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Prophylactic Effect of Selected Arthropod Haemolymph on *Trypanosoma brucei brucei* Experimentally Infected Mice

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Abstract

Trypanosomiasis is one of the World's Neglected Tropical Diseases (NTDs) that affects both man and animals. The current control measures are mostly chemotherapeutic which face numerous challenges such as non availability of drugs, toxicity and long term protocol. These challenges have made researchers look for alternative cure for this disease since no vaccine is available yet. Eight species of arthropods (scorpion, beetle, grasshoper, butterfly, cricket, cockroach, spider and crab) were collected from the wild using different sampling methods. Haemolymph was collected from sampled arthropods using novel Antennae method of haemolymph sampling. Trypanosoma brucei brucei was sourced, identified and propagated in rats. The prophylactic effect of arthropod haemolymph of selected arthropods on T. b. brucei was carried out using 20 groups of mice of 8-10 weeks old with each group having 4 mice that were randomly assigned. Mice in all groups except the control 1 and 2 were administered 0.3µl of either 100% or 50% concentrations of arthropod haemolymph, four days pre-infection, with T. b. brucei infected blood. The control 1 and 2 were administered 0.3µl diminiazene aceturate and distilled water respectively before infection. The effects of the haemolymph on the parasite count, weight and PCV were monitored for 21 days at 3 days interval. The results of the study showed the prophylactic ability of grasshopper, cricket, cockroach, crab and diminazene (control 1) which were able to clear T. b. brucei by the 15th day of experiment. While haemolymph of scorpion and beetle encouraged parasitaemia, mice in control group 2 died before the 9th day post treatment. It is recommended that arthropod's haemolymph of crab, cockroach and grasshopper can be harnessed for their prophylactic properties against T. b. brucei infection.

Keywords: Arthropod haemolymph, Prophylactic, Parasitaemia,*Trypanosoma brucei brucei*

INTRODUCTION

Trypanosomes are protozoan parasites of the family Trypanosomastidae that infect a variety of domestic and wild animals and also humans causing trypanosomiasis. There are several species of trypanosomes that cause significant diseases in humans and animals. Animal

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Africa Trypanosomiasis (AAT) majorly caused by *Trypanosoma brucei brucei* while Human Africa Trypanosomiasis (HAT) is caused by *Trypanosoma brucei gambiense* and *brucei rhodesiense*. *Trypanosoma brucei gambiense* occur in 24 countries of West and Central Africa and *Trypanosoma brucei rhodesiense* is found in 13 countries in East and South Africa causing 92% and 8% of the human form of the disease (WHO, 2023). At least sixty five million people are at risk of this disease and about 100,000-300,000 new cases are recorded per year with most of these in central Africa (WHO, 2021). The control of trypanosomiasis is based mainly on the use of drugs and vector control. Although drugs such as isometamidium chloride, quinapyramine sulphate and chloride are used as prophylaxis during transhumance or high seasonal parasitic pressure (Adeyemi and Sulaiman 2015), increased drug resistance due to the complex nature of the trypanosome parasites has hindered current control methods (Chitanga *et al*.,2011;Kazoni *et al*., 2019).

Worldwide, many natural products are used for medicinal purposes, the search for new drugs against trypanosomiasis and the quest for the next generation antibiotics is focused on natural peptides produced by animals or insects that ward off infection. Insects and other invertebrates since time immemorial have not only been used as an item of food, but have also played important role in the treatment of diseases and other dysfunctions. Arthropod possess haemolymph which is similar to the blood of vertebrate showing a variety of circulating cells called haemocytes and contains organic substances such as; haemocyanin, amino acids, carbohydrates, fatty acids (Tatiana *et al*., 2015), ions, lipids, glycerol, hormones as well as inorganic salt mostly sodium, chlorine, potassium, magnesium and calcium, water, some cells and pigment. The primary oxygen transporter molecule is haemocyanin (Sowers *et al*.,2006). Haemocyanins are proteins present in the haemocytes containing two copper atoms that reversibly bind to a single oxygen molecule and are found only in Mollusca and Arthropoda. They are composed of phenoloxidase, a protein responsible for sclerotization in arthropods (Soderhall and Cerenius, 1998; Terwilliger *et al*., 1998), hexamerins, a protein responsible for storage in insect and cryptocyanin, a crustacean molting protein (Aguinaldo *et al*., 1997; Burmester, 2001).

