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Naringin enhances reverse cholesterol transport in high fat/low streptozocin induced diabetic rats

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ABSTRACT

Naringin, a citrus-derived flavonoid with antihyperglycemic, antihyperlipidemic, and antioxidant properties, is reported to be a useful nutraceutical in the management of diabetes and its complications. This study investigated the mechanism of antiatherogenic properties of naringin in type 2 diabetes (T2DM) using high fat-low streptozocin rat model of T2DM. Rats were treated daily with 50, 100 and 200 mg/kg naringin orally for 21 days. Levels of biomarkers of T2DM, lipid profile and activity of paraoxonase (PON) were assayed spectrophotometrically. The levels of expression of hepatic 3-hydroxy-3-methyl-glutaryl-CoA reductase (*Hmgcr*), scavenger receptor class B member 1 (*Scarb1*), aryl hydrocarbon receptor (*Ahr*), hepatic Lipase (*Lipc*), and lecithin-cholesterol acyltransferase (*Lcat*) were assessed using relative reverse transcriptase polymerase chain reaction technique. Naringin treatment resulted in a dose-dependent significant ($p < 0.05$) decrease in the levels of plasma cholesterol and triglyceride from 84.84 ± 1.62 to 55.59 ± 1.50 mg/dL and 123.03 ± 15.11 to 55.00 ± 0.86 mg/dL, respectively, at 200 mg/kg naringin. In the liver, *Scarb1* and *Ahr* were significantly ($p < 0.05$) upregulated at 200 mg/kg naringin while *Lipc* and *Lcat* were significantly ($p < 0.05$) upregulated by 50 mg/kg naringin. T2DM-induced decrease in PON activities in the plasma, liver and HDL was significantly ($p < 0.05$) reversed by 200 mg/kg naringin treatment. These genes play critical roles in reverse cholesterol transport and hence our results showed that the antiatherogenic property of naringin in T2DM involves enhancement of reverse cholesterol transport and PON activity.

1. Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes and it is characterized by hyperglycemia associated with insulin resistance and impairment in insulin secretion with concomitant alteration in the intermediary metabolism of carbohydrate, protein and lipid [1,2]. The prevalence of T2DM has continued to be on the rise as a result of increase in obesity, which has been attributed to change in lifestyle and diet. Hence, the developing countries are expected to experience the greatest increase in the morbidity and mortality of this disease by the year 2025 [3].

T2DM is known to be associated with major complications like atherosclerotic coronary heart disease, cardiomyopathy, stroke and nephropathy. These complications are often responsible for increased mortality due to T2DM and they are associated with dyslipidemia and hypertension [2]. For example, atherosclerotic coronary heart disease is promoted by lipoprotein abnormalities which involve elevated very low density lipoproteins (VLDL) cholesterol and low high density lipoprotein (HDL) cholesterol [4]. Although the pathogenesis of atherosclerosis is complex, its development is dependent on the oxidation of LDL. This oxidation is prevented by paraoxonases, which are antioxidant protein component of lipoproteins, and are considered as better predictors of atherosclerotic risk than HDL in diabetes [5,6].

Although T2DM is a chronic disease, its management often involves control of both short-term and long-term diabetes-related problems [7]. Hence, the reduction of hyperglycemia and the risk of long-term complications is the target of any effective treatment regimen.

Although, there are a number of anti-diabetic pharmacological agents; they are however, limited by their unwanted side effects [8]. Thus, there has been a growing interest in nutraceuticals and functional

Abbreviations: T2DM, type 2 diabetes mellitus; PON, paraoxonase; *Hmgcr*, hepatic 3-hydroxy-3-methylglutaryl-CoA reductase; *Scarb1*, scavenger receptor class B member 1; *Ahr*, aryl hydrocarbon receptor; *Lipc*, hepatic Lipase; *Lcat*, lecithin-cholesterol acyltransferase; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoprotein; STZ, streptozotocin; HFD, high fat diet; DPP-IV, dipeptidyl peptidase-4; ELISA, enzyme linked immunosorbent assay; CPT, carnitine palmitoyl transferase; ACE, angiotensin converting enzyme; RT-PCR, reverse transcriptase polymerase chain reaction; GSP, gene specific primers

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food that can be useful in the management of T2DM and its associated complications.

Citrus is one of such functional foods that has been proposed to be beneficial in the management of diabetes mellitus and naringin is one of its active phytochemicals [9]. Naringin (PubChem CID: 442,428), a flavone glycoside, and aglycone of naringenin that gives grapefruit its bitter taste, has been reported for its antihyperglycemic, antioxidant and antihyperlipidemic properties [9,10]. For instance, Xulu and Oroma Owira [11] showed that naringin could ameliorate dyslipidemia in type I diabetic rats while Choi et al [10] further demonstrated the antioxidant effect of this flavonoid in rats fed with high cholesterol diet. Subsequently, the potential of naringin to improveT2DM in rats was demonstrated by Parmar et al. [12] using *in silico*, in vitro and in vivo inhibition of a biomarker of T2DM. Recently, Pari and Chandramohan [13] showed that the anti- T2DM property of naringin is due to the modulation of key carbohydrate metabolism enzymes in type 2 diabetic rat model.

