ORIGINAL ARTICLE

Ameliorative effects of Nigerian bitter honey on streptozotocin-induced hepatorenal damage in Wistar rats

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Abstract

Background: Mortalities resulting from liver and kidney failure are enormous. It is however worrisome that conventional medications are rarely available for their treatment. Honey is traditionally reputed as a natural supplement for relieving disease symptoms. The therapeutic values of honey vary widely from one botanical source to the other. *Aim and Objective:* To evaluate the beneficial effects of Nigerian Bitter Honey (BH) on hepatorenal damage induced by Streptozotocin (STZ). *Material and Methods:* Forty female Wistar rats (90-110 g) were randomly grouped into five (n=8 each) as follows: Group A (2 ml/kg distilled water), Group B (50 mg/kg BH), Group C (65 mg/kg STZ), Group D (STZ + BH), and Group E (BH + STZ). Hepatorenal damage was induced in rats by a single dose of STZ (65 mg/kg body weight). Animals were sacrificed after 28 days of treatment. Blood samples were collected into sterile plain bottles. Liver and kidney tissues were harvested and processed for histopathological assessment. Liver function tests (Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), total protein, albumin, bilirubin); kidney function tests (urea and creatinine) were carried out. *Results:* Bitter honey treatment (Group D) significantly ($p<0.05$) reduced AST, ALT, urea, creatinine and bilirubin levels while also increasing total protein and albumin concentrations. Also, BH significantly improved the histoarchitectural integrity of the liver and the kidney of the treated and pretreated rats. *Conclusion:* Bitter honey demonstrated outstanding hepatoprotective and renoprotective effects. It may therefore serve as a novel natural supplement for the management of liver and kidney diseases.

Keywords: Nigerian Bitter-honey, streptozotocin, hepatorenal, liver function tests, kidney function tests

Introduction

Hepatorenal damage is a pathological symptom with numerous etiologies. In Southeastern Nigeria, kidney disease was responsible for about 23% of all medical deaths across a period of 13 years [1], while hepatic disease accounted for 7.9% [2] of total hospital admissions. Patients with

hepatorenal failure usually experience poor quality of life and poor clinical outcomes during disease management. Streptozotocin-induced hepatorenal damage had been reported in several literatures [3]. A decline in hepatocellular function characterized by increased permeability is associated with elevated circulating levels of Aspartate Amino-transferase (AST), Alanine Aminotransferase (ALT) [4]. Clinically, these molecular markers are used to evaluate the extent of compromised hepatocellular integrity. In cases of renal failure, elevated urea and creatinine are frontline clinical indicators [5].

It is rather unfortunate that there is a dearth of conventional medication which can alleviate hepatorenal disorders in a single formulation. As a rich source of essential bioactive principles, honey is often used in medical nutrition therapy for relieving pathological symptoms. In folklore medicine, honey is highly reputed for its healing properties. Within the past decade, honey is often applied as a complement or an ideal alternative to conventional treatment regimen. Amongst other therapeutic benefits, it is routinely applied in wound dressing [6] because of its antibiotic potentials. Also, it is used for relieving symptoms of chronic illnesses such as nephrotic syndrome [7], hepatitis [8], metabolic syndrome [9] and cancer [10]. As a result, honey is presently regarded as a natural repository of essential phytochemicals. However, the bioavailability of these bioactive compounds in honey varies primarily because of the diversity of botanical precursors [11]. As a result, specific pharmaco-logical responses are exclusive to certain honey varieties and cannot be generalized across all kinds of honey. This is particularly so as some honey samples have proven toxicity in specific tissues and organs [12-13].

As of now, the possible hepatoprotective and reno-protective effects of a Bitter Honey (BH) native to a known botanical source in Nigeria is yet to be reported. Based on this premise, this study there-fore aimed at investigating the possible hepato-protective and renoprotective efficacy of Nigerian BH sample on the liver and kidney tissues using biochemical and histological approaches.

