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Original Article

Antioxidant and Antidiabetic Activities of Methanolic extract of Bark of *Cinnamomum zeylanicum* in Diabetic Rats

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ABSTRACT

Background: *Cinnamomum zeylanicum* or Ceylon cinnamon is used for the management of dyspepsia, dysmenorrhea, memory loss and tremor. It is also traditionally recommended for the management of diabetes. Further studies are required to explore the antioxidant and antidiabetic activity of various extracts of bark of *C. zeylanicum*. Hence, the present study is planned to investigate the antioxidant and antidiabetic activities of methanolic extract of barks of *C. zeylanicum*. **Methods:** Bark of *C. zeylanicum* was extracted with methanol, ethanol and acetone and its antioxidant activity was studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging assays. Acute toxic effect of methanolic extract of *C. zeylanicum* (MECZ) studied as per the method described in OECD Guideline for testing of chemicals (Test Guideline 423). MECZ was studied for its antidiabetic effect using streptozotocin-induced diabetic rats. **Results:** In both DPPH and ABTS free radical scavenging assay, methanolic and acetone extracts exhibited free radical scavenging activity, respectively. In acute toxicity testing, MECZ did not show any significant toxic signs up to 2000 mg/kg, hence the antidiabetic activity of MECZ was carried out at the dose levels of 125, 250 and 500 mg/kg. MECZ showed antidiabetic activity from 2nd week of the experiment onward. In the biochemical analysis, MECZ treated animals showed significant decreases in the levels of ALP and urea when compared with control. In lipid profile analysis, diabetic animals and diabetic animals treated glibenclamide showed significant increases in the levels of total cholesterol when compared with normal control and MECZ prevented the STZ-induced hyperlipidemia. At the end of the study, diabetic animals and diabetic animals treated with glibenclamide and MECZ showed significant decreases in the level of insulin when compared with the control group. In the histopathological analysis, sections from the liver, pancreas and kidney of the diabetic animals showed mid-to-moderate toxic effects and glibenclamide and MECZ 500 mg/kg prevented the STZ-induced cellular changes. **Conclusion:** The MECZ exhibited significant antioxidant and antidiabetic activities.

Key words: Diabetes mellitus, Free radicals, Insulin, Streptozotocin.

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INTRODUCTION

Plants and isolated phytochemicals are generally utilized for the counteractive action and treatment of different wellbeing infirmities from time immemorial interest for homegrown medications, natural wellbeing items, nutraceutical, sustenance supplement, homegrown pharmaceuticals and beauty care products are expanding all-inclusive.[1] There are approximately 5-15 % of the total species of higher plants which have been investigated intensively and these plants are identified as a good source of bioactive compounds.[2] Plant secondary metabolites such as taxol, lectinan, morphine and reserpine are good therapeutic agents and are incorporated in modern medicine.[3] *Cinnamomum zeylanicum* is one of the well-known plant species for its medicinal properties. It is native to Sri Lanka and is a small evergreen tree belonging to the Lauraceae family and was used in Asia long before it became known in Europe.[4] This family is well known for its aroma and thick bark and has over 2000 species of trees and shrubs.[5] The Lauraceae family is economically important as it is used extensively in the treatment of various diseases due to its active biological secondary metabolites such as terpenes, flavanoids, polyphenols and alkaloids that have significant antidiabetic, anti-inflammatory, antibacterial, antiviral and antifungal activities.[6,7] The health effects benefited from *C. zeylanicum* have been demonstrated *in vivo* and *in vitro* experiments. The leaf and the bark of the cinnamon species have higher commercial value compared to the root which has camphor as its main constituent.[4] Trans-cinnamaldehyde, eugenol and linalool are the main components of the *C. zeylanicum* bark.[8] Mesripour *et al.* demonstrated the antidiabetic effect of aqueous extract *C. zeylanicum* bark in alloxan-induced diabetic mice and reported

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that the aqueous extract *C. zeylanicum* has ability to reduce the blood glucose levels and also improves memory performance.[9] The further studies are required to explore the antioxidant and antidiabetic activity of various extracts of bark of *C. zeylanicum*. Hence the present study is planned to investigate the antioxidant and antidiabetic activities of bark of methanolic extract of bark of *C. zeylanicum*.

MATERIALS AND METHODS

Collection and identification of barks of *C. zeylanicum*

The cinnamon barks were obtained from Penang Spice Garden, Pulau Pinang and the authenticity of *C. zeylanicum* species was confirmed by Dr. Deivanai Subramanian, Faculty of Applied Sciences, AIMST University.

