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ORIGINAL ARTICLE

Antioxidant and Antidiabetic Activities of Methanolic Extract of *Cinnamomum Cassia*

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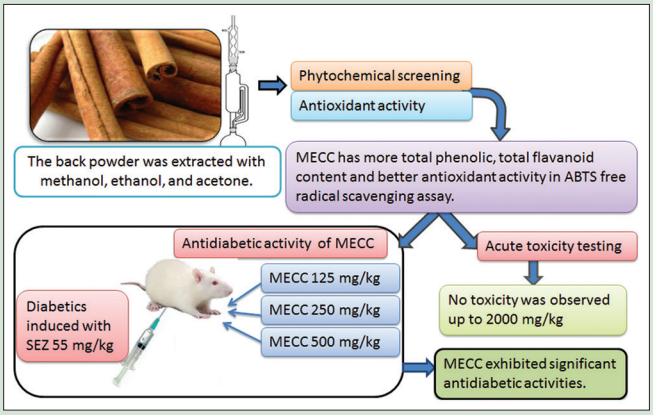
ABSTRACT

Background: *Cinnamomum cassia* or *Chinese cinnamon* is one of the fundamental herbs in traditional Chinese medicine. *C. cassia* is used as astringent, antiseptic, and used for the treatment of metabolic disorders. The antioxidant and antidiabetic effects of its extracts are unclear. Hence, the present study is planned to investigate the antioxidant and antidiabetic effects of methanolic extracts barks of *C. cassia*. **Materials and Methods:** Bark of *C. cassia* was extracted with methanol, ethanol, and acetone and its antioxidant activity was studied using 2,2-diphenyl-1-picrylhydrazyl(DPPH)and2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging assays. Acute toxic effect of methanolic extract of *C. cassia* (MECC) carried out as Organisation for Economic Co-operation and Development guidelines. MECC was studied for its antidiabetic effect using streptozotocin (STZ)-induced diabetic rats. **Results:** In both DPPH and ABTS free radical scavenging assay, methanolic and ethanolic extracts exhibited free radical scavenging activity. In acute toxicity testing, MECC did not show any significant toxic signs up to 2000 mg/kg, hence the antidiabetic activity of MECC was carried out at the dose levels of 125, 250, and 500 mg/kg. MECC showed antidiabetic activity from 2nd week of the experiment onward. At the end of the study, diabetic animals showed significant increases in the levels of total cholesterol (TC), very-low-density lipoprotein, and TC/high-density lipoprotein radio compare with that of normal control and MECC prevented the STZ-induced hyperlipidemia. In the histopathological analysis, sections from the liver, pancreas, and kidney of the diabetic animals and the animals treated with MECC 500 mg/kg showed mid-to-moderate toxic effects. **Conclusion:** The MECC exhibited significant antioxidant and antidiabetic activities.

Keywords: *Cinnamomum cassia,* diabetes, streptozotocin, Sprague-Dawley rats

SUMMARY

- Globally, the prevalence rate of diabetes is increasing, and diabetes mellitus is the ninth major cause of death. Diabetes mellitus also increase the risk of macro- and microvascular complications. In traditional medicinal system, herbs are used to managing diabetes mellitus and its complications. In traditional Chinese medicine, Cinnamomum Cassia (Lauraceae) is used for the treatment of diabetes mellitus. In this present study, the antioxidant and antidiabetic activities of methanolic extract of C. Cassia is evaluated.



Abbreviation Used: ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, ALP: Alkaline phosphatase, ANOVA: Analysis of variance, AUHAEC: AIMST University Human and Animal Ethics Committee, BW: Body weight, CKD: Chronic kidney disease, CMC: Carboxymethyl cellulose, DPPH: 2,2-diphenyl-1-picrylhydrazyl, GLP1: Glucagon-Like Peptide-1, HDL: High-density lipoprotein, MECC: Methanolic extract of *C. cassia,* OECD: Organisation for Economic Co-operation and Development, SD: Sprague-Dawley rats, S-GLUT1: Sodium glucose co-transporter 1, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, STZ: Streptozotocin, TC: Total cholesterol, VLDL: Very-low-density lipoprotein, WHO: World Health Organization.

