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Effect of cinnamon aqueous extracts on hyperglycemia in diabetes-induced wistar rat model

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Abstract

Diabetes mellitus is considered the epidemic of the century. Although various treatments for diabetes are available, the development of effective novel treatments based on medicinal plants has not yet been recognized. Therefore, this study focused on the ability of *Cinnamomum verum* to lower blood glucose levels in Wistar rats suffering from diabetes. The cinnamon bark extract was prepared by sonication in water for one hour. Wistar rats (weight 180-220 g) were kept alternately in the dark and light for 12 hours, with a standard diet and constant water supply. Diabetes was induced with streptozotocin (STZ), and the diabetic rats were divided into groups (6 rats/group), which were treated with cinnamon and metformin for 42 days. Fasting blood glucose levels were measured at regular intervals. After 42 days, the rats were sacrificed, and blood was collected by cardiac puncture to measure fasting blood glucose (FBG), insulin, lipid profile, renal function, and liver function tests. In the cinnamon-treated group, the mean FBG (IQR) at baseline and four weeks after treatment were 310 (256-352) mg/dL and 119 (105-147) mg/dL, respectively ($p = 0.02$). However, no significant difference in insulin resistance and lipid profile was observed. The biochemical tests, hematological analysis, and histopathological examinations showed that the 42-day treatment with cinnamon had no significant adverse effects on kidney, liver, and bone marrow function. It can be concluded that the aqueous cinnamon bark extracts effectively improve hyperglycemia without adversely affecting the liver, kidneys, or bone marrow.

Keywords

adverse effects; *Cinnamomum verum*; diabetes mellitus; glycemic control

Introduction

Diabetes mellitus is defined as a heterogeneous metabolic disorder that is mainly characterized by hyperglycemia (1). As glucose in chronic excess causes toxic effects, diabetes is linked with a number of metabolic complications. The prevalence of diabetes is increasing as an epidemic throughout the world. Asia has been named the epicenter of this global Type 2 Diabetes Mellitus (T2DM) epidemic (2). Diabetes is the ninth major killer in the world. In 2010, it was estimated that the global projection for diabetes in 2025 would be 438 million, but that prediction has already exceeded 25 million by 2020. Therefore, the International Diabetes Federation estimates that 578 million adults will be diagnosed with diabetes by 2030 and 700 million by 2045 (3).

Achieving optimal glycemic control is the most effective way of controlling the complications in both Type 1 and Type 2 diabetes. Even though various treatment options are available to treat hyperglycemia, scientists are looking for better treatments with fewer side effects. Further, demand for plant-based dietary supplements, herbal nutraceuticals, and functional foods has increased for managing diabetes in recent years as they offer new moieties with strong biological activities (4). According to the literature, more than 400 plant species have been reported to have the property of lowering hyperglycemia.

Among those plants, *Cinnamomum zeylanicum* (Cinnamon) (Laureaceae family) contains 250 species of shrubs and trees found in Southeast Asia, China and Australia. For centuries, cinnamon bark has been used as a spice in many countries across the globe (5). *C. zeylanicum* Blume (*Cinnamomum verum*/ true cinnamon/ Ceylon cinnamon) and Cinnamon cassia (*Cinnamomum aromaticum*/ Chinese cinnamon) are the two main varieties of cinnamon (6). Chemical constituents of cinnamon are known to have the ability to regulate blood glucose through insulin-mimetic properties (7). Therefore, this research aims to explore the effect of aqueous extract of cinnamon bark in hyperglycemia of diabetes-induced Wistar rat models and the acute and sub-acute adverse effects on the liver, kidney and bone marrow of rats with the intervention.

Materials and Methods

Ethical approval

The ethical approval was obtained from the Ethics Review Committee, Faculty of Medicine, the University of Ruhuna, Sri Lanka. (2020.p.112). All protocols used in this study were according to the standard guided by the Council for International Organizations of Medical Sciences international guiding principles of biomedical research involving animals.

Plant material

C. verum J. Presl (Family: Lauraceae) bark was collected from a cinnamon plantation in Karapitiya, Galle, Sri Lanka. The Department of National Botanic Gardens, National Herbarium, Peradeniya, Sri Lanka, identified and authenticated the species.

Preparation of aqueous extract

Dried Cinnamon inner barks were ground into a powder and five hundred grams (500 g) of powdered cinnamon barks were extracted into distilled water (1 L) using sonication for one hour (sonicator, ACP 250H model from MRC laboratory-instruments, UK) (8). The mixture was filtered through a cheesecloth, and the filtered aqueous extract was freeze-dried (Freeze dryer Model FE-10-MR, China) and stored at -20 ^{0}C until used.

Experimental animals

Healthy male Wistar rats (weight 180-220 g) were procured from the Medical Research Institute, Colombo, Sri Lanka. Animals were acclimatized in the animal house in the Medical Faculty of University of Ruhuna at 28±2^{0}C and relative humidity of 60-80% in polypropylene cages for two weeks. The animals were exposed to alternate 12 hours of darkness and light cycles. A standard diet was given to rats *ad libitum*. A constant supply of water was maintained throughout.

Sample size

Six rats per group were allocated according to the guidelines of animal ethics.

Experimental design

Albino male Wistar rats were randomly grouped into 6 rats per group. The rats in groups 1, 2 and 3 were induced diabetes with STZ. Group 4 rats were not treated with STZ (negative control). Groups 1 and 2 were treated with oral metformin (200 mg/kg/day dissolved in water) and *C. verum* bark extract (20 mg/kg/day), respectively, for 42 days. Groups 3 and 4 did not receive any treatment.

