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Research Article

EVALUATION OF ANTIGLYCATION ACTIVITY AND GLYCATION REVERTING POTENTIAL OF *CASSIA AURICULATA FLOWERS*

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ABSTRACT

Cassia auriculata Linn. belongs to the Family *Caesalpiniaceae and* it is commonly called as Avaram in local name. It possess number of medicinal properties, especially it is used in the treatment of hyperglycemia. In the present study, in vitro antiglycation activity and glycation reverting potential of the flower extract (ethyl acetate) of *Cassia auriculata* was investigated. Antiglycation and Glycation reverting activity was studied using NBT and BSA glucose model assay respectively. Phytochemical analysis revealed that the flower extract contains bioactive constituents such as flavonoids, glycosides, phenols, tannins, quinone, coumarins, saponins and carbohydrates. The antiglycation activity of ethyl acetate extract was studied for a period of 3 weeks. Metformin was used as standard. There is a marked decrease in the reduction of NBT and prevention of glycation on 16th day. After 3 weeks of incubation the extract and drug showed maximum antiglycation activity of 82% and 90% respectively. The glycation reversing ability by extract and metformin increases with increase in concentration. The glycation reverting potential of metformin at concentrations of 100-500µg was 15% -75 % respectively and for the extracts (100-500 µg concentration) the glycation reverting potential was found be 4- 25%. This is the primary study aimed to reveal the glycation reverting ability of the ethyl acetate extract of *Cassia auriculata*. From this study, it can be concluded that the consumption of *Cassia* flowers as a decoction may play an important role in the controlling diabetes and its complications.

Keywords: *Cassia auriculata,* antiglycation, glycation reverting potential, advanced glycation end products.

1. INTRODUCTION

Diabetes mellitus (DM) is a multisystemic endocrine disorder characterized by defects in Insulin secretion or action resulting in persistent hyperglycemia. Growing population, aging, obesity due to Sedentary lifestyle contribute to increased incidence of diabetes at an alarming rate both in developed and developing countries. Chronic hyperglycemia contributes to microvascular (damage to small blood vessels) and macrovascular (damage to larger blood vessels) complications. Retinopathy, nephropathy and neuropathy leading to impotence and diabetic foot disorders are the triads of Microvascular complications. Cardiovascular dysfunctions such as heart attacks, strokes and insufficient blood flow to legs considered as the foremost macrovascular complications.

Glycation is considered as one of the main molecular mechanisms in eliciting several diabetic complications like diabetic retinopathy, nephropathy, neuropathy and some cardiovascular diseases [1]. During persistent hyperglycemia plasma proteins and collagen undergo glycosylation to form Advanced glycated End products (AGE) [2]. Increased level of AGE in blood results in failure of vital organs such as eyes, heart, nerves, kidneys and the blood vessels.

Currently available drugs for the treatment of Diabetes pose undesirable side effects such as liver problems, diarrhea and hypoglycemia at higher dose. Hence, in recent days, the use of natural products are increasing as they are safe, effective, low cost. Medicinal plants contains components of therapeutic values and are used as remedies for human diseases since antiquity. India's biodiversity is a mega one with rich flora and fauna. Various medicinal plants found in India are used in formulation of medications by Ayurvedic, Homeopathy, Unani, Siddha. *Cassia auriculata* Linn is one such plant and it is commonly known as Tanner's Cassia or Avaram Poo in vernacular name. The fresh or powdered form of Cassia plant parts are profoundly used in Ayurvedic medicine as a tonic, astringent and as

a remedy for diabetes, conjunctivitis and rheumatism [3]. *Cassia auriculata* is one of the principal constituents in "Avaarai panchaga chooranam" a siddha herbal preparation which is used for the treatment of diabetes. There are substantial evidence that proved the anti-diabetic properties of *Cassia auriculata* flower extract. However, no scientific reports are available to study the effect of ethyl acetate extract of *Cassia auriculata* flower on antiglycation activity and glycation reverting potential. Hence, an attempt has been made to study the antiglycation and glycation reverting ability of ethylacetate extract of *Cassia auriculata* flower extract.

2. MATERIAL AND METHODS

The flowers of *Cassia auriculata* were collected, washed thoroughly with distilled water and shade dried. The shade dried flowers were pulverized and it was stored in an airtight container until further use.