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INTRODUCTION

Diabetes is a serious and chronic disease that occurs when the body does not produce sufficient insulin to regulate normal blood glucose levels and it is a common effect of uncontrolled diabetes. According to the World Health Organization global report on diabetes 2016, >400 million people live with diabetes.[1] The prevalence of diabetes is increasing dramatically in both developed and developing countries due to urbanization, population growth, aging, and increasing prevalence of obesity and physical inactivity. The International Diabetes Federation forecasts 334 million people will have diabetes in 2025 and major prevalence will be in India (79.4 million), China (42.3 million), and United States (30.3 million).[2] Globally, the public health burden and the human economic cost are increasing with diabetes, hence the search for an alternative for the treatment of diabetes is essential in the current era.

Traditional medicine, especially plant-based drugs are said to be an important alternative in the health-care system. Historically, natural products have been used for the treatment of many diseases and illnesses since ancient times.[3] Two-thirds of the world's population relied on plant-derived drugs.[4] It is estimated that 1% of all flowering plants including *Cinnamomum cassia* have gained recognition by modern

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scientist although local communities have used about 10%–12% of it to treat various infections.[5]

C. cassia (Lauraceae) also known *Chinese cassia* or *Chinese cinnamon*, is considered one of the fundamental herbs in traditional Chinese medicine.[6] Medicinal use of cinnamon dates back approximately 5000 years, and it is traditionally used as astringent, antiseptic, and used to treat nausea, flatulence, and diarrhea.[7,8] Kamble and Rambhimaiah studied the antidiabetic effect of aqueous extract of *C. cassia* (60 mg/kg) alone and supplemented with glibenclamide (5 mg/kg) in alloxan-induced diabetic rats, and the aqueous extract of *C. cassia* exert antidiabetic activity.[8] Khan *et al*. also studied the effect of cinnamon on glucose and lipids levels of people with type 2 diabetes and suggested that addition of 1, 3, or 6 g of cinnamon to the diet led to significant decreases in serum glucose levels after 40 days.[9] Cinnamon is suggested to have antifungal,[10] antiulcer,[11] chemopreventive,[12] antispasmodic,[13] and hypolipidemic[14] activities. The relationship between antioxidant properties and antidiabetic effect of various solvent extract of *C. cassia* remains unclear. Hence, the present study is planned to study the antioxidant properties and antidiabetic effect of various solvent extracts of *C. cassia*.

MATERIALS AND METHODS

Collection and identification of barks of *Cinnamomum cassia*

The bark of cinnamon was collected from the local market at Bedong and its authenticity was confirmed by Dr. Deivanai Subramanian. The bark was cleaned and dried in an oven for 24 h at 40°C. The dried bark was then powdered using a blender and the power was stored in an airtight container.

Extraction of barks of *Cinnamomum cassia*

The powdered barks of *C. cassia* was packed in thimble and placed in a Soxhlet apparatus. The back powder was extracted with methanolic, ethanolic, and acetone. The extraction was carried out for 24 h at about 55°C–80°C; the extract was filtered through muslin cloth. The filtrate was concentrated to a dry mass by evaporation under reduced pressure. The yield of methanolic, ethanolic, and acetone extract of barks of *C. cassia* was found to be 22.57, 17, and 15.3% w/v, respectively. The extracts of barks of *C. cassia* were stored in a desiccator at room temperature until further analysis.

Phytochemical screening

Qualitative phytochemical analysis of methanol, ethanol, and acetone extracts of *C. cassia* 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. cassia* determined by the method described by elsewhere.[15,16] Total flavonoid content and total phenolic content of various extracts of *C. cassia* compared with gallic acid and quercetin, respectively. All tests were done in triplicates.

