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Full Length Research Paper

Effects of honey on sialidase activities in blood and liver of adult wistar rats

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Honey is a sweet viscous fluid produced by bees. Honey which contains mainly fructose and glucose is either taken as curative agent or substitute for refined sugar, yet its chronic effect on blood and liver sialic acid levels have not been reported. This study reports an investigation into the sialic acid levels in liver, hemoglobin-free erythrocytes and plasma in adult Wistar rats exposed to chronic consumption of honey. Twenty adult Wistar rats, between 170 and 200 grams in weight were divided into four groups of five rats each. The rats were fed daily with 0%, 20%, 30%, and 40% of honey mixed with 100, 80, 70 and 60 grams of animal chow in groups I, II, III and IV, respectively for eight weeks. The Wistar rats were then sacrificed and blood and liver tissue samples were collected for sialic acid analyses. The sialic acid levels in the plasma free, haemoglobin-free erythrocytes and liver free sialic acid levels in groups II, III and IV induced by honey consumption showed statistically significant differences ($p<0.05$) when compared with the control group I. Chronic consumption of honey may increase the risk of hepatic damage.

Keywords: Erythrocytes, Honey, Liver, Sialic acid, Sialidase.

INTRODUCTION

Honey is a sweet and viscous fluid produced by honey bees (and some other species), and derived from the nectar of flowers. With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%), making it similar to the synthetically produced inverted sugar syrup which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates (Riddle, 2001).

Honey contains trace amounts of several vitamins and minerals (Standifer, 2007). Typical honey analysis shows the following : Fructose: 38.0% , Glucose: 31.0% , Sucrose: 1.0%, Water: 17.0%, Other sugars (maltose, melezitose): 9.0%, Ash: 0.17%, Other: 3.38% (Erguder, *et al.,* 2008).

Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Martos, *et al.*, 2000).

Honey provides antibacterial, anti-inflammatory, immune-stimulant, antiulcer and wound/burn healing (regenerative) effects (Fiorani, *et al.*, 2006). Free radicals lead to oxidative damage in many molecules, such as lipids, proteins and nucleic acids. Antioxidant foods that are rich in flavonoids are protective agents against ailments such as atherosclerosis, aging, and cancerous diseases because oxidative damage (Perez, *et al***.,** 2006).

Antioxidants in honey have also been implicated in reducing the damage done to the colon in colitis (Bilsel, *et al*., 2002). "Natural" unprocessed honey and honey from farmers who may have a small number of hives are more toxic. Commercial processing, with pooling of honey from numerous sources generally dilutes any toxins (Walderhaug, 2001). **]**. Antibacterial properties of honey are the result of the low water activity causing osmosis, hydrogen peroxide effect (Wahdan, 1998).

Sialic acid is a derivative of a nine-carbon monosaccharide and is widely distributed throughout human and animal tissues and found in several fluids, including serum, cerebrospinal fluid, saliva, urine, amniotic fluid, and mother's milk. It is the negative charge of this ubiquitous chemical that is responsible for the slippery feel of saliva and mucins coating the body's organs. As terminal sugars of an animal's cellular glycocalyx, sialic acid is one of the first substances a microbe "sees" when entering a host. As a regulator of

innate defense mechanisms, host sialic acid is an environmental substance to be copied or eaten up (Schauer, 1985).

Sialidases are believed to act as virulence factors, allowing successful competition with the host by alleviating their spread in host tissues (Godoy, *et al*., 1993). One of the effects of sialidase overflow is anaemia: red blood cells are covered by a dense coat of sialic acid molecules that is removed by this dysregulated sialidase action. As a consequence, the galactose residues are demasked on the red blood cell surface after removal of sialic acid, presenting a signal for degradation by liver hepatocytes (Schauer, 1985). Due to the resulting lack of negative charge on the erythrocyte surface, the cells tend to aggregate, which leads to thrombosis (Muller, 1974a).

