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Anti-Trypanosomal Activities of Natural Honey on *Trypanosoma congolense* Infected Wistar Rats

S.A. Ameen
Department of Animal Production and Health,
Ladoke Akintola University of Technology, P.M.B. 4000 Ogbomoso,
Oyo State, Nigeria

Abstract: An experiment was conducted to determine the antitrypanosomal activities of natural honey on wistar rats infected with *Trypanosoma congolense*. Twenty four albino rats (males and females) of wistar strain weighing about 90-110 g were used the animals were housed in well ventilated cages and kept under controlled environmental conditions. Each rat was inoculated intraperitoneally with 1.0×10^5 mL^{-1} of the *T. congolense*. The rats were divided into 3 groups A-C with 8 rats per group. Group A that served as the control were infected and given distilled water, group B and C were infected and treated with diminazine aceturate and natural honey, respectively at 7th day post infection. The parasitaemia levels, mean body weight changes and haematological parameters were analysed using one way ANOVA. The parasitaemia levels, mean body weight and haematological parameters were significantly different ($p<0.05$) in the three groups. There was a total clearance in the parasitemia level of the group B treated with diminazene aceturate while in group C there was reduction but no total clearance. It was concluded that natural honey even as part of regular diet could be a useful, cheap and readily available agent in the management of African trypanosomosis in an endemic area.

Key words: Natural honey, antitrypanosomal, Wistar rats, *Trypanosoma congolense*, animal

INTRODUCTION

Trypanosomosis has been reported to affect humans and domestic animals. It is described as a complex debilitating and often fatal condition caused by infection with one or more of the pathogenic tsetse-transmitted protozoan hemoflagellate parasites of the genus *Trypanosoma* (Anene *et al*., 2001). From all indications, the disease has been a great challenge to the livestock industry where the barrier imposed has been difficult to surmount by any form of chemotherapy, prophylaxis or control (Holmes *et al*., 2004; Van den Bossche and Doran, 2004).

Over four decades ago, the disease along with malaria, cancer and heart diseases was considered by the World Health Organization (WHO, 1998) as being among the ten major health problems facing mankind (Kershaw, 1970). According to Sekoni (1993), trypanosomosis is among the important diseases which cause various reproductive disorders in both male and female animals. Although, the protozoan parasites localize in internal organs of the infected host, the gonads are obviously their preferred site (Ashman and Seed, 1974). Sporadic cases of congenital transmission of trypanosomes have also been reported in humans (Rocha *et al*., 2004), sheep (Ikede and Losos, 1972) and mice (Ijagbone and Agbede, 2000). Among the three subspecies of *T. brucei*, *T. brucei brucei* is considered the most virulent in domestic animals (Ikede, 1983). Human trypanosomosis or sleeping sickness is caused by the other two subspecies namely *T.b. rhodesiense* and *T.b. gambiense*. While sleeping sickness associated with *T.b. rhodesiense* manifests in the form of acute inflammatory disease, the *T.b. gambiense* variant develops as a chronic autoimmune disease (Van Meirvenne, 1999).

Trypanosomal infections are known to cause immune suppression responsible for the host's inability to eliminate the trypanosomes even after administration of trypanocidal drugs (Godwin *et al*., 1972). Diminazene aceturate and isomethamidium chloride are the most currently used trypanocides used for prophylactics and curative purpose for the control of the disease in cattle (WHO, 1998). Unfortunately, the parasite has developed resistance to these drugs which makes the search for effacious chemotherapeautic agents form locally available natural honey used in folk medicine as a result of the presence of some anti bacterial and theurapetic properties.

