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BASIC INVESTIGATION

Antihyperglycemic and antihyperlipidemic action of cinnamaldehyde in C57blks/j Db/db mice

Juane Li, Tonghua Liu, Lei Wang, Xiangyu Guo, Tunhai Xu, Lili Wu, Lingling Qin, Wen Sun

Juane Li, Shaanxi Provincial People's Hospital, Xi'an, Shaanxi 710068, China
Tonghua Liu, Lei Wang, Xiangyu Guo, Tunhai Xu, Lili Wu, Lingling Qin, Wen Sun, Beijing University of Chinese Medicine, Beijing 100029, China
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Correspondence to: Prof. Tonghua Liu, Science and Technology Department, Beijing University of Chinese Medicine, Beijing 100029, China. thliu@163.net
Telephone: +86-10-64286642
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Abstract

OBJECTIVE: To investigate the effects of cinnamaldehyde (CA), an active and major compound in cinnamon, on glucose metabolism and insulin resistance in C57BLKS/J db/db mice.

METHODS: Sixteen male C57BLKS db/db mice were randomly divided into control and CA treatment groups. CA was given (20 mg·kg^{-1}·day^{-1}, p.o.) for 4 weeks. Pure water was given to control and db/+ mice. Subsequently, the levels of fasting blood glucose (FBG), fasting serum insulin, triglyeride, cholesterol, low-density lipoprotein -cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and free fatty acids (FFA), as well as the mRNA content of adiponectin and tumor necrosis factor (TNF)-α in adipose tissue, glucose transporter type 4 (GLUT-4) in skeletal muscle, and protein expressions of Akt, phospho-Akt (Thr308), AMPKα, phospho-AMPKα (Thr172) in skeletal muscle were measured.

RESULTS: 1) CA decreased serum levels of FBG and insulin as well as body weight in db/db mice; 2) CA increased serum HDL-C levels; 3) CA significantly decreased the mRNA expression of TNF-α in adipose tissue and upregulated mRNA expression of GLUT-4 in skeletal muscle; 4) protein expression of p-Akt was increased in CA-treated mice, but Akt, AMPKα and p-AMPKα showed no change.

CONCLUSION: CA has antihyperglycemic and antihyperlipidemic actions in db/db mice and could be useful in the treatment of type-2 diabetes.

Key words: Cinnamaldehyde; Diabetes mellitus, Type 2; Insulin resistance

INTRODUCTION

Type-2 diabetes mellitus (T2DM) is a chronic metabolic disorder known to have affected »4% of adults aged ≥ 20 years in 1995, and is expected to affect 380 million by 2025.[1] The cause of T2DM is not known, although genetic and environmental factors (e.g. obesity, lack of exercise) appear to play a part. T2DM occurs if the pancreas does not produce sufficient amounts of insulin or if the body cannot effectively use the insulin it produces.[2] Insulin resistance is a major contributor to the pathogenesis of T2DM and has a key role in associated metabolic abnormalities such as hyperlipidemia and hypertension.[3]

Several types of anti-diabetic medicines have been studied for their ability to lower blood glucose and ultimately lower the prevalence of secondary complications of this disease. There is growing interest in folk remedies using herbs due to the side effects associated

with the synthetic medicines used in the treatment of T2DM. Cinnamon is the bark of Cinnamomi cassise (Lauraceae). It is a traditional folk medicine used in China, Korea and Russia for the treatment of T2DM.4 Interest in this herb has increased since the discovery of its potential effects on insulin5 and its ability to reduce levels of fasting blood glucose (FBG) and plasma lipids in humans.[6] Subsequently, some studies have reported that cinnamon extract decreases blood glucose,[7] improves glucose uptake in adipocytes,[8] and improves the function of pancreatic islets[9] in Wistar rats, as well as slowing down absorption of carbohydrates in the small intestine.[10] Cinnamon extract significantly increases insulin sensitivity possibly by regulating the peroxisome proliferator-activated receptor (PPAR)-medicated metabolism of glucose and lipids[11] as well as increasing the amount of proteins involved in insulin signaling, glucose transport, and anti-inflammatory/anti-angiogenesis responses.[11,12]

The active compounds of cinnamon have been reported. These include water-soluble polyphenol type-A polymers,[8,11,13] cinnamaldehyde (CA)[14-17] and procyanidin oligomers.[18] As a major and effective compound isolated from cinnamon,[19,20] CA possesses anti-hypoglycemic and anti-hypolipidemic effects in streptozotocin (STZ)-induced T2DM in rats[14,17] and improves the function of pancreatic islets.[9] However, reports on the effect of CA on the metabolism of glucose and lipids in T2DM are lacking. The aim of the present study was to investigate the effect of CA in db/db mice, which are used as models of obesity, insulin resistance and T2DM.

