Natural honey reduced atherogenic and coronary risk indices in Wistar rats

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BACKGROUND
The biochemical mechanism underlying the nutraceutical effects of honey is poorly understood, thus making its functions more a matter of speculations. In this study, we investigated the effects of honey on the atherogenic and coronary risk indices in Wistar rats.

METHODS
An experimental design comprising two groups of rats fed with normal rat chow but with the experimental group receiving 10% honey in water and the control group water alone, for five weeks. Blood samples were collected weekly from each group, and the level of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and total protein were determined. The lipids profile (total cholesterol, total triglycerides, LDL and HDL) were also determined, and the atherogenic and coronary indices were estimated. Data were analyzed, and p<0.05 was considered significant.

RESULTS
There were no significant changes in both groups’ serum SOD and CAT across the weeks of study. The LDL cholesterol of the honey-treated rats, however, decreased significantly (9.95 mg/dL) compared to the controls (27.07 mg/dL) (p=0.000). In contrast, honey intake elevated the HDL cholesterol (18.37 mg/dL) relative to 12.25 mg/dL in the control group (p=0.003). Consequently, honey treatment caused significant depletion of atherogenic and coronary risk indices (76.13%, p=0.001) and (50.37%, p=0.023) respectively.

CONCLUSION
We show evidence that the regular intake of honey, at a concentration as low as ten percent of total water intake, may lower factors for the onset of hypertension and coronary diseases.

Keywords: Honey, atherosclerosis, coronary risk, anti-oxidant, oxidative stress, rats
INTRODUCTION

Cardiovascular diseases (CVDs) result from several metabolic disorders and the natural process of aging. During aging, metabolism abnormalities set in as a result of the oxidative attack of reactive oxygen species (ROS) on biomolecules generally and lipids in particular. The transport of lipids in the blood is in the form of lipoproteins such as the low density (LDL) and high density (HDL) lipoproteins. When oxidized, the ox-LDL formed are scavenged by the macrophages, resulting in serum lipoprotein imbalance measured as atherosclerosis or coronary risk indices. Macrophages, when heavily laden with oxidized lipids, form foam cells which later result in atheroma plaques and eventually atherosclerosis and coronary heart disease.

Thus, CVDs are often the outcome of oxidative stress resulting from ROS.

There are two main classes of ROS: non-radical species ($\text{H}_2\text{O}_2$) and free radical forms ($\text{O}_2^-$, $\text{OH}$, $\text{OH}_2$). Cellular defense mechanism against these radicals involves the use of enzymes such as superoxide dismutase (SOD) which scuffles the damaging reactions of superoxide, converting it to $\text{H}_2\text{O}_2$. The $\text{H}_2\text{O}_2$ produced is then removed by catalase (CAT).

Superoxide dismutase and CAT are commonly called antioxidant enzymes while the ROS are pro-oxidant. Another natural but non-enzymatic antioxidant is glutathione (GSH). Summarily, oxidative stress results from an imbalance between pro-oxidants and antioxidants.

Over the years, several antioxidants, both natural and synthetic, have been packaged as supplements; they are however expensive and not readily available. The use of nutrition then becomes an attractive alternative.

Nutrition is of immense importance in aging. Honey is a multi-nutrient food containing varying amounts of minerals ranging from 0.02 to 0.03 g/100 g, with aluminum, barium, boron, chlorine, fluoride, iodine, sulfur and potassium accounting for one third of the total elements. There are reports that honey stimulates insulin secretion resulting in decreased glucose blood levels in addition to its antibacterial, anti-inflammatory, and immune-stimulant activities. Furthermore, honey contains several physiologically important amino acids including all nine essential amino acids and all nonessential amino acids. Another useful group of compounds found in honey is the polyphenols, which are at concentrations of 56 to 500 mg/kg. These polyphenols are mainly flavonoids, phenolic acids, and phenolic acid derivatives, and they contribute to the antioxidant properties of honey.

The antioxidant activities are essential in the reduction of oxidative reactions and aid of human health promotion.