Aim: Since honey is widely used irrespective of the type, the aim was to study its effect on liver bound and free sialic acid levels, haemoglobin-free erythrocytes (ghosts) and plasma free sialic acid levels of adult Wistar rats after consumption of honey for eight weeks. The study was approved by the Ethics and Research Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka. Nigeria.

MATERIALS AND METHODS

Honey sample: The honey used was purchased from A and Shine International Limited, Abuja, Nigeria (undiluted, no artificial flavours or colours, no preservatives added) with National Agency for Food, Drugs, Administration and Control of Nigeria (NAFDAC) Registration No. 01-6025.

Animals

Twenty adult wistar rats, between 170 and 200 grams in weight were used for this study. They were divided into four groups of five rats each. Daily consumption of 0%, 20%, 30%, and 40% of honey were mixed with 100, 80, 70 and 60 grams of animal chow were used in feeding the rats in groups I, II, III and IV respectively for eight weeks. The wistar rats were sacrificed; blood and liver tissue samples were collected for sialic acid analysis. 1.0g of liver tissue collected was homogenized with 1ml of deionised water for determination of both free and bound liver sialic acid levels. Haemoglobin-free erythrocytes membranes (ghosts) sialic acid which represents the bound and plasma free sialic acid levels were measured using the thiobarbituric (TBA) assay (Aminoff 1961). Haemoglobin - free erythrocyte membranes were prepared as previously described (Dodge, et al., 1963).

Brief description of Preparation of Haemoglobin-Free Erythrocyte Membranes (Ghosts) according to Dodge et al (1963). 0.1ml of Acid Citrate Dextrose (ACD) was dispensed into a 5 ml capacity vacutainer to collect 0.3 ml of blood from mice. The erythrocytes were washed by suspending them in isotonic phosphate buffer, 310 ideal milliosmola (imOsm) at 1,500 x g for 20 minutes and the plasma/buffy coats were removed by aspiration using Pasteur pipette. The erythocytes were properly washed several times with isotonic phosphate buffer, 310 ideal milliosmola (imOsm), pH 7.4 until a completely clear supernatant was obtained, while erythrocytes settled at the bottom. The washed erythrocytes were immediately haemolysed by adding 3ml of hypotonic phosphate buffer (20imOsm, pH 7.4) to the washed erythrocytes and centrifuged at 20,000 xg for 40 minutes. The supernatant was decanted and ghosts cells were washed several times with the hypotonic phosphate buffer (20imOsm, pH 7.4) until they were clear, free of haemoglobin and whitish like wool.

Statistical analysis

SigmaStat 2.0 (Systat Software, Inc. Point Richmond, CA) and Microsoft excel were used for statistical analysis, graphs and charts. One way Analysis of variance (ANOVA) and All Pairwise Multiple Comparison Procedure – Turkey Test were used to evaluate the sialic acid levels of each group of mice as a result of the toxic effect of fluoride. Probability level of ≤0.05 was considered significant.

RESULTS

The results obtained are shown on figures I-IV.

A. Haemoglobin-free erythrocyte membranes (ghosts) Sialic acid

The mean values of sialic acid as contained in figure 1 in groups I (control), II, III and IV are 2.717 ± 0.291, 3.308 ± 0.150, 3.065 ± 0.0479 and 2.277 ± 0.191 respectively.

There was an increase of sialic acid level in group II (20% of honey). Thereafter, the levels decreased in groups III, and IV (30% and 40% respectively) when compared with the control group I (0% of honey). Specifically, there was statistically significant difference between groups II and IV ($p<0.05$).

