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Research Article

Assessment study of some hematological indices in cholesterol-induced hyperlipidemic rabbits treated with Nigerian honey

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ABSTRACT

Background: Honey, a product of honeybees is a natural and viscous liquid that is said to be a highly nutritive. It varies in physical and chemical properties depending on the temperature, method of cultivation and other elements. It was the aim of this study to assess possible changes in some hematological indices in cholesterol-induced hyperlipidemic rabbits fed with varying doses of Nigerian honey. **Methods:** Twenty-five female rabbits were randomly separated into 5 groups (A-E) with 5 animals per group. Groups A-D animals were fed on high cholesterol diet (HCD) orally daily for a period of 2 weeks, to induce hyperlipidemia; Groups A-C animals were fed daily with 10ml/kg, 5ml/kg and 1ml/kg body weight of honey respectively for another period of 2 weeks after hyperlipidemia had been observed. Group E served as control. Blood sample was collected from the marginal ear vein of the animals at baseline, after cholesterol induction and after honey administration. Results were expressed as mean ± standard error of mean and P-value ≤ 0.05 was considered as statistically significant. **Results:** With a high cholesterol diet (HCD), Red blood cell (RBC) count and Packed cell volume (PCV) were significantly decreased ($P \leq 0.05$) while platelet count was increased ($P \leq 0.001$). Honey administration for 2 weeks produced a significant increase in PCV ($P \leq 0.01$) and platelet count ($P \leq 0.001$). **Conclusions:** An increase in the rate of hematopoiesis mat be the mechanism through which honey increased blood parameters. The overall effect of honey on blood count as seen in rabbits if applicable to man may be a good remedy for the management of anemia.

INTRODUCTION

Honey is a viscous liquid, naturally sweet and is known to be a highly nutritive product made by honeybees. It is known that honey serves as source of food for the honeybees during cold weather or especially when fresh food sources are scarce (Molan, 1992; Manyi-Loh, *et al.,* 2011). It is well documented that honey possesses medicinal properties at both preventive and curative levels (Singh *et al.,* 2012). It has been demonstrated that honey varies in physical and chemical properties depending on the water content, type of flora used for its production (pasturage), temperature, quantity of specific sugars used during the manufacturing, the season and production methods of the bees. It has also been observed that due to the complexity and unusual nature of the honey, variations occur in the composition and its characteristics especially as it relates to the geographical and botanical origin of the nectar (Joseph *et al.,* 2007; Yaghoobi *et al.,* 2008). It is a well-known fact that honey has a long shelf fife as compared to most foods.

Honey is very well cultivated all over Nigeria and is also known to have a wide range of medicinal and other applications. In earlier times, local farmers harvested honey from the wild. Presently, apiculture has become a growing industry in many parts of Nigeria as well as in many other parts of the continent (Adesunkanmi and Oyelami, 1994). Most of the major constituents of honey have been determined and described as: sugar (fructose 30%, glucose 31%) and 17% water. Other constituents are: sucrose (1%), maltose (7%), melezitose, phenolic, flavonoids, terpenses, and hydrogen peroxide (Molan, 1992). In addition, honey has also been shown to contain acids, vitamins, proteins and minerals like phosphorous, calcium, magnesium

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(White, 1980) and the enzymes Diastase, Invertase and Glucose Oxidase (Bogdanov *et al.,* 2008). The pure honey was noted to contain alkaloids, auterquinone glycosides, cardiac glycosides, flavonoids and reducing compounds (Rakhi *et al.,* 2010). Other important components of honey that have been noted to act as antioxidants include: α-tocopherol, ascorbic acid, vitamins, organic acid, flavonoids and phenolic enzymes (Galal *et al.,* 2012). On the whole, honey has a glycemic index which ranges from 31 to 78 depending on the variety, and a density of about 1.36 kilograms per liter (36% denser than water). The pH of honey is commonly noted to be between 3.2 and 4.5.

