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Beebread and Honey improves some Haematologic Parameters in Male Albino Mice

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ABSTRACT

The effects of honey and beebread on some haematological parameters were investigated in fifteen apparently healthy adult albino male mice. The mice were divided into three groups of five mice each after a two-week period of acclimatization. The mice in group A were given distilled water orally to serve as a control while those in groups B and C were administered honey and beebread respectively by gavages at the dose rate of 1g/kg body weight and at a concentration of 40% w/v using water as a vehicle, once daily for 70 consecutive days. Micro-haematocrit and hematocytometry were used for determination of packed cell volume and erythrocyte and differential leucocyte counts, respectively. Haemoglobin concentration was estimated by using Cyanmethemoglobin method. There were significant (p <0.05) increases in the red blood cells count, packed cell volume and haemoglobin concentration in the treatment groups compared to the control group, with the most increase observed in the group treated with beebread. There was significant (p < 0.05) increase in total leucocyte count in the treated groups with the highest count recorded in the honey treated group than the control. Lymphocytes and neutrophils significantly (p < 0.05) increased in the treatment groups but there were no significant (P > 0.05) changes in the number of eosinophils, basophils and monocytes. The study demonstrates the positive haematopoietic effects of supplementation of feed with honey and beebread.

Keywords: Beebread; Erythrocytes; Honey; Leucocytes; Mice

INTRODUCTION

Honey varies in physical and chemical properties depending on the water content, type of flora used for its production (pasturage), temperature, the season and production methods of the bees (Joseph *et al.*, 2007). Yaghoobi *et al.* (2008) had observed that due to the complexity and unusual nature of honey, variations occur in the composition and its characteristics especially as it relates to the geographical and botanical origin of the nectar.

Daily consumption of honey could affect biochemical and haematological parameters depending on the concentration (Haro *et al.*, 2000). A previous study showed that haematologic parameters; haemoglobin concentration, packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and platelets count increased after the administration of honey in rats (Ekanem and Yusuf, 2007).

Honey has also been reported to stimulate the immune system by increasing monocytes and neutrophils (Kassim *et al.*, 2012). A decrease in prostaglandin levels, elevated nitric oxide production and increased levels of lymphocytes, platelets, serum protein, albumin and copper were reported in a 40year-old woman living with a long history of acquired

immune deficiency syndrome (AIDS) after treatment with 80g of natural honey daily for 21 days (Al-Waili, 2003).

Beebread is a product of the hive obtained from pollen collected by bees, to which they add honey and digestive enzymes and is subsequently stored in the combs. The bees transform the bee pollen into beebread by an anaerobic fermentation process. Like honey, the physical and chemical composition of bee bread depends on the soil, season, farm practice and geographical location (Mohammad *et al.*, 2020).

Honey contains a variety of biological and pharmacological properties that vary depending on its origin and processing. These compounds include vitamins, flavonoids, antioxidants as well as hydrogen peroxide (H_2O_2) (Muhammad *et al.*, 2012). Honey and beebread from different parts have been reported to have different characteristics, pollen digestibility and chrysin concentration. This study was conducted to establish if honey and beebread farmed in Askira Uba, Borno state of Nigeria have any haematological effect.

MATERIALS AND METHODS

Animals

Fifteen apparently healthy adult male mice purchased from the University of Jos, Plateau State, Nigeria were used for this study. The mice were kept for two weeks to acclimatize at the Animal House of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri. The mice were kept in plastic cages, fed with pelletized commercial feed (Vital Feed[®]) and water ad libitum daily. The mice were divided into three groups of five mice each: A, B and C and given distilled water, honey, and beebread by gavages respectively, once daily at the dose rate of 1g/kg body weight at the concentration of 40% w/v using water as a vehicle for a duration of 70 consecutive days.

Blood Collection and Preservation

At the end of the study, blood samples were collected from the jugular veins in labeled plastic tubes (Silver Health Diagnostics, Nigeria) containing EDTA. The samples were transported on ice (without direct contact) to the laboratory and analyzed within 2 hours of sample collection.

Source and Storage of Honey and Beebread

Honey and beebread were sourced directly from a bee farm in Askira Uba local government area of Borno State. They were separately put in tightly sealed glass jars and transported to Maiduguri within 12 hours. The jars were kept in a cool dry cabinet throughout the study period.

Determination of Packed Cell Volume, Erythrocyte and Leucocyte Counts and Haemoglobin Concentration

Packed cell volume (PCV), erythrocyte (RBC) and leucocyte counts (WBC) were determined for each blood sample using micro-haematocrit and haematocytometric methods, respectively (Schalm *et al.*, 2011). Haemoglobin concentration was determined using Cyanmethemoglobin method (by using spectrophotometer) (Gheldof *et al.*, 2002).

Statistical Analyses

Data obtained were summarized as means \pm standard deviations (SD). Differences between means were analyzed using analysis of variance (ANOVA; One way) followed by Tukey's post-hoc test and p< 0.05 was considered as statistically significant. Statistical analyses were done using computer software, GraphPad InStat[®] (2018).

