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Full Length Research Paper

Effects of honey-supplemented diet on the parasitemia and some enzymes of *Trypanosoma brucei*-infected rats

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***Trypanosoma brucei*-infected rats were treated with natural honey and honey-supplemented diet at three days before infection (prophylaxis), early and late stages of infection. Proximate percentage composition of the honey was 71.19±0.90% carbohydrate, 3.28±0.05% protein and 10.68±0.27% lipid among others. Prophylactic treatment with natural honey extended the lifespan of infected rats by 13 extra days from a control of 12 days post infection while early and late stage treatments extended the lifespan by 10 and 5 days, respectively. Prophylactic feeding with honey-supplemented diet extended the lifespan by 6 extra days while early and late stage feeding extended it for 5 and 3 days, respectively. A specific pattern could not be established for alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzyme activities in the liver and serum. We conclude that honey even as part of a regular diet could be a useful, cheap and readily presentable agent in the management of African sleeping sickness for residents of disease endemic areas.**

Key words: Honey, diet, sleeping sickness, management.

INTRODUCTION

Trypanosomosis, caused by African trypanosomes continues to be a public threat in tropical Africa (Abenga and Lawal, 2005; Chretien and Smoak, 2005). Drug regiments are toxic and cumbersome in addition to being expensive (Kioy and Mattock, 2005; Moore, 2005). The onus is to continue to seek inexpensive and less cumbersome approaches to the management and treatment of the disease (Jannin and Cattand, 2004; Moore, 2005).

Honey is a natural product that is widely available in different parts of the world (Terrab et al., 2003; Yao et al., 2003; Bonvehí et al., 2004; Sanz et al., 2004; Malacalza et al., 2005) and is readily obtained from natural or cultured bee colonies. It has been reported as having antimicrobial properties (Al-Waili and Saloom, 1999; Adebolu, 2005) as well as therapeutic effects on internal and external ailments (Okeniyi, 2005; Orsolic et al., 2005). Identification of natural products active against African trypanosomosis has been described as a step towards treatment and control of the disease (Hoet et al., 2004). We have earlier suggested that honey could be a potential agent in the management of African sleeping sickness (Ekanem and Yusuf, 2005). In this report, using infected but untreated rats as control, we confirm that honey could be a useful agent in the management of African trypanosomosis. We assess the possibility of making it a part of the diet for the infected individuals and also see how this affects the levels of some enzyme activities in the host.

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MATERIALS AND METHODS

Materials

Honey was obtained from Faculty of Agriculture, University of Ilorin, Nigeria. Rats were obtained from the Animal Holdings of the Department of Biochemistry, University of Ilorin, Nigeria.

Table 1. Composition of the diet used to maintain *T. brucei*-infected rats (g/Kg).

Feed component	Weight
Corn starch	516
Soybean	250
Oil	40
Cellulose (rice husk)	40
*Vitamin/Mineral mix	50
DL-methionine	4
Honey	100

*Vitamin/Mineral mix (per Kg of diet): Thiamin hydrochloride, 6 mg; Pyridoxin hydrochloride, 7 mg; nicotinic acid, 30 mg; folic acid, 2 mg; calcium pantothenate,16 mg; biotin, 0.2 mg; cyanocobalamin, 0.01 mg; retinal palmitate, 4000 IU; cholecalciferol, 1000 IU; tocopherol acetate 50 IU; menadione 0.05 mg; choline chloride, 2 g; $CoCl_2.6H_2O$, 0.001 g; $CuSO_4.5H_2O$, 0.079 g; $MnSO_4$, 0.178 g; KI, 0.032 g; KH_2PO_4, 5.559 g; $CaSO_4$, 5.25 g; NaCl, 5.573 g; $ZnCO_3$, 1.6 g; $FeSO_4.7H_2O$, 1.078 g; $MgSO_4.7H_2O$, 2.292 g.

Trypanosoma brucei brucei was collected from Veterinary and Livestock Studies Department, Nigeria Institute for Trypanosomiasis Research, Vom, near Jos, Nigeria. Glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) assay kits were products of Randox Laboratories Customer Technical Support, Northern Ireland. Alkaline Phosphatase (ALP) assay kit was a product of Teco Diagnostics, 1268 N. Lakeview Avenue, Anaehim, CA 92 807.

Inoculation of rats

Parasite infested blood was obtained from the tail of infected rats at high parasitemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritoneal cavity of uninfected rat weighing approximately 250 g. The suspension contained 3 or 4 trypanosomes per view at x100 magnification (approximately 10^6 cells per ml).

