

## Research Article

# Oral Glucose Tolerance Effect of *Ceasalpinia mimosoides* Leaf Extract and Role of Alpha Amylase Inhibitory Activity

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## ABSTRACT

The present study was to evaluate the oral glucose tolerance test in normal wistar albino rats and *invitro* alpha amylase inhibitory activity in aqueous extract and ethanolic extract of *Ceasalpinia mimosoides* leaves. The results shows that the plant extracts possess good alpha amylase inhibitory activity indicating dietary use in the management of diabetes. Oral glucose tolerance test in the *Ceasalpinia mimosoides* leaves extract is carried out in glucose loaded normal fasted rats results showed that they are highly significant ( $p < 0.001$ ) indicating the antihyperglycemic effect of plant extract.

**Keywords:** Alpha amylase enzyme, Glucose, Glibenclamide.

## INTRODUCTION

*Ceasalpinia mimosoides* is a Shrub and an armed straggler. This plant occurs all over asian countries, particularly in moist deciduous and degraded forests, plains to high altitude. In India it is seen in mostly in kerala ,with tuberous root stock, prickly all over; twigs glandular hairy. Leaves up to 40 cm long; pinnate, oblong, base unequal, apex obtuse, entire margin, rachis prickly. The plant has been used by folklore practioners and tribals for many diseases as an anthelmintic, antiarthritic, ulcer and wound management<sup>1-5</sup>. Been good in treatment of many diseases traditionally, the plant material is extracted for the study of alpha amylase inhibitory activity and oral glucose tolerance test.

## MATERIALS AND METHODS

The fresh leaves of *Ceasalpinia mimosoides* was collected from the locally growing area of Kottayam district, Kerala in February 2013. The plant was identified and authenticated by Raju Thomas, H.O.D, Department of Botany, BaseliusCollege, Kottayam, Kerala.

### Extraction of plant material

The powdered sample of *Ceasalpinia mimosoides* leaves of 120 gm were packed well in soxhlet apparatus separately and extracted with 500 ml ethanol and aqueous solvent continuously for 48 hours. Both extracts were collected separately and concentrated by using rotary evaporator and were stored in

a desiccators for experimental purposes and subjected to phytochemical screening<sup>6</sup>.

### Animals

Female rats of healthy twenty four Wistar albino rats weighing of 150-200gms and three female albino mice weighing between 20-25gms were obtained from the animal house of Department of Pharmacology, UCP ,RIMSR, puthupally which is housed in polycarbonate cages under standard conditions of temperature ( $25 \pm 2^{\circ}$  C) and relative humidity (30-70%) with a light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. Approved at the Institutional Animal Ethics Committee (IAEC), CPCSEA NO - 1702/PO/C/13/CPCSEA of UCP, DPS, RIMSR was taken for conducting study. After procurement, all the animals were divided into different groups and were left for one week for acclimatization and were maintained on standard conditions.

### Acute toxicity study

This was performed to ascertain safe dose by the Organization of Economic Cooperation and Development (OECD) 423 guidelines. Swiss Albino Mice weighing between 20-25 g .They were kept fasting four hours prior to the treatment. A single administration of starting dose of 2000 mg/kg body weight/ p.o of the extract (ethanolic extract and aqueous was suspended in 1% CMC solution) was

administered to three female mice. They were noted individually after dosing, at least once during the first 30 minutes, with special attention given during the first four hours and thereafter for a total of 14 days. There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. When the experiment was repeated again with same dose level, 2000 mg/kg body weight/ p.o of plant extract for 7 more days and observed for fourteen days, no change was observed.

### Experimental procedure

#### *Invitro*

#### Assay for $\alpha$ -amylase Inhibition

Twenty five microlitres of 10 mg/ml extract and 25 $\mu$ l of 25mM phosphate buffer pH 6.9, containing porcine  $\alpha$  amylase at a concentration of 0.5 mg/ml were incubated at 25 $^{\circ}$ C for 10 min. After pre incubation, 25 $\mu$ l of 0.5% starch solution in 25mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25 $^{\circ}$ C for 10 min. The reaction was stopped with 50 $\mu$ l of 96mM 3, 5 dinitrosalicylic acid colour reagent. The micro plate was then incubated in a boiling water bath for 5 min and cooled to room temperature. Acarbose was used as standard drug. Absorbance was measured at 540nm using a microplate reader<sup>7-8</sup>.

#### *Invivo*

#### Oral glucose tolerance test

Fast overnight (18 h) rats. Divide them into 4 groups of 6 rats each.

