INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level. Diabetes mellitus is caused due to relative or absolute deficiency of insulin or resistance to the action of insulin at the cellular level [1]. The abnormalities in metabolism of carbohydrate protein and fat are due to a deficient action of insulin on target tissues resulting from insensitivity or lack of insulin [2]. In developing countries, diabetes mellitus type 2 represents near about 90% of total people with diabetes. The percentage is much higher in developing countries [3]. One of the prominent and early symptoms of diabetes mellitus type 2 is postprandial hyperglycemia (PPHG). Postprandial hyperglycemia has been identified as an independent risk factor for developing cardiovascular disease in patients with or without diagnosed diabetes. Studies have shown that PPHG, instead of Fasting glucose, is a significant predictor of subsequent myocardial infarction and death in patients with newly diagnosed diabetes mellitus type 2 [4]. Drug with mild α amylase inhibition is considered as preferable for treatment of postprandial hyperglycaemia since the side effects related to very high inhibition of pancreatic α-amylase such as flatulence, abdominal distension, and diarrhoea etc caused by intake of drug acarbose, results in abnormal fermentation of undigested carbohydrate by of colon bacteria mark limitation in its use [5]. Therefore, α-glucosidase inhibitors are considered as better therapeutic to control the PPHG spike in diabetes mellitus type 2. Terminalia is a genus of large trees of the flowering plant, family Combretaceae, comprising around 100 species distributed in tropical regions of the world. Being a native plant of South East Asia, the dried ripe fruit of Terminalia chebula has traditionally been used to treat various ailments including diabetes [6-9]. Though several studies were conducted earlier upon the fruit part of T. chebula, the literature survey reveals that there is no previous report on the hypoglycemic evaluation of the leaf part of the plant T. chebula, both in vitro and in vivo. Hence, in the current study, the crude methanol extract of the plant was used to access its hypoglycemic potentiality in vitro and in vivo.

MATERIALS AND METHODS

Chemicals and reagents

Alpha-glucosidase (EC 3.2.1.20) porcine pancreatic alpha-amylase enzyme (3.2.1.1) and alloxan monohydrate were procured from Sigma Co. USA. Standard drug acarbose was purchased from a nearby pharmaceutical shop of Guwahati. For estimating the blood glucose level, Glucometer Select One-touch was used. All solvent used in this study were of analytical grade.

Preparation of plant extract

Collected leaves were shade dried, grounded to the fine powder and extracted subsequently in methanol, using a Soxhlet apparatus. The crude extract was concentrated using a rotary evaporator (BUCHI R II). The semisolid extract obtained was then stored at 4 °C until the assay.

In vitro Alpha amylase inhibition assay of T. chebula extract

α-amylase inhibition was determined using the modified version of the method according to Berendsen [10]. Briefly, 100 µl of test extract was allowed to react with 200 µl of the porcine pancreatic alpha-amylase enzyme (Sigma Aldrich-3176) of 0.5unit/ml and 100 µl of 2 mmol of sodium phosphate buffer (pH 6.9). After 20 min of incubation at 37 °C, 100 µl of 1% potato starch solution was then added. The same was performed for the blank, where 200 µl of an enzyme was replaced by the buffer. After incubation for 15 min, 500 µl of 3, 5 Dintro salicylic acid reagents were added to both control and test. They were kept in a boiling water bath for 10 min. The absorbance was recorded at 540 nm using a UV–VIS spectrophotometer and the percentage of inhibition of α-amylase enzyme was calculated using the formula:

\[
\text{Inhibition(%) } = \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100
\]
The crude methanol extract of *T. chebula* leaves was subjected to column chromatography using silica gel 60–120 mesh for the isolation of bioactive antidiabetic principles. The column (300 x 18 mm diameter) was packed with slurry of silica and petroleum ether and kept for overnight. Next morning plant sample (crude methanol extract of *T. chebula* leaves in powder form) was loaded over the packed column with the help of a spatula. The column was eluted with a solvent of increasing polarity. All fractions were analyzed in Merck TLC plates (20 cm x 20 cm) using a different proportion of hexane and ethyl acetate as mobile phase. Obtained fractions were tested for their in vitro hypoglycemic property using alpha-amylase and alpha-glucosidase enzymes using the same protocol mentioned earlier. The fraction showing highest α-glucosidase inhibitory activity was calculated by the following equation:

\[ \text{Inhibition} \% = \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100 \]

Where, \( \Delta A \) is the absorbance of the control reaction, \( \Delta A \) is absorbance of the test sample reaction.