Proximate analysis

Proximate analysis was carried out as described in AOAC (1999) to estimate moisture, crude protein, crude fibre, fat and ash content. The carbohydrate content was obtained by subtracting the sum of protein, fat, ash and fibre from the total dry matter.

Feed composition

Feed was formulated with the different classes of food: corn starch was used as carbohydrate, dried milled soybean was used as protein, pure soybean oil was used as a source of lipid and dried rice husk was used as fibre. Other ingredients of the feed include DL-methionine and vitamin/mineral mix. Honey was added as supplement. These are shown in Table 1. Commercial feed used as diet for the control rats was a product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria.

Tissue collection and preparation

Rats were anaesthetized using cotton wool soaked in chloroform. Blood samples were collected by cardiac puncture using needle and syringe from where they were transferred into centrifuge tubes. The blood was allowed to clot for 10 min at room temperature and then centrifuged using laboratory centrifuge (SM800B, Surgified Medicals, England) at 3000 rpm for 5 min. The serum was then separated with a clean Pasteur pipette and stored frozen until required for use (Ogbu and Okechukwu, 2001). The liver was excised, cleansed of blood and superficial connective tissues and transferred into ice cold 0.25 M sucrose solution. The liver was cut with a sterile blade and homogenized in an ice cold 0.25 M sucrose solution (1:5 w/v) using Teflon homogenizer. Maximal release of enzyme activities was ensured by freezing the homogenate (Ngaha et al., 1989).

Enzyme assay

ALP was assayed using the phenolphthalein monophosphate method of Klein et al (1960) as revised by Babson et al (1966). GOT and GPT were assayed following the method of Reitman and Frankel (1957). The protein content was determined using biuret reagent as reported by Gornall et al. (1949). Measurement of the activities was done using spectronic 21 digital spectrophotometer (Bausch and Lomb Rochester, New York).

Statistical analysis

Data are presented as mean of four replicates ± standard error of mean (SEM). Analysis of variance was carried out and complemented with student's t-test. Level of statistical significance was taken at $p<0.05$ (Adamu and Johnson, 1997).

Experimental design

In the first experiment, infected rats of four in each group were inoculated intraperitoneally with honey at 100 mg/kg body weight 72 h before infection for prophylactic assessment, on the first day of sighting parasite in the blood (3 to 4 days post infection) for early stage treatment and on day 9 for late stage treatment. In the second experiment, honey supplemented diet were fed to rats for prophylactic, early and late stage treatments. Serum and liver activities of ALP, GPT and GOT were also determined in the rats at high parasitemia (as confirmed by the death of an extra rat in the group).

RESULTS

Proximate composition of honey

The proximate composition of honey used for this work (Table 2) shows that carbohydrate forms 71.19 ± 0.90% of it. Lipid and protein among other contents were 10.61 ± 0.27 and 3.28 ± 0.05%, respectively.

Parasitaemia

Figure 1 shows the result of infected rats administered with honey intraperitoneally at 100 mg/Kg body weight for prophylactic, early and late stage treatments. Prophylactic treatment extended the lifespan by 13 days while early and late stage treatments extended the lifespan for 10 and 5 days, respectively. The progress of the infection in rats fed with honey-supplemented diet is shown in Figure 2.

Table 2. Proximate composition of honey used in this study.

Food Class	Percentage (%)
Carbohydrate	71.19 ± 0.90
Protein	3.28 ± 0.05
Lipid	10.68 ± 0.27
Crude fibre	0.01 ± 0.003
Ash	2.87 ± 0.39
Moisture content	12.75 ± 0.07

Each value is a mean of 4 determinations ± SEM.

