



STUDY OF ANTIDIABETIC AND FREE RADICAL SCAVENGING ACTIVITY OF THE SEED EXTRACT OF *STRYCHNOS NUXVOMICA*

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ABSTRACT

The Present investigation was carried out to study the antidiabetic and antioxidant effects of the methanolic extract of *Strychnos nuxvomica* in alloxan induced diabetic model. The antidiabetic activity of the methanolic extract of *Strychnos nuxvomica* was evaluated in normal & alloxan induced diabetic rats. Increased body weight and decreased blood glucose level of the test animals shows that the extract exhibited significant antidiabetic activity when compared to diabetic control group. The results also indicated dose dependent effect. The antidiabetic activity produced by the extract may be due to increased uptake of glucose at the tissue level or by an increase in pancreatic beta cell function or due to inhibition of intestinal absorption of glucose.

The extract also produces a significant increase in Super Oxide Dismutase(SOD), Catalase & Total Protein(TP) level and decrease in Lipid Peroxidation(LPO), Total cholesterol, serum creatinine & Blood Urea Nitrogen(BUN) level in alloxan induced diabetic rats, which clearly show the antioxidant property of the extract. In vitro antioxidant study of the methanolic extract of *Strychnos nuxvomica* was done by DPPH assay in which the percentage scavenging activity of methanolic extract of *Strychnos nuxvomica* was gradually increased. The study indicated that the methanolic extract of *Strychnos nuxvomica* is a potential antidiabetic and antioxidant agent and lends scientific support for its use in folk medicine.

**Keywords:** Diabetes mellitus, *Strychnos nuxvomica*, antioxidant property, DPPH

INTRODUCTION

Despite the great efforts that have been made in understanding the management of diabetes, and disease related complications are increasing unabated<sup>1</sup>. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease<sup>2</sup>. Many traditional treatments have been recommended in the complementary and alternative system of medicine for treatment of diabetes mellitus<sup>3</sup>. Diabetes mellitus is one of the major health problems in the world today .The incident of diabetes is affecting people from all walks of life.

Diabetes mellitus (DM) comprises a group of metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics, environmental factors and life style choices. The metabolic deregulation associated with DM causes secondary pathophysiological changes in multiple organ systems, which are associated with oxidative stress and damage to tissues .Excessive generation of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of

vascular diseases and the normal anti oxidant defense mechanism is insufficient in the regulation of this increased oxidative stress .Hence antioxidants from other sources are to be provided to counteract the oxidative stress<sup>4</sup>.

*Strychnos nuxvomica* Linn (Loganiaceae) is a medium sized tree, both wild and cultivated, throughout the India. The plant is popularly known as Snake wood in English. In the Indian system of medicine, the medicinal attribution of this species has be known for long time. As per the traditional claims ,the root bark is used in cholera ,leaves used in chronic wounds and ulcers, and seeds used as appetizer, antiperiodic, purgative, asthma, diabetes, skin diseases<sup>5</sup> etc,.

Phytochemically the plant has been reported to contain alkaloids like Strychnine ,brucine, and strychnine and glycosides like loganin, caffeotannic acid and also traces of copper<sup>6,7</sup>. In pharmacology, only few activities such as analgesic<sup>8</sup>, apoptotic effect, antidepressant<sup>9</sup> antidote for snake poisoning<sup>10</sup> antitumor<sup>11</sup> has been proved. Hence the present study was undertaken to evaluate is potential against Diabetes and Free radical scavenging activity.

## Materials and Methods

### Plant material

*Strychnos nux vomica* seeds were collected from Tambaram, Tamilnadu state in the month of January. Botanical identification was done by Prof. P.J ayaraman, Director, Plant Anatomy Research Centre, Medicinal plant research unit, West Tambaram, Chennai.