Antioxidant activity

Antioxidant activity of methanolic, ethanolic, and acetone of *C. cassia* was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging methods using method described by elsewhere.[17-19]

2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay

Briefly, a 0.1 mM DPPH solution was freshly prepared in methanol/ethanol/ acetone and 1 ml of this solution was vigorously mixed with of 50 μL of various concentrations (50–1000 μg/ml) of methanolic/ethanolic/acetone extract of *C. cassia*, respectively, of each extract. After 30 min incubation in the dark at room temperature, and absorbance was measured at 517 nm against a blank using a ultraviolet (UV)/visible spectrometer. All analysis was performed in triplicate. The DPPH free radical scavenging properties of various extract of *C. cassia* compared with ascorbic acid.[17,18]

2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid free radical scavenging assay

ABTS was prepared by the mixing of 7 mM ABTS with 2.45 mM potassium persulfate and allowed the mixture to stand in the dark at room temperature for 12 h. Before assay, the ABTS working reagent was diluted with deionized water to reach an absorbance of 0.68–0.72 at 734 nm. The assay was assessed by mixing 500 μL of ABTS radical solution with 500 μL of various dilutions of each extract, and this mixture was allowed stand for 8 min, and then the absorbance was measured at 734 nm against a blank using a UV/visible spectrometer. The ABTS free radical scavenging properties of various extract of *C. cassia* compared with trolox (a water-soluble analog of Vitamin E). All analysis was performed in triplicate.[18,19]

Animals

Healthy, adult, either gender of Sprague-Dawley (SD) rats (200 ± 10 g body weight [BW]) were obtained from Shafazz Enterprise, Malaysia. The animals were housed in large, spacious, polyacrylic cages at an 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 (AUHAEC/FAS/2016/03) of AIMST University and the study was conducted according to Animal Research Review Panel guidelines.

Acute toxicity testing

Methanolic extract of *C. cassia* (MECC) was used for pharmacological screening because it has more total phenolic and total flavanoid content and showed better antioxidant activity in ABTS free radical scavenging assay. Acute oral toxicity of the MECC was carried as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. Healthy, adult female SD rats weighing 200 ± 10 g (3 animals/dose) were used for the experiment. Overnight fasted rats were orally fed with MECC at the dose levels of 5, 50, 300, and 2000 mg/kg BW. The rats were then closely monitored for change in their behavioral, neurological, and autonomic profiles continuously for 24 h after dosing. After 24 h, the animals were observed (at least two times a day) for a period of 14 days to evaluate the changes on behavioral, neurological, autonomic profiles, and mortality.[20]

Antidiabetic activity of methanolic extract of *C. cassia*

Healthy, adult male SD rats weighing 200 ± 10 g was used for the experiment. Overnight-fasted rats were administered intraperitoneally with freshly prepared 55 mg/kg streptozotocin (STZ) in 0.1 M citrate buffer (pH 4.5) to induce diabetes mellitus. After 24 h of diabetes induction, the rats were fed with 5% of the glucose solution (2 ml/kg BW) to prevent hypoglycemic mortality. Diabetes mellitus was confirmed after 48 h of diabetes induction by measuring the fasting blood glucose level using the blood sample obtained from the tail vein. Rats with fasting glucose of > 200 mg/dl were confirmed as diabetic rats and they were used for further experiment. Diabetic animals were randomly divided into five groups (Group II–VI) each group of 6 rats as follows:

- Group I: Normal control rats

- Group II: Diabetic control rats
- Group III: Diabetic animals treated with glibenclamide 10 mg/kg
- Group IV: Diabetic animals treated with MECC125 mg/kg
- Group V: Diabetic animals treated with MECC 250 mg/kg
- Group VI: Diabetic animals treated with MECC 500 mg/kg

Animals in Group I (normal control) and Group II (diabetic control) animals were received 0.5% w/v carboxymethylcellulose (CMC). Animals in Group III were treated with 10 mg/kg BW of glibenclamide and animals in Group IV–VI were treated with MECC at dose levels of 125, 250, and 500 mg/kg BW. The dose of MECC for antidiabetic study was selected from the results of acute toxicity study. The standard and test drugs were suspended with 0.5% w/v CMC and were administered once daily through oral gavage for 21 consecutive days.[20,21]