There have been detailed phytochemical studies of the Nigerian honey. Results of these studies revealed that the Nigerian honey constitutes; 17.9% water, 28.3% glucose, 38.9% fructose, 4.4% maltose, 1.6% sucrose, 0.2% nitrogen and 8.5±2.7 mg/kg hydroxymethylfurfural, a pH of 3.9, 15mm^2/s viscosity and a total acidity of 21.5±5.6 meq/kg (Fasasi, 2012).

It has been noted that the constituents of blood may change in relation to physiological conditions of health and also that the constituents of blood are reliable indicators of the physiological status of the individual animal (Khan and Zafar, 2005). Thus, studies based on hematological parameters generally prove useful in the diagnosis of most diseases and in most cases, provide useful information that are beneficial to workers in investigative to ascertain the damage (or potential damage) to the blood (Onyeyili et al., 1992; Etim et al., 2014). Thus, the plasma and formed cellular elements of blood including the platelets (thrombocytes), have been the main area of focus by researchers in the past engaged in studies based on blood chemistry as a tool in diagnostic procedures.

A number of studies have shown that honey may have ameliorative effects on the symptoms of *Trypanosomosis brucei* in rats with trypanosome infection (Ekanem and Yusuf, 2007). Ameliorative effects in blood parameters in rats such as hemoglobin concentration, PCV, RBC, WBC and platelets count was also reported after the administration of honey (Ekanem and Yusuf, 2007). Honey has also been reported to stimulate the immune system; monocytes and neutrophils (Kassim *et al.,* 2012). In one other study conducted on a 40 year old woman living with a long history of Acquired Immune Deficiency Virus (AIDS) and treated with 80g of natural honey daily for 21 days, a decrease in prostaglandin levels, elevated nitric oxide production and increased levels of lymphocytes, platelets, serum protein, albumin and copper were reported (Al Waili et al., 2006). A daily treatment with natural raw honey for 3 months in a 30-year old HIV positive man also showed an elevated CD4, CD8, platelet, lymphocyte, neutrophil, red blood cell and white blood cell counts and hemoglobin levels (Heidari et al., 2012). These and several other similar reports are documented evidence that natural raw honey may have some clearly definable physiological mechanisms to improve the immune systems and functions in HIV positive patients.

Studies have also shown that honey possesses anti-inflammatory effects on both acute and chronic inflammations in rats (Owoyele *et al.,* 2011), anti-leukemic activity (Abubakar *et al.,* 2012), and anti-cancer properties (Fernandez-Cabezudo *et al.,* 2013).

It was the aim of this study to assess possible changes in some hematological indices in cholesterol-induced hiperlipidemic rabbits fed with varying doses of Nigerian honey.

METHODS

Nigerian honey was obtained from a farm, in Esan Central Local Government Area of Edo State, Nigeria. The indigenous honey marketed locally was certified as natural and pure.

A total of 25 female rabbits used in this study were divided into five groups of five animals per group. The animals were allowed to acclimatize for a period of two weeks, with standard feeds and clean water throughout the period of study. Groups A, B, C and D were the test animals, while group E animals served as the control group. All the animals, except those of control group were daily fed on high cholesterol diet (HCD) orally for a period of 2 weeks, to induce hyperlipidemia according to the method of Dhulasavant *et al.* (2010). Groups A, B and C rabbits received daily dose of 10ml/kg, 5ml/kg and 1ml/kg of honey, respectively, for another 2 weeks after hyperlipidemia had been confirmed. The animals were fasted prior to the administration of cholesterol and honey, to allow for a food-free bowel. The administration of cholesterol and honey was carried out using orogastric tube to ensure that the dose content allotted for each animal was taken fully.

Blood samples were collected at baseline, at week 2 (after the cholesterol induction) and at week 4 (after treatment with honey) from the marginal ear vein of the rabbit after overnight fasting. The ear vein of the animals was dilated using xylene and cotton wool to allow easy access to the veins. The samples were analyzed for RBC count, PCV, Total WBC count, WBC differential and platelet counts using an auto-analyser. All results were expressed as mean ± standard error of mean, and one-way analysis of variance as well as post hoc tests using the SPSS (statistical package, version 16.0), and P-value of $\leq$ 0.05 was regarded as statistically significant.

