Original Research Article

The Ameliorative effect of Honey on Lead (Pb) – Induced Nephrotoxicity in male wistar rats.

Abstract

Exposure to lead (Pb^{2+}) is known to portend serious damaging effects on the kidneys which are central to drug and substances excretion. Hence, a natural chelating agent such as honeyis sought to attenuate the deleterious effects of lead induced renal damage. This study investigates the ameliorative influence of honey on renal function in lead – induced nephrotoxicity in wistar rats. Twenty-four (24) adult male Wistar rats (weighing 180-200g) were divided into four groups of six (n=6) rats each. Group 1: served as (control) and received distilled water (10 mL/kg) alone and normal feed daily. Groups 2 to 4 served as test groups and received 10mg/kg/bw of body weight of Pb, 2ml/100mg/kg of body weight of honey and 1ml/10mg/kg/bw of body weight of Pb plus 2ml/100g of body weight of honey respectively. All treatments were daily administered orally using oral gavage and lasted for 28 days. At the end of drug administration, experimental animals were anaesthetized using ketamine cardiac puncture was used for blood collection for renal function parameters analysis. A significant ($p<0.05$) elevation in the serum potassium, uric acid, urea and creatinine was observed for the lead (Pb) treated group compared to the control. However, there was a significant ($p<0.05$) decrease in the serum levels of sodium, potassium, uric acid, urea and creatinine in the honey treated group and the honey + lead (Pb) group when compared with the lead (Pb) treated group. These results strongly suggest a possible tubular disruption and consequent alteration of ionic pumps, and ion channels within the renal tubules due to lead (Pb) exposure. This clearly points to the fact that honey may possess anti-inflammatory and antioxidant properties. It can be concluded that oral administration of honey confers a protective and ameliorative potentials against heavy metals (lead) induced kidney dysfunction in experimental animal models.However, lead (Pb^{2+}) toxicityseems to competitively inhibits the renal Na^{+}/K^{+} ATPase.

Keywords: Honey, Nephrotoxicity, Lead (Pb^{2+}), renal Na^{+}/K^{+} ATPase, male wistar rats.

Introduction

Industrialization and man's activities have contributed to promoting environmental pollution by introducing unwanted toxic compounds such as heavy metals, of which lead (Pb) is a leadingactor. Lead (Pb) is a ubiquitous, nonessential, and nonbiodegradable toxic heavy metal thatcauses numerous biochemical, behavioral, and physiological alterations in the biological system.[1]Acute and chronic exposure to lead is often associated with organ damage and dysfunction. Of note are the kidneys which are central to lead metabolism and excretion. However, the kidneys remain animportant target of damage due to lead (Pb) toxicity,[2] through its cascade of activities that alters the reduction – oxidation pathways in the kidneys, thereby increasing Reactive Oxygen Species (ROS) and diminishing the antioxidant systems.[3]These free radicals (ROS) devastate the lipid units of the cell membrane by denaturing proteins and peroxidation, causing enzymatic deactivation that lead to mitochondrial dysfunction.[4] Chelation therapy is a recommended strategy in the treatment and prevention of chronic Pb

poisoning.However, the major limiting factor linked with chelation therapy is that the action of some chelating agent used could have a nonspecific action resulting into the removal of essential metals from the body.[5]Several heavy metal chelating agents have been applied as therapeutic drugs for lead-exposed patients, such as succimer, dimercaprol, dimercaptosuccinic acid, and $CaNa_2$ -Ethylenediaminetetraacetic acid (EDTA). However, these chelators have multiple side effects.[6]Therefore, great attention has been given to natural compounds that could attenuate the deleterious effects of lead poisoning and protect the cell from lead-induced damage. One of such natural products is honey, which is a complex nutritional sweetener composed mainly of carbohydrates, water, organic acids, minerals, vitamins, enzymes, proteins, amino acids, volatile compounds, and several bioactive substances (phenols and flavonoids, among others), as well as pollen grains.[4]

Various experimental studies have indicated that honey diet have profound beneficial health effects against various pathologies.[7] For several years now, nutritional sciences haveimproved significantly on the greater understanding of biochemical and physiological roles and mechanisms of diets on diseases processes and wellbeing.So, a search fornatural products and or dietary additives with the ability to reverselead-induced nephrotoxicity constitutes an active interest of the present study.Therefore, this study sought to investigate the ameliorative effect of honey on lead – induced nephrotoxicity in male wistar rats.

