

ANTIDIABETIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF EXTRACTS OF THE LEAVES OF *CINNAMOMUM ZEYLANICUM* ON ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

The leaves of *Cinnamomum zeylanicum* have been used in traditional health systems to treat diabetes mellitus. However, the antidiabetic activity of this medicinal plant is not scientifically validated and authenticated. The present study aimed to investigate the *in vitro* and *in vivo* anti-diabetic activity of flower crude extract and solvent fractions of *Cinnamomum zeylanicum*. The *in vitro* α -amylase inhibition of the crude extract and solvent fractions of *Cinnamomum zeylanicum*. Blood glucose lowering activity of 80% Ethanolic crude extract and solvent fraction was studied in animal models: Hypoglycemic mice model, oral glucose loaded mice model, dose-treated Alloxan -induced diabetic mice model. The effect of the crude extract on diabetic lipid profile was studied. The acute toxicity study of *Cinnamomum zeylanicum* leaves extract did not show mortality in the animals at the limit dose during the observation period. The result of α -amylase enzyme inhibition activity was found in a dose-dependent manner, the strongest activity was shown by Crude extract fraction (89.60 % inhibition at 1000 μ g/mL) compared to the standard acarbose having 97.19% inhibition at 1000 μ g/mL. The crude extract of *Cinnamomum zeylanicum* showed significant blood glucose-lowering effect on hypoglycemic mice and oral glucose loaded mice. In Alloxan-induced diabetic mice model, the crude extract fraction significantly decreased the fasting blood glucose level after 14 days of treatment.

The result demonstrated the beneficial biochemical effects of *Cinnamomum zeylanicum* extract by inhibiting α -amylase improving serum lipid profile levels. The leaves crude extract are effective in lowering blood glucose levels in diabetic and hypoglycemic mice. The claimed traditional use as antidiabetic has scientific ground.

Keywords: Diabetes mellitus, Herbal medicine, *Cinnamomum zeylanicum*, Alloxan, Anti diabetic activity.

1. INTRODUCTION

Depressed patients commonly complain about feeling sad, lack of interest in their day to day work, inability to find pleasure in activities that would normally please others, and feelings of discontent. It is also associated with feeling of guilt or reduced self-worth, disturbed sleep, changes in appetite, and low energy. In severe forms of depression, patients are known to cause self harm or even suicide. Although drugs available for the therapy of depression, they have their limitations, such as delayed therapeutic response and low responders to these drugs, which poses a problem with patient compliance. Hence, there is a requirement for alternative treatment of depressive disorders with the use of medicinal plants.

The plant *Mimosa pudica* is a weed that grows in humid areas, open fields and by roadsides. It grows as a shrub, under 100 cm in height, and is easily identifiable by its characteristic 15 – 20 pairs of leaflets that folds when disturbed, and is hence known as “Lajwanti” in Hindi, and “Touch me not plant” in English. It is believed to be native to the Middle Americas and is now found in other in all tropical countries of the Asian subcontinent and South East Asia. It has been used as a folk lore medicine since many years because of its various medicinal properties, and because it is an easily cultivable plant and is abundantly available.

2. MATERIAL AND METHODS

2.1. Preparation of plant extracts

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered leaves was uniformly packed into a thimble in a Soxhlet apparatus and extracted with 1000ml Petroleum ether, Ethyl acetate and Ethanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in refrigerator at 4°C for their future use in phytochemical analysis and pharmacological screenings.

2.2. Alpha-amylase inhibition assay

The a-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method.⁵⁰ The crude and solvent fractions of *Cinnamomum zeylanicum* were dissolved in buffer ((Na₂HPO₄/ NaH₂PO₄ (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 50 to 1000 mg/mL. A volume of 200 mL of a-amylase solution (Molchem) (2 units/mL) was mixed with 200 mL of the extract and was incubated for 10 minutes at 30 C. Thereafter, 200 mL of the starch solution (1% in water w/v) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) and was boiled for 10 minutes in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technologies). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 mL of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (Bayer) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The inhibition of a-amylase was expressed as percentage of inhibition and was calculated by the following equation: Inhibition (%) $\frac{1}{4} \left[(Ac - Acb) / (Ac - Asb) \right] \times 100$, where Ac is the absorbance of control; Acb is the absorbance of control blank; As is the absorbance of sample; and Asb is the absorbance of sample blank. The % a-amylase inhibition was plotted against the extract concentration and the IC₅₀ values were obtained from the graph.

2.3 Induction of diabetes to animals

A single dose (100 mg/kg b.w., i.p.) of Alloxan dissolved in sodium citrate buffer was used for the induction of diabetes in Mice after overnight fasting. After 1 hr of Alloxan administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycaemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

2.4. Experimental design

Five groups of Mice six in each groups received the following treatment schedule for 14 days.

GROUP I - Normal control (normal saline 10 ml /kg, P.O)

GROUP II - Alloxan treated control (100 mg/kg, I.P)

GROUP III - Alloxan (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).