Extraction of barks of *C. zeylanicum*

C. zeylanicum bark was cleaned thoroughly and placed in an oven to be dried. The dried barks were grounded to the powder and placed in an oven at temperature 35°C for overnight to ensure maximum moisture was lost. The powered barks of *C. zeylanicum* was extracted with a hot percolation method. The powdered barks of *C. zeylanicum* was packed in a thimble and placed in a Soxhlet apparatus and extracted using methanol, ethanol and acetone. The extraction was carried out for 24 h at about 55°C-80°C; the extract was filtered through a muslin cloth. The filtrate was concentrated to a dry mass by evaporation under reduced pressure. The extracts of *C. zeylanicum* were stored in a desiccator at room temperature until further analysis. The percentage yield of methanolic, ethanolic and acetone extract of *C. zeylanicum* was found to be ≈21, ≈19 and ≈12% w/v, respectively.

Phytochemical screening

Qualitative phytochemical analysis of methanol, ethanol and acetone extracts of *C. zeylanicum* were carried out to test for the presence of constituents such as alkaloids, flavonoids, terpenoids, saponins, tannins and glycoside. Total flavonoid content and total phenolic content of methanolic, ethanolic and acetone extracts of *C. zeylanicum* determined by the method described by elsewhere.[10-12] Total flavonoid content and total phenolic content of various extracts of *C. zeylanicum* compared with gallic acid and quercetin, respectively. Gallic acid and quercetin were used as standards and the results were expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE). All tests were done in triplicates.

Antioxidant activity

Antioxidant activity of extracts of *C. zeylanicum* were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging methods using the method described elsewhere.[13,14]

DPPH free radical scavenging assay: A stock solution of 0.1 mM concentration DPPH was freshly prepared in methanol and kept in the dark at 4°C. A 2 ml of different concentrations (50, 200 ,400 ,600 ,800 and 1000 µg/ml) of plant extracts were added to 1 ml of DPPH stock solution and incubated at room temperature (23-27°C) in the dark for approximately 20 min. Later, the absorbance was recorded against a blank at 517 nm using an ultraviolet-visible (UV–vis) spectrophotometer (Model UV 1800, Shimadzu, Japan). All analysis was performed in triplicate. The DPPH free radical scavenging properties of extracts of *C. zeylanicum* was compared with ascorbic acid. Inhibition of DPPH radical in term of percentage (%) was calculated using the formula [(Ac-As)/Ac X 100; Ac = Absorbance of control and As = Absorbance of sample].

ABTS free radical scavenging activity: ABTS (7 mM) was mixed with 2.45 mM potassium persulfate to obtain the stock solution of ABTS. Before proceeding with the test, the mixture was left in the dark for 12 hr. Before ABTS was added to the sample, the samples had to be diluted to obtain an absorbance of 0.7 at 734 nm. The extracts were then dissolved in absolute ethanol with the concentrations of 100, 300, 500, 700, 900 and 1000 µg/ml. . Diluted ABTS (0.5 ml) solution was added to the 0.5 ml of sample and left for 8 min before the absorbance was taken again at 734 nm. All analysis was performed in triplicate. The ABTS free radical scavenging properties of extracts of *C. zeylanicum* was compared with trolox. Inhibition of ABTS radical in term of percentage (%) was calculated using the formula [(Ac-As)/Ac X 100; Ac = Absorbance of control and As = Absorbance of sample].

Antidiabetic activity

Animals: Healthy, adult, either gender of *Sprague-Dawley* (SD) rats (180 ± 15 g body weight [BW]) were obtained from Shafazz Enterprise, Malaysia. The animals were housed in large, spacious, polyacrylic cages at ambient room temperature with 12 h light/12 h dark cycle. Rats had free access to water and rodent pellets diet. The study was approved by the Human and Animal Ethics Committee of AIMST University and the study was conducted according to the Animal Research Review Panel Guidelines.

Acute toxicity testing: The healthy, adult, female SD rats were used for acute toxicity testing. The study was carried out according to the guidelines that were set by the Organization for Economic Co-operation and Development (OECD), Test Guideline 423. The rats (3 animals/dose) were overnight and orally fed with methanolic, ethanolic and acetone extracts of barks of *C. zeylanicum* in increasing dose of 5, 50, 300 and 2000 mg/kg BW. The rats were closely monitored for changes in their neurological, behavioral and autonomic profiles for 24 hr upon administration of doses. The animals were observed (at least two times a day) for a period of 14 days for any for toxicity signs.[15]

Antidiabetic activity: In acute toxicity testing methanolic extract of barks of *C. zeylanicum* (MECZ) did not show any toxic sings. Hence MECZ was used for antidiabetic screening. Healthy, adult, male SD rats were used in this study. Diabetes mellitus was induced in overnight-fasted rats by administration of intraperitoneal injection of freshly prepared 55 mg/kg streptozotocin (STZ) in 0.1 M citrate buffer (pH 4.5). After 24 hr of STZ administration, the rats were given with glucose solution (2 ml/kg BW) to avoid hypoglycemic mortality.[16] After 48 hr, the blood sample was collected from the tail vein and blood glucose was measured to confirm diabetes mellitus. Rats with fasting blood glucose of >11 mmol/L were considered to have diabetes and they were used for this experiment. These diabetic animals were randomly divided into five groups (Group II – VI) as follows ($n = 6$).

