Some liver function indices and blood parameters in T. brucei-infected rats treated with honey

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Abstract

Honey has been reported to clear infection through a number of properties including boosting the immune system, its anti-inflammatory action, antioxidant activity and stimulation of cell growth. Anaemia and serum biochemical changes are common features of African trypanosomosis. We investigated whether honey has protective effect on some liver functions and blood parameters affected by trypanosome infection. The serum albumin concentration in infected untreated rats increased significantly (p<0.05) compared with control whereas treatment with honey returned this effect to normal values. Anaemia which became severe by day 11 of post infection as measured by significant changes (p<0.05) in the haemoglobin, packed cell volume, red blood cell, white blood cell and platelets counts was ameliorated when compared with the control (p<0.05). There was a significant decrease (p<0.05) in liver gamma glutamyl transferase in infected untreated, prophylactic and late stage treated group compared with the control groups. We suggest that honey has ameliorative effects on symptoms and some biochemical effects of T. brucei infections in rats.

Keywords: Trypanosomosis, Biochemical changes, Honey, Amelioration, Rat

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INTRODUCTION

Trypanosomosis, caused by African trypanosomes has been a public threat to people of sub-Saharan Africa\(^1\)\(^-\)\(^4\). *Trypanosoma brucei* infection, like other trypanosome infections precipitate increased red blood cell destruction which results in anaemia\(^5\)\(^,\)\(^6\) as well as tissue damage\(^1\). These changes together with the need by the host to destroy the parasite\(^8\) are presumably responsible for the symptoms of African sleeping sickness\(^9\). Despite the prolific research on the subject, no single, complete explanation for the pathogenesis of the disease has emerged. This is a disease for which both man and other animals whether economic, domestic or wild stand the risk of epidemics\(^1\)\(^,\)\(^3\)\(^,\)\(^4\). Splenomegaly and hepatomegaly which have been reported in *T. brucei*-infections\(^10\) have been shown to be directly related to the severity of anaemia and levels of parasitaemia\(^11\).

We have earlier reported that the administration of honey was able to reduce the parasitaemia and significantly extended the lifespan of *T. brucei*-infected rats\(^12\)\(^,\)\(^13\), even when included as part of diet\(^13\). We further reported the effect of honey on liver and serum ALP, GOT and GPT\(^12\)\(^,\)\(^13\). In this study, we further investigate the effect of honey treatment on additional haematological and liver indices to assess the ameliorative effect of the treatment on some symptoms caused by *T. brucei* infection.

MATERIALS AND METHODS

Federe strain of *T. brucei* was obtained from the Veterinary and Livestock studies Department, Nigerian Institute for Trypanosomiasis Research, Vom, Plateau state, Nigeria. Honey used for this experiment was obtained from Faculty of Agriculture, University of Ilorin, Nigeria. Assay kits for albumin, total bilirubin and gamma glutamyl transferase were products of Randox laboratories Ltd, United Kingdom.

Inoculation of rats with parasite

Parasite infected blood was obtained from the tail of infected rats at high parasitaemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritoneal cavity of uninfected rats weighing approximately 250g. The suspension as earlier described\(^12\)\(^,\)\(^13\) contained 3 or 4 trypanosome per view at x100 magnification.

**Administration of honey**

Infected and uninfected rats were administered intraperitoneally with 0.5ml solution of honey in distilled water containing 3.0mg/kg body weight on the first day of sighting parasite (early), 72hrs before infection (prophylactic) and 72 hrs after the sighting of parasite (late) in the blood of infected rats. Administration of honey continued on daily basis until one of the infected untreated rats died. Previous experiments\(^12\)\(^,\)\(^13\) show that infected untreated rats die 11 to 12 days post infection.

**Haematological and liver function indices.**

The liver function test and blood parameters were determined on rats when the infection progressed to late stage of the disease (11 days). Serum and liver collection was carried out as described earlier\(^12\)\(^,\)\(^13\). Albumin concentration was determined based on its quantitative binding to the indicator 3,3,5,5 – tetrabromo-m-cresol sulphonaphthalein (bromocresol green, BCG), which absorb maximally at 578nm as described by Doumas *et al.*\(^14\). The method of Evelyn and Mallony\(^15\) was used to determine the total bilirubin. The Gamma glutamyl trasferase was assayed using the method described by Orlowski and Meister\(^16\). Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), and platelet count were determined using the automated haematologic analyzer SYSMEX KX21,a product of SYSMEX corporation, Japan employing the method described by Dacie and Lewis\(^17\). Protein concentrations were determined using biuret method\(^18\) as described by Plummer\(^19\).

**Statistical analysis**

The group mean + S.E.M was calculated for each analyst and significant difference between means evaluated by analysis of variance (ANOVA). Post test analysis was done using the Tukey- Krammer multiple comparism tests. Values of p< 0.05 were considered as statistically significant\(^20\).