GROUP IV - Alloxan (100 mg/kg, i.p.) + EECZ.(200 mg/kg, P.O)

GROUP V - Alloxan (100 mg/kg, i.p.)+ EECZ. (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control Mice. Group IV and Group V (which previously received Alloxan 100mg/kg) were given fixed doses of ethanol leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Cinnamomum zeylanicum* and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (EECZ- Ethanolic extract of *Cinnamomum zeylanicum* Leaves).

2.5. Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of Mice at weekly intervals till the end of the study 1, 7, and 14 days. Estimation of biochemical parameters Serum blood glucose. On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose

3. RESULTS AND DISCUSSION

3.1. Appearance and percentage yield of EECZ (Ethanolic Extract of *Cinnamomum zeylanicum* Leaves)

Table 1: a-Amylase Inhibitory Activities of the Crude Extract and Solvent Fractions.

Concentration (mg/mL)	Percentage inhibition				
	Chloroform fraction	Ethyl acetate fraction	Aqueous fraction	Crude extract	Acarbose
50	6.41 + 0.1	15.82 + 0.35	29.16 + 1.11	34.91 + 0.36	57.65 + 0.79
100	11.64 + 0.69	20.04 + 0.11	35.71 + 0.82	41.05 + 1.42	68.10 + 0.46
200	23.14 + 0.45	27.16 + 1.92	42.12 + 0.46	61.19 + 0.98	76.93 + 1.53

400	29.65 + 0.50	46.90 + 0.15	54.81 + 0.53	73.34 + 0.76	88.51 + 0.17
600	38.01 + 0.99	54.14 + 0.64	68.93 + 0.92	81.92 + 0.24	93.06 + 0.26
800	45.15 + 0.81	65.54 + 0.49	75.50 + 0.76	86.41 + 0.19	96.27 + 0.17
1000	53.34 + 0.76	74.77 + 0.12	83.19 + 0.81	89.60 + 0.74	97.19 + 0.92
IC50	31.14 + 0.12	21.80 + 0.71	14.24 + 0.64	7.21 + 0.91	3.34 + 0.14

In Vitro a-Amylase Inhibition Activity of Crude Extract and Solvent Fractions *In vitro* a-amylase inhibitory study evaluating the percent of a-amylase inhibition as a function of extract concentrations and the IC50 values were calculated (Figure). Concentration dependent inhibitions were observed for various concentrations of the tested extracts and the standard. Among the extracts, the crude extract exhibited the lowest IC50 of 67.21 + 0.91 mg/mL and the IC50 values of water fraction, ethyl acetate fraction, and the chloroform fraction were 14.24 + 0.64, 21.80 + 0.71, and 31.14 + 0.12 mg/mL, respectively. The standard positive control acarbose showed an IC50 of 3.34 + 0.14 mg/mL

Table 2: Hypoglycemic Test

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	30min	1hr
CONTROL Carboxyme Thyl Cellulose (CMC)	0.5%	69.15±2.451	68.14±4.320	71.19±2.129
Positive Control Glibenclamide	2	67.24±3.209	50.15±1.492**	30.96±3.298***
Aqueous Ethanolic Extract of <i>Cinnamomum zeylanicum</i>	200	66.87±1.251	57.91±3.482*	55.14±2.101*
Aqueous Ethanolic Extract of <i>Cinnamomum zeylanicum</i>	400	66.18±3.420	50.19±3.281**	34.2±1.921***

3.2. *Invivo antidiabetic study*

Table 3: Results of the effects of Ethanolic extract on blood Glucose levels

TREATMENT	BLOOD GLUCOSE LEVEL (mg/dl)		
	0 min	30min	1hr
Normal control 10 ml/kg P.O	77.29±3.104	73.1±3.219	72.2± 3.917
Negative control	261.1±2.91	267.2±4.1	271.3±2.1
Positive control (Glibenclamide 2mg/kg) P.O	251.18±3.156	136.98±2.4***	113±1.1***
EECZ 200 mg/kg P.O	256±2.1	245.1±2.154**	241.2±1.209**
EECZ 400 mg/kg P.O	260±1.10	170.2±1.72***	158.1±2.9***

4. CONCLUSION

This study revealed that the crude extract and solvent fractions of *Cinnamomum zeylanicum* have showed significant lowering of blood glucose level on diabetic, Hypoglycemic and oral glucose loaded mice and not permitted bodyweight loss of diabetic.

The results also verified that inhibition of intestinal α -amylase by the extracts may contribute to the anti hyperglycemic activity. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications. *Cinnamomum zeylanicum* would be promising for further clinical studies in the management of DM. Further studies to find out the mechanism of this plant for its antidiabetogenic effect and there is a need for bioactivity guided investigation to isolate the lead compound responsible for the antidiabetic activity.

The present study suggested that the isolation of active constituents from Ethanolic extract of *Cinnamomum zeylanicum* leaf and characterize the compounds by using preliminary phytochemical studies.

5. REFERENCES

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