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Investigation of Antioxidant and Antihyperglycemic Activities of Ethanol Leaves Extract of *Cinamomum tamala,* A Commonly Used Cooking Ingredient

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Abstract

Cinnamomum tamala is used traditionally to treat various ailments, also used as spice in daily cooking. The present study explored the possible antioxidant and antihyperglycemic activity of dried leaves extract of C. tamala. Antioxidant property was calculated using 1,1-diphenyl-2-picrylhydrazyl test. In vitro antidiaibetic activity was determined by pancreatic α-amylase and α-glucosidase enzyme inhibition assay. Furthermore, antihyperglycemic activity was also evaluated by oral glucose tolerance test (OGTT) and alloxan-induced diabetic experiment in mice. The extract revealed a notable amount of total phenolic content (119.6 ± 2.1 mg gallic acid equivalent per gram, and total flavonoid content (520.2 ± 3.4 mg QE/g. The extract demonstrated a significant α-amylase and α-glucosidase inhibitory activity and the IC_{50} value was 71.37µg/mL and 54.20 µg/mL. The extract lowered the blood sugar level significantly ($p<0.05$) in comparison to untreated mice. Moreover, the extract significantly ($p<0.05$) controlled elevated Fasting Blood Sugar (FBS) in blood and prevented the diabetogenic activity of alloxan in treated animals. The extract expressed the potential antioxidant and antihyperglycemic activity, and justifies their usages in the health care system.

Keywords: *Cinnamomum tamala*, DPPH, alpha-amylase, alpha-glucosidase, OGTT, Alloxan

1. Introduction

Oxidative stress is developed by an increased level of reactive oxygen species (ROS) in the body, macromolecules of the cell mainly deoxyribonucleic acid, proteins, and lipids become misaligned. Numerous chronic degenerative disorders are further exacerbated by ROS (Styskal et. al., 2012; Finkel and Holbrook, 2000). The antioxidant molecules of the body play a significant role by inhibiting ROS formation. Cellular dysfunction caused by an imbalance between ROS formation and endogenous antioxidant activity contributes to the initiation and maintenance of inflammation and, in turn, to the pathophysiology of several crippling diseases, such as cancer, heart disease, hyperglycemia, or neurological problems (Johansen et al., 2005; Lugrin et al., 2013). Diabetes mellitus (DM), an important financial and health burden, is considered to cause high blood sugar levels via impairing insulin production, increasing insulin resistance, or both (Golder et al., 2020). Diabetes affects 6 % of people globally and a notable number of patients are increasing mainly in developing countries (Zimmet et al., 2001; Harris et al., 1987). Patients with diabetes have been found to have several comorbidities such as cardiomyopathy, angiopathy, nephropathy, etc. that may result in morbidity. Additionally, patients with persistent hyperglycemia have shown evidence of antioxidant mechanism suppression (Gavin, 1998). Lifestyle modifications, regular exercise, and a healthy diet are very important parameters for controlling DM. Antioxidant contributes a crucial role in protecting our biological process against damage induced by ROS. The frequent consumption of naturally occurring antioxidant containing foods or additional nutraceuticals may also be highly beneficial for managing or controlling the DM brought on by oxidative stress (Lobo et al., 2010). Various modern drugs are commercially available to treat chronic hyperglycemia but prolonged administration of these marketed drugs causes serious complications such as cardiac failure, anemia, and lactic acidosis as well as develops sudden hypoglycemic conditions in patients (Bailey & Krentz, 2010). It has been reported that plant-based traditional medicines have shown promising evidence to be safe and effective for the treatment of various ailments (Van Wyk & Prinsloo, 2020). Decoctions, freshly blended liquids made from medicinal plants, or herbal remedies are used in the primary healthcare systems of developing nations like Sri Lanka, India, North Africa, China, and Bangladesh to manage various illnesses including malignancy, diabetes, and arthritis (Chaudhury & Rafei, 2002). Due to a lack of resources and the limits of traditional medicine, plants have a long

history of being utilized as a primary source of medicine to cure a variety of diseases in underdeveloped nations. Based on traditional uses, scientists are continuously searching to find safe and naturally active antidiabetic agents for the management of diabetes.

Cinnamomum tamala (Buch. -Ham.) Nees & Eberm is an 8 m tall, evergreen tree that can be found in Bangladesh, India, Nepal, Sri Lanka, Australia, and other countries. It is a member of the Lauraceae family. Tejpata, the name for dried *C. tamala* leaves, is a ubiquitous ingredient in daily cooking. It has several historical uses, including the usage of the leaves as an astringent, stimulant, and carminative as well as a treatment for diarrhea and cardiac problems (Maharjan et al., 2021). Leaves were added as a preservative when making pineapple juice (Kapoor et al., 2008). The dried leaf and bark of *C. tamala* were reportedly used to treat fever, anemia, and body odor in the first century according to ancient literature (Chakraborty U, 2010). Some reports of *C. tamala* have been published that it has antioxidant (Prasad et al., 2009), antimicrobial (Parekh, J., & Chanda, S.V. 2007), anticancer (Shahwar et al., 2015), and anti-inflammatory activity (Dumbre et al., 2014). The dried leaves of *C. tamala* are used in cooking in addition to their conventional and regular usage. The present investigation focuses on the possible antioxidant and anti-hyperglycemic effects of *C. tamala* (CTLE) leaves extract.

