

ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY EFFECTS OF *CALLIANDRA HAEMATOCEPHALA* AND ITS POTENTIAL ROLE IN DIABETES MELLITUSPUNNAGAI K¹, GLORY JOSEPHINE I^{2*}¹Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India. ²Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India. Email: dr.gloryj@gmail.com

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ABSTRACT

Objective: The global burden and the high prevalence of diabetes mellitus in India signify the importance of its control with effective measures. Particularly, intestinal alpha-amylase and alpha-glucosidase enzyme inhibitors reduce postprandial hyperglycemia and play a key role in preventing diabetic complications. Herbal plants also possess greater antioxidant, enzyme inhibitory, and antihyperglycemic activities with minimal side effects. Hence, this study is done to elucidate the inhibition of alpha-amylase and alpha-glucosidase enzyme activity and antidiabetic potential of *Calliandra haematocephala*.

Methods: The leaves of *C. haematocephala* extracted by ethanol as a solvent were used to elucidate the blocking of alpha-amylase and alpha-glucosidase enzymes for antidiabetic effect, and the absorbance was measured using spectrophotometry. The percentage of inhibition and half maximal inhibitory concentration (IC₅₀) value of the plant and control were calculated.

Results: The maximum percentage inhibition of *C. haematocephala* against alpha-amylase was 61% (IC₅₀ value of 82.18 µg/ml) and alpha-glucosidase was 71% (31.07 µg/ml). The maximum percentage inhibition of acarbose against alpha-amylase was 92% (IC₅₀ value of 42.65 µg/ml) and alpha-glucosidase was 89% (62.28 µg/ml).

Conclusion: Hence, the above study proved the antidiabetic activity of *C. haematocephala* and its significant role in the pharmacotherapy of diabetes mellitus.

Keywords: *Calliandra haematocephala*, Alpha-amylase, Alpha-glucosidase.

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INTRODUCTION

Diabetes mellitus presents with uncontrolled blood sugar levels due to lack of insulin or insulin resistance. For the past three decades, global prevalence of diabetes has almost doubled in the adult population, particularly in lower to middle socioeconomic group [1]. Hence, it is considered to be one of the four priority non-communicable diseases which are targeted for action. India shares the global diabetes burden because of early onset of disease, low awareness, and lifestyle modification [2]. Studies revealed the high prevalence of prediabetic state and rapid conversion of impaired glucose tolerance to diabetes in both South Indian urban and rural population [3]. Hence, the primary prevention and management are important because of rapid rise of vascular complications. Medicinal plants possess antidiabetic effect are used worldwide. They are effective in controlling plasma blood sugar and have minimal side effects. Considering the side effects of the antihyperglycemic drugs today, these plants could be a potential alternative or add-on drug and also reduce the economic burden. Moreover, the various chemical constituents target on different modes of mechanisms favor the treatment and prevention of complications [4,5].

The plant *Calliandra haematocephala* (red powder puff) is an evergreen deciduous and well-branched shrub with silky leaves. These shrubs are 10–15' (3–4.6 m) height and belong to the family Mimosaceae (Touch-me-not family). Red powder puff has attractive soft round brushes shaped flowers which bloom from October to April. The plant has been used in traditional medicine because of its blood purifying, anti-inflammatory, gastroprotective, hepatoprotective, immunomodulatory, and antiulcerogenic properties. The phytochemical investigations show the presence of tannins, flavonoids, and saponins which are responsible

for these activities. Studies proved that betulinic acid is responsible for its antitumor, anti-HIV, anti-rotavirus activities, and gallic acid for antibacterial activity [6-11].

Alpha-amylase and alpha-glucosidase inhibitors are used for the drug therapy of diabetes mellitus by reducing postprandial hyperglycemia. Many plants possess significant alpha-amylase and alpha-glucosidase curbing potential depicted by protection of the crops by suppressing the digestion of carbohydrate and change the feeding pattern of insects [12]. Studies proved about the bioactivity of polyphenols in plants and its correlation with their antioxidant and hypoglycemic properties [13]. Synthetic inhibitors such as acarbose and miglitol cause so many gastrointestinal side effects. Hence, the plant-derived alpha-amylase and alpha-glucosidase inhibitors could be a vital target for type 2 diabetes mellitus with minimal side effects. The two enzymes alpha-amylase and alpha-glucosidase and their inhibition by the ethanol extract of the study plant *C. haematocephala* are the design we postulated as the aim of the study.

METHODS

The *C. haematocephala* leaves were obtained in and around Madipakkam area and certified by Prof. P. Jayaraman, from Plant Anatomy Research Centre of West Tambaram. (Reg No. PARC/2017/3442).

Sample extraction

Soxhlet apparatus was used to extract the powdered sample (25 g) of the plant with ethanol used as a solvent. The extract was condensed under reduced pressure in rotary vacuum evaporator and stored at 4°C.

