



COMPARISON OF *IN-VITRO* ANTI HYPERGLYCEMIC ACTIVITY OF HYDRO ETHANOLIC FRUIT PULP EXTRACTS OF *TAMARINDUS INDICA L.* AND *GARCINIA GUMMI-GUTTA L.*

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Article Received on 28/04/2018

Article Revised on 18/05/2018

Article Accepted on 08/06/2018

ABSTRACT

The present study was designed to compare the anti hyperglycemic activity of 50% hydro ethanol fruit pulp extract of *Tamarindus indica L.* and *Garcinia gummi-gutta L.* The anti hyperglycemic activity was evaluated using various assays such as α -amylase inhibition assay (DNSA method), glucose diffusion inhibitory assay and non-enzymatic glycosylation of haemoglobin assay. The results revealed that *Tamarindus indica L.* and *Garcinia gummi-gutta L.* fruit pulp extracts were found to contain significant anti hyperglycemic activity. But on comparison it was observed that *Tamarindus indica L.* possessed more anti hyperglycemic potential than *Garcinia gummi-gutta L.* and can be utilized for better diabetic control.

KEYWORDS: *Tamarindus indica L.*, *Garcinia gummi-gutta L.*, anti hyperglycemic, α -amylase inhibition, glucose diffusion, glycosylation of haemoglobin.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder associated with hyperglycemia, abnormal elevated levels of lipid in blood and hypoinsulinemia.^[1] There are three major types of diabetes which includes type 1, type 2 and gestational diabetes mellitus.^[2,3] According to WHO, the incidence of diabetes mellitus is estimated to reach 5.4% by the year 2025 in most of the developing countries.^[4] A variety of synthetic drugs have been developed which can stimulate the β -cells of pancreas to secrete additional insulin and to serve as insulin sensitizers.^[5,6,7] One of the effective way for lowering the levels of post prandial hyperglycemia are provided by α -amylase and α -glucosidase inhibitors.^[8] Though the standard synthetic drugs have been in use for the treatment of diabetes, they cause a variety of side effects and thus suggest other effective alternatives.^[9] Several studies have shown that some of the plants in traditional medicine possess beneficial effects in diabetic patients.^[10,11]

Tamarindus indica L. is a large, long living evergreen tree which can grow up to 20-30m. The fruits are between 5-14cm in length with a hard brown shell called pods and the pulp has high acid and sugar content.^[12] Every part of *Tamarindus indica L.* has rich nutritional value and have broad spectrum applications in medicine.^[13] *Garcinia gummi-gutta L.* is widely distributed in semi-evergreen to evergreen forests. The fruits are yellow or red in colour and the pulp contains 5-

8 big seeds.^[14,15,16] It has been used traditionally as medicine for the treatment of various ailments.^[17] The present study was designed to investigate the scientific basis *in-vitro* for the use of these plants in the management of diabetes mellitus.

MATERIALS AND METHODS

Collection of plant materials

The fresh fruits of *Tamarindus indica L.* were collected from the local area of Coimbatore and *Garcinia gummi-gutta L.* were collected from Kerala. Both the plant materials were authenticated at the Botanical Survey of India (No.: BSI/SRC/5/23/2018/Tech./2171), Tamil Nadu Agricultural University, Coimbatore.

Preparation of extracts

The extracts were prepared by dissolving 10g of *Tamarindus indica L.* pulp and 10g of powdered fruits of *Garcinia gummi-gutta L.* in 100ml of 50% hydro ethanol in 250ml Erlenmeyer flasks. The flasks were then cotton plugged and kept for 48 hours at room temperature with intermittent shaking. After 48 hours, it was filtered through Whatmann No.1 filter paper, the filtrate concentrated under reduced pressure in a rotary vacuum evaporator and was stored in an air tight container for further use.

Methods

The extracts were used to study the anti hyperglycemic activity by

a) α -amylase inhibition assay-DNSA method^[18]

0.1-0.5ml of both plant extracts (100-500 μ g/ml) were taken into different test tubes. The volume in all the tubes was made up to 1.0ml with 20mM phosphate buffer of pH 6.9. Blank was measured by taking 1.0ml of phosphate buffer. Control was prepared without plant extract. To all the tubes 1.0ml of α -amylase (0.5mg/ml) was added and incubated at 25°C for 10minutes. After incubation, 1.0ml of 0.5% starch solution in 0.02M sodium phosphate buffer of pH 6.9 was added to all the tubes and incubated at 25°C for 10minutes. The reaction was stopped by adding 1.0ml of DNSA, the reaction mixture was kept in boiling water bath for 5minutes and cooled to room temperature. After cooling, the absorbance was measured colorimetrically at 565nm. Metformin (100-500 μ g/ml) was used as a standard drug and percentage inhibition was calculated using the formula,

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

b) Glucose diffusion inhibitory assay^[18]

