Niger. J. Physiol. Sci. 33(December 2018) 129-138
www.njps.com.ng

Niger. J. Physiol. Sci.

Effects of Honey on Postprandial Hyperlipidemia and Oxidative Stress in Wistar Rats: Role of HMG-CoA Reductase Inhibition and Antioxidant Effect

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Summary: Postprandial hyperlipidemia is associated with oxidative stress and is an important risk factor for atherosclerosis and cardiovascular disease. The aims of this study were to investigate the antihyperlipidemic effect of honey administered 5 or 60 minutes before a high-fat diet (HFD), to explore the role of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase in antihyperlipidemic effect of honey and to investigate the effect of honey on postprandial oxidative stress. Rats were fasted and randomized into 5 groups. Groups 1 and 2 were administered portable water. After 60 minutes, the groups were given portable water and HFD, respectively. Group 3 was administered honey. After 5 minutes, the rats were given HFD. Groups 4 and 5 were administered honey and simvastatin, respectively. After 60 minutes, the rats were given HFD. Four hours after portable water or HFD administration, the rats were sacrificed. Group 2 had significantly ($p < 0.01$) higher total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, catalase activity and significantly ($p < 0.05$) lower high density lipoprotein (HDL) cholesterol and HMG-CoA: mevalonate ($p < 0.001$) compared with Group 1. Group 3 had significantly ($p < 0.01$) higher TG and VLDL cholesterol and lower HMG-CoA: mevalonate compared with Group 1. Groups 4 and 5 exhibited significantly ($p < 0.05$ or $p < 0.001$) higher HDL cholesterol and HMG-CoA: mevalonate and lower LDL cholesterol compared with group 2. Honey pretreatment 60 minutes before HFD feeding exerts more significant antihyperlipidemic effect and attenuates more considerably postprandial hyperlipidemia-induced oxidative stress than honey administered 5 minutes before HFD in Wistar rats. This marked antihyperlipidemic effect of honey pretreatment is mediated in part via inhibition of HMG-CoA reductase.

Keywords*:* Honey, Postprandial hyperlipidemia, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, Oxidative stress

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Manuscript Accepted: August, 2018

INTRODUCTION

Hyperlipidemia is a main risk factor that triggers atherosclerosis and coronary heart disease (CHD) (Mensink *et al.*, 2003). Hence, reduction of hyperlipidemia is an important therapeutic strategy aimed at reducing atherosclerosis and preventing other vascular events including CHD and cerebrovascular disease (Collins *et al.*, 2004). Statins remain the most prescribed drugs for the treatment of hyperlipidemia. They competitively inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase (Goldstein and Brown, 1990). This enzyme is a rate-limiting enzyme which plays an important role in the biosynthesis pathway of cholesterol where it catalyzes the conversion of HMG-CoA to mevalonate (Friesen and Rodwell, 2004). To derive maximal therapeutic benefits, many patients require long-term statin therapy. However, prolonged treatment of statins is associated with serious adverse effects including muscle weakness, rhabdomyolysis, liver damage and cognitive problems (Golomb and Evans, 2008). Consequently, treatment of dyslipidemia and associated disorders with agents that are devoid of adverse effects or have fewer side effects is desirable (Peluso *et al.*, 2014). Besides fasting hyperlipidemia, postprandial hyperlipidemia is an important risk factor for cardiovascular diseases (Pirillo *et al.*, 2014). The lethality of postprandial hyperlipidemia is reinforced

by evidence showing that postprandial hyperlipidemia is a stronger predictor of cardiovascular risk than fasting hyperlipidemia (Mora *et al.*, 2008). Postprandial hyperlipidemia is associated with endothelial dysfunction and inflammation (Lee *et al.*, 2002, van Oostrom *et al.*, 2004). Available evidence suggests that the deleterious effect of postprandial hyperlipidemia is mediated via induction of oxidative stress (Bae *et al.*, 2001). Hence, the importance of dietary interventions aimed at mitigating postprandial hyperlipidemia and the accompanying oxidative stress cannot be overemphasized.