Group I : Normal control rats with 1ml of 1 % w/v c.m.c vehicle solution.

Group II : Normal rats with standard drug glibenclamide 5mg/kg body weight p.o

Group III : Normal rats with E.E.C.M 400mg/kg body weight p.o

Group IV : Normal rats with A.E.C.M 400 mg/kg body weight p.o

Glucose (2g/kg) is to be given 30 min after the administration of extracts, glibenclamide. Then blood is to be withdrawn from retro orbital sinus at 0, 30, 60 and 120 min of glucose administration and estimated blood glucose levels by using glucose oxidase-peroxidase reactive strips and a glucometer<sup>9-12</sup>.

#### Statistical analysis

Results were expressed as mean  $\pm$  SEM, (n=6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test. \*P<0.05, \*\*<0.01 and \*\*\*<0.001, show

statistical significance when compared treatment group with normal control.

### RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed that *Ceasalpinia mimosoides* leaves extracts shows the presence of carbohydrates, tannins, phenolic compound, flavonoids, saponins, terpenoides, steroids, alkaloids. The extracts were found to be safe up to 2000 mg/kg body weight since no death and signs of toxicity were observed while conducting acute toxicity test.  $\alpha$ -amylase is a digestive enzyme that hydrolyses alpha-1,4 bonds of large polysaccharides such as starch and glycogen, yielding the smaller by-products of glucose and maltose. Inhibition of  $\alpha$ -amylase enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose<sup>13-14</sup>. There was a dose dependent increase in percentage inhibition seen in the alpha amylase enzyme. At a concentration of 12.5  $\mu$ g/ml shows 48.1 % in EECM and 42 % in AECM, where at 100  $\mu$ g/ml shows 72.2 % at EECM and 67.5 % at AECM. Statistical evaluation shows a comparison between percentage inhibition of *Ceasalpinia mimosoides* leaves extract and standard corresponding to each concentration indicates that EECM and AECM possess significant alpha amylase inhibitory activity as compared to the standard acarbose.

OGTT determine how quickly glucose cleared from the blood after a given oral glucose dose. The test is usually used to test for diabetes, insulin resistance or rarer disorders of carbohydrate metabolism<sup>15</sup>. In this study it was observed that, with in 30 min of starting oral glucose tolerance test, the blood glucose levels are elevated which is higher than the 0 min . At 60 min onwards the blood glucose levels slightly comes to decreases. The treatment groups GLB 5mg/kg, EECM400 mg/kg, AECM 400 mg/kg reduces blood glucose levels, in which significant level of decrease in the glucose levels observed at 90 min. The level of significance were compared with the normal group in which glibenclamide significantly reduces the blood glucose levels, (p<0.001).The EECM shows a moderate level of significance, (p<0.01) and AECM shows (p<0.05). This investigation shows that both extract possesses glucose lowering effect indicating a better glucose utilization capacity. They may due to tissue glucose uptake and reduce hepatic glucose output, there by producing an antihyperglycemic effect.

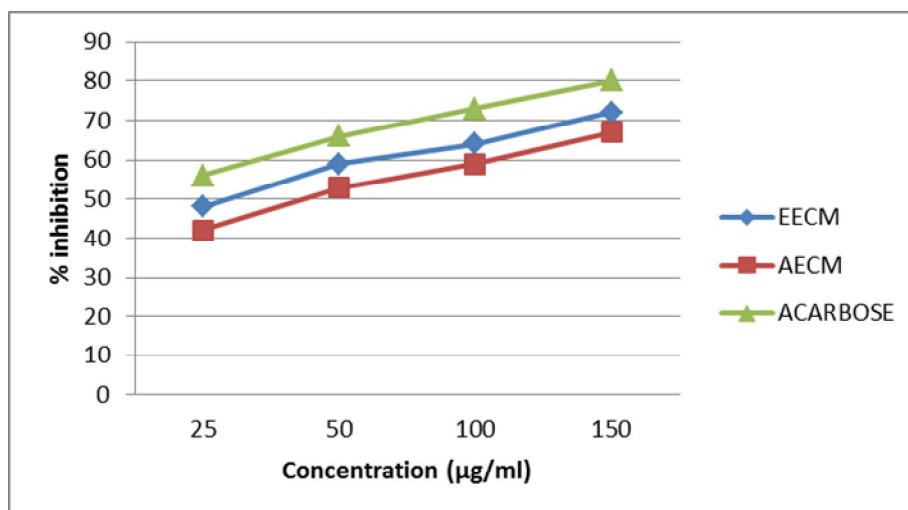
**CONCLUSION**

The present study showed that *ceasalpinia mimosoides* leaf extracts EECM and AECM have significant hypoglycemic activity. It proved an increased glucose tolerance of normal rats in the OGTT. *In vitro* study showed that plant proved a good alpha amylase

enzyme inhibitory activity suggest an use of dietary intervention in the clinical management of diabetes. We can assume that this may be due to the phytochemical constituents present in them. Future experiments to carry out the lead moiety responsible for plant hypoglycemic effect.

