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ORIGINAL ARTICLE

Hypoglycemic and Anti-lipidemic Properties of *Cinnamomum zeylanicum* ("Sri Wijaya" Accession) Water-soluble Nutraceutical in Streptozotocin-induced Diabetic and Healthy Wistar Rats

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ABSTRACT

Background: Hyperglycemia is a serious health crisis worldwide, and more than 90% of the Sri Lankan patients effected with the condition have diabetes mellitus type 2. Natural therapeutic agents can manage the progression of the disease. **Objectives:** To investigate the hypoglycemic and antilipidemic effects of *Cinnamomum zeylanicum* ("Sri Wijaya" accession) water-soluble nutraceuticals in diabetic-induced and healthy Wistar rats. **Materials and Methods:** The diabetic and nondiabetic Wistar rats were treated with Cinnamon pressured water, Cinnamon decoction, pretreated pressured water Cinnamon extract for 1 month. The results were compared with the group treated with the positive control, Acarbose untreated normal group. Blood glucose and other biochemical parameters were estimated using commercial test kits. **Results:** There was a significant difference in the fasting serum glucose, food consumption, and water consumption in rats with induced diabetes. The total cholesterol level was significantly decreased in the normal groups treated with Cinnamon extracts, compared with the untreated groups. There was a significant increase in high-density lipoprotein cholesterol levels in the normal group treated with pressured water Cinnamon extract and decoction when compared with the Acarbose-treated diabetic group. Alanine aminotransferase (ALT) levels were significantly higher in the diabetic group treated with Acarbose than in all Cinnamon-treated groups. However, no significant difference was shown in normal rat groups for aspartate aminotransferase and ALT. **Conclusion:** Cinnamon nutraceuticals have the potential to reduce hyperglycemia in diabetic rats. Cinnamon extracts may inhibit α-amylase and α-glucosidase enzymes in rat pancreatic tissues.

Key words: Blood glucose, *Cinnamomum zeylanicum*, diabetes mellitus, Wistar rats

SUMMARY

- Diabetes is a metabolic disease resulting from the deficiency of insulin secretion, action or both. Most studies on anti-diabetic properties of Cinnamon have been conducted using *Cinnamomum cassia* and no investigations have based on *Cinnamomum zeylaicum* Sri Wijaya accession
- Current study was aimed to conduct scientific investigation to establish the efficacy of Cinnamon Sri Wijaya quills extracts in controlling diabetics without adverse side effects
- FSG levels were significantly reduced in DM-PWE (30%) DM-DWE (30%), and DM-prePWE (22%) groups respectively and there were no any toxic effects of Cinnamon extracts observed in healthy Wistar rats
- Sri Wijaya accession of Cinnamon demonstrate ability to control hyperglycemia and cholesterol in the blood
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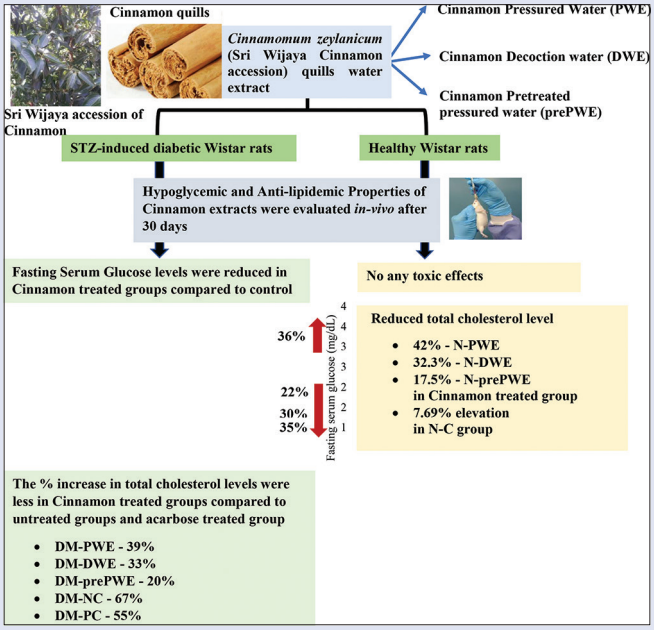

Abbreviations used: N-C: Normal control, N-PWE: Normal rats treated with pressured water Cinnamon extract, N-DWE: Normal rats treated with Cinnamon decoction, N-PrePWE: Normal rats treated with pretreated pressured water Cinnamon extract, DM-NC: Diabetic rats without any treatment, DM-PWE: Diabetic rats treated with pressured water Cinnamon extract, DM-DWE: Diabetic rats treated with Cinnamon decoction, DM-PrePWE: Diabetic rats treated with pretreated pressured water Cinnamon extract, DM-PC: Diabetic rats treated with Acarbose, FSG: Fasting serum glucose, TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, DM: Diabetes mellitus,

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DMT2: Diabetes mellitus type 2, CC: *Cinnamomum zeylanicum*, CCSW: *Cinnamomum zeylanicum* "Sri Wijaya" accession, CCSG: *Cinnamomum zeylanicum* "Sri Gemunu" accession, PWE: Cinnamon pressured water extract, DWE: Cinnamon decoction water extract, pre-PWE: Cinnamon pressured water extract with fungal pretreatments, STZ: streptozotocin, DMSO: Dimethyl sulfoxide.

