Pharmacological and phytochemical investigation of *Cestrum nocturnum* leaf extract for antihyperglycemic and antihyperlipidemic activity.

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ABSTRACT

**Background:** Plant derivatives with purported hypoglycemic properties have been used in traditional medicine around the world. The Leaves of *Cestrum nocturnum* (Raat rani) were reported to have good medicinal values in traditional system of medicine. In the present study, we investigated the phytochemical, pharmacological and acute toxicological properties of the extracts of *Cestrum nocturnum* leaves.

**Material and Methods:** Antidiabetic effect of hydro alcoholic extract and saponin fraction of *Cestrum nocturnum* leaves were evaluated in streptozotocin induced diabetic rats. The hydro alcoholic extract 200, 400 mg/kg and saponin fraction 50, 100 mg/kg was administered orally to streptozotocin induced diabetic rats for a period of three weeks. Various biochemical parameters such as blood glucose, lipids, creatinine, urea, alkaline phosphatase, total protein, albumin and liver glycogen content were evaluated. These stated parameters in the diabetic rats were reverted back approximately near to normal after treatment with the respective higher dose of leaf extract and saponin fraction of *Cestrum nocturnum*. Histological examination of the pancreas from diabetic rats showed degenerative changes in β-cells; whereas treatment significantly reversed the histopathological damage to the islets cells.

**Conclusion:** The results obtained in this study indicated that the hydro alcoholic extract and saponin fraction of *Cestrum nocturnum* leaves significantly improved the levels of various biochemical parameters. The findings could justify the role of this plant material in the management of diabetes. This property of the plant may be attributed to its antihyperlipidemic and hepato protective property.

**Keywords:** Streptozotocin, *Cestrum nocturnum*, Saponin, Biochemical parameters, Histopathology.

INTRODUCTION

The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity and treatment. In the natural system of medicine, many plants have been claimed to be useful for the treatment of diabetes mellitus. The dependence of large rural population on medicinal plants for treatment of diabetes is because of its availability and affordability.
Additionally, after the approbation made by World Health Organization on diabetes mellitus, exploration on hyperglycemic agents from medicinal plants has become more significant [2]. The *Cestrum nocturnum* is found to have many pharmacological activities such as antimalarial, antimicrobial, antifungal, larvicidal, antioxidant etc. The extract of the leaves is used to treat epilepsy and other seizure disorders, as well as headaches and nervous imbalances. The plant contains many saponins, flavonoids and sterols/triterpenoids as its main constituents, which are known bioactive principles for antidiabetic potential. The antidiabetic property of *Cestrum nocturnum* has been reported in ancient literature of Ayurveda. Hence, by considering stated activity of *Cestrum nocturnum* leaves, the present investigation was undertaken to evaluate antihyperglycemic activity of STZ induced diabetic rats. The present research work comprises of the phytochemical and pharmacological investigations of leaves of *Cestrum nocturnum* Linn. (raatrani) for antidiabetic activity through in vivo Streptozotocin induced type II diabetes model in rats.

**MATERIAL AND METHODS**

The leaves of *Cestrum nocturnum* were collected fresh from local market Pune, washed with distilled water to remove any traces of dust. The plant at flowering stage was authenticated by Botanical Survey of India (BSI), Pune.

**Extraction**

The leaves were shade dried and dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction. The powder was placed in a thimble and extracted using a soxhlet apparatus. Various solvents used for extraction were:

1. Petroleum ether
2. Hydro Alcohol
3. Methanol
4. Chloroform
5. Water

The extraction was continued until the powder was exhausted. The obtained extract was concentrated using rotary evaporator and the hot plate.

**Phytochemical analysis**

The various extracts were evaluated for the presence of carbohydrates, proteins, flavonoids, tannins, cardiac glycoside, saponins and alkaloids using standard procedures.

**Experimental animals**

Sprague Dawley rat of either sex, weighing 200-250 g were procured from National Institute of Bioscience, Pune. Rats were placed in polypropylene cages (two per cage) randomly with paddy husk as bedding. The animals were maintained throughout all the experiments. Animals had free access of water and standard laboratory feed (Amrut feed, Chakan, India) ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/2013/14/152) by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune, constituted under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Toxicity study**

Acute Oral Toxicity Studies were carried out in Sprague Dawley rats following OECD 423 Guidelines [3]. Dose limit at 2000 mg/kg (single dose) was administered to rat and observed for 14 days. The hydro alcoholic extract and saponin fraction of *Cestrum nocturnum* leaves did not produce toxic symptoms or changes in behavior or death and found to be safer in rat up to the dose of 2000 mg/kg body weight. Animals treated with hydro alcoholic extract and saponin fraction of *Cestrum nocturnum* leaves exhibited normal body weight gain and food intake throughout the study. Acute toxicity tests have shown that the LD50 of the extract and fraction in rat was higher than 2000 mg/kg and it is categorized under category 5 of GSH as per OECD guidelines 423. Two doses of extract (200 and 400mg/kg per day) and saponin fraction (50 and 100mg/kg per day) were used in all further experiments.