### Extraction procedure

The seeds were dried at room temperature until they were free from moisture. The seeds were then subjected to size reduction to get coarse powder of desired particle size. The coarse powder was then stored in a clean dry air tight container. The powdered material was first subjected to defatting by soxhlet apparatus using petroleum ether for 15 hrs. Then the maceration was subjected to extraction by soxhlet apparatus with methanol for 48 hrs. The obtained extract was finally dried at low temperature under reduced pressure in a rotary evaporator crude powder was obtained and used to prepare suspensions 0.16mg/kg concentration using water:Tween 80 (4:1) for treatment.

### Drugs

Alloxan was obtained from Sisco Research Lab pvt.Ltd, Mumbai, India. All other chemicals used for this study were of analytical grade.

### Animals

Albino rats (wistar stain) of either sex were utilized for employed in this study. The rats were maintained under standard laboratory conditions at  $25 \pm 2^\circ\text{C}$ , relative humidity  $50 \pm 15\%$  and normal photo period (12 hr dark and 12hr light) were used for the experiment. Commercial pellet diet and water were provided *ad libitum*. The experimental protocol has been approved by the institutional animal ethics committee, proposal number being IAEC.19/2008.

### Effects of *Strychnos nux vomica* seed Extracts on Alloxan -Induced Diabetes Mellitus

Diabetic mellitus in rats was induced by intra peritoneal injection of Alloxan 50 mg/kg dissolved in 1% citrate buffer (pH- 4.5) in male albino rats fasted for 12 hours. Animals are considered to be diabetic if they had plasma glucose level of 240 mg/dl in addition to polyuria, hyperphagia and decrease in body weight. After 96 hours of Alloxan injection, 1 ml of blood sample was withdrawn from animals by sinocular puncture under anaesthetized condition in tubes containing potassium oxalate and sodium fluoride as anticoagulant and plasma glucose level was determined using commercially available GOD POD Kit using auto analyzer.

Animals were grouped according to the approximately same plasma glucose level and body weight. The Wistar rats were divided into four groups:

Group I: Normal control rats received distilled water

Group II: Diabetic control rats received Alloxan (120mg/kg)

Group III: Diabetic rats received *Strychnos nux vomica* seed extract (0.16mg/kg) for three weeks from the third day of Alloxan treatment.

Group IV: Diabetic rats received standard drug Glipizide (0.5mg/kg) for three weeks from the third day of Alloxan treatment.

At the end of experiment rats were fasted overnight and scarified by cervical decapitation. Blood is collected; plasma and serum were obtained and used for determination of various biochemical parameters like Blood glucose level, total protein, Total cholesterol, Serum creatinine & Blood urea nitrogen. The liver was carefully removed, homogenized and the homogenate was used for the estimation of Lipid per oxidation, Super Oxide Dismutase & Catalase level. Body weight was noted on the day "0" and on the day "21".

### Statistical analysis

Results were expressed as mean  $\pm$  S.E.M. The significance of the data was evaluated using ANOVA and were considered statistically significant when  $P < 0.01$  and  $P < 0.05$ . The statistical analysis was carried out using SPSS software package (Statistical package for the Social Sciences, United States).

### Histopathological studies<sup>12</sup>

#### Light Microscopic studies

At the end of 21 days pancreas from control, diabetic control, seed extract treated (methanolic), and standard drug treated (Glipizide) rats were quickly removed from histopathological studies. Pancreatic tissues removed from control and treated rats were washed in saline, fixed in Hollande-Bouin fixative (16) for 48 Hrs and processed for paraffin embedding. The sections stained in Ehrlich haematoxylin (16), counterstained in eosin and mounted were observed under a leitz diaplano microscope (Leica, Germany).

#### Ultra structural studies

Pancreas from the normal, diabetic control, seed extract, standard drug treated rats were removed after three weeks treatment, rinsed in physiological saline (0.9% sodium chloride) and fixed in 2% glutaraldehyde (primary fixative) over night and then post fixed 1% osmium tetroxide (secondary fixative) for 2-3 hours. Subsequently, the tissues were washed thoroughly in the washing buffer to remove excess osmium tetroxide. Sections (thickness) were

obtained using Richer(Austria) ultra tome and stained in toluidine blue 0. Also appropriate regions were chosen for ultra structural study.

