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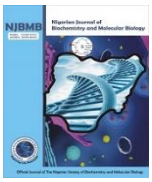

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Research Article

# A Comparative Study on the Effect of Honey and Sucrose Rich Diet on Key Tissue-Organ Function of Wistar Rats

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## ABSTRACT

Natural honey is considered a better alternative to table sugar, and particularly the Obudu Cattle Ranch honey that supplies much of the Nigerian markets. This work compared the effect of table sugar and the Obudu honey-sweetened diets on the functions of key organs of Wistar rats. Equivalent calories of the sweeteners were fed to 5 groups of rats: Normal control, 15% sugar, 30% sugar, 12% honey and 24% honey for 13 weeks. Food consumption and blood indices of liver, kidney and hematopoietic functions were determined. The results obtained show no marked effect of both sweeteners on measured liver function indices. Compared to the honey-based diet, the sugar-sweetened-diet did not cause any significant change in kidneys and blood functions. However, compared to the control, S15% diet was found to cause a 3.36% and 31.29% increase in serum sodium and chloride levels, respectively ($P < 0.05$). Similarly, the H12% and H24% diets caused 19.77% and 34.09% increase in serum potassium concentration and white blood cells counts respectively, relative to the control ($P < 0.05$). Therefore, natural honey may not have any advantage over table sugar in regards to tissue/organ functions and vice versa.

**Keywords**: *Obudu natural honey, Table sugar, Liver function, Kidney function, Haematological indices*

## INTRODUCTION

Obesity is a term used in describing a type of disease that occurs when an individual's weight exceeds the range considered healthy for his or her height (Center for Disease Control (CDC), 2022) Overweight and obesity are nutritional disorders that are endemic in many countries and reports have shown that the number of obese people is on a steady rise in low and middle income economies as result of a shift in dietary habit from traditional to westernized diet (Ford *et al*., 2017). An estimated mortality rate of 2.8 million people per year worldwide is attributed to overweight (Perez-Rodrigo, 2013). According the World Health Organization, (WHO) more than 1.9 billion adults aged 18 years and older were overweight in 2016, over 650 million out of this number were obese (WHO, 2021). This trend is worrisome particularly because obesity has been widely identified as a risk factor for debilitating conditions such as diabetes mellitus, cardiovascular disease and some forms of cancer (Atangwho *et al*., 2012), probably due to the proximity of visceral fat to vital tissues and organs in the body (Fox, 2011). Prominent among the myriad of pathogenic factors of these disorders is the consumption of high energy foods and highly palatable calorie-dense diets (Li *et al*., 2012) which tend to foster positive energy balance. Indeed, several lines of evidence have suggested that overweight or obesity adversely affect a lot of tissues/organs viz. liver, heart, kidneys, testes and blood (Noeman *et al*., 2011). For instance, being the major site for fatty acid biosynthesis, excess carbohydrate in diets

including beverages and caloric-sugar-sweetened foods, may constitute extra metabolic burden to the liver, which over time may result in wear and tear of the hepatocytes. Earlier, the association of overweight with fatty liver was demonstrated and reported (Kim *et al*., 2011).

Furthermore, prolonged and excessive sugar filtration can compromise kidney functions (Dovey, 2015) and this is usually accompanied by changes in blood chemistry (Hodgson, 2004). The constancy of blood composition is a function of perfusion through the organs that regulate its components (Rogers, 2011). Thus, plasma levels of certain biomarkers often reflect the functional activities of such secretory organs or glands (Nelson and Cox, 2013).

Sweeteners are often added to foods or drinks to impart taste thereby improving the palatability of such foods or drinks (Sheriff *et al*., 2011). The most commonly used caloric-sweeteners are honey and table sugar. Whereas honey is a viscous supersaturated sugar solution containing about 82.4 g/100g of carbohydrates and a glycemic index (GI) ranging from 32 to 85 (Bogdanov *et al*., 2008), table sugar is a processed substance, essentially sucrose with an approximate GI of 68 (Kappico *et al*., 2012). The glycemic indices of both sweeteners suggest their apparent contribution to prolonged postprandial hyperglycemia which can be detrimental to health (Chepulis and Starkey, 2008), particularly in persons with some underlying chronic pathologies. Moreover, excess glucose or hyperglycaemia is said to be toxic to the structure and functions of many organs/tissues via mechanisms such as oxidative stress (Morakinyo *et al*., 2013).