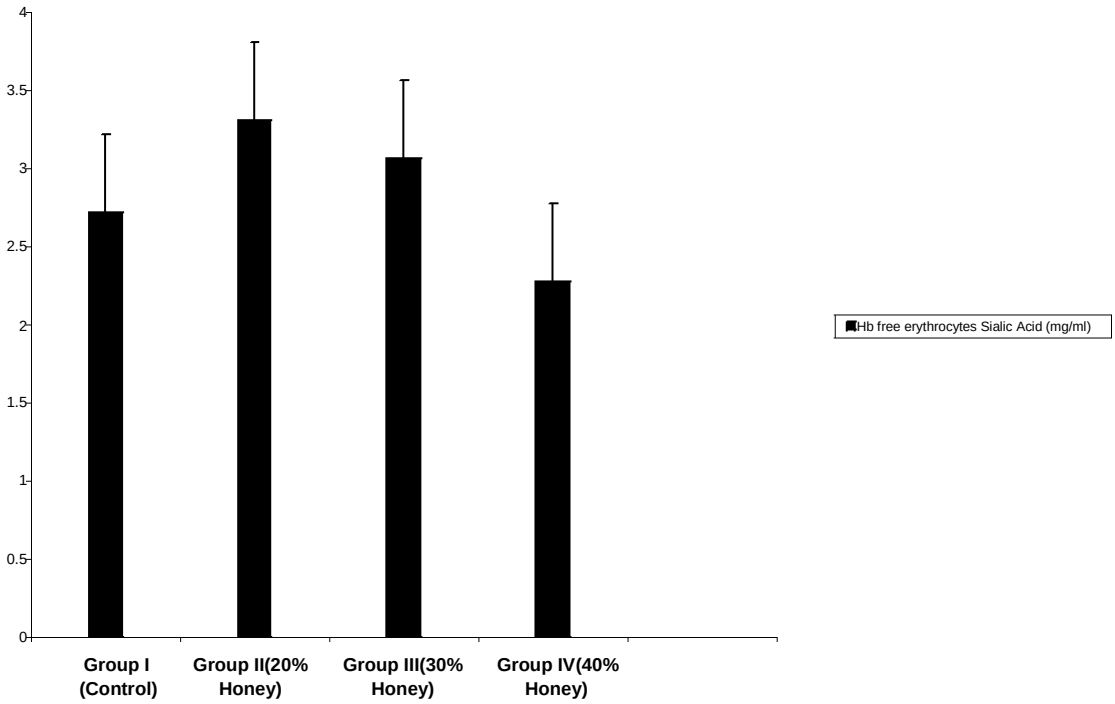

Figure 1, Sialic Acid of Haemoglobin--Free Erythrocyte Membranes of wistar rats after oral consumption of honey for 8 weeks.
Note: The increased activity of the enzyme sialidase is noted in groups III – IV. P= 0.013