The haemolymph which houses the immune system, play a role in the arthropods immunity and provides the first line of protection against bacteria, fungi and viral pathogens (Tatiana *et al*., 2015). The high diversity of invertebrates evidences the efficiency of their defense system and indicates that the absence of acquired immunity did not hinder their successes. This probably explains why haemolymph has been used extensively as anti-fungi, anti-bacteria, anti-cancer among other diseases (Tatiana *et al.,* 2015). Therefore, aim the aim of the study is to evaluate the prophylactic potential of the selected arthropod's haemolymph for the prevention of trypanosomiasis.

MATERIALS AND METHODS

Arthropod Sample Collection

Eight species of arthropods namely; scorpion, beetle, grass hopper, butterfly, cricket, cockroach, spider and crab used were collected using different sampling methods such as pitfall method (Mora-Aguilar *et al*., 2023), Hand net method (Gibb and Oseto 2006), Newspaper method (Schauff, 2001), Deep container and sweep methods **(**Adenusi *et al*.,2018). Arthropods collected were transferred separately into perforated plastic containers covered with lid before transporting them to the laboratory for identification and further study.

Collection of Arthropod Haemolymph

Haemolymph (1ml) was collected from each arthropod sampled using novel Antennae method of Haemolymph Sampling (Borsuk, 2017). In this method haemolymph were collected from selected arthropods that have been anesthetized on ice and carefully cleaned with 70% ethanol after excising the metathoracic legs and gently pressing the abdomen. Collection of haemolymph was carried out with micropipettes and drops collected were transferred to Eppendorf tubes (on ice) and kept in freezing (-11^{o}C) compartment (Duman and Horwath 1983) of refrigerator prior to analysis.

Sources and identification of *Trypanosoma brucei brucei*

Trypanosoma brucei brucei was sourced, identified and propagated by infecting four clean and healthy rats (donor rats) so as to produce more parasites to be used for the studies. Eighty (80) experimental mice were randomly grouped into 20 groups and each group of 4 mice were administered 0.3 µl of either 100% and 50% concentration of the different arthropod haemolymph intraperitoneally using 2ml syringe and a 25guage needle. The administration of haemolymph was carried out 4 days pre-infection of mice with *T. b. brucei* infected blood (2-3 parasites per microscopic field). Mice in the control group 1 were pre-treated 0.3µl with diminiazene aceturate 4 days before infection with *T. b. brucei* infected blood while mice in control group 2 were infected with *T. b. brucei* and no pre-treatment nor administration of haemolymph. Mice in all the groups were monitored every three days for a period of 21 days for parasitaemia. The number of parasites seen per field under the microscope was counted as described by Herbert and Lumsden (1976).

The parasite load of mice was monitored by microscopic examination of tail blood at X40 magnification under the light microscope, body weight in gram (g) of experimental mice was determined using Electronic Kitchen Scale (EK3250) and the Packed Cell Volume of mice was carried out using microhaematocrit method by Dacie and Lewis (1999).

Data Analysis

Data obtained were summarized using tables; T-test was used to compare the weight and PCV of mice in the prophylactic groups.

RESULTS

Prophylactic Effects of Sampled Arthropods Haemolymph in Mice

The prophylactic effects of haemolymph at 100% and 50% concentrations 4 days before infection with *T. b. brucei* is shown in Table1. All mice pretreated with arthropod haemolymph from grass hopper, cricket, cockroach, crab and diminazene (control 1)were able to clear *T. b. brucei* by the 15th day of experiment except mice pre-treated with haemolymph of scorpion and beetle before infection. In these 2 groups, the parasites were active till day 21, however, mice in the negative control group (2) died after day 6. Similarly, mice pretreated with 50% concentration of the various haemolymph 4 days before infection with *T. b. brucei* showed varied responses (Table1). Mice in group 3-7 administered grasshopper, cricket, cockroach, crab and diminazene (Control1) respectively 4 days before challenged with *T. b. brucei* recorded zero parasitemia by the 15th day of infection while mice in control group(2) that were not pre-administered standard drug nor arthropod haemolymph died by the 9th day.

Table 1: Effect of Sampled Arthropods Haemolymph at 100% and 50% Concentrations in Mice, pre-Treated then Infected with *Trypanosoma brucei brucei*

