Antidiabetic Potential of *Onosma hispidum* Roots

F Hussain, A Ali, M Shahid

ABSTRACT

Objective: To investigate the antihyperglycemic, antihyperlipidemic and pancreatic beta cells regeneration potentials of *Onosma hispidum* (ratanjot) root extracts in alloxan-induced diabetic rabbits.

Methods: Among treatment groups, group 1 was vehicle with normal healthy rabbits as negative control. Second group had diabetic control (diabetic rabbits as positive control) rabbits. Diabetic rabbits treated with 100 mg/kg body weight methanolic extract of *O. hispidum* and 100mg/kg body weight *n*-haxane extract of *O. hispidum* were third and fourth groups. The groups treated with *O. hispidum* extracts were also compared against glibenclamide (oral antidiabetic agent) as a standard. Blood glucose, triacylgylcerides, cholesterol, HDL-C and LDL-C were measured by standard methods.

Results: Diabetic group treated with methanol extract showed significant reduction in blood glucose levels (29.94%; $p < 0.05$) and TAG (40.08%; $p < 0.05$) as compared to treated group and controls. However, *O. hispidum* treatment did not normalize these parameters in diabetic animals as compared to synthetic drug. Histopathological examination showed trivial pancreatic beta cells restoration activity.

Conclusions: *O. hispidum* roots have the potential to lower blood glucose and lipid profile in diabetic condition. Extensive natural explorations are encouraged in the quest for dietary adjuncts for diabetes management.

Keywords: Alloxan, antihyperglycemia, diabetes mellitus, *Onosma hispidum*, pancreatic beta cells

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INTRODUCTION

Diabetes mellitus (DM) has reached pandemic proportions worldwide. Hyperglycemia, hyperlipidemia and beta cells impairment is central to the development and progression of DM, as a result of the combined effects of genetic and acquired factors (1, 2). DM related microvascular and macrovascular complications are the major causes of morbidity and mortality. Wound healing in people with DM is often a prolonged curative dare (3). Currently, botanical explorations are encouraged in the quest for natural dietary adjuncts as functional foods for diabetes management. Numerous medicinal plants have been used for the management of DM in traditional medicine as they are a great source of biological constituents. Medicinal plants with antidiabetic activities are more desired, owing to lesser side-effects and low cost. Literature surveys demonstrated the benefit of several ethnobotanicals with varying degrees of antidiabetic bioactivities and different mechanisms of action (4-6). Despite the tremendous developments in medicinal chemistry, traditional medicine is still a common practice for the treatment of diabetes (7, 8).

Onosma hispidum (Boraginaceae), perennial, found at dry, rocky slopes (1700 - 4000 metres height) in Kashmir, South East Asia is used as a medicinal plant. *Onosma hispidum* or ratanjot possess antiseptic, antipyretic, antibacterial, antihelmintic, hypoglycemic and cholinesterase inhibitory activities. Its role in foot ulcer, optical diseases, bronchitis and itch are well recognized (9-14). Although antihyperglycemic potential of *O. hispidum* has been extensively investigated, studies on some very important biochemical aspects are missing. It is hypothesized that it can also demonstrate a protective role against pancreatic beta cells damage. This study aims to assess hypoglycemic, hypolipidemic and pancreatic beta cells regenerative potentials of *O. hispidum* in alloxan induced diabetic rabbits.

MATERIALS AND METHODS

Plant material and preparation of extracts

Onosma hispidum Wallich roots were collected from Bara Dawakhana, Karkhana Bazaar, Faisalabad, Pakistan. Plant was identified by an expert botanist Dr. Mansoor Hameed, Associate Professor, Department of Botany, University of Agriculture, Faisalabad, Pakistan and voucher specimen was kept for future reference. Shade-dried and powdered *O.hispidum* (50 g) was homogenized with 150 mL methanol and *n*-hexane. The final yields of the extracts were 5.7% and 3.6% for methanol and *n*-hexane respectively. The suspensions of extracts were prepared in 0.5% w/v carboxymethylcellulose in normal saline solution.