However, more detailed studies are still needed to understand the mechanism by which naringin improves T2DM and prevents its associated complications. This present study therefore seeks to investigate the effects of naringin on a high fat fed/streptozotocin-induced T2DM rat model.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) was a product of Sigma-Aldrich (St. Loius, MO, USA) while Metformin was a product of Swipha Pharma, Lagos, Nigeria. Naringin was a product of Human Kang Biotechnology Company, Human Province, China. RNA later and RNA extraction spin column kits were products of Aidlab Biotechnologies Co. Ltd (Beijing, China) while TransGen Easy Script one-step RT-PCR kit was a product of TransGen Biotech Co. Ltd (Beijing, China). All other chemicals were products of Sigma-Aldrich (St. Loius, MO, USA).

2.2. Animals

Thirty male inbred albino rats weighing between 150 and 200 g were used for this study. The rats were housed in specific pathogen-free polypropylene cages under normal temperature (22 ± 2 °C) with 12-h light and dark cycle. The animals were allowed to acclimatize for three weeks before commencement of the experiment. The experiment was approved by the Covenant University Ethical Committee (CU/BIOSC-RECU/BIO/2015/004) and carried out according to the guidelines of the committee.

2.3. Experimental design

The rats were randomly divided into six groups of five rats each. Five of the groups were maintained on a high fat diet (HFD), which contained 45% fat (Table 1), throughout the period of the experiment (9 weeks) while the last group was maintained on normal pellet diet and served as the normal control. After 4 weeks, the HFD fed groups were given a low dose (35 mg/kg body weight) of STZ intraperitoneally while the normal control group was administered the vehicle (0.1 mL/kg body weight of 0.1 M citrate buffer), as described by Zhang et al. [14]. Another dose of STZ was administered at week 6 of the experiment, after which fasting blood glucose was checked and naringin was then administered orally for 21 days. The rats were grouped as shown below;

Group A: Diabetic Control: Rats fed HFD

Group B: Diabetic rats treated with 50 mg/kg naringin: Rats fed HFD

Group C: Diabetic rats treated with 100 mg/kg naringin: Rats fed HFD

Group D: Diabetic rats treated with 200 mg/kg naringin: Rats fed HFD

Table 1
Composition of diet.

Component	Level (g/100 g) in diet	
	Normal diet	High fat diet
Fish meal	25	25
Sucrose	10	10
Corn starch	49.5	4.5
Vegetable oil	5	5
Salt/mineral mix*	5.5	5.5
Cellulose	5	5
Tallow	–	45

* Salt/mineral mix contains the following (in g/100 g): calcium phosphate, 49.50; sodium powder, 11.80; potassium sulfate, 5.20; sodium chloride, 7.40; magnesium oxide, 2.40; potassium citrate, 22.40; ferric citrate, 0.60; manganous carbonate, 0.35; cupric carbonate, 0.03; zinc carbonate, 0.16; chromium potassium sulfate, 0.055; potassium iodate, 0.001; sodium selenate, 0.001; choline chloride, 0.50; thiamine HCl, 0.06; riboflavin, 0.06; niacin, 0.30; calcium pantothenate, 0.16; biotin, 0.01; vitamin B12, 0.10; vitamin D3, 0.025; vitamin E acetate, 1.00; pyridoxine, 0.07; folic acid, 0.02; vitamin A acetate, 0.08.

Group E: Diabetic rats treated with 50 mg/kg metformin: Rats fed HFD

Group F: Normal Control: Rats fed normal diet

Twenty-four hours after the last dose of naringin, the rats were euthanized under light ether anesthesia. Blood was collected from the anaesthetized animals by cardiac puncture while liver and kidney were also excised for biochemical and molecular analysis. The blood and organs were processed as previously described by Rotimi et al. [15], while portions of the liver were stabilized in RNAlater® for RNA analysis.

2.4. Biochemical analysis

Glucose, bicarbonate, α-amylase and α-hydroxyl butyrate dehydrogenase were determined spectrophotometrically in the plasma using commercially available kits (BioSino Biotechnology & Science Inc., Changping District Beijing, China) while insulin and dipeptidyl peptidase-4 (DPP-IV) were determined using enzyme linked immunosorbent assay (ELISA) kits (Hangzhou Eastbiopharm Co., Ltd. Hangzhou, China).

2.5. Plasma lipid profiles

Plasma cholesterol and triacylglycerols were determined spectrophotometrically using commercially available kits according to manufacturer's instructions. HDL and HDL_3 were obtained from the plasma using the dextran sulfate – $MgCl_2$ precipitation method as described by Rifai et al. [16]. The supernatant obtained after centrifugation contained the HDL and HDL_3, while the precipitate contained VLDL and $VLDL_3$ respectively. Free fatty acid was determined spectrophotometrically as described by Rotimi et al. [17].

