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Research Article

EFFECT OF STRYCHNOS NUX VOMICA LEAF EXTRACT ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Background: *Plants have been used as a source of drug for the treatment of Diabetes Mellitus in developing countries where the cost of conventional medicines represents a burden to population. Management of Diabetes Mellitus is a global problem and successful treatment is very essential for preventing or at least delaying the onset of long term complications of the disorder. Strychnos nuxvomica Linn is a plant used in the traditional system of medicine in India for the treatment of Diabetes mellitus.*

Aim: *The Anti-diabetic property of Strychnos nuxvomica along with its antioxidant property is studied.*

Materials and Methods: *The extract was initially subjected for preliminary phytochemical analysis followed by toxicological evaluation. Wister rats were made use of to study the antidiabetic activity.*

Result and Discussion: *Preliminary Phytochemical analysis of the methanolic extract of leaf showed that the plant has a rich possession of phytochemicals like alkaloids, reducing sugars, tannins and phenols. Terpenoids, steroids, gums and mucilage were absent in the extract. Acute oral toxicity studies reveal that methanolic leaf extract did not produce any mortality or signs of toxicity at the dose of 500mg/kg b.w.p.o in experimental rats.*

Conclusion: *The extract of Strychnos nuxvomica showed significant anti-diabetic activity with acute oral toxicity studies revealing that Strychnous Nuxvomica Methanolic leaf extract did not produce any mortality or signs of toxicity at the dose of 500mg/kg b.w.p.o in experimental rats.*

KEYWORDS: *Anti-diabetic, Strychnos nuxvomica, Toxicity, Phytochemicals.*

INTRODUCTION

In drug discovery and development, medicinal herbs have consistently been considered the leading source of pharmaceuticals employed in the treatment of various human diseases due to their chemical diversity and broad biological functionality [Dheer.R, Nema RK, Dheer M, 2006]. There is a worldwide "green revolution" and is reflected in belief that herbal remedies are safe and less damaging to the human body than synthetic drugs [Chakrabarti.S *et al*., 2005]. According to WHO herbal medicines are defined as finished, labeled medicinal products that contain active ingredient, aerial or underground part of plants or other plant material or their combinations [Kavitha J.V *et al*., 2007]. The annual herbs sales have skyrocketed and the global traditional market is growing at the rate of 7-15% annually. In the present scenario, herbal drugs are claimed for almost every disorder ranging from diabetes to rejuvenators [Nicholas A.B, Colledge, Walker R, 2008].

The use of medicinal plants for the treatment of Diabetes Mellitus dates back from the Ebers Papyrus of about 1550 BC. Ethno botanical knowledge played a particular important role in historical Diabetes [Vijayakumar M, Govindarajan R, Rao G, 2006]. The medicinal plants might provide a useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as a dietary adjuvant to existing therapies [Sy GY *et al*., 2005].

Insulin has been proved to be effective to some extent in increasing the life expectancy of diabetic patients but not a permanent solution since there are many drawbacks of this therapy [Lawrence L, Goodman & Gilman's, 11th edition]. Also the therapy with oral hypoglycemic agents is not satisfactory [Sathoskar, Kale, Bhandarkar's 7th edition]. Thus the search for a new therapeutic agent devoid of adverse effects originating from pants used in traditional medicines would be of interest [Chattopadhyay S, Ramanathan M, Das J, 1997].

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Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species [Rib G, Vogel F, 2nd edition]. Several antiiflammatory, digestive, antinecrotic, antidiabetic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant or radical scavenging mechanism as a part of their activity [Santhakurarai P, Prakasam A, Pugalendi KV, 2003]. Keeping this in view one such plant *Strychnos nuxvomica* which possess good antioxidant property was chosen for study and evaluated for its antidiabetic and antioxidant potential [T.E. Wallis, 5th edition].

Taxonomical Classification of *Strychnos Nuxvomica* [Kokate CK, 5th edition]:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliposida
Subclass : Asteridae
Order : Gentianales
Family : Loganiaceae
Genus : *Strychnos* L.
Species : *Strychnos nuxvomica* L.

Morphology and Chemical Constituents [Ecobichon DJ, 2nd edition]:

The tree grows wild on the hills of Malabar coast in Travancore and Cochin and also on the Coromandal coast in the district of Ganjan, Godavari and Nellore. It is a perennial tree with globular suit inside which is a seed with a silky surface due to radially arranged, densely covered, closely appressed unicellular lignified covering trichomes. The leaves are pinnate 5-10cm long. The flowers are small yellow colored. The plant parts used for extract are seeds and leaves. The leaves contain alkaloids strychnine and brucine. The seeds contain about 3% volatile oil and also chlorgenic acid. The leaves contain alkaloid strychnine and also glycoside loganoside to the extent of 4-5%.

