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PROTECTIVE EFFECT OF ETHANOLIC EXTRACT AND FRESH JUICE OF *CASSIA AURICULATA* LINN. ON THE BLOOD SUGAR OF ALBINO RATS

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ABSTRACT

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Lack of insulin affects the metabolism of carbohydrates, proteins, fats and causes significance disturbance of water and electrolyte homeostasis. Lot of plants is available to cure diabetes and also used in traditional system of medicines. Ancient period most of the herbal medicines were used as fresh materials like juice, paste. Later only developed into kashaya then some formulations without proper evaluation of the phytochemicals and proper dosage fixation. Few plants onlyused for the people after evaluation. The aim of study is to evaluate the physiochemical and phytochemical nature of the plant and comparatively study the antidiabetic activity of ethanolic extract and fresh juice of *Cassia auriculata* Linn. flowerin diabetic rats. *Cassia auriculata* Linn. commonly known as tanner's cassia, also known as “Avaram” in Tamil language is a shrub belongs to the Caesalpiniaceae family. The flowers are quantitatively evaluated for phytochemical analysis like Flavonoid content, total phenol content etc. and documented. The methanolic extract and fresh juice of the flowers were screened for their protective effect towards reduction in blood sugar level in albino rats by hypoglycemic activity in normal rats and OGTT methods. It shows a very good hypoglycemic activity.

KEYWORDS: Kashaya, hyperglycemia, Avaram, Caesalpiniaceae.

INTRODUCTION

Diabetes mellitus, the most common endocrine disease, is not a single disease but a group of disorders of varying etiology and pathogenesis. The management of diabetes is considered a global problem and a cure has yet to be discovered.[1,2,3] Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem.[4] The searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus.Hence the search for safer and more effective hypoglycemic agent has continued. Generally herbal drugs are non-toxic and safety.[5,6] The ethno botanical information reports about 800 plants that may possess anti-diabetic potential.[7] A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of diabetes.[8] Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes due to the fact that they are less toxic.[9]

The *Cassia auriculata* shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour.[10] It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes.[11] It has many medicinal properties.[14] Its bark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases.[12] It is also used for the treatment of ulcers, leprosy, liver disease, hyperlipidimia. It was also observed that flower and leaf extract of *Cassia auriculata* shown to have antipyretic activity.[13] The flowers are used widely used in Ayurveda tradition system used as avarai panchanga chooranum and the main constituents of Kalpa herbal tea.[14] The flowers are used to treat urinary discharges, nocturnal emissions and throat irritation.[15] Flowers crushed and taken with goat's milk to prevent white discharge in women.[16] The root of the plant is used in decoction as alternative as well as medicinal oil prepared from the bark in Tamil called as averai- yennai[17] Root powder acts as a coagulant, prevents diarrhea, dysentery and fruit juice indigestion. It gives relief against skin ailments.[18]

2. MATERIALS AND METHODS

2.1. Authentication of plant

Cassia auriculata Linn plant flowers are collected in Coimbatore District, and authenticated by Dr. G. V. S. Moorthy, Botanical survey of India, Coimbatore.V.No.is BSI/SRC/5/23/2012-13 Tech/496 and the specimen herbarium was submitted for reference.

2.2. Extraction process of plant material

The plants flowers were collected cleaned, except the floral parts like petioles other plant parts were separated, shade dried and powdered then the powder was passed through 40 mesh sizes and packed in an air tight container for further use. The air dried plant powder was used for the extraction. About 1kg of drugs powder extracted with ethanol by cold maceration method for 7 days. Then the extracts were filtered and the last trace of the solvent was evaporated under reduced pressure in a rotary evaporator. The yield of the dry extract was calculated. Extracts were kept in desiccators to avoid moisture absorption and keep the extract in dry condition.[19,20]

2.3. Determination of extractive value

Accurately weighed 5 g of air-dried powdered drug was macerated with 100 ml of 90% alcohol of the specified strength in a closed flask for 24 hrs, shaken frequently during first 6 hrs and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100^{o}C to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug .The same procedure was repeated with different solvents like chloroform, petroleum ether, benzene and water according to the standard procedure.[19, 20]

