

**NEPHROPROTECTIVE AND ANTIHYPERLIPEDEMIC ACTIVITY
OF HONEY IN ALLOXAN INDUCED DIABETIC WISTAR RATS**

ABSTRACT

Recently, there is increased interest in natural honey which is widely used in folklore practices because of its medicinal beneficial effects both at curative and preventive levels. It can prevent hyperglycaemia and thus may have potential therapeutic implications for diabetes complications. The aim of this work was to study the long-term effect of pure honey from Niger Delta region on alloxan induced toxicity of the kidney and serum lipid 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 animals were administered honey solution (50% v/v) at a dose of 10ml/Kg body weight/day. Diabetes was induced in the other two groups by administration of alloxan. Group III received no honey and served as diabetic control group. Group IV diabetic animals were administered honey solution (50% v/v) at a dose of 10ml/Kg body weight/day. Lipid profile (total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein) and kidney function (creatinine, urea and electrolytes) were investigated and compared between all test groups. Also, atherogenic index was calculated. The results obtained revealed alloxan induced diabetic renal dysfunction, as reflected by up-regulated kidney function parameters - urea (55.56%), creatinine (113.84%), and a decrease in sodium (4.91%) and bicarbonate (41.97%) levels while a non-significant difference between potassium in diabetic control and diabetic treated. Moreover, the non-diabetic administered honey showed decrease in sodium and potassium levels compared with the referents. Regarding serum lipid there was also up-regulated total cholesterol (55.56%), triglyceride (27.39%), low density lipoprotein (96.57%), atherogenic index and decreased high density lipoprotein (23.15%) levels. Honey administration for 56 days diminished the deleterious effects of alloxan induced diabetic kidney dysfunction and improved lipid profile parameters. It could be concluded that Niger Delta honey also has the potential medicinal properties to protect against alloxan induced renal dysfunction and hyperlipidemia in diabetic male wistar rats.

35 **KEYWORDS:** Niger Delta honey; kidney; lipid; alloxan; diabetic rat.

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37 **INTRODUCTION**

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

Page | 2

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

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64 **MATERIALS AND METHODS**

65 ***Sample Collection***

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

67 ***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-peroxidase 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.

Experimental animals grouping and treatment

Male adult wistar rats weighing 200-250g 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 10ml/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.

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 diacetyl 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.

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

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.

Conflict of interest

Authors declared no conflicts of interest regarding this manuscript. Also, there was no funding.

Statistical Analysis

The assays were carried out in triplicate, and the data were expressed as mean values and the standard deviation (mean $\pm$ SD). Statistical analysis was carried out using Scientific Package of Social Science software version 20.0 (SPSS, Inc. Chicago, IL) and Microsoft excel. Two different set of statistics, which is descriptive and analytical statistics was applied. The descriptive statistic was used to analyze mean and standard deviation (SD) whereby analytical statistics, one-way ANOVA was used to determine statistical significance among the groups. p value <0.05 were considered statistically significant.

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 nonsignificant difference in potassium levels in the serum of diabetic control and diabetic treated were observed. There was nonsignificant 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 antihyperlipedemic 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.

DISCUSSION

Evidence has revealed that the botanical and geographical origins determine the specific composition and potential medicinal properties of all types of honeys [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 nephrotoxic effect of alloxan diabetic. 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 front line defence against diabetes and its complications in agreement with plausibly its nephroprotective effects [19, 24].

Furthermore, in the current investigation, we observed 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 significance 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.

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 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 - 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.

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.

Page | 8

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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**

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