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Research Article

Evaluation of Anti diabetic activity of *Cassia surattensis* burm.F. Flower in Streptozotocin induced Diabetic rats

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ABSTRACT

Flowers of *Cassia Surattensis* Burm.f. (Piperaceae) possess several bioactivities and are used in traditional medicinal systems. However, its anti diabetic activity has not been scientifically investigated so far. The aim of this study therefore, was to investigate the anti diabetic activity of *Cassia Surattensis* flower. This was tested in normoglycaemic and streptozotocin (STZ)-induced diabetic rats using oral administration of hot water extract (HE) and ethanolic extract (EE). In normoglycaemic rats, both HE and EE significantly lowered the blood glucose level in a dose-dependent manner. In glucose tolerance test, both extracts markedly reduced the external glucose load. The anti diabetic activity of HE is comparable to that of EE. Both extracts were found to be non-toxic and well tolerated after following chronic oral administration (no overt signs of toxicity, hepatotoxicity or renotoxicity). It is concluded that HE and EE of *Cassia Surattensis* flower possess safe and strong anti diabetic activity and efficacy of both extracts was almost comparable to that of glybenclamide

Keywords: Cassia Surattensis Burm.f; Streptozotocin; hot water extract; Ethanolic extract.

INTRODUCTION

The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetic patients worldwide. (Wild S et al., 2004; Burrows NR et al., 2005; Ragunathan M et al., 1992). The current treatment although provides good glycemic control but does a little in preventing complications (Holman RR et al., 1991). Besides, these drugs are associated with side effects (Maclennan AHet al., 1996; Rang HP, Dale MM, 1991). Many herbal products including several metals and minerals have been described for the cure for diabetes mellitus in ancient literature. There is an increasing demand by patients to use the natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agent (Rang HP et al., Goodman LS, Gilman A, 2006) such as sulfonylureas, metformin, α-glucosidase inhibitors, trioglitazone, etc.

Herbal preparations alone or in combination oral hypoglycemic agents some time produce a good therapeutic response in some resistant cases where modern medicines alone fail. The available literature shows that there are more than 400 plant species showing hypoglycemic activity and presently several laboratories are involved in isolating new herbal hypoglycemic agents (Prout TE et al., 1974; Kameswara Rao B et al., 1997; Bei B Zhang et al., 2000; Anturlikar SD et al., 1995; Maclennan AH et al., Grover, J.K et al., 2002; Karunanayake E.et al., 1984) Though some of the plants are reputed in the indigenous system of medicine for their activities, it remains to be scientifically established.

Cassia is a large genus with some 500 species, among which are a number of highly attractive flowering trees. *Cassia Surattensisis* a glabrous tree belonging to family Caesalpiniacea, found throughout India, tropical Asia and Australia. The leaves are long linear, acute, curved in shape. The flower is yellow in color and shorter than the leaves. Phytochemical study of flower of *Cassia Surattensis* has been indicated the presence of chrysophenol, physcion, stearic acid, β-sitosterol and β- D glucoside. In folk medicine, bark and leaves of *Cassia Surattensis* are used for the treatment of diabetes (C.K.N.Nair et al., 1998) and gonorrhea.

The leaves are used for blennorrhagia. In Ayurvedic systems of medicine, herbal extracts but not purified compounds have been used from centuries because of many constituents are considered to be beneficial. Therefore, this study was undertaken to investigate the ability of *Cassia Surattensis* flowers to lower blood glucose levels. This was tested in normoglycaemic and streptozotocin (STZ)-induced diabetic rats using oral administration of hot water and cold ethanolic extracts. In addition, the toxicity of the extracts was also tested using chronic administration.

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Materials and Methods

The flowers of the plant were collected from the Alagarkovil region, Madurai District in the month of August and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. D. Stephen Department of Botany, American college of Arts and Science, Madurai. A voucher specimen (CS/AUG/BOT/02) has been kept in our museum for future reference. The flowers were dried in shade for 10 days and coarsely powdered with the help of a hand- grinding mill and the powder was passed through sieve No. 60

Animals

Healthy, adult male Wistar rats weighing 180-200 g were used for study. The animals were housed in large and spaces polypropylene cages, maintained under standard condition (12 h Light/12 h dark cycle, 25ºC and 30-35% humidity) and fed with standard pellet diet (M/s. Hindustan lever Ltd., Bangalore, India) and water *ad libitum*. The study was approved by institutional animal ethics committee of Ultra College of Pharmacy, Madurai. All the animals experimental procedure were carried out as per CPCSEA guideline. (CPCSEA No.890/ac/05/ CPCSEA).

