Comparative evaluation of anti-diabetic action and pancreatic histopathology of rats treated with two alkaloidal plant extracts

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**Abstract**
This study aimed to understand *Strychnos nux vomica* and *Holarrhena pubescens* stem bark extract action towards M3 receptor in controlling blood glucose levels. *Strychnos nux vomica* and *Holarrhena pubescens* are both alkaloidal drugs can help in controlling Hyperglycemic level. This will be useful in the formulation of a new herbal drug molecule for treating diabetes. Chloroform and ethanolic extracts of selected alkaloidal plants were extracted using the soxhlet apparatus and obtained quotes were tested for acute toxicity studies and carried out anti-diabetic action on Wister albino rats for 21 days. Results obtained from Blood glucose levels and histopathological study of test groups are compared with blood glucose levels of standard group, and highly significant action was identified by the chloroform extract of *Strychnos nux vomica* and *Holarrhena pubescens* group. Moderate anti-diabetic action was observed remaining two groups of ethanolic extracts. *Strychnos nux vomica* and *Holarrhena pubescens* ethanolic extract groups are acting on M3 receptors and controlling Hyperglycemic levels.

**INTRODUCTION**
Strychnine and brucine are the chief constituents of Nux vomica having the botanical name of *Strychnos nux vomica* belong to the family Loganaceae. The stem bark is having folklore usage of controlling blood sugar levels, preventing respiratory and gastrointestinal disorders. There is no scientific evidence for treating diabetes in the animal model. Hence, this research aims to screen the anti-diabetic activity of strychnos nux vomica stem bark activity. It contains total alkaloids 2.6%–3%; out of them, strychnine is 1.25%–1.5%, 1.7% of brucine (Nadkarni, 1954). Remaining alkaloids are α-Colubrine β-Colubrine, n-oxystrychnine, pseudo strychnine, isostrychnine, protostrychnine, 3-methoxyicajine, novacine, chlorogenic acid, and glycoside, loganin (Trease and Evans, 2000). Nux vomica in crude form can be used to treat constipation, anaemia, lumbar dysfunction, respiratory disorders, and gastrointestinal disorders (Gruenwald, 2000).
Himalayan ranges. Uses of this plant are mentioned in the classical Ayurvedic literature, and it has many folklore claims. *Holarrhena pubescens* is categorised as lactiferous, small deciduous tree, which grows to a height of 13 m, a width of 1.1 m and girth with 3–7 m. It is having leaf size of 15 to 13 cm length and 4 to 12 cm width; leaves are having the shape of obtuse, rounded or acute apex; its veins are around 10–14 in number, having opposite venation pattern; it is ovate, leaves have 1.5 cm size petioles, and its veins are 3–6 cm in diameter. Flowers are odourless and has a white colour and are in a terminal corymbose cyme. Corolla puberulous outside; 8–13 mm long tube, slightly inflated near the base over stamens (Gopal and Chauhan, 2006).

Weston et al. work is showing that acetylcholine Muscarinic three receptors (M3) on the pancreas are one of the reasons for the dysregulation of insulin. Stimulation of the M3 receptor located on the pancreas is involved in regulating the cholinergic pathway which is stimulated by glucose leads to insulin release from the beta cells of islets of langerhans (Singh et al., 2009). Receptors upon stimulation result in the release of insulin, chloroform extract of *Strychnos nux vomica* and *Holarrhena pubescens* Stem bark extracts have shown M3 receptors’ stimulation on the pancreas has caused the insulin release and controls the excess glucose levels in blood (Roca et al., 2004). This was further supported by our study based on alloxan-induced pancreatic damage model in Wister albino rats by biological parameters evaluation and histopathology of pancreatic cells.

**METHODOLOGY**

**Selected Plants**

*Strychnos nux-vomica* stem bark was processed from the Nirmal district, Telangana, India, during October 2018. The plant materials were authenticated by Dr. Prasanna PV scientist F and HOD at India’s botanical survey, Deccan regional centre, Hyderabad, Telangana, India. Voucher specimen No: BSI/DRC/18-19/Tech./589, has been deposited at the Botanical Survey of India (BSI).

The stem bark of *Holarrhena pubescens wall ex G.Don* was collected from Nirmal district, Telangana, India, during November 2016. Dr. Rasingam L did authentication of plant material, and scientist In-charge at the BSI, Deccan regional centre, Hyderabad, Telangana, India. Voucher specimen No: BSI/DRC/16-17/Tech./681, deposited at BSI

**Antihyperglycemic effect of plant extracts**

**Dose fixation studies**

Swiss albino mice of 20-25g were chosen for determining acute toxicity studies. Animals were categorised into control and test groups; each group contains six animals. 5% normal saline was given to the control group, and the test group was treated with chloroform and ethanolic extract of *S. Nux vomica* stem bark extracts and Holarrhena* pubescens* stem bark extracts respectively. Experimental animals were screened for 4 hours to 48 hours for behavioural and mortality changes; there, by LD50 value is calculated (Yeh et al., 2003).

