JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 14, ISSUE 1: 390 – 403 (2024)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Nutritional and phytochemical composition of honey obtained from bees in Benue State, Nigeria, and its effects on the body weights, haematology and antibody titres of albino rats

Onyekachi V. Asuzu [1,2], Ikenna M. Okpala [1], Sarah P. Kuhn [2], Ikechukwu J. Udeani [1], Chidiebere C. Ubachukwu [1], Chikaodili C. Ezeugwu [1], Blessing A. Ezeocha [1], Chidiebere Anyaoha [1], Obinna Onyejekwe [1], Lotanna G. Nwobi [1] and Okezie S. Ekere [1]

[1] Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[2] School of Medicine, Dentistry & Biomedical Sciences, Queen's University, Belfast, United Kingdom.

==========

Abstract

Honey has been used in traditional medicine worldwide. This study evaluated the nutritional and phytochemical composition of honey obtained from Benue State, Nigeria, and studied the effects of oral administration of the honey on the body weights, haematology and antibody titres of albino rats. The honey used for the study was obtained from an apiarist in Benue State, Nigeria in February 2019 (dry season). It was subjected to nutritional and phytochemical analysis following standard procedures. The *in vivo* study with rats utilized 20 albino rats randomly assigned to four groups (A, B, C, D) of five rats each. Rats in group A were the untreated controls, while rats in groups B, C, and D were given daily oral doses of the honey at 2.5, 5.0 and 7.5 mg/kg respectively for 28 days. Body weights and temperature of the rats were measured at weekly intervals, and blood samples were collected from the rats once a week for haematology and antibody titre determination. Results showed that the protein and carbohydrate content of the honey studied was higher than that of the European standard, while its moisture and hydroxymethylfurfural content was lower than that of the European standard. The honey had high levels of saponins, phenol and terpenoids, moderate levels of alkaloids and steroids, and low levels of flavonoids. Administration of the honey to rats led to significantly ($p < 0.05$) higher body weights, total leukocyte, neutrophil and erythrocyte counts in the treated groups in a dose dependent manner. Rats treated with honey further showed a significantly ($p < 0.05$) higher antibody titre than the untreated control. These results suggest that the honey used in this study is rich nutritionally and in valuable phytochemicals, and its oral administration to rats led to higher body weights, total leukocyte, erythrocyte and neutrophil counts and antibody titre.

Keywords: Nigerian honey; Nutritional composition; Phytochemicals; Haematology; Antibody titre; Albino rats.

***Correspondence:** Onyekachi V. Asuzu; Email: onyekachi.asuzu@unn.edu.ng; Phone: +2348026256828

Article History: Initial submission received: November 24, 2023; Final revised form received: February 05, 2024; Accepted for publication: February 09, 2024; Published: February 16, 2024.

Introduction

Nigerian honey is produced by bees from the family *Apis mellifera adansonii* a genetically close relative of *A. m. scutelleta* also known as the "African killer bees" (Akinwade *et al*., 2013). West African bees are known to be very aggressive but also more resistant to a number of known bee pests and diseases (McMenamin and Genersch, 2015; Ramos-Cuellar *et al*., 2022). Honeybees are the main pollinators of flowering plants and thus contribute to a greater degree to the presence and sustenance of food production.

Honey is a major product of honeybees, and from ages past even to current times, the global burden of diseases has been mitigated using honey. Honey is considered one of nature's wonders, as it is a natural, viscous, sweet yet acidic substance, produced by honeybees from nectar of blossoms or from secretions of living parts of plants, which they transform by mixing with their saliva and store up to mature within the honeycombs (Abeshu and Geleta, 2016; Bogdanov *et al.*, 2008). Honey is a simple, but complex mixture of compounds, and has been adopted from historian times to soothe aliments such as throat infections, tuberculosis, thirst, hiccups, treatment of eye diseases, bronchial asthma, fatigue, dizziness, hepatitis, constipation, worm infestation, piles, eczema, healing of ulcers, and wounds, and it is also used as a nutritional supplement which can be taken at any time (Krell, 1996; Eteraf-Oskouei and Najafi, 2013). Honey is composed primarily of fructose and glucose but also contains 4 - 5% fructo-oligosaccharides, which serve as prebiotic agents (Wang et al, 2017). It has been reported to contain over 180 constituents some of which includes amino acids, vitamins, minerals, and enzymes (Crane and Bee Research Association, 1975).

To date, there are massive amounts of information in available literature on the properties of honey from various sources. Natural honey has been reported to have a hundred-fold potency in anti-microbial activity (Mandal and Mandal, 2011). Honey has been used in the treatment of chronic skin ulcers, gastrointestinal conditions, respiratory diseases as well as in post-surgical procedures, as antiseptic, antibacterial and antifungal agent (Bogdanov *et al*, 2008). In addition to being used on surface conditions, its use has also been explored on systemic ailments (Mandal and Mandal, 2011). As food, honey is known to provide energy and nourishment as well as to enhance the functionality of vital organs in the body and to help the body fight off infections (Ajibola, 2016). Much of the therapeutic abilities of honey is believed to be due to the high sugar concentration and the resulting osmotic effect, acidity and due to hydrogen peroxide generated from the oxidative conversion of glucose to gluconic acid by glucose oxidase upon dilution (Ajibola, 2016; Krell, 1996; Osato *et al*, 1999).