In vitro methods for antidiabetic activity**Determination of alpha-amylase inhibitory activity**

The alpha-amylase inhibitory effects of the plant extracts were elucidated by the method of Rege and Chowdary, 2014 [14]. The assay mixture containing 200 µl of sodium phosphate buffer, 20 µl of enzyme, and 20 µl of extract was incubated for 10 min at room temperature followed by the addition of 200 µl of starch in all the tubes. The reaction was stopped with the addition of 400 µl of DNS reagent and kept in the boiling water bath for 5 min, cooled and diluted with 15 ml of distilled water and absorbance of the control, and the samples were measured at 540 nm. The control sample was devoid of the plant extract. The percentage inhibition was analyzed by the formula:

Calculation

Percentage inhibition (%) = $\frac{\text{Absorption of the control} - \text{Absorption of the sample}}{\text{Absorption of the control}} \times 100$.

Determination of alpha-glucosidase inhibitory activity

Reaction mixture containing 50 µl phosphate buffer, 10 µl alpha-glucosidase, and 20 µl of varying concentrations of extracts was preincubated at 37°C for 15 min. Then, 20 µl p-nitrophenyl-α-D-Glucopyranoside was added as a substrate and incubated further at PNPg for 30 min. The reaction was stopped by adding 50 µl sodium carbonate. The yellow color produced was observed at 405 nm. Each experiment was carried out along with appropriate blanks. Acarbose at various concentrations (20–100 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition [15].

Calculation

Inhibition (%) = $\frac{\text{Absorption of the control} - \text{Absorption of the sample}}{\text{Absorption of the control}} \times 100$.

50% inhibitory concentration (IC50) calculation [16]

The IC50 of the sample and control calculation = $\frac{AC-AS}{AC} \times 100$.

RESULTS**Alpha-amylase assay**

The maximum inhibitory activity of the sample was 61% (*C. haematocephala*) and IC50 at around 82.18 µg/ml. Whereas, the maximum inhibitory activity of control (acarbose) was 92% and IC50 was at around 42.65 µg/ml (Table 1). Then, the graph was plotted with the concentration of sample on X-axis and percentage of inhibition of alpha-amylase enzyme at Y-axis (Fig. 1).

Alpha-glucosidase assay

The sample showed inhibitory activity of 71% (*C. haematocephala*) and IC50 at around 82.18 µg/ml. Whereas, the maximum inhibitory activity of control (acarbose) was 89% and IC50 was at around 62.28 µg/ml (Table 2). Then, the graph was plotted with the concentration of sample on X-axis and percentage of inhibition of alpha-glucosidase enzyme at Y-axis (Fig. 2).

Table 1: In vitro antidiabetic activity of *C. haematocephala* by alpha-amylase method and IC50 calculation of both sample and control (acarbose)

Concentration of sample (µg/ml)	% inhibition sample	% inhibition of acarbose
20	15	36
40	23	47
60	35	59
80	50	78
100	61	92
IC50 (µg/ml)	82.18 (µg/ml)	42.65 (µg/ml)

C. haematocephala: *Calliandra haematocephala*, IC50: Half maximal inhibitory concentration

DISCUSSION

Postprandial hyperglycemia is the important key factor for glycemic control and predictor of micro- and macro-vascular complications in diabetes mellitus [17]. Drugs which inhibit the alpha-amylase and alpha-glucosidase enzymes and reduce the postprandial hyperglycemia could be a novel approach for this metabolic disorder. These drugs inhibit the conversion of postprandial starch into simple glucose and its intestinal absorption. In addition to it, alpha-

Table 2: In vitro antidiabetic activity of *C. haematocephala* by alpha-glucosidase method and IC50 calculation of both sample and control (acarbose)

Concentration of sample (µg/ml)	% inhibition sample	% inhibition of acarbose
20	29	45
40	37	53
60	49	67
80	58	77
100	71	89
IC50 (µg/ml)	31.07 (µg/ml)	62.28 (µg/ml)

C. haematocephala: *Calliandra haematocephala*, IC50: Half maximal inhibitory concentration

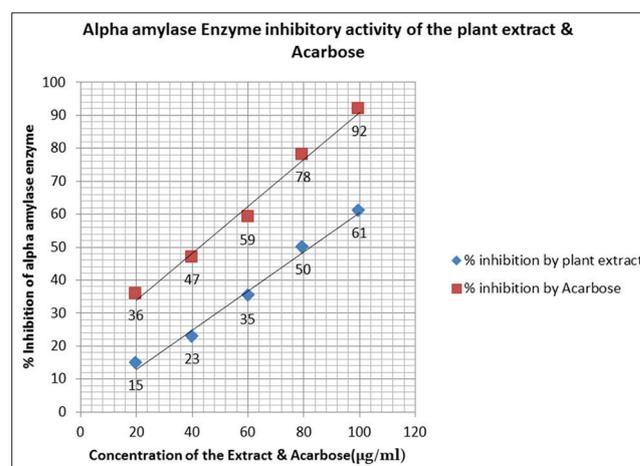


Fig. 1: In vitro antidiabetic activity of *Calliandra haematocephala* by alpha-amylase method and half maximal inhibitory concentration calculation of both sample and control (acarbose)