Ethical Statement

All animals were handled according to the International Guiding Principle for Biomedical Research Involving Animals (CIOMS, 1985).

RESULTS AND DISCUSSION

Red Blood Cell Count, Haemoglobin Concentration and Packed Cell Volume

The result for the effect of honey and beebread on RBC count is presented in Table 1. There were significant increases (p < 0.05) in the red blood cell count in the experimental groups (Honey: 7.53 ± 018 ; Beebread: 8.79 ± 0.63) compared to the control (6.08 ± 0.68). The highest increase (p < 0.05) was recorded in the beebread treated group (8.79 ± 0.63).

There was a significant increase (p< 0.05) in the haemoglobin concentration of treated groups with that of the beebread treatment group (17.12 ± 0.16) being higher than

that of the honey treatment group (15.04 \pm 0.29), when compared with the control group (13.58 \pm 0.38) as presented in Table 1.

The result for PCV is presented in Table 1. There was a significant increase (p < 0.05) in the PCV of treated groups when compared with the control group.

The highest percentage was observed in the beebread treated group (43.40 \pm 0.55) and a moderately higher percentage was recorded in the honey treated group (41.20 \pm 0.84) when compared with the lowest percentage recorded in the control group (40.80 \pm 1.10).

Beebread has been reported to have an ability to increase serum iron, zinc and magnesium levels and other trace elements in blood (Al-Waili *et al.*, 2006) and has a high antioxidant activity which confers protection on the erythrocyte membrane (Baltrusaityte *et al.*, 2007; Bakour *et al.*, 2017). Honey also contains iron and copper in addition to zinc and magnesium. Iron is very necessary for haemoglobin synthesis where it binds with protoporphyrin to form the haeme molecule a necessity for effective erythropoiesis (Al-Waili, 2003; Singh, 2018). Beebread has been shown to improve the digestive absorption of iron (Attia and Elnaggar, 2014). The iron present in beebread and honey could have attributed to the increased RBC, PCV and haemoglobin concentration observed.

Zinc, a constituent of beebread and honey stimulates the activity of vitamins that are involved in the formation of red blood cells (Fukushima *et al.*, 2009; Roohani *et al.*, 2013; Chen *et al.*, 2018). Zinc also enhances the synthesis of haemoglobin as well as erythropoietin (Konomi *et al.*, 2005; Baranauskas *et al.*, 2020) thereby improving erythropoiesis (Chen *et al.*, 2018). Zinc has been reported to have antioxidant properties which conferred protection against lysis in erythrocytes. The immunity of patients suffering from viral infections was boosted by zinc (Name *et al.*, 2021). Reduced haemolysis and increased erythropoiesis because of treatment with beebread or honey may have raised the population of erythrocytes in circulation in the mice studied (Name *et al.*, 2021).

Magnesium, another mineral contained in beebread and honey improves erythropoiesis, increases reticulocytosis, reduces oxidative stress and plays an active role in improving the immune system (Son *et al.*, 2007). Magnesium activates vitamin D needed for proliferation of burst-forming unit-erythroid and erythroid progenitor cells. Magnesium also activates many enzymes in the body, is essential for the stability of cell function and cell repair and maintains the antioxidant status of cells. The integrity of erythrocyte membrane is maintained by magnesium. Beebread and honey probably enhanced the process of erythropoiesis and reduced the susceptibility of erythrocytes to lysis and eryptosis (Son *et al.*, 2007).

Honey and beebread contain chrysin a flavonoid that improves absorption of vitamin B_{12} and iron. Chrysin may be responsible for increased erythropoiesis observed as elevations of erythrocyte count, packed cell volume and haemoglobin concentration. The antioxidant property of chrysin (Samarghandian *et al.*, 2019) may have improved erythrocyte membrane stability. Blasa *et al.* (2007) reported that honey flavonoids protected red blood cells from oxidative damage by interacting with the red blood cell membrane. These flavonoids donate electrons to the membrane oxidoreductase to efficiently reduce extracellular oxidants (Mara *et al.*, 2006). Phenolic, a flavonoid is also responsible for antioxidant behavior of honey (Jaromir *et al.*, 2009) and bee bread (Vilma *et al.*, 2007).

The treatment with beebread and honey might have protected blood cells from pathological consequences of free radical induced membrane lipid peroxidation such as increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity (Bhalchandra *et al.*, 2018) because they contain flavonoids, such as quercetin and rutin, which exert strong activity against oxidative damage and inflammatory response (Zuluaga *et al.*, 2015).

Total Leucocyte and Differential Leucocyte Counts

There was a significant increase (p< 0.05) in the leucocyte count of treated groups as compared to the control group (7.34 ± 0.74) with the highest count observed in the beebread treatment group (9.67 ± 0.42) as shown in Table 2.

The effect of honey and beebread on lymphocytes is presented in Table 2. Statistically significant (p < 0.05) differences were observed in lymphocyte count in the control group (5.18 ± 0.10) as compared to the experimental groups (Honey: 6.52 ± 0.40 ; Beebread: 6.54 ± 0.56).