2.6. Liver lipid profiles

Lipids were extracted from the liver according to the method of Folch et al. [18] and aliquots of the extract were used for determining cholesterol and triacylglycerol concentrations as previously described by Rotimi et al. [19].

2.7. Determination of paraoxonase activity

Paraoxonase was determined in the plasma, HDL, HDL_3, VLDL, $VLDL_3$ and liver homogenate as described by Afolabi et al. [20]. Briefly, phenylacetate was prepared freshly in 100 mM Tris-acetate buffer pH

7.4 containing 10 mM calcium chloride. The mixture was incubated at 37 °C with appropriate volumes of sample. The rate of phenol generation was monitored at 270 nm and the activity calculated using the molar extinction coefficient of 1480 M^{-1} cm^{-1}.

2.8. Determination of hepatic carnitine palmitoyl transferase (CPT) activity

Total CPT activity was determined spectrophotometrically according to the method of Ling et al. [21]. Briefly, aliquot of the liver homogenate equivalent to 20 μg protein was mixed in 200 μL of reaction buffer containing 20 mM HEPES, 1 mM EGTA, 220 mM sucrose, 40 mM KCl, 0.1 mM 5,5′-dithio-bis (2-nitrobenzoic acid), 1.3 mg/mL BSA, and 40 μM palmitoyl-CoA, pH 7.4. The reaction was initiated by adding 1 mM L-carnitine and read at 412 nm after 5 min incubation at 37 °C. The CPT activity was calculated as amount of CoASH released per min per mg protein using 13.6 mM/cm as the extinction coefficient for 5-thio-2-nitrobenzoate.

2.9. Determination of angiotensin converting enzyme (ACE) activity

ACE was determined in the kidney spectrophotometrically, using N-Hippuryl–His-Leu hydrate, hydrochloric acid and ethyl acetate at 228 nm according to the method of Cushman and Cheung [22].

2.10. Gene expression analysis

The levels of expression of certain genes involved in lipid homeostasis were quantified using relative reverse transcriptase polymerase chain reaction (RT-PCR) techniques as described by Chaudhry [23], with appropriate modifications. Briefly, RNA from the liver samples was extracted using the spin column kit obtained from Aidlab's EASY-spin Plus® according to the manufacturer's instructions. The RT-PCR was carried out with 500 ng RNA template using the Transgen *Easy-Script®* one-step RT-PCR supermix according to manufacturer's instructions. Samples were subjected to an initial incubation at 45 °C for 30 min for cDNA synthesis, followed by PCR amplification, using gene specific primers (GSP) (Table 2), 94 °C for 5 min followed by 40 cycles of 94 °C for 30s, 5 min at the annealing temperature of GSP and 1 min at 72 °C. All amplifications were carried out in C1000 Touch™ Thermal Cycler (BioRad, CA, USA). The intensity of the amplicon bands on 1.2% agarose was analyzed using Image J software as earlier described [15, 24].

2.11. Statistical analysis

Data were expressed as mean ± SEM of six replicates in each group. Analysis of variance (ANOVA) was carried out to test for the level of homogeneity at $p < 0.05$ among the groups. Duncan's multiple range test was used to separate the heterogeneous groups.

3. Results

3.1. Naringin improves biomarkers of diabetes in rats

The levels of glucose, insulin, bicarbonate, free fatty acids and DPP-IV in the plasma as well as the activities of plasma α-hydroxyl butyrate dehydrogenase, plasma amylase and kidney ACE were determined and the results depicted in Fig. 1(a–h). Diabetes significantly ($p < 0.05$) increased the levels of these biomarkers with naringin treatments resulting in a dose-dependent significant ($p < 0.05$) reduction. Naringin treatment also significantly ($p < 0.05$) reduced the level of plasma DPP-IV and the activity of kidney ACE than metformin did.

3.2. Modulation of paraoxonase activity by naringin

Diabetes resulted in significant ($p < 0.05$) decrease in the activities of PON in the plasma, liver and HDL with a concomitant increase in the VLDL and $VLDL_3$ (Fig. 2a–f). In the plasma, naringin significantly ($p < 0.05$) increased the activity of PON with 100 and 200 mg/kg having higher activities, but are still significantly ($p < 0.05$) lower than metformin and normal control. In the liver, naringin at 50 mg/kg significantly ($p < 0.05$) reversed the activity of PON to level that was not significantly ($p > 0.05$) different from normal control. Only 200 mg/kg naringin significantly ($p < 0.05$) increased the activity of PON in HDL. Diabetes resulted in the elevation of PON activities in VLDL and $VLDL_3$. This increase in the activity of PON was significantly ($p < 0.05$) reversed by naringin with 100 and 200 mg/kg having the lowest reduction in VLDL and $VLDL_3$, respectively.

