Research Article

The ameliorating effects of honey on some biochemical parameters on rats exposed to cyanide

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ABSTRACT: In this study, the ameliorating effect of honey on some biochemical parameters on rats exposed to cyanide (CN) was studied. Significant decreases in urea, creatinine and transaminases (AST and ALT) and alkaline phosphatase (ALP) activity were observed in all the groups treated with honey after exposure to cyanide when compared with the untreated group administered cyanide only. In addition, there was no significant difference in these parameters in the honey treated group when compared with the group given sodium thiosulphate (a known cyanide antidote) after exposure to cyanide. A significant decrease was also indicated in the superoxide Dismutase (SOD) and catalase activities in the liver and kidney of rats treated with honey when compared with the untreated group. A significant decrease in LDL-cholesterol and triglyceride levels and a significant increase (p<0.05) in HDL-cholesterol were observed in the honey treated rats when compared with the untreated group. The overall results indicate that honey acts as a good antidote to cyanide poisoning and is able to ameliorate the biochemical effects caused by exposing rats to cyanide.

KEYWORDS: Cyanide, honey, oxidative stress, lipid profile, transaminases

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INTRODUCTION

Cyanide is a known poisonous compound and has been indicated to induce oxidative stress in organisms (Okolie and Osagie 2000). Cyanide exist naturally as most plants and animals use them as their source of nitrogen and defense system (Moller, 2010). The primary effect of cyanide poisoning is impairment of oxidative phosphorylation (a process that involves the utilization of oxygen for the production of essential energy sources, which is mostly in the form of ATP (Ilona, et al., 2015). Ruminant animals are more susceptible to cyanide poisoning or exposure by cyanogenic plants than horses and pigs, since the enzymes that destroys hydrogen cyanide are found in the gastric cavity (Moller, 2010). The first signs of cyanide exposure include increased and deep breathing, which is as a result of the inhibition of cytochrome oxidase, loss of eye coloration, gasping for air due to the anoxic nature of the body, respiratory arrest and finally death (Ilona et al. 2015). The subjects (animals or humans) may last for only a couple of minutes before been found dead upon chronic exposure to cyanide (Brenan, et al, 1999). Several antidotes have equally been discovered in cyanide toxicity. Amongst which is the very common sodium thiosulphate, sodium nitrite, hydroxocobalamin (Vitamin B₁₂), etc.

Honey is a common sweetener of foods and sugar-sweetened beverages. It consists of two main sugars, which are glucose and fructose (Taherah and Moslem, 2013). Honey contains several biochemical and microbial constituents including glucose oxidase, flavonoids, peroxides, etc., which are known for their therapeutic roles (Hanna and Shynaa, 2011). The colour, mineral contents, flavors and vitamin constituent of honey depends mainly on the floral portion of the plant from which the honey bees gather their nectar. In modern medicine, honey is used in the treatment of several health conditions such as skin ulcers, respiratory and gastrointestinal conditions, dandruff, eczema etc. (Alvarez et al., 2010). Studies have shown that honey contains anti-oxidative properties which enable it to carry out its many functions (Yaghoobi et al, 2008).

It has been discovered from previous studies that increase in the consumption of honey also decreases serum triglycerides and low-density lipoprotein but increases high-density lipoprotein (HDL) (Mohammed et al., 2012). Hyperlipidemia is a common risk factor in the increment of most metabolic disease conditions including atherosclerosis, obesity, etc. HDL has been shown to reduce inflammation, protect oxidation of low-density lipoprotein as well as decreasing atherosclerosis accumulation in the arterial walls (Sirtori, 2006). Obesity-related disorders especially visceral adiposity is a common metabolic condition caused by increase in low density lipoprotein (LDL), in the blood. Taking cognizance of the toxic effect of cyanide it is therefore pertinent for us to determine the ameliorating effect of the components of honey on cyanide exposed rats

MATERIALS AND METHODS

Experimental Animals

A total of 28 male Wister rats, weighing between 120 g to 150 g were used for this study. They were acclimatized for 7 days, fed with pelletized mouse chow and water ad libitum. After the acclimatization period, the 28 wister rats, were divided into 4 groups each containing 7 rats: Group 1 (Control), given food and water only; Groups 2 – 4 were exposed to 9 mg CN/kg b.w in 100 ml of water (0.09mg CN/ml) daily. Potassium cyanide salt was used as the source of cyanide. In addition, rats in Group 3 were given honey (5 ml in 10 ml of drinking water) three times weekly. Group 4 were also injected with 0.25 ml of sodium thiosulphate three times weekly. The treatment was carried out for four weeks. All the animals were handled in accordance with the principles of laboratory animal care as documented in National Institute of Health (NIH) for laboratory animal welfare (National research council, 1996).