Natural honey has been used since, ancient times for the treatment of some respiratory diseases and for the healing of skin wounds (Tahany *et al*., 2009; Smith *et al*., 2009). Also, honey is recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds

(Brudzynski, 2006). Renewed interest in honey for various therapeutic purposes including treatment of infected wounds has led to the search for different types of honey with antibacterial activity (Mullai and Menon, 2007). The healing effect of honey could be due to various physical and chemical properties (Snow and Manley-Harris, 2004). The floral source of honey plays an important role on its biological properties (Molan, 2002). The use of honey as therapeutic substance has been rediscovered by medical provincials in more recent times and has been accepted as antibacterial agent for treatment of ulcers bed sore, surface wound infection and surface infections resulting from wounds (Brudzynski, 2006). Also, honey has been found to be effective in treating bacterial gastroenteritis ininfants (Haffeejee, 1985) and liver diseases (Yoirish, 1977). The honey has been reported to have some vitamins with antioxidant properties and the prominent of which are vitamins A, C, D, E, K and other B-complex with variable amounts of thiamine, riboflavin, pantothenic acid, nicotinic acid, pyridoxine, folic acid and biotin. (Haydak *et al*., 1942; Nalda *et al*., 2005; Bogdanov *et al*., 2007). It contains some mineral elements such as potassium, iron, copper and other trace elements which are important in erythropoiesis. There is relatively no enough information on antitrypanosomal activities of natural honey on trypanosomosis in albino rats, therefore, this study focuses on antitrypanosomal activities of honey on albino rats infected *Trypanosoma congolense*.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at the Ladoke Akintola University of Technology Ogbomoso Teaching and Research farm.

Collection of natural honey: Natural honey was collected from the Apiary Unit of the Ladoke Akintola University of Technology Ogbomoso Teaching and Research farm.

Animals and experimental design: Twenty four rats (males and females) of wistar strain weighing about 90-110 g were obtained from animal house of the Department of Veterinary Anatomy, University of Ibadan were used for the study. The animals were allowed to acclimatize for 3 weeks at the experimental laboratory of the Department of Animal Production and Health, LAUTECH. The animals were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25±5°C), relative humidity (50±5%) and 12 h light/dark cycle. The 24 rats were divided into 3 parallel groups consisting of the control and infected rats. The animals were fed and also watered *ad libitum*.

Parasitemia and inocula: *Trypanosoma congolense* infected rats were obtained from the Department of Veterinary Anatomy, University of Ibadan and the rats were inoculated Intraperitoneally (IP) via the blood collected from the infected rat. An estimation of parasitaemia was made on the pooled sample which was then diluted with Phosphate Buffered Saline (PBS) containing 1.5% glucose at PH 8.0 to give 1×10^5 trypanosomes in 1 mL of Phosphate Buffered Saline (PBS). The trypanosome was injected intraperitoneally at a rate of 1mL per each rat.

Infection of the rats: Blood was collected from a mice heavily infected with *T. congolense* and immediately diluted with sterile buffered phosphate saline (pH 7.2). Healthy rats were injected with 1×10^5 *T. congolense* per mL of blood intraperitoneally. Evaluation of parasitemia was done daily till parasites were detected microscopically as described by Herbert and Lumsden (1976).

Administration of drug: The rats were divided into three groups of eight rats per group. Group A (Control) received 0.5 mL/100 g average body weight of distilled water. Group B was treated with diminazene aceturate while Group C was treated with natural honey orally. The weights, haematological parameters and levels of parasitaemia were monitored daily for 14 days.

Determination of parasitaemia: Blood films were collected from the caudal vein of each rat and trypanosome count was determined by examination of the wet mount, microscopically at x40 magnification.

Determination of weight and haematological parameters: The weight of the rats were determined using Docbel Braun Model with minimum capacity of 50 g while Packed Cell Volume (PCV), Red Blood Cell (RBC) counts and Haemoglobin (Hb) concentrations were determined as described by Jain (1986).

Statistical analysis: Following the experimental design, all data collected were subjected to completely randomized Analysis of Variance (ANOVA). The $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The parasites introduced into the rats were successfully detected on the 7th day of post infection for the three groups, respectively. There was significant increase ($p<0.05$) in the parasitaemia level of group A from day 7-14 post infection with resultant mortality on day 14.