Inducing diabetes

The rats were kept fasting for 12 hours and diabetes was induced with STZ. “Negative controls” were injected with 0.2 mL of 0.9% sodium chloride in one group, and the other rats were injected once with STZ solution at a dose of 40 mg/kg body weight. Preparation of STZ solution: Citrate buffer (1 mL, 0.1 M, pH 4.5) was freshly prepared to dissolve STZ (8 mg), and the STZ solution was maintained on ice before use. Ten days after STZ treatment, fasting blood glucose (FBG) was measured. If the FBG of the rats was ≥ 215 mg/dl, those were identified as a diabetic model. If the rats did not achieve FBG ≥ 215 mg/dl after 10 days, they were treated again with the same dose of STZ.

Administration of metformin and cinnamon extracts to the rats

Powdered cinnamon aqueous extract and commercially available metformin tablets were dissolved in distilled water separately. Prepared cinnamon and metformin solutions were administered daily doses of 20 mg/kg and 200 mg/kg to the rats in groups 1 and 2 via oral gavage.

Collection of blood samples

Blood samples were collected at the baseline and fortnightly from the tail vein until 42 days to fluoride oxalate tubes (to measure FBG) and serum separator tubes (to measure total cholesterol (TC) and triglycerides). After 42 days, the rats were sacrificed by overdosing on chemical anaesthetics. They drew blood through the cardiac puncture to measure FBG, insulin, lipid profile, renal function, and liver function tests. Blood samples were centrifuged at 3500 rpm for 10 minutes at room temperature, serum was separated and stored at -20°C until analysis.

Determination of FBG

FBG was estimated by the GOD-PAP method at the baseline and fortnightly. The “Glucose Monoreagent LR” assay kit (Gensan Productions, Italy) was used to determine the blood glucose level. The quantity of 1000 µL of reagent from the assay kit was added to all test tubes (standard, blank, and samples tubes) followed by 10 µL of

standard in the kit, 10 µL of distilled water and 10 µL of extract respectively. After 10 minutes of incubation, the absorbance of samples and standard was measured against the reagent blank 510 nm wavelength. Finally, the FBG was calculated using the equation below (9):

$$Glucose\ \left(\frac{mg}{dL}\right) = \left(\frac{absorbance_{of\ sample}}{absorbance_{of\ standard}}\right) * Concentration\_of\_Standard$$

(Eqn.1)

Determination of components in lipid profile

Triglycerides and Total Cholesterol (TC) were measured at the baseline and fortnightly. Triglycerides were measured using the GPO-PAP-enzymatic colorimetric method using an assay kit (Gensan Productions, Italy). The amount of 1000 µL of assay kit reagent was added to each test tube of standard, blank, and samples, followed by adding 10 µL of standard from the kit, 10 µL of distilled water and 10 µL of extract. After incubation for 10 minutes, the absorbance of the samples and standard were measured at 510 nm against the reagent blank. The following equation was used to calculate the triglycerides in plasma samples:

$$Tryglyceride\ \left(\frac{mg}{dL}\right) = \left(\frac{absorbance_{of\ sample}}{absorbance_{of\ standard}}\right) * Concentration\_of\_Standard$$

(Eqn.2)

TC was measured using the CHOD-PAP-enzymatic colorimetric method and an assay kit (Gensan Productions, Italy). The assay kit's reagent 1000 µL was added to each test tube of standard, blank and sample, followed by 10 µL of the kit's standard, 10 µL of distilled water and 10 µL of extract, respectively. The absorbance of the samples and the standard were measured in comparison to the reagent blank at 510 nm after incubation for 10 minutes. TC was calculated using the following equation:

$$Total\ cholesterol\ \left(\frac{mg}{dL}\right) = \left(\frac{absorbance_{of\ sample}}{absorbance_{of\ standard}}\right) * Concentration\_of\_Standard$$

(Eqn.3)

The precipitation method of the assay kit was used to determine high-density lipoprotein (HDL) after 42 days. An assay kit (Gensan Productions, Italy) was used. About 0.5 mL of sample and 0.5 mL of reagent were added into each tube and mixed using vortex. The tubes were centrifuged at 3500 rpm for 10 minutes. The tubes were labeled as blank, standard, and samples accordingly, and 1000 µL of enzymatic cholesterol reagent was added to each tube. The amount of 10 µL of standard and supernatants of previously centrifuged samples were transferred into the tubes above. Then, all tubes were incubated for 10 minutes at 37^{0}C. The absorbance at 510 nm was measured, and HDL values were obtained using the equation below:

$$HDL\ \left(\frac{mg}{dL}\right) = \left(\frac{absorbance_{of\ sample}}{absorbance_{of\ standard}}\right) * Concentration\_of\_Standard \times 2$$

(Eqn.4)

Low-density lipoprotein (LDL) Cholesterol was determined using total triglycerides (TG), TC, and HDL values by the Friedewald equation mentioned below (10):

$$LDL = Total\ Cholesterol - HDL - \left(\frac{TG}{5}\right)$$

(Eqn.5)

Determination of renal function

The “Creatinine LR” Assay kit (Gensan Productions, Italy) was used to determine the creatinine content of the plasma using the colorimetric method without deproteinization. The assay kit's reagents R1 and R2 were mixed in equal parts to prepare a working solution. Then, 1000 µL of the working solution was added to each test tube (standard, blank and sample) followed by adding 10 µL of the kit's standard, 10 µL of distilled water and 10 µL of extract. The absorbance of the samples and the standard were measured in comparison to the blank at 510 nm after 30 seconds (first reading = A1) followed by the second reading after 2 minutes (second reading = A2). Creatinine was calculated using the equation below:

$$Creatinine\ \left(\frac{mg}{dl}\right) = \frac{A2sample - A1sample}{A2standard - A1standard} * concentration\ of\ the\ standard$$

