Short Communication

Preliminary assessment of *Calotropis gigantea* leaves extract for *in vitro* antidiabetic activity

Mohd Shahnawaz, Shambaditya Goswami, Ajay Kumar Shukla*

Department of Pharmaceutical Science, ITM College of Pharmacy, Gida Gorakhpur, India

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Abstract

Objective: The side effects related with the usage of synthetic antidiabetic drugs make it urgent to search for alternative drugs commencing medicinal plants. Therefore, this study was aimed to evaluating α -amylase inhibitory potential of *Calotropis gigantea* leaves extract as well as its possible mode of inhibiting these enzymes. **Material and methods:** Phytochemical investigation of petroleum ether, acetone and alcoholic extract were revealed that alcoholic extract having potent bioactive molecule therefore alcoholic extract was selected for further investigation of antidiabetic activity by using porcine pancreatic α -amylase method. **Results:** It was found to possess potential inhibitory effects on starch break down during *in-vitro*. Incubation of graded concentrations of the extracts (11.81-36.01 mg/ml) with α -amylase *in-vitro* resulted in a noticeable decrease in the enzyme activity (%) from 5-100 for *Calotropis gigantea*. The inhibition of α -amylase values for *Calotropis gigantea* was found 40.0 mg/ml, revealed that this extract inhibited to enzyme in a non-competitive manner. The α -amylase inhibitory properties of *Calotropis gigantea* leaves extract may be due to the presence of some phytochemicals such as flavonoids, tannins and saponins in the leaves. **Conclusion:** It can be concluded from this study that the *Calotropis gigantea* leaves extracts could serve as source of antidiabetic agents which may act through the inhibition of carbohydrate hydrolyzing enzymes α -amylase.

Keywords: *Calotropis gigantea*, diabetes mellitus, α -amylase

Introduction

The search for the discovery of antidiabetic drugs from medicinal plants is an important strategy that is required to combat the widespread nature of diabetes mellitus among all populations of the world. This is due to the fact that present synthetic drugs are bedeviled with many limitations ranging from limited efficacy and several side effects such as hypoglycaemia, weight gain and chronic tissue damage (Shukla et al., 2011; Lawal et al., 2010). Consequently, several plants with folkloric usage as antidiabetics are being verified with the aid of scientific methods. Some of these plants include *Blighia sapida*, *Morus alba*, *Spondias monbin*, *Treculia africana* and *Calotropis gigantea* leaves. *Calotropis gigantea* R.Br. (Asclepiadaceae) is small, erect and compact shrub of

about 4-6 M in height. It is a species of *calotropis* native commonly known as Mudar Yercum (milkweed or swallow-wort). It is a common wasteland weed. *Calotropis* belongs to Asclepiadaceae or Milkweed or Arka family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. Other familiar plants of *Calotropis* are Milk weed or Silk weed (*Asclepias syriaca* L.), Butterfly weed (*Asclepias tuberosa* L.) and *Calotropis procera* (Ait.) is native to India, *Calotropis* grows wild up to 900 meters throughout the country on a variety of soils in different climates, sometimes where nothing else grows (Hussein et al., 1994; Ajay et al., 2011; Iqbal et al., 2005; Roy et al., 2005). Arka (*Calotropis gigantea*) an important drug of Ayurveda is known in this country from the earliest time. It is mentioned by the earliest Hindu writers and the ancient name of the plant which occurs in the vedic literature was Arka alluding to the form of leaves, which was used in the sacrificial rites.

In Dhanvantari Nigantu three varieties of Arka are

*Address for Corresponding Author:

Dr. Ajay Kumar Shukla

Department of Pharmaceutical Science, ITM College of Pharmacy,
Gida Gorakhpur, India

Email: ashukla1007@gmail.com

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mentioned viz. Rajarkah, Suklarkah and Sveta mandarah. It has been widely used in the Sudanese, Unani, Arabic and Indian traditional medicinal system for the treatment of various diseases namely leprosy, ulcers, piles and diseases of the spleen, liver and abdomen (Roy et al., 2005; Charde et al., 2011).