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INTRODUCTION

Diabetes mellitus (DM) is considered a complex chronic disorder with elevated blood glucose levels. The prevalence of diabetes in the adult population in Sri Lanka is 8.7% in the year 2019.[1] Diabetes imposes a significant financial burden on the affected population. The estimated annual cost for diabetic treatments and prevalence in the world was $ 327 in the year 2017.[2] The demise of β-cell and/or their dysfunction, insulin secretion deficiency, insulin resistance, overweight/obesity, inadequate physical activity, age, and smoking are major risk factors for high glucose and altered blood lipid profiles.[3] Type 1 diabetes or insulin-dependent diabetes accounts for 5%–10% of the total diabetic population, while DM Type 2 (DMT2) diabetes constitutes 90%–95% of the selected population.[2]

Agents such as thiazolidinediones, sulfonylurea, metformin, α-glucosidase inhibitors, and glucogen-like peptide 1 receptor agonists have been approved for the management of type 2 diabetes.[4,5] However, the long-term usage of these agents is problematic because of their high cost, adverse side effects, and inefficiency after prolonged use. To overcome these deficiencies, attention has been given to a number of natural potential diabetic preventive agents. Plant-derived extracts or phytochemicals and lifestyle modification were evaluated as alternative therapies for the management of DMT2. Adequate physical activities, healthy body weight, avoiding tobacco usage, higher intake of nuts,[6] berries,[7] yogurt,[8] coffee, and tea[9] are associated with a reduction in blood glucose elevation. Hence, the use of synthetic drugs for the treatment for a diabetic has shifted to natural remedies due to its minor side effects, availability, low cost, and durability.

In this study, the effects of *Cinnamomum zeylanicum* (CC, family Lauraceae), commonly known as Ceylon Cinnamon, which is indigenous to Sri Lanka, were evaluated in lowering blood glucose and improving lipid profiles. The plant contains various kinds of beneficial health effects such as anti-microbial activity,[10,11] blood glucose-reducing properties,[12-16] cardiovascular disease reducing properties,[14] risk of reducing chronic cancers,[17,18] and antioxidant activities.[19,20] Biologically, active compounds in Cinnamon possess insulin-mimicking properties and these compounds enhance the glucose uptake by activating the insulin receptor kinase activity, auto-phosphorylation of the insulin receptor, and glycogen synthase activity.[21-25] Furthermore, the plant extract exhibited an anti-diabetic effect independent of insulin by upregulating uncoupling protein 1 and glucose transporter 4.[26]

Two accessions of *C. zeylanicum,* named "Sri Wijaya" (CCSW) and "Sri Gemunu" (CCSG), have been developed[27] for commercial cultivation based on their yield and quality performance (https://www.lankabusinessonline.com/sri-lanka-develops-new-cinnamon-varieties/, accessed, 19.06.2020). Our previous studies have revealed that pressurized water extract (PWE), decoction water extract (DWE), and pressurized water extract with *Trichoderma harrzianum* (MH298760) pretreatments (pre-PWE) of "Sri Wijaya" accession has significant α-glucosidase and α-amylase inhibition properties *in vitro* (unpublished observations). Our studies have identified that the major compound present in PWE, and DWE of SW is Cinnamyl alcohol (unpublished observations). Clinical evidence regarding the effects of the indigenous variety of Ceylon Cinnamon pressured water, decoction, and pretreated pressured water extracts on blood glucose and lipid profiles has not been reported. In this study, we investigated the anti-diabetic and anti-dyslipidemic effects of "Sri Wijaya" Cinnamon water extracts in normal and type 2 diabetic rat models, and explored whether the plant extracts could minimize diabetes-related complications in Wistar rat models.

MATERIALS AND METHODS

Sample collection

Dried quills of CCSW accession (10 kg) were collected from the Sub Cinnamon Research Station Wariyagala, Nillambe, Central Province, Sri Lanka. The botanist at the Department of Botany, University of Kelaniya, and the Sub Cinnamon Research Institute, Nillambe, have authenticated the plant material. The voucher specimen of CCSW was deposited at the publicly available herbarium, Department of Botany, the University of Kelaniya under the family Lauraceae (Deposition numbers is CIN-SV-001).