(Eqn.6)

The renal function of the rats was detected using test kits (Rat KIM1 ELISA Kit and Rat Lipocalin-2 ELISA Kit (NGAL, Elabscience Biotechnology Inc). The diluted standard, blank, and sample wells were determined in the test kit's given ELISA plates. Wash buffer, reference standards, biotinylated detection Ab working solution and HRP conjugate working solution were prepared according to the instructions in the test kit. Then, 100 µL of standards, blanks, and samples were added into the appropriate wells, and the plates were covered with sealer provided in the kit and incubated for 90 minutes at 37^{0}C. After incubation, liquid from each well was decanted, and 100 µL of biotinylated detection Ab working solution was added immediately to each and incubated for 1 hour. As the next step, 350 µL of wash buffer was added after decanting the liquids from each well. These washing steps were repeated three times, and 100 µL of HRP conjugate working solution was added to each well and allowed to incubate for 30 minutes. Solutions were decanted, and the washing process was repeated 5 times, followed by adding 90 µL of substrate reagent to each well and incubated for 15 minutes. Finally, each well was added to the kit's 50 µL of stop solution. Each well's optical density (OD value) was measured simultaneously with a microplate reader at 450 nm.

Determination of liver function

Alkaline phosphatase (ALP), aspartate transaminase (AST and alanine transaminase (ALT) assays were conducted according to the instructions of the manufacturer of the test kits. Working solutions for ALP, AST, and ALT tests were prepared by mixing R1 and R2 solutions, which were given separately for each kit at 4:1 proportion. For AST and ALT testing, 1000 µL of the working solution was

added to each test tube (blank, standard and sample), followed by 100 µL of distilled water and 100 µL of the sample. Then, it was allowed to incubate for 1 minute at 37^{0}C and absorbance of sample (EC) was measured at time 0 after 1,2 and 3 minutes. Calculations for AST and ALT were done according to the following equations

$$AST\left(\frac{U}{I}\right) = \left(\frac{\Delta E}{min}\right) * 1746$$

$$ALT\left(\frac{U}{I}\right) = \left(\frac{\Delta E}{min}\right) * 1746 \quad \text{(Eqn.7)}$$

ALP tests were performed by adding 1000 µL of the working solution to each test tube. According to the labels, 20 µL of distilled water and 20 µL of sample were added for blank and sample tubes respectively and incubated for a minute at 37°C. The absorbance (EC) was measured at 0 after 1, 2 and 3 minutes. Readings were taken to determine the absorbance variation ΔE/min. The following equation was used to calculate the ALP:

$$ALP\left(\frac{U}{I}\right) = \left(\frac{\Delta E}{min}\right) * 2757 \quad \text{(Eqn.8)}$$

Determination of insulin

Insulin ELISA kit manufactured (Elabscience Biotechnology Inc.) was used to determine the insulin in the plasma. The same ELISA procedure was used with the KIM 1 ELISA kit.

McAuley (McA), homeostasis model assessment (HOMA) and QUICKI were used in this study to assess insulin resistance. McA described a method for measurement of insulin resistance, which correlates with estimates of IR measured by the euglycemic clamp technique (11). Homeostasis model assessment (HOMA) calculates insulin resistance and beta-cell function from fasting glucose and insuln concentrations (12):

$$HOMA = \left(Insulin\left(\frac{mU}{ml}\right) \times Glucose\left(\frac{mmol}{l}\right)\right)/22.5 \quad \text{(Eqn.9)}$$

Histopathological examination of liver and renal specimens

Organs of rats after 42 days, namely liver and kidney, were collected after dissecting the rats and fixed in 10% formaldehyde. Trimmed specimens were placed in appropriately labeled tissue cassettes after fixation and the specimens were processed by dehydrating them with alcohol and clearing them with xylene. After, tissues were infiltrated with a paraffin wax embedding agent and the specimens were sectioned using a microtome. The tissue ribbons were carefully transferred to a warm water bath after being cut. They were allowed to float on the surface before being scooped onto a slide beneath the water level. After clearly labeling the slides, they were allowed to dry upright at 37^{0}C for a few hours to gently melt the excess paraffin wax while leaving the tissue section intact. Then, those tissue sections were stained using hematoxylin and eosin. A Clinical Histopathologist examined prepared slides. The microscope was used to observe the specimen slides and determined whether there were any significant histopathological differences compared to healthy histopathological specimens using an electronic microscope. The entire specimen was scanned on a monitor at X885. An area with maximum damage was chosen and photographed for each tissue block.

Bone marrow sampling, staining and microscopic examination

Bilateral hind limbs were disarticulated from the pelvis immediately after sacrificing Wistar rats. Skin and the muscles overlying the femur and tibia were dissected. The epiphyseal cartilage was removed using a 10 CC syringe with a 21G needle, bone marrow was aspirated by applying negative pressure, and direct smears were prepared with aspirate. The same procedure was performed for the other limb and slides were prepared. If the yield was unsatisfactory, aspirates were also obtained from the bilateral tibia. Peripheral blood smears are also prepared from the blood obtained from cardiac puncture.

The smears were allowed to air dry and stained with Leishman stain. A hematologist examined the slides of bone marrow smears using an Olympus CX 43 RF biological microscope under 40x and 100x magnifications.

Statistical Analysis

Wilcoxon Signed Rank and Krushkal-Wallis tests were used for statistical analysis using SPSS version 22. AAT Bioquest, Inc. (2022, November 13). Quest Graph™ Four Parameter Logistic (4PL) Curve Calculator. AAT Bioquest was used to generate the four-parameter logistic curve for analyzing ELISA (13).