2. Materials & Methods

2.1. Chemicals and reagents

Methanol, ethanol, n-hexane, ascorbic acid, hydrochloric acid, dimethyl ketone, chloroform, Gallic acid, quercetin, sodium carbonate, sodium dihydrogen phosphate, sodium trichloroacetate, sodium nitrite, aluminum chloride, sodium hydroxide, potassium ferricyanide, Ferric chloride, disodium hydrogen phosphate, iodine, 1,1-diphenyl-2-picrylhydrazine (DPPH) were collected from Mark, Germany. Alloxan, alpha-amylase, alpha-glucosidase, and Folin Ciocalteu reagent were purchased from Sigma Chemical Co. Ltd., St. Louis, MO, USA. Standard drugs (glibenclamide and acarbose) were obtained respectively from Incepta Pharmaceuticals Ltd., Bangladesh & Pacific Pharmaceuticals Ltd., Dhaka, Bangladesh.

2.2. Plant leaves and authentication

The *Cinnamomum tamala* leaves were collected in March 2020 from Narayangonj, Bangladesh. Dr. Mohammad Sayedur Rahman (Scientific Officer, Bangladesh National Herbarium) confirmed its identification and the specimens were deposited there for further reference (Voucher No.: 431022).

2.3. Extraction

Fresh leaves were kept in a dry, clean place with access to natural sunlight. After completing the drying of samples, it was ground to a coarse powder. To obtain the crude extract, cold extraction process was done. Initially, coarse powdered material (500 g) was taken and soaked with 1.5 ml of ethanol (90%) for 10 days at room temperature with irregular stirring and shaking. First, filtered the mixture with wodge of cotton and then filtered through Whatman filter paper to remove any residue. To get the crude extract, the ethanol was evaporated by using a water bath maintained at 60°C. *Cinnamomum tamala* leaves extract (CTLE) was used to conduct the present study.

2.4. Laboratory animals

Healthy, young female swiss mice (4-5 weeks, 20 to 25 g) were obtained from the Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh. The experimental mice were stored in the animal lab of R. P. Shaha University (RPSU) and maintained a specific temperature and Relative Humidity (25 ± 1°C, 56-60%) as well as 12:12 (light: dark) period along with proper supply of foods along with water. All animal studies were carried out on the regulations of animal ethical committee guidelines, RPSU Ethics Committee (Certificate Serial No.: RPSU/Registrar/ECR/Phr/2020/46)

2.5. Determination of secondary metabolites

2.5.1. Estimation of total phenolic content of CTLE:

The total phenolic content (TPC) in CTLE was measured by ultraviolet spectrophotometer (UV) by Folin-Ciocalteu (FC) chemical (Egharevba et al., 2019). To make calibration curve, several known concentrations of standard gallic acid were made (0.5–0.1 mg/mL). Solution (1 mL) from each concentration was combined with distilled water (9 mL) and 1 mL of 10% v/v FC reagent and waited for 5 min. Then, 10 mL of 7% sodium carbonate solution was taken to the test tube and the final volume was adjusted (25 mL) with distilled water. After incubation for 30 min, absorbance was read at 750 nm against blank. Gallic acid calibration curve was used to ascertain the TPC (Expressed as mg GAE/ g of dry extract) of CTLE.

2.5.2. Determination of total flavonoid content of CTLE:

The total flavonoid content (TFC) of CTLE was determined by using the aluminium chloride calorimetric method (Biswas et al., 2018). To conduct this experiment, 1 mL of sample was mixed with the solution of sodium nitrous (5% w/v, 0.3 mL), and distilled water (4 mL), allowing the mixer to react (5 min) at room temperature. After the incubation, aluminium chloride (0.3 mL, 10% w/v), and 2 mL of sodium hydroxide (1 M) were added to the test tube. The final volume of the test tube was adjusted to 10 mL. Blank was prepared and measured the absorbance at 510 nm against blank. Quercetin, a well-known flavonoid was considered as a standard. The TFC of CTLE was determined as mg QE/g of dry extract.

2.5.3. Estimation of total tannin content of CTLE:

The total tannin content (TTC) was estimated as described previously (Biswas et al., 2018). To estimate TTC, 10 mg CTLE was taken and dissolved with distilled water (10 mL) to make 1000 µg/mL. Several known concentrations of gallic acid were made for constructing the calibration curve. 7.5 mL distilled water, and Folin-ciocalteu's reagent (0.5 mL) were added to the ethanol extract (0.1 mL) followed by the addition of 1 mL solution of sodium carbonate (35%). After completing the reaction (0.5 H) at room temperature, the absorbance of the sample was recorded at 725 nm. The TTC of CTLE was expressed as gallic acid equivalent (mg per g of the extracted compound).