Group	P-Value	Haemolymph concentration (%)	Haemolymph	Parasitemia in days							
				Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
1	0.014	100	Scorpion	0	+	++	++	++	+	+	+
2			Beetle	0	+	+	++	++	++	+	++
3			G/ hopper	0	+	+	+	+	0	0	0
4			Butterfly	0	+	+	++	+	+	+	+
5			Cricket	0	+	+	+	+	0	0	0
6			Cockroach	0	+	+	+	+	0	0	0
7			Spider	0	+	++	+++	++	++	++	++
8			Crab	0	+	+	+	0	0	0	0
9			Diminiazene (Control 1)	0	+	+	+	0	0	0	0
10			Untreated (Control 2)	0	++	+++	-	-	-	-	-
1	0.0078	50	Scorpion	0	+	+	+	+	+	+	+
2			Beetle	0	+	+	+	+	++	+	+
3			G/ hopper	0	+	+	++	+	+	0	0
4			Butterfly	0	+	++	++	+	+	+	+
5			Cricket	0	+	+	+	+	0	0	0
6			Cockroach	0	+	+	+	+	0	0	0
7			Spider	0	+	++	++	+	+	+	+
8			Crab	0	+	+	+	+	0	0	0
9			Diminiazene (Control 1)	0	+	+	+	0	0	0	0
10			Untreated (Control 2)	0	++	+++	-	-	-	-	-

Key: 0 parasite (0), 2-4 parasites (+), 8-16 parasites (++), - parasite (no parasitemia observed due to death of mice

Effect of Arthropod Hemolymph on Weight of Mice

Mice in all groups that were administered 100% concentration haemolymph 4 days before infection with *T. b. brucei* showed a reduction in mean weight between Day 3 and Day9 except mice pre-treated with Diminiazene aceturate (Control 1) which maintained a slight increase in weight throughout the observation period (Table 2). However, mice in group 1-7 recorded slight decrease in weight between days 1-11 and an increase between day 12 and day 21while all mice in the untreated group (Control 2) died by day 9. Similarly, the weight of mice administered haemolymph at 50% concentration pre-infection with trypanosome parasites showed a slight change during the 21 days observation period (Table 2). Mice in all groups except untreated mice (control 2) recorded decrease in weight within the first 11 days and a slight rise in the weight from day 12. Mice that were not administered pre-infection treatment (control2) had reduced weight within the first 3 days and all died by the ninth day of infection. However, there is no significant difference in weight gain between the treated groups and control ($P<0.05$).

Table 2: Effect of Sampled Haemolymphat 100% and 50% Concentrations on Weight of Mice Pre- treated then Infected with *Trypanosoma brucei brucei*

Group	P-value	Haemolymph concentration (%)	Haemolymph	Mean Weight (g)in days							
				Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
1	0.99	100	Scorpion	22.2	22.0	21.8	21.6	21.6	21.7	21.9	21.9
2		100	Beetle	10.3	10.0	10.0	9.9	9.8	10.1	10.3	10.1
3		100	G/ hopper	21.8	21.4	21.2	21.3	21.6	21.6	21.3	21.5
4		100	Butterfly	22.0	21.3	20.4	19.2	19.8	20.2	21.1	21.8
5		100	Cricket	25.8	25.0	25.0	25.0	25.5	25.5	25.6	25.9
6		100	Cockroach	14.4	14.0	14.1	14.0	14.0	14.4	14.6	14.7
7		100	Spider	19.7	19.0	18.4	18.2	18.7	19.2	19.3	19.5
8		100	Crab	12.2	12.0	12.0	11.9	11.5	11.8	11.9	11.9
9			Diminiazene (Control 1)	25.2	25.0	25.5	25.5	25.7	25.8	25.8	25.9
10			Untreated (Control 2)	16.3	16.0	15.5	-	-	-	-	-
1	0.84	50	Scorpion	17.1	17.0	17.0	17.0	16.8	16.9	16.9	16.9
2		50	Beetle	14.0	14.1	14.1	13.8	13.8	13.9	14.0	13.9
3		50	G/ hopper	15.2	15.2	15.1	15.0	15.1	15.3	15.3	15.3
4		50	Butterfly	19.4	19.0	18.7	18.2	17.9	18.1	18.5	18.9
5		50	Cricket	12.3	12.0	11.8	11.8	11.7	11.8	11.8	11.8
6		50	Cockroach	15.1	15.0	15.0	15.0	14.9	14.9	14.8	14.8
7		50	Spider	14.5	14.1	13.8	13.5	13.2	13.4	13.9	14.1
8		50	Crab	13.2	13.0	13.3	13.2	13.0	13.1	13.2	13.1
9			Diminiazene (Control 1)	15.3	15.0	15.0	15.1	15.1	15.2	15.2	15.3
10			Untreated (Control 2)	15.9	15.8	15.3	-	-	-	-	-

Key: - parasite (no weight observed due to death of mice)