3.3. Effect of naringin on lipid metabolizing proteins in the diabetic rats

The activity of CPT and the levels of expression of *Hmgcr*, *Scarb1*, *Ahr*, *Lipc* and *Lcat* in the liver were depicted in Fig. 3a–f. The activity of CPT was significantly ($p < 0.05$) reduced in the diabetic control group. However, this reduction was significantly ($p < 0.05$) reversed in a dose-dependent pattern with 200 mg/kg having activity that was significantly ($p < 0.05$) higher than normal control. Naringin treatment did not significantly ($p > 0.05$) affect the level of expression of hepatic *Hmgcr*. The expression of hepatic *Scarb1*, *Ahr*, *Lipc* and *Lcat* were significantly ($p < 0.05$) reduced in the diabetic control. This diabetes-induced suppression was however significantly ($p < 0.05$) reversed by naringin. The upregulation of *Scarb1* and *Ahr* by naringin was dose-dependent. However, 50 mg/kg naringin resulted in the highest expression of *Lipc* among the three dosages. The effect of naringin on the expression of *Lcat* was not significantly ($p > 0.05$) affected by dosage. Metformin treatment resulted in the upregulation of *Lipc* and *Lcat* to levels that was significantly ($p < 0.05$) higher than all the naringin dosages but not significantly ($p > 0.05$) different from normal control.

Table 2
List and sequences of Gene Specific Primers.

Gene Code	Gene name	GSP Sequence (5′ - > 3′)	Template
Lipc	Hepatic lipase C	Forward: GAGCCCAGTCCCCCTTCA Reverse: ATGTCATTCTTTGCTGCGTCTC	NM_012597.2
Ahr	Aryl hydrocarbon receptor	Forward: GGGCCAAGAGCTTCTTTGATG Reverse: GCAAGTCCTGCCAGTCTCTGA	NM_001308255.1
Scarb1	Scavenger receptor class B, member 1	Forward: GGCAAATTTGGCCTGTTCGT Reverse: CCACAGCAATGGCAGGACTA	NM_031541.1
Lcat	Lecithin cholesterol acyltransferase	Forward: AACTGGCTGTGCTACCGAAA Reverse: TAGGTCTTGCCAAAGCCAGG	NM_017024.2
Hmgcr	3-hydroxy-3-methylglutaryl-CoA reductase	Forward: CCTCCATTGAGATCCGGAGG Reverse: TCAGCCAGACCCAAGGAAAC	NM_013134.2
β-ACTIN	Beta actin	Forward: GTCAGGTCATCACTATCGGCAAT Reverse: AGAGGTCTTTACGGATGTCAACGT	NM_031144.3