MATERIALS AND METHODS

Collection and authentication of plant material:

The leaf of *Strychnos nux vomica* was obtained from the plants which have grown in the wild Thiruvallur district. The plant material was identified and authenticated by Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy) Central Research Institute (Siddha) Aurambakkam, Chennai- 600106.

Preparation of plant extract:

The leaves were dried in the shade. The dried leaves were powdered and weighed quantity of the powder was subjected to cold maceration extraction. Methanol was used as the solvent. Before and after the extraction, the marc was completely dried and weighted. The extract was concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The methanol extract yielded brown semisolid residues and the extract was preserved in a refrigerator till further usage.

Experimental animals:

Inbred adult Wistar albino rats (150-280g) of both sex were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet fed and tap water was provided. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Fasting animals were deprived of food for 16 hours but were allowed to free access to water.

The project was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation of Animals). **IAEC Reference No: IAEC/XIII/03/CLBMCP/2008-2009 dated 16/06/2008.**

Preliminary Phytochemical Analysis:

Methanolic extract of Strychnos nux vomica leaves were subjected to preliminary analysis to test for the presence or absence of alkaloids, carbohydrates and reducing sugars, steroids, proteins, tannins, phenolic compounds, flavanoids, flavones, gums and mucilage, glycosides, saponins, triterpenoids by the usually performed standard tests.

Acute oral toxicity study:

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). The acute toxic class method is a stepwise procedure with three male animals per step. Depending upon the mortality and or moribund status of animals, an average of 2-3 steps may be necessary to allow judgment for the acute toxicity of test substance. The method used defined doses (5, 50, 500mg.kg body weight) and the results allow a substance to be as ranked and classified according to the Globally Harmonized System (GHS) for which causes acute toxicity.

Experimental procedure:

Wister rats of male sex weighing 100-280g were used for the study. The starting dose level of methanolic extract was 500mg/kg body weight p.o as most of crude extract poses LD 50 value more than 500mg/kg p.o. dose value was administered 0.5ml/mg body weight to overnight fasted rats with 0.5%w/v SCMC. Food was withheld for further 3-4 hours after administration of methanolic extract and observed for signs and toxicity. Body weights of rats before and after termination were noted and any changes in the skin and fur, eyes and also respiratory, circulatory, autonomic and CNS and somatomotor activity and behavior pattern were observed and also signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

Pharmacological studies:

Induction of Diabetes Mellitus in experimental animals:

Adult inbreed wister albino rats (32 number) of either sex were overnight fasted and received a freshly prepared solution of Streptozotocin 35mg/kg in Citro-phosphate buffer pH 4.5, injected intraperitonially. After injection the animals had a free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia, normal rats (6 rats) received 1ml citrate buffer as vehicle. The development of diabetes was confirmed after 48hours of the Streptozotocin injection. The animals fasting glucose levels were more than 200mg/dl selected for experimentation. Out of the 32 animals subjected for diabetes induction, 6 animals died before grouping and 2 animals were omitted from the study due to sub-diabetic condition. Of the remaining 24 animals 4 groups were formed and used for the experimentation. In the present study, Glibenclamide (0.4mg/kg body weight) was used as the standard drug.

Determination of blood glucose levels:

Blood was collected from tip of the tail vein and fasting glucose level was measured using single touch glucometer based on glucose oxidase method.

Effects of subacute treatment on blood glucose levels in STZ induce diabetic rats:

The animals were divided into 5 groups'

Group I: Normal control animals received 0.5% CMC 5ml/kg body weight for 14 days.

Group II: STZ (35mg/kg body weight) induced animals received 0.5% CMC 5ml/kg body weight for 14 days.

Group III: STZ (35mg/kg body weight) induced animals received glibenclamide 0.4mg/kg body weight for 14 days.

Group IV: STZ (35mg/kg body weight) induced animals received

Group V: STZ (35mg/kg body weight) induced animals received

The above mentioned treatment schedule was followed for the respective groups of animals for 14days. Blood samples were collected overnight fasted animals on the 0^{th}, 7^{th}, 15^{th} day to estimate blood glucose levels using glucometer.

RESULTS

Preliminary Phytochemical analysis of methanolic leaf extract of *Strychnos nuxvomica*:

The result of preliminary phytochemical analysis of leaf extract of *Strychnos nuxvomica* is shown in Table 1. Methanolic extract shows the presence of alkaloids, glycosides, tannins and sterols. However the extract shows negative test for proteins, saponins, carbohydrates, mucilage and gums.

Acute oral toxicity:

The acute oral toxicity was done according to OECD 423 guidelines (acute toxicity class method). A single administration of a starting dose of 500mg/kg bw/p.o of methanolic extract was administered to 3 male rats and observed for 14 days. There was no considerable change in body weight before and after treatment of the extract and no signs of toxicity were observed. The results are shown in Table 2.