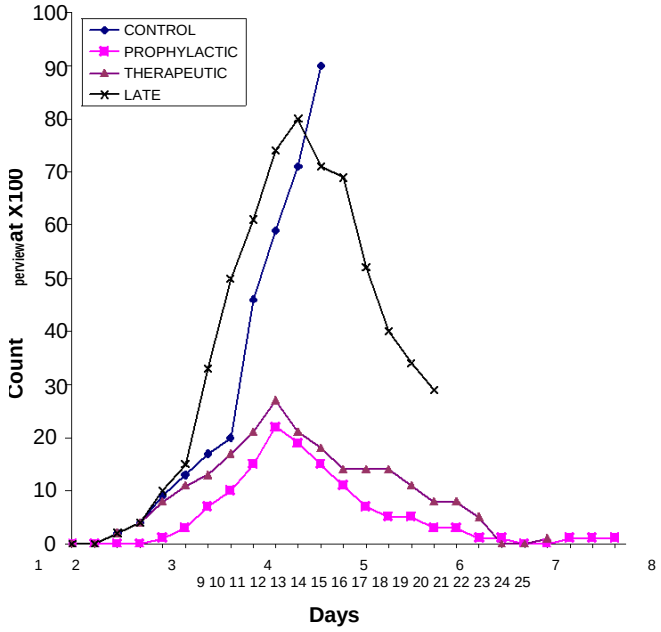

Figure 1. Trypanosome count for intraperitoneal treatment of infected rats with honey at 100 mg/Kg body weight of rat at 3 days before infection (prophylactic), early (therapeutic) and late stages. Each point is an average count from 4 rats.

Prophylactic feeding extended the lifespan for six days while early and late stage treatments extended it for 5 and 3 days, respectively. Parasitaemia was lowered in all cases (Figures 1 and 2) when compared with the control.

Alkaline phosphatase activities

The results of the activities of ALP in the liver and serum of infected rats fed with honey-supplemented diets are shown in Table 3. There were significant decreases ($p<0.05$) in liver ALP activities for early and late stage treatments in comparison with the infected non-treated contrary to the significant increase ($p<0.05$) observed with the prophylactic treatment. There were significant increases in serum ALP activities for early and late stage treatments whereas the activity was significantly lower ($p<0.05$) with prophylactic treatments.

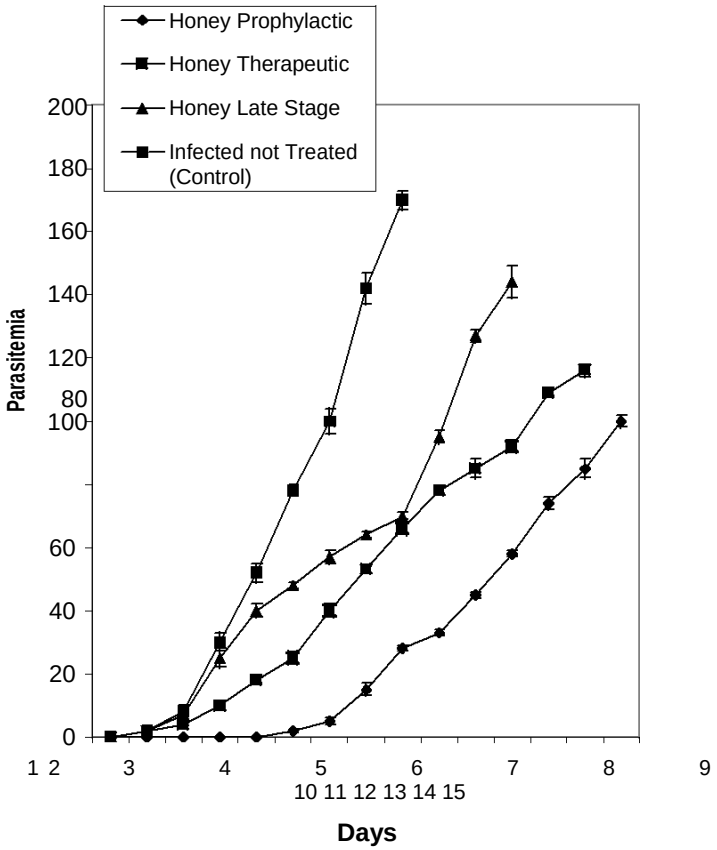

Figure 2. Trypanosome count for infected rats fed with honey supplemented diet at 3days before infection (prophylactic), early (therapeutic) and late stages. Each point is an average count from 4 rats.

Glutamate pyruvate transaminase activities

Table 4 shows the results of activities of GPT in the liver and serum. There were significant increases in the serum enzyme activities when compared with the infected but not treated rats. Increases were also observed in the liver enzyme activities for prophylactic and early stage treatments while it was significantly lower for late stage treatments.

Glutamate oxaloacetate transaminase activities

Results of GOT assay are shown in Table 5. Serum GOT activities were significantly higher in rats that received early and late stage treatments when compared with the activities in the infected but not treated rats. Significant increases were also observed in enzyme activities in the liver of rats fed with honey-supplemented diet for prophylatic, early and late stage treatments.