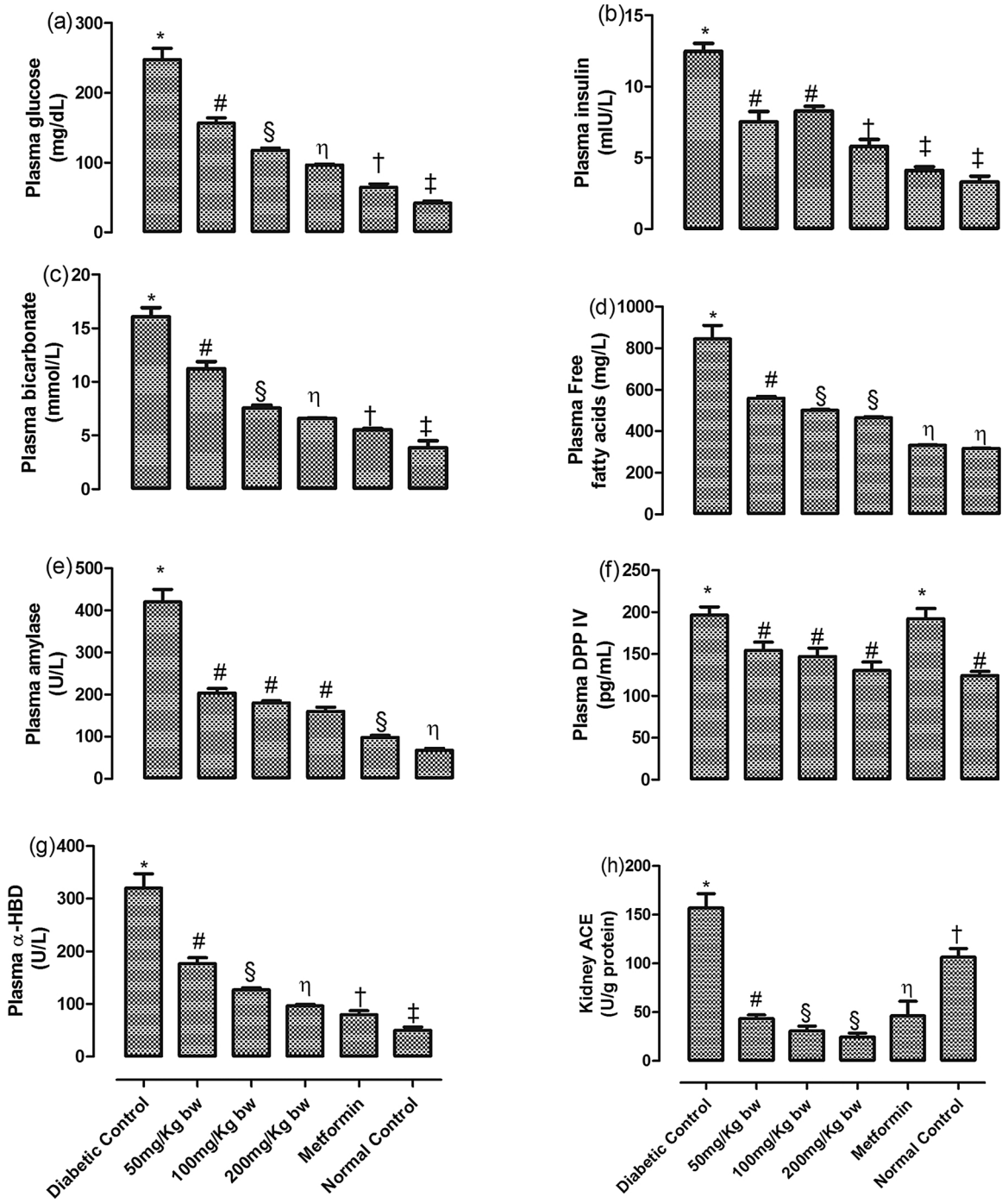

Fig. 1. (a–h): Effects of naringin on biomarkers of T2DM the experimental rats. (a) Level of plasma glucose, (b) level of plasma insulin, (c) level of plasma bicarbonate, (d) level of plasma free fatty acids, (e) activity of plasma amylase (f) level of plasma DPP IV, (g) activity of plasma α-HBD and (h) activity of kidney ACE.
Bars represent mean ± SEM (n = 5). Bars with different statistical markers are significantly different at $p < 0.05$.

3.4. Effect of naringin on plasma and hepatic levels of cholesterol and triacylglycerol

Table 3 shows the levels of cholesterol and triacylglycerol in the plasma, HDL, HDL_3 and liver. The diabetic untreated animals had elevated cholesterol, triacylglycerol in the plasma and liver. Naringin treatment was associated with a significant ($p < 0.05$) dose-dependent decrease in the level of plasma cholesterol. However, there was no significant ($p > 0.05$) difference in the level of plasma triacylglycerol among the groups treated with naringin. In the liver, a significant ($p < 0.05$) dose-dependent decrease was also observed in the level of cholesterol; however, 200 mg/kg naringin gave no further decrease. None of the treatments altered the elevated hepatic triacylglycerol level. Although the level of HDL cholesterol in the diabetic control was not significantly ($p > 0.05$) different from that of normal control, naringin at 200 mg/kg significantly ($p < 0.05$) increased its level. However, HDL triacylglycerol was significantly ($p < 0.05$) decreased in the diabetic control group and naringin caused a further decrease. Interestingly, the level of HDL_3 cholesterol which was significantly ($p < 0.05$) decreased in the diabetic control was significantly ($p < 0.05$) reversed by naringin in a dose-dependent pattern.

