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IN-VITRO EVALUATION OF ARISTOLOCHIA BRACTEOLATA FOR ITS ANTI-DIABETIC ACTIVITY

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ABSTRACT

INTRODUCTION:

Diabetes Mellitus (DM) is a non-communicable disease, which is considered one of the five principal causes of death in the world.The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world.

AIM:

In-vitro evaluation of Aristolochia bracteolata for it's anti-diabetic activity.

MATERIALS AND METHODS:

Aristolochia bracteolata shade-dried leaves weighing about 200g were placed in a soxhlet apparatus and extracted with 70% hydroalcoholic (70% ethanol and 30% water). The resulting extract was filtered using Whatman filter paper No. 1 before being concentrated on a rotary evaporator.

RESULTS:

The results indicated that Aristolochia bracteolata possesses significant anti-diabetic activity.

CONCLUSION:

With agreement to previously conducted studies our research can conclude that A.bracteolata demonstrates significant anti-diabetic activity .

Keywords: Aristolochia bracteolata , anti-diabetic activity , xanthine oxidase inhibitory activity , Alpha-glucosidase inhibitory activity.

INTRODUCTION

Hyperglycemia, a complication of diabetes mellitus caused by abnormal insulin production and/or action, as well as by impaired protein, lipid, and glucose metabolism, is a complicated illness.Due to its multifaceted effects on vital biochemical processes in nearly every cell in the body, it is rightfully acknowledged as a global public health concern and is regarded as the most difficult metabolic endemic of the 21st century.(1)

For the treatment of DM, a variety of contemporary medications including biguanides, sulfonylureas, and thiazolidinediones are available. The use of these synthetic medications is connected with negative side effects. As a result, using herbal remedies to treat diabetes mellitus has become more significant globally.

Aristolochia bracteolata is a shrub distributed throughout India, belonging to the family Aristolochiaceae . It is a creeper plant and slender perennial. Stems 30-45 cm. long, weak, prostrate, branched, striate, glabrous. Leaves 3.8-7.5 cm. long and as broad as long, broadly ovate. Aristolochia bracteolata is used to cure a number of ailments, including cancer, stomach stimulants, anti-inflammatory drugs, antifungal, and antibacterial properties.A literature survey revealed that methanolic extract of whole plant of Aristolochia bracteolata is endowed with various chemical components such as phenolic compounds, flavonoids, triterpenoids, alkaloids, steroids, cardiac glycosides, saponins, aristolochic acid-A & aristolochic acid-D.

AIM:

Evaluation of the in-vitro anti-diabetic activity, alpha-glucosidase , xanthine oxidase activity and DPP-IV inhibitory potential of leaves of *A.bracteolata.*

MATERIALS AND METHODS:

Materials and Methodology

Plant Material

The leaves of *Aristolochia bracteolata* were collected from Tirunelveli district and were authenticated by Dr.Chelladurai, Botanist. The leaves were thoroughly washed under running tap water and then shade dried at ambient temperature. Thereafter the dried leaves sample was pulverized into a coarse powder and ready for extraction.

Preparation of Plant Extract

About 200g of the shade dried leaves of *Aristolochia bracteolata* was packed in a soxhlet apparatus and extracted with 70% hydrolacohol (70% Ethanol and 30% water). The extract obtained was filtered using Whatman filter paper No.1 after which the filtrate was concentrated on a rotary evaporator. The hydroalcoholic extract of *A.bracteolata* thus prepared was used for further analysis.