Preparation of plant extracts

Preparation of hot water extract (HE)

Cassia Surattensis flowers were air dried for 3–5 days in the shade and cut into small pieces. Five hundred grams were boil with 2.5 L of distilled water for 4 h. The hot water extract was concentrated under vacuum at 60 ◦C, freeze-dried at −20 ◦C (yield 34.2%, w/w, dry weight basis) and stored at 4 ◦C until use.

Preparation of ethanolic extract (EE)

Cassia Surattensis flowers were air dried for 3–5 days in the shade and 500 grams were extracted with absolute ethanol 80% ethanol using Soxlet apparatus for 6 hrs. The extract was evaporated to dryness under reduced pressure at 60 ◦C (yield 25.6%, w/w, dry weight basis) and stored at 4 ◦C until use.

Phytochemical screening

The alcoholic extracts obtained were subjected to preliminary phytochemical screening (Kokate CK.,et al., 1994) to identify the chemical constituents. The methods of analysis employed were those described by (Harbone & Baxter,1993; Trease & Evans, 1983).

Acute toxicity studies

Healthy *Wistar* rats of either sex were used, starved overnight were orally fed with the aqueous and ethanolic extract in increasing dose levels of 500, 1000, 2000 and 4000 mg/kg body weight. The animals were observed continuously for 2 h under the following profiles:

(i) Behavioral profile: Alertness, restlessness, irritability and fearfulness

(ii) Neurological profile: Spontaneous activity, reactivity, touch response, pain response and gait

(iii) Autonomic profile: Defecation and urination. After a period of 24 and 72 h animals were observed for any lethality or death.

Induction of diabetes in rats

Diabetes was induced by single intraperitoneal injection of freshly prepared streptozotocin (50 mg/Kg, Sigma Chemical company St.Louis MO,USA) dissolved in 0.1 M citrate buffer (pH 4.5) after over- night fasting of 12 hr. The diabetes was assessed by determining the blood glucose concentration after 48 hrs of streptozotocin injection. The rats with blood glucose level above 200 mg/dL were selected for the experimental studies.To prevent the hypoglycemia which occurred during the first 24 h following the STZ administration, 5% glucose solution was orally given to the diabetic rats. In all experiments, rats were fasted for 16 h prior to STZ injection. Only rats found with permanent diabetic were used for the antidiabetic study.

Experimental design

In the experiment (Chattopadhyay S et al., 1997; Ghosh MN et al., 1994; Vogel.H et al., 2002; Kameswara Rao et al., Arul B et al., 2004)a total number of 30rats (24 diabetic rats, 6 normal rats) were used. The rats were divided into 4 groups of six each.

Group I: Control group (Vehicle treated)

Group II: Diabetic control (streptozotocin 50mg/kg b.w i.p)

Group III: Diabetic rats receiving "HE of *Cassia Surattensis*" (400 mg/kg b.w orally*)*

Group IV: Diabetic rats receiving "EE of *Cassia Surattensis"* (400 mg/kg b.w orally*)*

Group V: Diabetic rats receiving Glibenclamide (0.25mg /kg b.w orally)

Standard drug and extract were prepared in 0.5% Carboxy methyl cellulose Suspension as a vehicle and administered orally, Treatment of experimental animals with flower extracts and reference drug were initiated 2days post streptozotocin injection and was carried out once daily, by gavage, for 14 days. Food and water were made freely available.

The blood samples were drawn on 0^{th} , 7^{th} , 14^{th} and 21^{st} day from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged (2,500 rpm/10min) to get serum. The serum was used for biochemical estimation of fasting blood glucose level,(Cakici I et al., 1994; Venkatesh S, et al., 20003; Fings CS et al., 1970; Herbert V,et al., 1965)Hemoglobin, and Glycosylated hemoglobin, Liver

glycogen and serum insulin. The Blood glucose level was determined by using glucometer and blood glucose test strips (CONTOURTMTS).Hemoglobin leads to formation of hemoglobin throughout the circulatory life of RBC by addition of glucose to N- terminal of hemoglobin beta chain. This process is non enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. The insulin concentration was calculated by Enzyme linked immuno sorbent assay (ELISA).Initial and final changes in body weight was observed.

Glucose Tolerence Test

The blood glucose concentrations of the animals were measured at the beginning of the study. In brief, twenty four rats were fasted for 16 h and assigned randomly into 4 equal groups (*n* = 6/group). These rats were orally treated in the following manner: Group 1 (1 ml of DW), Group 2 (400 mg/kg of HE), Group 3 (400 mg/kg of EE) and Group 4 (0.25 mg/kg of Glibenclamide). Blood glucose level was measured by using glucometer at 0 hr (pre- study).One hour later, all these rats were orally loaded with 5 ml/kg of 50% (w/v) glucose solution. The blood samples were collected at 1hr, 2hr, 3hr intervals after the administration of the glucose .The blood samples were collected with potassium oxalate and sodium fluoride solution and glucose levels were estimated using a glucose oxidase–peroxidase reactive strips and a glucometer.