There are two broad categories of honey: the blossom or nectar honey, which originates from nectars of plants, and honeydew honey, that are mainly from excretions of plant sucking insects (Hemiptera) on the living parts of plants or secretions of living parts of plants. Different types of honey exert different degrees of health benefits even though they have similar composition and physicochemical properties such as high osmolarity, low moisture and acidity. This variation in health benefits is related to the honeys' geographical, seasonal and botanical origin as well as the harvesting, processing and storage conditions. A randomized controlled trial in 526 obese subjects and 710 normal subjects showed that supplementation of honey for 28 days significantly reduced total cholesterol (TC) and LDL together with increased HDL. However, these effects were strongly correlated with gender, BMI and ethnicity. Another study with a longer treatment period was conducted, whereby treatment of 10% honeydew honey (100 g/kg) mixed in diets was compared with sucrose for 365 days. Although there was no significant decrease in triglycerides and LDL, the honey-fed rats had significantly higher HDL levels ($2.82 \pm 0.30 \text{ mmol/L}$) than the rats on a sugar-free diet ($2.32 \pm 0.33 \text{ mmol/L}$) and sucrose diet ($2.44 \pm 0.51 \text{ mmol/L}$). This conflicting result needs another study to examine the protective effect of honey on lipid profile. The aim of the present study was to evaluate the effect of honey on the redox status, lipid profile as well as atherogenic indices in Wistar rats.
METHODS

Research design
A randomized controlled trial was conducted at the Redeemer’s University Animal House facility, Mowe, Ogun State, Nigeria from January to April 2017.

Animals and chemicals
Thirty male Wistar rats, about six months old, with an average weight of 209.6 ± 7.23g, purchased from Covenant Farms, Ibadan, were acclimatized in a well-ventilated animal room at 25 ± 2°C under controlled light cycles at the Redeemer’s University Animal House facility, Mowe, Ogun State, Nigeria. A total of thirty male Wistar rats were used in the study. The sample size was determined by multiplying the number of trials (3) with the sample periods, 5 times of once a week for five weeks. The rats were fed standard commercial chow and clean water ad libitum for at least a week before the experiment. Other reagents were of analytical grade and used as supplied.

Physicochemical characterization of honey
The moisture content of the honey was determined by the refractometer, and electrical conductivity using conductivity meter (Schott Instrument, Germany). Total acidity was determined by titration to pH 8.3 with 0.1 M sodium hydroxide; relative densities and glucose and fructose concentrations were determined using Benedict’s reagent. Cu²⁺ and Zn²⁺ contents were determined using atomic absorption spectroscopy (AAS-7000, Shimadzu, Japan). Vitamin C content was determined based on the decolorization of dichlorophenolindophenol.¹⁷

Honey
The honey used in this study is a natural (unprocessed) acacia honey, typically produced by bees that feed on flowers, mainly the Fabaceae and Sapotaceae plants in the rainforest zone of Nigeria. The honey was purchased from the local market at Oyigbo, Lagos Nigeria. It has no National Agency for Food and Drug Administration Control (NAFDAC) registered number, to eliminate the bias that might be introduced during processing. The honey was dark brown in color with relative density of 1.88 and an electrical conductivity of 0.0012 mS/cm; it was slightly acidic (0.093%/mass) with a moisture content of 14.4 g/100 g, fructose and glucose concentrations of 6.7 and 7.1 g/100 g respectively and relatively low copper and zinc level of 0.043 and 0.022 ppm, respectively. The honey was dissolved in drinking water and prepared freshly each time it was administered.

Specimen collection
Rats were sacrificed 12 h after the last treatment administration. Blood was collected by cardiac puncture into plain universal tubes and was then allowed to stand for 1 hour. Serum was prepared by centrifugation at 4000 rpm for 10 min. The clear supernatant was used to estimate the serum enzymes and other parameters.

Biochemical analysis
Serum total protein concentration was determined using the biuret reaction with bovine serum albumin as standard. Catalase (CAT) level was determined colorimetrically at 570 nm by measuring the chromic acid produced when dichromate in acetic acid is reduced to chromic
acetate in the presence of H$_2$O$_2$. The level of superoxide dismutase (SOD) activity was determined as the conversion of 50% adrenaline (0.3 mM epinephrine) to adrenochrome in 1 minute. Reduced glutathione level (GSH) was determined using the Ellman’s reagent: the reduced form of glutathione is the bulk of cellular non-protein sulfhydryl groups. These sulfhydryl compounds react with 5′5′-dithiobis-2-nitrobenzoic acid (Ellman’s reagent) to form a relatively stable yellow compound. Total cholesterol (TC), low-density lipoprotein-cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglyceride (TG) were measured spectrophotometrically using Randox commercial kits. Atherogenic index (AI) and coronary risk index (CRI) were estimated using the formulae:

\[ AI = \frac{LDL \text{ Cholesterol}}{HDL \text{ Cholesterol}} \quad \text{and} \quad CRI = \frac{Total \text{ Cholesterol}}{HDL \text{ Cholesterol}} \]

Data Analysis

Data were analyzed at a 95% level of significance using paired t-tests for within-group comparisons and independent t-tests for between-group comparisons. Further analysis was done in 2 (group) x 5 (week) mixed design ANOVA with repeated measures using SPSS Statistics 23 (IBM. Armonk, NY, USA) to evaluate the effect of honey intake on all biochemical parameters. All measured values are expressed as mean ± SEM of triplicates. Simple percentages were also used for the atherogenic and coronary risk indices.