Incidentally, some previous studies have indicated that honey can improve hepatic and renal functions in healthy individuals (Erejuwa *et al*., 2012), besides other reports of its health benefits. The presence of some essential nutrients in the honey is usually the main reason why people opt for it rather than table sugar. However, these nutrients are in such minute quantities that in order to derive their nutritional benefits from honey a person has to ingest as much as 50-80g per intake of honey (Bogdanov *et al*., 2008) and by so doing inadvertently consume more than required amount of its sugar. Hence a higher likelihood of being predisposed to overweight than table sugar.

There is therefore a dire need for scientific clarification of these misgivings, the purpose for which this study was designed. A few studies have in the past compared table sugar and honey, however, to our knowledge, none of such works studied natural honey from the Obudu Cattle Ranch which supplies particularly the entire South-South Region of Nigeria and most other parts of Nigeria as well as exported to a small extent. The composition of honey is variable and is primarily determined by the botanical source, seasonal, geographical, and environmental factors. Moreover, no previous studies have considered the implications of these sweeteners on the functions of the key organs involved in the metabolism, transport and excretion of these sweeteners and their metabolites; and in equal calorie.

## MATERIALS AND METHODS

### Materials

Natural honey was obtained from the Obudu Cattle Ranch in Obanliku, Cross River State, Nigeria. Whereas white granulated table sugar was procured from Golden Sugar Company (Apapa, Lagos State, Nigeria). All reagents used were of analytical grade.

### Experimental animals

Thirty-five (35) male Wistar rats (*Rattus norvegicus*) weighing 100 - 120g at the outset of the experiment were used. They were randomly divided into 5 treatment groups (n = 7), housed in standard laboratory cages at room temperature of 25 ± 2°C, humidity of 50 - 62% and were allowed to acclimatize for 4 weeks. The animals were maintained on a 12:12 hour light/dark cycle in the animal facility of the Department of Genetics and Biotechnology, University of Calabar, Nigeria. All experimental and surgical procedures were carried out in line with the University and International animal care guidelines. The use of animals and research procedures were approved by the Faculty Animal Research Ethics Committee, Faculty of Basic Medical Sciences (FAREC-FBMS), University of Calabar (Approval Number: 014B20117).

### Feeding protocol

The diets were formulated to simulate average sugar composition of common sweetened foods and beverages. Accordingly, the sugar and honey were incorporated in the animals' diets and administered to the 5 groups thus: NC (normal control; 100% rat chow), S15% (15% sugar + 85% rat chow), S30% (30% sugar + 70% rat chow), H12% (12% honey + 88% rat chow) and H24% (24% honey + 76% rat chow). The table sugar and honey were incorporated by wet mixing with the rat chow (w/w), then re-pelleted and fed to the rats as shown on Table 1. The percentages of these sweeteners are calorically equivalent based on a weight/weight honey to sugar ratio of 1:1¼ (Kappico et al., 2012), i.e. 12% and 24% of honey are equivalent in calories to 15% and 30% of sugar respectively. Fresh diets were prepared every other day to avoid spoilage. The animals were allowed access to the diets and clean drinking water ad libitum during the 13 weeks study period. The leftover diets were carefully collected and weighed every other day in

order to determine the animals' food consumption i.e. the difference between initial diet supplied and the leftover.