RESULTS:

All the animals fed daily on high cholesterol diet (HCD) orally for a period of 2 weeks developed

Table 1: Mean values of some hematological parameters at baseline, after cholesterol induction (week 2) and after treatment with honey (week 4) of normal and hyperlipidemic rabbits.

Parameters		Group A	Group B	Group C	Group D	Group E (Control)
Red Blood Cell Count (X 10^6\ul)	Baseline	5.54 ± 0.15	5.12 ± 0.28	5.91 ± 0.31	5.79 ± 0.14	5.19 ± 0.26
	2 Weeks	4.30 ± 0.23*	4.19 ± 0.14*	4.46 ± 0.32*	4.43 ± 0.32*	5.49 ± 0.18
	4 Weeks	5.08 ± 0.45	5.33 ± 0.44	5.32 ± 0.40	4.26 ± 0.27	5.26 ± 0.10
Packed Cell Volume (%)	Baseline	31.42 ± 1.93	31.38 ± 0.75	32.34 ± 1.26	32.06 ± 0.88	30.60 ± 0.76
	2 Weeks	26.30 ± 1.19***	23.64 ± 0.96***	26.46 ± 0.50***	27.58 ± 0.91***	31.58 ± 0.92
	4 Weeks	35.04 ± 1.76	34.31 ± 1.35	35.84 ± 1.03	29.06 ± 1.23**	31.68 ± 0.89
Platelet Count (X 10^3\ul)	Baseline	268.80 ± 13.55	296.20 ± 23.84	303.60 ± 13.19	270.60 ±22.60	296.80 ± 10.16
	2 Weeks	445.60 ± 13.21***	488.40 ± 31.60***	453.60 ± 19.64***	448.60 ± 11.37***	282.80 ± 8.52
	4 Weeks	454.60 ± 10.53***	482.20 ± 3.88***	466.80 ± 14.72***	384.60 ± 9.20	292.00 ± 16.39
White Blood Cell Count (X 10^3\ul)	Baseline	7.26 ± 0.73	7.01 ± 0.59	7.28 ± 0.50	7.00 ± 0.63	6.81 ± 0.38
	2 Weeks	5.74 ± 0.30	5.74 ± 0.21	5.94 ± 0.26	6.01 ± 0.17	6.61 ± 0.41
	4 Weeks	7.12 ± 0.11	6.65 ± 0.38	5.97 ± 0.55	5.67 ± 0.33	6.76 ± 0.43
Neutrophils (X 10^3\ul)	Baseline	4.70 ± 0.54	4.37 ± 0.44	4.72 ± 0.62	4.28 ± 0.62	4.02 ± 0.46
	2 Weeks	3.30 ± 0.27	3.38 ± 0.16	3.62 ± 0.30	3.56 ± 0.13	3.90 ± 0.10
	4 Weeks	4.26 ± 0.23	3.65 ± 0.31	3.32 ± 0.51	3.06 ± 0.24	4.04 ± 0.32
Eosinophils (X 10^3\ul)	Baseline	0.15 ± 0.02	0.14 ± 0.03	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.02
	2 Weeks	0.14 ± 0.01	0.14 ± 0.04	0.16 ± 0.04	0.18 ± 0.01	0.16 ± 0.03
	4 Weeks	0.15 ± 0.00	0.16 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.16 ± 0.02
Basophils (X 10^3\ul)	Baseline	0.15 ± 0.02	0.14 ± 0.03	0.15 ± 0.02	0.16 ± 0.02	0.15 ± 0.01
	2 Weeks	0.15 ± 0.02	0.15 ± 0.04	0.16 ± 0.04	0.17 ± 0.02	0.14 ± 0.01
	4 Weeks	0.15 ± 0.01	0.18 ± 0.03	0.15 ± 0.02	0.17 ± 0.02	0.15 ± 0.02
Monocytes (X 10^3\ul)	Baseline	0.16 ± 0.02	0.16 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.15 ± 0.02
	2 Weeks	0.19 ± 0.02	0.16 ± 0.02	0.18 ± 0.04	0.20 ± 0.01	0.16 ± 0.02
	4 Weeks	0.19 ± 0.00	0.20 ± 0.03	0.19 ± 0.04	0.22 ± 0.03	0.15 ± 0.02
Lymphocytes (X 10^3\ul)	Baseline	2.10 ± 0.29	2.20 ± 0.23	2.06 ± 0.14	2.24 ± 0.19	2.34 ± 0.10
	2 Weeks	1.96 ± 0.26	1.90 ± 0.21	1.82 ± 0.31	1.90 ± 0.26	2.24 ± 0.34
	4 Weeks	2.37 ± 0.22	2.46 ± 0.34	2.14 ± 0.17	1.82 ± 0.31	2.26 ± 0.19