2.1. Extraction of Plant Material

About 500g of the powder was delipidated with 1.5L petroleum ether (40°C) in an orbital shaker. The solvent was removed by filtration and the filtrate was extracted by Soxhlet apparatus with 500ml of ethyl acetate till exhaustion. The extract was filtered using Whatman No.1 filter paper and then concentrated using a rotary evaporator at 4°C.The residual extracts was stored at 4°C for further analysis.

2.2. Phytochemical Screening

Ethylacetate extract was assessed for the presence of phytochemicals using the standard methods [4,5].

2.3. Antiglycation activity

Ten ml of Bovine serum albumin (BSA, 20 mg/ml) was mixed with 5ml of 500mM glucose and 0.02g of sodium azide in 100ml of phosphate buffer (200 mM, pH 7.4). Known concentrations of the extract was dissolved in 5ml of 200mM phosphate buffer (pH maintained at 7.4) and it was added to the reaction mixture. The mixture was incubated at 37°C for 21 days to obtain glycated products. Metformin was used as the standard. Phosphate buffer saline alone serves as sample control. Aliquots of the reaction mixture was taken at different time periods (0, 4^{th}, 6^{th}, 9^{th}, 16^{th}, 21^{st} day after incubation). 0.5ml of aliquots was added to 2.0ml of NBT (0.3mM.) in 100 mM sodium carbonate buffer (pH 10.35),the same is repeated for metformin. The tubes were mixed well and incubated at room temperature for 15 min and the absorbance was read at 530nm against a blank [6].

% Inhibition of glycation = (A Sample - A Control)/A Sample X 100

2.4. Glycation reverting activity

Glycation reverting activity of extract was done according to the protocol of Premakumara et al [7] with minor alterations.

Half (0.5) ml of 1 % Bovine serum albumin was incubated with 0.4ml glucose (500 mM) and 0.1ml of phosphate buffer (pH 7.4) comprising 0.02% sodium azide. The reaction mixture was incubated at 60°C for 40 h. 0.6ml of aliquot was transferred to an Eppendorf tube and 60 µL of 100% (w/v) TCA was added, mixed well. The tubes were then centrifuged in microfuge (15,000 rpm) at 4°C for 4 min. The supernatant was decanted. The AGEs-BSA precipitate was suspended in 50mM phosphate buffer (pH 7.4). 100-500µg/ml of extract (n=3) was added to the test tube, incubated at 60°C for 40 h. The tubes are then cooled, 60 µl of 100% (w/v) TCA was added, mixed well and centrifuged at 15,000 rpm for 4 min(4°C).The precipitate obtained was dissolved in 3ml of phosphate buffer saline (pH 10). The fluorescence intensity was measured at an excitation wave length of 370 nm and emission wave length of 440 nm using micro plate reader. The same procedure is repeated using Metformin. Glucose BSA was used as control.

Inhibition (%) = $[(Fc\text{-}F\,b) - (F\,s\text{-}Fsb)\,(Fc\text{-}F\,b)]*100$

Where Fb is the florescence of incubated BSA alone (blank), Fc is the florescence of incubated BSA, glucose (control), Fs is the florescence of the incubated BSA, glucose, and extracts and Fsb is the florescence of incubated BSA with the extracts as the positive control.

3. RESULTS AND DISCUSSION

Preliminary qualitative phytochemical analysis of ethylacetate extract of flowers of Cassia auriculata is represented in table 1. The phytochemical analysis showed that the extract contains phytochemical constituents such as cardiac glycoside, phenolic compounds, flavonoids, and alkaloids, saponin except terpenoids, steroids. Secondary metabolites afford imperative pharmaceutical properties for human health [8]. Compounds belonging to the flavonoids and alkaloids family are used as drugs or as dietary supplements to heal or prevent various diseases [9]. Cardiac glycosides were useful in the treatment of

asthma, as stimulant in case of cardiac failure. Saponin can be used as lipid lowering agent. Research show that Phenolic compounds are present as large groups in all plants. Presence of phenolic compounds may play a key role in the prevention of several chronic complications such as diabetes, cancer, cardiovascular disease, microbial infections [10].

Table 1 depicts the phytochemical constituents of ethyl acetate extract of flowers of Cassia auriculata.