Biochemical Assays

Urea estimation was carried out using Urease-Berthelot Method (Weatherburn, 1967). Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Bethelot’s reaction.
Determination of serum aspartate aminotransferase and alanine aminotransferase was by the method of Reitman and Frankel, (1957) and determination of serum albumin was by the method of Doumas et al., (1971).

Triglycerides (trigs) level was determined according to the method of Buccolo and David (1973). Total cholesterol was assayed according to the method of Roeschlaub et al., (1974). Since serum LDL-C cannot be determined quantitatively it was calculated using the Friedewald equation:

$$LDL-C \text{ mg/dl} = \text{Total Cholesterol} – \text{HDL} – \left(\frac{\text{Triglycerides}}{5}\right)$$

Assay for superoxide dismutase (SOD) activity was by the method of Misra (1972). The method of Kaplan (1972) was adopted for catalase determination, while lipid peroxidation was determined by the method of Guteridge and Wilking (1982).

**Statistical Analysis**

The data obtained were subjected to statistical analysis using analysis of variance (ANOVA) and Least Significance Test. The level of significance used was $p< 0.05$.

**RESULTS AND DISCUSSION**

This study was carried out to determine the ameliorating effects of honey on some biochemical parameters in rats exposed to cyanide. Cyanide has been shown to be toxic to the liver resulting in leakage of its enzymes into the serum. AST and ALT are usually found in high amounts in the liver where they play important role in the metabolism of amino acid (Whitehead et al., 1999). However, damage or toxicity to the liver, leads to the leakage of these enzymes from the liver into the circulation where their levels become elevated (Whitehead et al., 1999, Harris, 2005).

In this study, significant increase in AST, ALT and ALP was indicated in all the groups exposed to cyanide when compared with the control animals (Group 1) that were not exposed to cyanide (Table 1). However, significant decrease in these parameters was indicated in the honey treated (Group 3) when compared with Group 2 that were not treated. Also, there was no significant difference in the AST, ALT, and ALP activities in the honey treated groups when compared with the group treated with sodium thiosulphate, a known cyanide antidote. Therefore, the elevated levels of AST and ALT in serum of rats maintained on cyanide alone suggest liver damage. High concentrations of these enzymes in the serum are indicative of liver injury or damage (Erejuwa et al., 2012). The study also showed an increased activity of ALP in serum of the rats maintained on cyanide alone. This is not surprising as hepato-cellular injury or damage is also expected given the fact that the liver is the main site of cyanide excretion (Shepherd, 2008). The present study goes to confirm previous studies that indicates the antioxidant activity of honey and its ability to reduce the values of the two transaminases and ALP in the serum (Al-Waili et al., 2006).

This study also examined the ameliorating effect of honey on some liver and kidney marker enzymes in rats exposed to cyanide. Albumin and total protein levels was significantly reduced ($P>0.05$) in rats exposed to cyanide only when compared with the control (Table 2). However, the groups treated with honey gave no significant difference when compared with the control and the group given a known cyanide antidote. Liver synthesizes plasma protein (Ganong, 2006) and lower protein level has been reported in compromised liver functions (Bass, 2003). Also, a decrease in the serum albumin and total protein have been proven to indicate kidney damage, resulting from the renal inability and its subsequent excretion in the urine (Albuminuria) (Vasilenko and Grebenev, 1990). Urea and creatinine levels were also significantly increased in the groups exposed to cyanide only when compared with control indicating a renal damage, however the groups treated with honey gave no significant difference when compared with the control. Urea is produced in the kidney as a bi-product of protein metabolism and creatinine which is used as an index of glomerular function (Treasure 2003)is also excreted in the kidney. Therefore, an increase in urea and creatinine in the blood has been indicated as indices for kidney damage (Muhammad et al 2013). The present study therefore indicates that honey helps to prevent damage to the liver and the kidney as a result of cyanide exposure.
The present study also indicated a significant increase in lipid peroxidation in Group 2 rats exposed to cyanide only without treatment when compared to the control (Table 3). However, the Group 3 rats treated with honey after cyanide exposure indicted no significant difference when compared with the control group and group 4 rats treated with a known cyanide antidote. Honey efficiently scavenged ROS generated in the tissue.

The increase in MDA level is an indication of cyanide induced oxidative stress. Oxidative stress referred to a reduction in the antioxidant defense system in cells due to an increase in ROS production in cell and tissues. Studies have shown that cyanide induces oxidative stress by increasing reactive oxygen species and nitric oxide (Gunasekar et al., 1996; Mills et al., 1996; Daya et al., 2002) as well as inhibition of antioxidant systems (Ardelt et al., 1989). Elevated levels of reactive oxygen species initiate lipid peroxidation (Frei, 1994; Val and Almeida-Val, 1999) that culminates in oxidative stress in tissues (Halliwell, 1989; 1994; Liu and Mori, 1994). The effect of reduced antioxidant capacity due to cyanide toxicity was reversed in group treated with honey and also compared favorably with those treated with sodium thiosulphate a known cyanide antidote. Honey efficiently scavenged ROS generated in the tissue.
Table 3: The effect of honey on the level of lipid peroxidation (MDA), superoxide dismutase (SOD) and catalase activity in the Liver and Kidney of rats exposed to cyanide