Chemicals and Reagents

Xanthine, α-glucosidase (1U/ml) from *Saccharomyces cerevisiae*, p-nitrophenyl- α -D-glucopyranoside (p-NPG), DPP-IV and synthetic substrate Gly-Pro-p-nitroanilide were procured from Sigma-aldrich, USA. Quercetin, Acarbose and Sitagliptin was purchased from TCI chemicals, India. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

Xanthine Oxidase Inhibitory Activity

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions, based on the procedure reported by Bustanji et al. 2011. The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50µL of different concentrations *Aristolochia bracteolata* (10-320µg/ml), different concentrations of Quercetin (10-320µM), 35µL of 0.1mM phosphate buffer (pH=7.5) and 30µL of enzyme solution (0.01units/ml of XO in 0.1mM phosphate buffer, pH=7.5), was prepared immediately before use. After 30mins of incubation at 25^{o}C, the reaction was initiated by the addition of 60µL of substrate solution (150mM of Xanthine in 0.1mM Phosphate buffer). The absorption at 295nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. A blank was prepared in the same manner. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid/minute at 25 °C. XO inhibitory activity is expressed as the percentage inhibition of XO in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of *A.bracteolata*. IC_{50} values were calculated from the mean values of data from three determinations. Quercetin was used as a reference standard.

A – Glucosidase Inhibitory Activity

The enzyme inhibition activity for α-glucosidase was evaluated according to the method previously reported by Shruthi et al. (2011) with minor modifications. The reaction mixture consisted of 50µL of 0.1M phosphate buffer (with pH of 7.0), 25µL of 0.5mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1M phosphate buffer, with pH of 7.0), 10µL of different concentrations of *A.bracteolata* (10-320µg/ml) and 25µL of α-glucosidase solution (a stock solution of 1mg/mL in 0.01M phosphate buffer, with pH of 7.0 was diluted to 0.1Unit/mL with the same buffer, with pH of 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100µL of 0.2M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using a microplate reader. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffers. Controls were conducted in an identical manner replacing the plant extracts with methanol. Acarbose was used as positive control. All experiments were carried out in triplicates.

In Vitro DPP-IV Inhibition Assay (Hamendra Singh Parmar et al., 2012)

Different sets of tubes each in triplicate were used for DPP-IV control; DPP-IV + different concentrations of sitagliptin (10-320nM), DPP-IV + *A.bracteolata* (10-320µg/ml). Briefly, the sitagliptin phosphate monohydrate and *A.bracteolata* were dissolved in dimethyl sulphoxide (DMSO) and diluted with tris buffer (pH 8.0, 50mM) to achieve the concentration the highest concentration. From the stock concentration of extract and standard, the working dilutions of 10, 20, 40, 80, 160, 320µg/ml and 10, 20, 40, 80, 160, 320nM were prepared. The assay was conducted using DPP-IV enzyme diluted with Tris buffer (0.05 U/ml) and pipette 50µl into clear microplate wells. Subsequently, 10µl of tris buffer or sitagliptin phosphate monohydrate or *A.bracteolata* was also added and incubated at 37°C for 20 min. Finally a 50µl of (0.20mM prepared in tris buffer) chromogenic substrate Gly-Prop-nitroanilide (Sigma–Aldrich, USA) was added into each tube. The hydrolysis of the substrate was monitored at 405 nm wavelength using a Microplate plate reader from Synergy Biotek, USA. Activity was expressed as ΔA_{405} nm/min. The experiment was performed in triplicate and compared with negative control (enzymatic solution without inhibition), while standard DPP-IV inhibitor drug sitagliptin employed as positive control.

RESULTS

Data were analyzed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC50 values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standards groups using the Holm-Sidak Test.