Material and Methods
Bitter honey
Nigerian BH was cultivated, harvested, and extracted at the Community Lifestyle Improve-ment Project (CLIP) farm. The farm is a registered farm with the Corporate Affairs Commission of Nigeria (RC: 0953750). The farm is located at Modakeke, Osun State, Nigeria. The freshly harvested BH was then stored in a pigmented bottle at room temperature until required for analysis. On each day of the treatment, BH was freshly dissolved in deionized water and administered immediately.

Drugs and reagents
Streptozotocin (STZ) purchased at Sigma Aldrich (MO, USA). AST, ALT, bilirubin, albumin, total protein, urea, creatinine assay kits were procured from Randox laboratory (Aldren, USA). All other reagents used were obtained from either the British Drug House (Poole, England) or Randox laboratory (Aldren, USA).

Animal procurement, grouping, and dosage regimen
Female Wistar rats (90-110 g) were purchased from the animal house facility of the Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife. The rats were kept in well-ventilated plastic cages (Mediwise animal cage, $430 \times 270 \times 15$ mm) under standard environmental conditions. A 12-h

light/dark cycle was ensured. The rats were given unrestricted access to water and chow. Ethical approval was acquired from Osun State Health Research Ethics Committee (OSHREC) with clearance number OSHREC/PRS/569T/158. Rats were given exclusive humane treatment in conformation with the principle of Laboratory Animal care of the National Society of Medical Research and Guides for the care and use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health Publication no. 80-23).

Rats were divided randomly into five groups (n = 8); Group A was administered 2 ml/kg/bw of distilled water for 28 days, Group B, was administered BH (50 mg/kg) only for 28 days, Group C was administered a single dose of STZ (65 mg/kg) only, Group D received a single dose of STZ (65 mg/kg) and then treated daily with BH (50 mg/kg) for 28 days, Group E was pretreated with BH (50 mg/kg) for 28 days and then administered a single dose of STZ (65 mg/kg). Distilled water, and BH was administered using an oral cannula. A dose of 50 mg/kg body weight BH was used as previously reported by [14]. The rat grouping, and treatment is shown in Table 1.

Induction of hepatorenal damage

Experimental and control rats were subjected to overnight fasting. Rats in negative control and BH treated groups were administered a single dose of STZ (65 mg/kg body weight) intraperitoneally. Hepatorenal damage induction in this study followed succinctly the method used by Uyar *et al*. [3]. On the 28th day following the pretreatment of rats in Group E with BH, the animals were fasted overnight, and then injected with STZ (65 mg/kg body weight).

Rat sacrifice

Rats were subjected to overnight fasting. On the following morning, rats (Groups A-D) were sacrificed through cardiac puncture after light diethyl ether anesthesia. Two days following STZ administration to rats in Group E, the animals were fasted overnight until the third day, blood sugar measurement was taken, and the animals were then sacrificed. Blood was collected into sterile centrifuge tubes without anticoagulants and allowed to clot at room temperature for about 30 minutes. The blood samples were centrifuged at $1500 \times$ g for 10 minutes. The sera were collected and stored at -20^0 C until use.

Table 1: Showing rat grouping, administration and dosage regimen

Groups	Administration	Dose regimen	Comment
A	Distilled Water	2 ml/kg	Normal Control
B	BH	50 mg/kg	BH-supplemented
C	STZ	65 mg/kg	Negative Control
D	STZ and BH	65 mg/kg and 50 mg/kg respectively	BH-treated
E	BH and STZ	50 mg/kg and 65 mg/kg respectively	BH Pretreatment

BH (bitter honey); STZ (streptozotocin)

Biochemical assay

Liver function markers

The activities of AST, ALT and the concentrations of bilirubin, total protein, and albumin were assayed using standard methods according to the manufacturer's instruction using Randox assay kits.

Renal function markers

Urea and creatinine concentrations were assayed using colorimetric methods according to the manufacturer's instruction using Randox assay kits.

Histopathological analysis

Tissue samples (liver and kidney) were carefully excised and fixed in 10% formolsaline, dehydrated through ascending grades of alcohol (50%, 70%, 90%, and absolute), cleared in xylene, and then impregnated in paraffin wax of melting point between $55^0C–56^0C$ for infiltration. Tissue sections at 5 microns were mounted on glass slides and stained in Hematoxylin and Eosin (H&E). The stained tissue sections were then observed under the microscope (Leica, DM 750) interfaced with a camera (Leica, ICC 50), and photomicrographs taken at ×40 objective lens were archived.