METHODS

Ethical approval of the study protocol

The experimental protocols were approved by the Animal Care and Use Committee of Beijing University of Chinese Medicine (Beijing, China).

Animals

Sixteen 7-week-old male BKS. Cg-+ Leprdb/+ Leprdb/Jcl (db/db) mice and 8 non-diabetic littermate control mice (db/+) were supplied by the Model Animal Resource Center of Nanjing University (Nanjing, China). The animals were kept in the air-conditioned Animal House of the Beijing University of Chinese Medicine at 25° C-30° C and 45%-55% relative humidity. They were fed standard food (Beijing Ke Ao Xie Li, Beijing, China) ad libitum under a 12 h light-dark cycle throughout the study.

Oral administration

After being fed for 1 week, 16 db/db mice were divided into two equal groups: CA and control. Mice in the CA group received CA solution (0.5% dimethyl sulfoxide (DMSO), 20 $mg \cdot kg^{-1} \cdot day^{-1}$; Shanghai Winherb Medical Science, Shanghai, China). Mice in the db/+ group and control group were given an equivalent volume of pure water alone (0.5% DMSO) by intragastric administration once a day for 4 weeks. The CA dosage was chosen based on that used in three reports.[10,14,17]

Measurement of FBG, serum insulin, HOMA-IR, blood lipids and body weight

FBG was measured immediately with a Glucometer (ACC U-Check; Roche, Basel, Switzerland) in tail-vein blood (≈5 μL each time) after fasting for 8 h on days 7, 14, 21 and 28. After treatment for 4 weeks, mice were fasted overnight and anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood was collected from the abdominal aorta. Serum levels of insulin were assayed with an enzyme-linked immunoassay (ELISA) kit (Linco Research, St Charles, MO, USA). Indices of insulin resistance were calculated using the homeostasis model of insulin resistance (HOMA-IR) index[21] whereby:

HOMA-IR=fasting blood glucose level (mmol/L) × serum insulin level (ng/mL)/22.5

Serum levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and free fatty acids (FFAs) were measured using commercial kits (Beijing North Institute of Biological Technology, Beijing, China) with an Automatic Biochemical Analyzer (AU400 Biochemistry Analyzer, Olympus, Tokyo, Japan). Body weight was measured on days 7, 14, 21 and 28.

Preparation of total RNA

Frozen samples of quadriceps muscle and epididymal fat tissues were crushed in liquid nitrogen. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The relative purity of RNA was assessed by spectrophotometric means. The ratio of absorbance at 260 nm to that at 280 nm was 1.7-2.0 for all preparations. The integrity of RNA was confirmed in 1% agarose gel.

mRNA analyses:

Total RNA was reverse-transcribed by a Reverse Transfer kit (Beijing North Institute of Biological Technology) with oligo dT primers following the manufacturer's instructions. The sequences of sense and antisense primers used for amplification are shown in Table 1.

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TNF-α, tumor necrosis factor-α, GLUT4, glucose transporter type 4.

Amplification was undertaken with an initial denaturation at 95°C for 5 min followed by 30 cycles at 95°C (30 s), 55°C (30 s), and then extension at 72°C (30 s). The polymerase chain reaction (PCR) products were separated on 1.0% agarose gel and stained with ethidium bromide. Glucose transporter type 4 (GLUT4) mRNA was measured by denaturation at 95°C for 2 min followed by 45 cycles of PCR (95°C for 20 s, 58°C for

25 s and 72°C for 30 s). Relative RNA levels were determined by analyzing the changes in SYBR green fluorescence during PCR using the $^{\Delta\Delta}$Ct method. To confirm amplification of specific transcripts, melting-curve profiles were produced at the end of each reaction. mRNA levels of all genes were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal control.