It is therefore not surprising that, in the recent decade, there has been an upsurge in the utilization of complementary and alternative medicines in the treatment of several diseases such as diabetes mellitus and hyperlipidemia (Nies *et al.*, 2006, Erejuwa, 2014). In addition to fewer episodes of side effects, most of the complementary, alternative or traditional medicine agents are of natural origin and cheaper though efficacy and safety remain major concerns (Qidwai *et al.*, 2014). Honey is one of such natural agents with several attributed pleiotropic effects (Erejuwa *et al.*, 2012; Kolawole *et al.*, 2015). Honey supplementation in diabetic rats was associated with amelioration of lipid abnormalities and reductions in coronary and cardiovascular risk indices (Erejuwa *et al.*, 2016). In combination with metformin or glibenclamide, honey administration markedly enhanced the antihyperlipidemic effect of these drugs (Erejuwa *et al.*, 2011, Nasrolahi *et al.*, 2012). Emerging evidence suggests honey has an ameliorative effect on obesity anthropometric parameters in rodent models of obesity (Erejuwa *et al.*, 2017a, Samat *et al.*, 2017). Recently, it was demonstrated that combination of honey with simvastatin resulted in augmented amelioration of body mass index and adiposity in HFD fed Wistar rats (Erejuwa *et al.*, 2017b). Currently, the mechanism of antihyperlipidemic and anti-obesity effects of honey is unknown. Likewise, the mechanism by which honey enhanced the effects of metformin, glibenclamide or simvastatin in ameliorating lipid abnormalities and adiposity is unclear. Therefore, this study was performed in Wistar rats with the following three aims: (i) To investigate and compare the antihyperlipidemic effect of honey administered 5 or 60 minutes before HFD feeding, (ii) To explore the potential role of HMG-CoA reductase in the antihyperlipidemic effect of honey, (iii) To investigate the effect of honey on postprandial hyperlipidemia-induced oxidative stress.

MATERIALS AND METHODS

Chemicals

Sodium arsenate, hydroxylamine hydrochloride and thiobarbituric acid were purchased from Sigma-Aldrich, MO, USA. All other reagents used were of analytical grade.

Animals

Male and female Wistar rats were obtained from animal house unit, Nsukka, Nigeria. The rats were allowed to acclimatize for 10 days. The rats were housed separately in plastic cages in an animal room. The animal house was well ventilated and had a temperature of 26 ± 2 °C and a 12-hour light:dark cycle. The rats were provided rat chow and drinking water *ad libitum*. The study protocol was approved by the Research Ethics Committee of Ebonyi State University (EBSU/DRIC/UREC/Vol. 04/005). The handling of rats strictly followed institutional and international guidelines on the Use and Handling of Laboratory Animals.

Honey

The honey was obtained from a bee farm in Abakaliki, Ebonyi State, Nigeria. The honey had a NAFDAC (National Agency for Food and Drug Administration Control) number. The honey was purchased from a bee farm registered with NAFDAC to ensure the honey used in the study was original, genuine and unadulterated. The honey was dissolved in drinking water (1:1) before administration. It was administered at a dose of 1.0 g/kg body weight (BW). The choice of this dose was based on findings from our previous studies in which 1.0 g/kg BW was demonstrated to be the optimal dose in ameliorating hyperlipidemia, excess weight gain, adiposity and body mass index in diabetic and obese rats (Erejuwa *et al.*, 2016, Erejuwa *et al.*, 2017a).

Treatment

The Wistar rats were fasted for about 36 hours. The fasted rats were randomly divided into 5 groups. Each group consisted of 5 rats. The rats were treated as follows:

Group 1: Fasted rats were administered portable water (1.0 ml/kg BW). After 60 minutes, the rats were given portable water (1.0 ml/kg BW) (control).

Group 2: Fasted rats were administered portable water (1.0 ml/kg BW). After 60 minutes, the rats were given HFD (5.0 ml/kg BW) (HFD only).

Group 3: Fasted rats were administered honey (1.0 g/kg BW). After 5 minutes, the rats were given HFD (5.0 ml/kg BW) (Honey5+HFD)

Group 4: Fasted rats were administered honey (1.0 g/kg BW). After 60 minutes, the rats were given HFD (5.0 ml/kg BW) (Honey60+HFD)

Group 5: Fasted rats were administered simvastatin (20 mg/kg BW). After 60 minutes, the rats were given HFD (5.0 ml/kg BW) (Simvastatin+HFD)

All the various agents (portable water, honey, simvastatin or HFD) were administered orally using a gavage needle. Before administering the agents, body weight was measured using a weight measuring scale.

The administered HFD was a mixture of olive oil and coconut oil (1:1). After 4 hours of HFD administration, the rats were sacrificed under diethyl ether anesthesia. Using plain tubes, blood samples were collected. The plain tubes containing the blood samples were left to stand at room temperature for about 3 hours. The clotted blood samples were centrifuged at 1500 rpm for 20 min. The serum samples were collected and used for analyses of lipid profile. A portion of the serum samples was used for the assay of catalase activity, malondialdehyde, total antioxidant status and total protein. The livers were rapidly harvested and used for the assay of HMG-CoA reductase activity.