2.6. DPPH radical scavenging activity

The antioxidant property of CTLE was measured according to the following method (Kumar. et al., 2014). Various concentrations (1024 to 1 µg/mL) of the plant extract were obtained by serial dilution with methanol. 1 mL of sample was taken and mixed with newly made DPPH solution (3 mL, 0.004% w/v). Incubation of test tubes was done in a dark place (30 min). UV-Visible spectrophotometer (Shimadzu, Japan) was used to measure the absorbance at 517 nm.
% inhibition was determined using the following formula:

$$\%\ \text{inhibition} = \left(1 - \frac{\text{Sample or standard absorbance}}{\text{Blank absorbance}}\right) \times 100$$

2.7. Determination of *in vitro* antidiabetic assay

2.7.1. α-Amylase inhibitory assay:

Starch-iodine test was employed to determine the inhibitory activity of α-Amylase by CTLE (Xiao et al., 2006). To conduct this test, various concentrations (512 to 4 µg/mL) of the extract were made using PBS (0.02 M, pH 7.0) containing dimethyl sulfoxide (2%). From each concentration, 1 ml of sample, α-amylase solution (20 µL, 2 units/mL), and 1 mL of phosphate buffer were taken and allowed the mixture for 37°C for 10 min. Then, freshly prepared starch solution (200 µL) was added to test tubes and again incubated for 60 min. Next, 1% iodine solution (200 µL, 5 mM I_2 and 5 mM KI) was added to the mixture and additionally, 10 mL of distilled water was added to each tube. A color change was observed and absorbance was read using UV

spectrophotometry at 610 nm. The result was compared with the standard drug acarbose. A similar process was conducted to make blank solutions.

Pancreatic α-amylase inhibitory activity was determined by:

$$\% \text{ enzyme inhibition activity} = \frac{\text{Sample Abs} - \text{Blank Abs (Without enzyme)}}{\text{Blank Abs (Without enzyme)} - \text{Control Abs}} \times 100$$

2.7.2. α-Glucosidase inhibitory assay:

α-Glucosidase enzyme inhibitory activity of the extract was determined as previously described method (Liu et al., 2016). The extract was dissolved in 2% DMSO and also made in various concentrations serially. Briefly, the mixture was prepared with 112 µL PBS (pH 6.8), α-glucosidase solution (20 µL, 1 unit/mL), and 10 µL of the sample. Then, the mixture was kept at 37°C for 15 min and added to 20 µL of *p*NPG (2.5 mmol/L). Further, the reaction was done at 37 °C for 15 min and the reaction was stopped using 80 µL of Na_2CO_3 solution (0.2 mol/L). Absorbance was noted using UV spectrophotometry at 405 nm, and acarbose was considered as a reference sample. Blank and control solutions were made using 10 µL of DMSO instead of the sample.

Enzyme inhibitory activity was calculated by:

$$\% \text{ enzyme inhibition activity} = \frac{\text{ODsample} - \text{ODsample blank}}{\text{ODcontrol} - \text{ODblank}} \times 100$$

Here, ODsample presented as absorbance of PBS + Enzyme + Sample + *p*NPG; ODsample blank presented as absorbance of PBS + Sample + *p*NPG; ODcontrol presented as absorbance of PBS + Enzyme + *p*NPG; ODblank presented as absorbance of PBS + *p*NPG

2.8. Physiological and pathological observations

The physio-pathological examinations were carried out using albino mice to know the animal behavior and side effects of plant extract. Randomly, five groups were made containing 6 mice in each group. To conduct the experiment, animals were overnight fasted, and only supplied water. Different doses such as 0.5, 1.0, 2.0, and 4.0 g per kg body weight (b.w.) of CTLE were administrated orally to treated groups, while the control group mice were given 10% Dimethyl sulfoxide (DMSO) solution. After administration of the extract, mice were comprehensively observed for 2 days and thereafter for 14 days. Different behavior of animals like convulsion, writhing reflex, movement, tremor, salivation, and urination was observed; also unwanted extract-induced effects such as body weight loss and diarrhea were investigated throughout the experiment (Javed et al., 2020).

2.9. Determination of antidiabetic activity assay of CTLE

2.9.1. Oral glucose tolerance test (OGTT):

OGTT is done to identify how fast the cells eliminate exogenous glucose from the blood (Golder et al., 2020). Before initiating the experiment, the mice were stored at room temperature without supplying food for 10 h to 16 h. The experimental mice were made into four groups containing six mice in each i.e. control (Mice treated with 1% tween 80 in water at a dose of 10 mg/kg b.w.), positive control (Mice treated with glibenclamide at a dose of 5 mg/kg b.w.), and test groups (One group mice received CTLE of 250 mg/kg b.w., whereas other group received CTLE of 500 mg/kg b.w.). Here, glibenclamide was used as a standard drug to treat hyperglycemia. Briefly, the mice received the standard and extract solution and 30 minutes later, the mice were administered a glucose solution of 2 g/kg orally. To observe the blood sugar level, blood was collected from the tail vein using a sterile needle. The glucose levels of animals were estimated at time intervals of 0, 30, 60, 90, 120 and 150 minutes by a glucometer.