**Table 1**. Experimental Design and Feed Formulation

| S/No. | Treatment group | Feed composition (per 100g) |
|---|---|---|
| 1 | Normal control | 100 % rat chow |
| 2 | Sugar-incorporated 1 (S15%) | 15 % sugar + 85 % rat chow |
| 3 | Sugar-incorporated 2 (S30%) | 30 % sugar + 70 % rat chow |
| 4 | Honey-incorporated 1 (H12%) | 12 % honey + 88 % rat chow |
| 5 | Honey-incorporated 2 (H24%) | 24 % honey + 76 % rat chow |

**Collection of samples**

At the end of the 13-week feeding, the rats were made to fast overnight (but had free access to water), afterwards euthanized and whole blood collected by cardiac puncture. The blood was divided into two portions each: The first portion into EDTA containers for use in determination of hematological indices based on non-cyanide haemoglobin analysis method and the remainder of the whole blood emptied into non-heparinized tubes. These second portions were allowed to stand for about an hour and centrifuged at 3000 rpm for 10 minutes to obtain serum used for liver and kidney function assays. The carcasses were dissected and livers were surgically removed, rinsed in normal saline and fixed in buffered 10% formalin preparatory to histopathological analysis.

**Biochemical assays**

Activities of alanine and aspartate aminotransferases i.e. ALT and AST respectively were determined using analytical kits obtained from Randox Laboratories Ltd. (Antrim, UK). The concentrations of sodium ($Na^+$) and potassium ($K^+$) were assayed using TECO diagnostic kits (Anaheim, USA). Furthermore, the serum chloride (Cl-) was analysed with DIALAB assay kits (Michigan, USA) and creatinine concentrations were evaluated using Agappe diagnostic kits (Knonauerstrasse, Switzerland).

**Histopathological studies**

Fixed liver tissues were sectioned with microtome (5 µm thickness) then fixed in slides. The slides were stained and counterstained according to Harry's Haematoxylin and Eosin staining procedures. Thereafter, the slides were mounted on Distrene, Plasticizer and Xylene (DPX) mountant and viewed under a light microscope (Olympus CKX41, Olympus, Japan) and photomicrographs were taken (400×).

**Statistical analysis**

Data are presented as the means ± SD. With the aid of SPSS software version 20.0, the data was subjected to one-way ANOVA and followed by least significant difference (LSD) post hoc comparison of means. Differences at $P < 0.05$ were considered significant

## RESULTS AND DISCUSSION

Result of the activities of serum aminotransferase is shown in Figure 1. There was no observed significant effect of the sweetened diets on the activities of the aminotransferase (ALT and AST) except as decrease in AST activity caused by the H12% diets when compared to the normal control ($P < 0.05$).

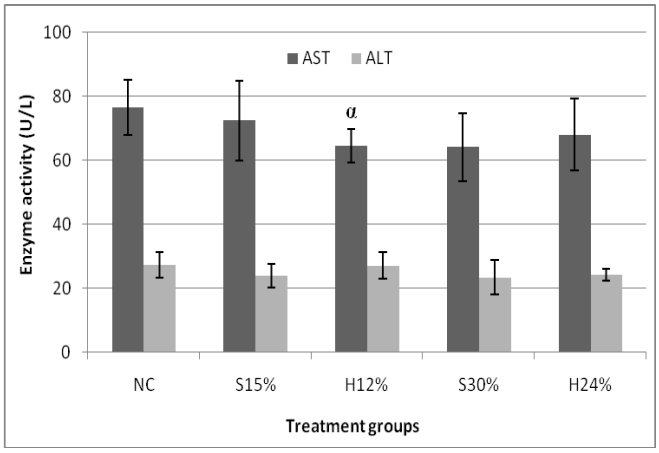


**Figure 1**. Activities of Serum Alanine and Aspartate Aminotransferases (ALT and AST) (U/L) of Rats Administered Honey and Sugar-Sweetened Diets.

NC = normal control, S15% = 15% sugar- sweetened diet, S30% = 30% sugar-sweetened diet, H12% = 12% honey-sweetened diet and H24% = 24% honey-sweetened diet. α = $P < 0.05$ vs NC. Values represent the means ± SD, n = 7.

**Serum electrolytes concentrations**

Result of the effect of the sweetened diets on serum electrolyte concentrations is shown in Figure 2. Compared to each other, the table sugar and honey-sweetened diets did not significantly alter the measured kidney function indices. However, when compared to the control, S15% diet was found to cause a 3.36% and 31.29% increase in sodium and chloride concentrations, respectively ($P < 0.05$). Similarly, H12% diet caused a 19.77% in serum potassium concentration relative to the control ($P < 0.05$).