In developing countries, and possibly throughout the world, natural therapies should be learned and applied to improve the possibilities of man's state of wellness. The aim of this study was to evaluate the nutritional composition and phytochemical constituents of local Nigerian honey sourced from Benue State, Nigeria, and to further evaluate the effects of its oral administration on the body weights, haematology, and antibody titres of albino rats.

Materials and Methods

The honey used for the study was obtained from an apiarist based in Benue State of Nigeria who keeps *Apis mellifera adansonii* (the prevalent bee species seen in West Africa) using a top-bar housing system within his apiary. The apiarist reported that the flora in the region was composed mainly of palm trees. We do not assume the honey to be mono-floral, because these bees were not

bred in an exclusive flora area. The honey used for the study was collected in February 2019, which is within the dry season in Nigeria. Samples were collected in sterile jars and sent to the laboratory for analysis and further experimental purposes.

The following methods were adopted for the nutritional evaluation of the moisture, fat, crude protein, ash, crude fibre, carbohydrate and hydroxymethylfurfural content of the honey sample:

Moisture determination: 2 g of the honey sample was placed in an aluminium dish. This was then transferred to a thermostatically controlled oven at a temperature of 105°C and normal atmospheric pressure. Drying continued until a constant mass was obtained. The moisture content was then calculated.

Fat determination: The solvent extraction method as described by Pearson (1976) was used for fat determination. 2 g of the honey sample was weighed into a filter paper and introduced into a paper thimble which was then transferred into a Soxhlet apparatus (Pearson, 1976). A round bottomed flask was fitted up to three quarters its volume with petroleum ether. This was connected to the extractor with the condenser properly fitted. The heating of the heating mantle was done at 50°C for 45 minutes. The solvent was recovered, and fat estimated by weight and the percentage fat calculated.

Crude protein determination: The micro Kjedahl method (AOAC, 1995) was used for crude protein determination. 1 g portion of the honey sample was weighed out and transferred into a Kjedahl digestion flask (Williams, 1986). 2 g of Na_2S0_4 was added followed by 0.5 g of $CuS0_4$ and 0.2 g selenium catalyst. The Kjedahl flask was then placed in the heating mantle of the digester. The mixture was heated gently at first after which the temperature was increased to the highest to complete the digestion. Digestion was fully achieved when the dark colour of the mixture became clear after which the Kjedahl flask was brought out and allowed to cool. The mixture was then diluted and made up to 100 ml using deionised water. An aliquot of 5 ml was taken from the diluted digestate for distillation.

For the distillation procedure, 10 ml of 50% NaOH and 5 ml aliquot of the digest was pipetted into the distilling unit of the Markham apparatus. The apparatus was switched on to steam distil the mixture in other to liberate NH_3. Distillation was completed when the purple colour of the mixed indicator turned green. 50 ml of the distillate was then collected and sent over to the burette for titration.

For the titration procedure, the 50 ml distillate was titrated against 0.01 NHCl until an end point was reached which is the appearance of a purple colour. The titre values were recorded afterwards. The crude protein content (N) was then calculated.

Ash content: The official method by the Association of Official Analytical Chemists (AOAC, 1995) was used for ash content determination. 2 g of the honey sample was weighed into a crucible which had been previously ignited and cooled prior to weighing. The crucible and its contents were heated on a muffle furnace at a temperature of 550°C then transferred into a desiccator and cooled for 15-30 min. It was then re-weighed, and percentage ash calculated.

Crude fibre determination procedure: The AOAC (1995) method was used for determination of crude fibre content. Oil was removed from 3 g of the honey sample, ground to pass through 1 mm mesh, either by stirring, setting, and decanting three times with petroleum spirit. The air-dried fat free sample was then transferred into a flask or beaker. 200 ml of this was measured at room temperature and mixed with 1.25% H_2S0_4 and boiled for 1 minute. Excess foaming was reduced by adding 1 ml of anti-foam solution and was boiled gently for 30 minutes,

maintaining a constant volume by the addition of water. An 11 cm Whatman No 42 grade filter paper was fitted in a Buchner funnel and hot water poured into the funnel and allowed to stand until the funnel became hot. At the end of the 30 minutes boiling, the acid mixture was allowed to stand for approximately one minute and poured into a shallow layer of hot water in a prepared funnel.

The suction of the filtration pump was adjusted in such a way that the bulk of 200 ml was filtered within 10 minutes. The insoluble matter was washed with boiling water until the washing were neutral to litmus paper. The residue was washed into the beaker using 200 ml of 1.25% NaOH. The solution was then boiled further for 30 minutes as described above and it was allowed to stand before being filtered through a filter crucible using gentle suction. The whole of the insoluble materials was transferred to the crucible with hot water and was washed with boiling water once with 10% HCl and then with H_20 again until the washing became neutral to litmus paper.

The crucible and its content were dried at 100°C and allowed to cool in a desiccator before being weighed. The crucible was placed in a mantle furnace set at 450°C and the temperature maintained until ashing was completed and finally the crucible was removed and cooled in a desiccator.

Calculation of results was done by dividing the loss in weight on ignition by 3 g to give the percentage fibre in the honey sample.

Carbohydrate content: The carbohydrate (CHO) content was calculated as follows: CHO + 100 – (Protein % + Ash % + Fibre % + Moiture % + Fat %).

Hydroxymethylfurfural content (Hmf): The Hmf content was determined by reading up the absorbance of a clarified sample against a reference solution of the same honey in which 284 nm chromophore was destroyed using bisulfite (Elmer, 2016).