## RESULTS

In the present study, the antidiabetic activity of methanolic seed extract of *Strychnos nux vomica* was evaluated in normal & Alloxan induced diabetic rats. The effects of *Strychnos nux vomica* extract on body weight are summarized in Table 1, in which there is significant increase ( $P>0.05$ ) in drug treated animals when compared to diabetic control. Table 2 shows the effect of methanolic seed extract of *Strychnos nux vomica* on blood glucose level in Alloxan induced diabetic rats. Diabetic rats treated with methanolic seed extract at doses of 100 and 200 mg/kg showed

significant decrease in blood glucose level at 2<sup>nd</sup> and 3<sup>rd</sup> week from initial levels ( $P<0.05$ ;  $P<0.01$ ). A significant time dependent hypoglycemic effect was shown throughout the period of study. Table 3 shows the effect of methanolic seed extract of *Strychnos nux vomica* on total protein, total cholesterol, Serum Creatinine, and BUN in Alloxan induced diabetic rats. There was a slight decrease in total Protein and increase in total cholesterol, serum creatinine & BUN level ( $P<0.05$ ;  $P<0.01$ ). Table 4 shows the effect of methanolic seed extract of *Strychnos nux vomica* on antioxidants level in liver homogenate of Alloxan induced diabetic rats. Super Oxide Dismutase & Catalase level was significantly increased and whereas Lipid Peroxide level was significantly decreased when compared to diabetic control.

**Table1: Effect of methanolic seed extract of *Strychnos nuxvomica* on the body weight (g) in Alloxan induced diabetic rats**

S.No	Groups	Initial body weight	Final body weight
1.	Normal control	150.67±1.15	175.67±3.44
2.	Diabetic control	170.83±2.32**	134.00±2.85**
3.	Standard	170.00±3.11*	127.40±1.71*
4.	Methanolic extract	200.33±4.12	159.50±2.12

Standard drug (Glipizide) – 0.5 mg/kg, Methanolic extract – 0.16mg/kg, Each value is represented as mean± SEM, No. of animals (n) = 6

**Table2: Effect of methanolic seed extract of *Strychnos nuxvomica* on blood glucose level BGL (mg/dl) Alloxan induced diabetic rats**

S.No	Groups	0 Week	1 Week	2 Week	3 Week
1	Normal control	101.46±3.81	132.92±2.86	124.70±3.23	120.45±2.23
2	Diabetic control	190.32±2.01	314.41±1.91	382.25±2.86	413±2.20**
3	Standard	407.20±2.48	308.34±3.47	236.99±5.91	160.47±2.37£
4	Ext-T	388.80±2.73	346.12±1.92	276.51±2.98	190.39±1.36££

Each value is represented as mean± SEM, No. of animals (n) = 6, \*\*p<0.01 Vs Normal control, ££ p<0.01 Vs Diabetic control, one way ANOVA followed by Dunnett's Test

**Table3: Effect of methanolic seed extract of *Strychnos nuxvomica* on Total protein, cholesterol, Serum, Creatinine, and BUN in Alloxan induced diabetic rats**

Groups	Total protein (g/dl)	Total cholesterol (mg/dl)	Serum creatinine (mg/dl)	BUN (mg/dl)
Normal control	6.96±0.02	61.28±1.79	0.50±0.01	42.66±1.22
Diabetic control	3.77±0.05**	81.60±1.15*	0.79±0.008**	70.79±0.88**
Standard	6.368±0.08££	61.948±0.56££	0.63±0.008££	50.83±0.87££
Ext-T	5.022±0.15££	67.78±1.34££	0.71±0.007£	61.83±1.35££

Each value is represented as mean± SEM, No of animals (n) = 6, \* p<0.05 VS Normal control, \*\* p<0.01VS Normal control, £ p<0.05 Vs diabetic control, ££ p<0.01 Vs Diabetic control, One way ANOVA followed by Dunnett's Test

