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IN VITRO ALPHA AMYLASE INHIBITORY ACTIVITY OF CRUDE ETHANOL EXTRACT OF *TERMINALIA CHEBULA* LEAVES

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ABSTRACT:
The present study was aimed to decrease post-prandial hyperglycemic levels in diabetic patients by inhibiting the enzyme alpha amylase using ethanol extract of *Terminalia chebula* leaves. *Terminalia chebula* leaves were subjected to soxhlet extraction using ethanol and studied wheat alpha amylase and alpha amylase inhibitory activity according to standard method. The results showed that ethanol leaf extract exhibit appreciable inhibition activity by dose dependent manner and the results were statistically significant with $p<0.001$ by using two way ANOVA followed by Bonferroni post test. *Terminalia chebula* may essentially contain herbal bioactive compounds that can mitigate post-prandial hyperglycemia and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents.

Key words: *Terminalia chebula*; wheat alpha amylase; alpha amylase; hyperglycemia; diabetes mellitus; soxhlet extractor.

INTRODUCTION:

Diabetes mellitus is a complex endocrine metabolic disorder that results in increased blood glucose levels due to absolute or relative deficiency of insulin.[1] It is one of the five leading causes of death worldwide, nearly 300 million Indians are expected to be diabetic by the year 2025.[2] Post-prandial serum hyperglycemic levels can be decreased through the inhibition of carbohydrate hydrolyzing enzyme like alpha amylase, which acts as target therapeutic advance for treating type II diabetes.[3] Inhibitors of pancreatic alpha amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels.[4] Synthetic alpha amylase inhibitors currently in clinical use are acarbose and miglitol. Their limitations include non-specificity, produce serious side effects such as bloating, abdominal discomfort, diarrhea and flatulence and failure to elevate diabetic complications.[5] Therefore, screening of alpha amylase inhibitors in medicinal plants has received immense attention.

For a long time natural products have been used for the treatment of diabetes, mainly in developing countries where the resources are limited and affordability and access to modern treatment is a problem.[6] *Terminalia chebula* also called as Black myrobalan, a traditional medicine extensively used in unani, ayurveda and homeopathic medicine. The biologically active chemicals of *Terminalia chebula* shows a wide spectrum of pharmacological activities and has been reported as antioxidant,[7] antidiabetic,[8] antibacterial,[9] antiviral[10] etc. The present study was carried out to investigate the alpha

amylase inhibitory activity of crude ethanol extract of *Terminalia chebula* leaves.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *Terminalia chebula* were collected from the herbal garden of Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam. The leaves were dried in shade for about a month, powdered, sieved and stored in air tight container for further studies.

Preparation of plant extract

The powdered and sieved leaf material of *Terminalia chebula* was extracted by using soxhlet apparatus.[11] 50 gm of dried powdered leaf material was packed in the soxhlet column and extracted for 10-12 hours using ethanol as solvent. Then the extracted fluid was concentrated by using distillation apparatus for a period of 2-3 hours and then air dried over night at room temperature and the percentage yield was calculated.

Extraction of wheat alpha amylase

500 gm of malted whole wheat flour was added slowly to 1 litre of 0.2% calcium acetate with continuous stirring for about 2 hours at room temperature. The suspension was cooled to 4°C and then centrifuged at 7500 rpm for 10 minutes. The clear brown supernatant was subjected to heat treatment at 70°C for 15 minutes in order to inactivate beta amylase because it may interferes with the enzymatic determination of alpha amylase. Alpha amylase was resistant to heat treatment and the pH was adjusted to 6.6 with 4% cold ammonium hydroxide. Heat treatment was carried out at 85 to 90°C with continuous stirring and then cooled to 2-3°C until use.[12]

In vitro wheat alpha amylase and alpha amylase inhibition assay

The wheat alpha amylase and alpha amylase inhibitory effect of ethanolic leaf extract of *Terminalia chebula* was determined according to standard method.[13] For wheat alpha amylase inhibition assay, the mixture containing 200 µl of 0.02M sodium phosphate buffer, 200 µl of wheat alpha amylase extract and 100 µl of different concentrations of ethanol extract of *Terminalia chebula* leaves were incubated for 10 minutes at room temperature and added 200 µl of 1% starch solution in all incubated test tubes. Then added 500 µl of dinitrosalicylic acid areagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control was prepared without any plant extracts. Acarbose was used as the reference standard. The percentage inhibition was calculated using the formula.

$$\% \text{ Inhibition} = \frac{\text{A540 control - A540 extract}}{\text{A540 control}} \times 100$$

Where, A540 = Absorbance at 540 nm.