Statistical analysis

Data were analyzed using a one-way Analysis of Variance (ANOVA). The *post hoc* analysis was carried out using Student Neuman-Keuls multiple comparison test on Graph Pad Prism version 5.03 (GraphPad Software, Inc. CA, 92037 USA). All data were presented as Mean ± Standard Error of the Mean (SEM). A significant level was set at $p < 0.05$.

Results

Effects of STZ and BH on organs weight

The weights of the liver (Figure 1) and kidney (Figure 2) was significantly higher in Group C (negative control) when compared to other groups. Whereas, Group D (BH-treated) rats had significantly lower liver and kidney weights relative to the rats in the negative control group.

Effects of STZ and bitter honey on aspartate transaminase and alanine aminotransferase activities

The data obtained on how BH (at a daily dose of 50 mg/kg BW of 20% BH) modulated biochemical markers of hepatic damage (induced with 65 mg/kg BW of STZ) are depicted in Figures 1-5. In each case, the BH treated group was compared with the negative control group. Results showed that AST (Figure 3) and ALT (Figure 4) were significantly different when experimental groups were compared. The BH treated groups presented with significantly ($p< 0.05$) lower activity of AST and ALT in comparison with the negative control group. There was no significant difference between AST and ALT activity in the normal control group (Group A) and the BH supplemented (Group B), BH treated (Group D) and BH pretreated (Group E) groups.

Effect of STZ and BH on total protein, albumin, and bilirubin

Comparison of total protein (Figure 5), albumin (Figure 6), and bilirubin (Figure 7), concentrations of the experimental groups also showed significant differences. Animals in the BH treated and pre-treated groups showed significant ($p<0.05$) decrease in bilirubin level while also showing significantly ($p< 0.05$) increased total protein and albumin concentrations compared to negative control group.

Effects of STZ and BH on urea and creatinine concentrations

Results depicting the ameliorative effects of BH on markers of renal damage areas are shown in Figures 8 and 9 respectively. There were significant differences in concentrations of urea and creatinine when all the groups were compared ($p<0.05$). Animals in both BH-treated and pretreated groups presented with lower concentrations of urea (Figure 8) and creatinine (Figure 9) when compared to the negative control group.