1.0ml of the extract was placed in a dialysis membrane along with a glucose solution (22mM in 0.15M sodium chloride). It was then tied at both ends using thread and immersed in a beaker containing 40.0ml of 0.15M sodium chloride and 10.0ml of distilled water. The control contained 1.0ml of 0.15M sodium chloride containing 22mM glucose and 1.0ml of distilled water alone. The beakers were then placed on orbital shaker at room temperature. The movement of glucose into the external solution was monitored every half an hour

namely 30mins, 60mins, 90mins, 120mins, 150mins and 180mins respectively. The amount of glucose diffused was estimated by o-toluidine method.

c) Non-enzymatic glycosylation of haemoglobin assay^[19]

Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01M, pH 7.4. 1.0ml each of the above solutions was mixed and different concentrations of extracts (100-500 μ g/ml) were added to it. The reaction mixture was incubated in dark at room temperature for 72hours and the degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin (100-500 μ g/ml) was used as a standard for assay and percentage inhibition was calculated using the formula,

$$\% \text{ Inhibition} = \frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of sample}} \times 100$$

Statistical analysis

All the assays were carried out in triplicates and the values are expressed as mean \pm S.D.

RESULTS AND DISCUSSION

a) α -amylase inhibition assay-DNSA method

α -amylase is the carbohydrate hydrolysing enzyme involved in hydrolysis of α -linked polysaccharides.^[20] This enzyme begins the process of carbohydrate digestion by breaking the 1,4-glycosidic linkages of polysaccharides to disaccharides such as maltose which is mainly responsible for post prandial hyperglycemia.^[21,22] Thus inhibitors of α -amylase may be useful in the control of hyperglycemia which delays carbohydrate digestion and thereby reduce the post prandial plasma glucose level.

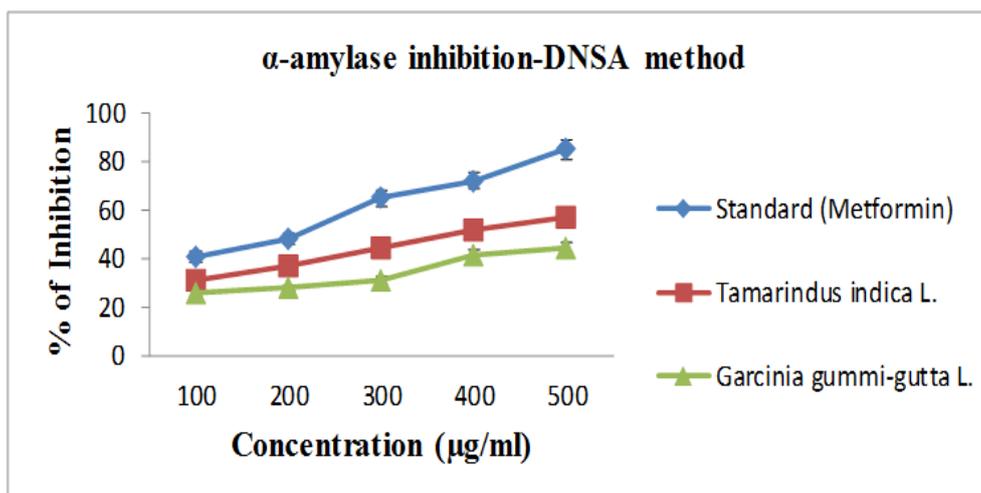


Fig. 1: α -amylase inhibitory activity of *Tamarindus indica* L. and *Garcinia gummi-gutta* L.

α -amylase inhibitory activity of both the fruit pulp extracts were found to increase with increase in concentration as shown in fig.1. The maximum inhibitory activity of 57.27 \pm 1.394% and 44.07 \pm 1.725% were found at a concentration of 500 μ g/ml for *Tamarindus indica* L. and *Garcinia gummi-gutta* L.

respectively. On comparison, *Tamarindus indica* L. showed more significant α -amylase inhibitory activity than *Garcinia gummi-gutta* L.

b) Glucose diffusion inhibitory assay

It is an effective assay to study the movement of glucose *in-vitro* using dialysis membrane. The glucose diffusion inhibitory effect of the fruit pulp extracts were assessed at an half an hour interval from 30-180mins and compared with that of the control which was prepared

without the plant extract. The ability of *Tamarindus indica L.* and *Garcinia gummi-gutta L.* extracts to alter glucose liberation from starch and their absorption prove them to be an attractive therapeutic agent in the management of diabetes mellitus.^[23]

