Hypoglycemic activity of *Onosma hispidum* (Ratanjot)

Neeraj Kumar, Ajay Kumar Gupta[1], Dhan Prakash, Pankaj Kumar[2]

Department of Pharmacy, Shri Ram Murti Smarak College of Engineering and Technology, Bareilly 243 202, [1]University Institute of Pharmacy. Chatrapati Sahuji Maharaj University, Kanpur, Uttar Pradesh, [2]Faculty of Pharmacy. Moradabad Educational Trust group of Institutions, Moradabad 244001, India

Background: *Onosma hispidum* (Ratanjot) belongs to the family Boraginaceae. The genus *Onosma* has about 150 known species in Asia. It has been found to be effective as an antioxidant, anti-inflammatory, and antimicrobial agent and promotes healing in burns, foot ulcers, and wounds. **Aim:** To study the effect of *O. hispidum* on blood glucose level in glucose-loaded, normal, and hyperglycemic rats. **Settings:** Department of Pharmacy, Bundelkhand University, Jhansi, Uttar Pradesh, India. **Design:** An experimental study was designed. **Materials and Methods:** The methanolic root extracts of *O. hispidum* are compared with glibenclamide for their influence on fasting blood glucose in glucose-loaded, normoglycemic, and alloxan-induced (120 mg/kg i.p) hyperglycemic rats. **Statistical Analysis:** The data were analysed by one way analysis of variance followed by Dunnett's test ($P < 0.01$). **Results:** In glucose-loaded, normal and hyperglycemic rats, the methanolic root extract of *O. hispidum* at the dose of 100 mg/kg p.o. reduced blood glucose significantly as compared to control, and it was almost as effective as glibenclamide. **Conclusion:** The methanolic root extract of *O. hispidum* has hypoglycemic action. As diabetes is associated with hyperglycemia and several other pathological changes such as infections, inflammation, foot ulcers and impairment of wound healing, these additional effects besides hypoglycemic effect of *O. hispidum* may be proved as a breakthrough in the treatment of diabetes. Biochemical and receptor oriented molecular studies are required to find out the exact mechanism of hypoglycemic action of *O. hispidum.*

KEY WORDS: Alloxan monohydrate, blood glucose level, diabetes mellitus, *Onosma hispidum*, Ratanjot

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Correspondence to: **Mr. Neeraj Kumar**, Department of Pharmacy, Shri Ram Murti Smarak College of Engineering and Technology, Bareilly - 243 202, Uttar Pradesh, India. E-mail: neerajsitm@yahoo.com

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Introduction

Diabetes is a major chronic endocrine disorder affecting approximately 10% of human population across the world. It is characterized by hyperglycemia that has reached to epidemic proportion in the present century.[1] According to WHO, diabetes mellitus is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycemia with disturbances in carbohydrate, fat, and protein metabolism,[2,3] the prevalence of diabetes is likely to increase by 35%. Currently, there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year of 2025.[4] In India, WHO suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in 2025.[5]

Several drugs such as biguanides and sulfonylurea[6] are presently available to reduce hyperglycemia in diabetes mellitus but have side effects such as hypoglycemic coma,[7] hepatorenal disturbance,[8] and also not safe for use during pregnancy.[9] Thus, search for a new class of compounds is essential to overcome these problems. The roots of *O. hispidum* are mainly used as edible part, coloring (dye), flavoring agent, folklore, and in traditional medicine.[10,11] Medicinally, the plant has cooling, laxative, anthelmintic, and alexipharmic effects and is also used to treat eye diseases, blood disorders, bronchitis, itch, and abdominal pain.[12] Recently, *O. hispidum* has been reported as antioxidant, antimicrobial, antiseptic, antibacterial and effective in the prevention of skin carcinogenesis, reducing tumor growth, oxidative stress,[13] and wound healing activity.[14]

Materials and Methods

Collection and identification of the plant

Dried roots of *O. hispidum* were purchased from the market of Amritsar, Punjab, India. Its identity was confirmed by Dr. S. Khatoon, Scientist, National Botanical Research Institute, Lucknow, India. The voucher specimen (BU/Pharma/07/1805) has been deposited in the

Department of Pharmacology, Bundelkhand University Jhansi, Uttar Pradesh, India.