Phytochemicals	Interpretation
Alkaloids	+
Saponin	+
Terpenoids	-
Phenols	+
Tannin	+
Steroids	-
Flavonoids	+
Cardiac Glycosides	+

+ Present, - absent

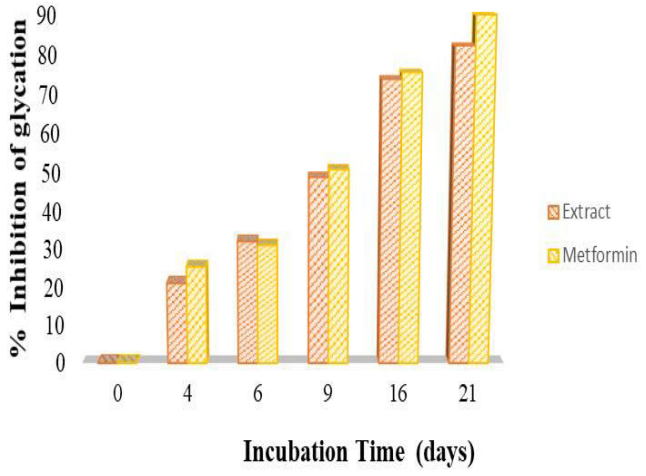

Fig. 1: Antiglycation activity of *Cassia* Extract and metformin

The effect of *Cassia* Extract against protein glycation was evaluated and its potency was compared with Metformin and it is represented in the Figure 1. On 4th day of incubation there was only around 20-26% inhibition by Extract and drug. Nearly 50% of inhibition of glycation by extract and drug was observed on the 9th day. After second week of incubation, 74-75% glycation inhibition was detected. On 3rd week of incubation, the extract and drug showed maximum antiglycation activity of 82% and 90% respectively.

Glycation of proteins in the body can lead to many complications and degenerative diseases [11]. Glycated products are formed when the extract is not able to inhibit the cross linking of albumin and glucose. NBT can be used as a mediator to study the inhibition of protein glycation. NBT is reduced at higher rate when there is increased glycated produts. From the data obtained, it is evident that the ethylacetate extract of *Cassia auriculata* has similar antiglycation activity as that of metformin.

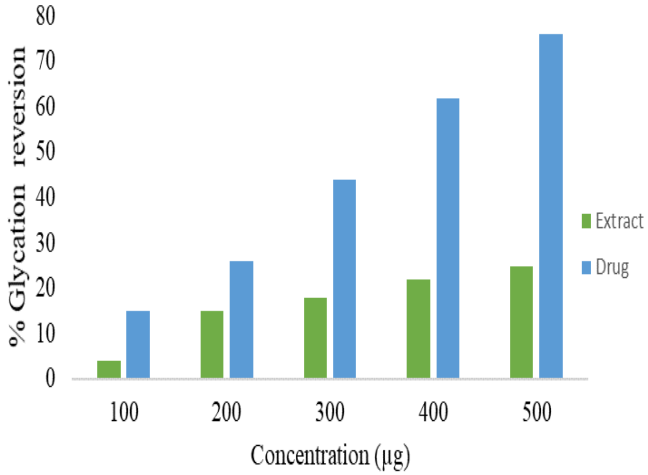

Fig. 2: Glycation reversion activity

Glycation reverting potential of the extract and Metformin is depicted in Figure 2.The glycation reversing ability was found be 4%, 15%,18%,22% and 25% for different concentration of the extract (100-500 µg), the glycation reverting potential of metformin is thrice as that of the extract. Metformin at various concentrations (100-500 µg) showed 15%, 26%, 44%, 62% and 75% glycation reverting ability. It was observed that Metformin possesses more glycation reverting activity when compared to extract.

An important way to alleviate AGE-related complications is to reverse the already formed AGE or break cross-links [12, 13]. Glycation reversing ability of the extract may be attributed by polyphenols that exert antioxidant activities. This may be the first work carried to assess the glycation reversing ability of *Cassia auriculata* flower extract.

4. CONCLUSION

The results of the present study indicate that the ethyl acetate extract of *Cassia auriculata* flowers ameliorate the diabetic complications which arise due to glycation. Discovery of more plant-based products which can

inhibit the distinctive phases of glycation or already formed AGEs can be used as target therapy in the prevention of diabetic complications in the future.

5. ACKNOWLEDGEMENTS

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Conflict of interest

The authors declare that there is no conflict of Interest.

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