Some plants are known to lower blood glucose levels and traditionally useful. So far reports on systematic evaluation and scientific investigation of these antidiabetic plant or their phytoconstituents pancreatic α -amylase inhibitors are scarce. This study was carried out not only to validate the traditional use of this plant in diabetes but also to initiate search for newer pharmacophores with specificity towards PAA. Structurally as well as mechanistically, porcine pancreatic α -Amylase is closely related to human pancreatic α -amylase. Hence sequential solvent extract of the *Calotropis gigantea* screened for the presence of PPA inhibitors, the lead extract quantified for PPA inhibition under in-vitro condition and subjected to phytochemical analysis in order to identify the probable inhibitory phytoconstituents. Therefore, the aim of this study was to evaluate the α -amylase inhibitory potential of *Calotropis gigantea* leaves with possible mechanism of hypoglycemic effect.

Plant material and other chemicals

Calotropis gigantea leaves were collected in February 2012 at Nandanagar, Kunraghat, Gorakhpur (U.P.). It was identified and authenticated at the Department of Pharmacognosy, ITM College, GIDA, Gorakhpur (U.P.) India. The plant material was washed with water and then dried in the sun light for 10-12 days. The leaves were grinded in to the mixer to make it fine powder and then passed through sieve no. 60. Further the fine powder was used for extraction by using Soxhlet apparatus (Bajaj et al., 2012).

Porcine pancreatic α -amylase and paranitrophenyl-glucopyranoside (PNPG) were products of Sigma-Adrich Co., St Louis, USA. Other chemicals and reagents were of analytical grade and water used was glass-distilled.

Preparation of plant extracts

Fresh leaves of *Calotropis gigantea* were cut and washed with water to remove all contaminants; they were dried under room temperature and grounded to powder. The powdered leaves were divided into three portions and hot extracted by using paterolium ether. The marc left after extraction of petroleum ether and acetone was dried and then extracted with ethanol 95% v/v, at 50-60°C. After completion of extraction, the solvent was removed by distillation. The residue was then stored in the desiccators for further use (Kokate et al., 2005).

Phytochemical screening

Phytochemical compositions of the leaves extract were determined using the various chemical tests (Mourya et al., 2017).

Preparation of various solutions (Sen et al., 1992)

Starch solution (0.5% w/v) was prepared by stirring and boiling 0.25 g of soluble potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α -amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The extracts were dissolved in DMSO to give concentrations from 11.8 to 40mg/ml (15, 20, 25, 30, 35, 40 mg/ml). The color reagent, was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml).

α -Amylase inhibitory assay

One ml of each plant extract and 1 ml enzyme solution were mixed in a tube and incubated at 25°C for 30 min (Mourya et al., 2017). To 1 ml of this mixture was added 1 ml of starch solution and the tube incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the closed tube placed into a 85°C water bath. After 15 min, the reaction mixture was removed from the water bath, cooled

Figure 1. Powdered leaves of *Calotropis gigantea*

and diluted with 9 ml distilled water and the absorbance value determined at 540 nm in UV spectrophotometer.

% Inhibition = $[(\text{Abs of control} - \text{Abs of extracts}) / \text{Abs of control}] \times 100$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

Preparation of control solution

In this case, the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as similar to above. Controls were conducted in an identical manner replacing the plant extracts with 1 ml DMSO. Acarbose solution (at the concentrations of 0.0094, 0.0118, 0.0147, 0.0184, 0.023, 0.036, 0.056, 0.07, 0.11, 0.21, 0.42 $\mu\text{g/ml}$) was used as positive control.

Percentage α -amylase inhibition = $100 \times (\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}) / \Delta A_{\text{Control}}$

$$\Delta A_{\text{Control}} = A_{\text{Test}} - A_{\text{Blank}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Test}} - A_{\text{Blank}}$$

The percentage inhibition was plotted against the sample concentration and a logarithmic regression curve established in order to calculate the IC_{50} value (inhibitory concentration). This would represent the concentration of sample (mg/ml) necessary to decrease the absorbance of α -amylase by 50%.

Results

The result of the phytochemical screening conducted on the various extracts of *Calotropis gigantea* leaves showed in table 1. It shows that flavonoids, reducing sugars and tannins are present in both the alcoholic and aqueous extracts while steroids was detected in petroleum ether and alcoholic extracts. However, saponin was present in alcoholic extract only (Gupta et al., 2005; Gupta et al., 2013).