- Group I: Normal control
- Group II: Diabetic control
- Group III: Diabetic animals treated with glibenclamide (20 mg/kg)
- Group IV: Diabetic animals treated with MECZ (125 mg/kg)
- Group V: Diabetic animals treated with MECZ (250 mg/kg)
- Group VI: Diabetic animals treated with MECZ (500 mg/kg)

The animals in group I and group II were administered with 0.5 % w/v carboxymethyl cellulose (CMC). Animals in group III were treated with 20 mg/kg BW of glibenclamide and animals in group IV – VI was treated with MECZ at dose levels of 125, 250 and 500 mg/kg BW, respectively. The dose of MECZ was derived from toxicological study. The standard and investigational drugs were suspended in 0.5% w/v CMC and administered once daily through oral gavage for 21 consecutive days. Few drops of venous blood was collected from the tail vein on the

2nd, 7th, 14th and 21st day of the experiment and immediately utilized for the estimation of blood glucose (whole blood) using an Blood Glucose Meters (Accu-Chek© Active glucose meter, Roche Diagnostics). Throughout the study, body weight changes were monitored at regular intervals. At the end of the study, blood sample was withdrawn from all the experimental animals through retro-orbital plexus puncture and the serum was separated and utilized for biochemical analysis.[17] Later, the rats were sacrificed and organs such as liver, pancreas, and kidney were collected for histopathological analysis and they were preserved in 10% v/v buffered neutral formalin.

Biochemical analysis

During the experiment, few drops of venous blood was collected from the tail vein and used for the estimation of blood glucose levels using Blood Glucose Meters. At the end of the study, a few milliliters of the blood sample were collected in a plain glass tube through retro-orbital plexus and the serum separated by centrifuging at 3000 RPM for 20 min. The serum sample was utilized for estimation of biochemical markers such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP), creatinine, urea, total protein, albumin, sodium, chloride and, potassium and lipid profile such as total cholesterol, triglyceride and high-density lipoprotein (HDL) cholesterol using performed using automated biochemistry analyzer (Olympus 640 Biochemistry Analyzer, Tokyo, Japan) at Gribbles Pathology Sdn. Bhd. (Sungai Petani, Kedah, Malaysia). The low-density lipoprotein (LDL) level, Very low-density lipoprotein (VLDL) and TC/HDL ratio were calculated mathematically.[17] Plasma insulin is Insulin is measured by ELISA method at Gribbles Pathology Sdn. Bhd. (Sungai Petani, Kedah, Malaysia).

Organ weight analysis

At the end of the study, all the experimental animals were sacrificed under mild ether anesthesia followed by cervical dislocation. The animal was dissected and the gross pathology was observed. The organs such as liver and kidney were harvested and absolute organ weights were measure and relative organ weight was calculated.

Histopathological analysis

Liver, pancreas, and kidney samples were excised from animals from group I, II, III & VI, and preserved in 10% v/v buffered neutral formalin. The liver, pancreas, and kidney sample were embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Liver, pancreas, and kidney sections of thickness 5 mm were prepared from paraffin blocks, stained with hematoxylin and eosin (H & E) and mounted in neutral DPX medium. The sections were examined under a light microscope.

Statistical analysis

The mean ± standard error of the mean (SEM) values was calculated for each group. Statistical analysis was carried out using One-way ANOVA followed by Tukey's *post hoc* test. $P < 0.05$ was considered to be significant.

RESULTS

Phytochemical test

Phytochemical analysis of methanolic, ethanolic and acetone extracts of *C. zeylanicum* were showed presence of glycosides, flavonoids, alkaloids, saponins, tannins and terpenoids.

Ethanolic extract of *C. zeylanicum* exhibits more phenolic content (551.67 mg GAE/g) followed by acetone extract (517 mg GAE/g) and methanolic extract (439.33 mg GAE/g) of *C. zeylanicum*. In total flavonoid content analysis, acetone extract of *C. zeylanicum* recorded the highest flavonoid content (634.2 mg QE/g) which was followed by methanolic (142.5 mg QE/g) and ethanolic extract (117.5 mg QE/g) of *C. zeylanicum*.