Animals

Healthy inbreed Albino Wister rats of either sex weighing from 200 to 280 g were procured from Central Drug Research Institute, Lucknow, Uttar Pradesh, India. They were fed on standard chow and given tap water *ad libitum*. The study was approved by Institutional Animal Ethical Committee for the purpose of control and supervision on experiments on animals (716/02/a/CPCSEA).

Out of 57 animals, 45 were segregated into nine groups of five animals each. Three groups were used for hypoglycemic activity in normal rats. Another three groups were used for glucose tolerance tests, and the remaining three groups were used to study the drug effect in diabetic rats. The rest 12 animals were used for toxicological study and LD_{50} estimation.

Preparation of plant extract

The roots of *O. hispidum* were coarsely powdered and extracted with methanol by using percolator extraction method. The extract was concentrated under reduced pressure using vacuum rotary evaporator (Buchi type) at 50 °C. The final yield of extract was 5.35%. The suspension of methanolic extract was prepared by using 0.5% w/v CMC (SD Fine Chemicals, Mumbai, India) in normal saline solution.[3,15]

Induction of diabetes

Animals of all the groups were weighed, and blood glucose was determined before inducing diabetes. Then diabetes was induced in the animals by administering a single dose of alloxan monohydrate (S.D. Fine Chemicals, Mumbai, India) (dose = 120 mg/kg i.p) intraperitoneally in 0.9% w/v of NaCl solution.[3,16] Fasting blood glucose was measured after 3 days to ensure the induction of diabetes. Blood glucose level was measured by using Accu Check Active™ test strips in Accu Check Active™ test meter. Rats having blood glucose level from 200 to 270 mg/dl were selected for experiments.

Acute oral toxicity study

Acute oral toxicity, at limit test of 2000 mg/kg lethality was observed where more than 70% of the animals died. Hence, next lower dose of 300 mg/kg was tested according to OECD/OCDE/423, and the cut off LD_{50} of extract of *O. hispidum* was found 1000 mg/kg. The sign of toxicity was first noticed after 4–8 h of extract administration.

Hypoglycemic study in alloxan-induced diabetic rats

The diabetic animals were divided into three groups of five animals each. Group 1 received 3% CMC suspension as the control group, Group 2 received glibenclamide 3.0 mg/kg body weight as the standard group, while Group 3 received root extract of *O. hispidum* 100 mg/kg body weight as the test group, in a single dose. The blood samples were collected at 0, 1, 2, and 3 h for estimation of blood glucose levels. Results are presented in Table 1 and Figure 1.

Hypoglycemic study in normal fasted rats

Animals were fasted overnight and were divided in to three groups of five animals each. Group 1 received 3% CMC suspension as control group, Group 2 received glibenclamide 3.0 mg/kg body weight as the standard group, while Group 3 received methanolic root extract of *O. hispidum* 100 mg/kg body weight as test group, in a single dose. The blood samples were collected at 0, 1, 2, and 3 h for estimation of blood glucose levels. Results are presented in Table 2 and Figure 2.

Study of oral glucose tolerance test in normal rats

The overnight fasted normal animals were divided into three groups of five animals each. Group 1 received 3% CMC suspension as control group, Group 2 received glibenclamide 3.0 mg/kg body weight as standard group, while Group 3 received methanolic extract of *O. hispidum* 100 mg/kg body weight as test group, in a single dose. All the groups were loaded with D-glucose (2.0 g/kg p.o.) solution after half an hour of drug administration. The blood samples were collected at 0, 1, 2, and 3 h for estimation of blood glucose levels. Results are elaborated in Table 3 and Figure 3.

Statistical analysis

Results are expressed as mean ± SD. The data were analysed by one way analysis of variance (ANOVA) followed by Dunnett's test, and the results were considered statistically significant at $P < 0.01$.