Effect of subacute treatment of methanolic extract of the leaves of *Strychnos nuxvomica* on blood glucose level in STZ induced diabetic rats:

In the subacute toxicity study STZ induced diabetic rats were treated with the methanolic extract 50mg and 100mg/kg bw/p.o, for a duration of 14 days. Treatment with 50mg significantly ($p < 0.01$) decreased the blood glucose level after 7^{th} day onwards. Treatment with methanolic extract 100mg produced significant ($p<0.01$) drop in blood glucose level after 7^{th} day onwards. Treatment with Glibenclamide 0.4mg/kg produced a significant ($p<0.05$) decrease in blood glucose level after 7^{th} day onwards and thereafter. Results are shown in Table 3.

Table No. 1: Preliminary Phytochemical analysis of methanolic leaf extract of *Strychnos nuxvomica*

S. No.	Constituents	Test	Methanol
1	**Alkaloids**	a. Mayer's reagent	Present
		b. Dragendroff's reagent	Present
		c. Hagner's reagent	Present
		d. Wagner's reagent	Present
2	**Carbohydrates**	a. Molisch's reagent	Absent
		b. Fehlingh's reagent	Absent
		c. Benedict's reagent	Absent
		d. Barfode's reagent	Absent
3	**Proteins**	a. Biuret test	Absent
		b. Millon's reagent	Absent
4	**Steroids**	a. Libermann's burchard test	Absent
		b. 5% Potassium Hydroxide	Absent
5	**Phenols**	a. Ferric chloride	Absent
		b. 10% sodium chloride	Absent
6	**Tannins**	a. 10% Lead acetate solution	Present
		b. 10% sodium chloride	Present
7	**Flavanoids**	a. Amyl alcohol + sodium acetate + ferric chloride	Present
		b. Conc. Sulphuric acid	Present
8	**Gums and mucilage**	a. Swelling test	Absent
9	**Glycosides**	a. Glacial acetic acid + ferric chloride + conc. Sulphuric acid	Present
10	**Sterols**	a. 5% potassium hydroxide	Present
11	**Saponins**	a. Foam test	Absent
12	**Terpenes**	a. Tin + thionyl chloride	Absent

Table No. 2: Results of Acute Oral Toxicity studies (OECD 423 guidelines)

S. No.	Treatment	Dose	Weight of animal (in gms)		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1	**SNM**	0.5g/kg	100	100	No signs of toxicity	Nil	Nil	14days
2	**SNM**	0.5g/kg	125	130	No signs of toxicity	Nil	Nil	14days

Table No. 3: Effect of subacute administration of SNM extract on STZ induced diabetic rats

S. No.	Groups	0th day	7th day	14th day
1	Group 1	67 + 2.3	92.31 ± 6.0	106.5 + 7.9
2	Group II	252.21 ± 6.3^{a*}	273.1 ± 3.31^{a**}	291.01 ±4.11^{a*}
3	Group III	240.0 ±6A^{b*}	176.1 ± 7.1^{b*}	116.0±2.1^{b*}
4	Group IV	246.3 ± 1.23^{b**}	198.6± 3.11^{b**}	154.4 ± 5.12^{b**}
5	Group V	241.1±6.13^{b*}	178.8±7.41^{b**}	120.0± 4.41^{b*}

The values are expressed as mean ± SME (Standard Mean Error) of four animals; a - Group II compared with Group I; b - Group III, IV, V as compared with Group II

Statistical significant test for comparing was done by one way ANOVA followed by Dunnet's multiple comparison test using Graph pad Prism software, inc version 4.03, 1992-2005. **P<0.01, *P<0.05.

DISCUSSION

Many species have been reported to present anti-diabetic activity. Working on the same line we have undertaken a study on *Strychnos nuxvomica* for its anti-diabetic property along with its antioxidant potential [Mustafa A, Didem D Orhan N, 2007]. Preliminary Phytochemical analysis of the methanolic extract of leaf showed that the plant has a rich possession of phytochemicals like alkaloids, reducing sugars, tannins and phenols. Terpenoids, steroids, gums and mucilage were absent in the extract.

Acute oral toxicity studies reveal that methanolic leaf extract did not produce any mortality or signs of toxicity at the dose of 500mg/kg b.w.p.o in experimental rats. The SNM extracts at doses 50 and 100mg/kg b.w.p.o did not significantly suppress blood glucose levels in overnight fasted normoglycemic animals but showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in rate of intestinal glucose absorption, achieved by an extra of a pancreatic action including stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process.

There is increasing evidence that Streptozotocin causes diabetes by rapid depletion of β cells through DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocytes in the inflammatory focus [Annie S, Rajendran K, Rakesh B, 2006].

