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Original Research Article

Extracts of *Neptunia prostrata* Linn. ameliorates progression of diabetes mellitus and hyperlipidemia in animal models

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ABSTRACT

Background: The herb *Neptunia prostrata Linn.* belonging to the family Mimosaceae has been used in folkloric medicine in the North-eastern states of India of Assam, Tripura and Meghalaya by indigenous herbal healers since time immemorial but there is a scarcity of any background study documenting its use as an antimicrobial herb. For the same, plants were collected and authenticated.
Methods: Following identification of these herbs methanolic, ethanolic, pet ether and chloroform extracts were prepared using soxhlation. Acute toxicity study as per OECD guidelines 420 was assessed in wistar albino rats and in swiss albino mice (n=5) of both sexes at doses of 2000 mg/kg body weight and did not reveal any morbidity or mortality in the animals within the stipulated period. Phytochemical screening was performed on all four extracts of *Neptunia prostrata*.
Results: Phytochemical constituents depicted presence of glycoside, flavonoids in only ethanolic, methanolic and chloroform extracts. Alkaloids were present in the chloroform extract. The antidiabetic and antihyperlipidemic activity was performed by HFD-STZ models in rats. The herbs showed antioxidant activity comparable to standard antioxidants in-vitro such as Ascorbic acid (Vitamin C) with comparable IC_{50} values.
Conclusions: Results of the antidiabetic shows immense potential in animal models and therapeutics and the antibacterial screening suggests conspicuous and potent putative role in the therapeutics of a vast plethora bacterial infections that need to be corroborated for the expansion of future prospective *in vivo* studies with larger sample size.

Keywords: *Neptunia prostrata*, Antioxidant, Antidiabetic, Antihyperlipidemic, HFD-STZ model, Antimicrobial

INTRODUCTION

The practice of indigenous medicine has time and again focused on herbs for their innate antimicrobial activity against a plethora of bacteria and moulds since ages.[1,2] *Neptunia Prostrata*, is one such herb growing in the states of Assam, Tripura of India and is regardedasa variant of the "Touch-me not plant" *Mimosa pudica* known locally by the synonym "Water-Mimosa". The leaves of this *herb* have found use in folkloric medicine and ancient Vedic texts since time immemorial.[3]

The herb being studied *Neptunia prostrata Linn.* (Synonym: *Neptunia oleracea* belonging to family *Mimosaceae*) is macroscopically a miniature aquatic herb that floats by its white spongy structure and has been reported to be a source of antioxidants.[4-8] Since traditionally antioxidants in aerial parts of many herbs have reported a close link to antimicrobial or antibacterial

properties this led us to objective of the study to elucidate any such properties associated with leaves this herb.

For the evaluation of the antimicrobial activity of the leaf extract of *Neptunia prostrata* literature review revealed that the indigenous ethnic groups of Tripura cultivate this plant both as vegetable as well as medicinal plant and prepare various tasty delicacy dishes with this vegetable. The plant has been used for different types of remedies like gastritis, acidity, constipation, dysentery, and also reported to possess hepatoprotective, analgesic and antimicrobial activity.[10,11]

METHODS

Acute toxicity study as per OECD guidelines 420

The standard method of oral acute toxicity study was contacted on rodents as per OECD guideline no 420. Wistar albino rats of both sexes (n=5) were administered 2000 mg/kg dose of the *Neptunia prostrata* extract (NP-1) used for the study and observed for 14 days for mortality of any behavioural abnormalities.

Identification of plant materials

The herbarium containing the dried plant materials were processed and identified as *Neptunia prostrata* respectively by the office of the botanical survey of India, Shibpur and a voucher was accorded the number BST/Herb/2019/003 respectively. A copy of the same was maintained for further use.

Phytochemical screening of extracts

Phytochemical screening is routinely performed in investigations to ascertain the first line of tests dealing with the chemical identification of the medicinally active substances found in medicinal herbs. Some of bioactive substances that can be derived from plants can be flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.[12-15] Table 1 showed phytochemical obtained from NP-1 and NP-2 extracts of the herb.

Dried leaf extracts (methanolic, ethanolic and hydro-alcoholic) of *Neptunia prostrata* were subjected to phytochemical screening to check for the broad chemical categorisation.