2.9.2 Alloxan induced antidiabetic test:

The mice were reserved overnight fasted only providing water. Before experimenting, the weight of the mice was noted. Then the mice were given freshly made alloxan (150 mg per kg body weight) intraperitoneally to develop diabetes. Alloxan solution was formulated in normal saline. The glucose solution was administered orally so that the hyperglycemic shock could be prevented in the experimental mice. Collection of blood was done by a sterile needle from the mice tail vein after 10 days of alloxan solution administration and using an electronic glucometer fasting blood sugar (FBS) was measured. The sugar level of mice higher than 10 mmol/L was considered for hyperglycemia and selected for this test. Finally, mice with diabetes were arranged into five groups containing six animals.

- Group 1 (Control): Mice received with normal saline orally.
- Group 2 (Diabetic control): Mice administrated alloxan solution orally.
- Group 3 (Positive control): Diabetic + Mice received the standard drug glibenclamide (5 mg/kg b.w.)
- Group 4 (Lower dose CTLE): Diabetic + CLTE low dose (250 mg/kg b.w.)
- Group 5 (Higher dose): Diabetic + CLTE high dose (500 mg/kg b.w.)

All these doses were given orally once per day for 31 days continuously. On the 10, 17, 24 and 31 days the blood glucose level of the mice was measured and recorded (Ahmed et al., 2010).

3. Statistical analysis

The results of the present studies are exhibited as mean ± standard deviation (SD) and statistical significance was done by one-way analysis of variance followed by Dunnett's test. The data were analyzed by using SPSS software (IBM Corporation, New York, USA). $P < 0.05$ was considered significant.

4. Results

4.1. Investigations of phytochemicals

The phytochemical evaluation showed CTLE contains alkaloids, flavonoids, tannins, glycoside, saponin, reducing sugar, and steroids (Table 1).

Table 1: Investigations of phytochemicals of CTLE

Phytochemicals	**Result**
Reducing sugar	+
Flavonoids	+
Tannins	+
Gums	-
Alkaloid	+
Saponin	+
Acidic compound	+
Phenolic compounds	+
Terpenoid	-
Steroid	+
Glycoside	+

+ indicates presence; - indicates absent

4.2. Assessment of plant secondary metabolites

TPC of CTLE was measured by a linear regression equation, y = 1.914x + 0.0488, R^2 = 0.9945 attained from the calibration curve of gallic acid and the value was found to be 119.6 ± 2.1 mg GAE/g of dry extract (Fig. 1A and D). TFC was measured by linear regression equation, y = 0.8115x −0.0847, R^2 = 0.9964, and the value was found to be 520.2 ± 3.4 mg QE/g of extract (Fig. 1B and D). Regarding the total tannin content (TTC) calculation, a formula obtained from the gallic acid calibration curve was used. The total tannin content of CTLE was found to be 16.5 ± 2.0 mg GAE/g of dry mass (Fig. 1C and D).