Table 1: Parasitaemia level ($\times 10^6$ mL^{-1}) from day 7 post infection of rats in groups A-C infected with *Trypanosoma congolense*

Groups	Day 7 p.i. (Day 1 of treatment)	Day 8 p.i. (Day 2 of treatment)	Day 9 p.i. (Day 3 of treatment)	Day 10 p.i. (Day 4 of treatment)	Day 11 p.i. (Day 5 of treatment)	Day 12 p.i. (Day 6 of treatment)	Day 13 p.i. (Day 7 of treatment)	Day 14 p.i. (Day 8 of treatment)
A	6.0±0.5	6.5±0.1	6.8±0.4	7.1±0.2	8.0±0.3	8.3±0.1	8.7±0.1	10.03±0.1
B	6.2±0.4	5.8±0.5	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.00±0.0*
C	6.3±0.1	5.9±0.0	5.7±0.2*	5.5±0.3*	5.3±0.2*	5.0±0.2*	4.8±0.2*	4.80±0.3*

Table 2: Mean body weight changes (g) from day 7 post infection (p.i.) of rats in groups A-C infected with *Trypanosoma congolense*

Groups	Day 7 p.i. (Day 1 of treatment)	Day 8 p.i. (Day 2 of treatment)	Day 9 p.i. (Day 3 of treatment)	Day 10 p.i. (Day 4 of treatment)	Day 11 p.i. (Day 5 of treatment)	Day 12 p.i. (Day 6 of treatment)	Day 13 p.i. (Day 7 of treatment)	Day 14 p.i. (Day 8 of treatment)
A	103±0.3	103±0.3	100±0.2	95±0.30	91±0.2	89±0.3	88±0.2	85±0.2
B	104±0.2	106±0.3	107±0.3*	109±0.2*	112±0.3*	114±0.2*	116±0.5*	118±0.1*
C	103±0.2	103±0.4	102±0.3*	101±0.2*	102±0.1*	103±0.4*	103±0.3*	103±0.1*

Table 3: Haematological parameters from day 7 post infection (p.i.) of rats in groups A-C infected with *Trypanosoma congolense*

Groups parameter/ Blood	Day 7 p.i. (Day 1 of treatment)	Day 8 p.i. (Day 2 of treatment)	Day 9 p.i. (Day 3 of treatment)	Day 10 p.i. (Day 4 of treatment)	Day 11 p.i. (Day 5 of treatment)	Day 12 p.i. (Day 6 of treatment)	Day 13 p.i. (Day 7 of treatment)	Day 14 p.i. (Day 8 of treatment)
A/PCV (%)	40.0±0.40	39.87±0.4	38.00±0.3	38.50±0.4	37.50±0.3	37.00±0.2	36.50±0.2	36.00±0.3
RBC$\times 10^6$ μL^{-1}	6.9±0.30	6.85±0.2	6.40±0.2	6.23±0.1	6.18±0.2	6.00±0.1	5.90±0.3	5.75±0.2
Hb (g dL^{-1})	13.4±0.20	13.20±0.3	12.90±0.3	12.52±0.2	12.40±0.1	12.23±0.2	12.18±0.3	12.00±0.3
B/PCV (%)	40.5±0.20	42.00±0.3	43.00±0.3*	44.50±0.3*	46.10±0.1*	47.00±0.2*	48.20±0.2*	48.50±0.3*
RBC$\times 10^6$ μL^{-1}	6.9±0.30	7.00±0.1	7.51±0.2*	7.80±0.2*	7.85±0.3*	7.90±0.3*	7.96±0.3*	7.99±0.2*
Hb (g dL^{-1})	13.3±0.20	14.26±0.2	15.00±0.3*	15.20±0.2*	15.44±0.2*	15.80±0.2*	15.90±0.2*	15.97±0.3*
C/PCV (%)	40.0±0.30	40.00±0.1	38.20±0.1	38.00±0.1	37.05±0.4	38.05±0.1*	39.08±0.3*	39.09±0.5*
RBC$\times 10^6$ μL^{-1}	6.8±0.40	6.80±0.4	6.70±0.3	6.60±0.3	6.60±0.3	6.80±0.0	6.80±0.2*	6.80±0.3*
Hb (g dL^{-1})	13.46±0.2	13.50±0.2	13.30±0.0	13.10±0.0	13.05±0.1*	13.09±0.3*	13.10±0.0*	13.30±0.1*

*Means significantly different from the control

There was reduction in the parasitaemia level from days 7-8 which was followed by total clearance of the parasites from days 9-14 in group B. Group C which was treated with honey showed that the parasite were not completely cleared but showed a significant reduction ($p<0.05$) in the parasitaemia level. All the infected rats showed progressive signs of weakness except in the group treated with diminazene aceturate (Table 1).