Quanitative Phytochemical Analysis

Determination of Alkaloid content of the honey: This was done by the alkaline precipitation gravimetric method as described by Harborne (1973). A measured weight of the honey sample was dispersed in 10% acetic acid solution in ethanol to make for a ratio of 1:10 (10%). The mixture was allowed to stand for 4 hours at 28°C. It was later filtered via Whatman No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analysed.

Determination of Flavonoid content of the honey: The flavonoid content of the honey was determined according to the method of Harborne (1973). 5 g of the honey sample was boiled in 50 ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered through Whatman No 42 grade of filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate. The flavonoid precipitate was recovered by filtration. The resulting weight difference gave the weight of flavonoids in the sample.

Determination of Tannin content of the honey: The tannin content was determined by the Folin-Denis colorimetric method, as described by Kirk and Sawyer (1998). 5 g sample was dispersed in 50 ml of distilled water and mixed. The mixture was allowed to stand for 30 minutes at 28°C before it was

filtered through Whatman No 42 grade of filter paper. 2 ml of the filtrate was dispersed into a 50 ml volumetric flask. Similarly, 2 ml standard tannin solution (tannic acid) and 2 ml of distilled water were put in separate volumetric flasks to serve as standard and blank respectively, and the reagent was added to each of the flasks and the 2.5 ml of saturated Na_2CO_3 solution added. The content of each flask was made up to 50 ml with distilled water and allowed to incubate at 28°C for 90 minutes. Their respective absorbances were measured with a spectrophotometer at 760 nm using the reagent blank to calibrate the instrument at zero, and the tannic acid content was deduced from a curve of the standard.

Determination of Cardiac Glycoside content of the honey: The glycoside content of the honey was determined using a spectrophotometric method as described by Onwuka (2005). 2 g of the honey sample was extracted with 20 ml distilled water over night. The mixture was then filtered with Whatman No 1 filter paper. The filtrate was made up to 100 ml with distilled water. 10 ml aliquot was taken from the diluted filtrate and placed in a 20 ml test tube. 2 ml alkaline picrate solution (prepared by massing out 1 g of picric acid, 5 g sodium carbonate and making up the mixture by diluting with hot distilled water to 200 ml) was added to the content of the test tube. A blank was run in the same manner alongside the sample. Both the blank and sample were read off in a Jenway 6305 spectrophotometer set at 490 nm wavelength. The cardiac glycoside concentration of the sample was then extrapolated from a glycoside standard curve.

Determination of Terpenoids content of the honey: The terpenoid content of the honey was determined using the gravimetric method, as described by Jiang *et al.*, (2016). 2 g of the sample was weighed into a 20 ml test tube. 20 ml analytical grade methanol was added. This was allowed to stand for three days. On the third day, the contents of the test tube was centrifuged at 3,000 rpm and the supernatant drained into a 100 ml pre-weighed beaker for onward drying in a hot air oven set at 70°C. The beaker and its dried content were weighed again and the percentage terpenoid calculated.

Determination of Steroid content of the honey: The steroid content of the honey was determined by the method described by Okeke and Elekwa (2003). A measured weight of the honey sample was dispersed in 100 ml freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered, and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). 2 ml of the eluate was put in a test tube and mixed with 2 ml of chloroform. 3 ml of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of concentrated H_2SO_4 were cautiously added until the solution was cool. A standard sterol solution was prepared and treated as described above. The absorbance of standard and prepared sample was measured using a spectrophotometer.

***In vivo* evaluation of the effects of oral administration of the honey to albino rats:**

Twenty male albino rats were used for the study. They were procured at eight weeks of age (126.9 – 142.6 g) from the Department of Zoology, University of Nigeria Nsukka. They were left to acclimatize for 14 days before commencement of the experiment. Throughout the use of these rats, they were fed with commercial feed (Vital feed®, Grand Cereal Oil Mill Ltd., Nigeria) and were provided with clean water *ad libitum*. The rats were humanely handled and cared for during the experiment. Ethical approval was sought for and obtained from the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (Approval Reference Number: FVM-UNN-IACUC-2023-09/117). The rats were randomly assigned into four groups (A, B, C, D)

comprising of five rats in each. Rats in group A served as the untreated control, while those in groups B, C and D were given daily oral doses of honey at 2.5, 5.0 and 7.5 mg/kg respectively for 28 days using gastric gavage.

The body weights and temperature of the rats were measured using a digital weighing balance and thermometer, respectively, at the beginning of the study (Week 0) and henceforth at weekly intervals all through the 28 days of the treatment. Change in body weight from the baseline was calculated for each rat. Also, blood samples for haematology and antibody assay were collected using sterile Bijou bottles at baseline (week 0) and at weekly intervals across the 28 days of the experiment. The blood samples meant for haematology were used to evaluate for the packed cell volume (PCV), haemoglobin concentration (Hbc), erythrocyte count (EC), total white blood cell count (TWBC), differential white blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Packed cell volume (PCV) was determined by the microhematocrit method, while the haemoglobin concentration was determined by the cyanmethemoglobin method (Schlam *et al*., 1975). The erythrocyte and total white blood cell counts were done by the haemocytometer method (Thrall and Weiser 2002). A thin blood smear made on a grease-free glass slide was prepared, air dried and stained following the Leishman technique for the differential white blood cell counts Thrall and Weiser 2002). The mean corpuscular values were calculated using the standard formula (Thrall and weiser, 2002; Stockham and Scott, 2008).