Biochemical analysis

The serum samples were used for the estimation of total cholesterol (TC), high density lipoprotein (HDL) cholesterol and triglycerides (TG). The TC, HDL cholesterol and TG were measured using commercially available kits (Agappe Diagnostics, Knonauerstrasse, Cham, Switzerland) on UV-Visible spectrophotometer (752 UV-VIS Spectrophotometer, China) based on the manufacturer's instructions. The LDL and VLDL cholesterol were calculated using the Friedewald equations (Friedewald *et al.*, 1972).

LDL cholesterol $= TC - HDLc - VLDLc$

VLDLc $= {}^{TG}/_{5}$

Estimation of non-HDL cholesterol, atherogenic index, coronary risk index and cardiovascular risk index

Non-HDL cholesterol, atherogenic index, coronary risk index and cardiovascular risk index were estimated from measured lipid profile parameters using the formulae below (Abbott *et al.*, 1988, Alladi *et al.*, 1989).

Non-HDLc $= TC - HDLc$

Atherogenic index $= {}^{LDLc}/_{HDLc}$

Coronary Risk Index $= {}^{TC}/_{HDLc}$

Cardiovascular Risk Index $= {}^{TG}/_{HDLc}$

Assay of HMG-CoA reductase activity

The activity of HMG-CoA reductase was determined in liver homogenate according to the method of Rao and Ramakrishnan, 1975 (Rao and Ramakrishnan, 1975). In this procedure, the concentrations of HMG-CoA and mevalonate in liver homogenates are assessed in terms of absorbances. The ratio of HMG-CoA to mevalonate is considered an index of HMG-CoA reductase activity that catalyzes the conversion of HMG-CoA to mevalonate. Briefly, equal volumes of 10% liver homogenate was mixed with dilute perchloric acid and kept for 5 min. The mixture was centrifuged at 2 000 × g for 10 min. The hepatic HMG-CoA was measured by its reaction with alkaline hydroxylamine reagent. Similarly, the hepatic mevalonate was determined by its reaction with acidic hydroxylamine reagent. After 10 min, the absorbance readings were taken at 540 nm against a similarly treated saline-arsenate blank. The HMG-CoA: mevalonate ratio is inversely proportional to the activity of HMG-CoA reductase. Higher HMG-CoA: mevalonate indicates lower activity of HMG-CoA reductase and vice versa.

Assay of catalase activity

The activity of catalase was determined as described by Goth (Gott, 1991). Briefly, the method entails incubating a test tube containing H_2O_2 (0.5 mL) and serum (0.1 mL). Addition of ammonium molybdate (0.5 mL) terminated the reaction after incubation at 37 °C for 60 seconds. This was followed by the measurement of absorbance of the yellow complex of ammonium molybdate and H_2O_2 at 405 nm using a spectrophotometer. One unit of catalase referred to the quantity of enzyme that catalyzes the breakdown of 1 μmol of H_2O_2/min.

Assay of malondialdehyde

Malondialdehyde (MDA) was assayed as thiobarbituric acid reactive substances (TBARS) using the method of Ohkawa and colleagues (Ohkawa *et al.*, 1979). In brief, the test tubes containing serum (100 μL) or MDA standards (100 μL), glacial acetic acid (pH 3.5; 1.5 mL), sodium dodecyl sulphate (200 μL), thiobarbituric acid (TBA) (1.5 mL) and portable water (700 μL) were incubated at 95 °C for 60 minutes. After incubation, the test tubes were allowed to cool and centrifuged at 3000 × g for 10 minutes. The MDA concentration was measured at 532 nm using a spectrophotometer. TBARS concentration was expressed as nmol of MDA per mg protein.

Assay of total antioxidant status

Total antioxidant status (TAS) was assayed using the method of Koracevic et al. (Koracevic *et al.*, 2000). Briefly, sodium benzoate (0.5 mL), Fe-EDTA complex (0.2 mL), and H_2O_2 (2 mL) were pipetted into a test tube containing serum (0.010 mL) and sodium phosphate buffer (0.49 mL). Control (blank) test was also performed for each sample. The assay tubes were vortexed and then incubated at 37 °C for 60 minutes. Acetic acid (1 mL) and TBA were added. The tubes were placed in a water bath containing boiling water for 10 minutes. The tubes were cooled to room temperature and the absorbance was measured at 532 nm against portable water using a spectrophotometer. Serum TAS was estimated using uric acid as standard.

Assay of total protein

The serum protein concentration was measured using Bradford's method (Bradford, 1976). Briefly, 100 uL of serum or protein standards were added to a tube

containing 5 mL of Coomassie Blue. The mixture was vortexed and incubated for 10 min. The absorbance was read at 595 nm against blank. Bovine serum albumin was used as the standard.