**Oral Glucose Tolerance Test (OGTT)**

Antihyperglycemic activity was studied in glucose-loaded hyperglycemic rats. Glibenclamide (5mg/kg) was used as the reference standard. The remaining
groups of rats were treated with 400mg/kg of methanolic, hydro alcoholic, aqueous, PET ether, chloroform extract and (100 mg/kg) Saponin Fraction. Blood sugar level was determined from overnight fasted animals at 0 hours. After 30 mins of the drug treatment, animals were fed with glucose (4g/kg) and blood glucose was determined at 1/2, 1, 2, and 3 hours after glucose load. Blood glucose concentration was estimated by using a commercial glucometer and test strips by collecting blood from the tail vein [4]

Study protocol

Induction of Experimental diabetes

All the animals had free access to water and food. A rat model of type 2 diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM) was induced in overnight-fasted rats by a single intraperitoneal injection of streptozotocin (60mg/kg).

Blood samples were obtained from the retro-orbital plexus in both Streptozotocin injected and control animals at 72 hours. Hyperglycemia was confirmed by elevated blood glucose levels determined at 72 hrs.

The rats were supplied with 10% glucose water and feed during the next 24 hours to avoid sudden hypoglycemia post-injection. On day 2, water was replaced with drinking water. Fasting blood glucose levels were determined by glucometer. Rats with fasting blood glucose levels above 250 mg/dL were considered diabetic.

Intervention of Study

The animals were grouped randomly each having six animals.

- Group-I: Normal Control (Vehicle p.o.)
- Group-II: Diabetic Control (DC + Vehicle p.o.)
- Group-III: Standard (DC + Glibenclamide 5 mg/kg, p.o.)
- Group-IV: HaECN (DC + HaECN 200 mg/kg, p.o.)
- Group-V: HaECN (DC + HaECN 400 mg/kg, p.o.)
- Group-VI: Fraction (DC + Saponin 50 mg/kg, p.o.)
- Group-VII: Fraction (DC + Saponin 100 mg/kg, p.o.)

The control group received Vehicle and in the treatment group the drug/extracts were administered orally for 21 days.

On the 0, 7, 14 and 21 day, the rats were fasted overnight and blood samples were withdrawn from the retro orbital plexus. Serum samples were used for the various biochemical estimations. At the end of experimental period, the respective rats were sacrificed and in-vitro test is carried out and the sample of pancreas was sent for histopathology studies.

Statistical analysis

All statistical analyses were made using the software Graph Pad Prism. All results were expressed as mean ± SEM, and compared with those of the control and diabetic groups using Tukey’s test and statistical significance was determined. The values were considered statistically significant when p<0.05.

RESULT

The extraction of *Cestrum nocturnum* leaves by using different solvent gives respective percent yield as stated in Table 1. The preliminary phytochemical analysis of different extracts of *Cestrum nocturnum* has revealed the presence of flavonoids, saponins, alkaloids, steroids, tannins, cardiac glycosides, proteins and carbohydrates. Saponins were most prevalent in near about all the extracts and particularly saponins as a phyto constituent were found to have the antidiabetic potential. The hydroalcoholic extract of *Cestrum nocturnum* was studied for antihyperglycemic activity [5]. Thus Saponins were isolated from hydroalcoholic extract of *Cestrum nocturnum*. 
Table 1: Phytochemical constituents and % yield of various extracts of *C. Nocturnum*

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>Percent (%)</th>
<th>CONSTITUENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract</td>
<td>13.12</td>
<td>Alkaloids, Carbohydrates, Proteins, Flavanoids, Saponins, Cardiac glycosides, Tannins, Steriods</td>
</tr>
<tr>
<td>Hydroalcoholic extract</td>
<td>16.08</td>
<td>Carbohydrates, Alkaloids, Flavanoids, Cardiac glycosides, Tannins, Steriods</td>
</tr>
<tr>
<td>PET ether extract</td>
<td>8.84</td>
<td>Carbohydrates, Tannins, Saponins, Cardiac glycosides, Steroids</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>9.44</td>
<td>Carbohydrates, Cardiac glycosides, Steroids, Saponins</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>19</td>
<td>Carbohydrates, Cardiac glycosides, Steroids, Saponins, tannins</td>
</tr>
</tbody>
</table>

Acute Oral Toxicity Studies were carried out in Sprague Dawley rats, the tests have shown that the LD50 of the extract and fraction in rat was higher than 2000 mg/kg and it is categorized under category 5 of GSH as per OECD guidelines 423.