A single dose of two concentration of methanolic extract did not bring about significant hypoglycemic action. In the sub-acute study, Glibenclamide treatment bought down the sugar levels from the first day of the treatment. SNM 50mg and 100mg treatment produces significant reduction in blood glucose levels from 7th day treatment and a steady decrease was observed thereafter. Another possibility for the activity may be due to presence of phytochemicals like flavanoids, tannins, glycosides and alkaloids. The study on the blood glucose level showed that the methanolic extract has a hypoglycemic activity in the Streptozotocin induced diabetic rats.

CONCLUSION

Treatment of SNM extract of leaf showed moderate hypoglycemic effects on normal animals and significant improvement in glucose tolerance on glucose fed hyperglycemic rats. Acute oral toxicity studies reveals that SNM leaf extract did not produce any mortality or signs of toxicity at the dose of 500mg/kg b.w.p.o in experimental rats. Based on the obtained results and observations we can infer that the leaf of the plant under study, *Strychnos nuxvomica* could be used for the treatment or supportive therapy for Diabetes mellitus. Further studies are required to establish the antihyperglycemic activity of *Strychnos nuxvomica* in terms of molecular mechanisms involved in the activity.

REFERENCES:

1. Rema Dheer, Nema RK, Manoj Dheer, *et al*. Healing power of herbs: let us not antagonize it. Plant Indica. **2006**;4:1-4.
2. Chakrabarti S, Kanti Biswas T, Tapan S, et al. Antidiabetic activity of *Caesalpinia bonducella* F in chronic type II diabetic model in long Evans rats and evaluation of insulin secretgogue property of its fractions on isolated islets. J Ehnopharmacol **2005**;97:117-122.
3. Kavitha JV, Joseph F, Rosario *et al*. Hypoglycemia and other related effect of *Boswelia Glabra* in alloxon induced diabetic rats. Ind J Physiol & Pharmacol **2007**;51(1):29-39.
4. Nicholas A Boon, Colledge, Walker R. David"s Principle and practice of Medicine Elsvier 14:806-846.
5. Vijayakumar M, Govindarajan R, Rao G. Action of *Hydrophilia auriculata* against strptozo to tin-induced oxidative stress. J Ethnopharmacol **2006**;11:356-361.
6. Arthur C, Guyton. Textbook of Medical Physiology 8th edition, Saunders Company. **2007**;855-867.
7. Sy GY, Cisse A, Nogonierma RB, Sarr M, Mbdj NA. Hypoglycemic and Antidiabetic activity of acetonic extract of *Vernonia Colorata* leaves in normoglycemic alloxan-induced diabetic rats. J Ethnopharmacol **2005**; 171-175.

8. Sutharason L, Hariharan RS, Vamsadhara. Drug utilization study in diabeteology outpatient setting of a tertiary hospital. Ind J Pharmacol **2003**;237-240.
9. Rang HP, Dale MM, Ritter JM et al. Pharmacology 4th edition, Churchchill Livingstone, Elsvier limited. **2003**; 380-385.
10. Lawrence L Goodman & Gilman's. The Pharmacological basis of therapeutics, 11th edition, McGRAWHILL, **2005**; 1613-1646.
11. Sathoskar, Kale, Bhandarkar's. Pharmacology and Pharmacotherapeutics, 7th editton popular prakashan, **2000**;901.
12. Chattopadhyay S, Ramanathan M, Das J, *et al*. Animal models in experimental diabetes. Ind J Exper Biol **1997**; 35:1141-1145.
13. Rib G, Vogel WF; Drug discovery and evaluation, 2nd edition; 947-1051.
14. Santhakuraari P, Prakasam A Pugalendi KV. Modulation of oxidative stress parameters by treatment with piper betle leaf in streptozocin induced diabetic rats. Ind J Pharmacol **2003**;35:373.
15. T.E.Wallis; Text book of Pharmacognosy 5th edition, CBS Publishers and Dsitributors, **2001**;205-207.
16. Kokate CK, Practical Pharmacognosy, 5th edition, Vallabh Prakasham, Delhi; **1991**;107-121.
17. Ecobichon DJ; The basis of toxicity testing, 2nd edition, CRC press, New York, **1997**;43-88.
18. Mustafa A, Didem D, Orhan N. Invivo antidiabetic and antioxidant potential of *Helichrysum Plicatum* sp. Plicatum capitulums in Streptozotocin induced diabetic rats. J Ethnopharmacol **2007**;109:54-59.
19. Annie S, Rajendran K Rakesh B. Effect of aqueous bark extract of *Garuga pinnata Roxb.* in Streptozocin-nicotinamide induced type II diabetic rats. J Ethnopharmacol **2006**;107:285-290.

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