There was significant reduction ($p<0.05$) in the body weight in group A from days 7-14 post infection followed by a significant increase in body weight in group B. Group C treated with honey shows an undulating effect in the level of the parameters (Table 2).

The red blood cell, packed cell volume and hemoglobin showed a significant reduction ($p<0.05$) in group A but show a significant increase ($p<0.05$) in the diminazene aceturate treated group and group C shows an undulating effect in the level of the parameters (Table 3).

The results obtained from this study showed that all infected wistar rats became parasitemic 7th days post infection and the rats in group A had persistent and increased parasitaemia through out the course of experiment while in group B there was total clearance of parasite from the blood of infected rats day 3 after treatment showing the efficacy of diminazene aceturate. In group C, there was reduction in levels of parasitaemia compared to group A. This is an indication that natural honey has trypanostatic effect. The initial increase and fluctuation in levels of parasitaemia could be attributed to Variant Surface Glycoprotein (VSG) coats of the parasites which make them elusive to host immune system and chemotherapy (Vickermann, 1985). The trypanostatic effect of honey observed in this study could be related to presence of antioxidants like vitamins which are the active compounds present in honey. It is hypothesized that this ability of honey could be attributed to their ability to aid total antioxidant defense system. This has been shown to reduce oxidative stress by protecting the defense system against the damaging effects of reactive oxygen species such as singlet oxygen, peroxyl radicals, nitric oxide and peroxynitrite. Indeed, nitric oxide has been implicated in the pathogenesis of trypanosomosis. It is therefore speculated that without the host immunological assistance, high concentration of concentration would be necessary to reduce or cause total clearance of *T. congolense* in the host (Mabbott and Sternberg, 1995; Sepulveda-Boza and Cassels, 1996).

There was a significant weight loss in group A may be due to the activities of the trypanosomes detected in the rats compared to other groups B and C treated with diminazene aceturate and natural honey, respectively. The weight loss has been reported as one of the clinical signs of trypanosomosis in rats infected with *T.b. brucei* Mann *et al.* (2003) and Raheem *et al.* (2009) in goat infected with *T. congolense*. Manifestation of various lesions in the tissues, organs, emaciation, anaemia as well

death has been reported in clinical trypanosomosis (Radostits *et al*., 2007). The improved weight gain in rats treated with natural honey may be associated with availability of monosaccarides, amino acids as well as protein in the natural honey.

It was recorded in group A that there was a decrease in the packed cell volume, red blood cell and haemoglobin level of the rats may be due to the activities of the trypanosomes detected in the rats compared to other groups B and C treated with diminazene aceturate and natural honey, respectively. The significant fall in PCV, RBC and Hb below the normal values in group A is sign of anaemia which is clinical sign of trypanosomosis. The moderate increase observed in group C may be associated with the facts that natural honey possess haemopoietic properties such as iron and copper (Haydak *et al*., 1942; Nalda *et al*., 2005; Bogdanov *et al*., 2007) which are very important in erythropoiesis and in the formation of haemoglobin. This beneficial influence of natural honey on haemoglobin concentration synthesis is a corrective measure of anaemia induced by trypanosome infection.

CONCLUSION

Although, natural honey has been useful in curing some diseases such as heart diseases, insect bites, arthrititis, hair loss, bladder-infection, cold, etc., however, it is recommended that natural honey even as a part of regular diet containing monosaccharides, aminoacids, protein, vitamins as well macro and trace minrals could be a useful, cheap and readily available agent in the management of African animal trypanosomosis in endemic area due to its trypanostatic effect.

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