Results

Fasting blood glucose levels

A significant reduction of FBG was observed after the cinnamon treatment. In the cinnamon-treated arm, median FBS (IQR) at baseline and 4 weeks after the treatment were 309.78 (255.54 -352.24) mg/dL and 119.13 (105.06-147.34) mg/dL (p = 0.02). The median (IQR) level of FBS during the treatment group in cinnamon and metformin arms were 169.06 (131.91-222.77) mg/dL and 77.88 (15.3100-113.43) mg/dL (P = 0.07). Interestingly, a significant difference between the cinnamon and metformin treatments was not observed.

Lipid profile

According to the Kruskal-Wallis Test, the distribution of triglycerides and HDL are the same across the categories of treatments after 42 days (p = 0.153 and p = 0.126, respectively). There was a significant difference across the categories of the treatments in TC (p = 0.021) and LDL (p = 0.021) levels after 42 days. Further, there was a significant median difference in TC values between baseline and after 04 weeks of cinnamon treatment (p = 0.02). However, there was no significant difference in the metformin-treated group (p = 0.07), Negative control (p = 0.3), and diabetes rats without any treatment (0.07) between

baseline TC values and 4 weeks after the treatments. Moreover, there was no significant difference between triglyceride values in the baseline and after 4 weeks of the cinnamon-treated group (p = 0.3), metformin-treated group (p = 0.07), and non-treated diabetic rats (p = 0.5). However, there is a significant difference in negative control (p = 0.03).

Renal function

Serum creatinine: The Median (IQR) serum creatinine value of cinnamon-treated arm has been reported as 1.24 (1.12-1.29) mg/dl after the treatment, which is higher than the reference value (0.2–0.8 mg/dl) while metformin-treated arm reported a median (IQR) value of 0.53 (0.45-0.61) mg/dl. (14).

KIM 1: The baseline KIM 1 values among treatments displayed no significant difference (p = 0.4). However, there is a significant difference among treatments after 42 days of the study, according to the Kruskal-Wallis Test (p = 0.03). Further, it can be determined that there is a significant difference between the baseline and end of the study in cinnamon treatment (Fig. 1). However, there is a significant difference between the baseline and 42 days after the treatment in the negative control group. At the same time, there is no significant difference in the metformin-treated group and diabetic non-treated group according to Wilcoxon signed ranked test (Fig. 1).

NGAL: NGAL concentrations showed significant differences among cinnamon-treated, negative control and metformin groups between the study's baseline and the end of the study (Fig. 2).

Insulin resistance

There were no significant differences between insulin resistance calculated according to the HOMA in baseline and after 42 days of treatments (Table 1). Further, insulin resistance among categories of treatments had no significant difference (p = 0.2).

Liver function

AST, ALT and ALP activities: According to the liver function tests, AST and ALT values of all treatment groups are within the reference range (Table 2). However, ALP value of the metformin-treated arm was lower than the reference value while other treatment groups were scattered within the reference range.

Histopathological examination of liver specimens: The liver tissue samples of negative controls (Fig. 3-A), the cinnamon-treated group (Fig. 3-B), the metformin-treated group (Fig. 3-C) and the diabetic non-treated group (Fig. 3-D) had preserved normal architecture. There was no fatty change, hepatocyte loss, lobular or portal inflammation and fibrosis in all the groups. However, passive hepatic congestion was observed in one cinnamon-treated rat.

Histopathological examination of renal specimens: Glomeruli in the cortex and renal tubules and interstitium of kidney tissues were normal in the negative control

