

**ANTI-HYPERGLYCEMIC AND ANTIOXIDANT PROPERTIES OF
CASSIA AURICULATA LEAVES AND FLOWERS ON ALLOXAN
INDUCED DIABETIC RATS**

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Summary

Oxidative stress occurs in diabetic patients and experimental models of diabetes. The present study investigated the effects of leaves and flowers parts of *Cassia auriculata* ethanolic extract on alloxan induced diabetic rats by measuring fasting blood glucose, lipid peroxidation and liver antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Treatment to diabetic rats with oral administration of *Cassia auriculata* at a dose of 120 mg/kg body weight for 15 days resulted in a significant alterations in comparison with diabetic control group. This results indicating that the underlying mechanism of the plants pharmacological action seems to be independent of insulin secretion. The result of the present study proved that the *Cassia auriculata* leaves and flowers possess significant anti-diabetic activity along with potent antioxidant potential in diabetic conditions.

Key words: Alloxan, antioxidants *Cassia auriculata*, diabetes and oxidative stress.

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Introduction

Diabetes mellitus arises from a deficient production of insulin by the beta cell of the pancreatic islets. Insufficient insulin results in hyperglycemia and the symptoms of diabetes, namely an excess sugar in the blood and urine, hunger, thirst and a gradual loss of weight. The disease is estimated to affect 4-5% of the population and patients are generally diabetes type 1 or type 2. Diabetes mellitus, characterized by hyperglycemia, is the most common serious metabolic disorder that is considered to be one of the five leading causes of death in the world (1).

Active oxygen metabolism plays a key role in the normal functioning of the beta cells of pancreas. Free oxygen radicals and oxidative stress appears to be an important element of the production of secondary complications in diabetes (2, 3). Hyperglycemia generates reactive oxygen species which in turn cause lipid peroxidation and membrane damage. Elevated levels of circulating lipid peroxides have been registered in diabetic patients and experimental animals.

Numerous studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) or reduction of antioxidant defense system (4, 5). Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired antioxidant enzyme, and formation of peroxides (6, 7). Lipid peroxidation (LPO) is a key marker of oxidative stress. It is a free radical-induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction. The significant extent of LPO products that was measured as thiobarbituric acid reactive substances (TBARS) has been reported in diabetes (7, 8). The formation of ROS was prevented by an antioxidant system that included non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants, and ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GPx) (9, 10).

Management of diabetes without any toxic side effects is still a challenge to the medical system. There is an increasing demand by patients to use natural products with anti-diabetic activity, because insulin and oral hypoglycemic agents have so many side effects. Hence, we are searching new treatment for diabetes mellitus without side effects. Herbal medicine has been long used for the treatment of diabetic patients and continues to be currently accepted as an alternative therapy (11).

After the introduction of insulin therapy the field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of than 400 plant species showing anti-diabetic activity, although some of these many remain to be scientifically established.

Cassia auriculata L commonly known as “Tanner’s cassia” (Ceasalpinaceae) is a medicinal plant, which grows abundantly all over India. It is widely used in Ayurvedic medicine as tonic, astringent and as remedy for diabetes, conjunctives and ophthalmia (12). Dried flower and leaf of the plants are being used for medical treatment (13).

Leaf extract has a protective action against alcohol induced oxidative stress to the cells as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymatic and non-enzymatic antioxidants (14) and experimentally induced alcohol related liver damage (15). The ethanol and methanol extracts of the flowers of *Cassia auriculata* showed antioxidant activity using improved assay based on the decolorization of the radical monocation of 2,2-azinobis –(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (16). *C. auriculata* has been shown to antiviral activity and anti spasmodic activity (17). The flower and leaf extract shown to anti pyretic activity (18). The leaf extracts also shows emollient effect (19). Compounds present in *C. auriculata* include an alkane-Nonacosane-6-one (20), Saponins (21) and tannins (22).

There are no available reports on the comparison studies of anti-diabetic and antioxidant action of *Cassia auriculata* leaves and flowers extract. Therefore the present study was undertaken to determine the effect of comparison studies of *Cassia auriculata* leaves and flowers extract on anti-diabetic and antioxidant properties in alloxan induced diabetic rats.

