Strychnos nux-vomica seeds: Pharmacognostical standardization, extraction, and antidiabetic activity

Rajesh Bhati¹, Anupama Singh², Vikas Anand Saharan³, Veerma Ram⁴, Anil Bhandari⁴
¹,⁴Department of Pharmaceutical Chemistry, ²Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan; ³Department of Pharmaceutical Sciences, Sardar Bhagwan Singh P.G. Institute of Biomedical Sciences & Research, Balawala, Dehradun, Uttarakhand, India

ABSTRACT

Background: Strychnos nux-vomica, commonly known as kuchla, contains strychnine and brucine as main constituents. Minor alkaloids present in the seeds are protostrychnine, vomicine, n-oxysterchnine, pseudostrychnine, isoxysterchnine, chlorogenic acid, and a glycoside. Seeds are used traditionally to treat diabetes, asthma, aphrodisiac and to improve appetite.

Objective: The present study was aimed to evaluate the various pharmacognostical characters and antidiabetic activity of S. nux-vomica seed. Materials and Methods: Pharmacognostical characters were performed as per the WHO guideline. Extraction was carried out in petroleum ether, chloroform, alcohol, hydroalcoholic, aqueous, and phytochemical constituents present in extracts were detected by different chemical tests. Among these extracts hydroalcoholic, aqueous extracts were evaluated for antidiabetic activity on the basis of extractive yield and phytoconstituents, in alloxan-induced diabetic rats using gliclazide as standard. Results: Various analytical values of S. nux-vomica extract were established. Phytoconstituents present in S. nux-vomica extracts were detected. Conclusion: S. nux-vomica extracts show antihyperglycemic activity in experimental animals.

Key words: Antidiabetic activity, alloxan, extract, kuchla

INTRODUCTION

The dried seeds of S. nux-vomica (family Loganiaceae) are commonly known as kuchla. Kuchla contain 2.6%–3% total alkaloids, out of which 1.25%–1.5% is strychnine, 1.7% is brucine, and the rest are vomicine and igasurine. Some other minor alkaloids are α-columbine, β-columbine, 3-methoxyicajine, protostrychnine, novacine, n-oxysterchnine, pseudostrychnine, isoxysterchnine, chlorogenic acid, and glycoside, loganin. Crude drug kuchla is used in the treatment of anemia, lumbago, asthma, bronchitis, constipation, diabetes, malarial fever, skin disease, paralysis, muscle weakness, and appetite loss.[8] Suppressive effect of S. nux-vomica was observed on induction of ovalbumin-specific IgE antibody response in mice.[9] Strychnine was identified and analyzed in detoxified kuchla seeds using liquid chromatography–electrospray mass spectrometry.[10] Strychnine and brucine were separated and quantified in S. nux-vomica by nonaqueous capillary electrophoresis.[11] Matrix-assisted laser desorption/ionization (MALDI) and-time of flight mass spectrometry (TOFMS) analysis of crude and processed S. nux-vomica seeds allowed rapid screening of alkaloid components.[12]

Literature review revealed that S. nux-vomica has been used traditionally for treating diabetes.[1,3,8-11] There has only been one paper reported describing the antidiabetic potential of Strycnos nux-vomica in methanolic extract.[12] This study was aimed to establish pharmacognostical parameters and explore antidiabetic potential of aqueous and hydroalcoholic extracts prepared from the seeds of S. nux-vomica. The antidiabetic activity was determined in alloxan-induced diabetic rats, and gliclazide was used as a positive control.
MATERIALS AND METHODS

Plant material
The seeds of *S. nux-vomica* were purchased from market of Jodhpur. The plant materials were authenticated by Dr. S. N. Mishra, senior scientist, medicinal and aromatic plants project, K.N.K. College of Horticulture, Mandsaur, Madhya Pradesh, India, and deposited at the Department of Pharmacognosy, Jodhpur National University, Jodhpur, India.

Pharmacognostical parameters
Pharmacognostical parameters, namely, powder characteristics, ash values, loss on drying (LOD), extractive values, and fluorescence analysis for *S. nux-vomica* were estimated as per WHO guidelines.[13]

Extraction and phytochemical investigation
The plant material was ground in mixer. The crude drug powder, passed through sieve no. 10 and retained on sieve no. 60, was used for extraction. The powdered drug was packed in Soxhlet extractor and extracted using solvents like petroleum ether, chloroform, ethanol, ethanol (50%) and water.[14] Different ash values were determined to find the inorganic content in the sample [Table 1]. Fluorescence analysis of *S. nux-vomica* seeds powder was done [Table 2]. Solvents of different polarity were used to find out the extractive value for seeds and rhizome of *S. nux-vomica* [Table 3]. Phytoconstituents present in extracts were detected by different chemical tests [Table 4].