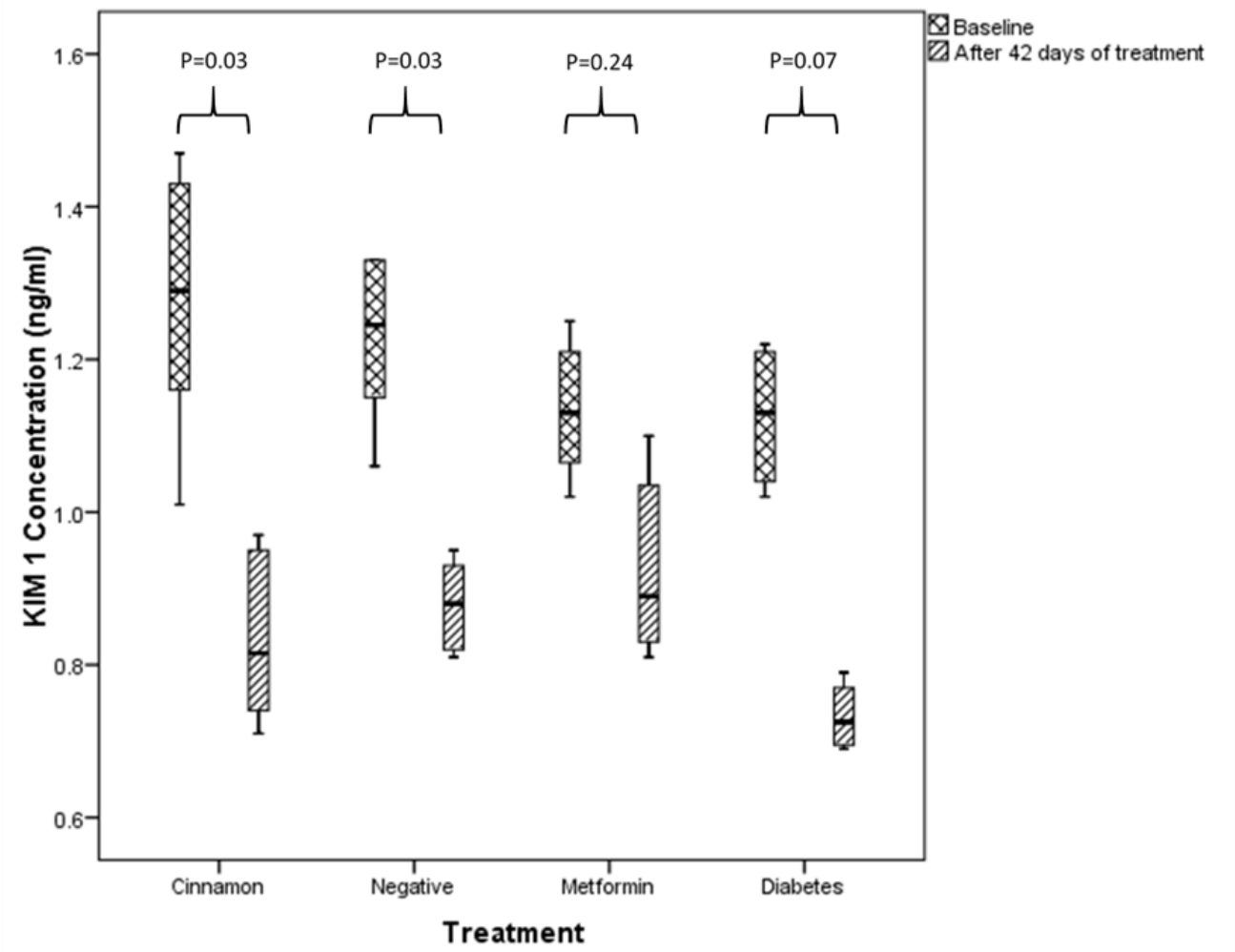

Fig. 1. KIM 1 concentration of the Wistar rats treated with cinnamon, metformin, negative control and diabetes-induced control for 42 days.

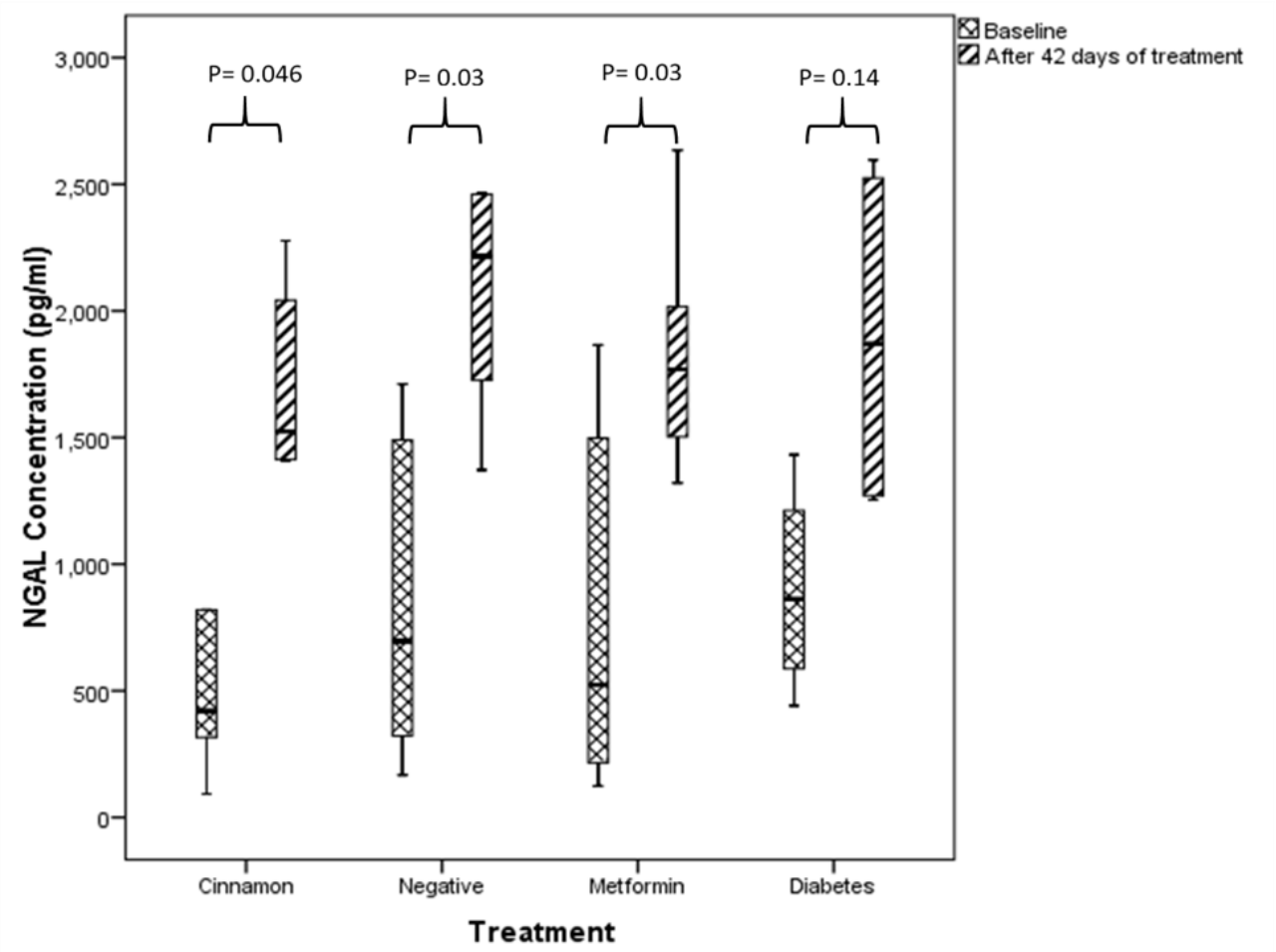

Fig. 2. NGAL concentration of the Wistar rats treated with cinnamon, metformin, negative control and diabetes-induced control for 42 days.