Histopathological study

On Twenty first day the animals were sacrificed by mild chloroform anaesthesia, the pancreas was excised and stored in 10% formalin after washing with normal saline. The tissues were washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial section of 5µm thickness were cut using a rotary microtome. The sections were deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haemotoxylin for 10min, followed by rinsing with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

Statistical analysis

Results were expressed as mean ± standard error. Statistical analysis was done by using repeated measure one way anova followed by Dunnett's multiple comparison test .P<0.05 was considered as significant.

RESULTS

Phytochemical screening

Phytochemical screening of both the plant extracts revealed that the presence of flavonoids, alkaloids, glycoside, tannins, saponins, phytosterols.

Acute Toxicity Study

Experiments were carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was seen even with the dose of 4 g/kg b.w. and there were no lethality in any of the group. Body weight was normal. Therefore, the cut off dose for effective dose (ED50) was taken as400 mg/kg b. w.which is the 1/10th of LD50.

Effect on Glucose Tolerance Test

Both HE and EE significantly ($P < 0.05$) improved the glucose tolerance test up to 3 h (Table 1). HE and EE showing approximately 14, 11, 10% and 16, 12, 11% reduction in glycaemia from control values at the 1, 2 and 3 h, respectively. Glybenclamide also improved the glucose tolerance test up to 3 h. This impairment was comparable to that of *Cassia Surattensis* extracts.

Effect of HE and EE on blood glucose level in normal fasted rats

Overnight fasted rats were divided into five groups of six rats each. Group I ,II and V were administered distilled water(control),diabetic(untreated control) and standard drug (Glybenclamide 0.25 mg/kg) by oral route. A dose 400 mg/kg and of HE and EE were suspended in drug vehicle and administered to group III and group IV orally. The drug vehicle, standard drug and test substance were administered once daily, per orally for the period of 21 days. All the drug administration procedure was carried out between 8-9:30 am of the day. The blood glucose levels were estimated on 0 (pre- study) and 7, 14, 21 day of the study. The rats were restrained in rat restrainer and blood samples were collected from the tail vein by making a small incision on the tail tip and 0.5-1.0 ml of the blood was collected for estimation of blood glucose, The results were tabulated in Table.2.

Changes of Serum Insulin, Liver Glycogen and Glycated Hemoglobin:

Since the Hot water Extract (HE) and Ethanolic Extract (EE) of *Cassia Surattensis* flower showed significant improvement in fasting blood glucose and OGTT of diabetic animals, it was intended to assess the effect of long-term treatment of the extract on serum insulin, liver glycogen and glycosylated hemoglobin in STZ-induced chronic diabetic rat model. Rats were treated with 400 mg/kg b.w. both extract once a day in the morning for 21 day. At the end of the treatment, the animals when compared with diabetic control, showed significant ($p < 0.01$) difference in serum insulin level and glycosylated hemoglobin level and significant ($p < 0.05$) effect on liver glycogen level (Table 3).

Effect of HE and EE on body weight in STZ induced diabetic rats:

There was a significant body weight loss in the diabetic rats (Diabetic control)during 21 days, Vehicle control animals were found to be stable in their body weight whereas animals treated with HE and EE at the doses of 400 mg/kg p.o. showed the significant increase in weight 14th day onwards, indicating that the HE and EE

Table 1: Effect of Hot water Extract (HE) and cold Ethanolic Extract (EE) of Cassia Surattensis flower on oral glucose tolerance test (mean ± SD, n = 6)

Group	Treatment	Glucose Concentration(mg/dl)			
		Pretreatment	Time following 50% oral Glucose load(h)		
		0hr	1hr	2hr	3hr
I	Control (1ml DW)	88.1±2.1	156.1±4.1	147.2±3.2	133.2±4.2
II	HE(400mg/Kg)	91.2±2.4	136.5±3.2*	126.2±4.2*	118.2±3.4*
III	EE(400mg/Kg)	92.6±.2.2	139.4±3.4*	128.9±2.3*	119.2±3.3*
IV	Glybenclamide(0.25mg/Kg)	89.4±2.5	132.1±2.4*	123.3±3.2*	105.2±3.5*