Ethical clearance

The study protocol was approved by the Research Ethics Committee of the Redeemer's University no RUN/IREC/17/1385.

RESULTS

The daily administration of ten percent honey in water to the rats for five weeks resulted in a significant decreased in total cholesterol, LDL-cholesterol and triglycerides, while the mean HDL-cholesterol showed a significant increase in both between the groups and across the weeks (p<0.001; p=0.000; p=0.007; p=0.003, respectively) (Table 1). The total cholesterol levels of the honey group at week 5 were significantly lower than those of the control group (p=0.032). There were initially non-significant higher values in the experimental group, after which there were significant reductions across the weeks (p=0.040) (Table 1). While the TG increased consistently and significantly in the control rats, honey consumption initiated an initial decrease in the TG till the third week which then remained constant after the fourth week. The TG was however significantly lower in the honey-consuming rats than in the controls after the five-week period of study (p=0.007). Conversely, the HDL decreased as the control rats aged, but increased in the honey-consuming group such that there was a highly significant difference at the end of five weeks of monitoring (p=0.003) and across the weeks (p=0.10). No between-group differences were observed for LDL until after the third week, but the difference became significant over time (p=0.000) since the LDL decreased consistently within the five weeks of research.

Generally, the antioxidant enzyme activities and GSH reduced as the rats aged, while the total protein increased. At the end of the five-week study, both the CAT and SOD were not significantly different between the two groups (p=1.000 and p=0.796, respectively). On a weekly basis, the CAT and SOD activities reduced significantly up to the fourth and third week, respectively. Although the GSH reduced as the rats aged, the honey group demonstrated significantly higher GSH values weekly and across the weeks (p=0.001). The total protein had an increasing tendency from baseline to week 5 in both the control and honey groups. There was no significant difference on a weekly basis but the difference was significant across the weeks (p=0.023) (Table 2).
Table 1. Distribution of lipid profile by treatment groups in rats

| Treatment | Total cholesterol (mg/dL) | Triglycerides (mg/dL) | HDL (mg/dL) | LDL (mg/dL) |
|-----------|---------------------------|-----------------------|-------------|-------------|
|           | Mean ± SEM                | Mean ± SEM            | Mean ± SEM  | Mean ± SEM  |
| Control   | 71.34 ± 8.34              | 69.42 ± 1.80          | 14.50 ± 1.13| 42.94 ± 7.15|
|            | (57.52 ± 1.99)           | (71.94 ± 1.64)        | (10.60 ± 0.35)| (32.54 ± 1.44)|
| p value   | 0.534                     | 0.047                 | 0.188       | 0.857       |
| Honey     | 75.09 ± 4.69              | 72.69 ± 3.09          | 16.74 ± 0.86| 43.90 ± 4.90|
|            | (62.97 ± 2.68)           | (64.97 ± 2.48)        | (13.99 ± 1.46)| (35.99 ± 1.89)|
| p value   | 0.021                     | 0.017                 | 0.052       | 0.087       |
|           | 79.69 ± 1.39              | 74.05 ± 3.39          | 17.87 ± 0.57| 23.92 ± 1.86|
|           | (79.69 ± 1.39)           | (70.87 ± 2.31)        | (17.87 ± 0.57)| (23.92 ± 1.86)|
|           | 58.01 ± 2.44              | 90.23 ± 4.56          | 19.37 ± 0.29| 13.46 ± 0.89|
|           | 58.01 ± 2.44              | (74.27 ± 4.86)        | (18.37 ± 1.46)| (9.95 ± 0.69)|
| p value   | 0.000                     | 0.012                 | 0.001       | 0.000       |

Values are expressed in mean ± SEM, p<0.05 significant as compared to control; HDL: high density lipoprotein; LDL: low density lipoprotein