Materials and Methods

Study Location

This study was conducted in the Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Science, University of Port Harcourt, Port Harcourt, Nigeria.

Experimental Animals

Twenty-four(24) adult maleWistar rats (weighing 180-200g) were obtained from the Physiology Department Central Animal House, University of Port Harcourt, Port Harcourt, Nigeria and managed under normal laboratory condition according to the University ethical guidelines. The rats were kept in clean cages and maintained at room temperature of 25°C ±2°C with a 12hours light-dark cycle, all rats had free access to food and water during the study period. The rats were allowed to acclimatize for two weeks before the commencement of the experiment.

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Experimental design

The animals were grouped into four groups of six (n=6) rats each. Group 1: served as (control) and received distilled water (10 mL/kg) alone and normal feed daily. Groups 2 to 4 served as test groups; and received 10mg/kg/bw of body weight of Pb, 2ml/100mg/kg of body weight of honey and 1ml/10mg/kg/bw of body weight of Pb plus 2ml/100g of body weight of honey respectively. All treatments were daily administered orally using oral gavage and lasted for 28 days. The doses selected for this study was according to Fasanmade and Alabi, (2008) with slight modifications.[8]

Ethics Statement

The experimental procedures, animal handling, sampling, and scarification were done according to the Natural Health Institute of Health Guidelines for Animal Care and approved by the "Institute Ethical Committee Guidelines" Council of European Communities.[9]

Preparation of Blood samples for Biochemical Analysis

At the end of drug administration, experimental animals were anaesthetized using 80% chloroform and then sacrificed through cervical dislodge. Cardiac puncture was used for blood collection for biochemical variables. Further, plasma was also obtained from the collected blood by centrifugation (3000 rpm; 15 minutes) at room temperature using a bench top centrifuge (Bosch, UK) for the assessment of electrolytes concentration, renal function markers.

Estimation of Kidney Function Markers

Assessment of Serum Creatinine Concentration:

Plasma (50 mL) was taken and mixed to a monoreagent (1000 lL) obtained from the assay kit. The mixture was then incubated for 60 seconds. Thereafter the absorbance was read (k 492 nm) twice within the interval of 1 min. Furthermore, the concentration of creatininewas calculated using the kit manufacturer method (Immunometrics Limited UK).

Assessment of Serum Urea Concentration:

Four parts of monoreagent taken from reagent I and one-part monoreagent taken from reagent II were mixed together and incubated at 15–25°C for 30 minutes. Following incubation, the mixture was kept in amber bottle prior to use. 10 mL of plasma sample and urea standard were further mixed to 1000 lLmonoreagent each and then further incubated at 20-25°C for 60 seconds. The absorbance was read at wavelength of 340nm twice within the interval of 1 minute. Finally, the concentration of urea was determined and calculated as proposed by the kit manufacturer (Immunometrics Limited, UK) using the formula:

Urea concentration = mg/dL

$$\frac{\text{Change in sample absorbance}}{\text{Change in standard absorbance}} \times \text{Standard concentration/Cal}$$

Assessment of Serum Albumin Concentration:

Plasma albumin was determined using RANDOX reagent kits as according to the instructions and method in the manual.[10]

Estimation of Plasma Electrolyte Concentration: The plasma electrolytes: Sodium, Potassium and Chlorides were assayed using their respective commercial kits. All assays were done using microplate reader SpectraMAX PLUS (a molecular Device product).

Statistical Analysis

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Comment [B3]: Plasma or serum? If Serum, the authors must indicate it under the section ''Preparation of blood samples for biochemical analysis'' in the methods

Comment [B4]: Plasma or serum? The title of this paragraph indicates ''Assessment of Serum Creatinine concentration'', yet the authors used plasma for the assay

Comment [B5]: Plasma or serum? The title of this paragraph indicates ''Assessment of Serum Urea concentration'', yet the authors used plasma

Comment [B6]: Kindly indicate the manufacturer and Country of origin

Comment [B7]: Be consistent, plasma or serum?

Comment [B8]: The authors must kindly indicate how they assessed the distribution (normality) of the data prior to analysis using one way ANOVA.

The data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) Version 21.0 software, and the results were presented as Mean ± Standard Error of Mean (SEM) and analysed with one-way analysis of variance (ANOVA) followed by Post Hoc. P<0.05 was considered statistically significant 0.05.