Table 1 Sequences of sense and antisense primers used for amplification

	Sense	Anti-sense
GAPDH (122 bp)	TGG AGA AAC CTG CCA AGT	GTT GCT GTT GAA GTC GCA
TNF-α (204 bp)	CGT AGC AAA CCA CCA AGT G	ATA GCA AAT CGG CTG ACG
Adiponectin (149 bp)	CAT TAT GAC GGC AGC ACT G	TCC TGA TAC TGG TCG TAG GTG
GLUT4 (100 bp)	GAG CCC CAG ATA CCT CTA CAT CAT	AGC TAG TGC GTC AGA CAC ATC AGA

Western blotting

The extracted protein samples of muscle tissues were separated on sodium dodecyl sulfate (SDS) polyacrylamide gels and electrotransferred onto polyvinylidene difluoride (PVDF) membranes. PVDF membranes were incubated with primary antibody (anti-Akt, anti-phospho-Akt (Thr 308), anti-AMPKα and antiphospho- AMPKα (Thr 172; Cell Signaling Technology, Danvers, MA, USA). Detection of immunoreactive bands was done using an Electrochemiluminescence kit (Beijing North Institute of Biological Technology). Densitometry was undertaken by scanning the radiographs using the Image J 1.4.3 system (Media Cybernetics, Bethesda, MD, USA).

Statistical analyses

Statistical analyses were undertaken using SPSS ver13.0 (SPSS, Chicago, IL, USA). Data are the mean± standard deviation. Comparisons among the groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. P<0.05 was considered significant.

RESULTS

Body weight

The changes in body weight after CA administration for 4 weeks are shown in Figure 1 Body weight in the db/+ group was significantly lower than that in the control and CA group during the 4-week treatment. Body weight in the control group was higher than that in the CA group on day 28 (P<0.05).

FBG levels

During the entire experiment, FBG levels in the control group and CA group were significantly higher that those in the db/+ group (Figure 2). On day 28, FBG levels in the CA group showed a significant reduction compared with the control group.

Serm levels of TG, TC, HDL-C, LDL-C and FFAs

There were significant differences in all of the lipid levels in the db/+ group compared with the control group and CA group (both P<0.01) (Figure 3). Serum concentrations of HDL-C in the CA group increased significantly as compared with the control group. However, no statistically significant differences in the levels of FFAs, TG, TC and LDL-C between the two groups was noted (P=0.16, 0.58, 0.07 and 0.40, respectively).

Serum levels of insulin and the HOMA-IR index

Serum levels of insulin and the HOMA-IR index in the CA group and control group were significantly higher than those in the db/+ group (Figure 4), and significant differences were observed between the CA group and control group.

Changes in the mRNA expression of adiponectin and tumor necrosis factor (TNF)-α in adipose tissue

Expression of adiponectin and TNF-α in white adipose tissue is shown in Figure 5. TNF-α mRNA levels in the CA group were significantly decreased as compared with those in the control group. However, there was no significant difference in adiponectin mRNA expression between the CA group and control group.

Changes in protein levels of Akt, p-Akt, AMPKα and p-AMPKα as well as mRNA expression of GLUT4 in muscle tissue

p-Akt protein expression and GLUT4 mRNA expression increased significantly in the CA group as compared with the control group (both P<0.05) (Figure 6). However, protein expression of Akt, p-AMPKα and AMPKα did not significantly change in the CA group as compared with the control group.