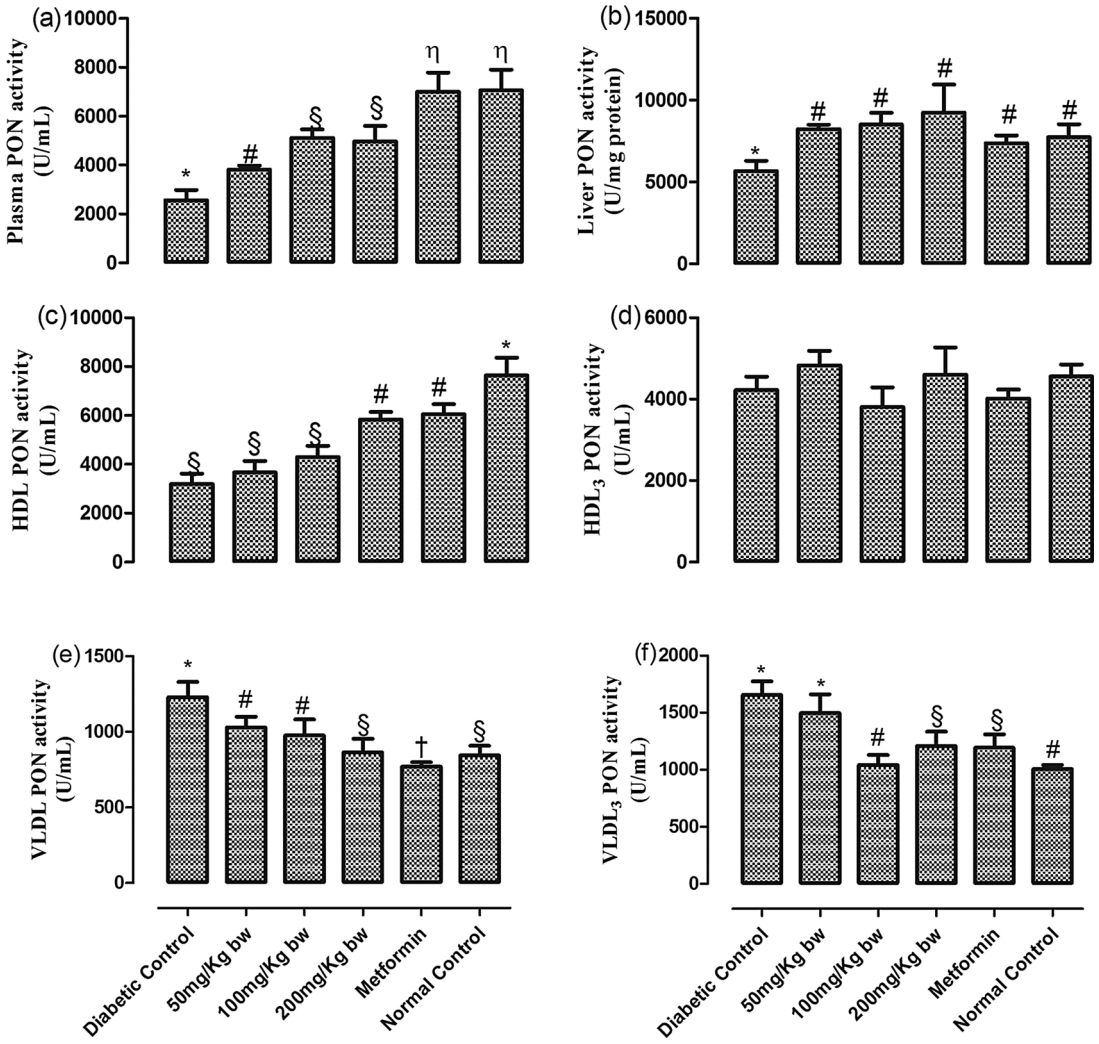

Fig. 2. (a–f): Effects of naringin on PON activities in T2DM rats. (a) Activity of PON in plasma, (b) activity of PON in liver, (c) activity of PON in HDL, (d) activity of PON in HDL_3, (e) activity of PON in VLDL and (f) activity of PON in $VLDL_3$.
Bars represent mean ± SEM (n = 5). Bars with different statistical markers are significantly different at $p < 0.05$.

4. Discussion

In this study, the rise in blood glucose was accompanied by elevated levels of plasma insulin, bicarbonate, FFA, and DPP-IV as well as the activities of plasma amylase and kidney ACE. This observation is consistent with T2DM and the model used in this study has been reported to simulate the human T2DM [25,26].

Insulin secretion following meal ingestion is stimulated by incretin hormones which are metabolized quickly by DPP-IV. The level of this enzyme is known to increase in type 2 diabetic patients and a previous study identified it as good target for antidiabetic therapy [27]. The naringin induced reduction in the level of DPP-IV observed in this study is consistent with the findings of Parmar et al. [12], who earlier reported the ability of naringin to inhibit this enzyme in vitro and in vivo. The role of DPP-IV in glucose metabolism involves the degradation of incretins-like glucagon like peptide-I and gastric inhibitory peptide thus affecting the effective functioning of insulin in mopping up glucose in the post-prandial state [28]. Interestingly, current pharmacotherapies of T2DM now focus on inhibiting DPP-IV and enhancing insulin availability and sensitivity of tissues to insulin [29,30].

Hyperglycemia in T2DM is due to increased body fat percentage and impaired glucose tolerance. The excessive fat intake accelerates insulin release as well as the influx of triglycerides into the blood. This activates the release of FFA into the blood stream through the action of lipoprotein lipase [31]. The increased plasma FFA level results in insulin resistance and that increase has been proposed as one of the mechanisms underlying the development of ketoacidosis, with concomitant increase in plasma amylase and bicarbonate level, in T2DM [32]. Naringin has recently been reported to reduced diabetic ketoacidosis in a type 1 model [33]; however, it of interest that our findings showed that naringin is also able to reverse ketoacidosis associated with T2DM. Apart from ketoacidosis, a major complication of T2DM is vascular complications, resulting in cardiovascular diseases which are accompanied with an increase in conversion of angiotensin I to angiotensin II by ACE. The elevation of the activity of this enzyme results in increased blood pressure and it's a major target of pharmacological therapy of hypertension [34]. Our findings showed that naringin could improve microvascular complications associated with T2DM by reducing the activity of ACE. The inhibition of the activity of this enzyme by naringin has been reported to be due to its flavonoid skeleton [35]. Our finding therefore provides experimental in vivo evidence for the ACE inhibitory property of naringin.