<table>
<thead>
<tr>
<th>Parameter / Group</th>
<th>Liver MDA</th>
<th>Kidney MDA</th>
<th>Liver SOD</th>
<th>Kidney SOD</th>
<th>Liver Catalase</th>
<th>Kidney Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1 (-Cyanide; CN)</td>
<td>1.01±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.4±1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.32±6.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.33±5.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.43±5.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP 2 (+Cyanide; CN)</td>
<td>7.49±1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.73±0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.53±2.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.45±2.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.28±2.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.42±1.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP 3 (CN + Honey)</td>
<td>1.10±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42±0.19&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>64.07±3.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.52±3.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.53±1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.43±1.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP 4 (Sodium Thiosulphate)</td>
<td>0.86±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03±0.31&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>63.18±10.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.30±3.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.48±11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.78±11.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean ± S. D, n=7. Values not sharing a common superscript on the same column differs significantly at (P< 0.05).

Table 4: The effects of honey on the lipid profile of rats exposed to cyanide toxicity

<table>
<thead>
<tr>
<th>Parameter / Group</th>
<th>Total Cholesterol</th>
<th>HDL-Cholesterol</th>
<th>LDL-Cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1 (-CN)</td>
<td>160.38±3.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.23±8.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.08±9.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210.38±10.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP 2 (+CN)</td>
<td>185.28±7.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.30±4.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.87±2.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>290.57±16.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP 3 (CN+Honey)</td>
<td>160.44±18.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.38±11.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.39±46.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263.32±8.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP 4 (Sodium Thiosulphate)</td>
<td>158.33±14.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.14±3.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.59±34.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>257.45±19.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean ± S. D, n=7. Values not sharing a common superscript on the same column differs significantly at (P< 0.05).
The present study also indicated that the liver and kidney SOD and catalase was significantly decreased (P>0.05) in group 2 rats exposed to cyanide only when compared to group 1 control group. However, the liver SOD and Catalase of honey treated group 3 rats indicated no significant difference when compared with the control and the known cyanide antidote sodium thiosulphate (Table 4). SOD and catalase are enzymes that help to protect the cells against free radicals. Hence it can be indicated that honey boosted the antioxidant capacity of the rats exposed to cyanide when the activity of SOD and Catalase decreased in the liver and kidney. Studies indicate that cyanide toxicity, leads to the production of free radicals which results in the development of oxidative stress, leading to an imbalance between the generation of reactive oxygen species and protective mechanisms (Antioxidants). The ameliorative effect of honey solution showed a better effect at mopping up the free radicals generated by cyanide. Honey has been shown to contain flavonoids, ascorbic acid, tocopherols, catalase and phenolic compounds. All of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals (Johnston et al., 2005).

The ameliorating effect of honey on the lipid profile of cyanide exposed rats was also examined in this study. The study indicated that cyanide toxicity, caused a significant increase in the level of triglycerides (Table 4) this was in accordance to an increase in the level of triglyceride observed by Dhas et al., (2011) in a study where he investigated the effects of hydrogen cyanide exposure in cassava workers. A significant decrease in HDL and a significant increase in LDL-cholesterol were also indicated in all rats exposed to cyanide. (Table 4) this is in agreement with the findings of Oyebami et al., (2014) in which the effect of Tapioca (a food source of cyanide) on the lipid profile of rats was investigated. However a significant increase in HDL (P<0.05) and a significant decrease in LDL were indicated in Group 3 rats treated with honey (Table 4). This is in agreement with the work of Muhammad et al., (2012).

The main function of lipoproteins is in the transport of cholesterol and triglycerides which are not water soluble, from the site of absorption and synthesis to the sites of utilization also HDL has been shown to reduce inflammation, protect oxidation of low density lipoprotein as well as decrease atherosclerosis accumulation in the arterial walls (Sirtori, 2006). This study therefore indicates that honey greatly improves the lipid profile of rats exposed to cyanide.

This study has also confirmed studies that the flavonoids present in honey apart from enhancing its antioxidant activities also play important role, in the synthesis of Niacin (vitamin A), thus it wouldn’t be out of place to say that due to the antioxidant properties which honey possess, it has the ability to reduce plasma cholesterol and thus, enhance the synthesis of HDL, in the liver reducing the risk of cardiovascular diseases as indicated in previous studies. (Gheldof and Engeseth, 2002; Gheldof et al., 2003).

**Conclusion**

The study indicates that honey acts as a good antidote to cyanide poisoning and is able to ameliorate the biochemical effects generated from cyanide exposure to rats.

**REFERENCES**