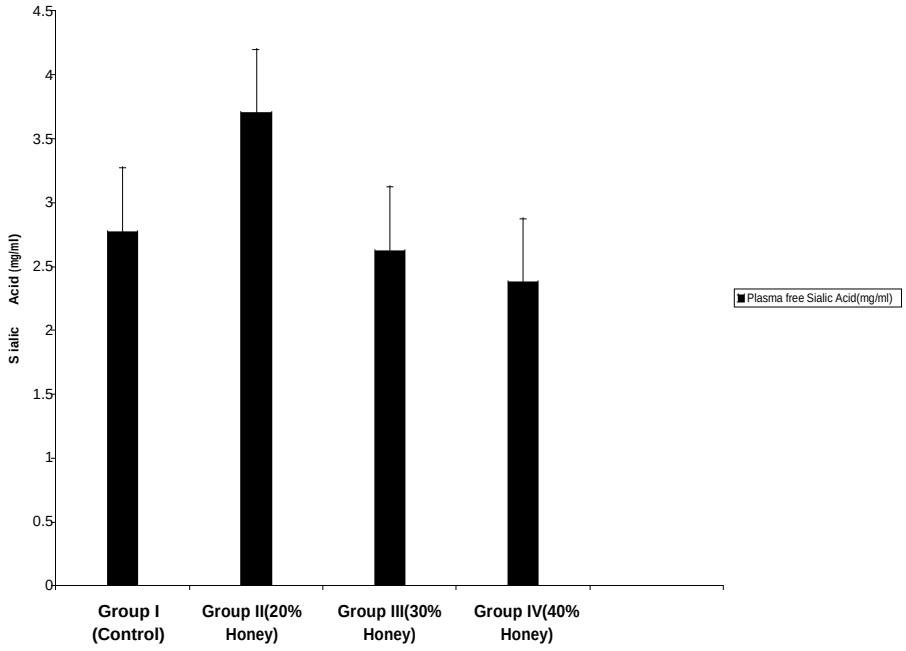

Figure 2. Plasma free sialic acid of wistar rats after oral consumption of honey for 8 weeks.
Note: The increased activity of the enzyme sialidase is noted in groups III – IV. P < 0.001.

B. **Plasma free sialic acid**

The mean values of plasma free sialic acid as contained in figure 2 in groups I (control), II, III and IV are 2.770 ± 0.293, 3.700 ± 0.0453, 2.623 ± 0.0832 and 2.377 ± 0.136 respectively.

There was an increase in sialic acid level in the group II (20% of honey). But sialic acid levels decreased in groups III and IV (30%and 40% of honey respectively) when compared with the control group. Specifically, there

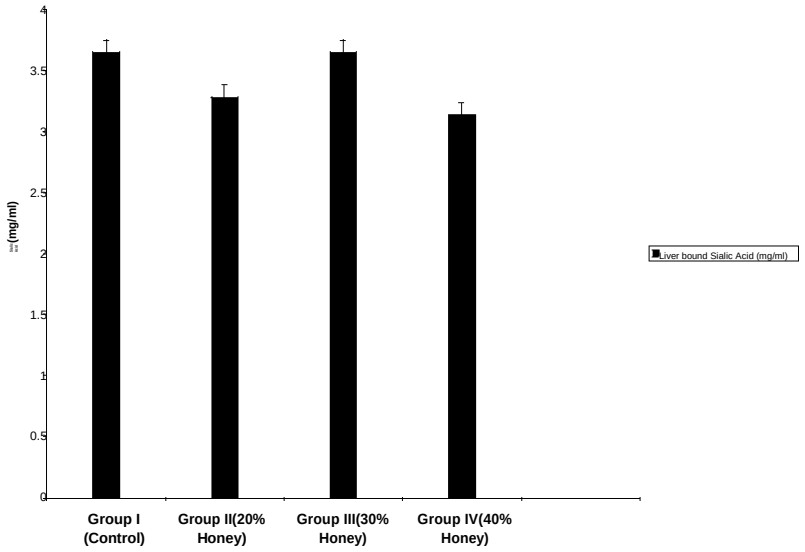

Figure 3. Liver bound sialic acid of wistar rats after oral consumption of honey for 8 weeks.
Note: There was no significant difference in the activity of the enzyme sialidase. P = 0.714