Histopathology of liver and kidney

Using H&E staining technique, extensive degeneration of the liver (Figure 10) and kidney (Figure 11) (induced by a single dose of 65 mg/kg BW of STZ) was observed. Sections also showed improvement following treatment with BH (50 mg/kg BW).
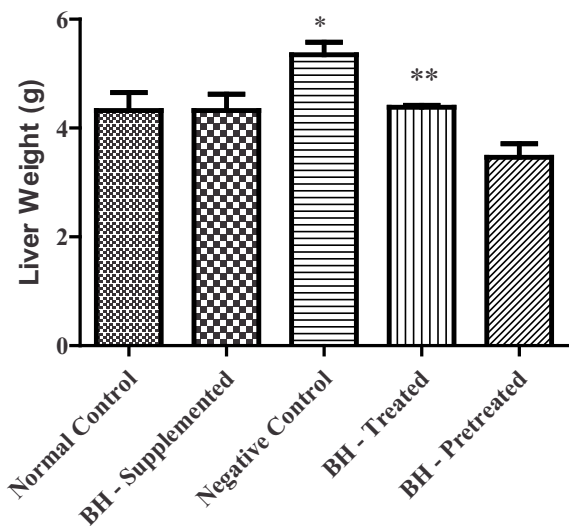
Figure 1: Showing the effects of BH on liver weights across the groups. BH (bitter honey); STZ (streptozotocin). Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher ($*p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower ($**p < 0.05$) compared to negative control.
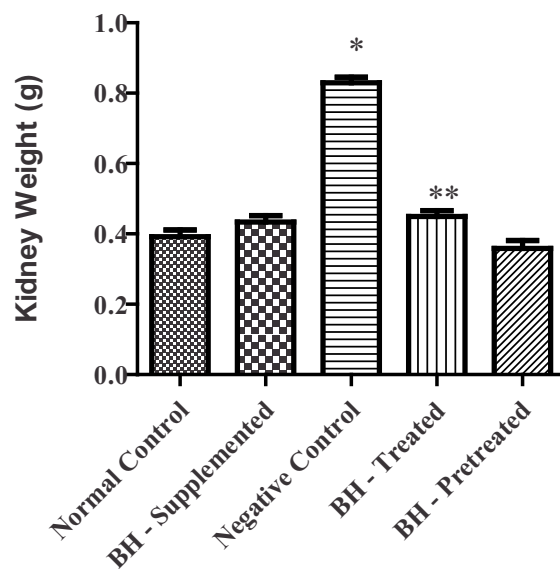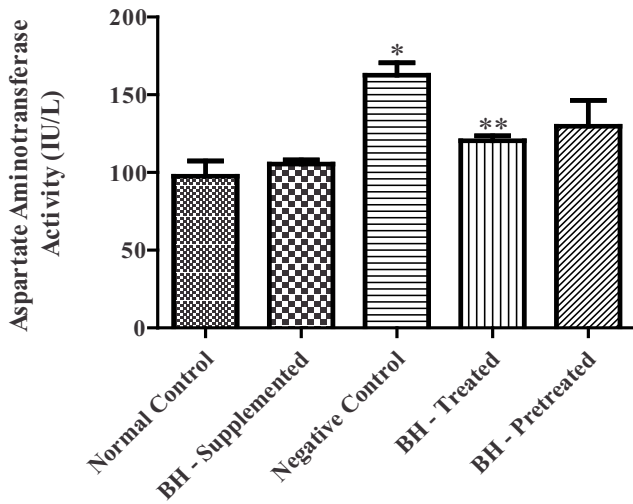

Figure 2: Effects of BH on kidney weight across the groups. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher ($*p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower ($**p < 0.05$) compared to negative control.
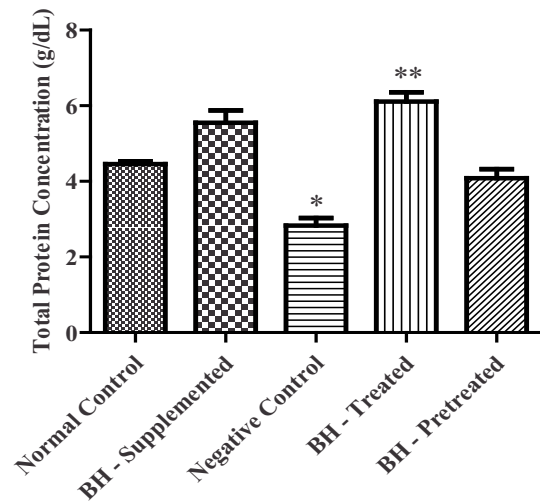
Figure 3: Effects of BH on aspartate trans-aminase activity. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher (*$p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower (**$p < 0.05$) compared to negative control.

Figure 5: Effect of BH on total protein concen-tration. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly lower (*$p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly higher (**$p < 0.05$) compared to negative control and BH pretreated groups.
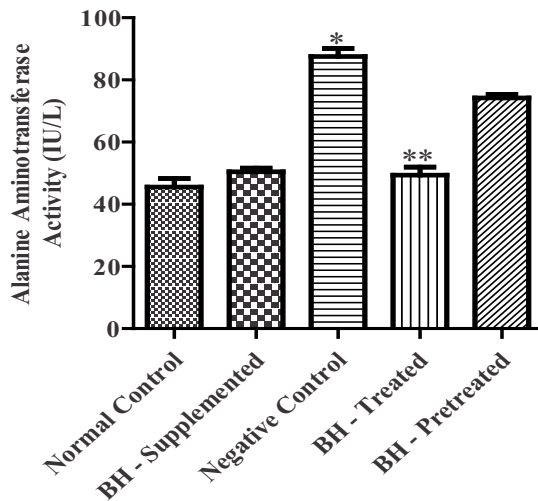