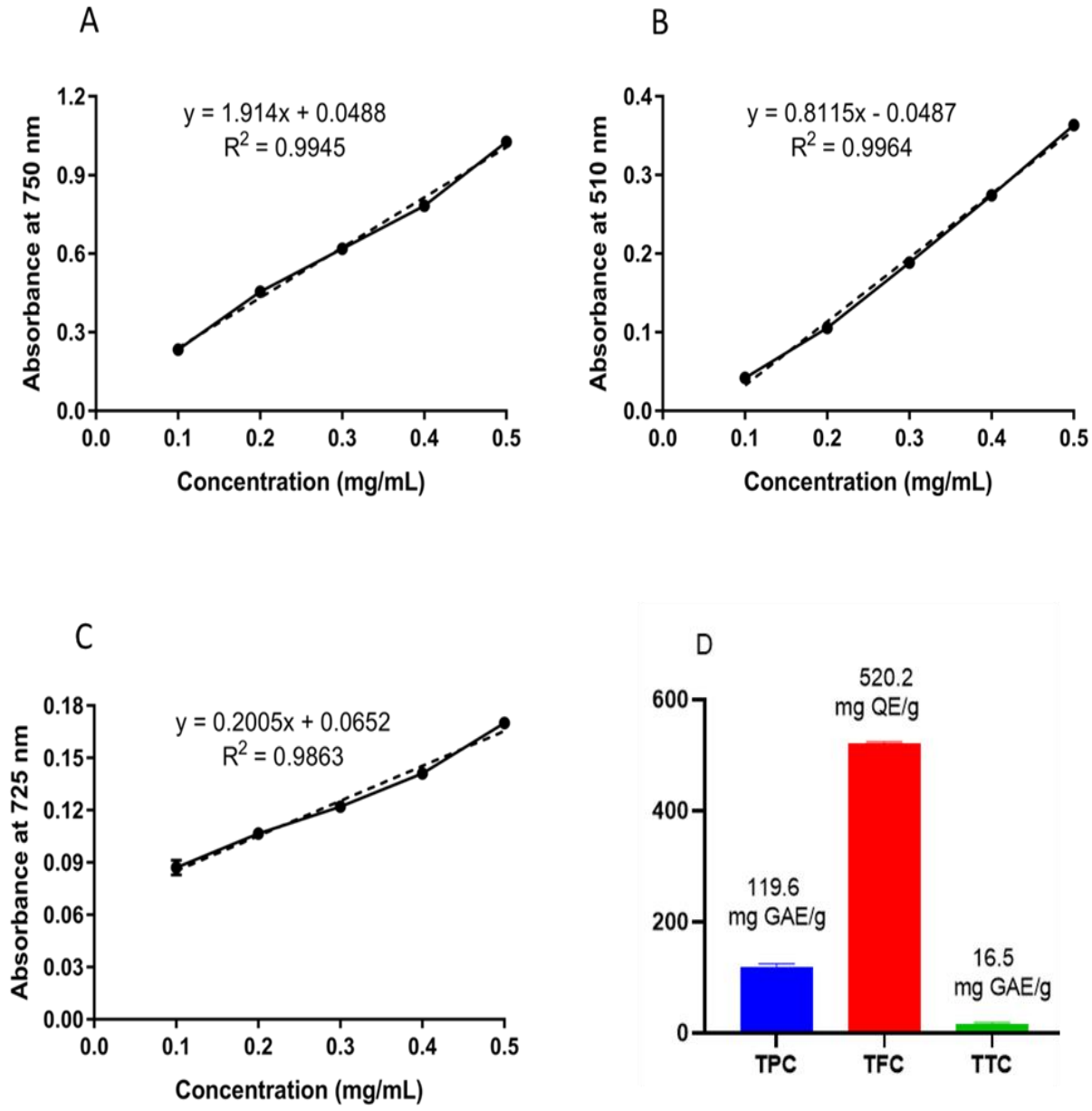

Figure 1: Investigation of plant secondary metabolites of CTLE. (A) Calibration curve of gallic acid for the estimation of TPC, (B) Calibration curve of quercetin for determination of TFC, (C) Calibration curve of standard gallic acid for the measurement of TTC, (D) Amount of secondary metabolites per gram of dry mass.

4.3. Evaluation of antioxidant activity

The antioxidant activity of CTLE was evaluated using the DPPH radical scavenging test. The IC_{50} value of CTLE was 20.41 µg/mL, while the IC_{50} value of reference ascorbic acid was 12.02 µg/mL (Fig. 2).

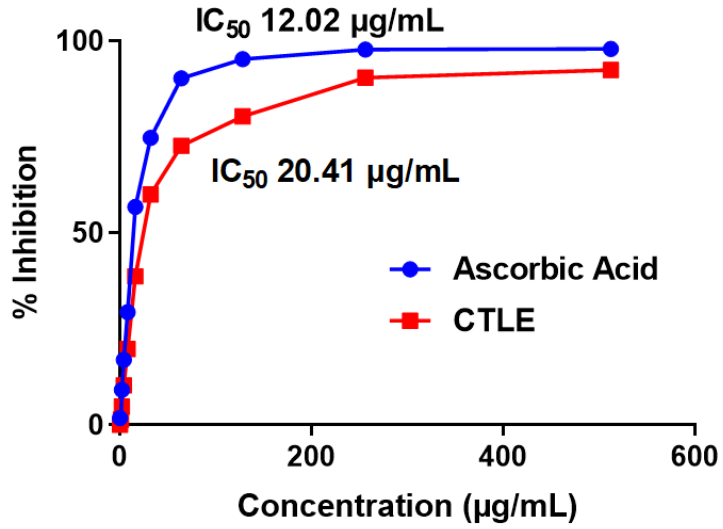

Figure 2: Determination of IC_{50} value of *Cinnamomum tamala* leaves extract (CTLE) and standard ascorbic acid in DPPH scavenging assay.

4.4. Evaluation of *in vitro* antidiabetic activity

The antidiabetic capacity of the plant extract was carried out using alpha-amylase and alpha-glucosidase inhibitory activity tests. Inhibition of alpha-amylase enzyme is a useful technique to know how quickly decrease the post-meal blood glucose level. Standard drug acarbose was considered to compare the result of alpha-amylase inhibitory activity by the extract. It was detected that the enzyme inhibition activity by the extract was concentration-dependent and the IC_{50} value of the plant extract was 70.37 µg/mL, while the IC_{50} value of acarbose was 14.53 µg/mL (Figure: 3). The alpha-glucosidase inhibitory activity was also measured. In this test, CTLE extract revealed the IC_{50} value of 54.20 µg/mL whereas the IC_{50} value of acarbose was shown 6.70 µg/mL (Figure: 3)