Table 2: Acute toxicity studies of extract and saponin fraction

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Dose level</th>
<th>LD50 Cut off value</th>
<th>Mortality at selected Dose</th>
<th>LD50 Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic extract</td>
<td>2000 mg/kg b.w.</td>
<td>5000 mg/kg b.w.</td>
<td>0/3</td>
<td>&gt;2000 mg/kg Category 5 of GSH</td>
</tr>
<tr>
<td>Saponin fraction</td>
<td>2000 mg/kg b.w.</td>
<td>5000 mg/kg b.w.</td>
<td>0/3</td>
<td>&gt;2000 mg/kg Category 5 of GSH</td>
</tr>
</tbody>
</table>

Note: b.w.: body weight
LD50: Lethal dose
GSH: Globally harmonized classification system

In glucose fed hyperglycemic rats, the oral administration of extract and saponin fraction significantly suppressed the rise in glucose level induced by glucose loading (Table 3).

Table 3: Effect of single dose treatment of various extracts and saponin fraction of *Cestrum nocturnum* leaves on Anti-hyperglycaemic activity in glucose-loaded hyperglycaemic rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood Glucose level (mg/dl) at time t (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Glu</td>
<td>-</td>
<td>75.50±2.41</td>
</tr>
<tr>
<td>Std</td>
<td>5</td>
<td>74.67±2.60</td>
</tr>
<tr>
<td>MECN</td>
<td>400</td>
<td>82.00±1.16</td>
</tr>
<tr>
<td>HaECN</td>
<td>400</td>
<td>73.83±2.09</td>
</tr>
<tr>
<td>PeECN</td>
<td>400</td>
<td>70.00±1.92</td>
</tr>
<tr>
<td>CECN</td>
<td>400</td>
<td>67.67±1.48</td>
</tr>
<tr>
<td>AECN</td>
<td>400</td>
<td>73.83±1.60</td>
</tr>
<tr>
<td>Saponin Fr.</td>
<td>100</td>
<td>80.21±1.23</td>
</tr>
</tbody>
</table>
It was observed that body weight of the rats in treatment groups with higher dose increased significantly after the completion of three weeks oral treatment (Figure 1).

The values are expressed as mean ± SEM, n = 6 *p<0.05, **p<0.01 when compared to Glucose Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

Glu.: Glucose Control  
Std.: Glebenclamide standard  
MECN: Methanolic extract of *Cestrum nocturnum*,  
HaECN: Hydroalcoholic extract of *Cestrum nocturnum*  
PeECN: Petroleum ether extract of *Cestrum nocturnum*  
CECN: Chloroform extract of *Cestrum nocturnum*  
AECN: Aqueous extract of *Cestrum nocturnum*  
Fr.: Fraction  

It was observed that body weight of the rats in treatment groups with higher dose increased significantly after the completion of three weeks oral treatment (Figure 1).

The values are expressed as mean ± SEM, n = 6 *p<0.001 when compared to Normal control, **p<0.001, *p<0.01,  p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test

The hydroalcoholic extract and saponins fraction of *Cestrum nocturnum* found to have decreased serum glucose activity (Figure 2) serum total cholesterol (Figure 3) and triglycerides levels (Figure 4) and elevated the HDL levels (Figure 5) in diabetic rats.
Figure 2 Effect of HaECN and saponin fraction on serum glucose

The values are expressed as mean ± SEM, n = 6 *p< 0.001 when compared to Normal control, "p< 0.001, ",p<0.01, "p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

Figure 3 Effect of HaECN and saponin fraction on serum cholesterol

The values are expressed as mean ± SEM, n = 6 *p< 0.001 compared to Normal control, "p< 0.001 compared to Normal control, "p<0.01, "p<0.05 compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.
**Figure 4** Effect of HaECN and saponin fraction on serum HDL.