Results

The median lethal dose (LD_{50}) in Albino Wister rats was calculated to be greater than 1000 mg/kg body weight (OECD). The methanolic root extract of *O. hispidum* roots produced hypoglycemia in alloxan-induced diabetic rats in acute study. Significant reduction (42.7%) in blood glucose level was observed at a dose of 100 mg/kg body weight as compared to control after 3 h. Glibenclamide also produced significant reduction (50.5%) in blood glucose level with a dose of 3.0 mg/kg body weight as compared to diabetic untreated control [Table 1 and

Table 1: Effect of methanolic root extract of *O. hispidum* on blood glucose levels in alloxan-induced diabetic rats

Group	Treatment	Blood glucose level (mg/dl)			
		0 h	1 h	2 h	3 h
1	Vehicle (control)	355.0 ± 22.1	354.3 ± 18.6	357.3 ± 17.8	356.7 ± 20.9
2	*Onosma hispidum*, 100 mg/kg of body weight	378.6 ± 8.2	291.6 ± 1.4*	213.3 ± 6.6**	204.3 ± 5.3**
3	Glibenclamide, 3.0 mg/kg of body weight	374.0 ± 6.6	287.3 ± 3.4*	236.0 ± 10.0**	176.6 ± 4.9**

Values are mean ± SEM, h = hour, wt = weight, n = 5 in each group; significant as compared to control *P < 0.01, **P < 0.001.

Table 2: Effect of methanolic root extract of *O. hispidum* on blood glucose levels in normal fasted rats

Group	Treatment	Blood glucose level (mg/dl)			
		0 h	1 h	2 h	3 h
1	Vehicle (Control)	109.1 ± 7.4	107.6 ± 2.1	105.9 ± 3.1	96.2 ± 3.2
2	*Onosma hispidum* 100 mg/kg of body weight	102.3 ± 2.9	97.8 ± 1.7	85.4 ± 2.7*	73.9 ± 1.6*
3	Glibenclamide, 3.0 mg/kg of body weight	99.2 ± 1.5	94.9 ± 2.8	81.7 ± 0.9*	71.8 ± 2.0*

Values are mean ± SEM, h = hour, wt = weight, n = 5 in each group; significant as compared to control *P < 0.01, **P < 0.001.

Table 3: Effect of methanolic root extract of *O. hispidum* on oral glucose tolerance test in normal rats

Groups	Treatment	Blood glucose level (mg/dl)			
		Fasting	1 h	2 h	3 h
1	Vehicle (control)	78.2 ± 1.6	207.7 ± 13.2	251.1 ± 9.1	198.4 ± 2.6
2	*Onosma hispidum* 100 mg/kg of body weight	78.9 ± 2.0	139.1 ± 8.8	100.6 ± 3.4	97.3 ± 2.6*
3	Glibenclamide, 3.0 mg/kg of body weight	90.5 ± 10.8	134.1 ± 6.9	95.9 ± 5.2	79.4 ± 2.5*

Values are mean ± SEM, h = hour, wt = weight, n = 5 in each group; significant as compared to control *P < 0.01, **P < 0.001.

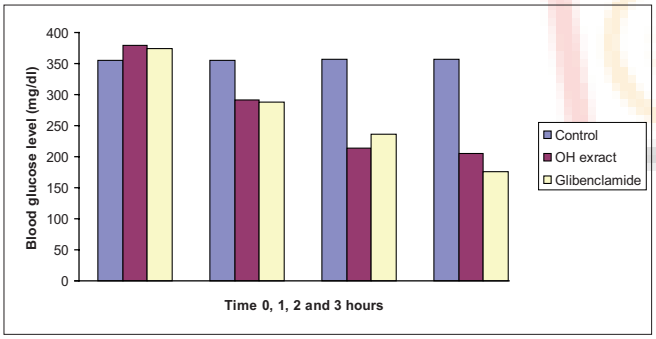

Figure 1: Effect of methanolic root extract of *O. hispidum* (OH) on blood glucose level in diabetic rats

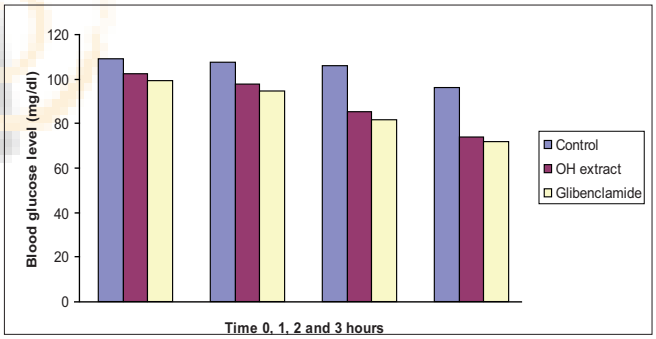

Figure 2: Effect of methanolic root extract of *O. hispidum* (OH) on blood glucose level in normal fasted rats