Statistical analysis

Data are expressed as mean ± SEM. The results were analyzed using SPSS 16.0. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of honey on HDL cholesterol

The data on the effect of honey on HDL cholesterol are presented in Figure 1. The HDL cholesterol was significantly ($p < 0.05$) lower in control HFD fed rats than in normal control rats. The levels of HDL cholesterol in rats administered honey or simvastatin 60 minutes before HFD feeding were significantly ($p < 0.05$) higher than the level in control HFD fed rats. The level of HDL cholesterol in rats administered honey 5 minutes before HFD feeding was not significantly ($p > 0.05$) different from that of control HFD fed rats.

Effect of honey on LDL cholesterol

The results on the effect of honey on LDL cholesterol are shown in Figure 2. The control HFD fed rats had significantly ($p < 0.05$) higher LDL cholesterol compared with normal control rats. The levels of LDL cholesterol in rats administered honey or simvastatin 60 minutes before HFD feeding were significantly ($p < 0.05$) lower than the level in control HFD fed rats. The level of LDL cholesterol in rats administered honey 5 minutes before HFD feeding was not significantly ($p > 0.05$) lower than that of control HFD fed rats.

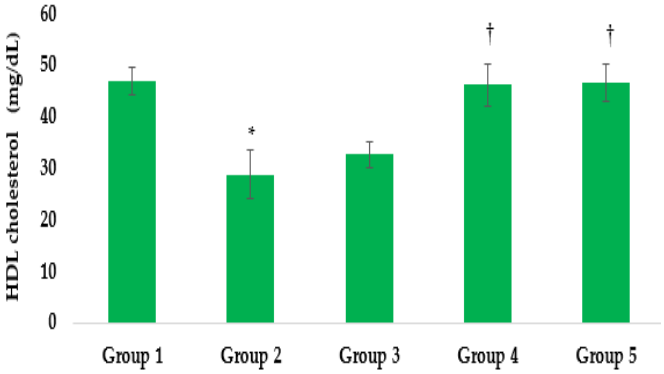

Figure 1. Effect of honey on HDL cholesterol in high fat diet fed rats. Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD); * $p < 0.05$ compared with Group 1; † $p < 0.05$ compared with Group 2.

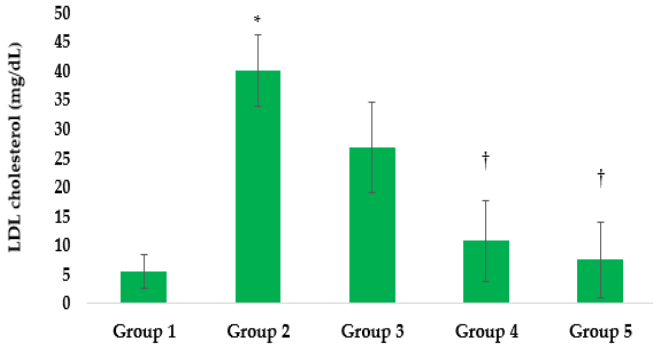

Figure 2. Effect of honey on LDL cholesterol in high fat diet fed rats. Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD); * $p < 0.05$ compared with Group 1; † $p < 0.05$ compared with Group 2.

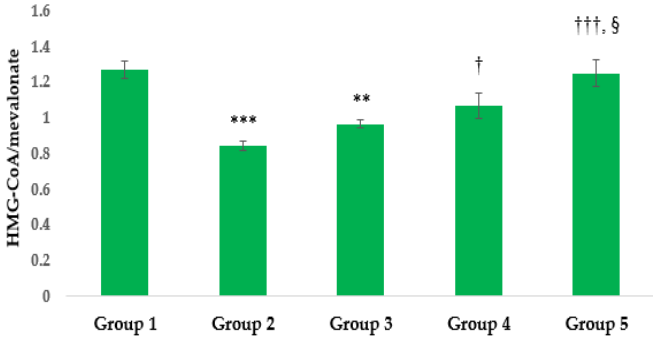

Figure 3. Effect of honey on HMG-CoA/mevalonate in high fat diet fed rats. Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD); ** $p < 0.01$ & *** $p < 0.001$ compared with Group 1; † $p < 0.05$ & ††† $p < 0.001$ compared with Group 2; § $p < 0.05$ compared with Group 3.

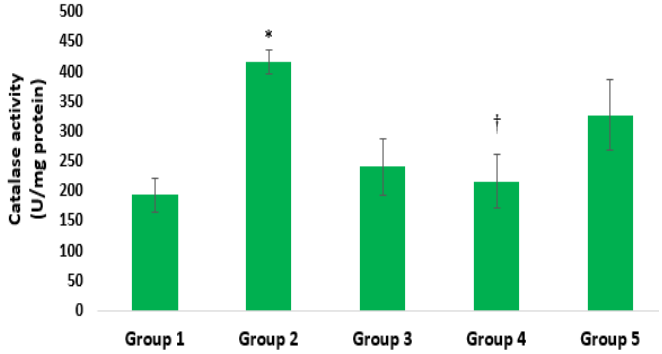

Figure 4. Effect of honey on serum catalase activity in high fat diet fed rats. Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD). * $p < 0.05$ compared with Group 1; † $p < 0.05$ compared with Group 2.