DISCUSSION

When genetic obesity is produced by complete knockout of the leptin receptor via the db mutation, db/db mice develop severe resistance to insulin, resulting in severe hyperglycemia and, ultimately, premature death due to insufficient insulin action. 23 In the KS genetic background, hyperglycemia secondary to severe obesi-

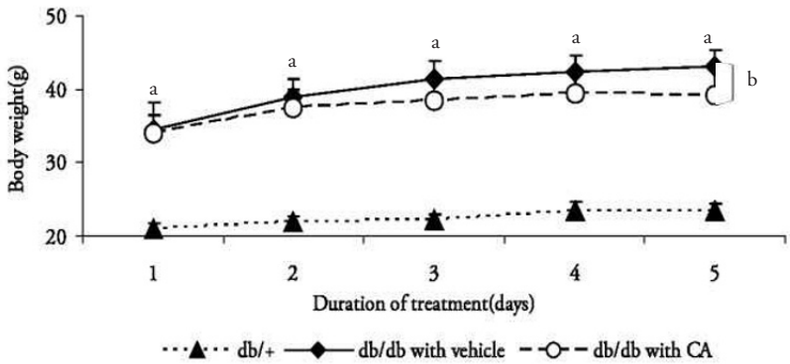

Figure 1 Effects of CA on body weight during 4-week treatment
CA group and control group compared with the db/+ group, [a]P<0.001; on day 28, the CA group compared with the control group, [b]*P*<0.05.

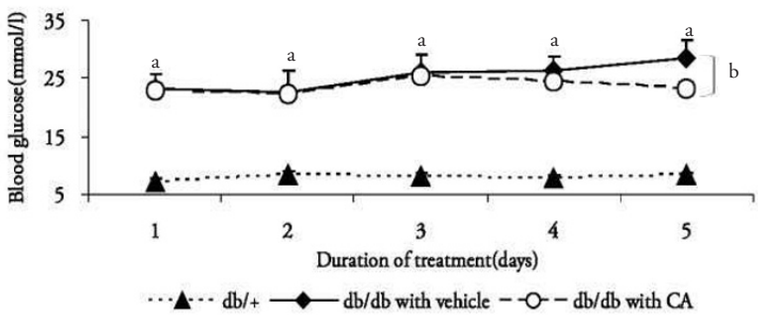

Figure 2 Effects of CA on fasting blood glucose levels during 4-week treatment
Control group and CA group compared with the db/+ group, [a]*P*<0.001; on day 28, the CA group compared with the control group, [b]*P*<0.05.

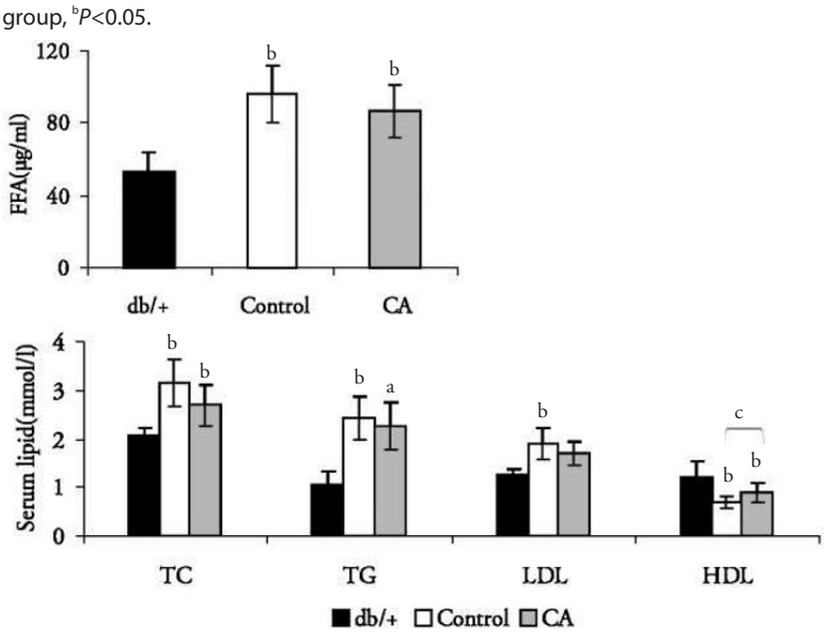

Figure 3 Serum levels of lipids and FFAs in the treatment groups
Compared with the db/+ group [b]*P*<0.01; compared with the control group, [c]*P*<0.05.

