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Evaluation of Antidiabetic activity of Ethanolic leaves Extract of *Ceasalpinia Mimosoides* in Alloxan induced Diabetic Rats

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ABSTRACT

In the present study the ethanolic extract of Ceasalpinia mimosoides Linn leaves were evaluated for antidiabetic activity on alloxan induced diabetic rats. The ethanolic leaves extract of Ceasalpinia mimosoides showed a highly reduction in blood glucose levels on compared with the diabetic control rats which is highly significant (p<0.001) which indicates a significant antidiabetic activity.

***Key words**: Alloxan monohydate, Antidiabetic, Ceasalpinia mimosoides.*

INTRODUCTION

Ceasalpinia mimosoides Linn is aso known as mimosa thorn (ceasalpiniacea). It is a Shrub and an armed straggler, to 6m with tuberous root stock, prickly all over; twigs glandular hairy. Leaves up to 40 cm long; pinnate 12-18 pairs; leaflets 10-15 pairs, 6 x 3 mm, oblong, base unequal, apex obtuse, entire margin; rachis prickly. It is seen in moist deciduous and degraded forests, plains to high altitude. The plant has been used by folklore practioners for many diseases as anthelmintic, antiarthritic, ulcer and wound management. Been well in treatment of many diseases traditionally its antidiabetic activity has not yet been reported while its related species show the same activity [1-7].

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.A herbarium specimen is deposited in our college museum (UCP/MGU/RIMSR/2013/herb7).

Preparation of extract:

The powdered sample of *Cesalpinia mimosoides* leaves of 120 gm were packed well in soxhlet apparatus and extracted with 500 ml ethanol for 48 hours. The extract obtained were collected and concentrated by using rotary evaporator and were stored in a desiccator, 14.3% w/v yield was obtained and subjected to phytochemical screening to identify various chemical constituents [8].

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 ± 2^0 C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. Approved at the Institutional Animal Ethics Committee (IAEC) of UCP, DPS, RIMSR was taken for conducting antidiabetic activity. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to room 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 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:

Diabetes was induced in rats by single intraperitonial injection of alloxan monohydrate 120 mg/kg dissolved in sterile distilled water to over-night fasted rats. After 72 h of injection, rats with marked hyperglycemia (fasting blood glucose > 250 mg/dl) were selected and used for the study.

Experimental design:

The animals were divided into five groups of six rats each.

Group I: Control normal rats administrated with 1ml 1% w/v c.m.c vehicle.
Group II: Diabetic control administrated with 1ml 1 % w/v c.m.c vehicle.
Group III: Diabetic rat received with glibenclamide 5 mg/kg/day p.o.
Group IV: Diabetic rat received with E.C.M 400mg/kg body weight p.o.

The diabetic rats were treated with test extract and standard drug continuously for 21 days. The effect of extract was determined by measuring the initial and final body weight, blood glucose level. 1, 7 , 14 , 21st days fasting blood glucose levels from retro orbital plexus puncture were estimated by using glucose estimating strips by a one touch glucometer (one touch horizon glucometer ,Johnson and Johnson) [9, 10].

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Statistical analysis:

Results were expressed as mean ± 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 diabetic control.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed the presence of carbohydrate, alkaloids, tannins and phenolic compounds, triterpenes, carbohydrate, flavanoids in ethanolic leaf extract (EECM). 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.

Alloxan is selectively toxic to pancreatic beta cells produces cell necrosis. The cytotoxic action is mediated by reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration, causing rapid destruction of beta cells leads to diabetes mellitus. Fasting blood glucose levels of animals in all groups were recorded on 1st, 7th, 14th, 21st days of the treatment period **Table No. 1**. A progressive decrease in blood glucose levels were seen during the time period of study. On the 1st day of treatment period a hyperglycemic state is observed in the alloxan induced groups. On the 7th day of treatment glib 5mg/kg , EECM 400 mg/kg , shows significance as compared with diabetic control group in which changes in the blood glucose levels were observed, EECM shows moderate level of significance(p<0.01). On the 14th day indicates a higher level of significance as compared with diabetic control group. At the end of the study the 21st day a higher reduction in blood glucose levels indicating a very higher level of significance (p<0.001) is observed in the EECM group **Fig. 1**. Induction of diabetes with alloxan is associated with decrease in body weight which is due to increased muscle wasting and due to loss of tissue proteins, lipolysis, fliud loss .Body weight of all group animals were recorded on 0th, 1st, 7th, 14th, 21st days of the treatment period **Table No. 2**. In the treated groups with GLB 5mg/kg, EECM 400 mg/kg on the 14th day shows a significant increase in body weight as compared to diabetic control group (p < 0.001). On the 21st day a higher level of significance is observed indicates its protective effect of controlling the glucose levels and improvement in the insulin secretion **Fig. 2**.