For the antibody assay, rats in all the groups were injected with 2 ml of washed sheep red blood cells at Day 0, and on days 7, 14, 21 and 28, blood samples were collected from them for serology. The blood collected was allowed to stand at room temperature to clot and serum was harvested from the clotted blood after centrifugation. The serum samples were used for the standard haemaglutination inhibition technique as described by Beard (1980). The geometric mean titre (GMT) was calculated mathematically for a two fold dilution (Villegas and Purchase, 1989).

Statistical analysis: Data generated from the study was analysed using a PRISM 9.0 statistical software. The data was subjected analysis of variance (ANOVA), and variant means were separated using Dunnett's multiple comparison test. Significance was accepted at probability less than 0.05.

Results

The protein content of the honey used for the study was 0.72g/100g and its carbohydrate content was 82.38g/100g; these values were comparatively higher than the minimum requirement as stipulated by the European Union (EU) (Bogdanov *et al*., 2013; Brodschneider *et al*., 2018) [Table 1]. The moisture content of the honey was 15.69 % while its hydroxymethylfurfural (Hmf) content was 11.69g/100g; the values obtained for moisture and hydroxymethylfurfural content of the honey sample were comparatively lower than the recommended standards by the EU, while the pH was of the same value with EU standards (Table 1).

The honey samples analysed had a high level of saponins (353.0 mg/100g), phenols (50.8 mg/100g) and terpenoids (41.7 mg/100g), and moderate levels of alkaloids (19.1 mg/100g), steroids (15.78 mg/100g) and tannins (7.3 mg/100g), but very low levels of flavonoids (0.748 mg/100g) and no glycoside (Table 2).

There were no significant ($p > 0.05$) changes in the body weight of the rat groups on week 1 of the experiment, but from week 2 to 4, the mean increase in the body weight of the rats

in the three treated groups (Groups B, C and D) were significantly ($p < 0.05$) higher than that of the untreated control in a dose dose-dependent manner (Figure 1). There was however no significant ($p > 0.05$) difference between the rat groups in their rectal temperature except on week 2 when the rectal temperature of Group B rats was significantly ($p < 0.05$) lower than that of Group A (Figure 2).

Table 1: Nutritional composition of honey obtained from Benue state, Nigeria, compared to a European standard *(Brodschneider et al., 2018)*.

Parameter	Honey sample used for the study	European standard
Protein (g/100g)	0.72	0.4
Carbohydrate (g/100g)	82.38	76.4
Moisture (%)	15.69	20
Ash (g/100g)	0.97	
Oils (g/100g)	0.24	
Hydroxymethylfurfural (mg/100g)	11.69	40-80
pH	3.90	3.9

Table 2: Quantitative phytochemical constituents of honey obtained from Benue State, Nigeria.

Parameter	Quantitative constituent (mg/100g)
Saponins	353
Alkaloids	19.1
Phenols	50.8
Glycosides	-
Tannins	7.3
Flavonoids	0.748
Steroids	15.78
Terpenoids	41.7

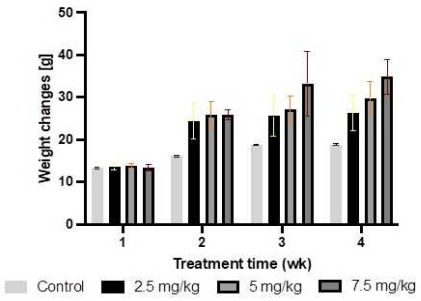

Figure 1. Body weight changes (mean with SEM bars) in albino rats given varied oral doses of Nigerian honey for four weeks.

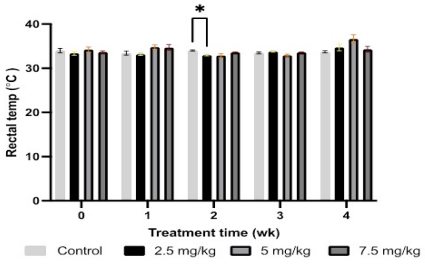

Figure 2. Rectal temperature (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

The total white blood cells (TWBC) counts of all the rat groups did not significantly vary ($p > 0.05$) on weeks 0 and 1, but on week 2, the TWBC counts of the Groups C and D rats were significantly ($p < 0.05$) higher than that of Group A, and further on weeks 3 and 4, the TWBC counts of all the treated rats groups (Group B, C and D) were significantly higher than that of the untreated control Group A (Figure 3).

The red blood cells (RBC) counts of all the rat groups did not significantly ($p > 0.05$) vary across the groups on weeks 0, 1 and 2, but on weeks 3 and 4, the RBC counts of the Groups C and D were significantly ($p < 0.05$) higher than those of Groups A and B rats (Figure 4).

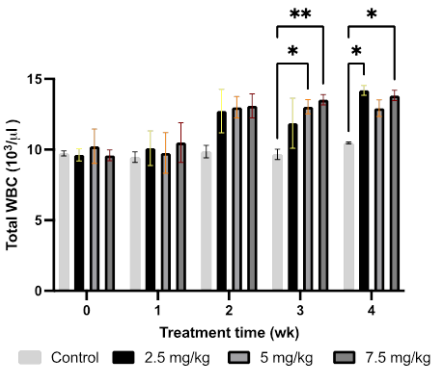

Figure 3. Total white blood cell counts (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

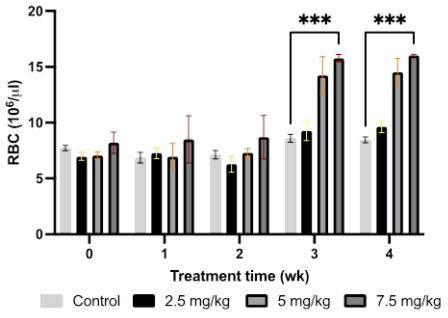

Figure 4. Red blood cell counts (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