Antioxidant activity

In DPPH free radical scavenging assay, MECZ exhibited the highest scavenging activity when compared against other extracts, recording activity of 94.97 ± 0.25% at concentrations of 1000 µg/ ml and the results were comparable with the same concentration of ascorbic acid (95.92 ± 0.29%). The acetone and ethanolic extracts of *C. zeylanicum* had the 92.32 ± 0.69% and 87.33 ± 0.42% scavenging activity at concentration of 1000 µg/ml, respectively. In ABTS free radical scavenging assay, acetone extract of *C. zeylanicum* exhibited the highest scavenging activity (94.55 ± 0.91%) at concentrations of 1000 µg/ ml and the same concentration of Trolox exhibited 64.45±0.43% scavenging activity. The methanolic and ethanolic extracts of *C. zeylanicum* had the 93.14 ± 0.40% and 75.56 ± 0.96% scavenging activity at a concentration of 1000 µg/ml, respectively. Since the MECZ exhibited the highest scavenging activity in DPPH method and relatively more free scavenging activity in the ABTS method, the further antidiabetic study was carried out with MECZ.

Acute toxicity testing

In acute toxicity testing, methanolic and ethanolic extracts of *C. zeylanicum* did not show any significant toxic effects up to 2000 mg/kg. The animals treated with acetone extract of *C. zeylanicum* at 2000 mg/kg showed mortality (one of three animals) on 14th day of the experiment.

Antidiabetic activity

Effect of MECZ on body weight: Throughout the study, the diabetic animals showed a reduction in body weight when compared with control. But the results were not significant. The animals treated with glibenclamide or MECZ at the dose levels of 125, 250 and 500 mg/kg did not showed any significant changes in body weight when compared with control (Figure 1).

Effect of MECZ on blood glucose: Throughout the study, diabetic control animals showed a significant increase in blood glucose levels ($P<0.001$), when compared with normal control. Whereas the diabetic animals treated with glibenclamide or MECZ at the dose levels of 250 mg/kg showed significant antidiabetic effect from day 7 onwards when compared with diabetic control. The animals treated with MECZ at 125 and 500 mg/kg treated animals showed antidiabetic activity from day 14 onwards, when compared with diabetic control (Table 1).

Effect of MECZ on biochemical parameters: Effect of MECZ on biochemical parameters were summarized in Table 2. The animals treated with glibenclamide or MECZ did not show any significant changes

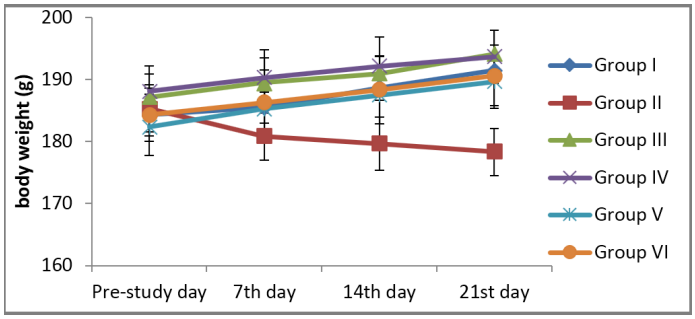

Figure 1: Effects of methanolic extract of barks *C. zeylanicum* on body weight of SD rats. Values are expressed as mean ± SEM ($n = 6$).

in the levels of SGOT, SGPT, total protein and total bilirubin when compared with control. The animals treated with glibenclamide showed a significant increase in the levels of urea and MECZ showed significant decreases in the levels of ALP (at 250 and 500 mg/kg dose levels) and urea (at 250 mg/kg) when compared with control. The animals treated with MECZ showed significant decreases in the levels of ALP and urea when compared with control, but the values are within normal limits.

Table 1: Effects of methanolic extract of barks *C. zeylanicum* on blood glucose levels of SD rats.

Group	Blood glucose (mmol/L)			
	0th day	7th day	14th day	21st day
Group I	5.80 ± 0.27	6.07 ± 0.27	5.78 ± 0.37	5.87 ± 0.41
Group II	11.53 ± 0.52***	12.37 ± 0.62***	13.07 ± 0.70***	12.93 ± 0.53***
Group III	11.35 ± 0.77***	9.67 ± 0.97*	7.25 ± 0.72###	6.62 ± 0.48###
Group IV	12.27 ± 0.72***	10.15 ± 0.66**	9.08 ± 1.04*##	8.62 ± 0.55*###
Group V	11.93 ± 0.92***	10.57 ± 0.52***##	7.93 ± 0.75###	7.53 ± 0.61###
Group VI	11.97 ± 0.81***	8.30 ± 0.86	6.98 ± 0.50###	7.10 ± 0.81###

Values are expressed as mean ± SEM ($n = 6$). *P<0.05; **P<0.01 and ***P<0.001 compare to normal control [group I]; ##P<0.01 and ###P<0.001 compare to diabetic control [Group II] (One-way ANOVA followed by Tukey's *post hoc* test).

In electrolyte analysis, the animals treated with MECZ at dose levels of 125 and 500 mg/kg showed a significant decrease in the level of sodium ion when compared with diabetic control. Also, no significant changes in the levels of chloride and potassium ions were observed (Table 3). At the end of the study, diabetic animals and diabetic animals treated with glibenclamide and MECZ showed significant decreases (P<0.001) in the level of insulin when compared with the control group. Both glibenclamide and MECZ have partially improved insulin levels, but the results were not significant (Table 3).