DW, Distilled water.*P<0.05, as compared with controls

Table 2: Effect of Hot water Extract (HE) and Ethanolic Extract (EE) of Cassia Surattensis flower on fasting blood glucose levels in streptozotocin induced diabetic rats (mean ± SD, n = 6)

Group	Treatment	plasma glucose Concentration(mg/dl)			
		0th day	7th day	14th day	21st day
I	Vehicle	77.14±1.23	79.16 ± 1.37	79.8 ± 1.69	80.4 ± 2.12
II	Diabetic control	215.1±1.29	249.2±2.31	279.2 ± 1.83	283.6 ± 1.38
III	HE (400mg/Kg)	211.21±2.77	172.2±1.05	130.33±1.78	112.2±2.26***
IV	EE (400mg/Kg)	217.83±3.20	162.9±2.11	137.73±2.54	111.44±2.46***
V	Glybenclamide(0.25mg/Kg)	213.23±3.12	162.03±1.43	114.13±2.92	93.93±2.43***

****p < 0.001* significant from diabetic control animals.

Table 3: Effect of Hot water Extract (HE) and cold Ethanolic Extract (EE) of Cassia Surattensis flower on serum insulin,liver glycogen and glycosylated hemoglobin levels in diabetic rats

Group	Treatment	Glucose Concentration(mg/dl)		
		Serum insulin (ng/ml)	Glycosylated Hemoglo-bin(%)	Liver glyco-gen(mg/g)
I	Control (1ml DW)	0.25 ± 0.22	3.23 ± 0.09	12.76±0.21
II	Diabetic control	0.16±0.01	6.68±0.25	5.12±0.12
III	Diabetic + HE(400mg/Kg)	0.26 ± 0.00**	3.35 ± 0.24***	12.64 ± 0.71*
IV	Diabetic + EE(400mg/Kg)	0.31 ± 0.00**	3.36 ± 0.24***	12.64 ± 0.71*
V	Glybenclamide(0.25mg/Kg)	0.32 ± 0.02**	3.80 ± 0.27***	13.98 ± 0.12*

Values are given as mean ± S.E.M from six rats in each group**p < 0.05* significant, ***p< 0.01* most significant, ****p< 0.001* highly significant from diabetic control animals.

had beneficial effects in preventing loss of body weight of diabetic rats. (Table.4)

Histopathological studies

Histopathology studies also support our findings. STZ was suspected to destroy pancreatic β cells. The histopathological examination revealed extensive alterations in pancreas of STZ-induced diabetic rats (Fig. 1 (A-E)). The pancreas of control rat (Fig. 1A) showing normal islets. Extensive damage to the islets of langerhans and reduced dimensions of islets (Fig. 1B) , restoration of normal cellular population size of islets with hyperplasia by glibenclamide (C) was also shown. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by Hot water and ethanolic extracts (Fig 1D-E).

Discussion and Conclusion

The STZ is a broad –Spectrum antibiotic extracted from Streptomyces acromogenes. The STZ induced diabetes causes the destruction of β cells of the islets, which leads to reduction in insulin release. An insufficient release of insulin, that leads high blood glucose namely hyperglycemia. The body weight of STZ – induced diabetic rats were reduced and also recovered by hypoglycemic treatment.

Literature survey indicates that there is no scientific evidence to support the antidiabetic effect of *Cassia Surattensis* . Therefore the present study is undertaken to investigate the action of aqueous extract of *Cassia Surattensis* leaves in different models of rats to ascertain the scientific basis for the use of these plants in the treatment of diabetes.

The preliminary acute toxicity revealed the nontoxic nature of *Cassia Surattensis*.The oral glucose tolerance

Table 4: Effect of Hot water Extract (HE) and Ethanolic Extract (EE) of Cassia Surattensis flower on body weight of rats

Group	Treatment	Body weight changes		
		1st Day	7th Day	21st Day
I	Control(1ml DW)	185.21±1.2	196.55±1.6	215.23±2.3
II	Diabetic control	197.53±1.1	181.21±1.4	169.24±2.6
III	Diabetic+HE(400mg/Kg)	189.12±2.3	178.23±1.7	185.23±1.4***
IV	Diabetic+EE(400mg/Kg)	196.35±1.5	184.53±1.5	192.23±1.2***
V	Glybenclamide(0.25mg /kg)	188.56±1.8	181.23±1.1	186.27±1.8***

DW, Distilled water, Values are given as mean ± S.E for six rats in each group* $p < 0.001$ highly significant from diabetic control animals.