The values are expressed as mean ± SEM, n = 6. 'p<0.001 when compared to Normal control, *p<0.001, 'p<0.01, 'p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

**Figure 5:** Effect of HaECN and saponin fraction on serum albumin

The values are expressed as mean ± SEM, n = 6. 'p<0.01, 'p<0.001 when compared to Normal control, 'p<0.01, 'p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

The treatment with extract and saponin fraction significantly restored the increased creatinine levels (Figure 6).
The values are expressed as mean ± SEM, n = 6. *p<0.001 when compared to Normal control, *p< 0.001, *p<0.01, *p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

The treatment groups were found to have positive effects with the decreased levels of alkaline phosphatase in the blood (Figure 7) and urea (Figure 8) as compared to the diabetic control.
Figure 8 Effect of HaECN and saponin fraction on serum urea

The values are expressed as mean ± SEM, n = 6. *p<0.001 when compared to Normal control, †p<0.001, ‡p<0.01, §p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

The oral treatment with extract and saponin fraction with higher dose (400 mg/kg of extract and 100 mg/kg of saponin fraction) for three weeks significantly increased serum total protein and albumin levels in diabetic rats, (Figure 9, 10).

Figure 9 Effect of HaECN and saponin fraction on serum alkaline phosphatase

The values are expressed as mean ± SEM, n = 6. *p<0.001 when compared to Normal control, †p<0.001, ‡p<0.01, §p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.
Figure 10 Effect of HaECN and saponin fraction on serum total proteins

The values are expressed as mean ± SEM, n = 6 *p< 0.01, *p<0.001 when compared to Normal control, *p< 0.001, *p<0.01, *p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

In the present study, Glibenclamide, extract and saponins approximately restored the decreased hepatic glycogen levels possibly by increasing the level of insulin (Figure 11).

Figure 11 Effect of HaECN and saponin fraction on Liver glycogen

The values are expressed as mean ± SEM, n = 6 *p< 0.001 when compared to Normal control, *p< 0.001, *p<0.01, *p<0.05 when compared to Diabetic Control. Statistical analysis was done by using one way ANOVA followed by Tuckey’s test.

The pancreas of the rats treated with HaECN 200 mg/kg (Figure 12 D), and fraction 50 mg/kg (Figure 16) show less effect as compared to standard treatment (Figure 14). The extract 400 mg/kg (Figure 17) and saponin fraction 100 mg/kg (Figure 18) showed significant improvement in necrosis (mild to moderate atrophy), fibrotic changes and reduced the injuries of pancreas.
Figure 12 A Normal control (Vehicle)

Figure 12 B Diabetic control (DC + Vehicle)

Figure 12 C Std (DC + Glibenclamide 5 mg/kg)

Figure 12 D Extract (DC + HaECN 200 mg/kg)

Figure 12 E Extract (DC + HaECN 400 mg/kg)

Figure 12 F Fraction (DC + Saponin 50 mg/kg)

Figure 12 G Fraction (DC + Saponin 100 mg/kg)
DISCUSSION
The research work encompasses a detailed and systematic phytochemical and pharmacological investigation of various extract/s and saponin fraction of leaves of Cestrum nocturnum for antidiabetic activity through in vivo Streptozotocin induced type II diabetes model in rats. The plant was authenticated and various properties were studied. Extraction of the plant material was carried out by soxhlet extraction method. Methanol, hydro alcohol, chloroform, petroleum ether and aqueous extracts of leaves were prepared. The extraction of Cestrum nocturnum leaves gives 19 \% yield in aqueous extract, 16.08 \% in hydroalcoholic extract, 13.12 \% yield in methanolic extract whereas 9.44 \% and 8.84 \% yield in chloroform and petroleum (Pet) ether extract respectively (Table 1). Qualitative phytochemical tests of the extracts of Cestrum nocturnum leaves revealed the presence of carbohydrates, proteins, saponins, flavonoids, alkaloids, cardiac glycosides, tannins and sterols. The presence of Carbohydrates, Cardiac glycosides, Steroids, Saponins is almost there in all extract whereas alkaloids, flavonoids are present in methanolic and hydroalcoholic extract respectively. Saponin fraction was isolated from hydroalcoholic extract of leaves.

In the present study intra-peritoneal injection of STZ (60 mg/kg) produced significant elevation in serum glucose level and produced hyperglycemia that is in agreement with previous study [6]. STZ-induced diabetic rats exhibited hyperglycemia, hyperlipidemia and decrease in body weight along with the fluctuation of various other parameters. The present study has demonstrated diminutions in serum glucose, cholesterol, triglyceride, creatinine, alkaline phosphatase and urea concentration and an increase in the albumin, total protein, HDL and body weight in diabetic rats treated with hydroalcoholic extract (200 mg/kg, 400 mg/kg) and saponin fraction (50 mg/kg and 100 mg/kg) of Cestrum nocturnum Leaves.