Nutraceutical preparation

Pulverized Cinnamon quills (10 g) were extracted with pressurized water (0.098 MPa, 200 mL for 10 min) for the preparation of PWE. For the preparation of the pre-PWE sample, pulverized Cinnamon quills (10 g) was treated with a pure culture of *Trichoderma harzianum* (MH298760) for 02 weeks. After the incubation period, the sample was extracted with pressurized water as above. For the preparation of DWE, Cinnamon quills (10 g) were boiled with water (200 mL) until the volume was reduced to 1/8. The water extract was filtered through a Walkman No. 1 filter paper and extracts were collected.

The pre-PWE nutraceutical was tested for any fungal contaminations with freshly prepared potato dextrose agar plates using the spread plate method.[28] Inoculated plates were incubated for five-seven days at room temperature (25°C ± 2°C) for the development of fungal colonies was observed. Samples without any fungal contamination were used for the animal experiments.

Animals and treatments

Wistar rat strain, (*Rattus norvegicus*, origin: Clea-Japan) bred at the Medical Research Institute (MRI) under aseptic conditions for research purposes were used for the animal experiments. Experimental rats were kept at 22°C–24°C and 40%–70% relative humidity. 12 h light-dark cycle was provided with filtered air by medium efficiency filters. Rat feed was prepared based on the WHO formula recommended by Saboudry[52] using locally available feed ingredients at the MRI. Sterilized wood shaving was used as the bedding material. All animal experiments were conducted in the sterilized animal experimental unit at the MRI.

Sample size calculation

Sample size per group = 2 SD^2 $(Z\alpha_{/2} + Z\beta)^2/d^2$[29]

SD: Standard deviation from the earlier study[30]

$Z\alpha_{/2} = Z_{0.05}/_2 = Z = 0.025 = 1.96$ (from the *Z* table) at the type 1 error of 5%

$Z\beta = Z = 0.20 = 0.842$ (from Z table) at the 80% power

d = effect size = the difference between mean values

Hence,

Sample size per group = 2 SD^2 $(1.96 + 0.842)^2/d^2$

$=2\ (74)^2\ (1.96 + 0.842)^2/100^2$

=8.59~9

Predictable attrition or death of animals: 10%

Corrected sample size = Sample size/(1− [% attrition/100])[29]

Corrected sample size = 9/0.9 = 10.

Inducing diabetes on Wistar rats

Healthy Wistar rats were induced with diabetes through a single intraperitoneal injection of newly prepared streptozotocin (STZ, Sigma-Aldrich, USA). The overnight fasted Wistar rats were injected with STZ at a single dose of 50 mg/kg.[31] Diabetes was confirmed 72 h after injecting STZ, in the STZ-treated Wistar rats by measuring the fasting blood glucose concentration as reported by Pushparaj *et al.* Wistar rats with fasting blood glucose levels above 200 mg/dL[32] were considered diabetic and they were used in the animal experiment.

Experimental design

Forty sex-balanced (1:1) 8 weeks old healthy Wistar rats, with a body weight range of 160 ± 2 g were selected. They were randomly divided into four groups using a random table (Group 1: normal control (N-C); Group 2: normal rats treated with pressured water Cinnamon extract (N-PWE); Group 3: normal rats treated with Cinnamon decoction (N-DWE); Group 4: normal rats treated with pretreated pressured water Cinnamon extract (N-prePWE), each experimental group had 10 rats. Fifty sex-balanced (1:1) 12 weeks old diabetic-induced Wistar rats, with a bodyweight range of 205 ± 3 g, were selected. Rats were randomly divided into five groups (Group 1: diabetic rats without any treatment (DM-NC); Group 2: diabetic-induced rats treated with pressured water Cinnamon extract (DM-PWE); Group 3: diabetic-induced rats treated with Cinnamon decoction (DM-DWE); Group 4: diabetic-induced rats treated with pretreated pressured water Cinnamon extract (DM-prePWE), and Group 5: diabetic-induced rats treated with Acarbose (DM-PC) with six rats per group.

All rats were kept in standard polypropylene rat cages and three-four Wistar rats were assigned to each experimental cage. All experimental rats had access to water from the respective water sources *ad libitum*.

The study was conducted in two stages (Stage 1 and Stage 2) as in healthy and diabetic-induced Wistar rats, respectively. Stage 1 evaluated the toxic effects on the liver and kidneys of healthy animals. Food consumption, water consumption, body weight, fasting serum glucose (FSG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum creatinine were evaluated over 1-month duration for both healthy as well as diabetic-induced rats. Stage 1 was conducted using four groups (groups 1, 2, 3, and 4), while five groups were recruited for stage 2 (groups 1, 2, 3, 4, and 5). Distilled water was administered for Group 1. Groups 2, 3, and 4 received Cinnamon nutraceuticals, extracted by pressurized water (PWE), decoction (DWE), and extracted by pretreated pressurized water (pre-PWE), respectively. Acarbose was given as a positive control for Group 5.[31]