Hyperinsulinemia contributes to altered lipid metabolism in T2DM through the inhibition of triglyceride hydrolysis, thus activating the production of malonyl CoA through the enzyme, acetyl CoA carboxylase. Malonyl CoA on the other hand allosterically inhibits CPT [36] and impedes the transfer of fatty acids to the mitochondria for β-oxidation resulting in the accumulation of FFA in the cytosol. Hence, our

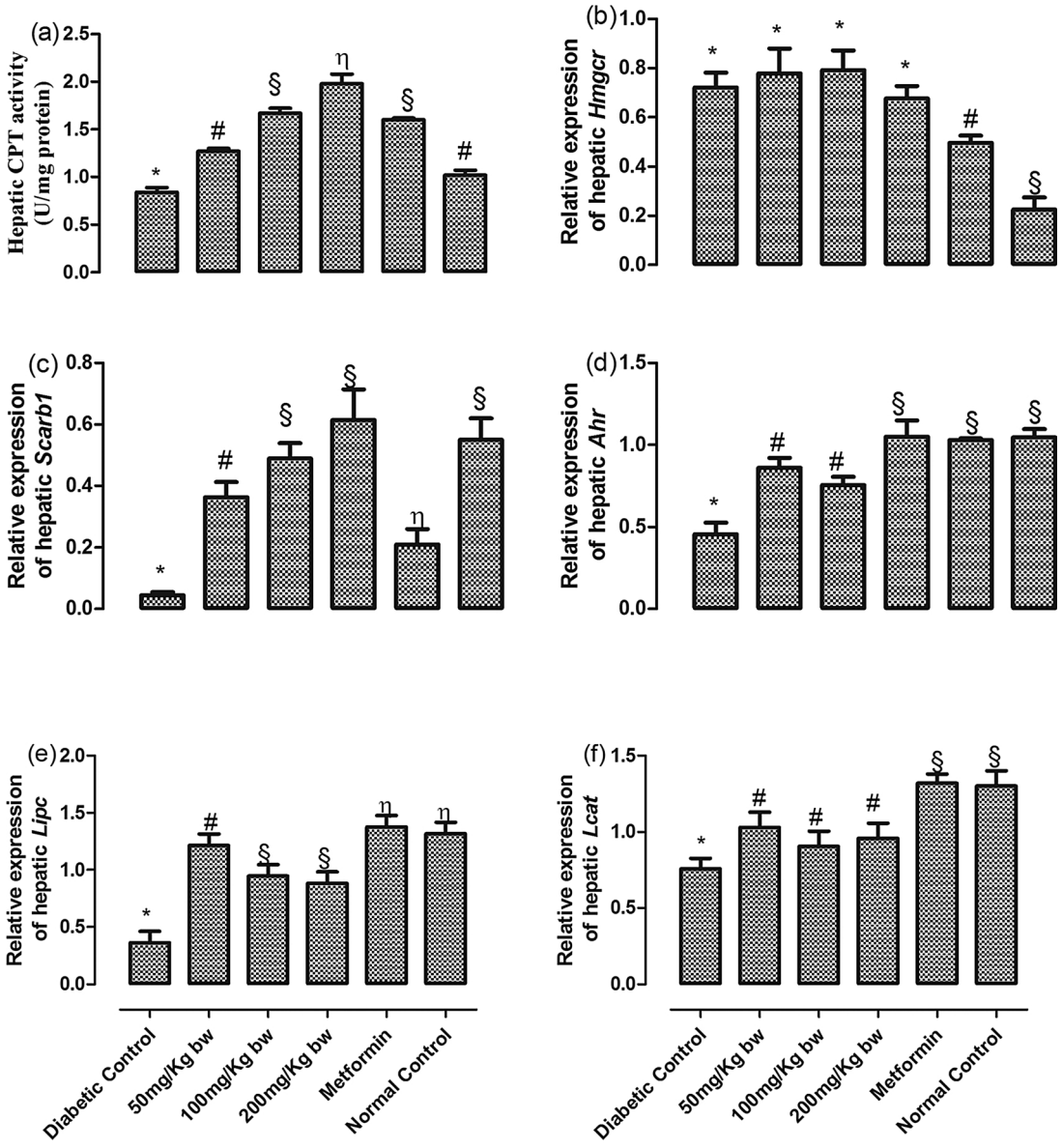

Fig. 3. (a–f): Effects of naringin on hepatic CPT activity and expression of cholesterol metabolizing genes in T2DM rats. (a) The activity of hepatic CPT, (b) the levels of expression of hepatic*Hmgcr* (c) the levels of expression of hepatic *Scarb1*, (d) the levels of expression of hepatic *Ahr*, (e) the levels of expression of hepatic *Lipc* and (f) the levels of expression of hepatic *Lcat*.

Bars represent mean ± SEM (n = 5). Bars with different statistical markers are significantly different at $p < 0.05$.