**Graph 1:** Graphical representation of Blood glucose in various drug treatment groups

**Figure 1:** Control group

**Figure 2:** Toxic group

**Animals selection for anti-diabetic action**

Wister albino rats of either sex of 150-250 g weight were taken for the study. The approval process...
Table 1: Drug protocol Treatment for anti-diabetic property

| S.No | Group               | Prior treatment | Drug treatment               | Number of days |
|------|---------------------|----------------|------------------------------|---------------|
| 1    | Control             | Water          | 5% gum acacia solution(P.O)  | 21            |
| 2    | Diabetic control    | Alloxan 110mg/kg b.w.i.p | 5% gum acacia solution(P.O)  | 21            |
| 3    | Standard            | Alloxan 110mg/kg b.w.i.p | Glibenclamide 1mg/kg b.w(P.O) | 21            |
| 4    | S.N.C.E             | Alloxan 110mg/kg b.w.i.p | 7.5mg/kg extract(P.O) S.N.C.E | 21            |
| 5    | S.N.E.E             | Alloxan 110mg/kg b.w.i.p | 7.5mg/kg extract(P.O) S.N.E.E | 21            |
| 6    | H.P.C.E             | Alloxan 110mg/kg b.w.i.p | 6mg/kg extract(P.O) H.P.C.E | 21            |
| 7    | H.P.E.E             | Alloxan 110mg/kg b.w.i.p | 6mg/kg extract(P.O) H.P.E.E | 21            |

Table 2: Results of blood glucose levels in various drugs treated groups

| Groups                  | 0 Day         | 7th Day        | 14th Day        | 21st Day        |
|-------------------------|---------------|----------------|-----------------|-----------------|
| Normal control          | 78.26±/-17.12 | 80.21±/-1.21   | 71+/-.24.52     | 74.24+/-17.63   |
| Toxic control (Diabetic)| 272.13+/-12.63| 298.25+/-3.56***| 305.25+/-4.87***| 360.23+/-45.85**|
| Standard (Glibenclamide 1mg/kg) | 250.17+/-1.26 | 169.27+/-2.45***| 120.27+/-1.23***| 92.12+/-1.56***|
| S.N.C.E                  | 258.45+/-1.47 | 241.48+/-0.64** | 160.87+/-0.48***| 145.37+/-1.12***|
| S.N.E.E                  | 297.64+/-2.9  | 285.59+/-4.5*  | 275.96+/-0.47** | 158.23+/-1.24**|
| H.P.C.E                  | 269.18+/-2.75 | 254.32+/-1.02**| 241.01+/-4.2*** | 211.64+/-23.23***|
| HPEE                     | 274.16+/-2.76 | 271.54+/-6.45* | 268.46+/-3.5**  | 260.15+/-0.24**|

All values are expressed as a Mean ±SEM,n=6; The test group’s results are considered significant at*P<0.05, ** P<0.01, ***P<0.001 compared to the diabetic control group.

Figure 3: Standard group

Figure 4: S.N.C.E-treated group

Induction of diabetes

Induction of Diabetes in experimental animals was carried by administering alloxan at a dose of 110 mg/kg b.w i.p, 24 hours fasting process was monitored for the experimental animals before the process.
administration of alloxan (Matkovic et al., 1996). Seventy-two hours after administration of alloxan blood samples were analysed for blood glucose levels. Animals which were exhibiting more than 200mg/dl of blood glucose levels were considered as diabetic and further used in the evaluation of anti-diabetic action of *Strychnos nux vomica* stem bark extracts and *H. pubescens* stem bark extracts simultaneously.

**Process of Experiment**

Animals which are having more 200mg/dl of blood glucose levels were categorised into seven groups consists of 6 animals in each group. Drug protocol treatment was given in Table 1.

**Biochemical analysis**

**Process of drug administration**

5% gum acacia was used as a suspending agent for mixing of selected stem bark extracts with distilled water which were administered to selected experimental animals using an oral feeding needle.

**Screening of biochemical parameters**

Anti-diabetic property of animals was determined by collecting the blood samples at 0, 7, 14 and 21 days from retro-orbital plexus of rats using micro-capillary tubes. Collected blood samples were centrifuged at 10,000rpm for 10 minutes, and glucose levels were estimated using Blood glucose-PAP (glucose oxidase/phenol/4-amino-antipyrine) method by using Elitech Glucose S.L. Kit in an auto-analysers. The blood glucose level was determined as mg/dl (Cheng, 2005) and the values of blood glucose levels were given in Table 2. Obtained results were graphically represented in Graph 1.