Results

Table 1: Effect of oral administration of Honey on some electrolytes in Lead (Pb) exposed male Wistar rats

Groups and Treatment	Na^+ (mmol/L)	K^+(mmol/L)	HCO^-_3(mmol/L)	Cl^-(mmol/L)
Group 1 (Control)	138.67 ± 4.25	4.40 ± 0.20	16.50 ± 2.01	76.83 ± 3.16
Group 2 (Pb only)	151.83 ± 13.81	5.38 ± 0.45 [a]	20.17 ± 0.65	82.00 ± 8.17
Group 3 (Honey only)	123.17 ± 3.91 [b]	4.13 ± 0.23 [b]	19.17 ± 1.60	97.67 ± 10.87
Group 4 (Honey + Pb)	125.17 ± 6.68 [b]	3.53 ± 0.43 [b]	22.50 ± 1.73 [a]	108.50 ± 7.44 [a, b]

Values are represented as mean ± SEM, n=6; [a] Significant at p<0.05 when compared to Group 1; [b] Significant at p<0.05 when compared to group 2.

Effect of Honey administration on some electrolytes in Lead (Pb) exposed male Wistar rats is as shown in table 1. A non-significant increasewas recorded in the serum levels of sodium, bicarbonate and chloride in the lead (Pb) treated group compared to the control. A significant (p<0.05) elevation in the serum potassium was observed for the lead (Pb) treated group compared to the control.
However, there was a significant (p<0.05) decreasein the serum levelof sodium and potassium in the honey treated group and the honey + lead (Pb) group when compared with the lead (Pb) treated group.

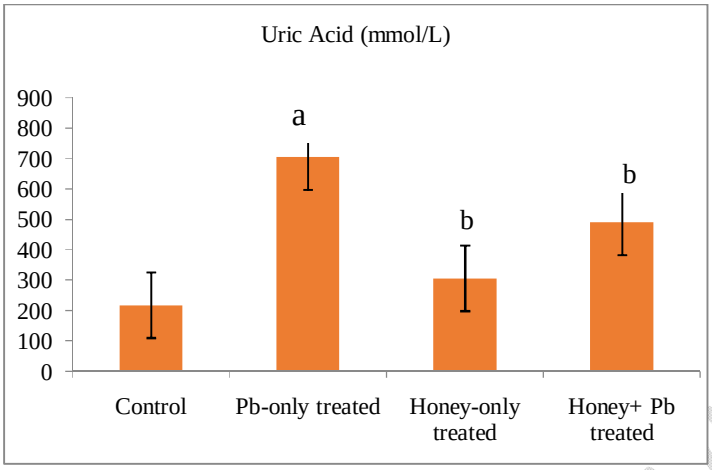

Figure 1: Effect of honey administration on uric acid level in Lead (Pb) exposed male Wistarrats

[a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to Pb treated group

Comment [B9]: Does the graph represent means±SEM? Kindly indicate this in the legend.

A highly significant (p<0.05) increase in the serum level of uric acid was recorded in the lead (Pb) group compared with the control (Figure 1). When compared with the lead treated group, the honey treated group and the honey + lead (Pb) treated group recorded a significant (p<0.05) reduction in the serum level of uric acid. With the honey treated group recording a more reduction in uric acid levels.