Figure 4: Effects of BH on alanine amino-transferase activity. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher (*$p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower (**$p < 0.05$) compared to negative control and BH pretreated groups.
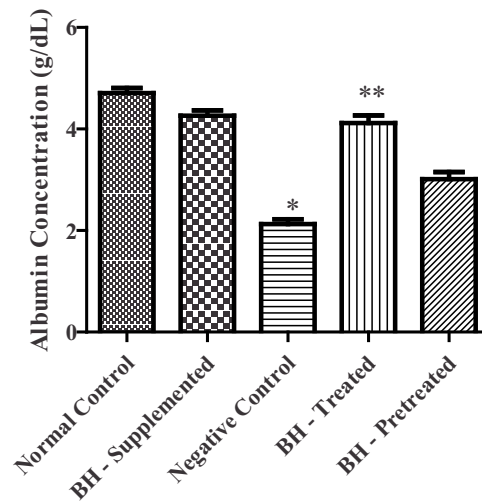

Figure 6: Effect of BH on albumin concen-tration. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly lower (*$p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly higher (**$p < 0.05$) compared to negative control and BH pretreated groups.
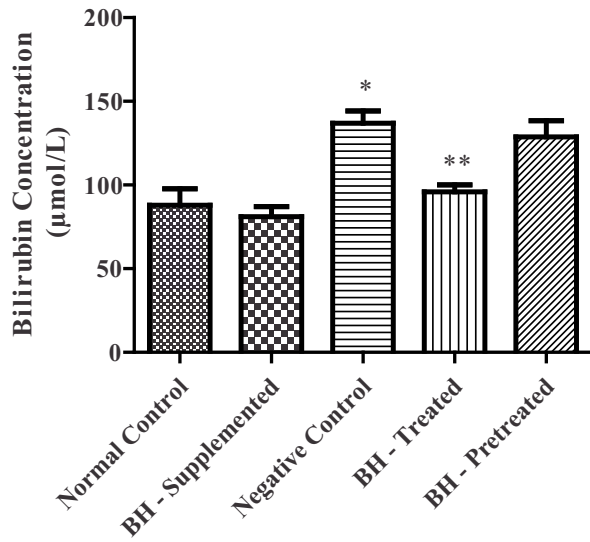
Figure 7: Effect of BH on bilirubin concentration. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher ($*p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower ($**p < 0.05$) compared to negative control and BH pretreated groups.

Figure 8: Effects of BH on urea concentration. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher ($*p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower ($**p < 0.05$) compared to negative control and BH pretreated groups.
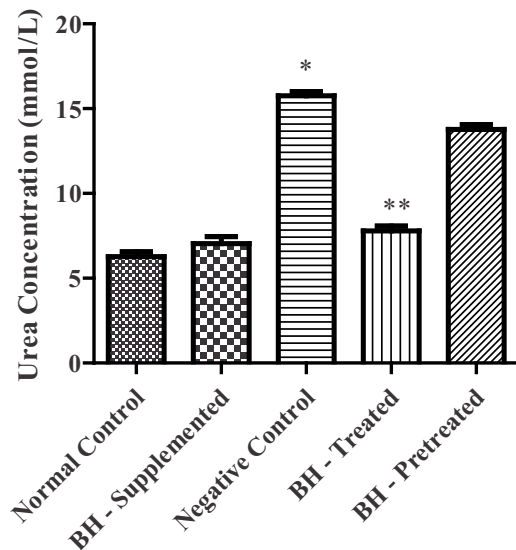