Effect of honey on HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A)/mevalonate

Figure 3 shows the results of the effect of honey on HMG-CoA/mevalonate. The rats administered portable water 60 minutes or honey 5 minutes before HFD feeding showed significantly ($p < 0.01$ or $p < 0.001$) lower HMG-CoA/mevalonate compared with normal control rats. The HMG-CoA/mevalonate in

Table 1. Effects of honey on serum total cholesterol, triglycerides, non-HDL cholesterol and VLDL cholesterol

	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Non-HDL cholesterol (mg/dL)	VLDL cholesterol (mg/dL)
Group 1	65.2 ± 3.5	65.8 ± 9.7	19.3 ± 3.5	13.2 ± 1.9
Group 2	96.9 ± 3.7 **	155.8 ± 22.5 **	63.4 ± 2.3 ***	31.2 ± 4.5 **
Group 3	85.1 ± 6.1	154.0 ± 15.7 **	56.0 ± 5.2 **	30.8 ± 3.1 **
Group 4	85.9 ± 4.9	110.8 ± 13.4	45.3 ± 8.3 *	20.0 ± 2.0
Group 5	86.7 ± 8.1	104.2 ± 10.4	34.6 ± 6.0 †	20.8 ± 2.1

Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD). * $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$ compared with Group 1; † $p < 0.05$ compared with Group 2

rats administered honey or simvastatin 60 minutes before HFD feeding was significantly ($p < 0.05$ or $p < 0.001$) higher than that in control HFD fed rats. The rats administered simvastatin 60 minutes before HFD feeding had significantly ($p < 0.05$) higher HMG-CoA/mevalonate than the rats administered honey 5 minutes before HFD feeding.

Effect of honey on serum catalase activity

The data on the effect of honey on serum catalase activity are shown in Figure 4. The control HFD fed rats had significantly ($p < 0.05$) higher serum catalase activity compared with normal control rats. The serum catalase activity in rats administered honey 5 minutes before HFD feeding was borderline ($p = 0.054$) lower than that of control HFD fed rats. The serum catalase activity in rats administered honey 60 minutes before HFD feeding was significantly ($p < 0.05$) lower than that of control HFD fed rats.

Effects of honey on serum total cholesterol, triglycerides, non-HDL cholesterol and VLDL cholesterol

The results of the effects of honey on total cholesterol, triglycerides, non-HDL cholesterol and VLDL cholesterol are shown in Table 1. The control HFD fed rats had significantly ($p < 0.01$) higher total cholesterol compared with normal control rats. The levels of total cholesterol in rats administered honey 5 or 60 minutes as well as simvastatin 60 minutes before HFD feeding were statistically non-significantly ($p > 0.05$) different from that of the normal control rats. The control HFD fed rats and rats administered honey 5 minutes before HFD feeding had significantly ($p < 0.01$) higher triglycerides compared with normal control rats. The levels of triglycerides in rats administered honey or simvastatin 60 minutes before HFD feeding were non-significantly ($p > 0.05$) different from that of the normal control rats.

The control rats and rats administered honey 5 or 60 minutes before HFD feeding had significantly ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively) higher non-HDL cholesterol compared with normal control rats. The non-HDL cholesterol in rats administered simvastatin 60 minutes before HFD feeding was significantly ($p < 0.05$) lower than that in control HFD fed rats. Rats of control HFD fed rats or rats administered honey 5 minutes before HFD feeding showed significantly ($p < 0.01$) elevated levels of VLDL cholesterol compared with normal control rats. The levels of VLDL cholesterol in rats administered honey or simvastatin 60 minutes before HFD feeding were non-significantly ($p > 0.05$) higher than the level in normal control rats but non-significantly ($p > 0.05$) lower than the level in control HFD fed rats.