The packed cell volume (PCV) of the rat groups did not significantly ($p > 0.05$) vary on weeks 0, 1 and 4, but on weeks 2 and 3, the mean PCV of the Group D rats was significantly ($p < 0.05$) higher than those of all other groups (Figure 5). In the same vein, the haemoglobin concentration (Hb) of the rat groups did not significantly ($p > 0.05$) vary on weeks 0, 1, 2 and 3, but on week 4, the mean Hb of Group B rats was significantly ($p < 0.05$) higher than that of Group A rats (Figure 6). The mean corpuscular volume (MCV) of the rat groups also did not significantly ($p > 0.05$) vary on weeks 0, 1 and 2, but on weeks 3 and 4, the MCV of rats in Group C and D were significantly ($p < 0.05$) lower than that of Group A rats (Figure 7). The MCH however did not significantly ($p > 0.05$) vary on weeks 0 and 1, but on week 2, the MCH of Group B, C and D were significantly ($p < 0.05$) higher than that of Group A, on week 3, the MCH of Groups B, C and D were significantly ($p < 0.05$) lower than that of Group A, while on week 4, it was the MCH of Groups C and D that was significantly ($p < 0.05$) lower than those of Group A and B (Figure 8). There were no significant ($p > 0.05$) variations in the mean corpuscular haemoglobin concentration (MCHC) all through the study (Figure 9).

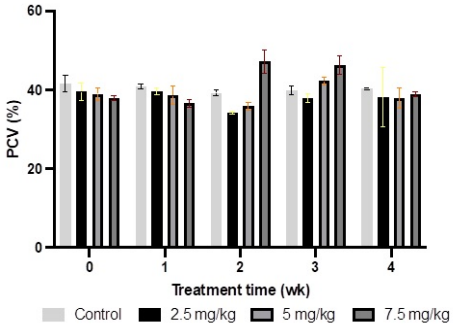

Figure 5. Packed cell volume (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

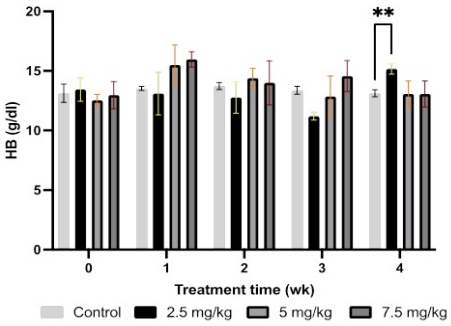

Figure 6. Haemoglobin concentration (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

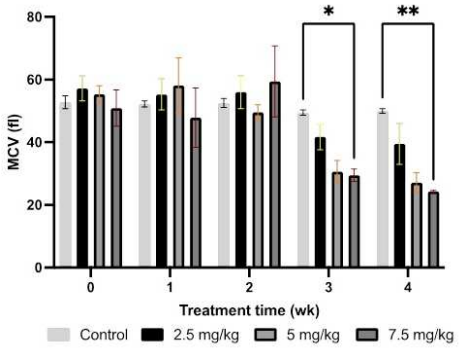

Figure 7. Mean corpuscular volume (MCV) [mean with SEM bars] of albino rats given varied oral doses of Nigerian honey for four weeks.

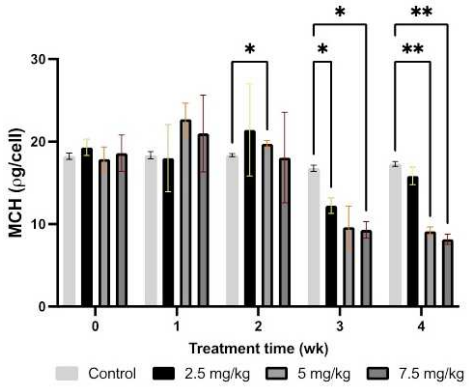

Figure 8. Mean corpuscular haemoglobin (MCH) [mean with SEM bars] of albino rats given varied oral doses of Nigerian honey for four weeks.

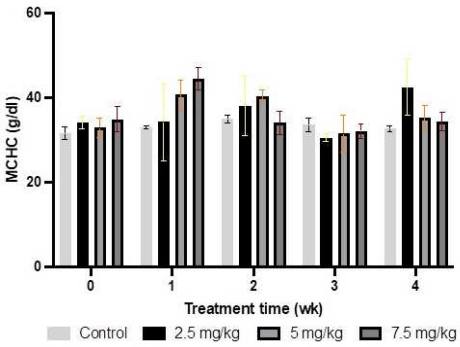

Figure 9. Mean corpuscular haemoglobin concentration (MCHC) [mean with SEM bars] of albino rats given varied oral doses of Nigerian honey for four weeks.

