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Effect of *Cassia auriculata* Linn flowers against alloxan-induced diabetes in rats

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Abstract

Objective : To evaluate the antidiabetic activity of flowers of *Cassia auriculata* Linn against alloxan-induced diabetes in rats and to isolate the bioactive constituents from the activity guided fraction. Materials and methods : The ethanol & aqueous extracts of flowers of *Cassia auriculata* were obtained by continuous soxhlet extraction & cold maceration respectively. Each extract was assessed for antidiabetic activity by estimating serum glucose level in diabetic rats. The active ethanol extract was subjected to qualitative chemical analysis to identify the active phytoconstituents. The sterol; β sitosterol, was isolated from ethanol extract by column chromatography. Results : In alloxan-induced diabetic rats the ethanol extract (250 mg / kg, p.o.) showed significant ($P<0.001$) antidiabetic activity as observed from serum glucose level in diabetic rats. However, the aqueous extract (250 mg / kg, p.o.) did not significantly reduce the serum glucose level in diabetic rats. The ethanol extract showed the presence of sterols, triterpenoids, flavonoids and tannins. Conclusion : From the results, it is revealed that, the active ethanol extract of *Cassia auriculata* flowers is worthwhile to develop the bioactive principle for diabetes mellitus and it is also concluded that the isolated sterol; β sitosterol, could be attributed for antidiabetic activity.

Keywords: *Cassia auriculata,* Flowers, Antidiabetic, Sterols, Ethanol Extract.

1. Introduction

In spite of the progress in treatment of Diabetes mellitus by introduction of various synthetic drugs & insulin, search for newer natural drugs continues because of several therapeutic complications associated with existing therapy. *Cassia auriculata* Linn. (Caesalpinaceae), commonly known as *Tanners Senna*, is a common, highly branched shrub with large bright yellow flowers distributed wildly in dry regions of the central provinces and western peninsula of India [1]. The plant as a whole has been used as antidiabetic, antidysentric, antimicrobial and for various skin diseases from ancient times [2]. In ayurveda, its seeds are used to treat various gastrointestinal disorders [2]. The flowers of the plant used as folk remedy

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for the treatment of Diabetes mellitus in southern parts of India [3]. However, no scientific study on the antidiabetic property of *Cassia auriculata* plant has been reported.

In the present study, we aimed at utilizing easily available plant to investigate the effect on serum glucose level in alloxan-induced diabetic rats and also to establish phyto-chemical and pharmacological profile in support of the folklore claim.

2. Materials and methods

Fresh flowers of *Cassia auriculata* were collected from local areas of North Karnataka between the month of October and November 2001 and were identified by Prof. Sasalatti, Dept of Botany, R. L.Science Institute, Belgaum. A voucher specimen [CG-05] has been deposited at the departmental herbarium, Belgaum.

2.1 Preparation of extracts

The plant flowers around (500 gm) were dried at room temperature and reduced to fine powder to particle size (#) 40 then subjected to continuous hot extraction with 90% ethanol in a soxhlet extractor for 48 h. The total ethanol extract was filtered and evaporated to dryness at 40°C under reduced pressure in a rota evaporator.

The fresh flowers (200 gm) were also subjected to cold maceration with chloroform-water (250 ml) to obtain aqueous extract and concentrated at room temperature. The yield of ethanol and aqueous extract was found to be 150 gm (30% w/w) and 16 gm (8% w/w) respectively. Both the extracts were kept in a desiccator till experimentation.

2.2 Preliminary phytochemical studies

The preliminary phytochemical screening of ethanol and aqueous extracts were performed to identify the presence of sterols, triterpenoids, flavonoids and tannins [5].

2.3 Isolation of β sitosterol

The ethanol extract was further subjected to chromatographic studies for separation and identification of sterols. The ethanol extract was saponified as per method mentioned in I.P (1996) to get unsaponifiable matter (USM). The USM (5 gm) obtained, was subjected to column chromatography over silica gel (250 gm) using petroleum ether-acetone gradients (80:20) as eluents to yield Fraction-A on the basis of TLC profile.

Further the Fraction-A was identified and confirmed as β sitosterol by performing Co-chromatography using TLC with standard; β sitosterol on adsorbant silica gel, Solvent system Petroleum ether: acetone (90:10), Anisaldehyde spray reagent [6].The development of thin layer chromatography displayed single spot R_f =[0.86].

2.4 Acute toxicity evaluation (LD_{50})

The acute toxicity of ethanol and aqueous extracts was evaluated in mice. The animals were fasted overnight prior to the acute toxicity study. Different groups containing 2 mice in each were orally administered with ethanol and aqueous extracts at 0.5, 1.0, 1.5, 2.0 gm/kg doses to different groups respectively. Control group received only propylene glycol. Drug treated and control groups were placed in polypropylene cages with free access to food and water.

Mortality and general behavior of the animals were observed continuously for initial 4 h and intermittently for next 6 h and then again at 24 h and 48 h after dosing. The parameter observed and recorded were sedation, hyperactivity, grooming, loss of righting reflex, respiratory rate and convulsion.1/10 th of lethal dose was taken as the screening dose [7].

2.5 Evaluation of antidiabetic activity

Antidiabetic activity was evaluated against alloxan-induced diabetes in rats [8]. The ethical

clearance was obtained by the Institutional Animal Ethics Committee (Registration No. 221/ CPCSEA) before experiment.