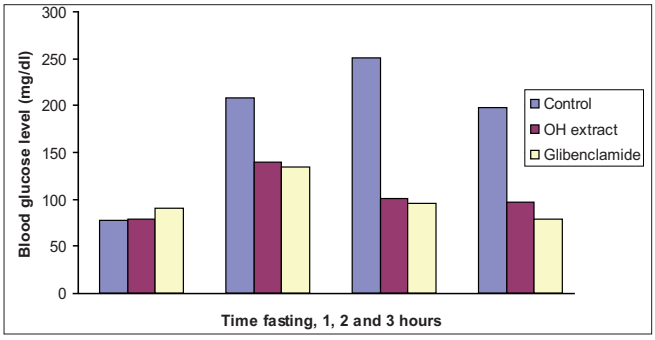

Figure 3: Effect of methanolic root extract of *O. hispidum* (OH) on oral glucose tolerance test in normal rats

Figure 1]. At a dose of 100 mg/kg, the hypoglycemic effect was observed up to 3 h after a single dose followed by a gradual increase in blood glucose level.

Administration of plant extract was found to reduce blood glucose level (23.2%) in normal fasted rats at a single dose after 3 h. The maximum reduction (25.3%) in blood glucose was observed after 3 h of standard drug glibenclamide administration as compared to the normal control [Table 2 and Figure 2].

The *O. hispidum* root extract at a dose of 100 mg/kg body weight reduced blood sugar (50.9%) significantly after 3 h as compared to normal control. Similarly, glibenclamide-treated rats also showed significant blood glucose lowering efficacy (60.0%) after 3 h in glucose-

loaded rats [Table 3 and Figure 3]. The administration of *O. hispidum* root extract showed a gradual decrease of blood sugar level in glucose-loaded normal rats. The initiation of hypoglycemic effect was observed after 1 h with maximum effects being seen at 3 h. The results also indicate the efficacy of methanolic root extract of *O. hispidum* in the maintenance of glucose levels in normal and alloxan-induced diabetic rats.

Discussion

The populations of developing and under developed countries widely use indigenous medicinal plants as their alternative therapy. In India, hundreds of plants or their combinations are used traditionally for the management of diabetes.[17] Ratanjot is widely used as spice in Indian kitchen for providing color and flavor to the foodstuffs and also permitted by food and drug administration. From various studies, it has been reported that *O. hispidum* has antibacterial,[13] cholinesterase inhibitory,[12] antitussive, and wound healing activity.[14] The results of present studies indicated that the root extract of *O. hispidum* is capable of reducing (23.2–50.9%) blood glucose level associated with diabetes in different experimental models [Tables 1–3]. The *O. hispidum* root extract showed a gradual decrease of blood glucose level after 1, 2, and 3 h of its administration in normal, glucose-loaded normal, and alloxan-induced diabetic rats. The maximum hypoglycemic effect was observed after 3 h followed by a gradual increase in all the three models.

Chemically, alloxan is 2,4,5,6-tetra-oxo-hexahydropyrimidine, a beta-cytotoxic substance that induces diabetes by librating free oxygen radicals, which cause lipid peroxidation-mediated pancreatic injury.[18] The root extract of *O. hispidum* may scavenge free radicals, facilitate regeneration of pancreatic cells, and to release more insulin, ultimately resulting in antidiabetic effect. The methanolic root extract of *O. hispidum* significantly decreases blood glucose level both in normal ($P < 0.01$) and alloxan-induced diabetic ($P < 0.01$) rats after 1, 2, and 3 h. The results showed significant hypoglycemic effect and glucose tolerance.

Conclusions

The methanolic root extract of *O. hispidum* showed promising antidiabetic activity as evident by its blood glucose lowering effect in diabetic as well as normal rats and increases glucose tolerance. Hence, long-term studies on *O. hispidum* and its purified individual phytochemicals are necessary to elucidate the exact mechanism of action and to find some potent hypoglycemic drug.

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