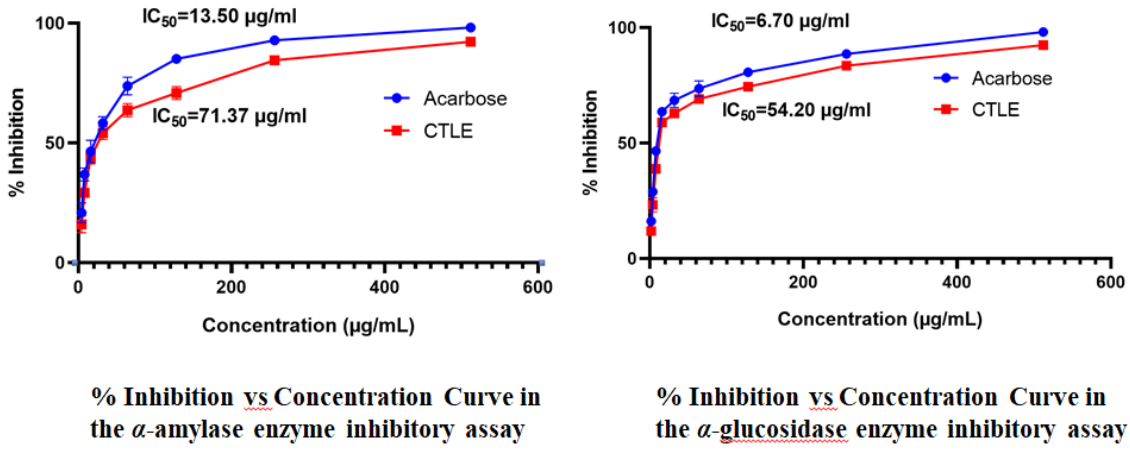

Figure 3: *In vitro* pancreatic enzyme α-amylase and α-glucosidase inhibition activity by CTLE.

4.5. Physiological and pathological observations

To evaluate the physio-pathological effects of CTLE in mice, experimental animals were given different doses of extract (0.5, 1.0, 2.0, and 4.0 g/kg b.w.). All treated mice showed normal behavior, also toxicity signs like changes in eyes, skin color and fur were remaining unchanged throughout the experiments in comparison to untreated and no mortality was noticed. Result expressed that the safe dose of CTLE was up to 4.0 g per kg body weight.

4.6. Determination of hypoglycemic activity

4.6.1. Evaluation of OGTT in mice:

The hypoglycemic activity of the extract was determined by oral glucose tolerance test. OGTT test determines the glucose tolerance, function of pancreatic *β*-cells, and insulin resistance. After administration of glucose, all mice's blood glucose level was elevated compared to fasting blood sugar level at 0 min (range of blood glucose level at 0 and 30 min: 5-5.30 and 17-18 mmol/L). CTLE exhibited a significant ($P < 0.05$) decrease in glucose level in blood in comparison to only glucose-treated mice (Fig. 4). Following the treatment of extracts, a marked decrease in blood glucose level was noticed at 90 min and the effect was unchanged till the end of the experiment (150 min). Extract at higher doses lowered 1.5-fold blood glucose level at 150 min (Control vs 500 mg/kg: 11.01 vs 6.56 mmol/L) in comparison to untreated animals (Fig. 4). At 250 mg/kg, it was observed that a marked reduction of blood glucose level on 90, 120, and 150 min following treatment compared to glucose-induced hyperglycemic mice (Fig. 4). Standard drug at a dose of 5 mg/kg body weight reduced the blood glucose remarkably at different time intervals compared to control groups (Fig. 4). Result indicated that glucose-induced high blood glucose level was well controlled by plant extract.

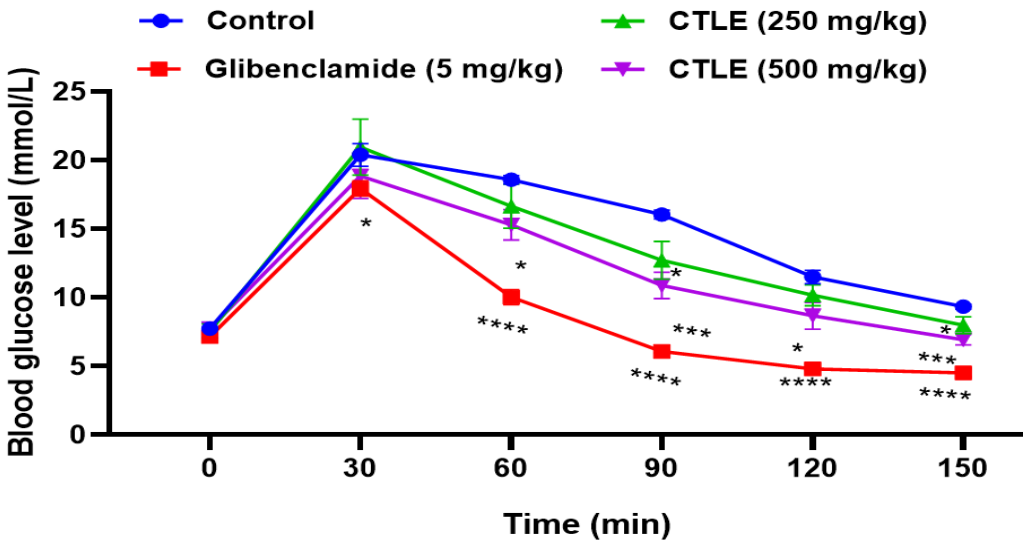

Figure 4: Observation of blood glucose level (mmol/L) by CTLE and glibenclamide in animals. Results are expressed as mean ± SEM; each group was 6 mice. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