Table 1. Preliminary phytochemical screening of the various extracts of *Calotropis gigantea* leaves

S. No.	Name of tests	Observations		
		Petroleum ether	Acetone	Alcohol
1.	Alkaloids (mayer's test)	-	-	+
2.	Cardiac glycoside (Keller- killani test)	-	+	+
3.	Anthraquinines test	-	-	+
4.	Tannins	-	-	+
5.	Saponins (forth test)	-	-	+
6.	Flavonoids	-	-	+
7.	Steroids	+	-	+
8.	Terpenoids (salkowski test)	+	-	+
9.	Reducing sugars	-	-	+
10.	Resins	-	-	+
11.	Phenols	-	+	+

Table 2. Results of % alpha amylase inhibition

S. No.	Concentration (mg/ml)	(%) α -Amylase inhibition
1.	15	7.692 $\pm$ 0.53
2.	20	15.348 $\pm$ 0.94
3.	25	23.076 $\pm$ 1.56
4.	30	32.692 $\pm$ 2.48
5.	35	42.307 $\pm$ 3.28
6.	40	50.020 $\pm$ 3.97

Figure 2. α -Amylase inhibitory activities of *Calotropis gigantea* extract

In the present study, *calotropis* species, was found to possess favorable inhibitory effects on starch break down *in vitro*. Incubation of graded concentrations of the extracts (11.8-36.0 mg/ml) with α -amylase and starch *in vitro* observed in a noticeable decrease in the enzyme activity (%) from 5-100 for *Calotropis gigantea*. Alpha-amylase inhibition results of *Calotropis gigantea* extract was found as 40.0 mg/ml (Table 2 and figure 2).

Discussion

Hyperglycemia disease is a state characterized by a quickly increase of blood glucose levels and is due to continuous hydrolysis of starch by pancreatic α -amylase and absorption of glucose by the intestinal α -amylase (Bever et al., 1979). One of the key therapeutic approaches for decreasing postprandial hyperglycemia is to retard digestion of glucose by the inhibition of these carbohydrate hydrolyzing enzymes, α -amylase, in the digestive tract (Bever et al., 1979). Consequently, inhibition of these carbohydrate-hydrolyzing enzymes can drastically decrease postprandial hyperglycemia after a mixed carbohydrate diet and can be an important strategy in the management of diabetes mellitus (Zacharias et al., 1980). In the present study, the results of the *in-vitro* α -amylase inhibitory assay showed that the alcoholic extracts of *Calotropis gigantea* leaves are a significantly inhibitor of α -amylase.

The percentage inhibitory effect of the alcoholic extracts of *Calotropis gigantea* leaves on α -amylase, could be as the presence of phytochemicals mainly are flavonoids, saponins and tannins etc. The tannins have been reported to stimulate phosphorylation of the insulin receptors as well as translocation of glucose transporter 4 (GluT4), a chief mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis. It also helps in the suppression of the key gene responsible for adipogenesis thereby helping to decrease blood glucose level without raising the adiposity (Ghos et al., 1988). Flavonoids have also been reported to preserve β -cell integrity and function by scavenging free radicals in the system and therefore protect against the progression of diabetes mellitus (Ferrington et al., 1990).

It can be concluded from this study that out of the three extracts of *Calotropis gigantea* leaves tested, the alcoholic extract displayed the most effective inhibition of α -amylase *in-vitro*. The potent inhibitory activities of this plant may be due to the synergistic effect of phytochemicals present in it.

Conclusion

The phytochemical and pharmacological studies on *Calotropis gigantea* were done. The phytochemical constituents were extracted by solvent extraction and identified by chemical test. Alcoholic extract showed the presence of majority of

phytoconstituents. Hence, it was selected for the *in vitro* studies. The results of study indicate that the alcoholic extract of *Calotropis gigantea* represented appreciable α -amylase inhibitory effects. This study supports the ayurvedic concept that the *Calotropis gigantea* could be useful in management of diabetes.

Conflict of interest

None

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