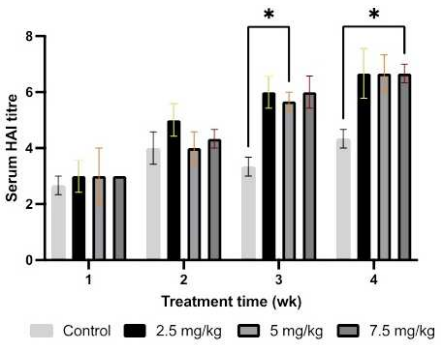

Figure 10. Serum haemagglutination inhibition titres (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

There was no significant ($p > 0.05$) variations in the mean serum haemagglutination inhibition (HI) titre for weeks 1 and 2; however on weeks 3 and 4, the serum HI titre in Groups B, C and D rats was significantly ($p < 0.05$) higher than that of Group A rats (Figure 10). There were no significant ($p > 0.05$) variations among the groups in their mean neutrophil counts at weeks 0 and 1, but at weeks 2, 3 and 4, the neutrophil counts of rats in Groups B, C and D were significantly ($p < 0.05$) higher than that of Group A rats in a dose dependent manner (Figure 11). The absolute lymphocyte count of the groups did not significantly ($p > 0.05$) vary at weeks 0, 1 and 3, but at week 2, Group D rats had a significantly ($p < 0.05$) lower lymphocyte count while at week 4,

Group B rats had a significantly ($p < 0.05$) higher lymphocyte count relative to other groups (Figure 12).

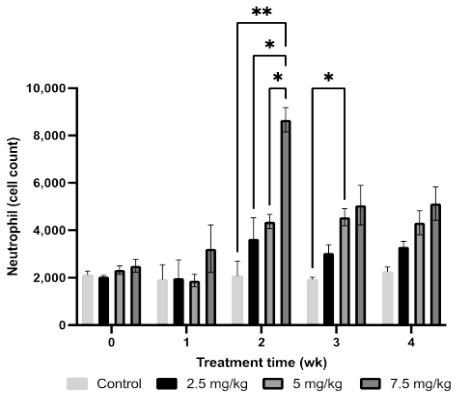

Figure 11. Neutrophil counts (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

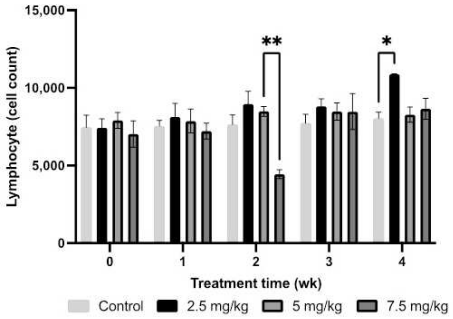

Figure 12. Lymphocyte counts (mean with SEM bars) of albino rats given varied oral doses of Nigerian honey for four weeks.

Discussion

The findings in the present study showed that the Nigerian honey had higher protein and carbohydrate values when compared with the minimum EU requirements; this suggests that the local Nigerian honey has an added benefit of providing additional nutritional value to its consumers. This high value of carbohydrate has been reported to be beneficial in providing energy for consumers and is known to be less toxic than table sugar (Pasupuleti *et al*., 2017). The protein content of the Nigerian honey (0.72g/100g) recorded in the present study was higher than what has been reported for other honey sources like Makuna honey (0.502 – 0.506g/100g), Tualang honey (0.36 – 0.66g/100g), and Sidr honey (0.15 – 0.506g/100g), but was comparable to what was reported for kelulut honey (0.39 – 0.85g/100g) (Al-Kafaween *et al*., 2021; 2023). These variations in protein content could be attributed to the bee species, geographical region, plants on which the bees forage on, etc. The relatively higher protein content of the Nigerian honey used for the study implies that it could offer improved nutritional benefits to the consumers.

The pH value of 3.9 recorded in this study and which is also that reported for the EU standard is worthy of note. Reports have shown that most bacterial organisms that contribute to reducing shelf-life of foods thrive best at neutral to slightly alkaline (Pavlova *et al*., 2018), whereas yeasts and mould rather prefer an acidic environments of pH 4.0 – 4.5 (El Sohaimy *et al*., 2015). It is believed that the pH value of honey could be a factor that prolongs its shelf-life and stability.

The moisture content of the honey used for the present study was lower than that of the EU standard (Brodschneider *et al*., 2018); this is thought to be due to the dry season during which the honey was harvested/collected. The moisture content of honey has been reported to be a primary quality indicator of honey (El Sohaimy *et al*., 2015). A moisture content above 20% has been reported to indicate that the honey is of low quality and could spoil more easily (Al-Kafaween *et al*., 2023).

The findings of varied quantities of a number of phytochemicals in the honey could be a good attribute of Nigerian honey (Khalil and Sulaiman, 2010). It has earlier been reported that the source of the phytochemicals found in honey is from the plants the bees forage on or visit, and as such, the type and quantity of these phytochemicals will vary based on the vegetation around which the bees forage and

live in (Pasupuleti *et al*., 2017; Al-Kafaween *et al*., 2023). These phytochemicals in their varied combinations are believed to be responsible for the health benefits attributed to honey consumption (Khalil *et al.*, 2011; Kishore *et al.*, 2011; Hossen *et al.*, 2017). It is also thought that the beneficial effects of these phytochemicals is responsible for the positive effects recorded in the rat groups treated with honey in the *in vivo* aspect of the present study.

The significantly higher gain in weight in the honey treated groups when compared with the untreated control in this study could be said to suggest an improved quality of life for the rats. It is thought that the high protein content of the honey used for the study may account for the higher weight gain in the honey treated rats. Though a significantly higher weight gain was recorded in this study for the honey treated rats, reports in literature have shown that the intake of honey reduces the incidence of obesity in honey consumers (Zulkifli *et al*., 2022). The lower incidence of obesity in honey consumers has been attributed to the ability of honey to reduce blood sugar, LDL, total cholesterol, systemic triglycerides levels, and improve the sensitivity of insulin towards lipids and triglyceride metabolism (Zulkifli *et al*., 2022).