**Statistical analysis**

Obtained results were subjected to One-way Analysis of variance (ANOVA) for comparison, the values $p<0.05$ was considered as significant, in Toxic group that is diabetic control group $***P<0.001$ compared to the normal control and test groups results are considered as significant at $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to standard groups results.

The result obtained from the studies was subjected to a one-way analysis of variance (ANOVA) for comparison among different groups. The minimum value of $p<0.05$ was considered as significant. The diabetic control group results were significant at $***P<0.001$ compared to the normal control group, the results of the test group are considered significant at $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to Diabetic control group.

**Histology Examination**

At the end of 21 days, all rats were sacrificed using chloroform, and the pancreatic tissues were collected from the animals and rapidly fixed in 10% normal saline for 48h. Selected tissue samples were then processed routinely by paraffin embedding and stained with hematoxylin and eosin. The slides were evaluated using a light microscope and photographed using an Olympus digital camera (Nikon 2). Histopathology images of rat’s pancreas in various groups were given in Figures 1, 2, 3, 4, 5, 6 and 7.
DISCUSSION

Anti Diabetic activity of *S. Nuxvomica*, *H. Pubescens* stem bark extracts on alloxan- treated diabetic rats, to find their In-vivo anti-diabetic action with their cell regeneration property on pancreatic islets. Results of acute toxicity studies LD50 value calculated as 750 mg/kg b.w for *S. Nuxvomica* stem bark extract and 600mg/kg b.w for *H. Pubescens* stem bark extract, by considering this 1/10th of LD50 value is taken into account to carry out In-Vivo anti-diabetic action. The results of Blood glucose levels indicate that blood glucose levels were continuously maintained in Normal control group animals during the study period in the range of 78.26 ± 17.12 to 74.24 ± 17.63. In contrast, Blood glucose of the diabetic control group has been increased to 272.13 ± 12.63 to 360.23 ± 45.85, which has been treated with alloxan (110 mg/kg b.w) only and not given any treatment. Standard drug Glibenclamide (1mg/kg b.w) has decreased the blood glucose levels from 250.17±1.26 to 92.12±1.56 during 21 days drug treatment, which is highly significant (p<0.001) when compared to Diabetic group. SNCE and SNEE group at a dose of 6.5 mg/kg b.w has shown significant and moderately significant values compared to standard groups. HPECE and HPEE treated group at a dose of 7mg/kg b.w have reduced the values from 269.18±2.75 to 211.64±23.23 and 274.16±2.76 to 260.15±0.24 respectively. Out of all the four extracts of *Strychnos nux vomica*, chloroform extract-treated groups have shown highly significant action compared to the standard group. From the histopathological studies results, the Control group pancreas histology reveals normal cell structure of islets and normal nucleus appearance [Figure 1]. Diabetic control group histological architecture cell damage, fewer islets of Langerhans, and degranulation of cells and necrosis [Figure 2]. The standard group has shown an increased number of islets of Langerhans, and cell regaining function was observed [Figure 3]. The chloroform extract of SN has shown highly significant action by increasing the number and decreased the necrotic changes than the diabetic control group [Figure 4]. Ethanolic extract of SN has shown moderate significant activity in islets regaining and nucleus structure [Figure 5]. HP’s chloroform extract has shown the rearrangement of beta cells and fibrotic growth, but the number of islets has been increased [Figure 7]. Ethanolic extract of HP has demonstrated the least significant action by less necrosis, degeneration, fibrosis and vacuolisation process [Weston-Green et al., 2013] [Figure 6]. Alloxan’s diabeticogenic action is due to oxidative stress, and histology changes were best described by (Coskun et al., 2005).

CONCLUSION

The present comparative studies on alkaloidal plants of *Strychnos nux vomica* and *Holarrhena pubescens* chloroform and ethanolic extracts were showing significant protection against alloxan-induced pancreatic cell damage. Further investigations need to be done to isolate the chemical constituents of selected plants; chloroform extracts need to be carried to determine the mechanism behind the hypoglycaemic activity. It was found that the selected alkaloidal stem bark especially chloroform extracts of both the selected plants were exhibiting significant anti-diabetic action by stimulating M3 receptors on the pancreas which causes insulin release and control the excess glucose in the blood.

ABBREVIATIONS

SNCE: Strychnosnuxvomica Chloroform extract; SNEE: Strychnosnuxvomica Ethanolic extract; HPECE: Holarrhanapubescens chloroform extract; HPEEE: Holarrhanapubescens Ethanolic extract; PO: per oral; Gm: grams; Mg: milligrams; Dl: deciliters; Kg: kilograms

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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