Calculation of dosage and administration

The typical human daily dose of acarbose is 300 mg/60 kg body weight.[31] According to the formula:

Drat = Dhuman × 0.71/0.11, the corresponding dose of acarbose for rats is 32.28 mg/kg/day.[31]

Cinnamon extracts were administered to experimental rats via a gastric lavage method using an oral sonde needle for a duration of 1 month by a qualified and experienced researcher. The daily dosage for Wistar rats is calculated according to the body surface area as shown below, using the human dosage (6 g) as per the previous study.[14]

Table 1: Effect of *Cinnamomum zeylanicum* extracts on blood glucose and lipid profile of healthy rats

Biochemical test	Mean (±SE)mg/dL			
	Group 1 (N-C)	Group 2 (N-PWE)	Group 3 (N-DWE)	Group 4 (N-prePWE)
FSG				
Day 0	81 (±3)	78 (±5)	94 (±3)	77 (±9)
Day 30	116 (±7)	141 (±5)	123 (±2)	115 (±13)
Difference mean value	35 (±8)[a]	64 (±2)[a]	32 (±4)[a]	43 (±5)[a]
TC				
Day 0	78 (±4)	69 (±5)	65 (±3)	57 (±4)
Day 30	84 (±6)	40 (±1)	44 (±3)	47 (±4)
Difference mean value	6 (±6)[a]	29 (±6)[b]	21 (±5)[b]	11 (±4)[b]
HDL-C				
Day 0	29 (±2)	25 (±2)	26 (±1)	27 (±1)
Day 30	25 (±2)	27 (±1)	29 (±2)	26 (±1)
Difference mean value	4 (±2)[a]	2 (±2)[b]	3 (±2)[b]	1 (±2)[a]
TG				
Day 0	212(±21)	142 (±4)	160 (±15)	138 (±9)
Day 30	212 (±6)	170 (±9)	177 (±9)	134 (±10)
Difference mean value	0.4 (±26)[a]	28 (±13)[b]	17 (±21)[b]	4 (±3)[a]
Creatinine				
Day 0	0.47 (±0.03)	0.46 (±0.02)	0.44 (±0.02)	0.43 (±0.02)
Day 30	0.55 (±0.02)	0.53 (±0.02)	0.5 (±0.00)	0.48 (±0.01)
Difference mean value	0.08 (±0.06)[a]	0.07 (±0.03)[a]	0.06 (±0.0)[a]	0.05 (±0.0)[a]

Data are presented as mean±SE (n=10); values within the same raw with different subscripts letters are significantly different at P<0.05. Normal control (N-C); Normal rats treated with pressured water Cinnamon extract (N-PWE); Normal rats treated with Cinnamon decoction (N-DWE); Normal rats treated with pretreated pressured water Cinnamon extract (N-prePWE); Fasting serum glucose (FSG); Total cholesterol (TC); Triglycerides (TG); High density lipoprotein cholesterol (HDL-C).

Table 2: Effect of *Cinnamomum zeylanicum* extracts on AST and ALT profile in healthy rats

Biochemical test	Mean (±SE)U/L			
	Group 1 (N-C)	Group 2 (N-PWE)	Group 3 (N-DWE)	Group 4 (N-prePWE)
AST				
Day 0	126 (± 7)	90 (±8)	112 (±5)	103 (±6)
Day 30	83 (±5)	76 (±3)	92 (±4)	76 (±2)
Difference mean value	43 (±10)[a]	14 (±10)[a]	20 (±9)[a]	27 (±8)[a]
ALT				
Day 0	44 (±3)	35 (±1)	45 (±3)	42 (±2)
Day 30	40 (±2)	38 (±2)	41 (±1)	32 (±1)
Difference mean value	4 (±3)[b]	3 (±1)[a]	4 (±3)[b]	10 (±3)[b]

Data are presented as mean±SE (n=10); values within the same raw with different subscripts letters are significantly different at P<0.05. Normal control (N-C); Normal rats treated with pressured water Cinnamon extract (N-PWE); Normal rats treated with Cinnamon decoction (N-DWE); Normal rats treated with pretreated pressured water Cinnamon extract (N-prePWE); Alanine Aminotransferase (ALT); Aspartate Aminotransferase (AST).

