



EVALUATION OF ANTIDIABETIC ACTIVITY OF *CASSIA SIAMEA* LEAVES IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

In the present study different extracts of *Cassia siamea* leaves were investigated for their antidiabetic activity. Ethanolic, Ethyl acetate and Hexane extracts of *Cassia siamea* leaves at doses 150 and 300 mg/kg were tested for antidiabetic activity in Alloxan induced diabetes model and the plasma blood glucose levels were estimated by GOD-POD method at 0, 2, 4, 6, 8 and 12hr. In Alloxan induced diabetes model, ethyl acetate extract of *Cassia siamea* leaves at two different doses produced significant ($P < 0.001$) reduction when compared to ethanol and hexane extracts. The results of this study explicate justification of the use of this plant in the treatment of diabetes. Among the three types of *Cassia siamea* leaves extracts, the ethyl acetate extract showed better activity than the other two extracts.

Key words: *Cassia siamea*, Leaves, Alloxan, Antidiabetic activity.

INTRODUCTION

Cassia siamea is commonly known as the Kassod tree, Cassia tree, belongs to family Fabaceae. Leaves are mainly used in the treatment of diabetes, bubo (lymph node swelling), urine stones, antihypertensive, insomnia (sleeplessness), dysentery and disorders of the large intestine (Hassain, 1999). The literature survey revealed that *C.siamea* contains different phytochemical compounds like lupeol, chrysophanol, cassiamin A, cassiamin, siameamin, siameadin, lupeone, rhein, chrysophanol- antrone, barakol, cassia chromone (5 acetonyl-7-hydroxy-2-methyl chromone), p-coumaric acid, apigenin-7-o-galactoside, beta-sitosterol, cassia chromone and cassiadinine (Chopra et al., 1956; Krishna Rao and Narayana Reddy, 1978; Hildebert et al., 1978; Kshetra et al., 1986; Ingkaninan et al., 1999) Since *C.siamea* has been reported to possess medicinal effects (Ahmed et al., 2012;

Chatsri D et al., 2005; Doughari et al., 2008; Ajaiyeoba et al., 2008; Nsonde Ntandou and Banzouzi et al., 2010) the present study was carried out to evaluate the antidiabetic activity of *C.siamea* leaves extracts. Diabetes is an example of a disease that has been treated with plant medicines (Bailey and Day, 1989). Research conducted in last few decades on plants mentioned in ancient literature that are used traditionally for diabetes have shown antidiabetic properties (Aboelsoud et al., 2007; Aboelsoud and Khalil, 2010).

MATERIALS AND METHODS

Preparation of extracts from leaves of *Cassia siamea*

The leaves of *Cassia siamea* were collected from Andhra University campus, Andhra Pradesh, India during the month of December 2011 and authenticated by Dr. P. Prayaga Murthy, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. Shade dried leaves of *Cassia siamea* was powdered and separately extracted in a Soxhlet apparatus for 6 hrs successively with hexane, ethyl acetate, and ethanol were concentrated to dryness under vacuum at temperature of 45°C by using rotary evaporator (Buchi, Switzerland), dried completely and stored in desiccator.

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Drug and Chemicals

Alloxan monohydrate was purchased from sigma-Aldrich, (St.Louis, USA), metformin and tween 20 were purchased from SD fine chemicals (India), blood glucose kit was purchased from auto span diagnostics Ltd. (India) and all other chemicals used in this experiment were of analytical grade.

Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250gm were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of $23\pm 2^{\circ}\text{C}$ having $50\pm 5\%$ relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Ajitkar et al., 2003).

Diabetes model: Alloxan induced Diabetes

Induction of diabetes

Animals were allowed to fast for 18h and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 100mg/kg b.w intraperitoneally. After stabilization of diabetes, the rats with blood glucose levels between 250-350mg/dl were used for the experiment (Ragavan and Krishnakumari, 2006).