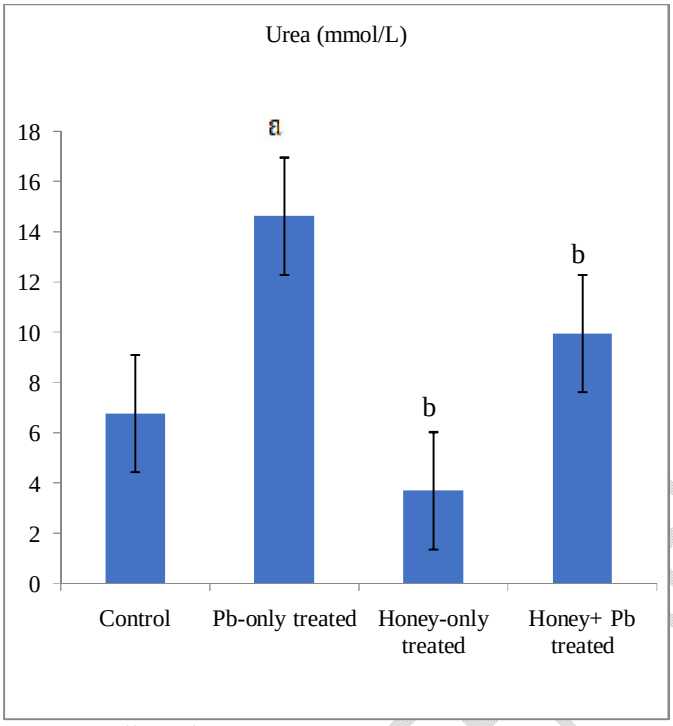

Figure 2: Effect of honey administration on urea level in Lead (Pb) exposed male Wistarrats

[a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to Pb treated group

Comment [B10]: What does the graph/plots represent? mean±SEM?

A significant (p<0.05) increase in the serum level of urea was recorded in the lead (Pb) group compared with the control (Figure 2). When compared with the lead treated group, the honey treated group and the honey + lead (Pb) treated group recorded a significant (p<0.05) reduction in the serum level of urea. With the honey treated group recording a more reduction in uric acid levels.

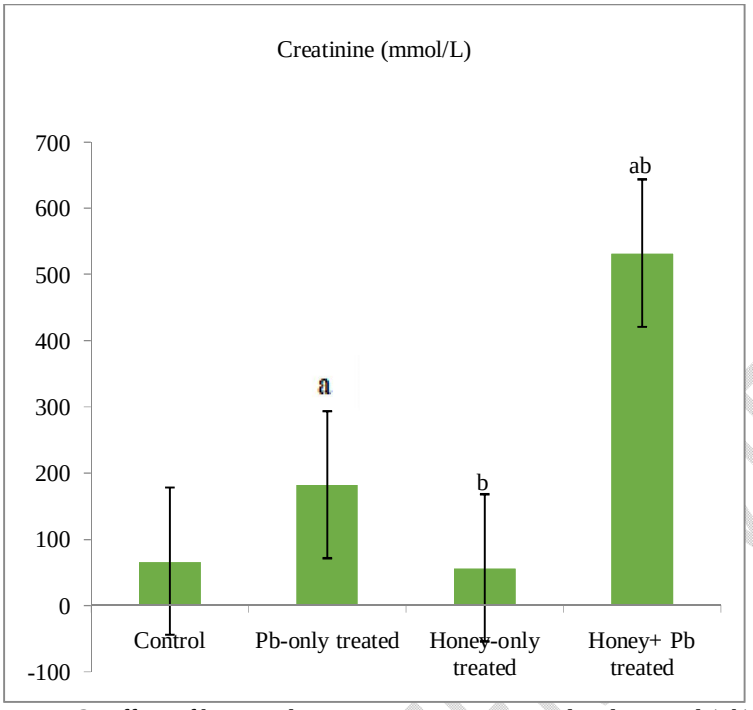

Figure 3: Effect of honey administration on creatinine level in Lead (Pb) exposed male wistarrats.
[a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to Pb treated group

Comment [B11]: What does the plot/graph represent? mean±SEM? Please indicate this in the figure legend

Figure3 shows the effect of honey administration on serum creatinine level in lead (Pb) exposed wistar rats. The serum creatinine level was significantly ($p<0.05$) increased in the lead (Pb) treated group and Honey + lead treated group when compared with the control. However, there was a significant ($p<0.05$) reduction in serum creatinine level with the group treated with honey compared with lead (Pb) treated group. The group treated with honey + lead (Pb) recorded asignificant ($p<0.05$) increase in the serum level of creatinine compared to the lead treated group.

Discussion

Lead is a common environmental and occupational toxic metal which can be found in water, food and as inhalational particles. Acute and chronic exposure to lead is often associated with organ damage and dysfunction withkidneys which are central to lead metabolism and excretion. However, the kidneys remain an important target of damage due to lead (Pb) toxicity,[2] through its cascade of activities that alters the reduction – oxidation pathways in the kidneys, thereby increasing Reactive Oxygen Species (ROS) and diminishing the antioxidant systems.[3]

Management of renal diseases with orthodox drugs poise serious economic challenges with adverse side effects to health, hence efforts have been increased to resort to natural compounds

such as honey that could attenuate the deleterious effects of lead poisoning and protect the cell from lead-induced damage. Honey mainly consists of sugars and water. Sugars in honey comprises predominantly of monosaccharides and oligosaccharides. The most abundant sugar in honey is fructose. Apart from sugars, honey also contains several vitamins, especially B complex and vitamin C, together with a lot of minerals. Some of the vitamins found in honey include ascorbic acid, pantothenic acid, niacin and riboflavin; while minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc are also present.[11] Honey contains at least 181 constituents.[12,13] The other constituents of honey are amino acids, antibiotic-rich inhibine, proteins and phenol antioxidants.[14] It also contains other bioactive substances such as phenolic constituents, flavonoids, organic acids, carotenoid-derived compounds, nitric oxide (NO) metabolites, amino acids and proteins.