Table 3: Effect of *Cinnamomum zeylanicum* extracts on blood glucose and lipid profile of diabetic rats

Biochemical test	Mean (±SE)mg/dL				
	Group 1 (DM-NC)	Group 2 (DM-PWE)	Group 3 (DM-DWE)	Group 4 (DM-prePWE)	Group 5 (DM-PC)
FSG					
Day 0	306 (±15)	340 (±22)	327 (±27)	363 (±12)	340 (±14)
Day 30	416 (±17)	238 (±17)	230 (±25)	285 (±17)	222 (±23)
Difference mean value	109 (±20)c	102 (±40)[a]	97 (±16)[a]	78 (±18)[a]	118 (±30)[b]
TC					
Day 0	36 (±1)	57 (±2)	43 (±3)	56 (±3)	40 (±3)
Day 30	60 (±3)	79 (±5)	57 (±4)	67 (±9)	62 (±7)
Difference mean value	24 (±4)[b]	23 (±5)[b]	14 (±3)[a]	11 (±11)[a]	22 (±5)[b]
HDL-C					
Day 0	27 (±1)	32 (±2)	32 (±1)	29 (±1)	27 (±1)
Day 30	33 (±3)	43 (±5)	34 (±2)	34 (±3)	31 (±1)
Difference mean value	6 (±2)[a]	11 (±5)[a]	1 (±3)[a]	5 (±3)[a]	4 (±2)[a]
TG					
Day 0	123 (±10)	158 (±8)	152 (±10)	173 (±11)	119 (±10)
Day 30	178(±15)	126 (±10)	124 (±16)	202 (±20)	124 (±16)
Difference mean value	55 (±13)[b]	32 (±16)[a]	28 (±13)[a]	25 (±32)[b]	5 (±124)[ab]
Creatinine					
Day 0	0.53 (±0.02)	0.55 (±0.02)	0.52 (±0.03)	0.57 (±0.05)	0.55 (±0.02)
Day 30	0.72 (±0.02)	0.58 (±0.04)	0.64 (±0.02)	0.77 (±0.05)	0.65 (±0.03)
Difference mean value	0.18 (±0.03)[ab]	0.03 (±0.03)[a]	0.11 (±0.03)[ab]	0.2 (±0.05)[b]	0.1 (±0.04)[ab]

Data are presented as mean±SE (n=6); values within the same raw with different subscripts letters are significantly different at P<0.05. Diabetic rats without any treatment (DM-NC); Diabetic rats treated with pressured water cinnamon extract (DM-PWE); Diabetic rats treated with Cinnamon decoction (DM-DWE); Diabetic rats treated with pretreated pressured water Cinnamon extract (DM-prePWE); Diabetic rats treated with Acarbose (DM-PC); Fasting serum glucose (FSG); Total cholesterol (TC); Triglycerides (TG); High density lipoprotein cholesterol (HDL-C)

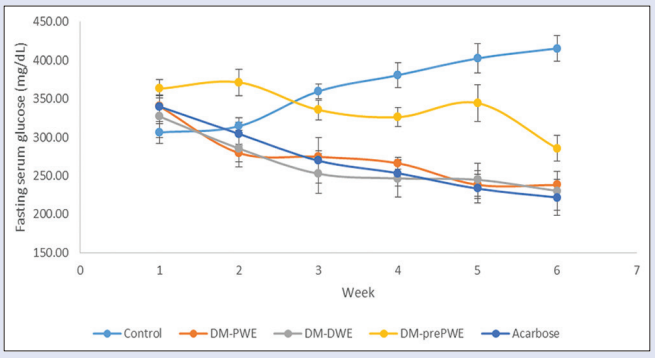

Figure 1: Mean fasting serum glucose of diabetic-induced rats during the study period. Data are presented as mean ± standard deviation (n = 6); DM-NC, Control: Diabetic rats without any treatment; DM-PWE: Diabetic rats treated with pressured water cinnamon extract; DM-DWE: Diabetic rats treated with Cinnamon decoction; DM-prePWE: Diabetic rats treated with pretreated pressured water Cinnamon extract; DM-PC: Diabetic rats treated with Acarbose

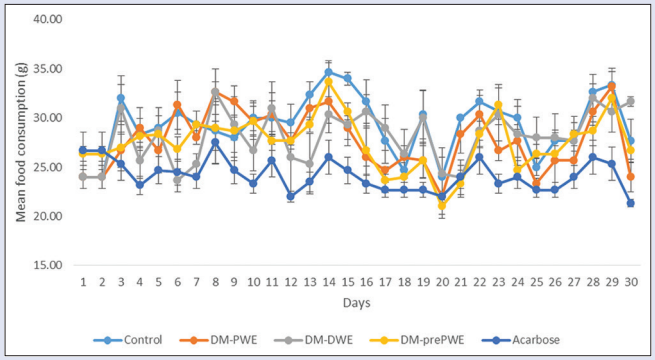

Figure 2: Mean food consumption of diabetes-induced rats during the study period. Data are presented as mean ± SE (n = 6); DM-NC, Control: Diabetic rats without any treatment; DM-PWE: Diabetic rats treated with pressured water cinnamon extract; DM-DWE: Diabetic rats treated with Cinnamon decoction; DM-prePWE: Diabetic rats treated with pretreated pressured water Cinnamon extract; DM-PC: Diabetic rats treated with Acarbose