4.6.2. Alloxan-induced anti-diabetic test

The *in vivo* antidiabetic activity by CTLE is presented in table 2. Animals that received alloxan solution showed a 3-fold greater fasting sugar level that was retained more than 3 weeks compared to untreated mice, clearly indicating a diabetic animal model by alloxan induction. Results indicated that administration of CTLE continuously down-regulated the increased blood glucose level on day 24th and 31st day compared to diabetic mice (DM). CTLE at a dose of 250 mg per kg lowered the sugar level significantly (On day 31: the sugar level in DM and CTLE at 250 mg per kg was 18.53 ± 3.42 mmol/Land 10.21 ± 3.45 mmol/L). The higher dose of CTLE (500 mg/kg) showed notable blood sugar levels falling from 14.98 ± 2.05 mmol/L to 7.82 ± 2.75 mmol/L from day 10 to day 31, respectively. Extract at 500 mg/kg showed more than 2-fold lowered blood sugar level at day 31 (sugar level of DM and CTLE at 500 mg/kg was 18.53 ± 3.42 mmol/L and 7.82 ± 2.75 mmol/L). Reference drug glibenclamide demonstrated a significant ($P < 0.001$) decrease in blood glucose level compared to the DM group (sugar level from 15.23 ± 2.41 mmol/L to 7.69 ± 1.16 mmol/L on day 10 to 31).

Table 2: Determination of CTLE activity on FBS in the diabetic model.

Groups	Day			
	10	17	24	31
Control	4.62 ± 0.88	4.97 ± 2.04	4.92 ± 2.20	4.63 ± 2.36
Diabetes mice (DM)	14.39 ± 1.59 *	18. 05 ± 2.33 *	18.35 ± 2.36 *■▲	18.53 ± 3.42 *■▲
Standard (5 mg/kg)	15.23 ± 2.41 *	14.06 ± 1.50 *	10.08 ± 3.46 θ	7.69 ± 1.16 θ
CTLE (250 mg/kg)	14.51 ± 1.75 *	14.21 ± 1.75 *	12.01 ± 3.18 *θ	10.21 ± 3.45 θ
CTLE (500 mg/kg)	14.98 ± 2.05 *	14.16 ± 1.06 *	10.56 ± 2.78 *θ	7.82 ± 2.75 θ

Results are expressed as mean ± SD; * $p < 0.05$ vs. Control (Dunnett's test); θ $p < 0.05$ vs. Standard; ■ $p <$ CTLE at 250 mg/kg; ▲ $p < 0.05$ vs CTLE at 500 mg/kg; the pair-wise comparison was done using Post-hoc Tukey test.

5. Discussion

Phytochemicals investigation of CTLE confirmed the presence of flavonoids, tannins, glycoside, saponin, reducing sugar, and steroids while gums and terpenoids were absent as shown in table 1. Numerous medications are currently on the market to treat both diabetes and oxidative stress, but patients have reported serious adverse effects including lactic acidosis, anemia, heart failure, and hypoglycemia (Bailey & Krentz, 2010). Regular consumption of naturally occurring antioxidant-rich foods and dietary supplements helps to reduce the production of free radicals as well as reactive oxygen species (ROS), which helps to prevent several classes of diseases including diabetes (Lobo et al., 2010). Different portions of *Cinnamomum tamala* have historically been employed as stimulants, carminatives, and anti-diabetics as primary treatments for a variety of illnesses in many different nations (Grover et al., 2002; Sharma & Rao, 2013). In addition, *C. tamala* dried leaves are a typical cooking component in Bangladesh. Because of this, we addressed in this evaluation the possible impact of CTLE on antioxidant and anti-diabetic properties.

In this study, we evaluated the secondary metabolites, which are frequently found in plant species and are essential for avoiding the development of free radicals. The TPC of CTLE was found to be 119.6 ± 2.1 mg gallic acid equilibrium per gram dry mass. TFC and TTC were found to be 520.2 ± 3.4 mg QE/g and 16.5 ± 2.0 mg gallic acid equilibrium per gram solid mass (Fig. 1). Polyphenols are common secondary metabolites that have antioxidant activities (Razali et al., 2008). Ingestion of antioxidant-containing foods in daily diet can contribute to boost our immune systems which enhance cellular protection from free radicals-induced effects (Lobo et al., 2010, Talukder et al., 2012). Phytochemical analysis exhibited that CTLE contains phenolics, saponins, flavonoids, tannins, and alkaloids. As shown in fig. 1 extract contains a considerable extent of phenolics, flavonoids but reasonable levels of tannins are available. Our data expresses the dried leaves of *Cinnamomum tamala* is a source of high antioxidants.

The extract expressed a remarkable DPPH scavenging activity as shown in figure 2 and the IC_{50} value of CTLE was 20.41 µg/mL, but the standard (ascorbic acid) was 12.02 µg/mL. DPPH is commonly recognized as a free radical. The reaction with antioxidant compounds and stable DPPH solution turns it from deep violet to light yellow color ((Biswas et al., 2018). The extract demonstrated a significant quantity of free radical scavenging activity in the DPPH experiment, highlighting its crucial antioxidant potential.

To conduct the animal experiment, it is important to determine the toxicity after the administration of plant extract. Results showed that after receiving only one dose of CTLE to experimental animals caused no mortality. No toxicity signs were observed in animals throughout the experiment. Our data showed that the extract is safe up to 4.0 g/kg and the oral lethal dose (LD50) was considered more than 4.0 g/kg.