Throughout the study, changes in animal BW were measured at regular intervals. On 7th and 14th day of the experiment, few drops of venous blood was collected from all the animals through tail vein and immediately blood glucose levels were measured using a glucometer (Accu-Chek Aviva meter, Roche Diagnostics (M) Sdn. Bhd., Selangor). At the end of the study (i.e., 21st day), blood sample was collected from all the experimental animals through retro-orbital plexus puncture under diethyl ether anesthesia for biochemical analysis. Later, all the experiential animals were euthanized by cervical dislocation and the organs such as lung, heart, liver and kidney were collected and relative weight was measured. A part of liver, pancreas and kidney tissues were preserved in10% v/v neutral formalin for histopathological examination.

Biochemical analysis

During the study, the few drops of blood sample were collected through rat tail vein and blood glucose was estimated immediately using glucometer. At the end of the experiment, few milliliter of the blood sample was collected in plain glass tube through retro-orbital plexus and the serum separated by centrifuging at 3000 RPM for 20 min. The serum sample was used for the estimation of biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total serum cholesterol, serum triglyceride, high-density lipoprotein (HDL) cholesterol, creatinine, urea, total protein, albumin, total bilirubin, insulin, sodium, and chloride. The samples were tested using biochemical analyzer (Cobas 6000 biochemical analyzer, Roche Diagnostics) and the level of insulin was estimated using electrochemiluminescence method in Clinipath Malaysia Sdn Bhd laboratory, Malaysia.

Low-density lipoprotein (LDL) cholesterol, very LDL (VLDL), and HDL ratio were calculated mathematically. The LDL cholesterol levels was calculated using Iranian formula (LDL = TC/1.19 + TG/1.9 − HDL/1.1–38 [mg/dL]); VLDL was calculated utilizing formula LDL/5 and HDL ratio was calculated using formula ([HDL-cholesterol/TC − HDL-cholesterol] ×100 [%]).[20,22]

Histopathological analysis

Organs such as liver, pancreas, and kidney were collected from normal control, diabetic control, glibenclamide, and MECC 500 mg/kg and they were preserved in 10% neutral formalin for histopathological analysis. The liver, pancreas, and kidney samples were embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Five-micrometer thickness of liver, pancreas, and kidney section was prepared from paraffin blocks and stained with hematoxylin and eosin and mounted in neutral DPX medium, and the sections were examined under a light microscope.

Statistical analysis

The results of free redial scavenging assay were expressed mean ± standard deviation and the antidiabetic activity of MECC was expressed as mean ± standard error of the mean. Statistical differences between the groups were determined using repeated measure analysis of variance, followed by Tukey's *post hoc* test. P <0.05 is considered statistically significant.

RESULTS

The methanolic, ethanolic, and acetone extracts of *C. cassia* showed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, and glycoside. MECC exhibits more phenolic content (675.33 mg GAE/g) followed by acetone extract (345.67 mg GAE/g) and ethanolic extract (85.33 mg GAE/g) of *C. cassia*. In total flavonoid content analysis also, MECC recorded the highest flavonoid content (5096.83 mg QE/g) which was followed by ethanolic (4974.17 mg QE/g) and acetone extract (4561.17 mg QE/g) of *C. cassia*.

In DPPH free radical scavenging assay, ethanolic extract exhibited the highest scavenging activity when compared against other extracts, recording an activity of 88.26% ±0.09% at concentrations of 1000 µg/ml and the results were comparable with the same concentration of ascorbic acid (87.66% ±0.52%). The methanolic and acetone extracts had the 63.08% ±0.16% and 70.79% ±0.20% scavenging activity at concentration of 1000 µg/ml, respectively.