The alpha amylase inhibition activity of the plant was assayed by the same method used for wheat alpha amylase inhibition activity.

Statistical analysis

Results were expressed as mean±standard error of mean (SEM), (n=3). Statistical analysis was carried out using two way ANOVA followed by Bonferroni post test in Graph Pad Prism 5 software. P values <0.001 were considered significant.

RESULTS AND DISCUSSION

The dried powdered leaves of *Terminalia chebula* were extracted with ethanol, percentage yield calculated was 23.08% and assayed for *in vitro* wheat alpha amylase and

alpha amylase inhibitory activity. The assay was carried out using five different concentrations (20, 40, 60, 80 and 100 µg/ml), the extract showed significant alpha amylase and wheat alpha amylase inhibitory activity at all the tested concentrations and the results have been tabulated in table 1 and 2 respectively. The results indicated that the ethanolic leaf extract exhibited appreciable inhibition activity against wheat alpha amylase and alpha amylase in comparison with acarbose. There was a dose dependent increase in percent inhibition against wheat alpha amylase and alpha amylase by ethanol extract of *Terminalia chebula* (figure 1 & 2). The present study indicated that *Terminalia chebula* could be useful in management of post-prandial hyperglycemia.

Table 1: Percentage inhibition of wheat alpha amylase

Concentration (µg/ml)	Acarbose (Standard)	Terminalia chebula (Ethanol extract)
100	90.53±0.67	45.14±0.85
80	81.63±0.85	32.67±0.49
60	63.17±0.87	22.55±0.89
40	55.21±1.32	10.41±0.49
20	41.08±1.03	6.53±0.33

NOTE: All values are represented as mean±SEM (n=3). All results were statistically significant with $p<0.001$ compared with standard drug.