Statistically significant (p < 0.05) differences were recorded in the neutrophil count of beebread and honey treated groups as compared to control. The highest count was in the beebread treated group (3.38 \pm 0.10) while moderately higher count was recorded in the honey treated group (3.06 \pm 0.00) when compared with the lowest in the control group (2.16 \pm 0.12) as presented in Table 2.

There were no statistically significant (p>0.05) differences in the number of eosinophils, basophils and monocytes when the control and treatment groups were compared as shown in Table 2.

The increase seen in total leucocyte count, lymphocyte, and neutrophil count after treatment with beebread could be attributed to the immunomodulatory properties of the flavonoid chrysin (Zeinali *et al.*, 2017; Salimi and Pourahmad, 2018) contained in beebread and honey (Wali *et al.*, 2020). Prolonged administration of honey and beebread in male mice revealed that there were increases in red and total white blood cell counts, packed cell volume and haemoglobin concentration in the treatment groups in comparison to the control group. The group of mice treated with beebread displayed the most significant increase in these haematological parameters indicative of a greater concentration, utilization and or absorption of these constituents than in honey.

Table 1: Effects of honey and beebread on red blood cell count, packed cell volume and haemoglobin concentration of male albino mice (Mean \pm SD; n=5)

	Treatment groups		
Haematological parameters	А	В	С
	(Distilled water)	(Honey 1g/kg)	(Bee Bread 1g/kg)
Red blood cell count ($\times 10^{12}/L$)	$6.08\pm0.68^{\rm a}$	7.53 ± 0.18^{b}	$8.79\pm0.63^{\circ}$
Haemoglobin concentration (g/dl)	$13.58\pm0.38^{\rm a}$	$15.04\pm0.29^{\text{b}}$	$17.12 \pm 0.16^{\circ}$
Packed cell volume (%)	$40.80 \pm 1.10^{\text{a}}$	41.20 ± 0.84^{b}	$43.40\pm0.55^{\circ}$
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a,b,c Means ± Standard deviations with different superscripts along rows are significantly (p<0.05) different

	Treatment groups		
White Blood Cells	А	В	С
	(Distilled water)	(Honey 1g/kg)	(Bee Bread 1g/kg)
Total Leucocyte count (×10 ³ /µl)	$7.34\pm0.74^{\rm a}$	$8.75\pm0.49^{\rm b}$	$9.67\pm0.42^{\rm b}$
Lymphocyte ($\times 10^{3}/\mu l$)	$5.18\pm0.10^{\mathrm{a}}$	6.52 ± 0.40^{b}	6.54 ± 0.56^{b}
Neutrophil (×10 ³ / μ l)	$2.16\pm0.12^{\rm a}$	3.06 ± 0.00^{b}	$3.38\pm0.10^{\circ}$
Eosinophil (× $10^3/\mu$ l)	$0.02\pm0.55^{\rm a}$	$0.02\pm0.45^{\rm a}$	$0.02\pm0.55^{\rm a}$
Basophil (×10 ³ / μ l)	$0.03\pm0.00^{\mathrm{a}}$	$0.03\pm0.00^{\mathrm{a}}$	$0.03\pm0.00^{\mathrm{a}}$
Monocyte (× $10^3/\mu$ l)	$0.09\pm0.00^{\mathrm{a}}$	$0.10\pm0.00^{\mathrm{a}}$	$0.10\pm0.00^{\mathrm{a}}$

a,b,c Means \pm Standard deviations with different superscripts along rows are significantly (p<0.05) different

Chicks fed 0.6% bee-pollen (beebread) diet were found to have the highest relative weights of thymus, bursa and spleen and the highest values for packed cell volume, haemoglobin concentration, red blood cells, white blood cells, neutrophils and lymphocytes when compared with the control group. Thus, suggesting that, the supplementation of bee-pollen to the diets of broiler chicks improved their performance, carcass traits and blood parameters (Farag and El-Rayes, 2016). Other studies have shown that treatment with honey increased RBC, WBC, PCV and HB in male albino rats (Berene *et al.*, 2015; Bhalchandra *et al.*, 2018). Increased PCV, mean corpuscular haemoglobin and WBC was reported by Muhammad *et al.* (2012). Bakour *et al.* (2022) demonstrated the antioxidant effects of beebread extracts in ameliorating oxidative stress and their ability to inhibit alpha amylase and Angiotensin I-converting enzymes in vitro.

The increased values of all the haematological parameters reported in this study fell within the normal reference value for these species (Ihedioha *et al.*, 2012) suggestive that the values only increased in comparison to mice not treated with beebread or honey and not due to stress or any related pathology.

Conclusion

The study demonstrated some of the positive haematopoietic effects of honey and beebread farmed in Uba, Borno state Nigeria.

Acknowledgement

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors' Contribution

INA contributed to designing, analysis of data, drafting and final approval of the work. OIJ and KG contributed to analysing and interpretation of data, drafting and final approval of the work. All authors have read and approved the final manuscript.

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