Animals and induction of diabetes mellitus

The animal experiments were approved by the Bioethical Committee, University of Agriculture, Faisalabad, Pakistan. Healthy adult male rabbits (*Oryctolagus cuniculus*) weighing 1.5 kg were procured from Jhang Bazar, Faisalabad, Pakistan. Animals were housed in the metal cages (three per cage) with free access to standard feed and tap water *ad libitum.* The animals were acclimatized to the laboratory conditions prior to experimentation in the animal house, Institute of Microbiology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan. Diabetes mellitus was induced by administering alloxan monohydrate (80 mg/kg body weight) in 5% normal saline through marginal ear vein. Alloxan monohydrate was procured from Sigma Chemical Co., St. Louis, MO, USA.

Fasting blood glucose levels were documented on alternative days until hyperglycemia was observed. The rabbits having fasting blood glucose levels more than 150 mg/dL were considered as diabetic.

Experimental setup and biochemical analyses

The overnight fasted animals were randomly divided into five groups (seven animals per group). Group 1 was vehicle with normal healthy rabbits as negative control. Second group had diabetic control (diabetic rabbits as positive control) rabbits. Diabetic rabbits treated with 100 mg/kg body weight methanolic extract of *O. hispidum* (D+OHM) and 100mg/kg body weight *n*-haxane extract of *O. hispidum* (D+OHH) were third and fourth groups. The groups treated with *O. hispidum* extracts were also compared against glibenclamide (oral antidiabetic agent) as a standard (D+G group). Biochemical assays were performed at Clinico-Medical Laboratory and Bioassay Section, Protein Molecular Biology Laboratory (PMBL), Department of Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan

Blood glucose, triacylglycerides (TAG), total cholesterol (TC), HDL- Cholesterol (HDL-C) were measured by kit methods (Ecoline Diasys GmbH Merck, Germany). LDL-C concentrations were calculated (15) as: Total cholesterol- HDL.Cholesterol- VLDL.Cholesterol. Whereas, VLDL-Cholesterol was calculated (Singh et al. 2008) as: Triacylglyceride/5.

Immediately after the slaughtering, the pancreas was removed and placed in 10 % formalin to fix the tissue for the histopathological examination. The paraffin sections were prepared in an automatic tissue processor and sliced into 5 mm thick sections in a rotary microtome and then stained with hematoxylin-eosin dye (Merck, Germany) and mounted with Canada balsam. The histopathological examination of the slides was performed under a photomicroscope and photographed (16). The parameters examined were degeneration, infiltration, proliferation and bleeding in pancreas.

Statistical Analyses

Data was expressed as mean ± S.D or mean and analyzed by t-test with significance level set at P<0.05 (SPSS; version 12.0, 2003 ® SPSS Inc., Chicago, IL, USA).

RESULTS

In present investigation, main focal points were antidiabetic (hypoglycemic, hypolipidemic), pancreatic beta cells regenerative efficacy of *O. hispidum* extracts. Alloxan administration induced expected increase in blood glucose in diabetic animals as compared to the vehicle. The extracts of *O. hispidum* showed hypoglycemic activity in tested animals (table 1). Administration of methanolic and *n*-hexane plant extracts reduced blood glucose levels up to 29.94% and 27.11% respectively. The maximum reduction (47.48%) in blood glucose was observed after glibenclamide administration ($p < 0.05$) as compared to the normal control.

Diabetic group treated with methanol extract showed significant reduction in TAG (40.08%; $p < 0.05$) as compared to *n*-hexane treated group (26.33%) and controls. Changes in TC by both extracts were comparable (22.47 *vs.* 24.59%). However, hypotriacylglyceridemia (50.97%) and hypocholesterolemia (37.89%) induced by glibenclamide was more pronounced ($p < 0.05$) than plant extracts. About 40.34%, 38.08% and -17.94% ($p < 0.05$) changes in HDL-C by methanol, *n*-hexane plant extracts and glibenclamide were determined. *O. hispidum* extracts treatments declined HDL-C. Contrary to that, antidiabetic drug raised HDL-C but lowered LDL-C concentrations (table 1).