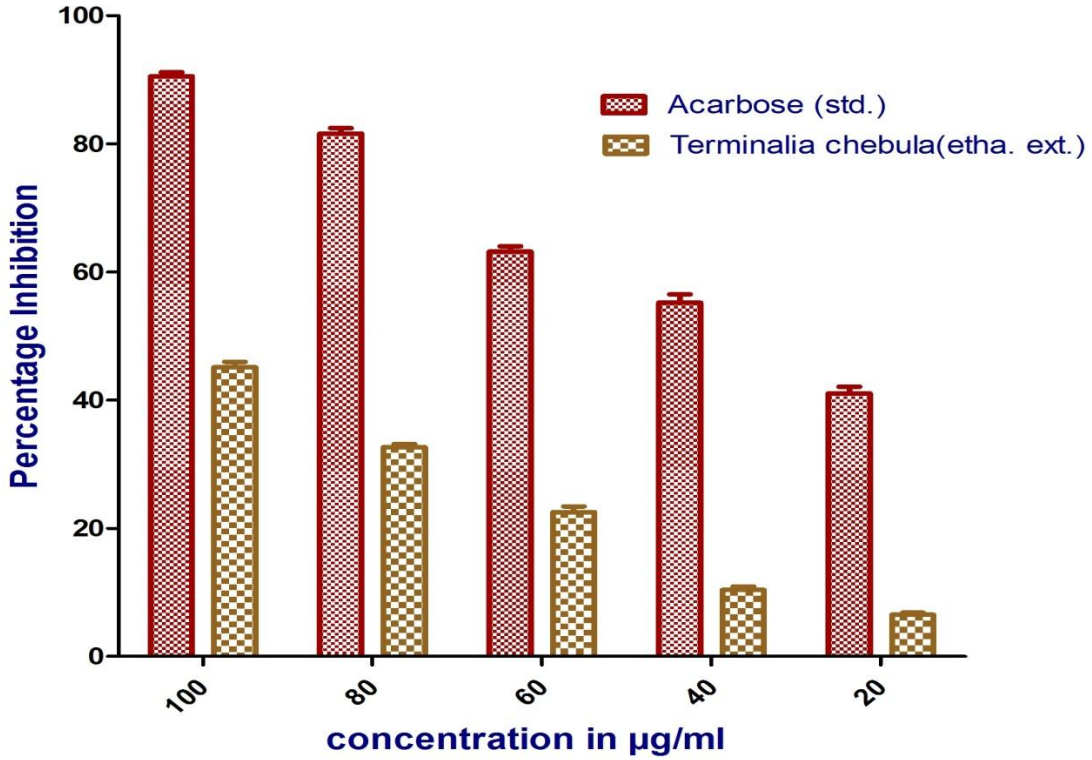

Fig.1: Percentage inhibition of wheat alpha amylase

Table 2: Percentage inhibition of alpha amylase

Concentration (µg/ml)	Acarbose (Standard)	Terminalia chebula (Ethanol extract)
100	89.33±1.10	50.85±0.96
80	77.43±0.85	38.31±0.87
60	63.85±0.78	27.20±0.69
40	53.60±0.84	21.21±1.22
20	42.52±0.70	11.02±0.23

NOTE: All values are represented as mean±SEM (n=3). All results were statistically significant with p<0.001 compared with standard drug.

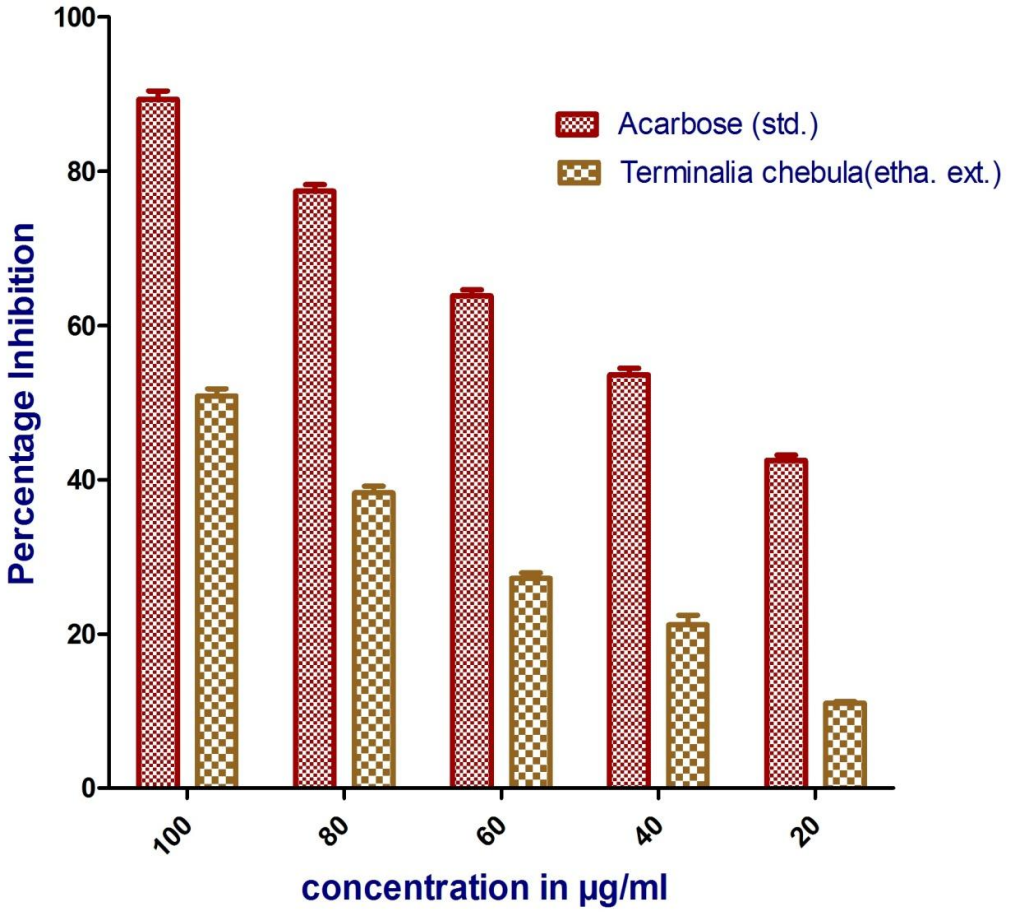

Fig. 2: Percentage inhibition of alpha amylase

CONCLUSION

The results of the present study indicated that the ethanolic leaf extract of *Terminali chebula* showed the appreciable wheat alpha amylase and alpha amylase inhibitory activity compared to the standard and can mitigate post-prandial hyperglycemia. *Terminalia chebula* may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and

characterization methodologies have to be carried out in order to identify the bioactive constituents. Based on the present investigation, it can be concluded that the selected plant leaves are containing chemical constituents that shows promising treatment for post-prandial hyperglycemia.

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