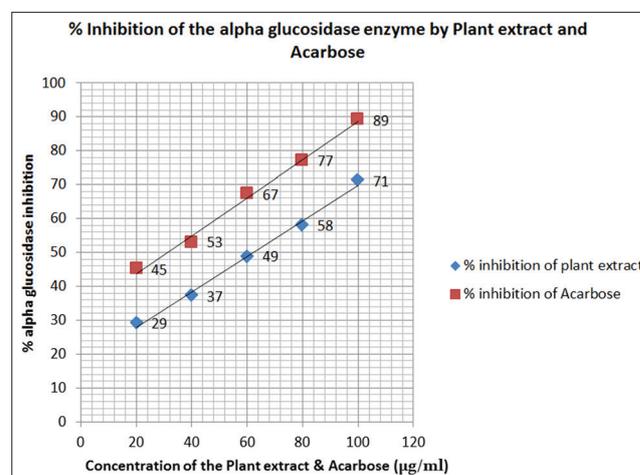


Fig. 2: In vitro antidiabetic activity of *Calliandra haematocephala* by alpha-glucosidase method and half maximal inhibitory concentration calculation of both sample and control (acarbose)

glucosidase inhibitors increase the release of the glucoregulatory hormone glucagon-like peptide-1 (GLP-1) into the circulation which contributes the glucose-lowering effect further [18]. Hence, they are effective as GLP-1 analogs and play in major therapeutic intervention of Type 2 diabetes mellitus. Acarbose, miglitol, and voglibose are used in newly diagnosed Type 2 diabetes and as a add-on drug with sulfonylureas and metformin to control the postprandial surge. However, gastrointestinal side effects are more with these synthetic inhibitors [19].

Herbal medicines have been used successfully in managing diabetes because of its ready availability, lesser side effects, and cost-effectiveness. Plants are good inhibitors of alpha-amylase and alpha-glucosidase and also have insulin-like activity [12]. In diabetes, increased lipid peroxidation leads to significant structural changes and alters lipid metabolism. Chronic hyperglycemia increases the production of reactive oxygen species that leads to the vascular complications through lipid peroxidation [20]. It is established that plant-derived bioactive phytochemicals have antidiabetic potential and reduce hyperglycemia. Hence, plants possess both antioxidant and antidiabetic properties could be a novel approach to prevent diabetic complications [21,22]. Docking studies also proved and isolated the active ingredients from the promising antidiabetic plants [23].

Flavonoids particularly isoquercitrin inhibit the sodium-dependent glucose uptake in the intestinal mucosa through sodium-dependent glucose transporter-1, delay the gastric emptying, stimulating glucose transporter 4 expression in skeletal muscles, and increase insulin sensitivity [24,25]. The antioxidant and anti-inflammatory actions of flavonoids protect the blood vessels and prevent the micro- and macro-vascular complications. Myricetin also possesses antioxidant, anti-inflammatory, and ameliorates insulin resistance [26]. One study showed that the leaves and bark of *Calliandra surinamensis* a different species of *Calliandra* were assessed for antidiabetic potential and found to be effective [27]. The phytochemical analysis of the *C. haematocephala* leaves extract shows the presence of alkaloids, tannins, flavonoids, saponin, and glycosides. It is proved that flavonoids, tannins, and glycosides isolated from *C. haematocephala* found to possess greater antioxidant activities [28]. Compounds such as myricetin and quercetin isolated from bark and leaves of *C. haematocephala* are found to possess strong radical scavenging properties [29,30]. Moreover, several studies supported the positive correlation of antioxidant properties with alpha-amylase and alpha-glucosidase inhibitory activities [12,13]. Hence, the antioxidant and alpha-amylase and alpha-glucosidase enzyme inhibitory properties ideally support the antihyperglycemic activity of *C. haematocephala*.

CONCLUSION

Hence, this study proved the antihyperglycemic activity and antidiabetic potential of *C. haematocephala* in type 2 diabetes mellitus. In comparison to synthetic alpha-glucosidase enzyme inhibitors, this plant could be a better alternative or adjuvant by increasing the efficacy of other antidiabetic drugs. However, isolating the compound which is responsible for the enzyme inhibitory activity would be a better lead and adjuvant in the control of diabetes mellitus.

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AUTHORS' CONTRIBUTION

Dr. Punnagai K (First author) - Concept, planned the experiments, analyzed the data and graph, and manuscript editing.

Dr. Glory Josephine I (Second and corresponding author) - Planned and conducted the experiments and manuscript writing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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