Effect of MECZ on lipid profile: The diabetic animals and diabetic animals treated with glibenclamide showed the significant increases in the levels of total cholesterol when compared with control. Whereas the animals treated with MECZ didn't show any significant changes in the levels of total cholesterol when compared with control. No significant changes in the levels of triglyceride, HDL, LDL, VLDL and TC/HDL ratio were observed with any of the treatment groups when compared with control and diabetic control (Table 4).

Effect of MECZ on organ weight analysis: The diabetic animals and diabetic animals treated with glibenclamide and MECZ didn't show any significant changes in organ weights of liver and kidney when compared with normal control. No significant changes in the organ weights of liver and kidney were observed with glibenclamide and MECZ treated groups when compared with normal and diabetic control.

Effect of MECZ on histology: In histopathological analysis, sections from liver, pancreas and kidney of diabetic control, glibenclamide and MECZ 500 mg/kg treated animals showed mid-to-moderate toxic effects (Figure 2).

Table 2: Effects of methanolic extract of barks *C. zeylanicum* on biochemical parameters of SD rats.

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein (g/L)	Albumin (g/L)	Total bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Group I	210.67±8.39	80.43±3.69	64±1.77	62.98±1.45	39.5±1.84	1.03±0.11	39.5±1.03	0.65±0.07
Group II	205.67±5.56	81.35±6.85	71.5±3.74	47.43±2.03	36.33±2.17	1.45±0.15	43.85±1.84	0.87±0.08
Group III	227.83±6.03	71.38±4.94	66.5±4.33	64.92±1.76	43.83±4.7	1.23±0.13	31.82±2.37	0.91±0.07*
Group IV	204.83±3.59	71.25±6.21	55.33±3.89	61.93±2.23	42.5±1.41	0.88±0.11	29.42±2.24*	0.69±0.04
Group V	211.83±2.80	72.67±3.97	48.33±2.91*	64.67±2.73	41.33±1.28	0.96±0.03	27.70±2.00**	0.68±0.05
Group VI	226.83±4.21	64.5±3.56	49.5±1.77*	64.33±2.8	39.67±2.46	0.90±0.04	32.35±2.4	0.64±0.05

Values are expressed as mean ± SEM (n = 6). *P<0.05; **P<0.01 and ***P<0.001 compare to normal control (One-way ANOVA followed by Tukey's *post-hoc* test).

Table 3: Effects of methanolic extract of barks *C. zeylanicum* on biochemical parameters of SD rats.

Groups	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Insulin (mU/L)
Group I	146± 4.66	73.5± 3.17	5± 0.28	1.38± 0.14
Group II	161.67± 4.94	79.83± 3.24	5.24± 0.50	0.35± 0.14***
Group III	138.83± 4.69	68.17± 5.61	4.52± 0.29	0.52± 0.14***
Group IV	135.67± 5.12#	70.33± 6.59	4.93± 0.34	0.57± 0.12**
Group V	135± 5.86	74.5± 4.88	5.38± 0.29	0.68± 0.14**
Group VI	126.5± 4.82#	76.83± 5.00	5.07± 0.28	0.77± 0.11*

Values are expressed as mean ± SEM ($n = 6$). *P<0.05; **P<0.01 and ***P<0.001 compare to normal control; #P<0.05 compare to diabetic control (One-way ANOVA followed by Tukey's *post-hoc* test).

Table 4: Effects of methanolic extract of barks *C. zeylanicum* on lipid profile of SD rats.

Groups	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	TC/HDL ratio
Group I	2.56±0.09	0.89±0.06	0.35±0.03	1.8±0.1	0.36±0.02	7.48±0.68
Group II	3.43±0.25**	1.05±0.08	0.3±0.04	2.68±0.27	0.54±0.05	12.14±1.57
Group III	3.41±0.22*	0.98±0.07	0.35±0.02	2.58±0.23	0.52±0.05	10.21±1.18
Group IV	2.78±0.14	0.74±0.03	0.41±0.03	2.03±0.13	0.41±0.03	6.98±0.6
Group V	2.76±0.1	0.77±0.03	0.43±0.03	1.99±0.11	0.4±0.02	6.62±0.46
Group VI	2.57±0.15	0.73±0.04	0.37±0.02	1.86±0.15	0.37±0.03	6.89±0.16

Values are expressed as mean ± SEM (n = 6). *P<0.05 and **P<0.01 compare to normal control (One-way ANOVA followed by Tukey's *post hoc* test).