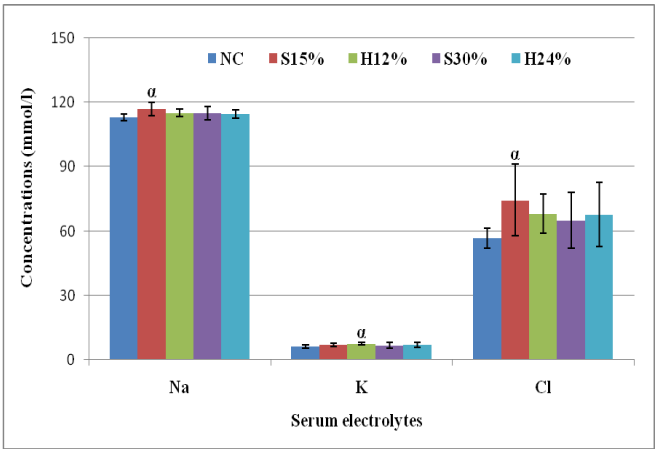


**Figure 2**. Serum Levels of Sodium (Na), Potassium (K), and Chloride (Cl) of Rats Administered Honey and Sugar-Sweetened

Diets. NC = normal control, S15% = 15% sugar sweetened diet, S30% = 30% sugar sweetened diet, H12% = 12% honey sweetened diet and H24% = 24% honey sweetened diet. α = P < 0.05 vs NC. Values represent the means ± SD, n = 7

**Serum creatinine concentrations**

Data obtained indicate that serum creatinine, a chief marker of kidney function did not differ significantly in the sugar-sweetened diets compared to both honey-sweetened diet and the control group (P > 0.05) i.e. values of the experimental rats were in the same range with the control as shown in Figure 3.

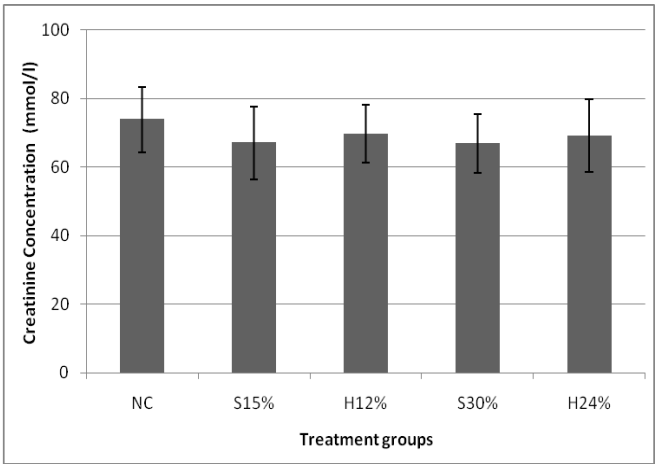


**Figure 3**. Serum Concentration of Creatinine (mmol/l) of Rats Administered Honey and Sugar Sweetened

Diets. NC = normal control, S15% = 15% sugar sweetened diet group, S30% = 30% sugar sweetened diet group, H12% = 12% honey sweetened diet group and H24% = 24% honey sweetened diet group. Values represent the means ± SD, n = 7.

**Hematological indices**

The red blood cell (RBC); haemoglobin (HGB); Hematocrit (HCT), mean cell haemoglobin concentration (MCHC) and lymphocyte (LYM) concentrations measured in the honey-administered rats were not significantly different from those of sugar-administered and normal control rats (Figure 4). However, the white blood cell (WBC) count of the H24% fed rats was found to be higher than the control by 34.09% (P < 0.05) suggesting a possible immunological response.