Antihyperglycemic effect of plant extracts

**Animals**
The study was conducted on healthy albino rats of either sex weighing 150–250 g. Prior approval by institutional animal ethical committee was obtained for conduct of experiments vide protocol number 2009–10/09. The animals were kept in separate cages in animal house under standard conditions.

**Induction of diabetes**
Diabetes was induced in the rats by administering 110 mg/kg of alloxan intraperitoneally to the rats, which were kept for 24 h fasting prior to administration. After 72 h, the blood samples were collected and analyzed for blood glucose. Albino rats which showed more than 200 mg/dL blood glucose were considered as diabetic and further used in our studies.

**Experimental procedure**
Healthy albino rats were marked and randomly distributed into 5 groups each consisting of 7 animals in each group. The blood samples were collected randomly to avoid any bias. The groups were divided as following:
- Group I: Normal Control
- Group II: Diabetic Control
- Group III: Standard drug (Gliclazide, 10 mg/kg)
- Group IV: Diabetic rats, treated with 50% Ethanolic extract of *S. nux-vomica* (3.6 mg/kg).
- Group V: Diabetic rats, treated with aqueous extract of *S. nux-vomica* (3.6 mg/kg).

**Blood sampling and biochemical analysis**

**Preparation and administration of drug.**
Distilled water was used as vehicle used for administration of aqueous and 50% ethanolic extract of *S. nux-vomica*. Drug was administered using oral feeding needle.

Blood samples were collected at day 0, 4, and 10 from animals by retro-orbital plexus of rats with the help of microcapillary tubes. The blood samples were collected in microcentrifuge tubes (Eppendorf tubes) and centrifuged for 10 min at 10,000 rpm. Blood glucose was estimated by GOD–PAP (glucose oxidase/phenol/4-amino-antipyrine) method using Elitech glucose SL kit with autoanalyzer (EC-5 plus V2 model, Erba, Mannheim, Germany) at filter range 500–550 nm. The blood glucose level was expressed as mg/dL of blood.

**Statistical analysis**
The data obtained in the studies was subjected to one-way analysis of variance (ANOVA) for comparison among different groups. P value <0.05 was considered significant and results were expressed as mean±SD [Figure 1].

RESULTS

**Results of pharmacognostical, extraction and phytochemical investigations of *S. nux-vomica***
The powder characteristics show the presence of lignified trichomes, aleurone grains, oil globules, and endosperm with plasmodesma [Figure 2].

Various analytical values and fluorescence analysis are presented in [Table 1 and 2] respectively. Extractive values and extractive yields of *S. nux-vomica* seeds in solvents of different polarity are reported in [Table 3]. Carbohydrate, protein, oil, steroid, alkaloid, resin, strychnine and brucine were detected in phytochemical screening [Table 4].

Thin-layer chromatography and high-performance liquid chromatography analysis with chemical controls may be conducted in ongoing studies for thorough phytochemical investigations.

**Antihyperglycemic effect of plant extracts**
The hydroalcoholic and aqueous extract of *S. nux-vomica*
(3.6 mg/kg) had shown significant reduction in blood glucose level in alloxan-induced diabetic rats [Figure 1]. The percent reduction of blood glucose levels in groups III, IV, and V at day 4 and 10 showed significant difference (P<0.01) when compared with percentage of blood glucose reduction at day 0 within the same group. Groups IV and V showed a significant difference in reducing blood glucose level when compared with standard control group III at P<0.05.

**DISCUSSION**

Medicinal plants have been widely claimed useful and found effective in the treatment of diabetes mellitus in various traditional systems of medicine. Various species of *Strychnos* have been reported traditionally for the treatment of diabetes.[1,3,8] The other species of the same genus, that is, *S. potatorum*, was used in the treatment of diabetes and it was proved effective.[15,16] In a recent published report, methanolic extract of *S. nux-vomica* has been proved as having a potential antidiabetic activity and antioxidant property. Considering these facts, the present study deals with the evaluation of diabetic activity of the aqueous and hydroalcoholic (50% ethanol) extract of seed powder of *S. nux-vomica* in alloxan-induced diabetic rats.