Table 2. Effects of honey on serum TC/HDL cholesterol, TG/HDL cholesterol and LDL/HDL cholesterol

Group	TC/HDL cholesterol	TG/HDL cholesterol	LDL/HDL cholesterol
1	1.42 ± 0.08	1.82 ± 0.40	0.11 ± 0.06
2	3.27 ± 0.40**	5.21 ± 0.70**	1.34 ± 0.40
3	2.65 ± 0.19*	4.81 ± 0.58**	0.85 ± 0.25
4	2.33 ± 0.32	3.29 ± 0.51	0.54 ± 0.41
5	1.74 ± 0.12 ††	2.34 ± 0.34††,§	0.28 ± 0.16

Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD). * $p < 0.05$, ** $p < 0.01$ compared with Group 1; †† $p < 0.01$ compared with Group 2; § $p < 0.05$ compared with Group 3

Effects of honey on TC/HDL cholesterol, TG/HDL cholesterol and LDL/HDL cholesterol

The data on the effects of honey on TC/HDL cholesterol, TG/HDL cholesterol and LDL/HDL cholesterol are presented in Table 2. The control HFD fed rats and rats administered honey 5 minutes before HFD feeding had significantly ($p < 0.01$ and $p < 0.05$, respectively) higher TC/HDL cholesterol compared with normal control rats. The TC/HDL cholesterol was significantly ($p < 0.01$) lower in rats administered simvastatin 60 minutes before HFD feeding than in control HFD fed rats. The control HFD fed rats and rats administered honey 5 minutes before HFD feeding had significantly ($p < 0.01$) higher TG/HDL cholesterol compared with normal control rats. The TG/HDL cholesterol was significantly ($p < 0.01$ or $p < 0.05$) lower in rats administered simvastatin 60 minutes before HFD feeding than in control HFD fed rats or rats administered honey 5 minutes before HFD feeding. The LDL/HDL cholesterol was non-significantly ($p = 0.057$) higher in control HFD fed rats than in normal control rats. Rats administered honey or simvastatin had non-significantly ($p > 0.05$) lower LDL/HDL cholesterol compared with control HFD fed rats.

Table 3. Effects of honey on serum TAS and MDA

	TAS (U/mg protein)	MDA (U/mg protein)
Group 1	3.63 ± 0.41	4.28 ± 0.30
Group 2	4.17 ± 0.32	4.11 ± 0.25
Group 3	3.71 ± 0.38	4.04 ± 0.21
Group 4	3.65 ± 0.49	4.23 ± 0.45
Group 5	4.66 ± 0.39	4.25 ± 0.55

Group 1 (control); Group 2 (HFD only); Group 3 (Honey5+HFD); Group 4 (Honey60+HFD); Group 5 (Simvastatin+HFD).

Effects of honey on serum total antioxidant status (TAS) and malondialdehyde (MDA)

The results on the effects of honey on serum TAS and MDA are presented in Table 3. Though statistically ($p > 0.05$) insignificant, the control HFD fed rats and rats administered simvastatin 60 minutes before HFD feeding had slightly higher TAS compared with rats of other groups. The levels of serum MDA were comparable in all the groups.

DISCUSSION

The control HFD fed rats had marked elevated levels of total cholesterol and triglycerides indicating development of hypercholesterolemia and dyslipidemia in these rats. The moderate hypercholesterolemia in rats administered simvastatin or honey 5 or 60 minutes before HFD feeding suggests that pre-treatment with honey or simvastatin suppressed elevations in total cholesterol and thus confirms the anti-hypercholesterolemic effect of honey (Al-Waili, 2004). The similarity in the levels of triglycerides in control HFD fed rats and rats fed honey 5 minutes before HFD indicates honey administered 5 minutes before HFD did not exert anti-hypertriglyceridemic effect. The considerably higher levels of triglycerides than that of the normal control group further confirms the lack of anti-hypertriglyceridemic effect of honey administered 5 minutes before HFD. In contrast, the levels of triglycerides in rats administered honey or simvastatin 60 minutes before HFD feeding were moderately but insignificantly higher than that of the normal control group. This indicates that honey administered 60 minutes before HFD exerted anti-hypertriglyceridemic effect in rats. Previous studies have demonstrated the anti-hypertriglyceridemic effect of honey (Erejuwa *et al.*, 2016; Samat *et al.*, 2017). Phenolic-rich compounds have been shown to inhibit hyperlipidemia (Tuzcu *et al.*, 2017, Qinna *et al.*, 2012). Honey is rich in phenolic acids and flavonoids (Erejuwa *et al.*, 2012). Therefore, the effect of honey in suppressing hypercholesterolemia and hypertriglyceridemia may be attributed to honey phenolic and flavonoid content.