2.4. Determination of crude fiber content

Weighed 3-4gm of finely powdered crude drug and extracted with petroleum ether at room temperature. Filter and dried the marc. Boiled 2gm of dried material with 200ml sulphuric acid for 30 minutes. Filtered the extracted material and washed with boiling water. Then boiled the material with 200ml of sodium hydroxide for 30 minutes. Filtered the solution again through muslin cloth and washed with 25ml of 1.25% H_2SO_4, 50ml of water and 25ml of alcohol successively. After washing the residue transfer to a tarred silica crucible (w1). Dried the residue for 2-3 hours at 140^{0}c and cool the crucible in a desiccator and weigh again

(w2). Then incinerated the residue for 30 minutes at 600°C, cooled and weighed again (w3). The fiber content was calculated by using the following formula.[19]

$$\text{Percentage of crude fiber in test sample} = \frac{(W2-W1)-(W3-W1)}{\text{Weight of test sample}} \times 100$$

2.5. Moisture content determination

Weighedaccurately 5g of the finely powdered drug in a flat bottomed dish. Dried in oven at 100-105°c for 3 hours then cool in a desicator over anhydrous silica gel. Weighed the drug again, calculated the weight difference after drying and calculate the percentage of loss on drying.[19,20]

2.6. Determination of total phenol content

Plant extracts where dissolved in methanol to obtain a concentration of 500µg per ml. about 3.9ml of distilled water and 0.5ml of folin-ciocalteu reagent where added to 0.1ml of the extract in a tube and incubated at room temperature for 3 minutes, 2ml of 20% sodium carbonate was added to these and kept on boiling water bath fora minute. The blue color formed was read at 650nm. The experiment was performed in triplicate a caliberation curve was constructor, using Gallic acid(100-500microgram per ml) as standard and total phenolic content of the extract (microgram per ml) expressed as Gallic acid equivalence.[22]

2.7. Determination of total flavanoid content

Plant extract dissolved in methanol to obtain a concentration of 500microgram per ml. one ml of test sample and 4ml of distilled water where\added to a volumetric flask(10ml capacity**)** and kept for 5minutes then adding 0.3ml of 5%sodium hydroxide**,** 0.3ml of 10% aluminium chloride where added. After 6 minutes of incubation at room temperature, 2ml of 1M NaOH was added to the reaction mixture. Immediately the funnel volume was made up to 10ml of distilled water. The absorbance was measured at 510nm. The experiment was performed in triplicate. A calibration curve was constructed, using quercetin (100-500 microgram per ml) as standard and total flavanoid content of the extract(µg/ ml) expressed as Rutine equivalent.[23]

2.8. Preliminary Phytochemical Analysis

The extracts prepared in different solvents were taken and standard procedure was followed to detect the nature of phytoconstituents present in them.[19,20,21]

2.9. Hypoglycemic activity

2.9.1. Animal Ethical Committie Approval

Animals were collected from Karpagam University animal house and IAEC approval was received from the Institutional Animal Ethical Committee (IAEC), Karpagam University, Coimbatore. Approval number is KU/ IAEC/ B.Pharm/ 150.

2.9.2. Animals selection

Inbreed adult male *wistar rats* (160-200g) were used for the experiment. The animals were maintained in a well ventilated room at a temperature of 25 ± 2º c with 12:12 hour / dark cycle in poly propylene cages. The standard pellet feed and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory condition 10 days prior initiation of experiments.[24]

2.9.3. Chemicals and Drugs

0.3% Carboxy methyl cellulose- 1 ml.

Glibenclamide -2mg/kg.

Ethanolic extract - 200 and 400 mg/ kg.

Fresh juice - 0.3 ml/kg.

2.9.4. Treatment protocol

Group I (control)- animals were treated with CMC 0.5% (0.3 ml/ 100g rat).

Group II (positive control) - animals were treated with Glibenclamide (2mg/ kg).

Group III (ethanol extract)- animals were treated with 200mg/kg ethanol extract.

Group IV (ethanol extract) - animals were treated with 400 mg/kg ethanol extract.