Table 3
Effects of naringin on cholesterol and triglycerides levels in plasma, HDL, HDL_3 and Liver.

	Plasma		HDL		HDL_3		Liver	
	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/100g tissue)	Triglyceride (mg/100g tissue)
Diabetic Control	84.84 ± 1.62^{f}	123.03 ± 15.11^{d}	44.47 ± 3.94^{b}	67.00 ± 10.77^{c}	25.63 ± 1.43^{a}	69.50 ± 14.67	22.69 ± 1.02^{a}	49.79 ± 2.61^{b}
50 mg/Kg bw	71.82 ± 1.82^{e}	72.99 ± 2.43^{c}	42.28 ± 2.25^{b}	45.91 ± 3.40^{a}	30.70 ± 0.46^{b}	51.38 ± 5.77	24.44 ± 2.70^{a}	47.69 ± 12.00^{b}
100 mg/Kg bw	64.50 ± 1.00^{d}	63.89 ± 1.57^{c}	35.01 ± 1.74^{a}	48.10 ± 5.22^{d}	33.69 ± 0.43^{c}	66.97 ± 11.47	17.57 ± 1.08^{b}	43.36 ± 54.71^{b}
200 mg/Kg bw	55.59 ± 1.50^{c}	55.00 ± 0.86^{c}	50.50 ± 5.30^{c}	44.39 ± 2.98^{a}	36.19 ± 0.33^{d}	64.59 ± 8.05	19.15 ± 1.30^{b}	49.56 ± 4.97^{b}
Metformin	49.92 ± 0.76^{b}	47.78 ± 1.77^{b}	51.89 ± 6.46^{c}	43.68 ± 4.69^{a}	40.22 ± 0.78^{e}	62.02 ± 11.00	23.13 ± 5.29^{a}	49.75 ± 4.97^{b}
Normal Control	40.88 ± 1.94^{a}	25.93 ± 4.30^{a}	46.91 ± 5.13^{b}	72.22 ± 10.22^{b}	50.84 ± 2.85^{f}	58.10 ± 8.39	13.62 ± 1.27^{c}	31.78 ± 3.61^{a}

Each value represents the mean ± SEM (n = 5). Values within the same column with different superscripts are significantly different at $p < 0.05$.

finding and that of [37] suggest that the elevation of plasma FFA in diabetic control group could be linked to the decrease in the activity of CPT. This inhibition of CPT, however, allows for more storage of fatty acids as triglyceride through esterification, which forms cholesteryl esters. This may account for the high level of hepatic cholesterol as observed in this study. Naringin-induced increase in CPT activity and expression of hepatic lipase could have resulted in increased lipolysis. The subsequent reduction in the level of plasma triacyclglycerols could have also resulted from the upregulation of *Ahr* by naringin. This receptor is known to repress the expression of genes involved in reductive biosynthesis of fatty acid thereby decreasing the overall fatty acid synthesis and secretion in hepatocytes; and it has been suggested to be a therapeutic target [38].

The findings of this study also demonstrated that naringin improves general lipid profile and metabolism in the T2DM animals by significantly increasing the expression of *Scarb1* and *Lcat* in the liver. Although previous studies have reported the ability of naringin to reverse dyslipidemia [33], its ability to increase the levels of plasma HDL cholesterol and HDL_3 cholesterol and increase the expression of *Scarb1* in a dose-dependent manner indicates that the enhancement of reverse cholesterol transport could be one of the mechanisms of its anti-dyslipidemia property. This finding is of interest because naringin did not show any significant effect in reducing the expression of *Hmgcr*.

Lecithin cholesterol acyltransferase enhances the accumulation of cholesterol in HDL and the fate of the cholesterol could be for: (1) transfer to triglyceride-rich lipoproteins which are subsequently taken up by hepatic LDL receptor as part of HDL containing apolipoprotein (particle uptake) or by (2) a selective uptake of HDL cholesteryl ester in liver involving *Scarb1* [39]. Our findings suggest the later to be the mechanism by which naringin induced cholesterol clearance in this study. Scavenger receptor class B, member 1, a cell surface glycoprotein, plays a key role in reverse cholesterol transport by mediating the uptake of cholesterol from HDL-cholesterol by the liver for metabolism and biliary excretion [39].

There is considerable evidence that the anti-atherogenic property of HDL is also due to the antioxidant property of its paraoxonase-1 component [5]. This apoprotein component of the lipoprotein is a hydrolytic enzyme that protects against lipid oxidation and several bioactive compounds have been reported to stimulate it [40,41]. The ability of naringin to enhance the activities of PON in the liver, plasma and HDL is a major contribution to reversing atherosclerosis, - an associated complication of T2DM

5. Conclusion

The data presented in this study suggest that naringin could reverse atherosclerosis associated with T2DM by reducing dyslipidemia via HDL mediated reverse cholesterol transport and protection of lipoprotein from oxidation by enhancing the activities of PON.

Conflict of interest

The authors declare that they have no competing interests.

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