**Results**

Albumin concentration was observed in the serum and liver prepared from *T. brucei* infected rats (Table 1).
The serum albumin concentration of infected untreated rats increased significantly (p < 0.05) when compared to control (normal) rats whereas there was no significant (p< 0.05) difference in that of the control, uninfected treated, prophylactic and late stage treatment rats. Significant differences were however observed when these values were compared with that of infected untreated rats. In the liver, there was no significant (p< 0.05) difference in albumin concentration of all experimental group when compared to the control (normal) rats.

Total bilirubin concentration was observed in the serum and liver prepared from T. brucei infected rats (Table 2). There was no significant difference (p<0.05) when compared to control (normal) rats and infected untreated rats. In the liver, observed when these values were compared with that of infected untreated rats. Significant differences were however marked significant decrease(p<0.05) when compared to the control, uninfected treated and early stage treated rats (Table 3).

**Table 1:** Albumin (g/dl) concentration at 11days post infection

<table>
<thead>
<tr>
<th>Rat grouping</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal)</td>
<td>0.792 ±0.178</td>
<td>0.290 ± 0.091</td>
</tr>
<tr>
<td>Infected untreated</td>
<td>1.725 ± 0.029 a</td>
<td>0.258 ± 0.040</td>
</tr>
<tr>
<td>Uninfected treated</td>
<td>0.716 ± 0.183 b</td>
<td>0.253 ± 0.147</td>
</tr>
<tr>
<td>Prophylactic treated</td>
<td>0.733 ± 0.205</td>
<td>0.306 ± 0.115</td>
</tr>
<tr>
<td>Early stage treated</td>
<td>0.725 ± 0.123 b</td>
<td>0.309 ± 0.127</td>
</tr>
<tr>
<td>Late stage treated</td>
<td>0.762 ± 0.162 b</td>
<td>0.289 ±0.000</td>
</tr>
</tbody>
</table>

Each concentration is an average of five determinations ± SEM. Values are significantly different in comparison with “control (normal) rats and “infected untreated rats at p<0.05.

The serum and liver of the entire experimental group when compared to the control (normal) group.

**Table 2:** Total bilirubin (µmol/L) concentration at 11days post infection

<table>
<thead>
<tr>
<th>Rat grouping</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal)</td>
<td>0.767 ± 0.232</td>
<td>1.541 ± 0.438</td>
</tr>
<tr>
<td>Infected untreated</td>
<td>0.803 ± 0.102</td>
<td>1.818 ± 0.383</td>
</tr>
<tr>
<td>Uninfected treated</td>
<td>0.975 ± 0.150</td>
<td>1.663 ± 0.506</td>
</tr>
<tr>
<td>Prophylactic treated</td>
<td>0.632 ± 0.202</td>
<td>1.339 ± 0.313</td>
</tr>
<tr>
<td>Early stage treated</td>
<td>0.691 ± 0.295</td>
<td>1.580 ± 0.476</td>
</tr>
<tr>
<td>Late stage treated</td>
<td>0.729 ± 0.065</td>
<td>1.551 ±0.000</td>
</tr>
</tbody>
</table>

Each concentration is an average of five determinations ± SEM.

Table 3 shows the gamma glutamyl transferase (U/L) for the serum and liver in six experimental groups. One unit is defined as the enzyme activity which will liberate 1 mol of p-nitroaniline under assay conditions. The specific activity of gamma GT in serum of all experimental group are significantly (p<0.05) the same except for the late stage treated which show maxima significant increase in specific activity. In contrast, the activity of Gamma GT in liver of infected untreated, prophylactic and late stage treated shows a marked significant decrease(p<0.05) when compared to the control (normal),uninfected treated and early stage treated rats(Table 3).

**Table 3:** Specific Activities of gamma glutamyl transferase (U/L) at 11days post infection

<table>
<thead>
<tr>
<th>Rat grouping</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal)</td>
<td>223.35±45.986</td>
<td>209.080 ± 92.873</td>
</tr>
<tr>
<td>Infected untreated</td>
<td>280.38±46.250</td>
<td>96.250 ±31.190 a</td>
</tr>
<tr>
<td>Uninfected treated</td>
<td>266.64±6.09</td>
<td>198.520 ±65.959</td>
</tr>
<tr>
<td>Prophylactic treated</td>
<td>175.91±46.290</td>
<td>96.405 ±31.235 ab</td>
</tr>
<tr>
<td>Early stage treated</td>
<td>225.49±71.295</td>
<td>227.436 ±66.584</td>
</tr>
<tr>
<td>Late stage treated</td>
<td>703.63±4.843 ae</td>
<td>23.033 ±10.981 abc</td>
</tr>
</tbody>
</table>

Each specific enzyme activity is an average of five determinations ± SEM. Values are significantly different in comparison with “control (normal) rat,”infected untreated rats and “uninfected treated rats at p < 0.05.