It is extremely difficult to treat diabetes using the available oral antidiabetic medications without experiencing adverse effects, which might include hypoglycemia and decreased efficacy after prolonged use. The World Health Organization (WHO) has advocated a continuous search for traditional therapies to properly treat this metabolic disease. To control or treat postprandial hyperglycemia, it may be crucial to look for novel natural alpha-amylase and alpha-glucosidase from plant species. Some therapeutic strategies delay the digestion of absorbable monosaccharides by intestinal pancreatic alpha-glucosidase and alpha-amylase, which results in a decrease in postprandial hyperglycemia (Aloulou et al., 2012). In this current study, the CTLE and reference drug acarbose showed a remarkable α-amylase inhibitory effect in the starch-iodine assay. Figure 3 exemplifies that the extract IC_{50} value was 71.37 µg/mL while the standard was 13.50 µg/mL. Furthermore, ethanol leaves extract showed a considerable α-glucosidase inhibition as shown in figure 3. Previously reported that secondary metabolites such as phenolics, quercetin, rutin, catechin, and flavonoids are important phytochemicals that act as α-amylase and α-glucosidase inhibitory activity (Kim et al., 2000) by interaction with proteins that leads to the complex formation and changing conformation (Tadera et al., 2006, McDougall et al., 2008). The observed pancreatic α-amylase and α-glucosidase enzyme inhibition activity by CTLE extract because it mainly contains various compounds like phenolics, flavonoids, etc. as compared to the pure inhibitor of acarbose drug.

Diabetes is a metabolic condition defined by elevated blood sugar levels brought on by insufficient beta cell release and activity of the hormone insulin. We performed both an alloxan-induced antidiabetic test and an *in vivo* oral glucose tolerance test to examine the antihyperglycemic effect of CTLE. Results from the OGTT test showed that the extract significantly decreased blood glucose levels ($p < 0.05$) compared to the control at 60, 90, 120, and 150 minutes (Figure 4). When compared to the control group, CTLE (500 mg/kg) showed significant antidiabetic activity at 90 minutes and significantly decreased blood sugar levels at 120 and 150 minutes. Blood glucose levels were significantly ($p < 0.05$) declined by the common medication, glibenclamide. The antidiabetic activity of CLTE on the alloxan-induced diabetic model is shown in table 2. Our data demonstrated that blood glucose level was elevated more than 3 times after administration of alloxan compared to untreated mice which clearly indicates alloxan induced diabetic model. After administration of samples in experimental mice, it was observed that blood glucose levels reduced continuously on days 24 and 31 compared to diabetic mice. As shown in table 2, CTLE at a dose of 250 mg per kg significantly ($P < 0.001$) lowered glucose in the blood (Sugar level of DM and CTLE at 250 mg/kg was 17.53 ± 2.42 mmol/Land 9.21 ± 2.45 mmol/L on day 31). The higher dose of CTLE (500 mg/kg) showed notable blood sugar levels falling from 14.98 ± 2.05 mmol/L to 7.82 ± 2.75 mmol/L from day 10 to day 31, respectively. On day 31, extract (500 mg/kg) lowered fasting sugar level more than 2-fold compared to DM.

Alloxan, a compound is commonly employed in experiments to develop diabetes in mice. Hyperglycemia is developed in mice after injection of alloxan due to its ability to form free radical that leads to damage of pancreatic β-cells of mice, ultimately low secretion of insulin (Szkudelski, 2001). It was observed that blood glucose level was changed in treated mice by CTLE, clearly indicating the inhibitory effect of diabetogenic activity by alloxan. Extract revealed notable antidiabetic effects throughout the experiment. Our result confirmed that CTLE can lower the high sugar level. Our data demonstrated extract can increase the function of β-cells or glucose consumption in mice. This result may be due to the existence of antioxidant constituents in the extract that prevents the free radical occurrence. It has been reported that phenolics, flavonoids, and tannins are responsible for antidiabetic activity (Olmedilla et al., 1997). Based on recent investigations, alloxan-induced diabetic experimental animals' pancreatic β-cell production may be increased by flavonoids (Kumari et al., 2012). Additionally, it is asserted that phenolics can decrease oxidative stress, which raises insulin sensitivity, and protect cells from alloxan-induced damage (Choi et al., 2011; Chakravarthy et al., 1980). Thus, the noticeable antihyperglycemic property of *C. tamala* leaves is recognized by the suppression of pancreatic α-amylase and α-glucosidase action as well as the prevention of oxidative stress.

6. Conclusion

Our data concluded that leaves extract of *C. tamala* reveals a potent antioxidant property along with antihyperglycemic activity through the inhibition of pancreatic alpha-amylase and alpha-glucosidase enzyme activity which justifies its contribution to the health care systems. Thus, it is suggested that future investigations are required to isolate and characterize the bioactive components which are accountable for contributing to the antidiabetic activity, also needed to its kinetic study of CTLE should find out for actual mechanism for antidiabetic activity.

Conflict of interest

No conflict of interest

Authors' declaration

We announce that this research paper is an original work. We will be held liable for any claims made over its content.

Author's contributions

The study was designed by AKB. Data interpretation, drafting, and revision were done by AKB, MIS, and RNA. AH, TS, SR managed the experiments and organized the data analysis.

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