Group V (fresh juice)- animals were treated with 0.3ml/kg fresh juice.

2.9.5. Study on Normoglycemic in rats

Normoglycemic studies were carried out in overnight fasted normal rats, which where, equally divided into 5 groups of 6 rats each. Normally control group received normal standard diets and vehicle only (1ml of 0.3% CMC; p.o.) and the standard group received reference drug Glibenclamidesuspended in the vehicle (2mg/kg, p.o.), while group from third and four where administered with 0.3ml of 200mg and 400mg of ethanolic extract of drug and group five received 0.3ml of fresh juice respectively. Blood sample where collected from tail vein by vein puncture method prior to dosing ie. 0 hour and then at regular intervals of

1hr, 2hr and 4th hr respectively and subjected to fasting blood glucose level. The results were documented and given in the figure.[25,26]

2.10. Anti-hyperglycemic assay (OGTT Method)

2.10.1. Animals selection

Inbreed adult male *wistar rats* (160-200g) were used for the experiment. The animals were maintained in a well ventilated room at a temperature of 25 ± 2º c with 12:12 hour / dark cycle in poly propylene cages. The standard pellet feed and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory condition 10 days prior initiation of experiments.[27]

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Group IV (ethanol extract) - animals were treated with 400 mg/kg ethanol extract.

Group V (fresh juice)- animals were treated with 0.3ml/kg fresh juice.

2.10.4. Study on oral glucose tolerance test (OGTT)

Initially, hypoglycaemic activity of plant extracts was carried out in overnight fasted normal rats, which were equally divided into 5 groups of 6 rats each. The blood glucose level was analyzed in fasting condition using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diabnostics, and GmbH, (Glucometer).Normal control group received only vehicle (1ml of 0.3% CMC; p.o.) and standard group received 1ml of reference drug Glibenclamide suspended in the vehicle, while group from third and fourth where administered with 0.3ml of 200mg and 400mg of ethanolic extract of drug and fifth group animals received 0.3ml of fresh juice respectively. Following 30 minutes post extract administration hyperglycaemia was then induced by oral administration of all the animals where fed with glucose (2g/kg)

when (t= 0min). Blood samples were obtained by the tail vein puncture method from tail vein prior to dosing and then at 30, 60, 90,120,150,180,210 and 240 minutes after glucose administration.[28,29]

2.10.5. Analytical Methods

The blood glucose level was analyzed immediately drawing the blood from tail vein in fasting condition as well as after drug administration using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diagnostics, and GmbH, (Digital Glucometer). Results were tabulated in table. The percent induced glycemia (%IG) following oral glucose load at different time intervals was calculated for the control and treated groups as follows: [30]

$$\% IG = \frac{Gx - G0}{G0} \times 100$$, Where *G*o is the initial glycemia (mg/dl) and *Gx* the glycemia

(mg/dl) at different time intervals after the oral glucose load (Braun mnitest REZ, Germany).[31]

2.10.6. Statistical Analysis

All the values of fasting blood sugar and after drug administration of drug in blood sugar were expressed as mean ± standard error of mean (SEM) n=6. Statistical difference was evaluated by using oneway analysis of variance (ANOVA) followed by Turkey's Dunnett's test. Data were considered statistically significant at p value ≤ 0.001, 0.01 and 0.05. Statistical analysis was performed using Graph Pad statistical software.

3. RESULT AND DISCUSSION

3.1. Physiochemical Evaluation

The flowers of *Cassia auriculata* was subjected for the analysis of physiochemical parameters like extractive values, ash values, moisture content and total fibre content. The results for physiochemical parameters are expressed in the table 1 and 2.

Table 1: Ash Values, fibre content and Moisture content.

PARAMETERS	VALUES IN % w/w
Total ash	3.24%
Water solubleash	2.05%
Acid insoluble ash	1.28%
Sulphated ash	2.37%
Total fiber content	2.16%
Moisture content	1.04%

Table 2: Extractive values.