Table 1. Insulin resistance of treatment groups

Treatment group	Insulin resistance at baseline*	Insulin resistance after 42 days of treatments*	P value (baseline vs 42 days after the treatments)
Cinnamon	0.0015 (0.0012-0.0026)	0.0016 (0.0006-0.0045)	0.8
Metformin	0.0007 (0.0005 -0.0018)	0.0015 (0.0009-0.0033)	0.3
Negative Control	0.0005 (0.0004 -0.0008)	0.0010 (0.0008 -0.0018)	0.1
Diabetes rats without treatments	0.0016 (0.0010-0.0045)	0.0064 (0.0019-0.0115)	0.1

* Values are as Median (IQR)

Table 2. Results of liver function tests (14)

Liver function tests with reference values	Median (IQR)			
	Cinnamon-treated group	Metformin-treated group	Negative Control	Non-treated Diabetes rats
AST (50-150)* (U/l)	50.63 (30.85-54.13)	67.51 (59.36-77.12)	73.9 (63.6 - 91.1)	40.2 (18.8-51.5)
ALT (10-40)* (U/l)	21.83 (13.97-29.1)	20.95 (18.33-34.92)	38.9 (35.9 -41.2)	29.4 (22.3-40.0)
ALP (30-130)* (U/l)	92.82 (36.76-117.63)	39.52 (28.49-78.12)	57.4 (29.2-110.5)	38.59 (9.2-79)

group (Fig. 4-A), cinnamon-treated group (Fig. 4-B), the metformin-treated group (Fig. 4-C) and the diabetic non-treated group (Fig. 4-D). Further, no proteinaceous secretion within tubules and no inflammatory cell infiltrate were observed in all groups. No thickening of vessel walls or alterations in blood vessels were observed in all treatment groups. In contrast, congested blood vessels in the interstitium of the kidney were observed in one rat in the cinnamon-treated group.

Bone marrow microscopic examination: Peripheral blood smears and smears of bone marrow aspirate were examined. A comprehensive morphological examination revealed no dysplasia, maturation arrest, hypoplasia, aplasia, necrosis, or increased blasts in any of the smears examined.

Discussion

This study revealed that dried cinnamon bark aqueous

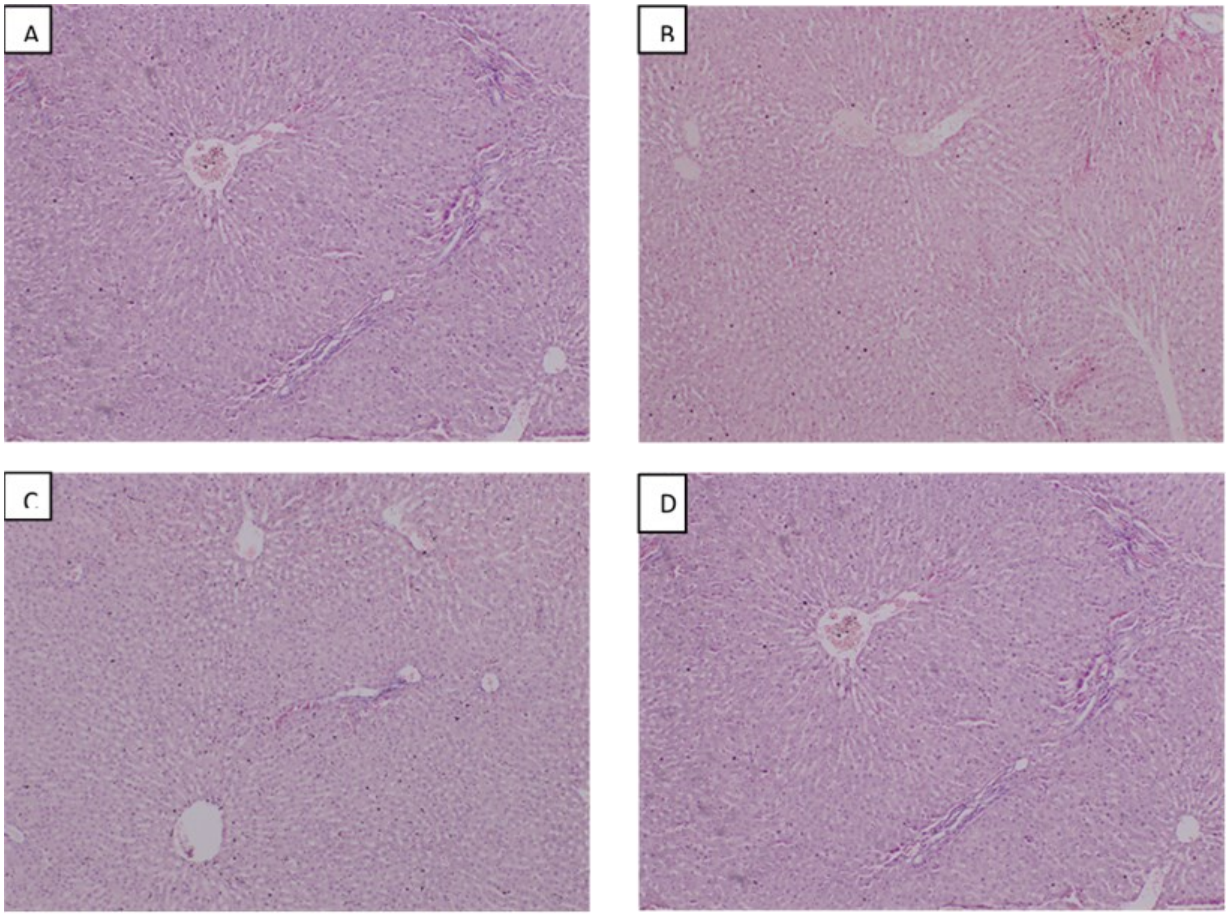

Fig. 3. Microscopic observation of liver (x10). **A**- Negative control; **B**- Cinnamon-treated group; **C**- Metformin-treated group; **D**- Diabetes non-treated group.