In acute toxicity testing, MECC did not showed any significant toxic effects up to 2000 mg/kg, hence the antidiabetic activity of MECC was carried out at the dose levels of 125, 250, and 500 mg/kg. In ABTS free radical scavenging assay, methanolic extract exhibited the highest scavenging activity when compared against other extracts, recording an activity of 65.40% ±0.42% at concentrations of 1000 µg/ml and the results were comparable with the same concentration of trolox (64.60% ±0.34%) at the same concentration. The ethanolic and acetone extracts had the 63.00% ±0.08% and 62.50% ±0.32% scavenging activity at concentration of 1000 µg/ml, respectively.

In antidiabetic study, the animals were injected with STZ to induce diabetes mellitus. After STZ injection, 80% animals were developed diabetes and 10% animals were died due to STZ induced hypoglycemia.

Throughout the study, diabetic animals showed significant increases in water intake (data not presented). Diabetic animals showed inhibition of regular BW gain when compared with control animals. However,

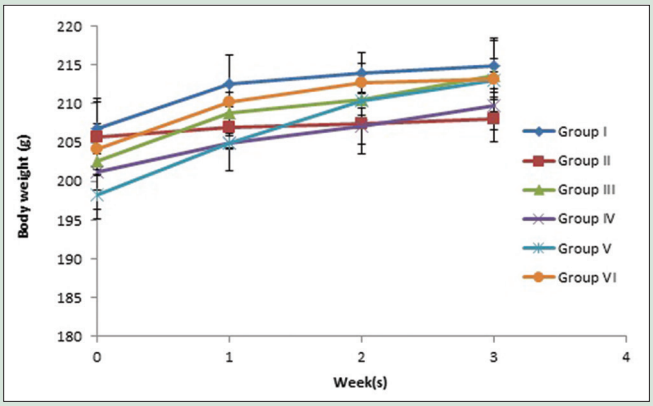

Figure 1: Effect of methanolic extract of *Cinnamomum cassia* on body weight of Sprague-Dawley rat. Values are expressed as mean ± standard error of the mean (n = 6)

the diabetes mellitus-induced BW reduction was inhibited by the glibenclamide and MECC [Figure 1]. Throughout the experiment, diabetic control animals showed significant increases in blood glucose levels compare with control animals and MECC showed significant reduction in STZ-induced hyperglycemia from 2nd week onward [Table 1].

In biochemical parameter analysis, diabetic animals showed significant increased levels of serum creatinine levels ($P < 0.05$) and MECC at 125 mg/kg showed significant increase level of ALP ($P < 0.001$) when compared with normal control. Biochemical parameters and mineral (sodium, potassium, and chloride) levels are found to be with in normal rage in the animals treated with MECC 250 and 500 mg/kg [Tables 2 and 3]. MECC-treated animals showed significant depletion in the levels of insulin when compared with control animals [Table 3]. At the end of the study, diabetic animals showed significant increases in the levels of total cholesterol (TC) ($P < 0.001$), VLDL ($P < 0.001$), and TC/HDL ($P < 0.001$) radio compare with normal control. Glibenclamide also significantly increased the levels of TC ($P < 0.01$), triglyceride ($P < 0.05$), and VLDL ($P < 0.05$) and MECC prevented the STZ-induced hyperlipidemia in rats [Table 4]. Organ weight analysis did not showed any significant variations in the absolute and relative weights of liver and kidney. In histopathological analysis, sections from liver, pancreas, and kidney of diabetic control, glibenclamide and MECC 500 mg/kg-treated animals showed mid-to-moderate toxic effects [Figure 2].