The significantly higher TWBC, neutrophil and RBC counts recorded in the treated groups when compared with the untreated control group was dose dependent and is worthy of note. It is suggestive that oral honey administration as used in this study may have enhanced the white and red blood cell production or helped spare their destruction. Knowing the role that white blood cells play in the immune protection of the body and the role that red blood cells play in gaseous exchange and overall health of the body (Weiss and Wardrop 2010), the dose dependent higher TWBC, neutrophil and RBC recorded for the honey treated groups is a big plus for the use of honey to stay or improve the state of health (Ranneh *et al*.., 2021). The higher TWBC and neutrophil counts concurred with the higher HI titres recorded for the honey treated group and imply that consumption of honey can enhance antibody response and thus immune status for honey consumers. This higher HI titre/enhanced antibody response may partly be responsible for the reported high therapeutic and health benefits of honey consumption (Ibraguren *et al.*, 2010; Aween *et al.*, 2012; Rani *et al.*, 2017; Mohammed *et al*., 2019; Al-kafaween *et al.*, 2021).

Conclusion: The results of this study have shown that the protein and carbohydrate content of the Nigerian honey studied was higher than that of the European standard, while its moisture and hydroxymethylfurfural content was lower than that of the European standards. It also further showed that the Nigerian honey studied had high levels of saponins, phenol and terpenoids, moderate levels of alkaloids and steroids, and low levels of flavonoids, and its administration to rats led to a significantly ($p < 0.05$) higher body weights, TWBC, neutrophils and RBC counts in a dose dependent manner. Also, the rat groups treated with honey showed significantly ($p < 0.05$) higher antibody titre when compared with untreated rats. All these taken together, suggests that the Nigerian honey used for the study is nutritionally rich and has beneficial bioactive phytochemicals that translated to improved health status in the rats used for the study.

Conflict of Interests

The authors declare no conflict of interest.

References

Abeshu MA, and Geleta B. (2016) Medicinal uses of honey. *Biology and Medicine*, *8*(2). https://doi.org/10.4172/0974-8369.1000276

Akinwande KL Badejo MA and Ogbogu SS (2013). Morphometrics and parasitic load of Varroa mites (Acari : Varroidea) on colonies of *Apis mellifera adansonii* in Southwest Nigeria. *Acarina*, 21(1): 17 – 25.

Ajibola A (2016) Physico-Chemical and Physiological Values of Honey and Its Importance as a Functional Food. *International Journal of Food and Nutritional Science*, *2*(6): 1 – 9. https://doi.org/10.15436/2377-0619.15.040

Al-Kafaween MA, Alwahsh M, Mohd Hilmi AB, and Abulebdah DH (2023) Physicochemical characteristics and bioactive compounds of different types of honey and their biological and therapeutic properties: A comprehensive review. *Antibiotics (Basel, Switzerland)*, *12*(2). https://doi.org/10.3390/antibiotics12020337

Al-Kafaween MA, Hilmi ABM and Al-Jamal HAN (2021). The Beneficial Effects of Stingless Bee Kelulut Honey against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. *Tropical Journal of Natural Products Research, 5*: 1788 – 1796.

AOAC (1995) Official Methods of Analysis. 16th ed., Association of Official Analytical Chemists, Washington DC.

Aween MM, Hassan Z, Muhialdin BJ, Noor HM and Eljamel YA (2012). Evaluation on antibacterial activity of *Lactobacillus acidophilus* strains isolated from honey. *American Journal of Applied Science,* 9: 807 – 817.

Beard, C.W. (1980) Isolation and Identification of Avian Pathogens. 2nd Edition, American Association of Avian Pathologists, pp. 129 – 135.

Bogdanov S, Jurendic T, Sieber R, and Gallmann P (2008) Honey for nutrition and health: a review. *Journal of the American College of Nutrition*, *27*(6): 677 – 689. https://doi.org/10.1080/07315724.2008.10719745

Brodschneider R, Gray A, Adjlane N, Ballis A, Brusbardis V, Charrière JD, Chlebo R, Coffey MF, Dahle B, de Graaf DC, Maja Dražić M, Evans G, Fedoriak M, Forsythe I, Gregorc A, Grzęda U, Hetzroni A, Kauko L, Kristiansen P, and Danihlík J (2018) Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. *Journal of Apicultural Research*, *57*(3), 452–457. https://doi.org/10.1080/00218839.2018.1460911

Crane E. Bee Research Association (1975). *Honey: a comprehensive survey*, p. 608.

El Sohaimy SA, Masry S, and Shehata M (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Science*, 60: 279–287.

Elmer P (2016) UV/Visible Spectroscopy Determination of Hydroxymethylfurfural (HMF) in honey using the LAMBDA spectrophotometer. *PerkinElmer, Inc.*, https://resources.perkinelmer.com

Eteraf-Oskouei T and Najafi M (2013). Traditional and modern uses of natural honey in human diseases: A review. *Iranian Journal of Basic Medical Sciences*, *16*(6), 731.

Harborne, J.B. (1973) Phytochemical Methods. Chapman and Hall Ltd., London, 49 – 188.

Hossen MS, Ali MY, Jahurul M, Abdel-Daim MM, Gan SH and Khalil MI (2017). Beneficial roles of honey polyphenols against some human degenerative diseases: A review. *Pharmacological Reports,* 69: 1194 – 1205.

Ibarguren C, Raya RR, Apella MC and Audisio MC (2010). Enterococcus faecium isolated from honey synthesized bacteriocin-like substances active against different Listeria monocytogenes strains. *Journal of Microbiology,* 48: 44 – 52.