The micro-anatomical changes in pancreatic tissues are presented in Fig 1(a-d). In normal group, pancreas was composed of serous acini and they were arranged in distinct lobules, interlobules, interlobular connective tissues, ducts and pancreatic islets of Langerhans. Pancreatic

islets composed of alpha and beta cells could be easily differentiated (a). In diabetic control group, the islet is atrophic with a central hyaline degenerative core. The beta cells are scattered and irregularly shaped and the usually orderly cell-to-cell relations within the islets are disrupted (b). In *O. hispidum* extracts treated groups, complete histopathological picture of pancreas was damaged. Pancreatic acini and islets were degenerated showing vacuoles in cytoplasm. There was uniform deposition of amyloid in degenerated pancreatic islets (c). In glibenclamide treated group, majority of the pancreatic beta cells were damaged. Serous acini and pancreatic islets of Langerhans were degenerated. Only some ducts were distinct in connective tissue (d).

DISCUSSION

Hypoglycemic action of *n*-hexane and methanolic extracts of *O.hispidum* (ratanjot) was investigated in alloxan induced diabetic rabbits. A comparable hypoglycemic effect was evidenced from the data obtained after oral administration of the extracts and glibenclamide. The treatments produced significant attenuation of the glucose ($P < 0.05$), suggesting that the hypoglycemic effect may be mediated through stimulating insulin synthesis and/or secretion from the pancreatic cells of langerhans. The hypoglycemic effect was observed to be slow but sustained, without any risk of developing severe hypoglycemia. However, it was not in compliance with the findings of Kumar et al. (14). They observed 42.7% reduction in blood glucose levels in rats by methanolic root extract of *O. hispidum*. This proved that *O.hispidum* could play an important role in treating diabetic/hyperglycemic patients. A short period of drug therapy improved glucose levels better than plant extracts and glibenclamide produced significant reduction ($P < 0.05$) in blood glucose

level as compared to the diabetic untreated control which received only vehicle suspension. The hypoglycemic activity of *O.hispidum* extracts is not comparable to that of glibenclamide.

Antidiabetic plants bestow diverse hypoglycemic mechanisms due to bioactive chemical compounds such as mucilages, glycans, proteins, pectins, flavonoids, steroids, triterpenoids, alkaloids, other nitrogen compounds and miscellaneous diverse bioactive compounds. At present, it is not known which phytoconstituents contributed to hypoglycemic action of *O.hispidum*. Some feasible mechanisms attribute it to the presence of flavonoids, alkaloids, steroid and glycoside principles, though this requires further investigation to sort out the bioactive lead compound and its mechanism (17, 18).

Hyperlipidemia is a common complication of alloxan-induced diabetes mellitus in experimental animals. Expected rise in TAG under diabetic conditions was lessened after *O. hispidum* treatment with methanolic extract being more effective than *n*-hexane. Furthermore, both solvents instigated almost parallel changes in TC. Profound decline in TAG and TC were observed by reference drug administration. HDL-C and LDL-C were decreased by plant treatment in tested animals. This was a quite surprising response, as former should elevate being good cholesterol. Glibenclamide enhanced HDL-C and declined LDL-C in diabetic rabbits as compared to negative, positive controls and treated groups. This can be inferred that synthetic drug has the better ability to normalized hyperlipidemia. Rectification of dyslipidemia is another feature of the study. The hypolipidemic effect may be due to elevation in HDL-C levels as this is good cholesterol.Some polysaccharides and flavonoids are identified as bioactive components responsible for hypolipidemic effects (19, 20).