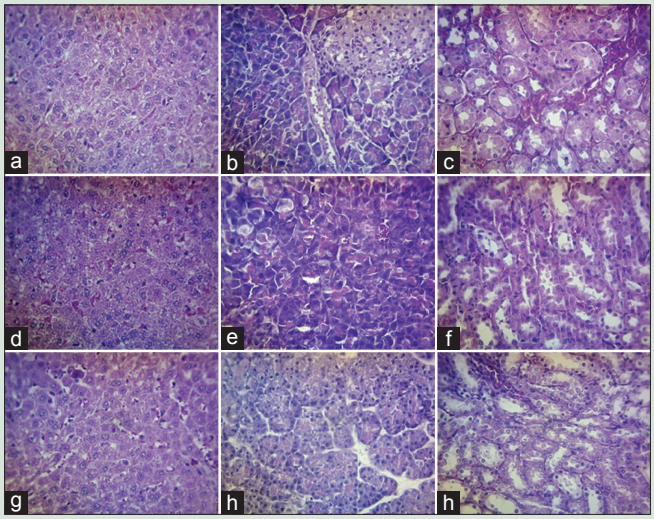

Figure 2: Photomicrograph of a section of organs of diabetic animals and animals treated with glibenclamide and methanolic extract of *Cinnamomum cassia* 500 mg/kg (H and E, ×400). (a) Liver of diabetic rat showing normal configuration of liver lobules with patchy feathery degeneration of hepatocytes and mild sinusoidal congestion; (b) pancreas of diabetic rat showing permanent vesicular nuclei with well-defined nucleoli and indicative of focal toxic changes; (c) kidney of diabetic rat showing congested glomeruli, regenerative tubules, and granular degeneration of the tubular epithelial cells; (d) liver of glibenclamide-treated rat showing mottled and speckled appearance of individual hepatocytes; (e) pancreas of glibenclamide-treated rat showing acute congestion vacuolated cytoplasmic appearance; (f) kidney of glibenclamide-treated rat showing mild vacuolar degeneration of tubules; (g) liver of methanolic extract of *Cinnamomum cassia* 500 mg/kg-treated rat showing mild kupffer cell hyperplasia and foci sinusoidal congestion; (h) pancreas of methanolic extract of *Cinnamomum cassia* 500 mg/kg-treated rat showing no apparent degeneration changes of pancreatic acini and small well-delineated lymph nodes; and (i) kidney of methanolic extract of *Cinnamomum cassia* 500 mg/kg-treated rat showing congested glomeruli and focal areas of toxic changes within the renal tubules exhibiting vasculation

DISCUSSION

MECC has rich total phenolic and total flavanoid content and showed the presence of antioxidant activity in both DPPH and ABTS free radical scavenging assays. Hence, MECC was used for the pharmacological studies. The phenolic substances and flavonoids are most important bioactive constituents of plants and which may have vast potential to being an important source of phytomedicine.[21]

Natural antioxidants can be classified accordingly by its different mechanism of action. In the present study, the various solvent extracts of *C. cassia* were tested for their free radical scavenging capacity and concentration-dependent radical scavenging activity

Table 1: Effect of methanolic extract of *Cinnamomum cassia* on blood glucose (mmol/L)

Groups	2nd day	7th day	14th day	21st day
Group I	5.82±0.25	5.70±0.21	5.73±0.14	5.78±0.30
Group II	10.68±0.41***	10.55±0.67***	11.17±1.05**	12.72±0.46***
Group III	10.55±0.45***	8.90±0.75	8.28±0.73	6.45±0.41
Group IV	11.47±0.52***	10.52±0.53**	10.18±1.38*	9.23±1.34
Group V	12.40±0.73***	10.92±1.23***	9.67±0.65*	8.88±1.51
Group VI	12.17±0.40***	10.67±0.70***	8.88±0.94	8.85±0.69

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). SEM: Standard error of the mean; ANOVA: Analysis of variance

Table 2: Effect of methanolic extract of *Cinnamomum cassia* on biochemical parameters