A dose-dependent α-amylase and α-glucosidase inhibitory activities were measured using an increasing concentration of plant sample (2, 4, 6, 8, 10 mg/ml) and the IC\(_{50}\) was calculated. IC\(_{50}\) denotes the concentration of plant extract that is required to inhibit 50% of enzyme activity.

### Oral maltose tolerance test

Six days after performing the glucose tolerance test, maltose tolerance test was performed in the same group of rats. The procedure for performing the maltose tolerance test was similar with glucose tolerance except that instead of glucose, maltose (3g/kg body weight) was orally administrated to all groups of rats, 30 min after administration of the plant extract.

### Oral starch tolerance test

Six days after performing the maltose tolerance test, starch tolerance test was performed in the same group of rats. Starch (3g/kg body weight) was orally administrated to all groups of rats, 30 min after administration of the plant extract.

### Bioassay-guided fractionation and partial characterization of crude methanol extract of *T. chebula* leave

The crude methanol extract of *T. chebula* leaves was subjected to column chromatography using silica gel 60–120 mesh for the isolation of bioactive antidiabetic principles. The column (300 x 18 mm diameter) was packed with slurry of silica and petroleum ether and kept for overnight. Next morning plant sample (crude methanol extract of *T. chebula* leaves in powder form) was loaded over the packed column with the help of a spatula. The column was eluted with a solvent of increasing polarity. All fractions were analyzed in Merck TLC plates (20 cm x 20 cm) using a different proportion of hexane and ethyl acetate as mobile phase. Obtained fractions were tested for their in vitro hypoglycemic property using alpha-amylase and alpha-glucosidase enzymes using the same protocol mentioned earlier. The fraction showing highest in vitro hypoglycemic activity was finally accessed for its maltose tolerance in vivo in an allophan-induced diabetic rat model using the pre mention protocol. The isolated active fractions or band was further characterized using FTIR analysis for the identification of the active principle group involved in retarding the postprandial hyperglycemia.

### Statistical analysis

The results obtained were expressed in mean±SEM. The studied groups were compared using ANOVA test and Post Hoc Turkey HSD analysis was done to compare the mean. Values were considered to be significant when the p-value was less than 0.05.

### RESULTS AND DISCUSSION

When accessed for α-amylase inhibitory activity at the concentration of 10 mg/ml, a mild inhibition of 70.46% was demonstrated by the extract compared to standard drug acarbose 80.21%. Whereas in case of enzyme alpha-glucosidase the same leave extract demonstrated a remarkable 100% inhibition compared to standard drug acarbose with 85.34% with a very minimum IC50 value of 0.956±0.342 mg/ml (table 1).
Oral carbohydrate tolerance test

The effect of the crude extract of *T. chebula* leaves, and acarbose on oral carbohydrate tolerance test was performed in both normal and alloxan-induced diabetic rats using monosaccharide (glucose), disaccharide (maltose) and polysaccharide (starch).

Oral glucose tolerance test

A total of 36 rats were used for the carbohydrate tolerance test. The postprandial glucose variation was measured by loading both the experimental groups with glucose (2 gm/kg body weight). In glucose tolerance test, we found that the oral administration of acarbose (10 mg/body weight), 30 min before oral administration of glucose to 16 h fasted normal and diabetic rats were capable of suppressing the postprandial blood glucose level at 60 and 120 min compared to methanol extract of *T. chebula* leaves (fig. 1 and 2).

Oral maltose tolerance test

A week after performing the oral glucose tolerance test, all the three groups were loaded with maltose (3 gm/kg body weight). In maltose tolerance test, oral administration of methanol extract of *T. chebula* leaves (300 mg/kg b. w) to diabetic rats significantly (*P<0.05*) suppressed the rise of postprandial blood glucose level compared to the standard drug acarbose (fig. 3 and 4).

Oral starch tolerance test

A week after performing the maltose tolerance test, all the three groups were loaded with starch (3 gm/kg body weight). In starch tolerance test, oral administration of standard drug acarbose (10 mg/kg body weight), 30 min before oral administration of glucose to normal and diabetic rats was showed higher capability of suppressing the postprandial blood glucose level at 60 and 120 min compared to methanol extract of *T. chebula* leaves (fig. 5 and 6).
Bioassay-guided fractionation of crude methanol extract of *T. chebula* leaves

The crude methanol extract being the most efficacious extract was later subjected to column chromatography for isolation of the active fraction using different solvent systems. The column chromatography of crude methanol extract of *T. chebula* leaves using different solvent systems yielded 18 fractions. The collected fractions were later combined into six main fractions based on the RF (Retention factor) value obtained by analytical thin layer chromatography. The in vitro enzyme inhibition study revealed that Fraction 5 (f5) demonstrated moderate alpha-amylase inhibition (IC50–42.86±0.56 µg/ml) compared to acarbose (IC50–45.06±1.01 µg/ml). However, the active fraction (f5) showed highest (P<0.01) alpha-glucosidase inhibition with a very minimum IC50 value of 39.58±0.98 µg/ml compared to acarbose (IC50–55.56±1.07 µg/ml) (fig. 7 and 8).