The levels of HDL and non-HDL cholesterol in rats pretreated with honey 5 minutes before HFD feeding were similar to that of control HFD fed rats. The non-HDL cholesterol reflects the cholesterol in VLDL, IDL (intermediate density lipoprotein) and LDL particles (Millan *et al.*, 2009). These data therefore suggest that honey pretreatment 5 minutes before HFD did not mitigate against HFD-induced reduction of HDL cholesterol and elevation of non-HDL cholesterol. The significantly higher HDL cholesterol level in rats pretreated with honey 60 minutes before HFD feeding indicates that honey pretreatment 60 minutes before HFD ingestion prevented against HFD-induced reduction of HDL cholesterol. The LDL cholesterol is highly susceptible to oxidation and oxidized LDL is atherogenic (Parthasarathy *et al.*, 2010). On the other hand, VLDL cholesterol is converted to LDL cholesterol via the action of lipoprotein lipase and thus further aggravates atherogenicity (Toth, 2016). Hence, the elevated levels of LDL and VLDL cholesterol in control HFD group and rats pretreated with honey 5 minutes before HFD feeding imply that the rats were highly vulnerable to developing atherosclerosis. The findings, as demonstrated by lower levels of LDL and VLDL cholesterol, also suggest that administering honey 60 minutes before HFD feeding suppressed HFD-induced elevations in LDL and VLDL cholesterol and thus may offer protection against cytotoxicity of oxidized LDL cholesterol. The ameliorative effect of honey on hyperlipidemia may be attributed to diverse constituents in honey. Honey is enriched in numerous bioactive substances including phytosterols which have been shown to enhance cholesterol metabolism (Howell *et al.*, 1998). Epidemiological evidence associates high concentrations of HDL cholesterol with several health beneficial effects including antiatherogenic effect, inhibition of LDL oxidation and healthy endothelial function (Chapman *et al.*, 2011). Therefore, the significant increase in HDL cholesterol and marked reduction in LDL cholesterol in rats pretreated with honey 60 minutes before HFD imply these rats were better protected against atherogenicity, LDL oxidation and impaired endothelial function.

In many disorders such as metabolic syndrome, diabetes mellitus and obesity, evaluation of lipid ratios is considered a stronger predictor of disease risks (Gasevic *et al.*, 2014). The TC/HDL cholesterol, for instance, is a coronary risk index which is used to predict risk of CHD (Ingelsson *et al.*, 2007) whereas the TG/HDL cholesterol reflects cardiovascular disease risk (de Giorgis *et al.*, 2014). The markedly elevated levels of TC/HDL cholesterol and TG/HDL cholesterol in control HFD fed rats and rats pretreated with honey 5 minutes before HFD suggest that honey administered 5 minutes before HFD did not prevent against risks of HFD-induced CHD and cardiovascular disease. The lack of significant differences in TC/HDL

cholesterol and TG/HDL cholesterol in rats pretreated with honey 60 minutes before HFD and normal control group imply the protective effect of honey against HFD-induced cardiovascular disease and CHD risks. The effects of honey in ameliorating postprandial hyperlipidemia and lipid ratios (risk indices) corroborate those of previous studies in diabetic and obese rats (Erejuwa *et al.*, 2011, Nasrolahi *et al.*, 2012, Erejuwa *et al.*, 2016, Samat *et al.*, 2017).

To the best of our knowledge, this is the first study in the literature to explore the potential role of HMG-CoA reductase in mediating the antihyperlipidemic effect of honey. In this study, lower HMG-CoA/mevalonate corresponds to a higher HMG-CoA reductase activity and vice versa. The fact that the levels of HMG-CoA/mevalonate in control HFD fed group and rats pretreated with honey 5 minutes before HFD were significantly lower than the level in the normal control rats suggest that honey administered 5 minutes before HFD feeding did not prevent HFD-enhanced HMG-CoA reductase activity. On the other hand, honey or simvastatin administered 60 minutes before HFD feeding was associated with higher levels of HMG-CoA/mevalonate in Wistar rats. Higher HMG-CoA/mevalonate signifies lower HMG-CoA reductase activity and, thus, implies inhibition of HMG-CoA reductase in these rats. Since the literature lacks data on the effect of honey on HMG-CoA reductase, we are unable to compare our findings with those of other researchers. Plant extracts with phytochemicals including phenolic compounds and flavonoids have been shown to inhibit HMG-CoA reductase *ex vivo* (Kwon *et al.*, 2010, Reddy *et al.*, 2014) and *in vivo* (Qinna *et al.*, 2012). Honey consists of numerous bioactive substances such as phenolic compounds which play an important role in its biological effects (Erejuwa *et al.*, 2012). Therefore, the suppression/inhibition of HMG-CoA reductase following honey pretreatment may be attributed to the high phenolic and flavonoid constituents in honey. Our findings, which showed that simvastatin pretreatment caused vast inhibition of HMG-CoA reductase, agrees with data from previous studies demonstrating statins as potent inhibitors of HMG-CoA reductase (Qinna *et al.*, 2012). The findings also revealed that honey pretreatment 60 minutes before HFD was more effective than honey administered 5 minutes before HFD in inhibiting hepatic HMG-CoA reductase. This inference is also substantiated by the serum data which indicated that honey pretreatment 5 minutes before HFD was less effective in ameliorating lipid abnormalities and disease risk indices.