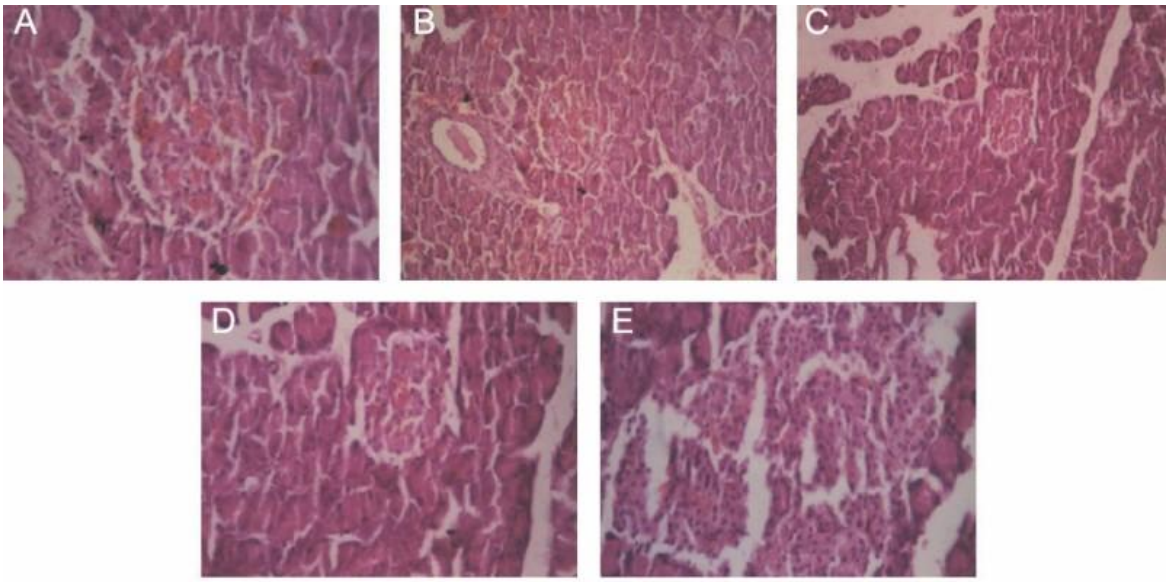

Figure 1: Hisology of rat pancreas stained by haematoxylin and eosin of (A) untreated and (B) STZ induced diabetic rats and effects of(C) glibenclamide, (D) Hot water extract, (E) ethanolic extract of flowers of Cassia Surattensis Microscope magnification: 400x

test showed that both extracts of *Cassia Surattensis* gave significant effect lower blood glucose level at the end of 60 min after glucose loaded and even lower level at the end of 120min and 180 min. The HE and EE of *Cassia Surattensis* enhanced glucose utilization, so the blood glucose level was significantly decreased in glucose loaded rats.

The present investigation reports the body weight was gained by both extracts of *Cassia Surattensis* flowers and glybenclamide treated diabetic rats due to increases glucose metabolism.

Hence, it can be said that *Cassia Surattensis* flowers does not have any effect on degradation of depot fat and it can maintain the body weight in type 2 diabetic state. Serum glucose level was 172.2±1.05mg/dl on day 7 and it decreased significantly ($p < 0.05$) to 185.23±1.4mg/dl with hot water extract of Cassia Surattensis flowers and from 162.9±2.11mg/dl on day 7 decreased significantly ($p < 0.05$) to 111.44±2.46mg/dl with ethanolic extract of *Cassia Surattensis* flowers . This phenomenon clearly indicates that both extracts of *Cassia Surattensis* flowers can potentially control the hyperglycemic state of diabetes.

Administration of both extracts of *Cassia Surattensis* flowers showed significant ($p < 0.01$) increase in the levels of serum insulin. The possible mechanism by which aqueous extract brings about its hypoglycemic action in diabetic rats may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. The significant increase in the glycogen level of the aqueous and ethanolic extract-treated diabetic animals may be because of the reactivation of glycogen synthase system. This focuses the one possible way of antidiabetic action of this both extracts by improvement of glycogenesis process.

The excess of glucose is present in the blood during diabetes, which react with hemoglobin and form glycosylated hemoglobin. Glycated hemoglobin levels were found to be increased in the untreated diabetic control group. Treatment with both extracts of *Cassia Surattensis* flowers showed a significant decrease in the glycated hemoglobin levels, which could be due to an

improvement in insulin secretion, which confirms the antidiabetogenic action of both extracts. The both extracts did not produce any significant effects on normal animals.

The histopathological investigation along with the biochemical evauation suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in both hot water and ethanolic extracts of cassia surattensis treated group. So, the flowers of *Cassia Surattensis* possess the significant antidiabetic activity and acts by stimulating the insulin production from the pancreas and it supports to control the diabetes and its complications.

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