

**Research Article****Inhibitory effect of hydroalcoholic extract of *Cestrum Nocturnum* on  $\alpha$ -amylase activity****Anil Kamboj\***

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**Keywords:** $\alpha$ -amylase; *C. nocturnum*; hyperglycemia; hydroalcoholic extracts**Abstract**

Inhibition of  $\alpha$ - amylase play a vital role in the clinical management of postprandial hyperglycemia. Although, powerful synthetic inhibitors are available, natural inhibitors are potentially safer.

The present study was carried out to evaluate  $\alpha$ - amylase inhibition activity from hydroalcoholic extracts from aerial parts of *Cestrum nocturnum*. Hydroalcoholic extract was prepared by Soxhletation Method. The extract showed strong inhibition towards  $\alpha$ - amylase activity and IC<sub>50</sub> value were 45.9  $\mu$ g. This *In vitro* studies indicate the potential of *C. nocturnum* in the development of effective anti-diabetic agents.

**Introduction**

Diabetes mellitus (DM) is heterogeneous primary disorder of carbohydrate with multiple etiological factors that generally involves absolute or relative insulin deficiency (type 1 DM) or combined resistance to insulin action and the insulin secretory response (type 2 DM)<sup>[1]</sup>. Although hypoglycemic agents such as insulin are commonly use for treating diabetes mellitus through the control of hyperglycemia, drugs therapies either alone or in combination fail to significantly alter the course of diabetic complications, and many limitations exist in their use<sup>[2]</sup>. One of the therapeutic approaches for decreasing post-prandial hyperglycemia is to prevent absorption of glucose by the inhibition of carbohydrate- hydrolyzing enzymes, such as  $\alpha$ -glucosidase and  $\alpha$ -amylase<sup>[3,4]</sup>. Thus, the retardation of action of  $\alpha$ -glucosidase and  $\alpha$ -amylase by inhibitors might be the most effective approaches to control type-2 diabetes mellitus.

*Cestrum nocturnum* L. is a garden shrub from Solanaceae family, and its flowers exude a special sweet fragrance at night, the main reason for the folknames, Night Cestrum, Lady of the Night and Night Blooming Jasmine<sup>[5]</sup>. It is widely naturalized in tropical and subtropical regions throughout the world, including Australia, southern China India and the southernmost United States. In traditional medicine, leaves of *C. nocturnum* have been used for their pharmacological significance in burns and swellings. It is also used for treating epilepsy and as stupefying charm medicine in West Indian Islands<sup>[6]</sup>. The volatile oil is known to be mosquito-repellent and hence *C. Nocturnum* is used to prevent malaria in several African Nations<sup>[7]</sup>. Pharmacological studies on the plant proved that the leaves have significant analgesic and

bactericidal activity [8]. Local anesthetic effect, inhibitory effect on central nerve system and cardiac arrhythmic effect of plant are also documented [9]. Hydroalcoholic extract of *C. Nocturnum* also reported for their antidiabetic activity [10]. Furthermore, *n*-butanol and polysaccharides extracts from *C. nocturnum* had obvious *invivo* effects on tumor inhibition<sup>[11]</sup>. Some phytochemical studies on leaves from *C. nocturnum* improved the presence of a calcinogenic glycoside<sup>[12]</sup> and other glycosides such as nocturnoside A and nocturnoside B<sup>[13]</sup>, phenol glucosides (cesternosides A and B)<sup>[14]</sup>, flavonol glycosides and steroidal saponins and glycosides<sup>[15], [16]</sup>. The present study was designated to investigate the inhibitory effect of hydroalcoholic extract of *C. nocturnum* on  $\alpha$ -amylase activity.

**Material and Methods****Collection of plant material**

The aerial parts of plant was collected from Kurukshetra University campus, Kurukshetra, during November 2012 and identified as *Cestrum nocturnum* (Family: Solanaceae) by Dr. H.B. Singh, Scientist Incharge, Raw Materials and Museum, National Institute of Science Communication And Information Resources, New Delhi where a voucher specimen (No.: NISCAIR/RHMD /Consult/-2011-12/ 2004/12 ) had deposited.

**Chemicals**

CNPG3 was purchased from Chemadiagnostics, Italy. Acarbose was purchased from Glucobay, Bayer Pharma, India, sodium dihydrogen orthophosphate dehydrate, and disodium hydrogen phosphate dehydrates (Himedia, India).

Trichloroacetic acid, alpha- amylase, bovine serum albumin, 5,5' dithio-bis-2-nitrobenzoic acid, reduced glutathione were procured from Sigma-Aldrich, St. Louis, USA. All the other chemicals used were of analytical grade.

#### Preparation of extracts

Aerial parts of *C. nocturnum* were dried in shade for two weeks. Dried parts was crushed and stored in an air tight container at room temperature. Dried powder was then extracted with hydroalcoholic (70:30) solution using Soxhlet's apparatus.

#### Preliminary Phytochemical screening

The extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, sterols and triterpenoids in leave extracts [17].