Due to its mild alpha-amylase and high alpha-glucosidase inhibition activity, the fraction (f5) further selected for in vivo study in alloxan-induced diabetic rat model. In vivo maltose tolerance test of (f5) revealed that the leaves of *T. chebula* was capable of retarding the postprandial hyperglycemia significantly (*P<0.05, **P<0.01) from 245 mg/dL (reading taken at 30 min) to 172 mg/dL (reading taken120 min) interval of time (fig. 9) compared to acarbose (230 mg/dL to 197 mg/dL) during the studied time interval (fig. 10).

The fraction (f5) demonstrated better result than acarbose in a diabetic group, however, when accessed in normal rat loaded with maltose, it was not found to be significant when compared statistically. The IR spectrum of the fraction exhibited broadband in the range, 3000–3500 cm⁻¹ which are generally attributed to the OH
stretching while the band observed at 1652 cm⁻¹ corresponds to C=O stretching (fig. 11). The bands observed in the range, 2833.99-2947.68 contribute to alkane C-H bond, 1540.54-1652.69-1500 cm⁻¹ are the due presence of N-H bonding, 1418.14-1506.68 are due to alkane C-H bond, while the ones at 1113.97 are due to ester linkage and that of 1000 cm⁻¹ to 500 cm⁻¹ are assigned to aromatic C-H bending vibration. The FTIR analysis demonstrated the presence of several functional groups in the most active fraction (f5). Thin layer chromatographic separation of (f5) yielded another subfraction (f5a). The subfraction on HPLC analysis revealed the presence of the gallic acid as a major constituent (fig. 12 and 13).

Fig. 11: FTIR spectra obtained for the active fraction (f5)

Fig. 12: HPLC spectra of gallic acid standard

Fig. 13: HPLC spectra of gallic acid isolated from T. chebula Retz leaves

Diabetes mellitus is one of the fast-growing health problems in both developing and developed nations.

Postprandial hyperglycemia that occurs due to impaired glucose tolerance (IGT) is alone a factor to double the risk of cardiovascular disease (CVD) [12-15]. There are many previous reports on the potentiality of the fruit part of T. chebula to inhibit enzyme alpha-glucosidase [16-18]. Several earlier studies conducted on the fruit part of T. chebula demonstrated potent maltase inhibitory activity due to the presence of three active ellagitannin (chebulanin, chebulagic acid and chebulinic acid [19]. Plant-derived hydrolyzable tannin is known to be responsible for varied pharmacological
activity including antidiabetic [20–24]. Gallic acid being one of the widely spread hydrolyzable tannins of *T. chebula* possesses very high antioxidant and hypoglycemic property [25, 26]. Recent studies indicate that plant-derived ploy phenols, because of its antioxidant and anti-inflammatory properties, attribute maximum towards the hypoglycemic effect via several modes like reduction of the intestinal absorption of dietary carbohydrate, improvement of β-cell function, improvement of insulin action, modulation of the some enzymes involved in glucose metabolism [27–30]. The enzyme α-glucosidase inhibitors fall under one of the categories of oral hypoglycemic agents that are generally used for the treatment of diabetes. α-glucosidase inhibitory compounds are abundant in nature, and those with very promising inhibitory potentiality can be clinically employed for treating diabetes mellitus type 2.

CONCLUSION

The present study concludes that a leaf of *T. chebula* is a potential inhibitor of enzyme alpha-glucosidase that plays a crucial role in intensifying the postprandial hyperglycemic condition. In future, more vigorous and authentic screening of ethnic knowledge-based antidiabetic plants is needed to be done, for the development of some effective bio formulations. Such formulations in the coming future will definitely combat metabolic syndrome like diabetes and complications associated with the disease.

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AUTHORS CONTRIBUTIONS

Jayashree Dutta has designed and performed the experiments. M. C Kalita assisted in the preparation of the manuscript. Both the authors have read and approved the content of the manuscript.

ABBREVIATION


CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES