Non-Alcoholic Fatty Liver Disease Following Administration of Unprocessed Nigerian Honey

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Abstract: Honey is a thick, viscous and sweet liquid made by bees from the nectar of flowers, transformed and stored in the honeycombs. Nectar is almost 80% water with some complex sugars, such as fructose (about 38.5%) and glucose (about 31.0%). Others include maltose, sucrose, other complex carbohydrates, trace amounts of vitamins and minerals. Twenty male albino rats were used for the experiment. The rats were randomly divided into 4 groups of 5 each and tested as follows:-Group O (Control) – water orally, Group A – 0.1ml/kg of unprocessed honey orally, Group B – 0.15ml/kg of unprocessed honey orally, Group C – 0.2ml/kg of unprocessed honey orally. The unprocessed honey was given once a day for forty two days (six weeks). At the end of the treatment; all the rats were sacrificed, the liver was dissected for histological examination and blood was taken for biochemical analysis. There were infiltrations of fat cells in the liver tissue at all doses which increased according to the order of administration. AST was elevated and was statistically different from the control. Honey which is a good food supplement may turn out to be harmful to health when abused or adulterated with other sweeter substances containing large amount of fructose.

Key word: Honey • Liver • Fatty • Biochemistry • Histopathology

INTRODUCTION

Honey is a thick, viscous and sweet liquid [1] made by bees from the nectar of flowers [2], transformed and stored in the honeycombs. Nectar is almost 80% water with some complex sugars [3], such as fructose (about 38.5%) and glucose (about 31.0%). Others include maltose, sucrose and some complex carbohydrates [4]. Trace amounts of vitamins and minerals can also be found in honey [5-7].

Honey is considered as medicine [3, 8, 9] and has a long history in traditional medical systems. It was used by the ancient Greeks, Sumerians and Egyptians [3, 10, 11]. Reports show that honey is not only used as a dietary supplement but also effective for treating wound infections [12-14] and post-radiotherapies mucosal trauma [15]. It is an antibacterial, anti-inflammatory, immune-stimulant, antiulcer [9, 16-18], anti-fungi [19] and an antioxidant [20]. Honey increases antibody titre against T-dependent and T-independent antigens during primary and secondary immune responses [21] and stimulates proliferation of B and T lymphocytes in cell cultures. It also stimulates monocytes to release cytokines, which activate immune responses [22, 23]. In addition, honey shows antitumor and antimetastatic effect and potentiates the antitumor effects of cytotoxic drugs [24]. Hippocrates recommended honey and vinegar for pain, a mixture of honey, water and other substances to treat acute fevers, as well as recommending its use to treat ulcers [25]. It can also be used in treating diarrhea [26] as well as a preservative for herbal medicines [27].

The use of honey for medicinal purpose cuts across a wide range of diseases and ailments globally; there have been little or no information on the constituents of unprocessed Nigerian honey. Hence the aim of this research was to evaluate the effect of unprocessed Nigerian honey on the liver of Wistar rats.
MATERIALS AND METHODS

Honey Collection: Unprocessed honey was bought in the month of January, 2012 in Wilberforce Island Bayelsa State, Nigeria. The honey was identified and confirmed by a honey trader in Amassoma community in Bayelsa State.

Animals: Twenty male Wistar albino rats were used for the experiment. They were obtained from the animal house of College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria. The rats were grouped into 4 consisting of 5 animals each and maintained under standard laboratory conditions of 27±2°C, relative humidity 50±15% and normal photo period (12h dark/12h light) [28] and were supplied with standard pellet food with tap water. All rats received human care according to the criteria outlined in the "Guide for care and use of laboratory animals" prepared by the National Academy of Science and published by The National Institutes of Health.

Experimental Design: Twenty male Wistar albino rats were used for the experiment. The rats were randomly divided into 4 groups of 5 each and fed with honey as follows:

Group O (Control) - water orally
Group A - 0.1ml/kg of unprocessed honey orally
Group B - 0.15ml/kg of unprocessed honey orally
Group C - 0.2ml/kg of unprocessed honey orally

The unprocessed honey was given once a day for forty two days (six weeks). At the end of the treatment; all the rats were sacrificed, the liver was dissected for histological examination and blood was taken for biochemical analysis.

Biochemical Analysis: Blood was obtained from the rats through cardiac puncture and allowed to clot. Serum samples were extracted by centrifuging the clotted blood at 3000g for 10 min. The serum samples were analyzed for Total protein (TP), Albumin (ALB), Aspartate transaminase (AST), Alanine transaminase (ALT) using automated Medical analyzer.

Histopathology: Immediately after dissection, the sections of the liver were placed in a tissue cassette and fixed in 10% buffered formalin for 24 h after which they were processed using standard histopathological methods. The processed tissues were then embedded in paraffin.

Sections of 5 μm thickness were cut on a rotary microtome and stained with haematoxylin and eosin for microscopic assessment [29].

Statistical Analysis: Values were represented as mean±SD. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparison Test using GraphPadInstat® software.

RESULTS

The gross anatomy of the liver showed milk colour protruded surfaces, making that portion of the liver more enlarged.

Histologically, there were infiltrations of fat cells in the liver tissue at all doses which increased according to the order of administration. Slides 1-4.

In parameters such as ALT, Albumin and Total protein were not statistically different from the control. Meanwhile AST was elevated and was statistically different from the control. Table 1.

Slide 1: Control group Liver
CV= Central vein, SS= Sinusoids, H= Hepatocyte

Slide 2: (Group A) Liver (0.1ml/kg). HV= Hepatic vein, HA= Hepatic artery.
There are infiltration of fat cells (FC), Sinusoidal closure and loss of tissue architecture.
Table 1: Biochemical analysis of rats administered with unprocessed honey for 42 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>0.1ml/kg</th>
<th>0.15ml/kg</th>
<th>0.2ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>67±8.63</td>
<td>58.3±3.27</td>
<td>65±3.80</td>
<td>73.5±4.71</td>
</tr>
<tr>
<td>AST</td>
<td>154.5±4.0</td>
<td>171±1.50</td>
<td>182.4±22.00</td>
<td>203±17.80***</td>
</tr>
<tr>
<td>Albumin</td>
<td>40.5±1.80</td>
<td>40.5±3.35</td>
<td>41.5±1.50</td>
<td>42.5±1.80</td>
</tr>
<tr>
<td>Total protein</td>
<td>139.5±25.12</td>
<td>118.5±25.12</td>
<td>139.5±1.11</td>
<td>145.5±8.84</td>
</tr>
</tbody>
</table>

Each value represents the mean±standard deviation (n = 5), values are statistically different from control at p< 0.05* and 0.001*** one way analysis of variance (ANOVA) + Tukey Multiple Comparison Test, using GraphPadInstat® software

Slide 3: Group B Liver (0.15ml/kg). There is intense infiltration of fat cells (FC), Sinusoidal closure, loss of radial arrangement of sinusoids from the central vein of the liver, leading to general loss of tissue architecture.

Slide 4: Group C Liver (0.2ml/kg)
Heavy infiltration of fat cells scattered all over the parenchymal of the liver, closure of both sinusoids as well as vessels of the liver by fat cells. The infiltration of fat cells is an evidence of non-alcoholic fatty disease

DISCUSSION

Honey is a food supplement and medicinal product for many and it is from a natural source, but unfortunately this has been adulterated by some honey farmers and business merchants in the quest to make more money, by adding substances such as hot water, sugar, sugar cane extract heated to high temperature etc.

In humans and rats there is no conversion of fructose to glucose in the intestine during absorption due to lack of converting enzymes, unlike other species. That is why fructose (in fruits and honey) was initially thought to be advisable for patients with diabetes due to its low glycemic index (it is about 23% compared to 100% in glucose) [30, 31].

Fructose is extracted by the liver from the portal circulation via glucose transporters-5 (GLUT5) in an insulin-independent mechanism. Fructose is immediately converted into pyruvate and lactate that enter the mitochondria, increasing acetyl CoA and lipogenesis. This causes shift in the balance from oxidation to esterification of non esterified fatty acids resulting in increased lipogenesis [31, 32]. In contrast to glucose, fructose in the liver can’t be stored as glycogen and only stored as lipid. This characteristic makes fructose a highly lipogenic nutrient [33].

It was interesting to identify that an unprocessed honey, widely and regularly distributed and consumed caused infiltration of fat cells in the liver tissue in all the doses administered, slides 1-5. This finding agrees with Bataa et al. [30] where fructose was used to induce non-alcoholic fatty liver disease and also with Wilson et al. [1] who reported that chronic use of honey may increase the risk of hepatic damage especially at higher doses. The findings also agree with Botezelli, et al., St. George et al. and Basciano, et al. [32-34]. Infiltration of fat cells is a key pointer of a disease known as Non-alcoholic Fatty Liver Disease (NAFLD) [35].

Aminotransferases are commonly used markers of hepatocyte injury. AST is present in blood cells and many tissues, including liver, muscle, brain, pancreas and lung [36]. AST elevations often predominate in patients with cirrhosis and even in liver diseases [37]. Our findings revealed elevation of serum level of aspartate aminotransferase (AST) in the group administered with 0.15ml/kg and 0.2ml/kg when compared with the control, which is the most common and often the only laboratory abnormality found in patients with non alcoholic fatty liver disease [38].
CONCLUSION

Honey which is a good food [39] supplement and medicine may turn out to be dangerous when abused or adulterated with other sweeter substances containing large amounts of fructose. Honey contains 38.5% of fructose [4], as such more fructose should not be added to it since it has been reported that fructose is the key cause of non-alcoholic fatty liver disease [35]. Therefore, there should be reduced intake of food or any substance with high content of fructose. Regulatory bodies in charge of food and other substances should also check the level of fructose in products before it is approved for use.

REFERENCES