Materials and Methods

Plant material

Fresh leaves and flowers of *Cassia auriculata* were collected from the local areas of Coimbatore district, Tamilnadu, India. The plant was identified and authenticated by a botanist.

Preparation of leaves and flowers extract

The *Cassia auriculata* leaves and flowers were dried at room temperature and powdered in an electrical grinder and stored at 5°C until further use. The powdered leaves and flowers were extracted with ethanol by immersing for 72 hours. The extracts were filtered and air dried to obtain the residue. The phytochemical screenings of solvent free extracts were qualitatively carried out by the method of Harborne (23).

Toxicity Studies

To study any possible toxic effects and / or changes in behavioural pattern, rats were treated with graded dose of *cassia auriculata* leaves and flowers extracts (100-1000 mg/kg b.w./rat/day) and kept under close observation for 8 hrs daily for 30 days. All symptoms including changes in awareness, mood, motor activity, posture, motor-co-ordination, muscle tone and reflexes were recorded for 30 days.

Experimental animals

Male Wistar albino rats weighing between 125-150g were obtained from Perundurai Medical College, Perundari. The animals were housed in large spacious cages and they were given food (Sai foods Pvt Ltd., Bangalore) and water *ad libitum* during the course of the experiment.

Experimental induction of diabetes in rats

The overnight fasted rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 160 mg/kg body weight (24). The control rats received the same amount of saline solution.

After one hour of alloxan administration, animals were given fed *ad libitum* and 1ml of (100mg/ml) glucose i.e., combats ensuring severe hyperglycemia. After 72 hrs of the alloxan injection, the animals were tested for evidence of diabetes by estimating their blood glucose level. The blood glucose level more than 150mg/100 ml of blood was criteria.

Experimental set up

Animals used for the present study were divided into 4 groups, each consisting of six animals.

- Group I : Control rats.
- Group II : Diabetic control rats.
- Group III : Diabetic rats treated with ethanolic extract of leaves of *Cassia auriculata* (120 mg/kg/b.w) in aqueous solution orally for 15 days.
- Group VI : Diabetic rats treated with ethanolic extract of flowers of *Cassia auriculata* (120 mg/kg/b.w) in aqueous solution orally for 15 days.

At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical dislocation. Blood was collected in tubes containing EDTA for the estimation of glucose by O-toluidine method (25).

The liver tissue was excised, rinsed in ice-cold saline and then homogenized in Tris-HCl buffer of pH 7.4 using a Teflon homogenizer. The liver homogenate were then centrifuged in a cooling centrifuge at 5000 x g to remove the debris and the supernatant was used for the analysis of biochemical parameters. The tissue homogenate was placed at -20°C until further use.

The levels of vitamin C and glutathione were estimated according to methods of Omaye et al. (26). The level of lipid peroxidation in tissue homogenate was estimated.

The activities of superoxide dismutase (SOD) and catalase (CAT) were assayed according to the methods of Marklunds and Marklund, (27) and Sinha, (28).The activity

of glutathione peroxidase was assayed according to the method of Rotruck *et al.* (29). Protein content in tissue homogenate was measured by the method of Lowry *et al.* (30).

Statistical analysis

The values were expressed as mean \pm SD. All the values were compared by students 't' test. P values less than 0.001 were considered to indicate statistical significant.

Results

The preliminary phytochemical screening of the ethanolic extracts of leaves and flowers of *Cassia auriculata* revealed the presence of biologically active ingredients such as alkaloids, flavanoids, saponins, carbohydrates, phenols, glycosides and tannins (Table 1).

Table 1: Qualitative screening of phytochemicals in ethanolic extracts of *Cassia auriculata* leaves and flowers.

Phytochemicals	Leaves	Flowers
	Inference	
Alkaloids	+	+
Phenols	+	+
Glycosides	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Proteins	+	+
Carbohydrates	+	+

+ = Presence; - = Absence

Acute toxicity studies conducted by us (data not shown) revealed that the administration of graded doses of *Cassia auriculata* leaves and flowers extracts (up to a dosage of 1000 mg/kg b.w./day) for 30 days produced no effect on the general behaviour or appearance of the animals and all the rats survived the test period. There were no signs of symptoms such as restlessness, respiratory distress, diarrhea, convulsions and coma.