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/L)	Total protein (g/L)	Total bilirubin (mg/dL)
Group I	110.00±7.40	60.00±5.57	51.67±4.19	28.65±3.47	0.62±0.04	41.50±2.04	62.83±1.23	1.03±0.11
Group II	139.50±7.83	75.13±7.49	63.67±4.91	39.48±2.86	0.98±0.13*	36.33±2.32	70.61±2.09	1.48±0.16
Group III	130.80±7.30	64.60±5.70	63.67±3.74***	31.38±2.86	0.85±0.10	42.83±4.07	65.86±1.89	1.16±0.13
Group IV	120.70±13.11	62.12±5.29	58.33±5.03	29.02±2.16	0.75±0.04	39.50±1.31	60.83±2.58	0.95±0.07
Group V	114.20±9.00	65.33±3.58	50.33±3.32	29.27±3.31	0.72±0.03	41.16±1.70	64.33±2.44	0.99±0.02
Group VI	104.70±11.08	59.50±3.39	53.00±4.95	32.88±3.11	0.72±0.02	39.16±1.96	60.66±3.73	0.98±0.04

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). SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; ALP: Alkaline phosphatase; SEM: Standard error of the mean; ANOVA: Analysis of variance

was observed in DPPH and ABTS methods. The phenolic and flavonoid compounds also responsible for the antioxidant activity of plants.[23] The antioxidant properties of the different extracts of *C. cassia* may be influenced by solubility, pH, and radical quenching by antioxidants.[24,25]

STZ is toxic glycoside obtained from *Streptomyces achromogenes*, a Gram-positive bacterium and it is toxic to the insulin-producing beta cells of the pancreas in mammals. The alkylating properties of the STZ modify the biological macromolecules, fragment DNA, and destroy the β-cells.[26] In the diabetic control group, reduction in BW was observed, which may be due to severe muscle degeneration, increased muscle wasting, and loss of tissue proteins.[26,27] The protective mechanism of the plant fraction on BW of diabetic rats may be due to its capacity to lower and regulate hyperglycemia. MECC exhibited significant antidiabetic activity against STZ-induced diabetes in rats at the end of 2nd week of the experiment onward, and the results were comparable with that of glibenclamide. This finding suggested that *C. cassia* has a beneficial effect on maintaining BW of diabetic rats. The flavonoids present in the plant may contribute for its antidiabetic activity.[28] The antidiabetic effect of flavonoids and polyphenolic compounds are may be due to regeneration of damaged beta cells of pancreases and inhibition of glucose transport by inhibiting the sodium-glucose cotransporter 1 (S-GLUT1) in the intestine, respectively.[29]

Diabetic animals showed significant increase of serum creatinine level and which can lead to the development of chronic kidney disease (CKD) and kidney failure. Lower serum creatinine independently increased the risk of diabetes and the reason is unclear. Harita *et al*. hypothesized that the lower serum creatinine might reflect a lower volume of skeletal muscle. Skeletal muscles are major target tissue of insulin and lower volume of skeletal muscle may have increase chances of development of insulin resistance, which may cause diabetes.[30,31] In the present study, increase in the level of serum creatinine level was observed in diabetic rats.

Significant increases of ALP level was found in glibenclamide-treated group which may be due to drug effect or diseases. Glibenclamide has antioxidant properties and has hepatoprotective effect through upregulating the intracellular reactive oxygen species and subsequently activating Akt-NF-κB pathway.[32] Hence, we hypothesis that impairment of liver enzyme in glibenclamide may be due to STZ-induced diabetes. In both, diabetic control and MECC-treated groups, significant reduction in the levels of insulin were observed, whereas glibenclamide improved the insulin secretions in diabetic animals. Li *et al*. also reported the effect of glibenclamide on insulin and this effect is due to increases in insulin sensitivity index level, moderately enlarged the islets and significantly reduced the scores of the injuries of the pancreas by glibenclamide.[33] STZ also induced hyperlipidemia and this may be due to exogenous fat loading, abnormal increase in the small intestinal acyl-coenzyme A: Cholesterol acyltransferase activity and enhancement of intestinal CoA-dependent esterification.[22] The animals treated with MECC reversed the STZ-induced hyperlipidemia.