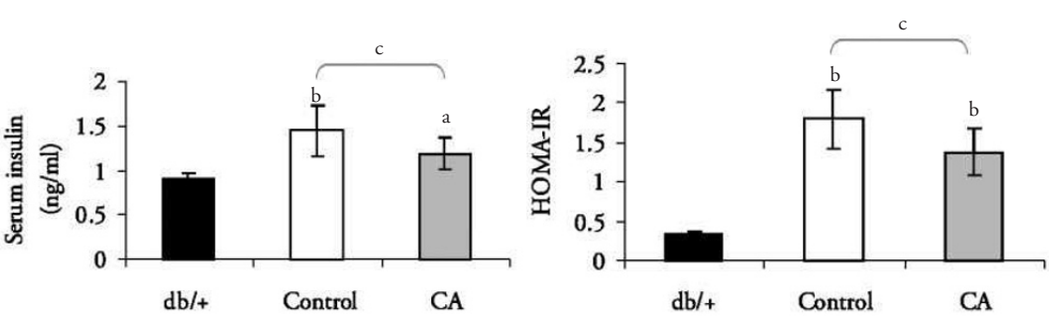

Figure 4 Effects of CA on serum levels of insulin and the HOMA-IR index
[a]*P*<0.05, [b]*P*<0.01 vs. the db/+ group; [c]*P*<0.05 vs. the control group.

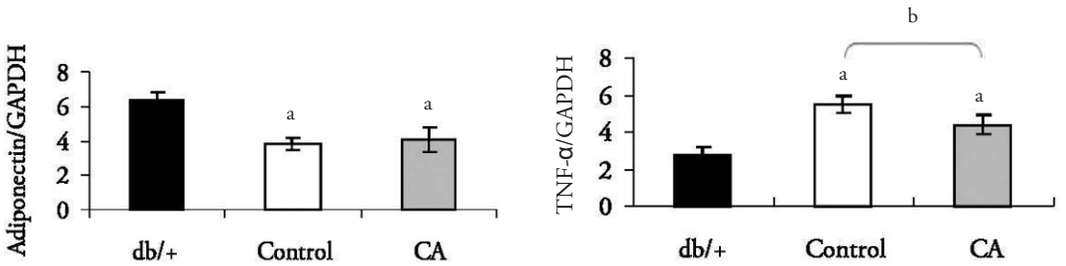

Figure 5 Effects of CA on mRNA expressions of adiponectin and TNF-α in adipose tissue
[a]*P*<0.01 vs. the db/+ group; [b]*P*<0.05 vs. the control group.

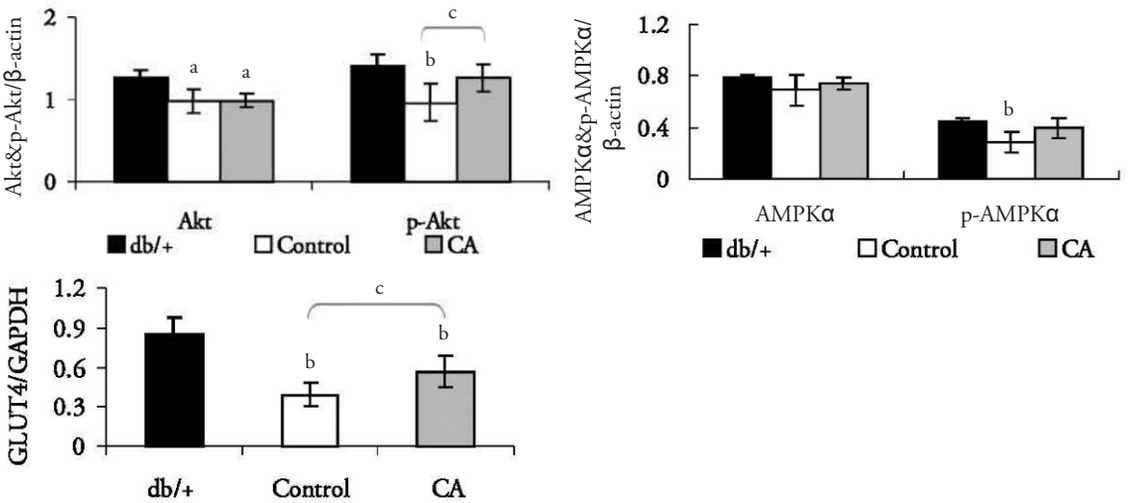

Figure 6 Effects of CA on protein expressions of Akt, p-Akt, AMPKα and p-AMPKα and mRNA of GLUT4 in skeletal muscle tissue
[a]*P*<0.05, [b]*P*<0.01 vs the db/+ group; [c]*P*<0.05 vs the control group.

ty-induced insulin resistance worsens with increase in age.[23] This particular inbred strain is an ideal model to investigate the effect of CA on glucose metabolism and insulin resistance in T2DM.