Experimental design

In the experiment a total number of 48 rats were used. The rats were divided into 8 groups; each group consists of six rats. Group I rats were treated with vehicle (2% tween 20) and served as control, group II rats were treated with metformin (3mg/kg b.w), group III and IV rats were treated with hexane extract of *c.siamea* leaves at a doses of 150 and 300 mg/kg b. w respectively, group V and VI rats were treated with ethyl acetate extract of *c. siamea* leaves at a doses of 150 and 300 mg/kg b. w respectively. Group VII and VIII rats were treated with ethanol extract of *c.siamea* leaves at doses of 150 and 300

mg/kg b. w respectively. All the doses were administered orally (Chattopadhyay, 1997).

Collection of blood samples and estimation of blood glucose

The animal was restrained in such a way that loose skin of the neck was tightened while handling the head with the left hand. With the help of index finger the eye was pressed just behind the angle of the jaw resulting in the engorgement of the retro orbital plexus. Then tip of the capillary was inserted at the medial canthus into the retro orbital plexus with gentle rotation by the other hand. As the vessels are ruptured, blood wells up in the retro orbital space. The tip of the capillary was then slightly with drawn, so that the blood flows into the capillary, which was collected in micro centrifuge tube containing small quantity of potassium oxalate and sodium fluoride as anticoagulant. Blood samples were collected from retro orbital plexus at 0, 2,4,6,8 and 12hr. The plasma blood glucose levels were estimated by GOD-POD method (Tomita et al., 1974).

RESULTS

Alloxan induced Diabetes

The mean percent decrease in blood glucose levels produced by standard drug metformin at the dose of 3mg/k.g was 22.99, 25.58, 24.86, 30.63, 21.4 at 2,4,6,8 and 12 h respectively. The mean percent decrease in blood glucose levels in diabetes induced rats produced by 150 and 300 mg/kg b.w of ethanolic extract of *c.siamea* leaves were 86.33, 84.83, 84.3, 83.50, 80.16, 88.6, 87.5, 83.5, 83.16 and 80 at 2,4,6,8 and 12 h respectively. The results were showed in Table 1 and Fig 1. The mean percent decrease in blood glucose levels in diabetes induced rats produced by 150 and 300 mg/kg b.w of ethyl acetate extract of *c.siamea* leaves were 96.16, 93, 90, 86.66, 84.16, 92.6, 86.8, 82.66, 81.16 and 77.3 at 2, 4, 6, 8 and 12 h respectively. The results were showed in Table 2 and Fig 2. The mean percent decrease in blood glucose levels in diabetes induced rats produced by 150 and 300 mg/kg b.w of hexane extract of *c.siamea* leaves were 84.66, 80.83, 78.5, 75.33, 73, 6.98, 12.77, 18.09, 25.06, and 21.92 at 2, 4, 6, 8 and 12 h respectively. The results were showed in Table 3 and Fig 3.

Table 1. Effect of ethanol extract of *Cassia siamea* leaves on blood glucose levels in diabetic rats

