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Nephroprotective and Antihyperlipedemic Activity of Honey in Alloxan Induced Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OO designed the study and performed statistical analysis. Author ANC wrote the Protocol and the first draft of the manuscript. Author JEO managed the literature searches. Authors OO, JEO and ANC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

There is increasing evidence to support the health benefits of natural honey. However, its use in the dietary management of diabetes mellitus is still evolving. The present study was aimed at investigating the long-term effects of Nigerian honey of Niger Delta origin on alloxan induced renal and serum lipid dysfunctions in diabetic wistar rats. Four groups of adult male wistar rats were used; 8 rats each. The first group received no honey but were given saline and served as normal control. Group II were non-diabetic and received honey solution (50% v/v) at a dose of 10ml/kg body weight/day. Diabetes was induced in groups III and IV by intra-peritoneal administration of 200mg/kg alloxan solution. Group III served as diabetic control. Group IV received a honey solution. At the end of 56 days, lipid profile and renal function were assessed. Also, atherogenic index was calculated. Results obtained revealed alloxan induced diabetic renal dysfunction, as reflected by up-regulated kidney function parameters–urea, creatinine, and a decrease in sodium, and bicarbonate, levels while a non-significant difference between potassium in diabetic control and diabetic treated. Regarding serum lipid, there was up-regulated total cholesterol, triglyceride, low-density lipoprotein, atherogenic index and decreased high-density lipoprotein levels. Therefore, oral administration of

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honey diminished the deleterious effects of alloxan-induced diabetes on renal function and improved lipid profile parameters. We conclude that Niger Delta honey has the potential medicinal properties to protect against alloxan induced renal dysfunction and hyperlipedemia in diabetic male wistar rats.

Keywords: Niger Delta honey; kidney; lipid; alloxan; diabetic rat.

1. INTRODUCTION

Diabetes mellitus is a chronic endocrine disease characterized by hyperglycaemia due to low or no insulin production in the body, and leads to enormous human and socioeconomic burden. Evidence indicates that the prevalence of non-communicable diseases especially diabetes mellitus is more common in developing countries than the developed countries [1-6]. Moreover, diabetes is expected to become significant over time due to increasing undesirable result of risk factors such as physical inactive and sedentary lifestyle with unhealthy dietary habits and to demographic changes [1-4].

Honey is a complex compound that has been reported to contain nearly 200 components, with fructose, glucose, and water as main substances [7-10]. Several studies have reported that honey contains numerous nutritional values and potential medicinal properties and had shown encouraging results on its health beneficial effects even in diabetes mellitus [7-15]. These effects of honey had led to multiple hypotheses on the mechanisms of action, which might be influenced by the botanical and geographical origins of the honey. Little is currently known about the potential medicinal beneficial effects of natural honey from the Niger Delta region of Nigeria. In our previous study, we reported that honey administered groups adopted in the study of the alloxan diabetic models gave positive results according to the parameters studied [15, 16]. Therefore, there is renewed interest in health benefits of honey as well as believe of the efficacious, safer, relatively fewer side effects and reduction in its cost effectiveness treatment. So, we have extended our previous studies (15, 16) to show the effect of long-term dietary honey on kidney function and lipid metabolism in normal and alloxan induced diabetic rats, for better understanding of the medicinal properties of Niger Delta honey.

2. MATERIALS AND METHODS

2.1 Study Period

This study was carried out during the months of June to October, 2016 in the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

2.2 Sample Collection

Natural honey was obtained from 'Divine honey' bee farm in Delta state, Nigeria.

2.3 Induction of Diabetes

Diabetes Mellitus was induced in overnight fasted rats via intraperitoneal administration of 200 mg/kg body weight of alloxan dissolved in normal saline. Three days after alloxan injection, development of diabetes was confirmed by measuring blood glucose levels in blood samples taken from tail vein and analyzed based on glucose oxidase-perioxidase principles [17] using digital glucometer (Accu-Chek Aviva, Germany) [18]. Blood glucose concentrations of ≥12.0 mmol/L was taken to be alloxan induced diabetic rats.

2.4 Experimental Animals Grouping and Treatment

Male adult wistar rats weighing 200-250 g were used in this study. They were kept under normal laboratory conditions and given free access of food and water. They were left for acclimation for one week before starting the experiment. Rats were randomly arranged into four groups; 8 each. Using oral cannula, once each morning (9-10 am) the rats were treated with honey for 56 days. Honey was diluted using distilled water and administered at a dose of 50% v/v. Each animal was administered honey solution at a dose of 10 ml/Kg body weight/day. The animals were grouped as follows:

Group I: the rats in this first group were non-diabetic and were given normal saline and served as the negative untreated general control group.
Group II: the rats in the second group were also non-diabetic but were administered honey solution (50%, v/v) at a dose of 10ml/Kg body weight/day.
Group III: the rats in the third group were alloxan induced diabetic and served as positive treated control group.