Histopathology of the pancreas, liver, and kidney of STZ diabetic animals showed moderate-to-severe toxic effects, and this may be due to hypertrophy of hepatocytes by an increase in the intracytoplasmic eosinophilic granules in hepatic cells, tubular atrophy and necrosis in renal cells, and increased level of general oxidative stress.[34,35] The animals treated with glibenclamide and MECC reversed the STZ-induced cellular damage.

C. cassia is known to have many pharmacological properties, and it is commonly used as astringent. In acute and repeated dose toxicity testing (data not presented), MECC did not showed any marked toxic effects. In a preclinical studies, hydroalcoholic extract of *C. cassia* showed antidiabetic activity in alloxan-induced diabetes.[36] In a clinical study, daily intake of 6 g of *C. cassia* over 40 days did not any significant adverse effect and which indicates that the *C. cassia* is well tolerable in human.[37] In another study, 1 g cinnamon from *C. cassia* did reduced blood glucose of type II diabetes patients.[38] Mang *et al*., also studied the effect of cinnamon on type 2 diabetes mellitus and concluded that the cinnamon extract has moderate effect on reducing fasting plasma glucose levels with poor glycemic control.[39] The antioxidant and antidiabetic activity of MECC is may be due to the presence of flavonoids and polyphenols. Flavonoids present in the plant regenerate the damaged beta cells of pancrease; polyphenols, a secondary metabolite of plant, increase glucagon-like peptide-1 (GLP-1) secretion, inhibit dipeptidyl peptidase-4, and exert an indirect effect on insulin secretion by modulation of gut microbiota.[20,40]

Table 3: Effect of methanolic extract of *Cinnamomum cassia* on minerals and insulin hormone

Groups	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Insulin (mU/L)
Group I	140.83±5.06	72.50±3.81	4.86±0.33	1.28±0.11
Group II	156.83±5.08	82.16±4.62	5.81±0.58	0.20±0.04***
Group III	141.33±5.56	68.50±6.61	4.45±0.35	0.78±0.14
Group IV	130.17±7.57	74.00±6.92	4.70±0.41	0.50±0.12***
Group V	136.50±8.09	76.66±5.37	4.95±0.33	0.56±0.12**
Group VI	128.00±6.29	76.16±6.64	5.10±0.28	0.61±0.14**

Values are expressed as mean±SEM (n=6). **P<0.01 and ***P<0.001 compare to normal control (one-way ANOVA followed by Tukey's *post hoc* test).
SEM: Standard error of the mean; ANOVA: Analysis of variance

CONCLUSION

The MECC exerts antioxidant activity against DPPH and ABTS free radical, and antidiabetic activity against STZ-induced diabetes mellitus in rats. The MECC also did not showed any toxicity or toxic signs in acute toxicity testing. Further, it also observed that MECC

Table 4: Effect of methanolic extract of *Cinnamomum cassia* on lipid profile

Groups	TC (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	TC/HDL ratio
Group I	2.56±0.09	0.71±0.03	0.42±0.03	1.82±0.10	0.36±0.02	6.28±0.46
Group II	4.01±0.07***	0.93±0.05	0.27±0.02	3.32±0.08*	0.66±0.02***	15.70±1.35***
Group III	3.56±0.26**	1.01±0.11*	0.36±0.03	2.72±0.26	0.55±0.05*	9.71±1.09
Group IV	2.62±0.22	0.78±0.04	0.40±0.03	1.86±0.23	0.37±0.05	6.73±0.85
Group V	2.78±0.18	0.75±0.04	0.39±0.04	2.05±0.20	0.41±0.04	7.75±1.32
Group VI	2.60±0.16	0.77±0.04	0.39±0.04	1.86±0.19	0.37±0.04	7.15±0.99

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).
SEM: Standard error of the mean; ANOVA: Analysis of variance; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very-low-density lipoprotein; TC: Total cholesterol

inhibits STZ-induced hyperlipidemia, which may help to prevent the complications associated with diabetes mellitus such as hyperlipidemia and hypercholesterolemia.

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Conflicts of interest

There are no conflicts of interest.

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