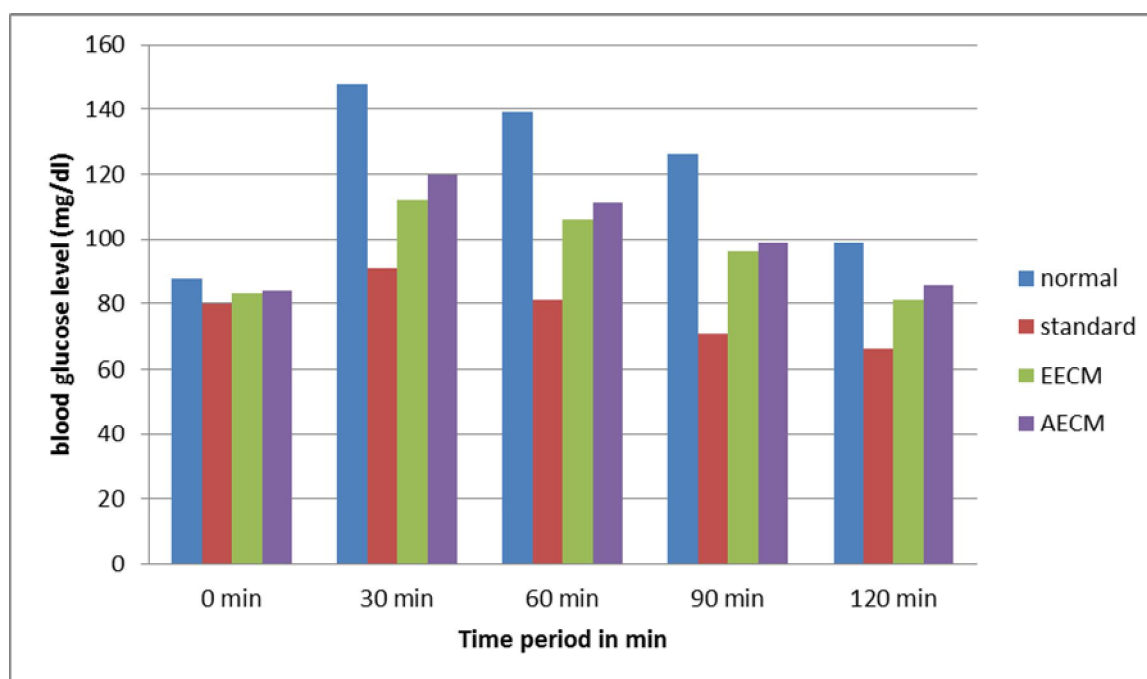
**Table 1: Alpha amylase inhibition assay**

S. No	Concentration (µg/ml)	% inhibition		
		EECM	AECM	Acarbose
1	12.5	48.1±1.025	42.0±0.75	56.12±1.085
2	25	59.2±1.085	53.23±1.4	66.18±1.728
3	50	64.5±1.626	59.4±1.004	73.26±1.041
4	100	72.2±1.952	67.5±2.055	80.01±1.549

**Graph. 1: Effect of alpha amylase inhibitory activity of ceasalpinia mimosoides leaves extract****Table 2: Effect of ceasalpinia mimosoides leaves extract in oral glucose tolerance test in normal rats**

Groups	Blood glucose level (mg/dl)				
	0min	30 min	60 min	90 min	120 min
Normal	88.33±1.856	148.66±1.76	139.66±2.18	126.66±2.02	99.33±4.37
GLB	80.33±2.333	91.66±2.603***	81.66±2.02***	71±1.15***	66.66±1.45***
EECM	83.33±5.925	112.66±2.333***	106±2.08***	96.33±1.45***	81.33±1.45**
AECM	84.66±5.207	120.33±1.764***	111±2.64***	99.33±1.45***	86.33±1.45*

All values are Mean±SEM, n=6, \*P<0.05, \*\*P<0.01 and \*\*\* P<0.001 represents statistical significance of treatment group Vs normal control group.



**Graph. 2: Effect of *caesalpinia mimosoides* leaves extract in oral glucose tolerance test in normal rats**

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