#### 2.5 Inhibition assay for Alpha-amylase activity

The  $\alpha$ -amylase inhibitory activity of hydroalcoholic extract of *C. Nocturnum* was determined based on the spectrophotometric assay using acarbose as the reference compound [18]. The extract was dissolved in DMSO to give concentrations from 10, 50 and 100  $\mu\text{g/ml}$ . The enzyme  $\alpha$ -amylase solution (0.5 U/ml) was prepared by mixing 3.246 mg of  $\alpha$ - amylase in 100 ml of 40mM phosphate buffer ph 6.9. Add 60  $\mu\text{l}$  of 40 mM phosphate buffer (ph 6.9)/acarbose/extract and 30  $\mu\text{l}$  of  $\alpha$ - amylase enzyme and are preincubated at 37°C for 10 min and then 120  $\mu\text{l}$  of CNPG3 was added, mixed and incubated at 37°C for 8 min. The absorbance was measured at 405 nm and control reaction was carried out without the extract. Percentage inhibition was calculated by the expression:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance test}}{\text{Absorbance control}} \times 100$$

#### Statistical analysis

Results were expressed as the mean  $\pm$  SEM, for statistical analysis of the data group, means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-test for multiple comparison.  $p < 0.001$  was considered to be statistically significant.

Table 1. Alpha amylase inhibition assay of hydroalcoholic extract of *C. nocturnum*

Sample IC <sub>50</sub> ( $\mu\text{g/ml}$ )	Concentration ( $\mu\text{g/ml}$ ) % inhibition		
Acarbose	0.1	32.41 $\pm$ 0.1	0.317
	0.5	73.36 $\pm$ 0.15	
	1.0	84.02 $\pm$ 0.13	
<i>C. nocturnum</i> extract	10	37.02 $\pm$ 0.16	45.9
	50	56.3 $\pm$ 0.11	
	100	62.9 $\pm$ 0.1	

The data are expressed in means  $\pm$  S.E.M. n = 3 in each group 0

## Results

### Preliminary phytochemical analysis

Preliminary phytochemical analysis showed the presence of carbohydrate, steroids, alkaloids, phenolic compounds, flavonoids, saponins and amino acids in hydroalcoholic extract of *C. nocturnum*.

### Alpha-amylase inhibition assay of hydroalcoholic extract of *C. nocturnum*

Table 1 results revealed that hydroalcoholic extract of *C. nocturnum* showed a significance inhibition of  $\alpha$ - amylase enzyme. The different concentration of extract ie. 10, 50, 100  $\mu\text{g/ml}$  showed 37.02  $\pm$  0.16%, 56.3  $\pm$  0.11%, 62.9  $\pm$  0.1% of  $\alpha$ - amylase enzyme inhibition and IC<sub>50</sub> value was 45.9  $\mu\text{g/ml}$ . The acarbose used as a reference standard at concentration of 0.1, 0.5, and 1.0  $\mu\text{g/ml}$  showed 32.41  $\pm$  0.1%, 73.36  $\pm$  0.15%, and 84.02  $\pm$  0.13% inhibition of  $\alpha$ -amylase activity and IC<sub>50</sub> value was 0.317  $\mu\text{g/ml}$ .

## Discussion

The 70% hydroalcoholic extract of *C. nocturnum* was tested for  $\alpha$ - amylase inhibition activity.

Drugs that inhibit carbohydrate hydrolyzing enzymes have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting the insulin secretion of NIDDM patients. The results of *in vitro* studies showed that *C. nocturnum* extract inhibits  $\alpha$ -amylase activity to a significant extent. Natural health products of vegetable origin were clearly indicated as a promising avenue for the prevention of chronic diseases [19].

The inhibition of  $\alpha$ -amylase activity is considered to be effective strategy for the control of diabetes by diminishing the absorption of glucose [20]

Many medicinal plants such as herbs have been reported to have anti-diabetic activity when assessed using presently available experimental techniques [21, 22, 23, 24]. For example, polyphenols from tea [22], sweet potato [25], berry [26] and Vietnamese edible plants such as *Syzygium zeylanicum*, *Cleistocalyx operaculatus*, and *Careya arborea* [24] exhibited an inhibitory effect on  $\alpha$ -amylase. Since various phenolic compounds have been generally accepted as anti-oxidant agents, it has been showed that the activity of  $\alpha$ -amylase is effectively inhibited by flavonoids, such as naringenin, luteolin, apigenin, diadzein and epigallocatechin gallate [27], indicating that polyphenolic compounds are able to inhibit the activities of carbohydrate-hydrolysing enzymes, due to their ability to bind with protein [28, 29, 4]. In our previous study, it has been showed that hydroalcoholic extract of *C. nocturnum* contains various phenolic compounds which are generally responsible for  $\alpha$ -amylase inhibition. Further study can be undertaken at cellular and molecular levels, which may further elucidate its mechanism in detail.

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