Table No. 1: Effect of *ceasalpinia mimosoides* leaves extract on blood glucose levels (mg/dl) of diabetic rats

Groups	1st day	7th day	14th day	21st day
Normal	93.16 ± 2.48	89.50 ± 3.69	97.5 ± 3.60	94.5 ± 4.36
Diabetic	394.33 ± 10.04	398.17 ± 10.19	402.3 ± 9.60	413.33 ± 7.20
Glibenclamide 5mg/kg	392.67 ± 10.59	288.6 ± 16.25***	176 ± 6.66***	120.17 ± 1.79***
EECM 400mg/kg	388.50 ± 8.63	309.00 ± 20.28**	194.3 ± 6.49 ***	123.8 ± 2.53 ***

All values are Mean±SEM, n=6, **P<0.01 and *** P<0.001 represents stastical significance of treatment group Vs Diabetic control

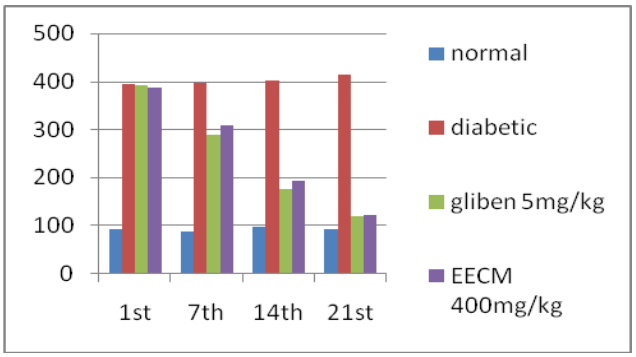

Fig. 1: Effect of *ceasalpinia mimosoides* leaves extract on blood glucose levels (mg/dl) of diabetic rats

Table No. 2: Effect of *ceasalpinia mimosoides* leaves extract in change in body weight of diabetic rats

Groups	1st day	7th day	14th day	21st day
Normal	154.07 ± 3.25	155.37 ± 3.26	156.72 ± 3.18	159.12 ± 2.91
Diabetic	139.33 ± 1.60	133.33 ± 1.94	125.83 ± 1.79	119.67 ± 1.52
Glibenclamide 5mg/kg	142.00 ± 2.06	144.17 ± 2.07*	146.67 ± 1.66***	149.50 ± 1.33***
EECM 400mg/kg	135.17 ± 0.83	136.67 ± 0.95	139.62 ± 1.92***	142.38 ± 1.88***

All values are Mean±SEM, n=6, **P<0.01, *** P<0.001 represents stastical significance of treatment group Vs Diabetic control.

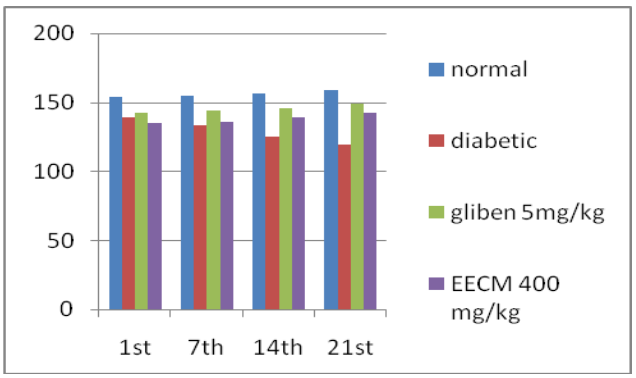

Fig. 2: Effect of ceasalpinia *mimosoides* leaves extract in change in body weight of diabetic rats

CONCLUSION

The present study indicates that the *ceasalpinia mimosides* leaves their ethanolic extract possess significant antidiabetic. We can assume that the antidiabetic activity of pant extract may due to the phytochemical constituents present in them. Further experiments should be carried out for the isolation of the components responsible for the hypoglycemic and hyolipidemic activity and explore the mechanism behind their activity.

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