion of the period of study. Extract concentration of 500 mg/kg body weight produced the best increase in the glucose threshold, 30-min post–glucose loading in normal mice (Table 1). Consequently, further diabetic mice treatment tests were carried out by means of this dosage.

Table 1: Effect of *A. hygrometricus* on oral glucose tolerance test (OGTT) in normal mice.

Treatment (mg/kg, p.o.)	Mean fasting glucose level (mg/dl) ± SD				
	Before glucose	0 min	30 min	60 min	120 min
Vehicle	108±4	310±15	203±6	141±8	114±9
Glyburide (10)	105±6	294±10	214±14	161±10	122±12
Extract (250)	101±8	311±14	294±7	136±15	139±7
Extract (500)	104±9	293±8	108±12	126±11	112±4
Extract (1000)	99±12	238±9	139±9	119±6	103±5

Values are mean ± SD from 6 mice.

Single administrations of the ethanolic extract of *A. hygrometricus* (500 mg/kg) as well as glyburide (10mg/kg) reduced the levels of blood glucose at 2, 4, and 6 h subsequent to administration (Table 2). Greatest diminution in the blood glucose level was observed at 6 h following the administration of the extract. Subacute administration (once a day for 28 days) of the extract as well as glyburide caused decline in the blood glucose levels as compared with the vehicle-treated set. Highest action of the extract (cutback from 345 to 165 mg/dl) was seen with a decline in blood glucose levels at the dosage of 500 mg/kg on the 21st day (Table 3).

Table 2: Effect of *A. hygrometricus* on blood glucose level in alloxan-induced diabetic mice (acute study).

Treatment (mg/kg, p.o.)	Mean fasting glucose level (mg/dl) ± SD				
	0 h	2 h	4 h	6 h	24 h
Vehicle	319±18	448±30	438±05	457±17	325±21
Glyburide (10)	322±24	217±21	193±27	176±16	245±22
Extract (500)	345±33	177±28	169±23	130±34	222±30

Values are mean ± SD from 6 mice.

Table 3: Effect of *A. hygrometricus* on blood glucose level in alloxan-induced diabetic mice (subacute study). Values are mean ± SD from 6 mice.

Treatment (mg/kg, p.o.)	Mean fasting glucose level (mg/dl) ± SD				
	Day 0	Day 7	Day 14	Day 21	Day 28
Vehicle	319±18	332±12	438±33	245±26	254±15
Glyburide (10)	322±24	204±32	184±22	160±16	163±27
Extract (500)	345±33	214±31	186±12	165±36	176±18

Administration of the vehicle (distilled water, 10 ml/kg, p.o.) in the alloxan-induced diabetic mice resulted in a decline in the body weight all through the phase of 28 days (Table 4). The extract (500 mg/kg) prohibited the reduction in body weight in the alloxan-treated mice. In contrast, the mice gained body weight as compared with the vehicle-treated set that indicated the positive

effect of the extract in preventing further loss of body weight. Administration of the vehicle in alloxan-induced diabetic mice resulted in the demise of 33.3% of the total animals through the 28-day study time. Administration of the extract (500 mg/kg) reduced mortality (16.6%) in mice. It was consequently evident that while no drug was administered, progression of diabetes resulted in death of mice, while treatment by the extract resulted in the cutback of mortality.

Table 4: Effect of extract on body weight in alloxan-induced diabetic mice

Treatment (mg/kg, p.o.)	Mean body weight (g) ± SD				
	Day 0	Day 7	Day 14	Day 21	Day 28
Vehicle	29.708±2.489	29.600±2.902	27.827±2.056	24.252±0.576	18.676±2.218
Glyburide (10)	29.644±1.643	28.952±1.919	29.617±1.574	30.661±1.462	32.053±0.926
Extract (500)	31.424±2.332	30.959±1.689	32.612±2.930	33.409±3.649	31.091±2.609

Values are mean ± SD from 6 mice.

DISCUSSION

In the past, several mushroom varieties had been reported to have hypoglycemic activities in animals [18, 22] and in diabetic patients [23]. It was revealed earlier that *Agaricus bisporus* (J. Lange) Imbach retarded the development of hyperglycemia, body weight loss, polydipsia, hyperphagia, and glycated hemoglobin in the streptozotocin-treated diabetic mice by counteracting decrease in plasma and pancreatic insulin concentration and by improving the hypoglycemic effect of insulin applied exogenously [18]. Gray & Flatt [22] showed that *Agaricus campestris* L. Fr. countered hyperglycemia of streptozotocin induced diabetic mice most likely by an insulin-releasing system. Aqueous fraction obtained from maitake mushrooms was reported to lessen fasting levels of blood glucose in animals [24, 25] and diabetic patients [23]. This effect was suggested to be via insulin or glucose metabolism and/or as a result of enhancing peripheral insulin sensitivity. Huge quantity of glycogen was also observed subsequent to treatment of rats, suggesting the prospect of enhanced formation of glycogen by the mushroom as a possible mechanism of its hypoglycemic effect. The ability of lectins obtained from *A. bisporus* and *A. campestris* to augment the release of insulin by isolated islets of Langerhans from rats had been well-documented [26]. In addition, some assortments of mushrooms were shown to have antihypertensive property in spontaneous hypertensive rats indicative of the likelihood of antidiabetic potential of mushrooms for hypertensive patients [25].

In sufficient quantities and being low in sugars, mushrooms may act as therapeutic foods for patients with diabetes [27]. Phytochemical investigation of *A. hygrometricus* shows the incidence of proteins, minerals, vitamins, carbohydrates, antioxidant phenolic compounds including flavonoids [28, 29]. The hepatoprotective effect of ethanolic extract of the mushroom is mediated through this antioxidant defense mechanism [30]. Moreover, the ethanolic extract is also capable of imparting protection against cardiovascular and cancerous diseases [31, 32]. Two new leishmanicidal and anticandidal compounds from *A. hygrometricus* have also been reported [33]. It also shows antimicrobial activities against other pathogens [34].

In the present study, the hypoglycemic action of the ethanolic extract of *A. hygrometricus* was evaluated in alloxan-induced diabetic mice. Admirable decrease in the blood glucose levels was seen at the second hour, and highest decline occurred at the sixth hour by the action of the extract in case of both acute and subacute study. The extract exhibited short inception and extended duration of hypoglycemic activity. A dose of 500 mg/kg of the extract showed finest action. Subacute treatment for 28 days with the extract in the tested doses brought about improvement in the body weights of diabetic mice, demonstrating its favorable effect in preventing weight loss. Administration of the extract lowered mortality (16.6%) relative to the diabetic mice (33.3%). The protecting effect against diabetes-induced weight loss is supported by prior studies [18]. In the oral glucose tolerance test, the doses improved the tolerance for glucose signifying augmented peripheral glucose utilization in mice. The extract showed the most favorable activity at the dose of 500 mg/kg.

CONCLUSION

To sum up, an oral dose of 500 mg/kg of the ethanolic extract of *A. hygrometricus* possesses strong hypoglycemic action against alloxan-induced diabetes and improved oral glucose tolerance (OGTT model) in mice.

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