Group IV: the rats in the fourth group were alloxan induced diabetic and were administered honey solution (50%, v/v) at a dose of 10ml/Kg body weight/day. The rats were housed in the laboratory cages and were provided with standard feed and allowed access to water *ad libitum* during the 56 days observation period.

2.5 Blood Collection and Analysis

At the end of the treatment period of 56 days, the animals were anaesthetised using urethane, sacrificed and blood samples were collected using 5ml syringe via cardiac puncture.

Urea was analyzed by diacethyl monoxyl method using BSA-3000 chemistry full automatic analyzer (SFRI, France) according to manufacturer's protocols.

Creatinine was analyzed by Jaffe method (kinetic method) using BSA-3000 chemistry full automatic analyzer (SFRI, France), according to manufacturer's protocols.

Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), were measured by enzymatic methods using a BSA 3000 chemical analyser by SFRI, France, according to manufacturer's protocols.

AIP (Atherogenic index of plasma) was calculated according to the following equation: AIP =log (TG/HDL-C) with units for TG and HDL-C in mmol/L.

2.6 Estimation of Serum Electrolytes

Serum electrolytes (Na, K, Cl, and HCO_3) were analyzed using Ion-selective Electrode (ISE) 4000 analyzer, by SFRI, France according to manufacturer's protocols

2.7 Ethical Approval

The Institutional animal ethics committee approved the experimental protocol. All animal experiments were carried out according to the National Research Council guide lines for the care and use of laboratory animals.

2.8 Statistical Analysis

The assays were carried out in triplicate, and the data were expressed as mean values and standard error of mean (mean ± SD). Statistical analysis was carried out using Statistical Package for Social Sciences software version 20.0 (SPSS, Inc. Chicago, IL) and Microsoft excel. Tables and graphs were used to represent data. Comparison of means was done using one-way ANOVA test. Differences in values were considered statistically significant at p <0.05.

3. RESULTS

The effect of honey on the treatment of the toxic effect of alloxan-induced diabetes on the kidney was revealed by a study of the kidney function parameters- creatinine and urea and electrolytes-sodium, potassium, bicarbonate and chloride in rats supplemented for 56 days, as illustrated in Table 1. Evidently diabetic control (positive control group) up-regulated urea (55.56%) and creatinine (113.84%) levels compared with the negative control (general control). However, the administration of honey to diabetic rats produced an improvement in kidney function by reduction in urea (22.22%) and creatinine (23.81%) levels as compared with negative control or by decreasing urea (50%) and creatinine (64.37%) compared with diabetic control. Similarly, honey fed non-diabetic rats produced significant decrease in urea (11.11%) and creatinine (25.58%) levels compared to negative control, or decrease in urea (42.85%) and creatinine (65.16%) levels in comparison with diabetic control.

The mean values of sodium, potassium, bicarbonate and chloride levels in the serum of diabetic and non-diabetic rats treated with honey for 56 days are also shown in Table 1. Evidently, administration of honey to diabetic rats produced significant increase in sodium (19.09%) and bicarbonate (34.67%) compared with the negative control as well as increased sodium (25.25%) and bicarbonate (132.35%) compared with the positive control (diabetic control). While honey administration in non-diabetic produced a decrease in sodium (10.92%), potassium (8.89%) and an increase in bicarbonate (21.33%) in comparison with negative control (normal rats) but a decrease in sodium (6.23%) and potassium (19.23%) and an increase in bicarbonate (109.09%) compared with the positive control (diabetic control). A non-significant difference in potassium levels in the serum of diabetic control and diabetic treated were observed. There was the non-significant difference in chloride levels between test groups and the referents. However, in comparison with the positive control, there was an increase of 1.48% in chloride level in non-diabetic and 0.13% in diabetic honey-fed rats.

Table 2 summaries the antihyperlipidemic activity of honey. It is evident that honey fed diabetic rats showed significant decrease in total cholesterol (42.86%) triglyceride (34.72%) and low-density lipoprotein (48.69%) and an increase in high-density lipoprotein (20.0%). Similarly, in non-diabetic rats, honey produced decrease in total cholesterol (30.63%), triglyceride (20.11%), and low-density lipoprotein (14.86%) and an increase in high-density lipoprotein (48.67%) in comparison with diabetic control. However, in comparison with negative control (normal rats), non-diabetic honey-fed rats produced increased total cholesterol (7.82),triglyceride (1.17%), low density lipoprotein (14.28%) and high density lipoprotein (12.27%) while diabetic fed honey produced decreased total cholesterol (11.11%),triglyceride (17.05%), low-density lipoprotein (0.86%) and high-density lipoprotein (7.79%). When compared statistically, high-density lipoprotein was significantly increased in diabetic fed honey than that of the diabetic control (p<0.05). Table 2 columns 6 show also the derived values for the atherogenic index. Honey treated was significantly (p<0.05) lower compared to diabetic control.

4. DISCUSSION

Evidence has revealed that the botanical and geographical origins determine the species composition and potential medicinal properties of all types of honey [7,13]. So, the present study was designed to investigate the potential beneficial effects of Niger Delta honey for 56 days aimed at protection of renal functions and lipid profile in alloxan-induced diabetic rats.