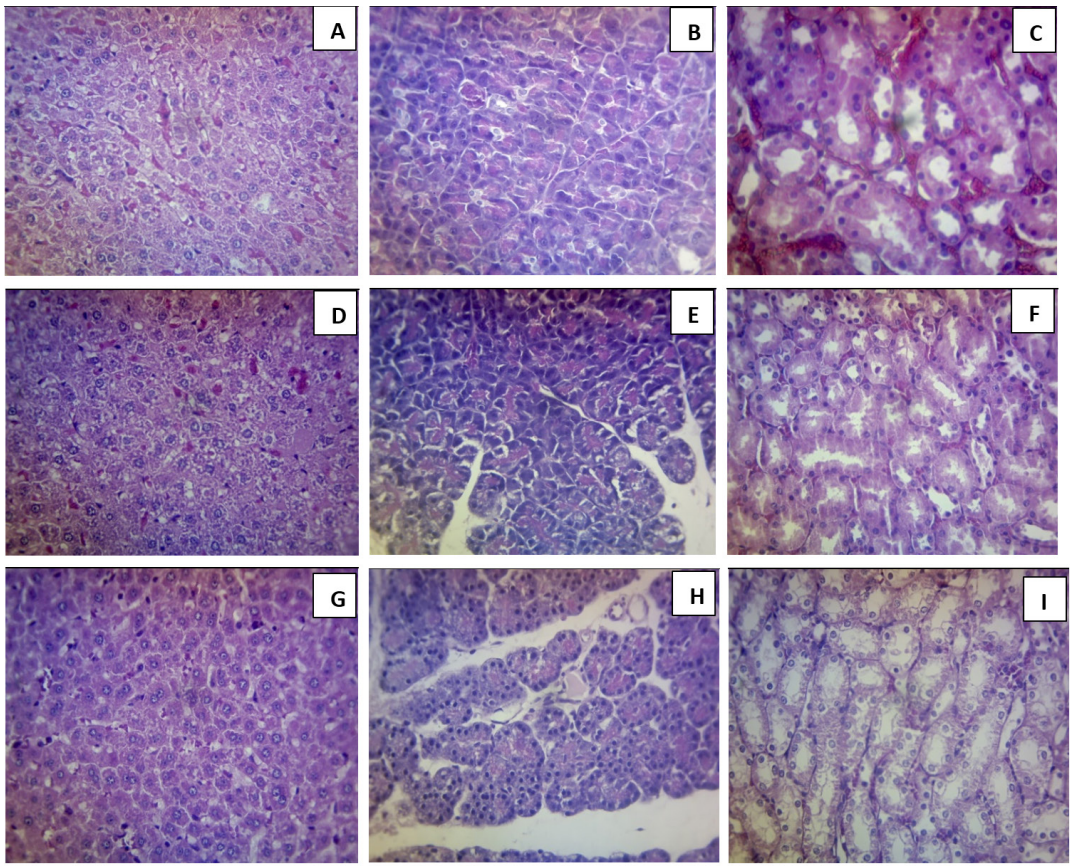

Figure 2: Photomicrograph of a section of organs of diabetic animals and animals treated with glibenclamide and methanolic extract of *Cinnamomum zeylanicum* 500 mg/kg (H & E, ×400). (A) Liver of diabetic rat showing normal configuration of liver lobules with patchy feathery degeneration of hepatocytes; (B) pancreas of diabetic rat showing permanent vesicular nuclei, no evidence of damage to the individual acinar cells and focal toxic changes. (C) kidney of diabetic rat showing congested glomeruli and granular degeneration of the tubular epithelial cells; (D) liver of glibenclamide-treated rat showing congested hepatocytes; (E) pancreas of glibenclamide-treated rat showing fetal pancreatic islets of Langerhans with few irregular interlobular ducts; (F) kidney of glibenclamide-treated rat showing mild vascular degeneration of tubules and acute patchy haemorrhagic congestion; (G) liver of methanolic extract of *Cinnamomum zeylanicum* 500 mg/kg-treated rat showing degeneration of hepatocytes and mortaled speckled appearance of the cytoplasm with the nuclei intact; (H) pancreas of methanolic extract of *Cinnamomum zeylanicum* 500 mg/kg-treated rat showing no apparent degeneration changes of pancreatic acini; and (I) kidney of methanolic extract of *Cinnamomum zeylanicum* 500 mg/kg-treated rat showing regenerative tubules and interstitial congestion.