Values are Means ± SEM asterisks denote statistically-significant differences (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$) compared to controls (n=5).

hyperlipidemia according as reported by Dhulasavant *et al.* (2010). Significant alterations in haematological parameters (RBC count, PCV, Total WBC count, WBC differential and platelet counts) were observed following administration of different doses of honey to the hyperlipidemic rabbits, as shown in Table 1.

DISCUSSION

This work was carried out to evaluate the possible effect of natural honey on some hematological parameters. Cholesterol, a core part of all cell membrane, is an essential molecule without which all living beings would not exist. The highest

concentrations of cholesterol are found in breast milk, where it is essential for infant nourishment and brain development. Cholesterol helps in the healing and repair of blood vessels. Blood vessels have a delicate lining and in the event of turbulence, the lining becomes damaged and has to be repaired (Bhavana, 2004).

Result obtained from this study as shown in Table 1 reveals that the administration of HCD significantly reduced RBC count ($P \leq 0.001$). PCV was reduced after a 2-week HCD administration ($P \leq 0.001$) and honey administration increased PCV in groups A-C ($P \leq 0.01$) but not dose dependent. These results are in agreement with that of Fiorani et al. (2006) and Aliyu et al. (2012). Platelet count showed a significant increase after HCD ($P \leq 0.001$) and this increase was sustained with 2 weeks administration of honey ($P \leq 0.001$) as compared to control. This is therefore in agreement with the study by Aliyu et al. (2012) and El-Rabey (2013). The increase in platelet count after cholesterol induction could be linked to the fact that an increase in cholesterol concentration in the body culminates into the formation of plagues.

Hypercholesterolemia has been shown from studies to play a role in the pathogenesis of deep vein thrombosis (Kawasaki et al., 1997). Findings have also shown that in patients with atherosclerosis, activation of both platelets and blood coagulation and an increase in fibrin turnover are detectable which may lead to thrombotic complications (Holvoet and Collen, 1997; FitzGerald et al., 1997).

WBC differentials (neutrophil, eosinophil, basophil and monocyte) were found to remain within normal range with the administration of either HCD or honey. Lymphocyte count was observed to increase after 2 weeks of HCD administration and a reduction after 2 weeks of although these observations were not statistically significant. Increase in lymphocyte count after two weeks of honey administration shows its possible effect in immunity as stated earlier by Al-Waili et al. (2006) and Heidari et al. (2012).

Some of the constituents of honey like folic acid and other vitamins are hematinics and as such they help in hemopoiesis. This may be the cause of the increased count as observed in this study. Vitamin C has been linked from previous studies to be involved in lymphocyte proliferation (Shaik-Dasthaqirisaheb et al., 2013).

CONCLUSION

It is possible that the mechanism by which honey increased these blood parameters in this study was through an increase in the rate of hematopoiesis. This is likely in view of the presence blood forming agents normally present in honey. The overall effect of honey on blood parameters in rabbits reported in this study, is of potential benefit in the management of anaemia, thereby boosting immunity, as well as understanding the pathogenesis of ischemic heart diseases caused by hyperlipidemia

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