Table 4: Effect of *Cinnamomum zeylanicum* extracts on AST and ALT profile in diabetic rats

Biochemical test	Mean (±SE)U/L				
	Group 1 (DM-NC)	Group 2 (DM-PWE)	Group 3 (DM-DWE)	Group 4 (DM-prePWE)	Group 5 (DM-PC)
AST					
Day 0	93 (±3)	69 (±5)	78 (±3)	132 (±10)	76 (±3)
Day 30	188(±22)	145 (±26)	228 (±21)	155 (±16)	98 (±14)
Difference mean value	95 (±23)^{ab}	76 (±29)^{ab}	150 (±22)^{b}	23 (±24)^{a}	23 (±16)^{a}
ALT					
Day 0	51 (±2)	54 (±3)	52 (±2)	70 (±2)	50 (±1)
Day 30	84 (±6)	85 (±9)	117 (±13)	125 (±9)	267 (±17)
Difference mean value	34 (±8)^{a}	30 (±10)^{a}	47 (±15)^{a}	62 (±12)^{a}	217 (±19)^{b}

Data are presented as mean±SE (n=6); values within the same raw with different subscripts letters are significantly different at P<0.05. Diabetic rats without any treatment (DM-NC); Diabetic rats treated with pressured water cinnamon extract (DM-PWE); Diabetic rats treated with Cinnamon decoction (DM-DWE); Diabetic rats treated with pretreated pressured water Cinnamon extract (DM-prePWE); Diabetic rats treated with Acarbose (DM-PC); Alanine Aminotransferase (ALT); Aspartate Aminotransferase (AST).

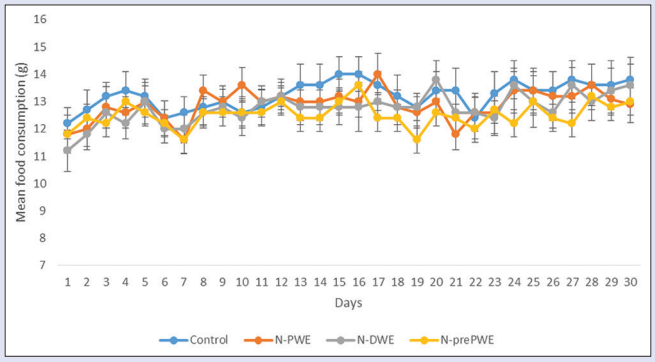

Figure 3: Mean food consumption of healthy rats during the study period. Data are existing as mean ± standard error (n = 10); N-C: Normal control; N-PWE: Normal rats treated with pressured water Cinnamon extract; N-DWE: Normal rats treated with Cinnamon decoction; N-pre-PWE: Normal rats treated with pretreated pressured water Cinnamon extract

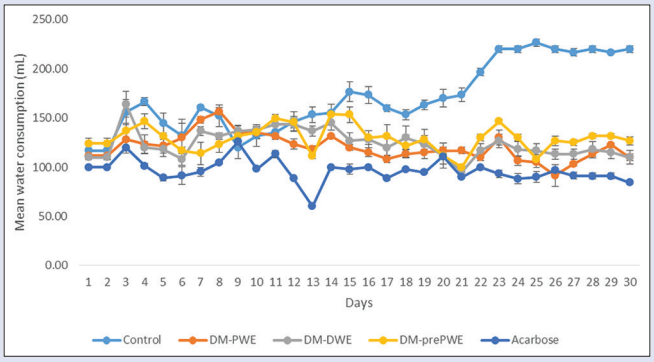

Figure 4: Mean water consumption of diabetes-induced rats during the study period. Data are presented as mean ± standard deviation (n = 6); DM-NC, Control: Diabetic rats without any treatment; DM-PWE: Diabetic rats treated with pressured water cinnamon extract; DM-DWE: Diabetic rats treated with Cinnamon decoction; DM-pre-PWE: Diabetic rats treated with pretreated pressured water Cinnamon extract; DM-PC: Diabetic rats treated with Acarbose

Rat dose (mg/kg) = Human dose (mg/kg) × (Human *Km* factor/Rat *Km* factor)[30,33]

Biochemical study

Experimental rats were sedated using isoflurane anesthesia to immobilize them and the rat tail was inserted into a clean container containing slightly warm water (40°C) for one min.[34] Mildly sedated rats were placed in a rat holder. One mL blood was drawn from the lateral tail vein of each Wistar rat, using a 23G sterilized needle and a one mL sterilized syringe as Waynforth and Flecknell.[49] Blood samples were collected at day zero (D_0, before the treatments) and Day 30 (D_{30}, 1 month after the treatment) by a qualified veterinarian. A one mL blood aliquot was collected into a sterilized Eppendorf tube and subsequently, serum was separated by centrifugation at 12,000 rpm for five min. The serum samples were stored at −20°C until further analysis. All experiments were carried at the noninfectious animal experimental laboratory in the MRI. Serum samples were used for testing, fasting serum glucose (FSG), creatinine, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) using standard test kits. At the end of the experimental period, all the experimental rats were humanely euthanized using CO_2 anesthesia.