Oxidative stress is associated with several diseases including diabetes mellitus and obesity and is considered a potential therapeutic target in these disorders (Erejuwa, 2012). The role of oxidative stress is implicated in the injurious effects of postprandial hyperlipidemia (Bae *et al.*, 2001). Oxidative stress has been demonstrated in HFD fed rats (Venkateshan *et al.*, 2016). In both healthy and obese subjects, postprandial hyperlipidemia has been shown to generate reactive oxygen species (ROS) and cause oxidative damage (Patel *et al.*, 2007, Stojiljkovic *et al.*, 2002). Some of the commonly generated ROS include superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). Superoxide dismutase catalyzes the conversion of $O_2^{\bullet-}$ to H_2O_2. Though H_2O_2 is more stable than $O_2^{\bullet}$, it generates a highly reactive hydroxyl radical ($^{\bullet}OH$). H_2O_2 is converted to water and oxygen in the presence of catalase (Haber and Weiss, 1934). The catalysis of H_2O_2 by catalase, therefore, helps to prevent the formation and cellular buildup of $^{\bullet}OH$. The results showed that serum catalase activity was significantly higher in control HFD fed rats than in normal control group. Enhanced activity or expression of certain enzymes including catalase and nitric oxide synthase has been demonstrated following increased levels of H_2O_2 (Tiedge *et al.*, 1999, Cao *et al.*, 2011). Therefore, the higher catalase activity in portable water-pretreated HFD fed rats may imply catalase induction in response to acute production of H_2O_2. The levels of serum catalase activity in rats pretreated with honey 5 or 60 minutes before HFD were similar compared with normal control rats. Hence, it can be inferred that honey pretreatment suppressed postprandial hyperlipidemia-enhanced catalase activity in rats. This is a confirmation that induction of catalase in portable water-pretreated HFD fed rats is an adaptive mechanism in response to increased ROS formation (Crawford and Davies, 1994). This is usually a protective mechanism aimed at preventing or reducing ROS-mediated damage. Compelling evidence indicates honey is a novel antioxidant (Erejuwa *et al.*, 2012). It is therefore not surprising that honey pretreatment prevented HFD-induction of catalase activity or restored catalase activity towards that of normal control group. The antioxidant effect of honey is due to its high phenolic and flavonoid constituents (Erejuwa *et al.*, 2012). These bioactive constituents are free radical scavengers.

In comparison with honey administered 5 minutes before HFD, honey pretreatment 60 minutes before HFD was more effective in preventing postprandial HFD induction of catalase activity in rats. Plant extracts rich in phenolic acids have also been reported to ameliorate hyperlipidemia-induced oxidative stress in HFD fed rats (Sarega *et al.*, 2016). In addition to catalase activity, this study explored the effect of honey on postprandial oxidative stress by assessing TAS and MDA. The study revealed that there was no significant difference in TAS among the groups. The evaluation of TAS reflects the overall contribution of the distinct antioxidants (enzymatic and non-enzymatic) present in a sample (Ghiselli *et al.*, 2000).

A slight induction of TAS in control HFD and simvastatin-pretreated HFD fed rats was however observed. This trend was similar to what was detected in catalase activity. Therefore, this slight induction of TAS in the two groups may be a contributory role of catalase in the assay of TAS. Determination of MDA is usually performed in oxidative stress studies to assess the extent of lipid peroxidation (Halliwell and Gutteridge, 1984). The results indicated that the levels of MDA were comparable among the groups. The lack of significant increase in MDA concentrations in control HFD fed rats, despite considerable postprandial hyperlipidemia, may be a consequence of enhanced catalase activity. Induction of catalase activity, an adaptive or defense mechanism, would result in increased catalysis of accumulated H_2O_2 to water and oxygen. This would invariably prevent or diminish the production of $^{\bullet}OH$. In the absence or low level of $^{\bullet}OH$, there would be no further propagation of ROS nor ROS mediated-lipid peroxidative damage and thus no change in MDA. Therefore, the lack of significant change in MDA in control HFD fed rats may imply that the induced activity of catalase was protective against lipid peroxidation.

In conclusion, honey administered 60 minutes before HFD feeding exerts more profound antihyperlipidemic effect than honey administered 5 minutes before HFD in Wistar rats. This marked antihyperlipidemic effect of honey pretreatment is mediated in part via inhibition of HMG-CoA reductase. Honey pretreatment also attenuates postprandial hyperlipidemia-induced oxidative stress in rats.

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