Figure 9: Effects of BH on creatinine concentration. Values are expressed as mean ± SEM (n=8). Statistical significance ($p < 0.05$). Value is significantly higher ($*p < 0.05$) compared to normal control and BH supplemented groups. Value is significantly lower ($**p < 0.05$) compared to negative control and BH pretreated groups.
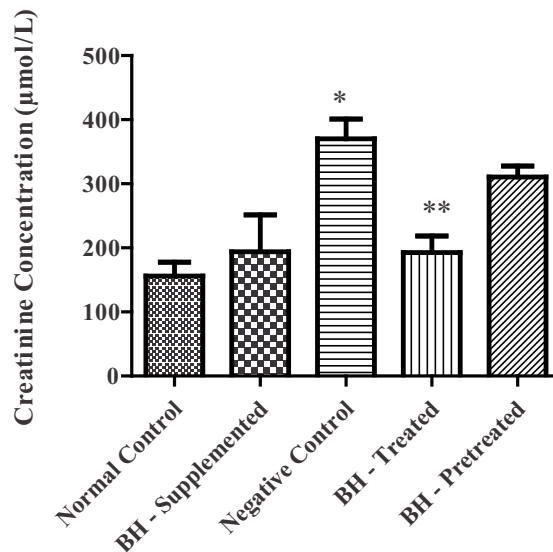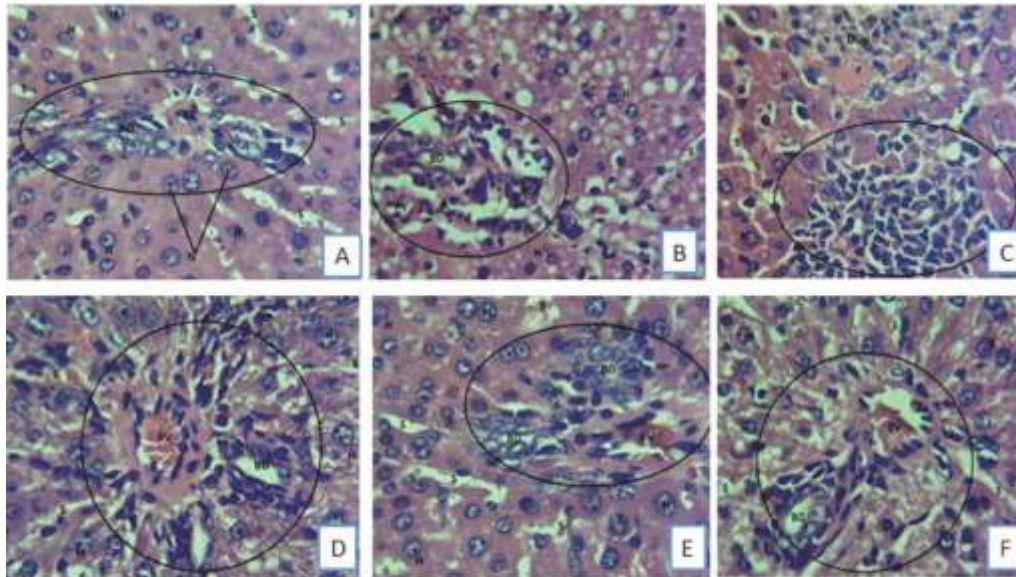
Figure 10: Representative light photomicrographs of liver sections subjected to H&E stain. The portal
triad area is circled. The BH treated group (D) had a normal bile duct (BD) section compared to the negative
control (C) having bile duct section showing marked periportal hepatitis and foci of degenerating hepatocytes
(DGH). Mag (x40)
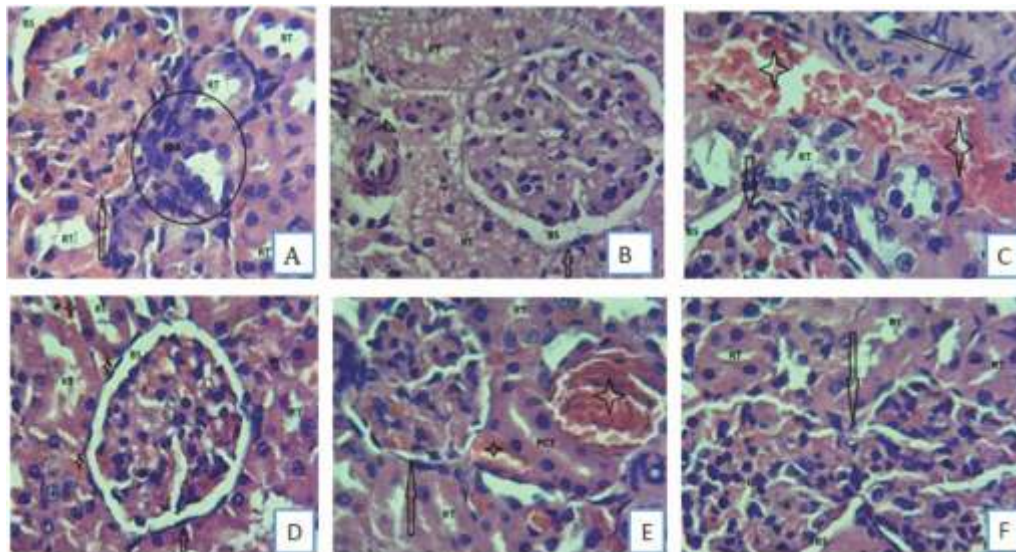**Abbreviations:** Bile Duct (BD); Portal Vessels (PV); Sinusoids (S); Hepatocytes (H).