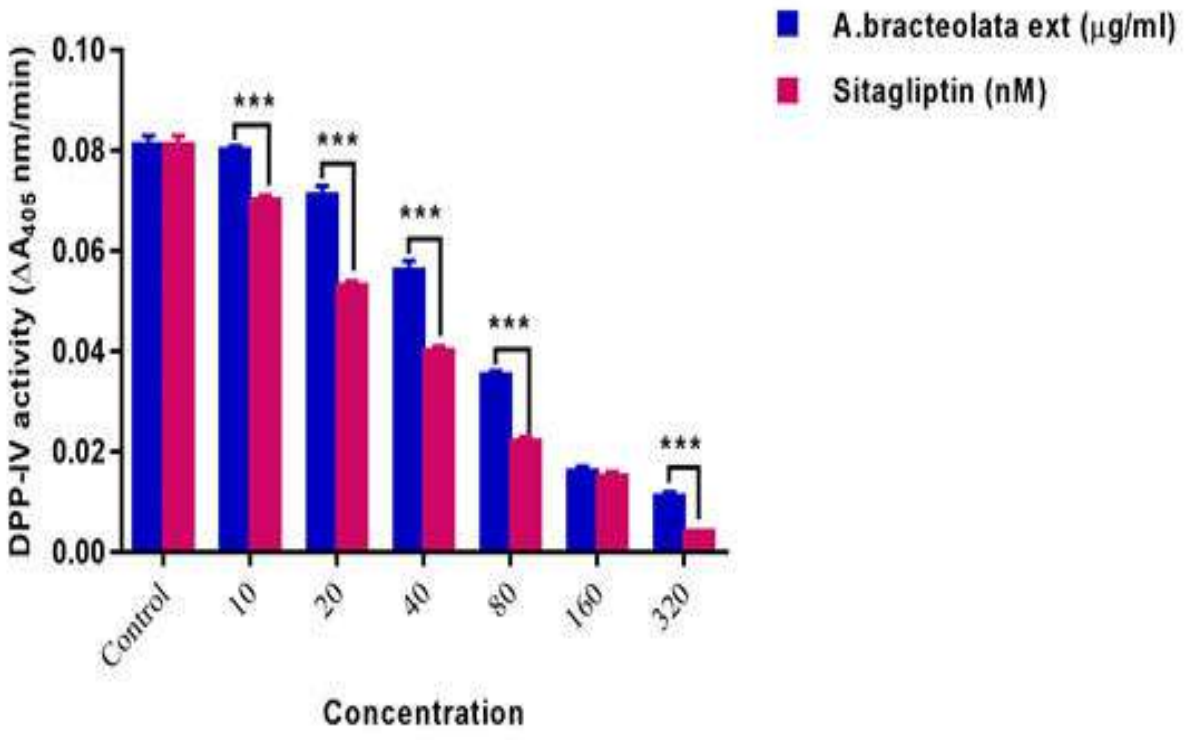

Fig.1: In the above graph, the x-axis denotes the DPP-IV activity and the y-axis denotes the concentration of the extract (blue) and the control (red).

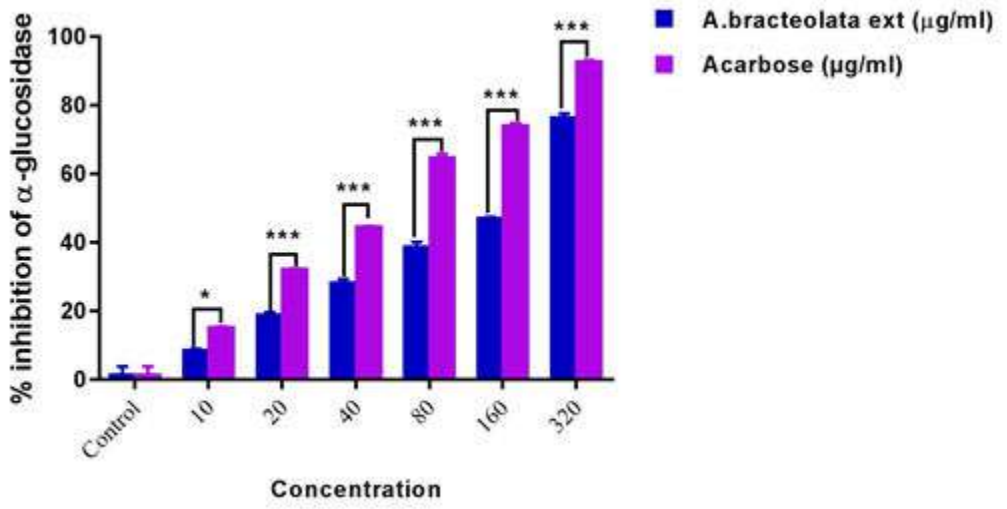

Fig.2 : In the above graph, the x-axis denotes the percentage inhibition of alpha-glucosidase and the y-axis denotes the concentration of the extract (blue) and the control (purple).