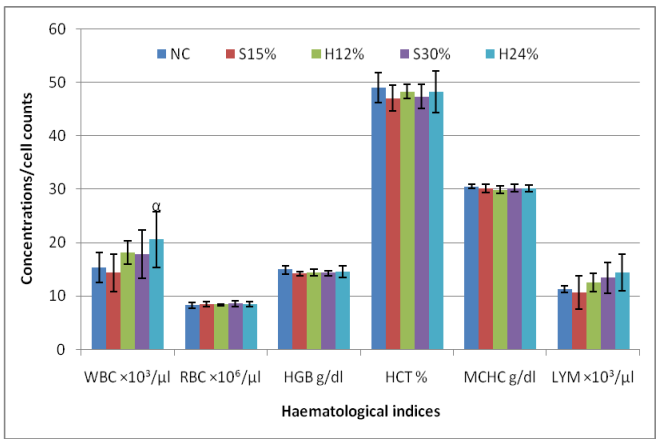


**Figure 4**. Hematological Indices of Rats fed Natural Honey and Table Sugar-Sweetened Diets.

White blood cell (WBC); red blood cell (RBC); haemoglobin (HGB); Hematocrit (HCT); mean cell haemoglobin concentration (MCHC) and lymphocytes (LYM). NC = normal control, S15% = 15% sugar sweetened diet, S30% = 30% sugar sweetened diet, H12% = 12% honey sweetened diet and H24% = 24% honey sweetened diet. Values represent the means ± SD, n = 7. α = P < 0.05 vs NC.

**Liver histology**

The cellular architecture and integrity of the hepatocytes were examined in this research and the photomicrographs shown in Figure 5a-e. Figure 5a is the histology of the normal control rats, showing the hepatic lobule with its central vein. The sinusoid radiates out from the central vein in hexagonal pattern characteristic of the normal liver cells. The hepatocytes were demonstrated with open faced multi-nuclei. Histology of S15% diet fed rats (Figure 5b) was quite similar to that of the normal control, except that the hepatocytes and their nuclei were slightly enlarged thus occluding the sinusoid. The sinusoids of the S30% diet fed rats appeared dilated (wider) than any other group with nuclei enlarged than those of control and S15% groups (Figure 5c). The H12% diet treated rats also showed section of hepatic lobule with enlarged central vein; sinusoids radiate hexagonally but appear constricted. Metaplasia of the hepatic nuclei was found (Figure 5d). In the H24% diet fed rats, the hepatocytes were well demonstrated with open faced multi-nuclei that appeared enlarged than any other group, even though its sinusoid radiates out from the central vessel in hexagonal pattern similar to control group. However, these minor changes were physiological responses, not significant enough to impact liver function or influence biochemical data.

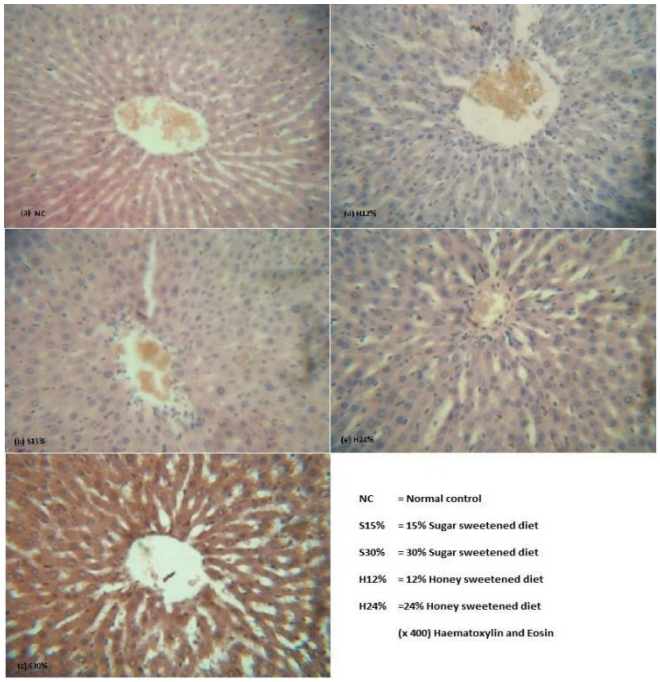


**Plate 1**. Photomicrograph of liver for groups I (A), II (B), III (C), IV (D), V (E) viewed with HE staining by light microscope