Sl. No	Solvent	Extractive values %w/w
1	Benzene	2.4%
2	Acetone	1.5%
3	Ethyl acetate	6.2%
4	Chloroform	4.2%
5	Ethanol	2.3%
6	Methanol	7.1%
7	Water	12.4%

3.2. Phyto Chemical Evaluation

Qualitative phyto chemical analysis of *Cassia auriculata* was done with 6 different solvents extracts showed the presence of majority of the compounds including alkaloids, carbohydrates, tannins, pyhto sterols and glycosides. The results with different solvents were given in the table 3.

Table 3: Preliminary Phytochemical evaluation of *Cassia auriculata* flowers.

S.no	Chemical test	Benzene	Acetone	Chloroform	Methanol	Ethanol	Water
1.	Alkaloids	+	+	+	+	+	+
2.	Carbohydrate	+	+	+	+	+	+
3.	Proteins	-	-	-	-	-	-
4.	Tannin	+	+	+	+	+	+
5.	Phytosterol	+	+	+	+	+	-
6.	Glycosides	+	+	+	+	+	+
7.	Saponins	-	-	-	-	-	-

3.4. Quantitative Estimation

Total Phenol and Flavonoid Content

The plant flower powder was estimated for their total phenolic content and total flavanoid content and it was found to be11.04 ±2.5 (mg TAE/g extract) and3.62±0.20(mg RE/g)respectively**.**

3.5. Antidiabetic activity

In the present study, induced diabetes produced a significant increase in blood glucose levels in the diabetic rats. In diabetic rats treated with *Cassia auriculata* extract and fresh juice there was a greater reduction in the blood glucose level and also the extracts at a concentration of 400 mg/kg when compared to 200 mg/kg. This shows that the effect of activity towards the ethanol extract was dose dependent. Moreover, the results on flower extract also indicate a

prolonged duration of antidiabetic action in this study which could be due to multiple sites of action possessed by the active principle of *Cassia auriculata*. The antidiabetic effect of the extract may be due to the presence of antihyperglycemic principle and their synergistic properties as contains polyphenols, flavonoids and diterpenoids as the major bioactive components.[32] The possible mode of action may be the extra-pancreatic action such as increased glucose uptake or α-glucosidase inhibition or mediation of β-endomorphin.[33]

According to theory, the hypoglycemic plants act through a variety of mechanisms such as improving insulin sensitivity, augment in glucose dependent insulin secretion and stimulating the regeneration of islets of langerhans in pancreas of diabeteic induced diabetic rats.[34] Moreover, the role of antioxidant compounds in both protection and therapy of diabetics have been considered in various scientific researches. Treatment of diabetic animals with antioxidant, prevent hyperglycemia through reduced oxidative stress and restoring beta cell function.[35] In this study our data suggests that the ethanol extract and the fresh juice of flowers of *Cassia auriculata* shows significant antidiabetic potential as seen by the lowering of blood glucose levels in diabetic rats. The reductions in the blood glucose level were shown in the figure 1, 2, 3 and 4.

3.5.1. Hypoglycemic Activity

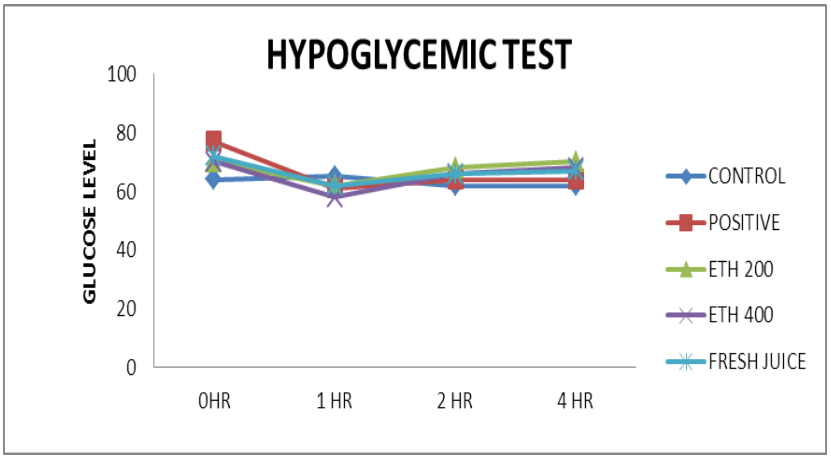

Fig1: Hypoglycemic activity of drug in normal rats.