Table 2. Distribution of catalase, glutathione and superoxide dismutase by treatment groups in rats

| Parameters | Treatment | week1 | week2 | week3 | week4 | week5 | Across weeks |
|------------|-----------|-------|-------|-------|-------|-------|--------------|
|            | CAT       | 18.22 ± 0.82 | 2.68 ± 0.39 | 7.83 ± 0.71 | 1.89 ± 0.21 | 2.49 ± 0.28 | p=1.000      |
| (µmol/mL/mg protein) | Honey     | 17.62 ± 1.54 | 3.61 ± 0.65 | 4.57 ± 0.64 | 3.84 ± 1.00 | 2.45 ± 0.68 | p=0.096      |
| p value    | 0.583     | 0.101 | 0.004 | 0.029 | 0.001 | 0.010 | 0.028        |
| SOD        | Control   | 0.44 ± 0.04 | 0.19 ± 0.02 | 0.02 ± 0.01 | 0.17 ± 0.01 | 0.11 ± 0.01 | p=0.089      |
| (U/min/mg protein) | Honey     | 0.32 ± 0.4 | 0.29 ± 0.03 | 0.13 ± 0.01 | 0.16 ± 0.01 | 0.11 ± 0.02 | p=0.089      |
| p value    | 0.021     | 0.009 | 0.001 | 0.288 | 1.000 | 0.001 | 0.045        |
| GSH        | Control   | 0.70 ± 0.07 | 0.49 ± 0.04 | 0.31 ± 0.03 | 0.29 ± 0.01 | 0.53 ± 0.03 | p=0.001      |
| (µg/mL/mg protein) | Honey     | 0.99 ± 0.11 | 0.57 ± 0.02 | 0.49 ± 0.02 | 0.48 ± 0.01 | 0.59 ± 0.02 | p=0.001      |
| p value    | 0.018     | 0.036 | 0.001 | 0.000 | 0.045 | 0.058 | 0.023        |
| Total protein | Control   | 23.90 ± 4.31 | 53.13 ± 1.78 | 63.65 ± 8.15 | 63.97 ± 1.77 | 50.00 ± 1.87 | p=0.023      |
| (mg/mL)    | Honey     | 32.25 ± 2.56 | 47.14 ± 3.87 | 53.69 ± 4.01 | 63.02 ± 3.83 | 53.13 ± 1.73 | p=0.023      |
| p value    | 0.527     | 0.361 | 0.417 | 0.367 | 0.958 | 0.058 | 0.023        |

Values are expressed in mean ± SEM; SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione
Reported in Table 3 were the atherogenic and coronary risk indices of the rats after five weeks of treatments. The honey consumption for five weeks drastically reduced the AI (p=0.020) as well as the CRI (p=0.000) when compared with the control group. The reductions were also significant across the five weeks of experimental period, both for AI (p=0.001) and for CRI (p=0.037). The percentage reductions in the atherogenic index and the coronary risk index respectively in honey treatment were 76.13% and 50.41%.

**DISCUSSION**

The administration of honey reduced atherosclerotic and coronary risk indices in Wistar rats by lowering the plasma levels of TC, TG, and LDL, but increased the level of HDL in the studied rats. Honey is rich in flavonoids, mainly polyphenols and phenolic compounds. Some of these flavonoids such as the honey polyphenols quercetin, apigenin, chrysin and luteolin are known hydrogen methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors.\(^{(18,19)}\) Hydroxymethylglutaryl-coenzyme A is a prominent enzyme in the de novo synthesis of cholesterol; its inhibitors thus prevent the accumulation of blood cholesterol and LDL in particular. On the other hand, the non-significant changes observed in the levels of CAT and SOD and the relative increase in GSH are an indication that the enzymes may not be central to the nutraceutical properties of honey, and that GSH may be important in the antioxidant property. However, more investigations are required in this direction to explain further the likely mechanism of actions of these agents. Previous findings lend credence to the therapeutic application of honey and offer prospects for its use as an adjuvant to promote cardiac health.\(^{(11,20)}\)

The physiochemical analysis of the honey showed that it is rich in ascorbate, fructose, and glucose; and that it contains trace elements such as copper and zinc. Generally, honey is rich in monosaccharides and vitamin C.\(^{(20,21)}\) Honey has also been reported to contain several trace elements\(^{(22)}\) and phenolic compounds,\(^{(9)}\) which are generally potent antioxidants. Al-Sabaaw\(y^{,(23)}\) in a study on relating lipid profile to selected trace elements, reported a negative correlation between serum Zn and TC, LDL, TG and LDL-C/HDL-C ratio and a significant positive correlation between serum Zn and HDL-C. The author further linked these activities to the antioxidant role of Zn. This implies that decrease in serum Zn level may lead to increased lipid peroxidation with consequent increase in the levels of TC, TG and LDL-C according to the results of earlier studies.\(^{(24,25)}\) Furthermore, copper and zinc are antioxidant trace metals that have previously been reported to stimulate the production of Cu-/Zn-SOD, a type of antioxidant enzyme.\(^{(26)}\)