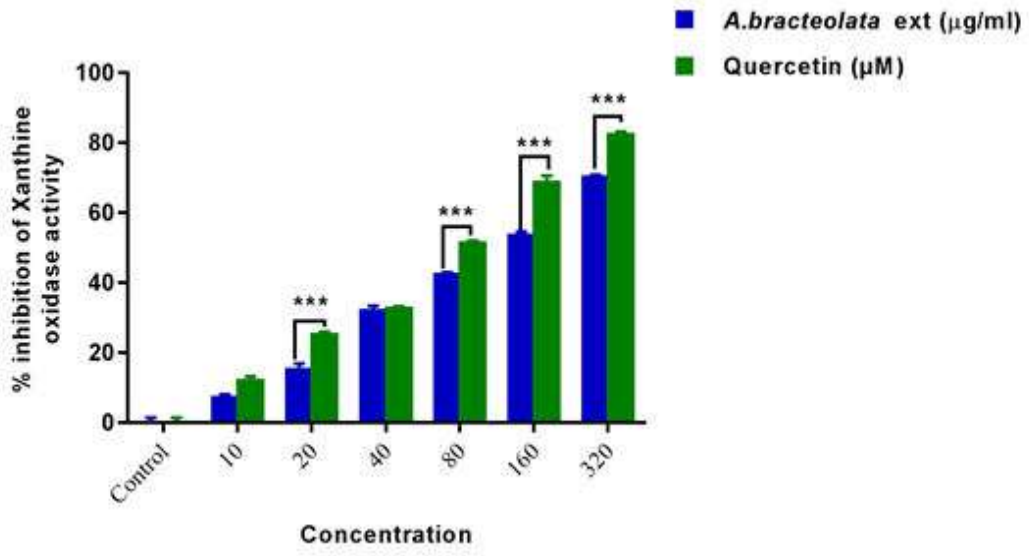

Fig.3: In the above graph, the x-axis denotes the percentage inhibition of xanthine oxidase and the y-axis denotes the concentration of the extract (blue) and the control (green).

DISCUSSION

In terms of DPP-IV activity it is seen that as the concentration is increased the activity of A.bracteolata extract is slightly more significant than the control, sitagliptin [Fig.1].

When comparing the percentage inhibition of alpha -glucosidase it is observed that A.bracteolata extract exhibits almost equal significance as that of the control, acarbose [Fig.2].

Furthermore, when the percentage inhibition of xanthine oxidase is compared between A.bracteolata extract and the control, quercetin ,it was observed that as the concentration is increased A.bracteolata extract exhibits almost equal significance to that of control.

Earlier studies done by El Omari N, Sayah K et al exhibited significant antioxidant and anti diabetic activity of Aristolochia longa plant extract.The extract also improved insulin sensitivity and restored the altered levels of biochemical markers associated with diabetes.(9)

In a previous study by Badami S, Jose CK et al showed that the ethanolic extract of A.bracteolata exhibits wound healing, antiinflammatory and antioxidant properties.It demonstrated significant anti-diabetic effects by reducing blood glucose levels and improving glucose tolerance.

In a review conducted by Piyathilaka KH, Karunaratne GH et al , it was observed that methanolic extract of the Aristolochiacaeae family had a very significant glucose lowering potency. A.bracteolata showed the highest glucose reduction when compared to other species of the family.

In agreement with previously conducted studies, our present study shows that A.bracteolata demonstrates potent anti-diabetic action.(10)

CONCLUSION

In summary the findings of the present study demonstrated that the hydro alcoholic extract of Aristolochia bracteolata has exerted significant anti diabetic activity. Overall, the plant would be a good candidate for further exploration as alternative therapy for the management of type 2 diabetes.However further studies are required to confirm the exact mechanism of action and to isolate the phytochemical constituents responsible for these activities.

CONFLICT OF INTEREST:

No conflict of interest.

ACKNOWLEDGEMENT:

FUNDING:

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ETHICAL CLEARANCE NUMBER:

Since this study is an in vitro study there is no requirement of ethical clearance.

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