**Figure 1:** Blood glucose levels in different groups on different days of treatment. Notations on x axis: I: Normal control; II: Diabetic control; III: Standard drug; IV: *Strychnos nux-vomica* ethanolic (50%) extract; V: *Strychnos nux-vomica* aqueous extract. Blood glucose values are presented as mean±SD; *represents significant percentage reduction of blood glucose level when compared with day 0 at P<0.01

**Table 1: Analytical values**

|                | Total ash | Acid-insoluble ash | Water-soluble ash | Loss on drying | Swelling index |
|----------------|-----------|--------------------|-------------------|----------------|---------------|
| **S. nux-vomica Seeds** | 4.23 ± 0.25 % w/w | 3.06 ± 0.05% w/w | 0.93 ± 0.05% w/w | 7.83 ± 0.28% w/w | 53.33 ± 11.54% v/v |
| **S. nux-vomica Rhizome** | 1.16 ± 0.12 % w/w | 2.56 ± 0.19% w/w | 2.85 ± 0.22% w/w | 22.29 ± 0.58% w/w | 24.63 ± 0.40% w/w |

**Table 2: Fluorescence analysis of *S. nux-vomica* seeds powder**

| Drug+solvent | Visible | Short UV | Long UV |
|--------------|---------|----------|---------|
| Drug         | No      | No       | Green   |
| Drug+ethanol | No      | No       | Green   |
| Drug+ethanolic sodium hydroxide solution | No | Green | Green |
| Drug+di. hydrochloric acid | No | No | No |
| Drug+di. nitric acid | No | No | No |
| Drug+di. sulfuric acid | No | No | No |
| Drug+petroleum ether | No | No | Green |
| Drug+chloroform | No | No | No |
| Drug+potassium hydroxide solution | Green | Green | No |
| Drug+acetic acid | No | Green | Green |
| Drug+water | No | No | No |
| Drug+sodium hydroxide solution | Green | Green | No |

**Table 3: Extractive values of *S. nux-vomica* seeds and rhizome in different solvents**

| Solvent       | Petroleum ether | Chloroform | Ethanol | Ethanol (50%) | Water |
|---------------|-----------------|------------|--------|---------------|-------|
| *S. nux-vomica Seeds* | 1.1 ± 0.10 %w/w | 1.6 ± 0.10 %w/w | 2.2 ± 0.15 %w/w | 9.1 ± 0.50 %w/w | 8.4 ± 0.36 %w/w |
| *S. nux-vomica Rhizome* | 1.16 ± 0.12 %w/w | 2.56 ± 0.19 %w/w | 2.85 ± 0.22 %w/w | 22.29 ± 0.58 %w/w | 24.63 ± 0.40 %w/w |
extract of *S. nux-vomica* may be due to the presence of these active ingredients. In our studies, significant reduction of blood glucose was observed on day 4 and 10 of treatment in groups III, IV, and V, which proved significant antidiabetic activity with the gliclazide, 50% ethanolic extract of *S. nux-vomica*, and aqueous extract of *S. nux-vomica* when compared with group II. Both the extracts were found effective and significantly (*P*<0.05) superior in reducing the blood glucose when compared with standard (gliclazide) control drug on day 10.

There was a significant increase in the superoxide dismutase and catalase levels and a significant decrease in the lipid peroxide level after the administration of methanolic seed extract of *S. nux-vomica*. Thus *S. nux-vomica* possesses antioxidant property, which prevents lipid peroxidation that leads to protein modification and damage.[12]

The hypoglycemic activity of ethanol extract of *S. potatorum* was related to the effect of ethanol extract on diabetic-induced rat liver alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels, which significantly reduced to almost normal level after 15 and 30 days of treatment.[16] The serum insulin and cholesterol levels were not significantly modified upon treatment with the plant extract, suggesting that the hypoglycemic activity of *S. potatorum* is independent of insulin secretion.

Therefore, it is anticipated that lipid peroxidation may provide scientific rationale for the use of *S. nux-vomica* as an antidiabetic plant.

**CONCLUSIONS**

Pharmacognostic parameters for *S. nux-vomica* seeds were estimated, which are helpful in identification and standardization. The aqueous and hydroalcoholic extracts showed the presence of steroids, alkaloids, and glycosides in *S. nux-vomica* seeds. Aqueous and hydroalcoholic extracts were superior to positive diabetic control in controlling blood glucose level on day 10. In conclusion, the present study indicates that hydroalcoholic and aqueous *S. nux-vomica* seed extracts, administered *per os*, are effective in controlling diabetes.

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