Jiang Z, Kempinski C and Chappell J (2016). Extraction and analysis of terpenes/terpenoids. *Current Protocols in Plant Biology,* 1: 345 – 358.

Khalil MI and Sulaiman SA (2010). The potential role of honey and its polyphenols in preventing heart diseases: A review. *African Journal of Traditional, Complementary and Alternative Medicines*, *7*(4): 315 – 321. https://doi.org/10.4314/AJTCAM.V7I4.56693

Khalil M, Alam N, Moniruzzaman M, Sulaiman S and Gan S (2011). Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science,* 76: C921 – C928.

Kishore RK, Halim AS, Syazana MN and Sirajudeen K (2011). Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutrition Research,* 31: 322 – 325.

Kirk RS and Sawyer R (1998). Pearson's composition and composition analysis of foods. 9thed. Churchill Livingstone, Edinburgh, pp. 615 – 616.

Krell R (1996) Value-added products from beekeeping. *FAO Agriculture Services Bulletin*, 16.

Mandal MD and Mandal S (2011) Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(2), 154.

McMenamin AJ and Genersch E (2015) Honeybee colony losses and associated viruses. *Current Opinion in Insect Science*, *8*: 121 – 129. https://doi.org/10.1016/J.COIS.2015.01.015

Mohammed SEA, Kabbashi AS, Koko WS, Ansari MJ, Adgaba N and Al-Ghamdi A (2019). *In vitro* activity of some natural honeys against *Entamoeba histolytica* and *Giardia lamblia* trophozoites. *Saudi Journal of Biological Sciences,* 26: 238 – 243.

Okeke CL and Elekwa I (2003). A phytochemical study of the extract of *Gongronema latifolium* Benth. (Ascalepiadaceae). *Journal of Health and Visual Sciences, 5*.

Onwuka GI (2005). Food Analysis and Instrumentation: Theory and Practice. Naphthali Print, Lagos, pp. 133 – 137.

Osato MS, Reddy SG and Graham DY (1999) Osmotic effect of honey on growth and viability of *Helicobacter pylori*. *Digestive Diseases and Sciences*, *44*(3): 462 – 464. https://doi.org/10.1023/A:1026676517213

Pasupuleti VR, Sammugam L, Ramesh N, and Gan SH (2017) Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. *Oxidative Medicine and Cellular Longevity*, *2017*: 1259510. https://doi.org/10.1155/2017/1259510

Pavlova T, Stamatovska V, Kalevska T, Dimov I, Assistant G and Nakov G (2018). Quality characteristics of honey: A review. *Proceedings of the University of Ruse, 57*: 31 – 37.

Pearson DA (1976) Chemical Analysis of Foods. *Churchill Livingstone, New York, 1: 422 – 511.* http://www.sciepub.com/reference/157376

Ramos-Cuellar AK, De la Mora A, Contreras-Escareño F, Morfin N, Tapia-González JM, Macías-Macías JO, Petukhova T, Correa-Benítez A and Guzman-Novoa E (2022). Genotype, but not climate, affects the resistance of honeybees (*Apis mellifera*) to viral infections and to the mite *Varroa destructor*. *Veterinary Sciences*, *9*(7). https://doi.org/10.3390/VETSCI9070358/S1

Ranneh Y, Akim AM, Hamid HA, Khazaai H, Fadel A, Zakaria ZA, Albujja M and Bakar MFA (2021). Honey and its nutritional and anti-inflammatory value. *BMC Complementary Medicine and Therapies, 21*(1): 1 – 17. https://doi.org/10.1186/S12906-020-03170-5

Rani GN, Budumuru R and Bandaru NR (2017). Antimicrobial activity of honey with special reference to methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA). *Journal of Clinical and Diagnostic Research,* 11: DC05.

Schlam OW, Jain NC and Carol EI (1975). Veterinary Haematology, 3rd Ed. Lea and Febiger, Philadelphia, pp. 144 – 167.

Stockham SL and Scott MA (2008). Fundamentals of Veterinary Clinical Pathology, 2nd Edition, Blackwell Publishing, Ames, pp. 61 – 64.

Thrall MA and Weiser MG (2002). Hematology. In: Hendrix, C.M., Ed., Laboratory Procedures for Veterinary Technicians, 4th Edition, Mosby Inc., Missouri, pp. 29 – 74.

Villegas P. and Purchase H.G. (1989). Titration of Biological Suspension. In: *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*, USA Kendal Hunt American Association of Avian Pathologists, Iowa, pp. 186 – 190.

Wang Q, Cai WJ, Yu L, Ding J and Feng YQ (2017) Comprehensive profiling of phytohormones in honey by sequential liquid-liquid extraction coupled with liquid chromatography – mass spectrometry. *Journal of Agricultural and Food Chemistry*, *65*(3): 575 – 585. https://doi.org/10.1021/ACS.JAFC.6B04234/SUPPL_FILE/JF6B04234_SI_001.PDF

Weiss D. J. and Wardrop K. J. (2010). *Schalm's Veterinary Hematology*, 6th Edition, Wiley-Blackwell, Iowa.

Williams S (1986). Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA. *Journal of Chromatography*, 351: 608 – 609.

Zulkifli MF, Radzi MNFM, Saludes JP, Dalisay DS and Ismail WIW (2022) Potential of Natural Honey in Controlling Obesity and its Related Complications. *Journal of Evidence-Based Integrative Medicine*, *27*. https://doi.org/10.1177/2515690X221103304