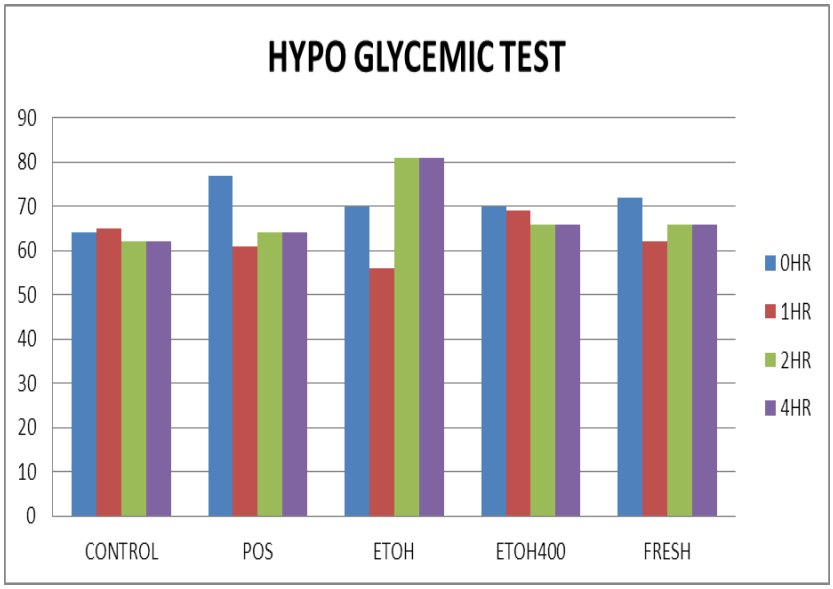

Fig 2: Hypoglycemic activity of drug in normal rats.

3.5.2. Effect of *Cassia auriculata* on glucose loaded rats

The ethanolic extract with both 200 mg/kg and 400 mg/kg body weight doses and fresh juice were subjected to screening for anti hyperglycemic activity by oral glucose tolerance test in albino rats possessed significant anti-hyperglycemic activity the blood glucose levels and the results are showed in figure3and 4.

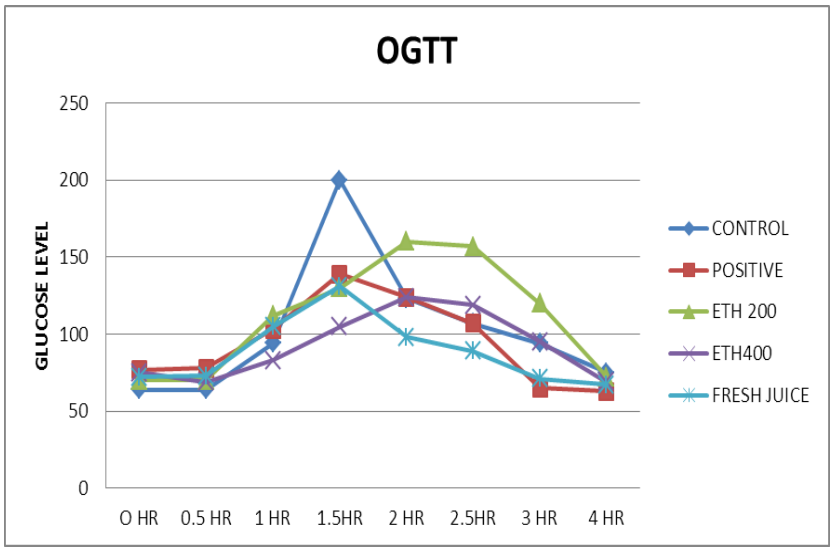

Fig 3: Antihyperglycemic effect of *Cassia auriculata* on glucose loaded rats.