## Discussion

The impact of equivalent calories of natural honey and table sugar incorporated diets on the hepatocyte integrity and functions of key organs/tissues in male Wistar rats was evaluated in this study. The formulation of the diets was simulated average sugar composition of common sweetened foods and beverages. The sweetened diets were found not to significantly alter serum liver enzyme activities except decreased activity of aspartate aminotransferase (AST) in H12% fed animals when compared to the control. Although hepatic fat deposition is known to disrupt hepatic cell integrity which can reflect as alteration in enzyme activities (Atangwho *et al*., 2012), the current observed decrease in AST activity does not suggest altered liver function. Damage to hepatocytes often correlates with high activities of AST and ALT in the blood (Murray *et al*., 2012), and liver impairment would also simultaneously alter both ALT and AST activities along with other liver function indices (Choudhary and Deevi, 2014). In the current data, there was no concomitant decrease observed in the ALT activity neither was there a follow up effect in the H24% indicating that the hepatic cells were not adversely affected by the honey diets. The result of liver histology also corroborates our submission of a null effect of the sweeteners on liver function, as no significant pathologic lesions were observed in the liver histology.

Furthermore, the serum concentrations of potassium ($K^+$), sodium ($Na^+$) and chloride ($Cl^-$) of the sugar-fed groups were not considerably different from those of the corresponding honey-fed groups, but the levels of $Na^+$ and $Cl^-$ in S15%-fed animals were significantly higher than the control. Effective homeostatic mechanisms in the kidneys may account for the obtained results because the kidneys' functions amongst others are to maintain these electrolytes at physiological ranges in the blood (Choudhary and Deevi, 2014). The noticeable increase in the concentration of $K^+$ in honey-fed rats (H12%) relative to the control may have been a transient rather than a sustained effect because a similar effect was not demonstrated by the group administered with twice the volume of honey (i.e. H24%), more so as the serum $K^+$ levels in the energy-equivalent sugar-fed (i.e. S15%) rats were not comparatively different from those in H12% rats. Moreover, the renal excretory function was not affected in both test and control groups as evidenced by the non-significant changes in serum creatinine, a remarkable marker of excretory efficiency of the kidneys. In an earlier study (Onyije *et al*., 2012) reported a slight elevation of serum creatinine concentration in male Wistar rats after 6 weeks administration of unprocessed honey compared to the control. The geographical source, study duration and percent honey incorporation in the diet, were different in looking at the study and this present one, hence may be responsible for the difference in the effect on creatinine. Be that as it may, the elevation in the previous result was not significant; moreover, result from chronic exposure as in the current design suffices over short-term report.

In contrast to an earlier report that honey caused an increase in lymphocyte counts (a type of WBC) in rats after long-term feeding for 52 weeks compared to rats administered sucrose-based diet (Chepulis, 2007), data from the current study revealed that neither the honey nor sugar based diets significantly impacted the lymphocyte counts of the rats. This was paralleled by null effect of the sweeteners on their respective group total WBC counts. Also, contrary to the reports of Eteraf-Oskouei and Najafi (2013) that honey elevates haemoglobin (HGB) concentrations, there was neither observable significant effect of honey on HGB and haematocrit (HCT) levels nor the haemoglobin derived factor, MCHC levels as compared to the sugar-fed groups. The difference in the source of honey and the length of feeding may be responsible for this difference in observations. These haematological results of the present study suggest that the blood composition and immunity of the rats were not compromised by the sweeteners.

In summary, results derived from this study have indicated that 3-month feeding of calorie-equivalent amount of natural honey and table sugar based diets separately is not likely to exert any adverse effect on the organs and tissues in the body, at least in the rat.

## AUTHORS' CONTRIBUTIONS

All authors participated in the research design. Author IFA, MAE & IJA conducted the research work, Author IFA, IJA and ARA wrote the manuscript. While Authors IJA, EIA and ARA revised the manuscript and the research design. All authors read and approved the final manuscript.

## CONFLICT OF INTEREST

None

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