The preliminary phytochemical analysis of different extracts of Cestrum nocturnum has revealed the presence of flavonoids, saponins, alkaloids, steriods, tannins, cardiac glycosides, proteins and carbohydrates. Saponins were most prevalent in near about all the extracts and particularly saponins as a phytoconstituent were found to have the antidiabetic potential. The hydroalcholic extract of Cestrum nocturnum was studied for antihyperglycemic activity [5]. Thus Saponins were isolated from hydroalcoholic extract of Cestrum nocturnum. Acute Oral Toxicity Studies were carried out in Sprague Dawley rats following OECD 423 Guidelines. Dose limit at 2000 mg/kg (single dose) was administrated to rat and observed for 14 days (Table 2). The hydroalcoholic extract and saponin fraction of Cestrum nocturnum leaves did not produce toxic symptoms or changes in behavior or death and found to be safer in rat upto the dose of 2000 mg/kg body weight. Animals treated with hydroalcoholic extract and saponin fraction of Cestrum nocturnum leaves exhibited normal body weight gain and food intake throughout the study. Acute toxicity tests have shown that the LD50 of the extract and fraction in rat was higher than 2000 mg/kg and it is categorized under category 5 of GSH as per OECD guidelines 423 [3].

The hypoglycemiac potential of extract and saponin fraction of Cestrum nocturnum was confirmed by its action on glucose loaded rats. The relationship between blood levels of glucose and insulin after an external load of glucose can be studied using OGTT [4] (Table 3). In glucose fed hyperglycemic rats, the oral administration of extract and saponin fraction significantly suppressed the rise in glucose level induced by glucose loading. Such an effect might be due to decrease in the rate of intestinal glucose absorption or by potentiation of pancreatic secretions or increasing the glucose uptake.

Diabetes mellitus causes failure to use glucose for energy which may leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins [7]. It was observed that body weight of the rats in treatment groups with higher dose increased significantly after the completion of three weeks oral treatment (P<0.001). The results of the present study indicated that the restoration of body weight might be contributed by reduced depletion of the body proteins (Figure 1).

Different mechanisms of action to reduce blood glucose levels with the help of plant extracts already exist. Some plants exhibit properties similar to the
well-known sulfonylurea drugs like glibenclamide; they reduce serum glucose in normoglycemic in all types of diabetic animals [8]. We hypothesized that the hydroalcoholic extract and saponins fraction of *Cestrum nocturnum* could have a sulfonylurea-like mechanism since there is significantly decreased serum glucose in normoglycemic rats like glibenclamide (P<0.001) (Figure 2). Sulfonylurea compounds lower serum glucose in normal and type 2 diabetic animals by stimulating insulin release from β-pancreatic cells.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats. Excess of fatty acids in serum produced during diabetes promotes conversion of excess fatty acids into phospholipids and cholesterol in liver [9]. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins [10]. In the present study, STZ induced diabetic rats showed abnormalities in lipid metabolism as evidenced from the significant elevation of serum total cholesterol and triglycerides. Three weeks repeated dose treatment significantly reduced serum total cholesterol (Figure 3) and triglycerides levels (Figure 4) and elevated the HDL levels (Figure 5) in diabetic rats indicating its anti-hyperlipidemic activity (P<0.001).

In diabetes mellitus there is decrease in body weight, which may due to decrease in muscle mass. This decrease in muscle mass occurs due to muscle wasting which cause increase in serum creatinine levels in diabetic rats. The treatment with extract and saponin fraction significantly restored the increased creatinine levels [11] (P<0.001) (Figure 6).

Alkaline phosphatase is an enzyme found throughout the body. However it tends to be most concentrated in liver, the bile ducts, bones and placenta. Disease that damage and destroy the cells of these organs lead to release of alkaline phosphatase into blood; which raises the blood level of alkaline phosphatase in diabetic rats[12]. The treatment with glibenclamide, extract and saponin fraction reduced the levels of alkaline phosphatase in the blood (P<0.001) (Figure 7).

An increase in urea level is seen when there is damage to kidney or kidney is not functioning properly. Increment of blood urea level with the
STZ induced diabetic rat by its antidiabetic, antihyperlipidemic and hepatoprotective activity.

ACKNOWLEDGEMENT

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DECLARATION OF INTEREST

There are no conflicts of interest.

REFERENCE