Figure 11: Representative light photomicrographs of kidney sections subjected to H&E stain. The BH
treated group (D) did not present with medial hypertrophy (line) of the branch of renal artery compared to negative
control (C). Also, the BH treated group (C) had only a mild congestion of the interstitium (star), unlike the negative
control group (D) which showed marked congestion of the interstitium (star) with mild infiltration of
inflammatory cells (arrow head).
Abbreviation: Glomerulus (G); Bowman's space (BS) Renal tubules, (RT); Juxta glomerular apparatus (JGA);
Proximal convoluted tubule (PCT); Distal convoluted tubule (DCT). Mag (x40)

Discussion

The present study investigated the pharmacological roles of BH from Nigeria in curtailing metabolic derangements associated with STZ-induced liver and kidney injury in the Wistar rat model using biochemical and histological approaches. Assessment of the organ weights revealed that STZ administration elicited a significant increase in the weights of the liver and the kidney. However, treatment of STZ-induced rats with BH significantly reduced the weights of the liver and kidney. Similar to our observations [15], obtained a significant reduction in testicular and epidydimal weights while also improving gonadal indices among rats exposed to prenatal stress. According to our previous findings [16], the botanical source of our BH was characterized by various medicinal plants such as *Chromolaena odorata*, *Blighia sapida, Irvingia gabonensis, Elaeis guineensis,* etc. Interestingly, some of these native plants are reputed for their potency in reducing body and organ weight. The weight-reducing efficacy of *Irvingia gabonensis* has been patented [17]. It is, therefore, possible that *Irvingia gabonensis* is the determinant of this inherent pharmacological property in bitter honey.

Biochemical and histopathological assessments showed that STZ administration elicited significant toxicity in the liver of the negative control group. Improvement in hepatocellular integrity by BH was observed. This was indicated by a significant reduction of serum AST, bilirubin and ALT levels and a significant increase of total protein and albumin among the treatment and pretreatment groups relative to the negative control group. This suggests that BH was effective in enhancing the regeneration of hepatocellular degeneration induced by STZ

A reduced level of albumin in the blood has been implicated in numerous pathologies. Since albumin synthesis is peculiar to the hepatocytes, a low albumin level in the blood may likely indicate impaired synthesis or excessive catabolism [18]. Therefore, improvement in albumin concentration among the BH treated rats relative to negative control group may signify a possible improvement in the synthetic functions of the liver or inhibition of extreme albumin catabolism. Similar to our findings, a Nigerian honey was also reported to enhance albumin and total protein levels [19]. Consistent with results from like studies, the protective advantage of honey on hepatocytes in STZ induced toxicity has been reported using Indian [20], and Bangladeshi [21] honey samples. A Yemeni honey sample from *Ziziphus spina-christi* was reported to significantly lower the serum values of AST and ALT in Melamine induced hepatotoxicity [22]. On the contrary, Sahin *et al*. [23] reported that bitter mad honey from the black sea region of Turkey predisposed to hepatotoxicity by increasing serum AST and ALT activities dose-dependently. Moreover, Manggau *et al*. [24] reported that BH significantly reduced ALT activity and creatinine concentrations but increased AST among centrally obese subjects. These variations in the hepatoprotective properties of honey further confirm the influence of vegetal precursors as a determinant of its inherent bioactivity.