The SOD and CAT enzymes are essential in the regulation of oxidative stress; altered activities of these antioxidant enzymes are an adaptive mechanism to protect cells against the toxic radicals. Since SOD converts superoxide anions to hydrogen peroxide (H\(_2\)O\(_2\)), an enhanced SOD activity might lead to increased turnover of H\(_2\)O\(_2\)\(^{(20)}\) which usually results in elevated activities of CAT. Normally, H\(_2\)O\(_2\) is further metabolized to H\(_2\)O and O\(_2\) by CAT, an enzyme that is highly susceptible to increased superoxide anions.\(^{(26)}\)

Data obtained in this study revealed no significant decrease in the activities of plasma SOD and CAT in the honey-treated group relative to the

| Parameters                  | Control        | Honey          | Across weeks* |
|-----------------------------|----------------|----------------|---------------|
| Atherogenic index (AI)      | 2.43 ± 0.82    | 0.58 ± 0.25 (76.13%)\(^{a}\) | p = 0.001     |
| Coronary risk index (CRI)   | 4.82 ± 0.87    | 2.39 ± 0.30 (50.41%)\(^{a}\) | p = 0.023     |

Values are expressed as mean ± SEM; \(^{a}\)p values across the five weeks of study; \(^{a}\)-% reduction following honey intervention in parentheses
control group. These results agree with a previous report by Erejuwa et al. (20) which demonstrated that honey treatment did not decrease the antioxidant enzymes in diabetic rats.

Hyperlipidemia has been associated with aging. Amelioration of this dyslipidemia is vital in the reduction of the onset of atherosclerosis and CVDs. (26) It is widely accepted that elevations in cholesterol and LDL plasma levels are major risk factors for coronary heart disease, (26,27) This study revealed that 10 % honey, in daily water, significantly reduced total cholesterol, triglycerides, and LDL levels but increased the HDL. The results were in line with previous results that suggested the hypocholesterolemic effect of honey. (28,29) There was a relationship between elevated LDL and atherosclerosis, since LDL in the blood gets deposited in the walls of the blood vessels and becomes the major component of the atherosclerotic plaque.

Furthermore, the 70% reduction in AI and the 50% reduction in CRI are clear evidences that honey have an ameliorative effect in reducing the onset of CVD and CHDs. Bahrami et al. (30) have reported the ameliorative effects of honey in patients with elevated risk factors and a separate study on diabetic rats by Erejuwa et al. (28) showed that honey caused a reduction in AI and CRI. In another study, Afroz et al. (31) reported that honey acted as an anti-hepatotoxic and anti-nephrotoxic agent by successfully altering the antioxidant enzyme activities of rats exposed to paracetamol. The ability of honey to enhance the antioxidant effect of hypoglycemic drugs such as metformin or glibenclamide has also been reported. (20) Furthermore, several studies have shown that increased dietary intake of natural phenolics correlates with reduced coronary heart disease and cancer mortality, with longer life expectancy. (17) Honey is generally rich in these polyphenolic compounds. In summary, the antioxidant properties of honey could be linked to its medicinal benefit. In this study we assumed that honey as a nutrient could not elicit any adverse effect on organs and tissues, so physiopathology was not conducted on organs and tissues of both the control and experimental rats, and phytochemical analyses of the honey were also not considered. Future studies may look into the impact of honey consumption on stomach lining and organ physiology, and qualitative and quantitative analyses of the phytochemicals may unravel the mechanism of action of honey.

CONCLUSION

This study showed that a 10 % honey intake reduced the levels of TC, TG, and LDL, but increased the level of HDL in healthy rats. On the other hand, the unaltered activities of CAT and SOD is an indication that the antioxidant enzymes may not take part in the prophylactic nutraceutical properties of honey, and that GSH is a candidate molecule in this regard.

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CONFLICTS OF INTEREST

No conflict of interest

CONTRIBUTORS

ODO and ATO contributed to research design, implementation and manuscript development; YOA and OTA contributed to implementation and data collection; GGD and OSA contributed to physiochemical and data analysis. All authors have read and approved the final manuscript.

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