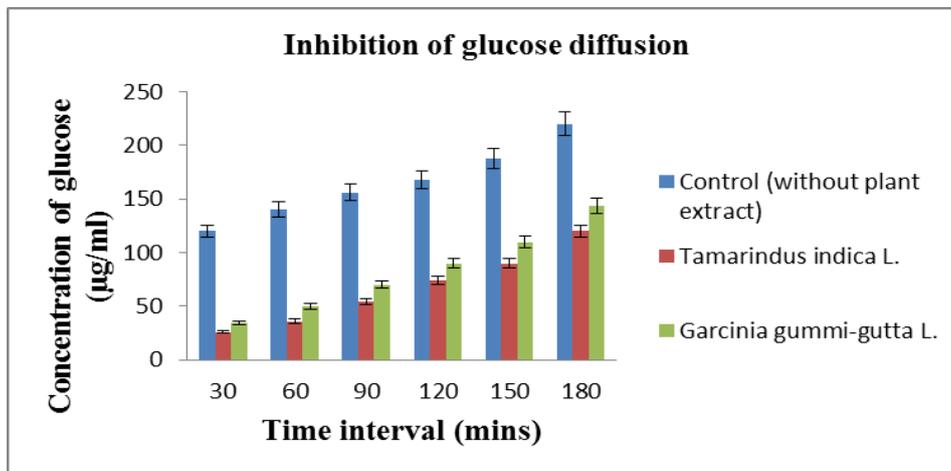


Fig. 2: Effect of *Tamarindus indica L.* and *Garcinia gummi-gutta L.* extracts on inhibition of glucose movement across dialysis membrane.

It is evident from fig.2 that both the extracts showed their inhibition on the movement of glucose across the dialysis membrane when compared to that of the control (without plant extract). In the control, there was no restriction to the movement of glucose across the dialysis membrane. On comparison, it was observed that *Tamarindus indica L.* fruit pulp extract was very much effective in inhibiting the movement of glucose into external solution across the dialysis membrane for as long as 180mins than that of *Garcinia gummi-gutta L.* fruit pulp extract.

Tamarindus indica L. and *Garcinia gummi-gutta L.* on the glycosylation of haemoglobin. The haemoglobin present in the red blood corpuscles has the ability to bind to glucose.^[24] This binding result in the formation of glycated end products which serves as a source of free radicals in diabetes mellitus.^[25] Inhibiting this binding of glucose to haemoglobin may lead to reduced level of free radicals in diabetes and reduces diabetic complications.^[26]

c) Non-enzymatic glycosylation of haemoglobin assay

This assay was performed to determine the inhibitory effect of 50% hydro ethanol fruit pulp extracts of

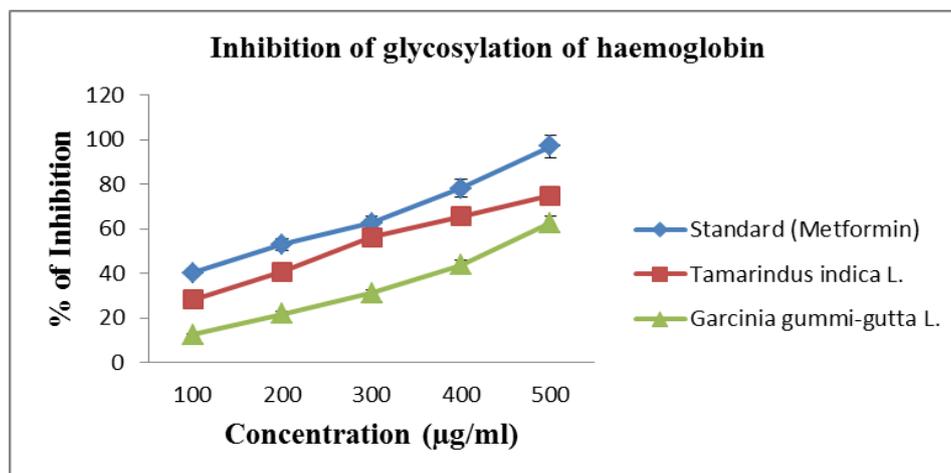


Fig. 3: Inhibitory effect of glycosylation of haemoglobin of *Tamarindus indica L.* and *Garcinia gummi-gutta L.*

It is observed from fig. 3 that the inhibition of glycosylation of haemoglobin for both the extracts were

found to increase with increase in concentration. The maximum % of inhibition was found at a concentration

of 500 μ g/ml as 75.12 \pm 0.866% and 62.52 \pm 1.065% for *Tamarindus indica L.* and *Garcinia gummi-gutta L.* respectively. It is evident from this study that the *Tamarindus indica L.* was found to possess potent inhibitory effect on the formation of glycated end products and can reduce various diabetic complications than *Garcinia gummi-gutta L.* Thus using various *in-vitro* anti diabetic assays, *Tamarindus indica L.* fruit pulp extract showed significant anti hyperglycemic activity.

CONCLUSION

It can be concluded that the hydro ethanolic fruit pulp extract of *Tamarindus indica L.* possess more anti hyperglycemic activity than *Garcinia gummi-gutta L.* hydro ethanolic fruit pulp extract.

ACKNOWLEDGEMENT

We are very thankful to the management of PSG College of Arts & Science, Coimbatore for providing necessary facilities to carry out this research work.

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