The present study investigates the ameliorative effects of honey on renal function in lead – induced nephrotoxicity in male wistar rats. A significant ($p<0.05$) elevation in the serum potassium was observed for the lead (Pb) treated group compared to the control. However, there was a significant ($p<0.05$) decrease in the serum levels of sodium and potassium in the honey treated group and the honey + lead (Pb) group when compared with the lead (Pb) treated group.These results strongly suggest a possible tubular disruption and consequent alteration of ionic pumps, and ion channels within the renal tubules due to lead (Pb) exposure. This assertion is consistent with the report of a scientific literature[15] whichobserved that heavy metals, such as Pb^{2+} competitively inhibits the Na^{+}/K^{+} ATPase. However, the mechanism by which lead (Pb) inhibits renal Na^{+}/K^{+} ATPase pump is unclear. Inhibition of the renal Na^{+}/K^{+} ATPase pump leads to an increase in extracellular fluid potassium concentration with associated reduction in sodium levels. However, the significant decrease in the serum levels of sodium and potassium observed in this study after the administration of honey to the experimental animals, may have occurred due to the action of flavonoids and other bioactive constituents contained in honey which acts as antioxidant and anti-inflammatory activity. Other studies have reported anti–inflammatory, antimicrobial, and antioxidant activities of honey.[16]

The impact of administration of lead (Pb) and oral supplementation of honey on serum uric acid, urea and creatinine levels were evaluated (figures 1,2 and 3). The kidneys provide the final common pathway for the excretion of many drugs and their metabolites. Drugs and their metabolites are taken up selectively and concentrated by the renal tubular cells before excretion into the urine.

In fact, urea is the first acute renal marker which increases when the kidney suffers any kind of injury. Otherwise, creatinine and uric acid are the most trustable renal marker and increases only when the majority of renal function is lost (Borge*et al.*,).[17]

The findings of the present study recorded a significant increase in the serum levels of uric acid, urea and creatinine in the lead (Pb) group compared with the control. When compared with the lead treated group, the honey treated group and the honey + lead (Pb) treated group recorded a significant reduction in the serum level of urea. The trend in these results clearly points to the fact that honey may possess anti-inflammatory and antioxidant properties which is consistent with the findings of Wafaa and Hemmat.[18] Hence, this strengthens various reports of literatures on the anti – inflammatory, antioxidant and other potencies of honey owing to its active biomarkers.[19]

Many studies reported that honey has a protective role against the effects of many drugs on kidney function.[19]The oral supplementation of honey has a protective and curative role on

Comment [B12]: In addition to the discussion on Na+ and K+ data, can the authors discuss the results for Cl- and HC03- as shown in table 1?

Comment [B13]: In figure 3, the level of Creatinine was rather significantly increased in honey+lead(Pb) treated group compared to Ph only treated group. Can the authors discuss this observation?

alteration caused by heavy metals on the kidney function parameters. However, these observations may be attributed to the antioxidant properties of honey which contain zinc and selenium,[20] in addition to many forms of flavonoid compounds. These compounds were known for their hydrogen donating antioxidant property.

Conclusion

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Itis concluded that the oral administration of honey confers a protective and ameliorative potentials against heavy metals (lead) induced kidney dysfunction in experimental animal models. This is in view of the effects of the doses administered in this study on the renal function parameters. Therefore, it is hereby suggested that honey could form part of treatment regime in the management of patients with lead or heavy metals induced nephropathy due to its possible chelating, antioxidant and anti-inflammatory potential.
It is important to state that lead (Pb^{2+}) seem to competitively inhibits the renal Na^{+}/K^{+} ATPase. This opens a novel quest for its possible role in extracellular volume control., however, a clear mechanism of this effect needs more clarification.

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