The level of blood glucose and activity of glucose 6 phosphate dehydrogenase in control and experimental groups were presented in Table 2. The level of blood glucose and activity of glucose 6 phosphate dehydrogenase were significantly altered in alloxan induced diabetic rats when compared to control rats. Altered levels were reverted near normal after the treatment of *Cassia auriculata* leaves and flowers extract to diabetic rats.

Table 2. Levels of blood glucose and glucose-6-phosphate dehydrogenase in control and experimental groups of rats

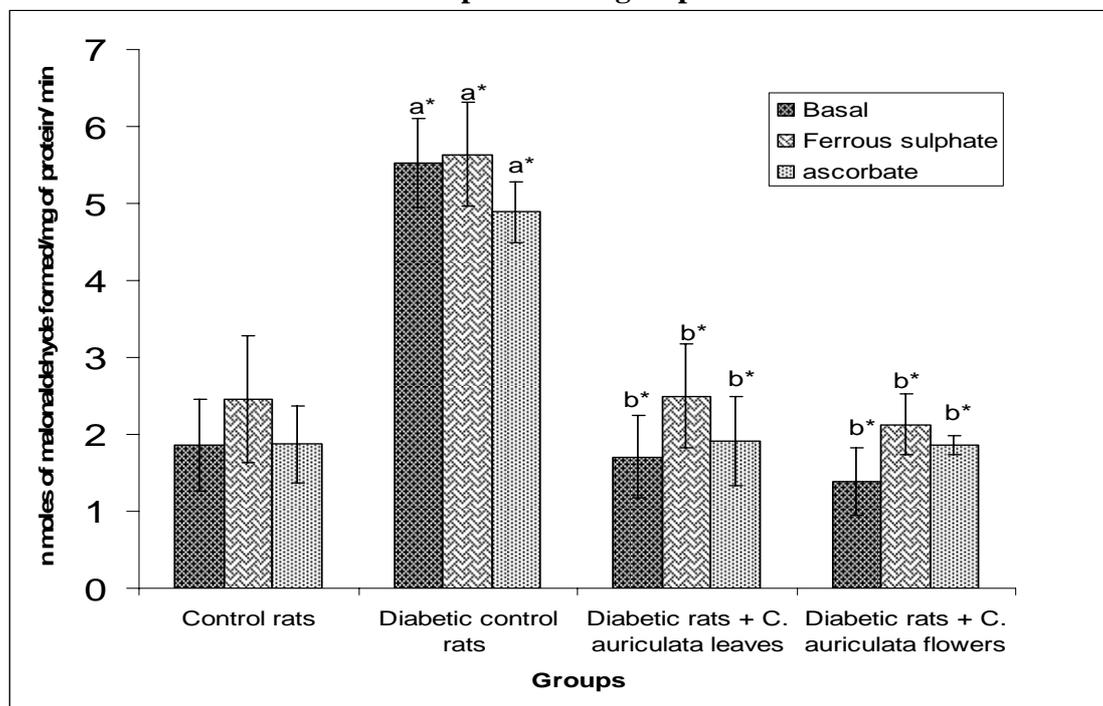
Groups	Blood glucose (mg/dl)	glucose-6-phosphate dehydrogenase (Units/ mg of protein)
Control rats	89.60 ± 18.98	2.37 ± 1.10
Diabetic control rats	184.52 ± 5.33 ^{a*}	1.90 ± 0.95 ^{a*}
Diabetic rats + <i>C. auriculata</i> leaves	86.11 ± 17.11 ^{b*}	2.07 ± 1.11 ^{b*}
Diabetic rats + <i>C. auriculata</i> flowers	98.49 ± 6.11 ^{b*}	1.92 ± 0.13 ^{b*}

Values are mean ± SD for six rats in each group; Values are statistically significant at * $p < 0.001$. All the values were compared by students' t' test. ^{a*} Diabetic control rats were compared with control rats; ^{b*} *C.auriculata leaves* and *C.auriculata flowers* treated groups of rats were compared with diabetic control rats.

Figure 1 represents the levels of basal, ferrous sulphate and ascorbate induced lipid peroxidation in the liver homogenate of control and experimental groups of rats. The significant increased levels of lipid peroxidation in alloxan induced diabetic rats. The levels were restored near control after the treatment of *Cassia auriculata* leaves and flowers extract to diabetic rats as compared to normal rats.