DISCUSSION

MECZ has rich total phenolic and total flavanoid content and showed the presence of antioxidant activity. Hence, MECZ was used for pharmacological studies. The total phenolic substances and flavonoids are the most important bioactive constituents of plants and may have vast potential to be an important source of phytomedicine.[18]

In the present study, the various solvent extracts of *C. zeylanicum* were tested for their free radical scavenging activity and concentration-dependent radical scavenging activity was observed in DPPH and ABTS methods. The various solvent extracts of *C. zeylanicum* were also showed the presence of total phenolics and flavonoids contents and this phytochemical may be responsible for its antioxidant activity. The flavonoids and phenolic compounds are the natural antioxidants that neutralizes the deleterious action of reactive species by hydrogen atom transfer, electron transfer and the ability to chelate transition metals.[19]

Streptozotocin, also known as streptozocin is a glucosamine nitrosourea compound derived from a gram-positive bacterium (*Streptomyces achromogenes*) and is toxic to the insulin-producing beta cells of the pancreas in mammals. The damage caused to the pancreatic beta cells is severe to a stage where repair is impossible in most cases.[20]

Diabetic animals exhibited a loss in body weight and diabetic animals treated with glibenclamide and MECZ prevented weight loss as well as to regulate the blood glucose level primarily. This finding suggests *C. zeylanicum* has the ability and beneficial aspects in regulating the body weight as well as controlling hyperglycemia of diabetic rats. Severe weight loss occurred in diabetes mellitus rats due to the increased muscle breakdown which is part of the characterization of STZ-induced diabetes mellitus.[21]

The MECZ is showing the significant antidiabetic activity and this effect was comparable with glibenclamide. The constituents present in the plant species, individually or synergistically, is the contributing factor of the antidiabetic activity.[22] The presence of phytoconstituents such as alkaloids, terpenoids, glycosides, flavonoids are possess antioxidant activity and contribute to the antidiabetic activity. The phytochemical constituents of *C. zeylanicum* are cinnamaldehyde, eugenol, *trans*-cinnamyl acetate, *trans*-caryophyllene, linalool, eugenol acetate, isoeugenol, benzyl benzoate, α pinene, β pinene, β caryophyllene, p-cymene, camphor and cinnamyl acetate.[23] In that, the antidiabetic activity of cinnamaldehyde, linalool and β caryophyllene was explored by the various researchers. Plaisier *et al*. repeated the antidiabetic activity of cinnamaldehyde and this activity may be due to inhibition of the activation of glucose uptake by glucose deprivation in a dose-dependent manner.[24] Cinnamaldehyde also, increased plasma insulin, hepatic glycogen and HDL levels and restored the altered hepatic enzyme levels.[25] In both *in vitro* and *in vivo* studies, linalool showed antidiabetic activity and enhances insulin resistance.[26] In a preclinical study, the diabetic rats treated with β caryophyllene for 45 days showed significant decreases in the levels of glucose with increased plasma insulin levels and ameliorated the altered activities of carbohydrate metabolic enzymes.[27] Cinnamon has the ability to enhance insulin signaling by potentiating insulin-regulated glucose utilization.[28] The hypoglycemic effect of cinnamon is may be due to the increase of the pancreatic secretion of insulin from the beta cells.[29]

In the biochemical analysis, the animals treated with glibenclamide showed significant increases in the levels of creatinine and MECZ (125 and 250 mg/kg) showed decreases in the levels of urea. Kidney function is assessed by determining the levels of creatinine, urea, uric acid and electrolytes. Creatinine is a metabolic by-product and its concentration is increased in the blood indicating kidney dysfunction.[30] The diabetic animals showed increases in the level of creatinine, but the values are not significant. In general, STZ increases the levels of SGOT, SGPT, ALP, urea and creatinine.[31] Glibenclamide is had antioxidant properties and has the hepatoprotective and neuroprotective effects that are mediated through upregulation of intracellular reactive oxygen species and inhibit of apoptosis pathway, respectively.[32,33] Diabetic animals showed increases in the levels of serum sodium levels that indicating the risk of development of renal failure.[34] The animals treated with MECZ, showed a significant reduction in serum sodium level compared with diabetic animals, which indicates that this plant has a nephroprotective effect. Ullah *et al*. reported the nephroprotective effect of *C. zeylanicum*.[35] The reason for significant reduction of urea levels in MECZ is not clear. Postprandial hyperlipidemia observed in diabetic animals and this indicating that diabetic animals having a risk of developing cardiovascular disease.[36] Glibenclamide and MECZ prevented STZ-induced postprandial hyperlipidemia, indicating that the investigational compound reducing the risk of development of the cardiovascular disease.

The reduction in insulin levels in the diabetic control group is due to the increased heme oxygenase activity in the liver of rats.[37] Furthermore, damage to the β-cells in the pancreas may lower the insulin levels compared with the normal control group. The rats treated with glibenclamide and MECZ try to restore the insulin levels, but the results were not significant.

Histopathology of the liver, pancreas and kidney of diabetic animals showed moderate-to-severe toxic effects and this may be due to degeneration of hepatocytes, intracellular changes in pancreatic cells and degeneration of the tubular epithelial cells respectively. The cellular damage in the liver, pancreas and kidney induced by STZ also reported elsewhere.[38,39] The animals treated with glibenclamide and MECC reversed the STZ−induced cellular damage.