Results of this study indicated that tested *O.hispidum* extracts not only possessed significant hypoglycemic effect but also had remarkable hypolipidemic effect in alloxan diabetic

rabbits. The actual ingredient present in *O.hispidum* extracts for blood glucose and lipid lowering potentials is not delineated in this study. Further investigations are desired to elucidate the comprehensive course of antidiabetic impact of ratanjot.

Onosma hispidum is well known for its medicinal potential, but the mechanism underlying its amelioration of type 2 diabetes is still elusive. Antidiabetic activity of most plants is attributed to the presence of polyphenols, flavonoids, terpenoids, coumarins and other phytoconstituents. Their mechanisms of action include insulin secretagogue activity, stimulation of synthesis and release of insulin from pancreas, insulin like activity, enhanced insulin binding on insulin receptor, beta cells rejuvenation, regeneration and stimulation (20, 21). Antidiabetic potential of *O. hispidum* appears to extrahepatic in present study, as it did not restore beta cell derangements in diabetic animals. Extracts either did not have the phyto-constituents responsible for pancreatic beta cells restoration or proper solvent and treatment time were required for obvious histopathological improvements.

Limitations of the present study include the fact that constant extract dosage was used. Variable test compound concentrations and thus increase in sample population could give more comprehensive data. The inferences of the study, nonetheless gives valuable information on *O. hispidum* that can widen the natural products domains.

CONCLUSION

In summary, the present study has shown that *O. hispidum* extracts demonstrate clear hypoglycemic and hypolipidemic effects in alloxan-induced diabetic rabbits. Although major

bioactive principles involved are yet to be determined, this investigation reveals the potential of *O. hispidum* for use as a natural antidiabetic agent.

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AUTHORS' NOTE

F Hussain conceived paper, finalized study design, supervised data collection and analyses, wrote manuscript and finalized it. A Ali participated in study design, samples collection and analyses, approval of final manuscript version. M Shahid participated in data analysis, revision of manuscript and approval of final version.

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Table 1: Comparative glucose and lipid profile of control and treatment groups

		Study Groups				
		Vehicle	**Diabetic**	**D+OHM**	**D+OHH**	**D+G**
Glucose	**Initial**	99.5 ± 13.12	178.6 ± 8.50	169.3 ± 12.89	177.0 ± 1.13	198.6 ± 8.14
	Final*	104.7 ± 13.23	187.3 ± 9.60	118.6 ± 8.96	129.0 ± 7.54	104.3 ± 10.40
TAG	**Initial**	88.19 ± 5.19	154.66 ± 6.02	171.33 ± 9.42	162.00 ± 7.81	159.74 ± 9.25
	Final*	89.15 ± 6.37	149.33 ± 7.50	102.6 ± 10.96*	119.33 ± 8.73	78.32 ± 5.7
TC	**Initial**	53.14 ± 10.58	200.33 ± 15.01	207.66 ± 8.08	225.00 ± 5.29	199.25 ± 6.38
	Final*	54.67 ± 7.64	221.33 ± 13.20	161.00 ± 7.0	169.66 ± 7.5	123.75 ± 2.80
HDL-C	**Initial**	28.66 ± 3.7	45.33 ± 8.5	39.66 ± 7.38	41.30 ± 3.05	43.7 ± 2.85
	Final*	27.99 ± 7.21	43.66 ± 10.21	23.41 ± 3.05	25.57 ± 2.07	51.54 ± 1.67
LDL-C	**Initial**	6.85 ± 1.92	124.07 ± 2.2	133.73 ± 1.57	151.27 ± 3.12	123.61 ± 2.99
	Final*	8.83 ± 5.61	132.98 ± 0.99	116.80 ± 2.43	120.47 ± 2.11	56.55 ± 2.97

*$p < 0.05$ as compared to diabetic control.
Data expressed as mean (mg/dL) ± SD of triplicate measurements for groups of seven animals each.
TAG: triacylglyceride; TC: total cholesterol; D+OHM: Diabetic group given O. *hispidum* methanol extract; D+OHH: Diabetic group given *O. hispidum n*-hexane extract; D+G: Diabetic group given glibenclamide.