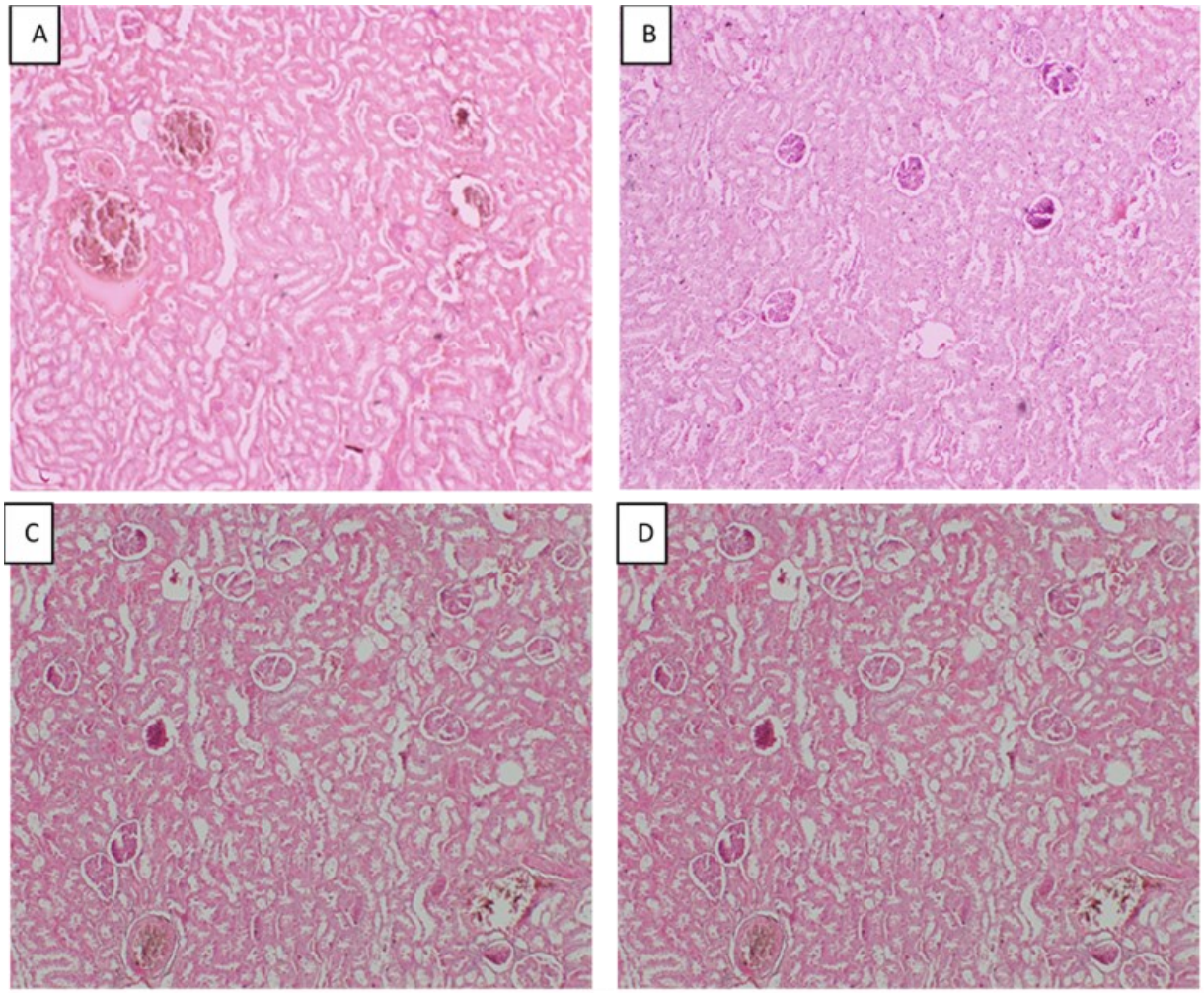

Fig. 4. Microscopic observation of kidney (x10). **A**-Negative control; **B**- Cinnamon-treated group; **C**-Metformin-treated group; **D**-Diabetes non-treated group.

extract at a dose of 20 mg/kg/day can reduce FBG of diabetes-induced rats. It is important to see that the reduction of FBG from cinnamon bark powder is not significantly different from that of FBG with metformin (positive standard). Furthermore, a study reported that a daily oral dose of 20 mg/kg of cinnamaldehyde one week before mating showed that cinnamaldehyde has a safe anti-diabetic action on gestational diabetes (15). Moreover, it was also reported from another study that treatment with cinnamon extract led to a significant decrease in FBG levels in the diabetic rats in the fourth week of the trial and in both at 0.20 g/kg bw and at 0.10 g/kg bw diabetes induced rat groups (16). Previously, It was also found that cinnamon extract improved glucose tolerance in type 2 diabetes rats treated with *C. zeylanicum* using oral glucose tolerance tests at 100 mg/kg/day (17). Another study has concluded that supplementation with cinnamon, in concentrations of 3 grams per day, without added sugar, can reduce HbA1c, fasting venous glycemia, and HOMA-IR index levels in type 2 diabetes patients (18).