Male Wister albino rats, each weighing about 200-220 gm, were divided into 4 groups containing 6 animals each. They were kept in polypropylene cages and fed on standard laboratory diet (Lipton India Ltd) and water *ad libitum*, maintained at 24-28°C temperature and exposing them to alternate cycle of 12 h of darkness and light each. Diabetes was induced to all grouped animals by injecting alloxan (60 mg / kg, i.v) dissolved in phosphate buffer (1ml) P^H 6.4.

Two days later blood samples were drawn and serum glucose levels determined to confirm the development of diabetes. The animals having serum glucose level above 250 mg/dl were selected for experiment [9]. All diabetic animals were fasted, had been deprived of food for at least 16 h but were allowed free access to water before experiment. Group 1 (control) was given with 1ml of propylene glycol orally, Group 2 and 3 received ethanol extract (250 mg/kg, p.o.) and aqueous extract (250 mg/kg, p.o.) respectively. Group 4 received standard drug Tolbutamide (500 mg/kg) orally.

All the test materials used were suspended in propylene glycol (1ml). The blood samples of all grouped animals were collected from the orbital plexus and serum glucose level was measured at 0, 0.5, 1, 2 and 4 h after treatment by glucose oxidase peroxidase method [9,10].

2.6 Statistical analysis

All the results are expressed in mg/100ml±S.E. The difference in the serum glucose levels at different time intervals between the test groups and diabetic control group was analysed for statistical significance by performing one-way ANOVA followed by Post-hoc Dunnett's test . $P<0.001$ implies significance [11].

3. Results and discussion

The effects of different extracts of *Cassia auriculata* flowers on serum glucose level at different time intervals are represented in [Table -1]. In alloxan-induced diabetic rats the ethanol extract (250 mg/kg, p.o.) exhibited significant

Table 1.
Effect of the extracts of *Cassia auriculata* Linn flowers on serum glucose level in alloxan - induced diabetic rats.

Gr.	Treatment and Dose	Serum glucose concentration (mg / dl) after treatment at				
		0	30 min	1 h	2h	4h
1	Diabetic control (Vehicle)	286.50±1.522	282.17±0.945	281.00±0.577	276.30±0.614	271.50±0.619
2	Ethanol extract (250 mg/kg)	285.33±0.802	255.33±1.66** (10.51)	243.33±1.308** (14.71)	223.00±1.238** (21.84)	202.33±1.430** (29.09)
3	Aqueous extract (250 mg / kg)	294.67±1.22	290.17±0.654 (1.52)	281.33±0.614 (4.53)	274.83±1.579 (6.73)	270.83±1.302* (8.09)
4	Tolbutamide (500 mg / kg)	303.16 ± 2.040	283.67±1.542 (6.42)	270.17±0.703** (10.88)	249.33±0.983** (17.75)	234.83±1.740** (22.53)

Each value represents Mean ± S.E (n = 6)
Number in the Parenthesis denotes percentage of reduction in Serum Glucose level.
** p< 0.001,*p< 0.01 *v/s* Diabetic control Group (One-way ANOVA followed by Post-hoc Dunnett's test).

serum glucose lowering effect after its oral administration and was stable up to 4 h. The maximum percent reduction (29.09%) of serum glucose was observed with ethanol extract at 4 h while the standard drug, Tolbutamide (500 mg/kg, p.o.) exhibited 22.53% reduction of serum glucose at the same time interval. The aqueous extract (250 mg/kg, p.o) showed less significant reduction in the serum glucose levels at 4 h in experimental animals.

The acute toxicity study of *Cassia auriculata* flower extracts revealed no mortality when administered orally up to a maximum dose of 2 g/kg body weight. At this dose there was no gross behavioral changes. From the phyto-chemical investigation of all the extracts, the ethanol extract showed the presence of sterols, flavonoids, triterpenoid and tannins. The β Sitosterol was isolated by column chromatography and identified by comparison of their TLC profile and spectral data with standard literature data [12,13].

This study reports for the first time the antidiabetic activity of flowers of *Cassia auriculata* which supports it's traditional and folklore claim to possess potential antidiabetic property.

Alloxan induces diabetes by increasing hydrogen peroxide and super oxide anions concentration, which may destroy pancreatic β cells and impair renal function. Tolbutamide reduces serum glucose level by stimulating β - cells to release insulin [14]. In the present study it is observed that Tolbutamide exhibited less significant effect compared to ethanol extract (250 mg/kg, p.o.).

This reveals that the observed serum glucose lowering effect of ethanol extract may not be due to potentiation of insulin and also suggest the drug may be effective in Non-insulin Dependent Diabetes Mellitus (NIDDM). With the significant antidiabetic activity of the ethanol extract of *Cassia auriculata* flowers in alloxan induced diabetic rats, the mechanism of action could, possibly, be due to increased peripheral glucose utilization, by inhibition of proximal tubular reabsorbtion mechanism in the kidney, inhibition of peripheral glucose release or by any other mechanism [15,16].

From the phytochemical investigation it was found that the major chemical constituents of the ethanol extract were sterols, flavonoids, triterpenoid and tannins. The plants containing sterols are reported to possess antidiabetic activity [17]. The presence of sterol; β Sitosterol in ethanol extract could be attributed for the observed Antidiabetic activity. However, studies are in progress in our laboratory to elucidate the detailed mechanism of action for antidiabetic activity.

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