Bilirubin, with its antioxidant and anti-inflammatory potentials, confers beneficial effects in many tissues. In a study by Xu *et al*. [25], the group of animals induced by STZ and treated with bilirubin had a significantly low level of urea and creatinine. Studies have also shown that bilirubin

can potentially scavenge free radicals in the endothelial vasculature, thereby protecting against microvascular complications [26]. However, elevation of bilirubin above the reference range is often used clinically as a biomarker of hepatic damage. The attenuation of STZ induced elevation of bilirubin by BH further suggests its hepato-protective property.

The biochemical analysis correlated well with histological evaluation, where the liver tissue section showed preservation in the histoarchitectural organization of the hepatic tissue in the BH treated group unlike the negative control group which presented with distortion in the normal histoarchitectural pattern as evident by marked periportal hepatitis and focal areas of degenerating hepatocytes.

An extract from *C. odorata* has been reported to selectively improve indices of hepatic damage by increasing total protein levels as well as circulating levels of albumin while consequently lowering the activities of AST and ALP in rat model of STZ induced hepatotoxicity [27]. Thus, the hepatoprotective property of the BH may likely be a function of bioactive components from *C. odorata*.

The result also showed that treatment with BH remediated STZ induced renal damage, and equally prevented renal damage in the pretreatment group. This was indicated by significantly low urea and creatinine level relative to the negative control group. This result is consistent with those obtained from honey samples native to other botanical sources. For example, Acacia honey has been reported to improve biochemical indices of hepatic [28] and renal [29] damage in a rat model. Arigela *et al*. [30] reported that bitter gourd honey from Malaysia ameliorated STZ-induced hepatorenal damage by reducing oxidative stress and inflammatory response. Also, Erejuwa *et al.* [9] documented the renoprotective function of a particular honey sample in a high fat diet-induced chronic kidney disease. However, the BH used in the present study showed better nephroprotective outcome compared to tualang honey. Also, Mohamed *et al*. [31] reported that, despite a significant reduction of creatinine, tualang honey did not restore distortions to renal histoarchitecture improvement in a high cholesterol diet-induced model of acute kidney disease.

This demonstrates the influence of plant precursors on variations in nutrient quality and the corresponding pharmacological property of honey. The anti-inflammatory property of plant steroids has been reviewed [32]. Therefore, the exclusive potency of our BH to counteract the inflammatory cell infiltration may be exclusive to its steroid content as shown by our previous study [16].

Furthermore, results obtained from the bio-chemical and histopathological assessments showed that BH treatment remediated STZ induced kidney dysfunction. The renoprotective efficacy of BH was also confirmed through histopathological analysis. While increased serum creatinine might have resulted from increased catabolism of structural proteins in the body, together with elevated urea in the negative control group, they may signify an impaired excretory capacity of the renal compartments. Interestingly, the degenerative changes were not manifested by the bitter honey-treated rats. This shows that the BH used in this study contains essential bioactive principles that can neutralize the debilitating effects of poisonous agents which can potentially impair the physio-logical activities of the kidney.

Notably, some of the plant precursors of our BH are well reputed for their remarkable roles in curtailing experimental models of acute and chronic nephrotoxicity. For example, Varatharajan *et al*. [33] documented the nephroprotective properties of leaf extracts of *E. guineensis* in STZ-induced kidney failure. Moreover, other botanical resources such as *I. gabonensis* [34] and *C. odorata* [27] have also been screened for their respective reno-protective effects. These observations signal a red flag against indiscriminate consumption and commercialization of honey, especially honey samples whose botanical source is unknown.

Conclusion

It may be concluded that Nigerian BH has demon-strated ameliorative and protective potentials against STZ-induced hepatorenal injury. The BH may therefore be a novel functional food as an adjunct for the management of hepatic as well as renal disorders. Further study is needed to identify the actual compounds eliciting the observed pharmacological responses.

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