Statistical analysis

Statistical analysis was performed using the standard Statistical Package for the Social Sciences (Armonk, New York, United States) (version 23). Baseline parameters of biochemical data (D_0) were compared with the values obtained after 1 month (D_{30}) of the experimental period using the paired t-test. The comparison of the data obtained from different test groups and control groups after 1 month of the experimental period was performed using one-way ANOVA with *post hoc*.

Ethics clearance

Ethics approval for the animal study was obtained from the Ethics Review Committee of the MRI, Colombo 8, Sri Lanka, and the ethics approval number was 20/2018. Rats were handled in accordance with the guiding principle provided by the International Council for Laboratory Animal Science (ICLAS) and animal experiments were conducted at the local settings in Sri Lanka.

RESULTS

Experimental animal's gender variation was not considered, as no significant differences were observed in the biochemical parameters. The values of FSG, TC, HDL-C, TG, and creatinine for the healthy and diabetic-induced rats, are shown in Tables 1 and 3, respectively. AST and ALT values are shown in Tables 2 and 4 for healthy and diabetic-induced rats, respectively.

The major reduction in TC was observed in the groups of rats treated with N-PWE (42%) followed by the groups, N-DWE (32.3%), and N-prePWE (17.5%), respectively [Table 3]. A 7.69% elevation in TC was

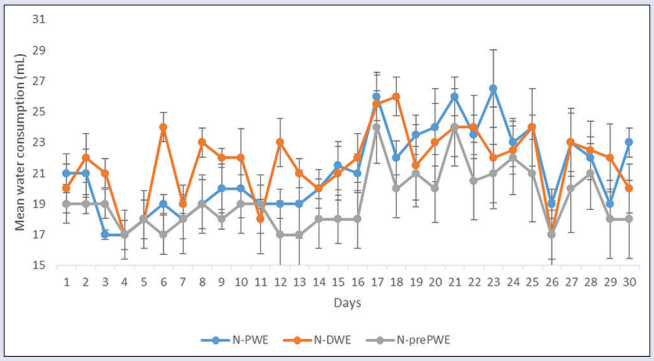

Figure 5: Mean water consumption of healthy rats during the study period. Data are existing as mean ± standard deviation (n = 10); N-C: Normal control; N-PWE: Normal rats treated with pressured water Cinnamon extract; N-DWE: Normal rats treated with Cinnamon decoction; N-pre-PWE: Normal rats treated with pretreated pressured water Cinnamon extract

shown in the N-C group. A significant increase was observed in HDL-C in all the Cinnamon-extract treated normal groups. The values were 8%, 11.5%, and 3.7% for the N-PWE, N-DWE, and N-prePWE, respectively. This is in comparison to, a 13.79% reduction in HDL-C observed in the N-C group [Table 1]. A significant difference was not observed in fasting serum glucose FSG, TG, creatinine, AST, and ALT [Tab*les 1* and *2*] for normal rats treated with Cinnamon extracts.

The FSG level was significantly decreased in DM-PWE (30%) DM-DWE (30%), and DM-pre-PWE (22%). The results were compared with the DM-PC group (35%). There was a significant increase in FSG in DM-NC (36%). The results are graphically illustrated in Figure 1.

The TC levels in all diabetes-induced rats were elevated after 30 days. However, the treatments were able to restore the elevation of cholesterol as the percent increase was significantly less in the treated group (DM-PWE (39%), DM-DWE (33%), and DM-prePWE (20%) compared to DM-NC and DM-PC (NC (67%) and DM-PC (55%), [Table 3]. A two-fold increase was shown in the DM-prePWE group for AST, while a fourfold significant increase was obtained for the DM-PC group for ALT [Table 4]. There were no major differences observed for HDL-C and creatinine in diabetic-induced rats.

Major differences in body weight, food consumption, and water consumption were observed only in diabetic-induced rats [Figures 2-4]. A reduction in body weight was shown in the DM-NC (3% reduction) than in the Cinnamon-treated groups and the Acarbose-treated group. The highest water consumption was shown in DM-NC (89%) and no significant changes were observed in other groups [Figures 2, 4 and 5]. Food intake was increased in DM-NC (15%) and DM-DWE (32%). However, a reduction in food consumption was observed in DM-PC (20%) [Figure 2].