Antioxidant assay

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced antioxidants like vitamin C, vitamin E; carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.[16,17]

The free radical scavenging activity was found to be high in methanolic extract for DPPH, hydroxyl, nitric oxide, superoxide, hydrogen peroxide (H_2O_2) radical. The content of total phenols, flavonoids and tannins was also found to be high in methanolic extract which may be correlated to its antioxidant activity.[18- 20]

Determination of DPPH radical scavenging activity

DPPH radical scavenging assay is a widely used method to evaluate the free radical scavenging ability of natural compounds. This assay is based on the measurement of the scavenging ability of antioxidant substances towards stable radical. The free radical scavenging activity of the extracts was examined in vitro using DPPH radical.[21,22]

The free radical DPPH (1,1-Diphenyl 1-2-pieryl-hydrazil) 0.1 mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3ml of various concentrations of extracts of *Neptunia prostrata* (25, 50, 75, 100, 125, 150 µgm/ml) of ethanol extracts. After thirty minutes after being put in the incubators. Then absorbance was measured at 517 nm. Percentage of inhibition calculated by comparing absorbance of controls and test samples.[23]

The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.[24]

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} values are defined as the concentrations in (microgram/ml) of extracts that inhibits the formation of DPPH radicals by 50%.[25,26]

RESULTS

Diphenyl pycrylhydrazyl (DPPH) assay of the extracts

The IC_{50} value of the extract was 11.2 µg/ml whereas the same for ascorbic acid was 18.02 µg/ml. Table 1 summarizes the phytochemical from NP-1 and NP-2 extracts of the herb.

Table 1: Extracts used for phytochemical screening.

Phytoconstituents	NP1 extract	NP2 extract
Tanins	-	-
Alkaloids	++	++
Flavonoids	+++	+
Glycosides	+++	++

Whereas Table 2 summarizes the indications of the antidiabetic effect of intervention and control groups across treatment groups and depicts prominent antidiabetic effect of the extracts. Tables 3-5 respectively shows the

effect of the extracts on lipid profile parameters like cholesterol, triglycerides with prominent statistically significant changes observed in metformin, glimepiride and crude test drug extract groups. Indicated the presence of alkaloids, glycosides and flavonoids in NP 1 and NP 2 leaf extracts respectively.

Table 2: Antidiabetic effect of intervention across treatment groups, (n=6).

Variables	Control	Diabetic control	Metformin	Glimepiride	Extract
Before induced diabetes	103.0±3.404	98.10±3.843	101.9±1.651	104.8±2.304	105.6±1.408
After induced HFD	103.2±3.272	130.2±3.428***	119.3±3.674*	118.7±4.848*	121.2±3.170**
STZ induced after HFD	103.2±3.272	305.7±5.863***	298.0±10.50***	301.4±10.29***	306.0±10.39***
After treatment	103.0±3.404***	302.6±11.29	106.8±2.081***	98.94±1.647***	97.19±1.186***

N=5, *p<0.05, **p<0.01, ***p<0.0001

Table 3: Effect of interventions of serum cholesterol levels.

Variables	Control	Diabetic control	Metformin	Glimepiride	Extract
Before induced diabetes	149.6±7.102	159.5±5.735	160.3±4.503	172.0±5.120*	172.4±3.645*
After induced diabetes	149.9±7.402	242.9±4.233***	238.9±3.356***	247.4±4.193***	230.6±1.878**
After treatment of diabetes	149.6±7.102***	250.3±4.366	219.4±5.053**	225.1±2.941**	207.8±2.419***

N=5, *p<0.05, **p<0.01, ***p<0.0001

Table 4: Effect of interventions of serum triglyceride levels.

Variables	Control	Diabetic control	Metformin	Glimepiride	Extract
Before induced diabetes	74.22±3.234	73.77±2.677	75.63±3.976	81.35±2.762	77.99±2.330
After induced diabetes	74.21±3.238	227.7±5.937***	222.2±3.631***	232.3±4.195***	229.9±5.111***
After treatment of diabetes	74.02±3.054***	232.9±6.763	211.0±4.347*	213.5±2.907*	209.4±3.059*

N=5, *p<0.05, **p<0.01, ***p<0.0001

Table 5: Effect of interventions of serum triglyceride levels.