**Table4: Effect of methanolic seed extract of *Strychnos nuxvomica* on Antioxidant levels in Liver homogenate of Alloxan induced diabetic rats**

Groups	SOD	CAT	LPO
Normal control	16.54±0.15	6.32±0.15	0.43±0.07
Diabetic control	11.32±0.18	2.68±0.19	1.89±0.13
Standard	15.148±0.33	5.225±0.14	0.34±0.01
Ext-T	13.859±0.21	4.653±0.14	0.23±0.01

Each value is represented as mean± SEM, No. of animals (n) = 6, SOD (U/mg protein), CAT (k/mg protein),LPO (µ mole/g protein), \*\*P<0.01 Diabetic control Vs Standard, \*\* P<0.01Diabetic control Vs extract, one way ANOVA followed by Dunett's Test

## DISCUSSION

It was observed that, treatment of diabetic rats with *Strychnos nux vomica*, had not only shown a significant antidiabetic activity but also possess an effective antioxidant activity which an evident from the observations.

Diabetes inducing agents like Alloxan and Streptozotocin are reported to induce diabetes with generation of free radicals; a significant reduction in antioxidant enzyme levels is indicated as the potential reason for the susceptibility of organs to atrophy in diabetic states<sup>13</sup>.

Alloxan induced diabetes by destroying  $\beta$ -cells of pancreas, through production of reactive oxygen species. In Alloxan induced diabetic rats, increased food consumption and decreased body weight were observed. This indicates polyphagic condition and loss of weight due to excessive break down of tissue proteins. *Strychnos nux vomica* seed extract improved the glucose tolerance in Alloxan induced diabetes in rats as compared to control; the alcoholic extract shows dose dependent effect, and 0.16mg/kg dose shows reduction in glucose level. *Strychnos nux vomica* extract show significant reduction in blood urea and creatinine in treated rats as compared to diabetic rats but significantly increased protein level.

Possible sources of oxidative stress and damage to proteins in diabetes induced free radicals generated by autoxidation reactions of sugars and sugar adducts to protein and by autoxidation of unsaturated lipids in plasma and membrane proteins. The oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage, and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems<sup>14</sup>.

Lipid Peroxidation is a free radical mediated oxidative to poly unsaturated fatty acids involving several types of free radical and termination occurs through enzymatic means or by free radical scavenging by anti oxidants. Under condition of severe oxidative stress, free radical generation leads to protein modification. Proteins may be damaged directly by specific interactions of oxidants free radicals with particular susceptible amino acids. The level of total plasma protein was found to be decreased in this study. This could be due to increased Peroxidation in the diabetic rats. *Strychnos nux vomica* extract significantly reduced the elevated LPO and significantly improved SOD and CAT<sup>15</sup> activity

In histopathological study the light microscopic photograph islet from control rat appeared circular with granulated beta cells appearing darker. Small &

shrunken islets & destruction of beta cells were observed in the diabetic condition well formed islets increased cell number observed in diabetic rats

In diabetes, there is a relation between glucose homeostasis, lipid metabolism later renal function and enzyme activities. It was found that a 21 day administration of *Strychnos nux vomica* seeds shows equal effectiveness in controlling diabetics when compared with diabetics rats treated with standard drug (Glipizide). Methanolic extract of *Strychnos nux vomica* seed proved to have hypoglycemic effect on Alloxan induced diabetic rats; a fact indicated that there was a repair/regeneration of the beta cells of the islets of langerhans. As a result there was a increase in insulin level, which brought a homeostasis in the above mentioned biochemical parameters such as cholesterol, urea, creatinine total protein, and in the enzyme activities.

## CONCLUSION

The present study indicated a significant anti diabetic effect of the methanol seed extract of *Strychnos nux vomica* and support its traditional usage in the control of diabetes. It is also shown strong anti-oxidant potential activity by in vivo studies.

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