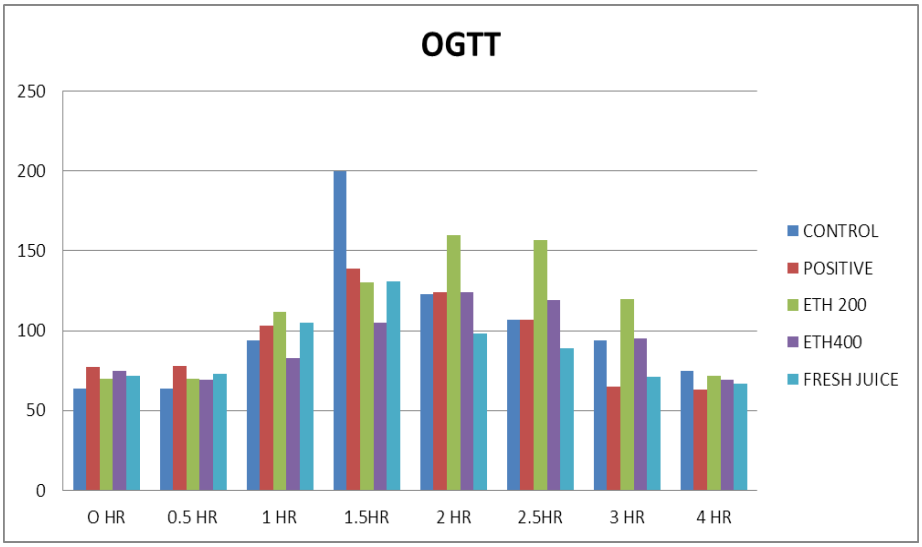

Fig 4: Antihyperglycemic effect of *Cassia auriculata* on glucose loaded rats.

Medicinal plant extracts have been valuable anti-diabetic agents and may involve one or more active components responsible for blood glucose reduction. Over 400 medicinal plants are available globally for the medication of *diabetes mellitus*, with a few having been subjected to scientific authentication to ascertain their effectiveness as anti-diabetic agents.

In this present study *cassia auriculata* was evaluated for its anti diabetic activity was evaluated by OGTT method and Hypoglycemic activity test method. In both methods it gave significant results, which confirmsthe anti diabetic activity.

In hypo glycemic test, after 1 hour of the administration of drug it shows degrees in glucose level. In standard drug treated animals the sugar level reduces upto 61.00±0.55mg/ml, in the ethanolic extract treated animals also decreases the sugar level that also more in 400mg/ml treated than in 200 mg/ml. After 2 hour slightly decreasing the glucose level in all groups after 4th hour very little raise in the glucose level, that also lower than that of the initial '0' hour sugar level. The animals treated with fresh juice shows better glucose level after 4thhour and it was nearer to that of the standard. So this result indicates the drugs activity is more and immediate effect at within one hour and remaining upto 4 hour.

In the OGTT method after the administration of glucose in all groups of animals a linear increment of glucose in the blood serum, but after 2 hours it starts to reduce the glucose level in serum. In the normal control group after 3rd hour it reaches 94 mg/ml. The positive control and the fresh juice showed significant reduction in blood glucose level. After 4th hour also better reduction in fresh juice treated animals that is also nearer to that of the standard drug

glibenclaimide. The animals treated with ethanolic extract 200mg/dl and 400 mg/dl also starts to give reduction in blood glucose level after 2nd hour then at 4th hour they showed significant reduction in the blood glucose level. Here the glucose level reduction is also dose dependent because animals treated with extract 400 mg/dl showed better reduction than 200mg/dl extract treatment.

CONCLUSION

This study conclud that the ethanolic extract and fresh juice of *Cassia auriculata* linn., produced a significant decrease of plasma glucose level, this effect was more potent after single oral administration. The fresh juice is having more potent effect than the extract. These results suggest that the effectiveness of the drugs depend probably, on the accumulative effect of active principles. In contrast, the significant increase in plasma glucose levels of untreated diabetic rats may be due to a progressive severity of untreated diabetes. Summarizing, it could be proved that the traditional use of *Cassia auriculata* linn. is an antidiabetic agent is justified and that extracts from this plant show a dose-dependent activity which is comparable to the standard antidiabetic drug Glibenclamide. Further, studies with isolated and purified constituents are needed to understand the complete mechanism of antidiabetic activity of this plant.

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