Variables	Control	Diabetic control	Metformin	Glimepiride	Extract
1st day wt.	155.0±9.354	158.0±15.86	174.0±12.98	196.0±14.27*	171.0±12.39
After 14 days wt	154.0±9.138	193.0±13.56*	198.0±14.97*	214.0±5.788***	201.0±7.483**
After 28 days wt.	156.0±8.718	230.0±14.58**	231.0±9.274***	236.0±4.848***	229.0±8.426***
After treatment wt.	157.0±9.165	254.0±8.860***	198.0±10.32*	227.0±4.359***	204.0±7.649**

N=5, *p<0.05, **p<0.01, ***p<0.0001

DISCUSSIONS

There is indeed a great dearth of credible literature related to reporting about the use of *Neptunia prostrata* from North eastern states of India. Only a handful number of studies focussed on the antioxidant nature of the leaves and other aerial parts but real novelty of study lies in linking the antidiabetic effect and antimicrobial effects on select strains of bacteria (Both gram positive and negative) to its already known pharmacological properties which may justify *in-vitro* prominent antioxidant profile of herb.[24,25]

Previous studies on *Neptunia prostrata* focussed on the anthelminthic and antimicrobial property of the herb but this is the first study on the putative antidiabetic potential of the herbs of *Neptunia prostrata*. The herb is edible and easily available making it an automatic choice for use by the local inhabitations of North-eastern states of India like Assam, Tripura, Manipur and Meghalaya where it is found abundantly.

The study was primarily aimed at assessing the plausible antidiabetic role of extracts of leaves of *Neptunia prostrata Linn* (NP). This facet of the medicinal herb has not found mention in reports and studies. Study by Juliana et al proposed a similar study methodology which was also used during our study design preparation. Gross phytochemical screening tests revealed the presence of

flavonoids and tannins predominantly in the leaves of the plant. However, as limitation of study may be not isolating and characterising any lead moiety that is responsible for bioactivity of NP and study of any isolated medicinal lead moiety could have been facilitated and ground for connecting potential antioxidant nature and antibacterial property of herb extract on basis of its structure and chemistry as reported in previous studies.[26] Findings reported clearly a definite antidiabetic effect of the extracts as seen in fasting (FBS) and post prandial (PPBS) and glycosylated haemoglobin (HbA1c) parameters.

The study stands out to report the safety of the herb through oral acute toxicity study as per OECD guidelines-420 and secondly with respect to the strong antioxidant and practically comparable IC_{50} values with standard antioxidants (such as ascorbic acid) and significant antihyperlipidemic effects (based on effects on cholesterol, triglyceride and body weight).

From the above-mentioned study, we came to know about the preliminary phytochemical screening of the extracts of the plant and also the antidiabetic effects of the plant in preclinical murine models. The IC_{50} value of the extract was 11.2 µg/ml whereas the same for Ascorbic acid was 18.02 µg/ml for the DPPH assay. With regards to the hydrogen peroxide antioxidant assay IC_{50} value of extract of *Neptunia prostrata* (NP-2) was 101.03 µg/ml while IC_{50} value of ascorbic acid (Vitamin C) was found to be about 93 µg/ml.

Acute toxicity study as per OECD guidelines 420 was assessed in Wistar albino rats and in Swiss albino mice (n=5) of both sexes at doses of 2000 mg/kg body weight and did not reveal any morbidity or mortality in the animals within the stipulated period pointing out towards its possible safety profile. This backs up the study findings which revealed *Neptunia prosatrata* as a safe drug with a high LD_{50} and underscores the need for such relatively non-deleterious natural products in therapeutics for the management of diabetes mellitus in humans.

Possible limitations of the study may lie in translational value of these preclinical findings and the absence of genetical models of diabetes in rats for induction and testing and possibly a larger sample size of animals for the work. The pharmacological in-vivo activities of the plant and its toxicity studies as per OECD guidelines need to be ascertained using a larger sample size using rodent model of diabetes mellitus to corroborate the present findings and reaffirm its putative role in therapeutics.

CONCLUSION

The extract of *Neptunia prostrata* showed pronounced and clinically significant antidiabetic, antihyperglycemic and antihyperlipidemic effects. It is to be seen, however, whether the same extract at this experimental or elevated doses can retard long standing diabetic complications namely retinopathy, neuropathy, nephropathy and microvascular and macrovascular complications usually seen in clinical settings. Further studies will be warranted to decipher its true role in phytotherapeutics.

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