The results of the present study clearly showed that CA produced a mild hypoglycemic effect after 4-week treatment in mice. More importantly, CA treatment significantly decreased the body weight, serum insulin level and HOMA-IR index along with improvement in serum levels of HDL-C. The anti-diabetic effect of CA in db/db mice may be due to an improvement in insulin sensitivity and loss of body weight. These results suggest that CA has mild hypoglycemic and hypolipidemic effects in obesity and insulin resistance, in contrast to findings from previous reports.[14]

Insulin resistance is a major factor in the pathogenesis of T2DM. It is induced primarily by decreases in the insulin stimulation of glucose transport and metabolism in muscles and adipocytes.[24,25] Insulin-stimulated glucose transport is known to occur through translocation of GLUT4 from the intracellular pool to the plasma membrane. Signaling downstream of PI3-kinase (PI3K) to GLUT4 translocation appears to be mediated (at least in part) by serine/threonine protein kinase B (PKB/Akt)[26] which is activated by phosphorylation at Thr308 residues.[27] In db/db mice, glucose utilization in skeletal muscle decreases significantly due to major defects in the activities of PI3K, PKB/Akt as well as atypical protein kinase C (aPKC) activation accompanied by a significant reduction in GLUT-4 mRNA expression in the quadriceps muscle.[28,29] Although we did not observe insulin-stimulated Akt kinase activity or GLUT4 translocation, increases in the mRNA expression of GLUT4 and protein expression of p-Akt(Thr 308) in skeletal muscle suggested that the anti-diabetic effects of CA could be achieved by influencing the stimulation and translocation of GLUT4 in insulin-resistant skeletal muscle. These results may be consistent with those of Anand et al., which showed that CA results in the translocation of membrane GLUT4 in skeletal muscle after 60-day treatment in STZ-induced T2DM in rats.[17]

The adenosine monophosphate-activated protein kinase (AMPK) pathway plays an important part in regulation of the metabolism of lipids and glucose. In addition, AMPK promotes glucose uptake into skeletal muscle and suppresses glucose output from the liver via insulin-independent mechanisms.[30] In our previous study, western blotting analyses showed that the level of phosphorylated AMPK (Tyr172)[31] in the skeletal muscle of db/db mice was significantly lower than that in db/+ mice, and was not significantly enhanced by CA treatment. It has been suggested that CA may not increase AMPK activity in insulin-resistant muscles.

Adipose tissue is increasingly recognized as an active endocrine organ with many secretory products and to be part of the innate immune system. With obesity, macrophages infiltrate adipose tissue, and numerous adipocytokines are released by macrophages and adipocytes.[32] Adipocytokines have important roles in the

pathogenesis of insulin resistance and associated metabolic complications such as dyslipidemia, hypertension, and premature heart disease. In addition to the production of pro-inflammatory cytokines that promote metabolic complications, adipose tissue is the sole source of adiponectin, which has anti-inflammatory actions and is associated with atherosclerosis.[33,34] Circulating levels of FFAs are increased in obesity and can induce insulin resistance.[35] The present study showed that CA decreased the expression of TNF-α in adipose tissue and serum levels of FFAs, which are possibly induced by a loss in body weight, but CA acts as an "insulin sensitizer" according to our analysis on the transcriptional level of PPARs (manuscript in preparation). This was the first study to explore the effects and mechanisms of CA on an obesity and insulin-resistance model of T2DM. This is in contrast to a STZ-induced model of T2DM, which is characterized by beta-cell damage.[36] In conclusion, CA exhibited hypoglycemic and hypolipidemic effects in db/db mice along with loss in body weight. This investigation revealed the potential use of CA or cinnamon as a natural alternative treatment for T2DM and/or metabolic syndrome.

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