In the current study, the changes in the reliable renal biomarkers - urea and creatinine levels are an indication of the severe injured renal function [19,20]. Moreover, our results agree with other studies that showed increases in the serum urea nitrogen and creatinine levels in animals exposed to alloxan or streptozotocin [21,22]. Furthermore, in the present study, honey administration in diabetic rats significantly ameliorated increased plasma creatinine and urea levels in consonance that it has a protective role against kidney dysfunction [19,23].

In this work, serum electrolytes, sodium and bicarbonate in the alloxan diabetic were significantly reduced compared with the control group, denoting the presence of kidney dysfunction. Evidently, in the administration of honey, the levels of the electrolytes were markedly up-regulated compared with diabetic control group. These findings further indicated the protective effects of honey in ameliorating the nephrotoxic effect of alloxan diabetic. The preponderance of sodium bicarbonate is indicative that honey may have probably created an alkaline environment that can help to reverse the damages caused by acidity in alloxan diabetic kidney dysfunction. As an elixir, honey thus is capable of slowing the progression of kidney diseases and it could be a frontline defence against diabetes and its complications in agreement with plausibly its nephroprotective effects [19,24].

Furthermore, in the current investigation, we observed the differential distribution of sodium and potassium in the honey fed diabetic rats. It could be possible that the cell membranes were not damaged, and that alloxan did not cause disturbances in Na+ and K+ pumping and disorders in membrane permeability. Moreover, the changes in serum potassium levels in diabetic control and honey fed diabetic did not achieve any statistical significant difference compared with those of the negative control. In the controversy, the serum potassium and sodium levels were significantly decreased in honey fed non-diabetic rats compared with referents which, however, needed further studies to elucidate the molecular mechanism of actions.

Table 1. Honey effect on renal functions

Treatments	Electrolytes					
	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Bicarbonate (mmol/l)	Urea (mmol/l)	Creatinine (μmol/l)
Non-diabetic Control	218.63±8.54	5.75±0.56	95.00±1.19	18.75±1.22	2.25±0.62	41.50±4.70
Non-diabetic + honey	194.75±3.78*	5.25±0.16*	93.75±1.25	22.75±0.37*	2.00±0.72	31.63±2.20
Diabetic Control	207.88±2.66	6.51±0.56*	92.38±1.99	10.88±1.16*	3.50±0.57	90.88±5.83*
Diabetic + Honey	260.38±6.67**	6.50±0.42**	92.50±1.49	25.25±1.07**	1.75±0.16**	32.38±1.33**

*N=8, *Significant change compared to non-diabetic control (p<0.05); **Significant change compared to diabetic control (p<0.05)*

Table 2. Honey effect on lipid profile

Treatments	Lipid indices		Atherogenic index		
	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High density protein (mg/dl)	Low density lipoprotein (mg/dl)	Atherogenic Index
Non-diabetic Control	218.63±8.54	14.13±0.88	4.88±0.35	3.50±0.65	0.21±0.16
Non-diabetic + honey	194.75±3.78*	14.38±0.46	5.50±0.73	4.00±0.91*	0.20±0.16
Diabetic Control	207.88±2.66	18.00±1.00*	3.75±0.31	6.88±1.42*	0.75±0.16*
Diabetic + Honey	260.38±6.67**	11.75±1.15**	4.50±0.19	3.53±0.19**	0.00±0.00**

*N=8, *Significant change compared to non-diabetic control (p<0.05); **Significant change compared to diabetic control (p<0.05)*

In our study, the effects of honey on the plasma levels of lipid bio-molecules have been recorded. An indicative of antilipidemic effect was observed as total cholesterol, triglyceride, and low-density lipoprotein showed significant decrease whereas high-density protein showed a significant increase by the treatment compared to diabetic group substantiating the work done by other researchers [25-28]. The findings are consistent with the view that natural honey can reduce the complications of diabetes and cardiovascular risk factors caused by abnormal lipid profile [29-34]. In a contradictory 7 day study [35] it was revealed that there were no detectable effects of honey treatment on cholesterol, high-density protein, low-density lipoprotein and cholesterol-high-density protein ratio; but a decrease in the level of triglyceride.

Besides, honey in the current study caused a marked increase in high-density protein, an indication of having a low glycaemic index [29]. It is known that foods with high glycaemic index are associated with decreased high-density protein levels [36]. The derived values for the atherogenic index of the honey-treated were significantly lower compared to diabetic control or general control collaborating with the hypothesis that honey has beneficial effects in diabetes by its anti-atherogenic effect [12]. Collectively, our results are consistent with those obtained in other studies and also support the reports elsewhere that longer period of honey feeding must be used, to obtain significant results.

5. CONCLUSION

It can be concluded that this study evidently has demonstrated that long-term dietary honey can cause reduction of deleterious effects on lipid metabolism and kidney functions which produced diabetic complications in alloxan-induced diabetic rats.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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