DISCUSSION

Cinnamon products are popular as beneficial health-promoting functional foods around the globe. However, its beneficial and/or adverse side effects on human health have not been clinically investigated. Systematic analysis and evaluation of the antidiabetic properties of Sri Wijaya accession of Cinnamon have not been previously reported. The current study was the first effort to investigate the hypoglycemic effects of aqueous Cinnamon extracts and its effect on the liver, kidney, and pancreas using an *in vivo* rat model

It is a well-known fact that intraperitoneal administration of STZ in rats can significantly increase blood glucose levels four days after injection. In addition, other diabetes-related symptoms, such as reduction in body weight, polyurea, and polydipsia are common pathological signs that are induced. The diabetes-induced animals showed a significant reduction in mean food consumption and no differences were observed in water consumption at Day 0 and Day 30 in Cinnamon-treated groups. The results correlate with previous observations in similar models.[35-37]

Animal studies have demonstrated that Cinnamon and its active constituent cinnamaldehyde can improve hyperglycemia and hyperlipidemia in a dose-dependent manner in healthy and streptozotocin-induced diabetic rats.[13,38,39] Kwon *et al.* recently demonstrated that Cinnamon oil can protect the pancreas from streptozotocin-induced β-cell damage *in vivo* and *in vitro*.[40]

The fasting blood glucose level was increased by 8% in the diabetic-control group and a 5% reduction was observed in the Cinnamon-treated group.[30] In the current study the percentage decreased in FSG levels were significantly much higher (DM-PWE (30%) DM-DWE (30%), and DM-prePWE (22%), respectively. The reason for the difference could be attributed to the change in the method of preparation of the extracts. In our studies, all the extracts were prepared under mild conditions using short heating cycles compared to Soxhlet extraction. Therefore, heat-sensitive phytochemicals may have been preserved in the extract. Khan *et al.*, have used *Cinnamon cassia* powder to treat patients with Type II diabetic and the percentage reduction in FSG levels was comparable to the current study. However, in this study, significant differences of the FSG level were not observed in healthy controls. Similar to the observations of Khan *et al.*, a significant difference was not observed in FSG in the healthy rat groups treated with Cinnamon extracts.

Ranasinghe *et al.*, have evaluated the effects of *C. zeylanicum* crude water extract on diabetic, and lipids in both healthy and diabetes-induced rats. The results demonstrated that the total cholesterol level was lower after 30 days of treatment in both healthy (20.5%) and diabetes-induced (21%) animals after treatment with Cinnamon-extracts.[30] In our study, some treatment groups demonstrated better percentage reduction of TC, (42%, 32.3%, in N-PWE, N-DWE, respectively) and 17.5% in, N-prePWE-treated group. Hypocholesteremia is often associated with hyperglycemia and this implies that the Cinnamon extracts can reduce some of the complications associated with hyperglycemia.

The cholesterol-lowering properties of Cinnamon can be correlated with its antioxidant properties.[41] Several studies have confirmed that there is a positive correlation between antioxidants, such as polyphenols and the inhibition of α-glucosidase and α-amylase activities.[42,43] In our previous study, high contents of proanthocyanidin and total phenolic content were observed in PWE and DWE and can be correlated well with the FSG and TC-lowering effects observed in the current study. Hence, Cinnamon nutraceuticals can be effective as a food supplement in patients with hyperlipidemia and cardiovascular diseases.

Natural products are an abundant source of hypoglycemic agents. In screening for potential hypoglycemic agents from natural products, α-amylase and α-glucosidase inhibitory potentials are assessed. Enzymes such as α-amylase, alpha-glucosidase, and lipase are known to digest dietary polysaccharides and lipids into monosaccharides and free fatty acids. Actions of these enzymes cause postprandial hyperglycemia in humans.[44,45] Our previous studies have indicated that Cinnamon extracted using pressured water, Cinnamon decoction and pretreated Cinnamon with the fungus *T. harzianum* remarkably improved the α-glucosidase and α-amylase inhibitory potential. The novel forms of extractions used in the current study are safer and environmentally friendly methods to prepare Cinnamon extracts for the control of

postprandial hyperglycemia in an animal model.

Most studies on the medicinal properties of Cinnamon have been conducted using Chinese cassia (*Cinnamomum cassia*), and relatively few investigations have been based on *C. zeylanicum*. *Cinnamomum cassia* contains a high percentage of the alkaloid coumarin, which is a liver toxin and carcinogenic.[46] In contrast, the percentage of coumarin in Ceylon Cinnamon is low.[47,48] Hence, Ceylon Cinnamon has a very high potential as a pharmaceutical ingredient. Furthermore, the pathological investigation of a recent study demonstrated that there are no toxic effects on Ceylon Cinnamon.[30]

CONCLUSIONS

The outcomes of the present study revealed that Cinnamon nutraceuticals have the potential to reduce hyperglycemia in STZ induced diabetic rats. Cinnamon extracts may inhibit α-amylase and α-glucosidase enzymes in rat pancreatic tissues. This therapeutic approach may help to control and prevent hyperglycemia in human beings.

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Conflicts of interest

There are no conflicts of interest.

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