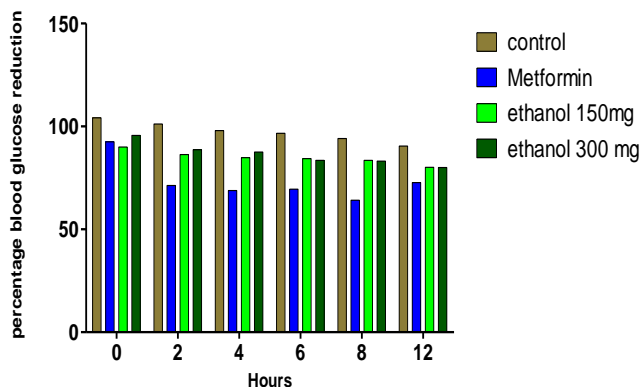
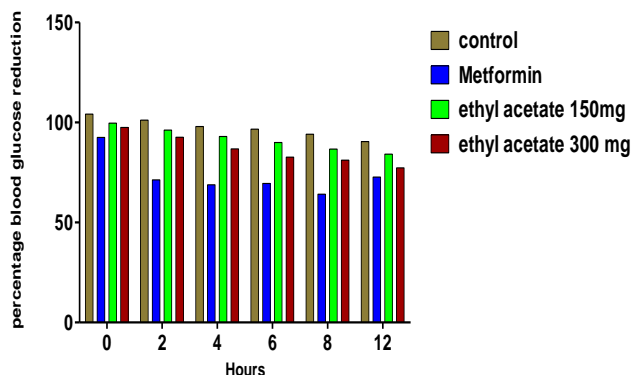
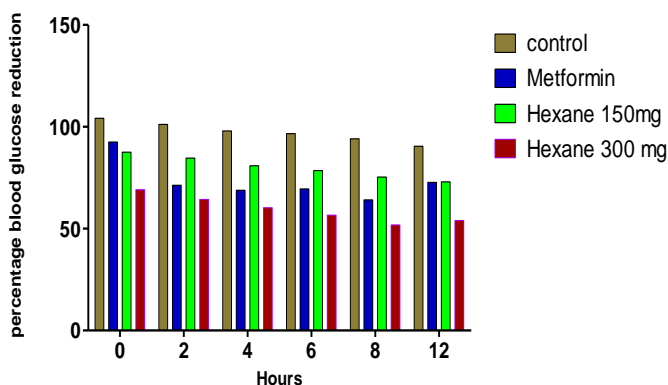
Group	Dose mg/k.g b.w	Blood glucose levels (mg/dl) at different time intervals					
		0h	2h	4h	6h	8h	12h
Control (drug vehicle)	Tween 20	104.16	101.16	98	96.66	94.16	90.5
<i>C.siamea</i> ethanol extract	150	90	86.33	84.83	84.3	83.5	80.16
<i>C.siamea</i> ethanol extract	300	95.66	88.6	87.5	83.5	83.16	80
Metformin	3	92.5	71.33	68.83	69.5	64.16	72.66

Table 2. Effect of ethyl acetate extract of *Cassia siamea* leaves on blood glucose levels in diabetic rats

Group	Dose mg/k.g b.w	Blood glucose levels (mg/dl) at different time intervals					
		0h	2h	4h	6h	8h	12h
Control (drug vehicle)	Tween 20	104.16	101.16	98	96.66	94.16	90.5
<i>C.siamea</i> ethyl acetate extract	150	99.66	96.16	93	90	86.66	84.16
<i>C.siamea</i> ethyl acetate extract	300	97.5	92.6	86.8	82.66	81.16	77.3
Metformin	3	92.5	71.33	68.83	69.5	64.16	72.66

Table 3. Effect of hexane extract of *Cassia siamea* leaves on blood glucose levels in diabetic rats

Group	Dose mg/k.g b.w	Blood glucose levels (mg/dl) at different time intervals					
		0h	2h	4h	6h	8h	12h
Control(DrugVehicle)	Tween 20	104.17	101.17	98	96.66	94.16	90.5
<i>C.siamea</i> hexane extract	150	87.5	84.66	80.83	78.5	75.33	73
<i>C.siamea</i> hexane extract	300	69.16	64.33	60.33	56.66	51.83	54
Metformin	3mg/kg	92.5	71.33	68.83	69.5	64.16	72.66

Fig 1. Effect of ethanolic extract of *Cassia siamea* leaves on blood glucose levels in diabetic rats**Fig 2. Effect of ethyl acetate extract of *Cassia siamea* leaves on blood glucose levels in diabetic rats****Fig 3. Effect of hexane extract of *Cassia siamea* leaves on blood glucose levels in diabetic rats**

DISCUSSION

Diabetes mellitus is a heterogeneous disease characterized by impaired insulin secretion often combined with insulin resistance (Cavaghan *et al.*, 2000). In this study the ethanol, ethyl acetate, hexane extracts of *C.siamea* leaves produced dose dependent blood glucose reduction in diabetic group. In diabetic group the

percentage blood glucose reduction with *C. siamea* leaves was observed up to 12h and maximum at 8h .The percentage blood glucose reduction produced by *C.siamea* leaf extracts at 300mg/k.g b.w in diabetic group were significant and were nearly equal with metformin (standard) treated group.

CONCLUSION

From the results we can conclude that the *C.siamea* leaves possess antihyperglycemic activity and it is further needed to isolate bioactive molecule responsible for antidiabetic activity.

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