The levels of plasma vitamin-C and glutathione in control and experimental groups of rats are presented in Table 3. The significant decreased levels of vitamin C and glutathione were observed in alloxan induced diabetic rats. Administration of *Cassia auriculata* leaves and flowers extracts to diabetic rats the levels were significantly increased when compared to normal rats.

Figure 1. Levels of lipid peroxidation such as basal, ferrous sulphate and ascorbate in liver of control and experimental groups of rats



Values are mean \pm SD for six rats in each group; Values are statistically significant at $^*p < 0.001$. All the values were compared by students 't' test. ^{a*}Diabetic control rats were compared with control rats; ^{b*}*C. auriculata leaves* and *C. auriculata flowers* treated groups of rats were compared with diabetic control rats.

Table 3. Levels of vitamin and glutathione in plasma of control and experimental groups of rats

Groups	Vitamin C (mg/dl)	Glutathione (nm/mg of protein)
Control rats	3.14 \pm 1.02	5.15 \pm 0.19
Diabetic control rats	2.66 \pm 1.06 ^{a*}	1.8 \pm 0.08 ^{a*}
Diabetic rats + <i>C. auriculata leaves</i>	3.04 \pm 1.10 ^{b*}	3.56 \pm 0.05 ^{b*}
Diabetic rats + <i>C. auriculata flowers</i>	3.09 \pm 0.57 ^{b*}	4.7 \pm 0.08 ^{b*}

Values are mean \pm SD for six rats in each group; Values are statistically significant at $^*p < 0.001$. All the values were compared by students 't' test. ^{a*}Diabetic control rats were compared with control rats; ^{b*}*C. auriculata leaves* and *C. auriculata flowers* treated groups of rats were compared with diabetic control rats.

The activities of enzymatic antioxidants such as SOD, CAT and GPx are shown in Table 4. During diabetes, there was a significant reduction in the activities of SOD, CAT and GPx. Treatment with *Cassia auriculata* leaves and flowers extracts to diabetic rats were significantly increased antioxidant enzymes when compared to normal rats.

Table 4. Activities of superoxide dismutase, catalase and glutathione peroxidase in control and experimental groups of rats

Groups	SOD	CAT	GPx
Control rats	13.52 ± 3.21	111.25 ± 10.58	42.29 ± 1.35
Diabetic control rats	6.05 ± 1.17 ^{a*}	158.36 ± 28.2 ^{a*}	20.38 ± 0.88 ^{a*}
Diabetic rats + <i>C. auriculata</i> leaves	10.35 ± 1.49 ^{b*}	207.83 ± 25.11 ^{b*}	28.71 ± 3.23 ^{b*}
Diabetic rats + <i>C. auriculata</i> flowers	9.37 ± 0.31 ^{b*}	165.37 ± 28.11 ^{b*}	22.97 ± 1.18 ^{b*}

Values are mean ± SD for six rats in each group; Values are statistically significant at *p<0.001. Units: SOD: Units/ mg of protein, CAT: μmole of H₂O₂ consumed / mg of protein, GPx: μg of reduced glutathione utilized/ mg of protein/ min. All the values were compared by students't test. ^{a*} Diabetic control rats were compared with control rats; ^{b*} *C.auriculata* leaves and *C.auriculata* flowers treated groups of rats were compared with diabetic control rats.

Discussion

Diabetogenic effect of alloxan is due to excess production of reactive oxygen species (ROS) leading to cytotoxicity in pancreatic beta cells which reduces the synthesis and the release of insulin (31), while affecting organs such as liver, kidney and haemopoietic system (32).

Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose levels in alloxan-induced diabetic animals (33, 34). The present study was conducted to assess the hypoglycemic properties of *cassia auriculata* leaves and flowers extracts in alloxan-induced diabetic rats. The ability of *cassia auriculata* leaves and flowers extracts in significantly controlling the increase in blood glucose levels in diabetic group of rats may be attributed to its antihyperglycemic effects. This results suggest that *cassia auriculata* leaves and flowers extracts has stimulate the insulin secretion from the remnant beta cells and/or regenerate the beta cells in alloxan induced diabetic rats

Glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting enzyme of the pentose phosphate pathway, which results in the production of ribose-5-phosphate

and NADPH. In the diabetic state, there are alterations in the specific activities of several glycolytic, NADPH generating and gluconeogenic enzymes in the liver (35). The liver is insulin-independent and requires insulin for glucose uptake, glucose phosphorylation and the entry of glucose-6-phosphate into the metabolic pathway. The reversal of the changes in this enzyme activity achieved by the extracts in the diabetic rats, revealed improvement in the formation of NADPH, favouring lipogenesis and the use of an alternative channel to dispose excess glucose via the HMP pathway (36).

Being a potent generator of reactive oxygen species, alloxan destroyed insulin-producing beta cells by an oxidative mechanism. This process also involves superoxide radical and hydrogen peroxide production by alloxan (37). Moreover, free radicals are intensively formed in diabetes during auto-oxidation of glucose and glycosylated proteins (38). Antioxidants prevent alloxan-induced beta cell damage and restore the blood glucose concentration and the antioxidant status to nearly normal levels.

Oxidative stress that leads to an increased production of reactive oxygen species (ROS) and finally cellular lipid peroxidation has been found to play an important role in the development of diabetes mellitus (7, 8). LPO is one of the cellular features of chronic diabetes. In diabetes, it is thought that hypoinsulinemia increases the activity of the enzyme, fatty acyl coenzyme A oxidase, which initiates beta-oxidation of fatty acids, resulting in LPO (4). Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (39). LPO will in turn result in elevated production of free radicals that are harmful to cells in the body (40). Moreover, lipid peroxide-mediated tissue damage has been observed in the development of both type I and II diabetes mellitus and insulin secretion is closely associated with lipoxygenase-derived peroxides. The increased LPO leads to cellular infiltration and islet cell damage in diabetes (9).

Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species (41). The observed decrease in plasma Vitamin C may be due to increased utilization as an antioxidant defense against increased reactive oxygen species or due to a decrease in GSH level, since GSH is required for the recycling of Vitamin C (42). Similar observations were reported in experimental diabetes (43). The *Cassia auriculata* leaves and flowers extracts treated diabetic rats showed decreased lipid peroxidation associated with increased levels of non enzymatic antioxidants. Studies have shown that alkaloids, flavanoids, saponins, carbohydrates, phenols, glycosides and tannins have antioxidant effect which may be due to the presence of these chemical constituents in the leaves and flowers of *Cassia auriculata*.

GSH is an important inhibitor of free radical mediated lipid peroxidation. The decreased levels of plasma GSH in diabetes may be due to increased utilization in trapping the oxy radicals. In this context, several researchers have also reported decreased levels of plasma GSH in experimental diabetic rats (44, 45). Administration of *Cassia auriculata* leaves and flowers extracts increased the level of GSH in alloxan-induced diabetic rats.

Superoxide dismutase catalyses the dismutaion of the highly reactive Superoxide anion to oxygen and hydrogen peroxide. Catalase and glutathione peroxidase are considered biologically essential in the reduction of hydrogen peroxide (46). Reports have shown that the activities of SOD, catalase and glutathione peroxidase were lowered in diabetic rats (47). However oral administration of *Cassia auriculata* leaves and flowers extracts reversed the activities of these enzymatic antioxidants. This suggests direct or indirect antioxidant nature of *Cassia auriculata* leaves and flowers extracts, which could be due to the free radical scavenging of phytochemicals present in the *Cassia auriculata* leaves and flowers acting as a strong free radical scavenger, thereby improving the antioxidant nature in alloxan-diabetic rats.

Conclusion

Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Our present investigation also confirms the antioxidant and antidiabetic effects of *Cassia auriculata* leaves and flowers. The preventative effects of *Cassia auriculata* may be due to inhibition of lipid peroxidation by its antioxidant nature. The results of the present studies have indicated significant antidiabetic effects with the ethanolic extracts of leaves and flowers of *Cassia auriculata* and support its traditional usage of both plant parts in the control of diabetes and its complications. Further investigations to identify the active principle (s) are obviously needed together with a detailed evaluation on the mechanisms involved in the observed activities.

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