In a clinical trial, water extract of *C. zeylanicum* significantly reduced the systolic & diastolic blood pressure and reduced the levels of TC & LDL.[40] Ranjbar *et al*. studied the antioxidative stress potential of *C. zeylanicum* in humans and found that decease in the levels of lipid peroxidation and increase in total antioxidant power in individuals who received cinnamon tea (100 mg/30 ml of cinnamon with tea daily for two weeks) compare to those who receive regular tea.[41]

CONCLUSION

Methanolic extract of *C. zeylanicum* has an ameliorative effect on STZ-induced diabetic's mellitus and this effect may be through its antioxidant defense mechanism. Methanolic extract of *C. zeylanicum* prevents the cellar damage in the liver, pancreas and kidney of STZ- induced diabetic rats. In addition, the methanolic extract of *C. zeylanicum* has stimulated insulin secretion from the pancreatic islets of the diabetic rat.

CONFLICT OF INTEREST

The authors declare no Conflict of interest.

ABBREVIATIONS

ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; **ALP:** Alkaline phosphatase; **ANOVA:** Analysis of variance; **CMC:** Carboxymethyl cellulose; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ELISA:** Enzyme-linked immunosorbent assay; **GAE:** Gallic acid equivalents; **H and E:** Haemotoxylin and Eosin; **HDL:** High-density lipoprotein; **LDL:** Low-density lipoprotein; **MECZ:** Methanolic extract of *Cinnamomum zeylanicum*; **OECD:** Organisation for Economic Co-operation and Development; **QE:** Quercetin equivalents; **SD:** Sprague-Dawley; **SEM:** Standard error of the mean; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamic-pyruvic transaminase; **STZ:** Streptozotocin; **UV–vis:** Ultraviolet-visible; **VLDL:** Very low-density lipoprotein.

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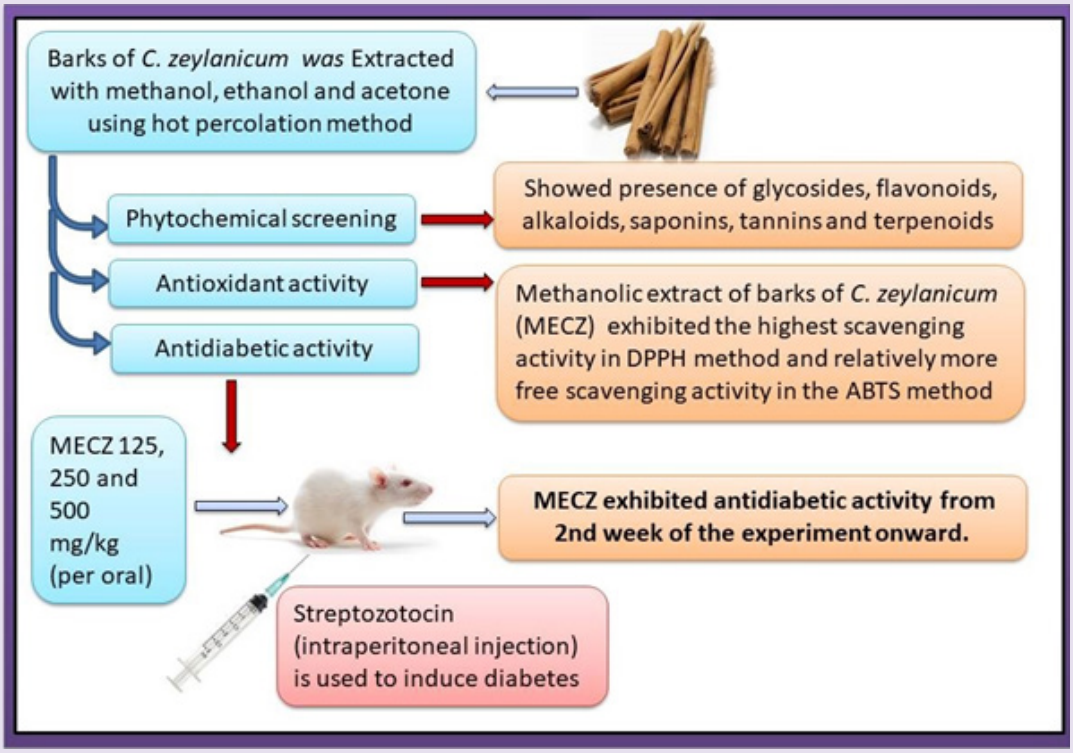
GRAPHICAL ABSTRACT



SUMMARY

- *Cinnamomum zeylanicum* is one of the well-known plant species for its medicinal properties.
- Methanolic, ethanol and acetone extract of *Cinnamomum zeylanicum* exhibited free radical scavenging properties.
- Methanolic extract of Bark of *Cinnamomum zeylanicum* prevented streptozotocin-induced diabetes mellitus in rats.

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