In another study, it was observed that administration of 4 g/kg body weight of cinnamon (*C. cassia*) extract powder can reduce serum TC, triglyceride, and LDL by 31.22%, 24.05%,and 43.49%, respectively (19). Further, HDL has been increased by 30.16% in adult male rats. Another study found that the TG and TC levels in control and treated diabetic rats differed significantly after adding cinnamon powder in a 5% dose to the rats' regular diet for 28 days (20). In contrast, the current study did not show significant differences across categories of treatments in lipid profile. As the chemical composition of the cinnamon bark powder varies with the variety, geographical area and the growth stage of the plantation, which might be the reason for the the difference in results. However, a recent study suggests that cinnamon improves the glycemic and lipid profile and reduces the BMI, particularly in type 2 diabetic patients who receive cinnamon supplementation in capsule form and at a dose of ≤2 g/day (21).

To evaluate the adverse effects on the liver with the intervention, AST, ALT, and ALP were performed. The findings of the liver function tests were within the reference range (Table 2). A previous study has examined the effect of three dosages of 1, 2, and 4 mL of water extract of cinnamon (*C. zeylanicum*) on blood glucose (mg/dL), AST, ALT, and ALP (U/L) of diabetic obese rats (22). They have obtained the results showing that cinnamon supplementation in a dose-dependent decrease in serum glucose, AST, ALT, and ALP levels in obese diabetic rats.

The median (IQR) serum creatinine value of the cinnamon-treated arm was higher than the reference range. However, KIM-1 concentrations have reduced during the study (Fig. 1). KIM-1, also known as Kidney Injury Molecule-1, is a protein widely expressed in the renal proximal tubular epithelium. The concentration of KIM-1 in blood is a reliable indicator of acute kidney injury (AKI) and has become a promising biomarker for early detection and diagnosis of AKI (23). Elevated KIM-1 levels in blood are thought to result from increased release from damaged renal proximal tubular cells into the circulate (23). KIM-1 has been found to have higher sensitivity and specificity compared to traditional biomarkers for detecting kidney injuries, such as creatinine and blood urea nitrogen (24). However, this study showed a significant reduction between the KIM 1 concentration of the cinnamon-treated arm and the negative control. Therefore, it was determined that there was no renal damage because of the cinnamon treatment. In another study, it was shown that STZ-induced diabetic rats treated with cinnamon (*C. zeylanicum*) extract at a level of more than 30 mg/kg/d could recover from hyperglycemia and nephropathy (25). Further, no significant toxicity had been observed by cinnamon aqueous extracts during many studies related to cinnamon (26-28).

In recent years, NGAL has gained significant attention as a biomarker for various diseases, especially kidney injury and dysfunction. The concentration of NGAL in blood has been shown to increase rapidly (Fig. 2) and significantly in cases of AKI, making it a useful diagnostic tool for detecting an early onset of AKI. The increase in NGAL levels in blood is thought to reflect the damage to renal tubular cells, which are the main site of NGAL production (29). However, there is a significant increase in NGAL concentration in all treatment groups except the non-treated diabetes rat group. In contrast, the histopathology investigations of this study indicate that there are no visible changes in liver and kidney tissues due to the effects of toxicity. It is studied that there was only a weak relationship between the pathological pattern of injury and the biochemical categorization (30). Only one rat was observed with congested blood vessels in the kidney congested blood vessels, and focal hemorrhages surrounding the central vein in the liver due to heart failure.

It is known that active bone marrow is present in cancerous bones of the epiphysis and the medullary cavity of the diaphysis of long bones of rats (31). Therefore, an attempt was made to obtain satisfactory samples from the epiphysis of the femur and medullary cavities of the femur and tibia. There was no morphological evidence of adverse effects on the bone marrow of the study's Wistar rats, either from the diabetes-induced drug STZ, the antidiabetic medication metformin, or the herbal extracts at the dose given during the study.

However, the study was conducted using 6 Wistar rats per group. Therefore, it is necessary to repeat the experiments with a larger group of animals before going for clinical studies. HDL, Creatinine, AST, ALT and ALP were tested only after 42 days of the treatment as the blood volume obtained from the tail vein blood drawing technique is limited at baseline. Further, the authors would like to suggest that further investigations have to be conducted to determine whether the renal function alters because of long-term cinnamon consumption, even though no significant toxicity was observed during the study.

Conclusion

It can be concluded that crude cinnamon aqueous extracts can reduce FBG in diabetes-induced rats. The study revealed that aqueous extract of cinnamon bark powder at a dose of 20 mg/kg/day can reduce FBG of diabetes-induced rats. Notably, the reduction of FBG from cinnamon bark powder is not significantly different from the reduction of FBG with metformin. According to the liver function tests, it has been evident from the results that there is no liver toxicity of rats caused by cinnamon treatment.

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Authors' Contributions

All authors contributed to the study's conception, design, and supervision. DW and SJ were major contributors in planning the experiments, preparing first draft of the manuscript, and interpreting the data. VPB was the project's principal investigator, contributor in research progressing and was the major contributor in editing the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: The study adhered to strict ethical standards, as evidenced by the approval received from the Ethics Review Committee at the Faculty of Medicine, University of Ruhuna, Sri Lanka (2020, p. 112). All animal research protocols followed the guidelines outlined by the Council for International Organizations of Medical Sciences (CIOMS) international guiding principles for biomedical research involving animals in Sri Lanka.

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