DISCUSSION

It is well documented that honey has antimicrobial and

Table 3. Specific activities of alkaline phosphatase in the liver and serum of *T. brucei*-infected rats.

Rat groupings	Enzyme activity (IU/L)	
	Liver	Serum
Infected but not treated (Control)	26.92 ± 0.85	366.41 ± 8.05
Prophylactic honey-treated	43.70 ± 1.18*	325.60 ± 12.52*
Early stage honey-treated	16.68 ± 0.30*	575.55 ± 7.57*
Late stage honey-treated	10.22 ± 0.025*	837.12 ± 3.55*

Each value is an average of four determinations ± S.E.M.
*Values are significantly different when compared with infected but not treated (control) at p<0.05.

Table 4. Specific activities of glutamate pyruvate transaminase in the liver and serum of *T. brucei*-infected rats.

Rat groupings	Enzyme activity (IU/L)	
	Liver	Serum
Infected but not treated	185.30 ± 3.25	2470.40 ± 5.05
Prophylactic honey-treated	314.77 ± 1.96*	2797.57 ± 0.91*
Early stage honey- treated	215.40 ± 0.78*	4018.18 ± 6.09*
Late stage honey-treated	120.33 ± 5.00*	5741.67 ± 5.47*

Each value is an average of four determinations ± S.E.M.
*Values are significantly different when compared with infected but not treated (control) at p<0.05

Table 5. Specific activities of glutamate oxaloacetate transaminase in the liver and serum of *T. brucei*-infected rats.

Rat groupings	Enzyme activity (IU/L)	
	Liver	Serum
Infected but not treated	5.86 ± 0.4	421.53 ± 0.31
Prophylactic honey-treated	122.51 ± 0.66*	679.09 ± 1.85*
Early stage honey-treated	62.05 ± 0.60*	860.9 ± 6.82*
Late stage honey-treated	15.61 ± 0.34*	1237.63 ± 2.24*

Each value is an average of four determinations ± S.E.M.
*Values are significantly different when compared with infected but not treated (control) at p<0.05.

therapeutic properties (Adebolu, 2005; Okeniyi, 2005; Orsolic et al., 2005). We have earlier reported that the administration of honey at 3 mg/kg body weight to infected rats was able to reduce the parasitaemia and extend the life span of rats by 7 days when compared to infected non-treated rats (Ekanem and Yusuf, 2005). The extended life span in this report for prophylactic, early and late stages of honey treated infected rats (Figure 1) confirms that honey administered intraperitoneally could be a useful and cheap agent in the management of African trypanosomosis. The extended life span of 10 days for prophylactic treatment suggests that routine consumption of honey in trypanosomosis endemic regions, where administration of toxic and expensive drugs is a problem could have some preventive and control implications for African trypanosomosis. It is perhaps the large carbohydrate content 71.19% (Table 2) and possibly in combination with other substances in the honey that confers the trypanocidal properties since the nature of the carbohydrate content may have implications for glucose consumption and the synthesis of the essential variable surface glycoprotein required for antibody evasion (Smith et al., 2004; Dinglasan et al., 2005; Roper et al., 2005).

The attempt to present honey in a simple and conducive form in the form of feed to the infected rats also shows that the most effective treatment was the prophylactic one (Figure 2) extending the lifespan for six days. Even though this is not as effective as intraperitoneal administration, it equally suggests that routine consumption of honey could have preventive implications.

The activities of enzymes such as ALP, GOT and GPT could be important in the diagnosis of diseases as well as in the investigation and thorough assessment of feed, drugs and extracts used in the treatment as these could give indications of progressive toxicity long before the actual manifestation of the toxic effects (Hanley et al., 1986). Damage done to tissues or organs often result in increased level of these enzymes in the blood. A specific pattern could not be established for these enzyme activities in the rat administered with honey-supplemented diets (Tables 3-5). The general significant increases observed in the serum enzyme activities imply progres-

sive damage to organs not necessarily the liver only. It is however possible that the observed results were due to tissue destruction by the parasites (Pentreath and Kennedy, 2004).

Honey has been useful in curing some diseases (Adebolu, 2005; Okeniyi, 2005; Orsolic et al., 2005). We have not succeeded in using it to cure African trypanosomosis. We however suggest that honey, even as part of a regular diet, could be a useful, cheap and readily presentable agent in the management of African trypanosomosis for residents of disease endemic areas.

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