**Table 4:** Haematological studies of T. brucei infected rats for 11days post infection

<table>
<thead>
<tr>
<th>Rat Groupings</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>RBC (x10⁸/L)</th>
<th>WBC (x10⁹/L)</th>
<th>Platelet (x10⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control normal</td>
<td>12.9±1.45</td>
<td>39.50±3.50</td>
<td>6.48±0.250</td>
<td>16.80±3.20</td>
<td>899±50±1.50</td>
</tr>
<tr>
<td>Infected untreated</td>
<td>7.20±0.00 a</td>
<td>30.50±4.50 a</td>
<td>4.06±0.70 a</td>
<td>7.97±1.14 a</td>
<td>319.67±49.59 a</td>
</tr>
<tr>
<td>Uninfected treated</td>
<td>13.07±0.521</td>
<td>39.67±1.76</td>
<td>6.56±0.24 a</td>
<td>10.97±4.82</td>
<td>689.00±31.81</td>
</tr>
<tr>
<td>Prophylactic treated</td>
<td>10.70±0.00</td>
<td>37.00±0.00</td>
<td>5.87±0.00</td>
<td>16.10±4.00</td>
<td>376.00±0.00 abc</td>
</tr>
<tr>
<td>Early stage treated</td>
<td>8.93±1.10 ac</td>
<td>34.67±3.48 ac</td>
<td>5.23±0.56 ab</td>
<td>9.40±2.20 ac</td>
<td>672.67±19.34 ac</td>
</tr>
<tr>
<td>Late stage treated</td>
<td>9.25±0.75 ac</td>
<td>33.50±2.50 ac</td>
<td>5.01±0.77</td>
<td>11.90±4.90 ab</td>
<td>462.67±1.61 abc</td>
</tr>
</tbody>
</table>

Each value is an average of five determinations ± SEM. Values are significantly different in comparison with “control (normal) rats,”infected untreated rats and “uninfected treated rats at p<0.05.
There was a marked decrease in specific activity of late stage treated rats when compared with infected untreated and prophylactic treated which group which also shows significant decrease (p<0.05).

Table 4 shows the result of haematological parameters monitor. The haemoglobin concentration, PCV, RBC, WBC and platelet count of infected untreated rats were significantly decreased (p<0.05) compared to the control (normal) rats. Platelet counts of infected untreated, prophylactic and late stage treated rats show significant decrease when compared to the control (normal) rats.

DISCUSSION

A number of studies have reported that honey has antimicrobial therapeutic properties, especially in situations where the body’s immune response is insufficient to clear infection21-23. However, we have earlier reported that the administration of honey at 3mg/kg body weight to infected rats was able to reduce the parasitaemia and extend the life span of rats when compared to infected non-infected rats12. It has been reported that honey stimulates T- lymphocytes in cell culture to multiply. It also activates neutrophil24.

Honey stimulates monocytes in cell cultures to release the cytokines, TNF-alpha, IL -1 and IL-6, the cell messengers that activate many facets of the immune response to infection25. In stimulation of these leucocytes, honey provide supply of glucose which is essential for the respiratory burst in macrophages that produce hydrogen peroxide the main component that attacked cell membrane of the parasite25.

Parasitaemia correlates with the severity of infection7. The disease is further complicated by anaemia, thrombocytopenia and leucopenia5,6,9 all or some of which may be related to breakdown of the immune system and the observable pathological consequences of infection.

Albumin binds and transports metal ions, bilirubin, drugs etc. Its levels maybe use to assess the synthetic function of the liver26. The result of albumin concentration in the serum and liver of uninfected–treated, prophylactic and late stage treatment were unaltered when compared to the control (normal) rats. Whereas the infected untreated shows significant increase in serum albumin when compared with other experimental groups. This result implies that the increase in albumin may be as a result of infection which was reduced to normal values by honey treatment when used.

Bilirubin is transported to the liver bound to albumin. High plasma conjugated bilirubin concentration indicates impaired hepatic excretory function26. In our investigation, there was no significant change in both the serum and liver of all the experimental groups which align with earlier report7.

Gamma glutamyl transferase (GGT) is an enzyme derived from endoplasmic reticulum of the cells of the hepabiliary tract. As this reticulum proliferates, for example in response to drugs, synthesis of the enzyme is induced and plasma GGT activity increase. Therefore a raised plasma activity does not necessarily indicate hepatocellular damage26.

The late stage treated group shows a significant increase in serum GGT when compared to others groups. In the liver there was significant decrease in infected untreated, prophylactic and late stage treated rats when compared with the control, uninfected and early stage treated rats. This implies that there was no leakage of the enzyme into serum but induction of the enzyme synthesis in the liver which may be caused by the infection.

It has been reported that the measurement of anaemia gives an indication of the severity of the disease7,27. The decrease in haemoglobin, PCV, RBC, WBC and platelet count of the infected untreated rats confirm earlier reports of anaemic condition in trypanosomosis. Honey was able to ameliorate the disease condition in prophylactic group with reduced effects on late and early stages.

We conclude again that honey has a potential in the management of African trypanosomosis as we earlier reported its trypanocidal capabilities. We further suggest from the results in this work that honey ameliorates the effects and symptoms of T. brucei infection in rats.
REFERENCES