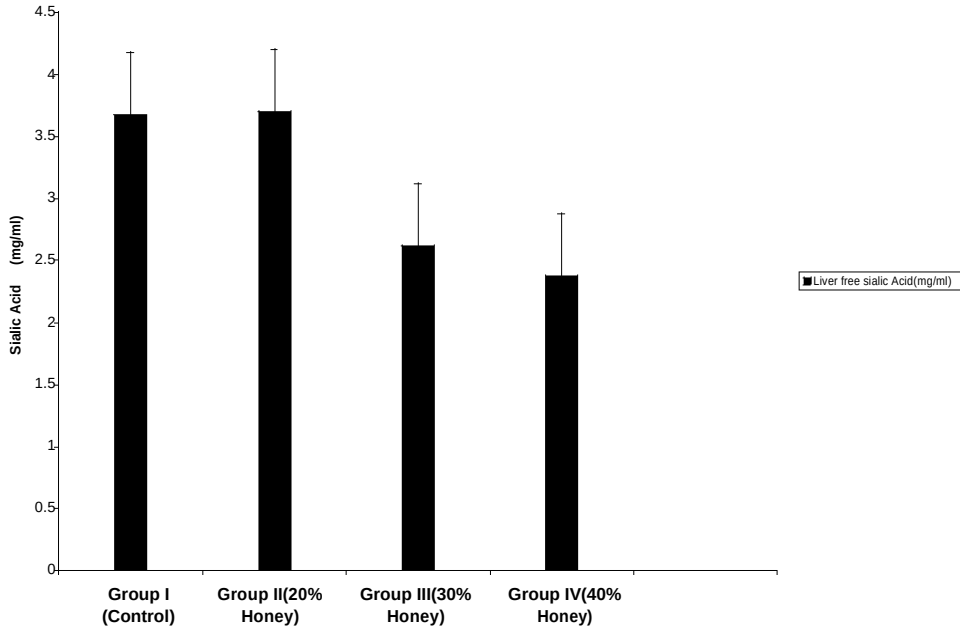

Figure 4. Liver free sialic acid of wistar rats after oral consumption of honey for 8 weeks.
Note: The increased activity of the enzyme sialidase was noted in groups III – IV. P < 0.001.

was statistically significant difference between groups I and II, II and III, II and IV ($p<0.05$).

C. Liver Bound sialic acid

The mean values of sialic acid contained in figure 3 in groups I (control), II, III and IV are 3.653 ± 0.464, 3.283 ± 0.396, 3.650 ± 0.0453 and 3.138 ± 0.468 respectively.

There was no statistically significant difference between the experimental groups II, III and IV that had 20%, 30% and 40% of honey when compared with the control group I that had 0% of honey.

D. Liver free sialic acid

The mean values of liver free sialic acid as contained in figure 4 in groups I (control), II, III and IV were 3.675 ±.207, 3.700 ± 0.0453, 2.623 ± 0.0832 and 2.377 ± 0.136

0 respectively.

Sialic acid levels decreased in groups III and IV (30%and 40% of honey, respectively) when compared with the control group I that had 0% of honey. Specifically, there was statistically significant difference between groups I and III, I and IV, II and III, II and IV ($p < 0.05$)

DISCUSSION

Sialic acid levels of haemoglobin-free erythrocytes (bound or ghosts cells) and liver free sialic acid of group II that had 20% of honey increased, indicating a decreased activity of the enzyme sialidase. The plasma free sialic acid and liver free sialic acid levels decreased in groups III and IV (30% and 40% of honey) respectively, showing a high activity of the enzyme sialidase, which cleaves the circulating sialic acid from the plasma and liver respectively.

The liver bound sialic acid levels was not affected may probably be due to the process of resialylation by sialyltransferase, the enzyme that attaches sialic acid to the liver, although, this was not investigated.

In an earlier histological study, we reported distortion of the radial arrangement of the sinusoids from the central vein, the distortion of the hexagonal shape of the hepatocytes with evidence of hepatic necrosis characterized by karyolytic and karyorrhexic cells and the desquamation of the wall of the central vein of the liver may be due to the cleaving of sialic acid by the enzyme sialidase from the haemoglobin-free erythrocytes, plasma and the liver, thus exposing the liver to the damage noticed when rats were exposed to chronic consumption of honey (Wilson et al., 2011).

The duration of consumption of honey and the dose may play a key role in the outcome of the results. In this study, oral consumption of honey for eight weeks showed statistically significant differences in the sialic acid levels in the plasma, haemoglobin-free erythrocytes and liver between the experimental groups ($p < 0.001$, $p= 0.013$ and $p < 0.001$) respectively. The activity of the enzyme sialidase was dose dependent.

CONCLUSION

Chronic use of honey may increase the risk of hepatic damage especially at higher doses. I am recommending that investigations should be carried out on the effect of honey on the liver at lower doses to establish the beneficial effects in order not to expose the liver and blood to unnecessary risk and damage.

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