Effect of Arthropod Hemolymph on Packed Cell Volume of Mice

The mean PCV of mice administered with 100% haemolymph of sampled arthropods at 4 days pre-infection with *T. b. brucei* is presented in Table 3 Mice in groups 1-8 recorded decreased PCV levels from Day 3 through to Day 12 and slight rise in PCV levels from Day 15 through to Day 21. Same pattern was also observed in mice treated with Diminiazene aceturate (Control 1) while in negative control (Control 2), mice recorded continuous decrease in PCV level till death on Day 9. Similarly, mice in groups 1-9 that were administered 50% haemolymph 4 days prior to challenge with *T. b. brucei* recorded decreased PCV from Day3- 12 while all the mice in the non-treated (Control 2) group died on Day 9 (Table 3). However, the PCV levels of mice rose steadily from Day 15 through Day 18 and 21 in all the treated groups (1-9). Statistical analysis shows significant difference in mean PCV levels of mice that were administered 50% of the various haemolymph 4 days prior to infection ($P>0.05$).

Table 3: The Mean PCV Level of Mice Administered 100% and 50% Haemolymph Concentration Pre-infection with *Trypanosoma brucei brucei*

Group	P-Value	Haemolymph concentration (%)	Haemolymph	Mean PCV (%) in days							
				Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
1	0.62	100	Scorpion	41.5	40.5	39.3	37.0	36.1	36.8	36.9	37.5
2		100	Beetle	47.0	46.5	35.5	34.2	35.8	36.1	36.5	36.8
3		100	Grass hopper	52.5	52.0	50.0	49.0	49.1	49.4	49.9	51.3
4		100	Butterfly	50.4	48.9	48.2	48.0	48.4	49.0	49.2	49,3
5		100	Cricket	51.5	49.5	49.0	48.0	48.3	48.5	48.9	49.1
6		100	Cockroach	46.5	44.0	43.1	42.0	42.4	42.8	42.9	43.9
7		100	Spider	48.3	48.0	47.1	46.8	47.0	47.6	47.9	48.0
8		100	Crab	47.5	46.0	45.1	44.0	44.8	45.3	46.1	46.8
9			Diminiazene (Control 1)	42.0	42.0	39.5	37.5	37.9	38.1	38.6	38.9
10			Untreated (Control 2)	42.0	41.4	38.1	-	-	-	-	-
1	0.34	50	Scorpion	41.5	40.5	39.5	39.0	38.3	38.5	38.9	39.1
2		50	Beetle	47.0	46.3	43.5	38.5	37.9	38.4	38.9	39.7
3		50	Grass hopper	52.5	51.5	42.5	40.5	40.4	41.5	42.7	43.4
4		50	Butterfly	50.3	50.1	49.3	48.7	48.2	48.6	49.1	49.7
5		50	Cricket	51.5	50.3	47.5	46.0	46.6	46.9	47.3	48.0
6		50	Cockroach	46.5	45.2	44.0	42.0	42.8	43.2	44.0	44.5
7		50	Spider	45.9	44.1	43.7	43.1	42.8	43.2	43.7	43.9
8		50	Crab	47.5	46.9	43.0	43.3	43.0	43.8	44.7	45.5
9			Diminiazene (Control 1)	42.0	41.3	39.5	37.5	37.3	37.5	37.8	38.2
10			Untreated (Control 2)	44.0	43.6	41.9	-	-	-	-	-

Key: - parasite (no PCV observed due to death of mice)

DISCUSSION

Recent studies have shown the use of natural products in the production of drugs (Sadek *et al*., 2024). Haemolymphs have been studied for their anti-parasitic activities (Gressler *et al*., 2012 and Shaymaa *et al*. 2020). In this work, the trypanocidal ability of mice administered haemolymph of crab, cockroach before infection with *T. b. brucei* is in agreement with the work of Sadek *et al*. (2024), who reported *Sarcophaga argyostoma* larval haemolymph as a natural alternative to berenil in treating *Trypanosoma evansi in vivo*. Despite the trypanocidal ability possessed by these arthropod haemolymph in mice, they could not satisfy conditions for a good vaccine such as achieving 60% weight gain despite recording zero mortality in such groups. According to Kurata (2006), a good vaccine should produce up to 60% weight gain in experimental animals. However, the failure of these haemolymphs to meet the conditions for a good vaccine may probably be due to the fact that only a single dose of the arthropod haemolymph was administered to the mice few days before infection which may not have been enough for the mice to develop immunity against the parasite. Furthermore, route of administration of vaccine influences the quality and quantity of vaccine- induced immunity (Natalija *et al*., 2013; Husseini *et al*., 2019). Yang *et al*. (2020) suggested that immunization through intraperitoneal route results in superior antitumor immune response and tumor suppression when compared with other routes (subcutaneous, intratumoral). For full protection to be achieved vaccine should be administered at intervals for some days before infection because regardless of which vaccine is taken, full protection will not be achieved until after two weeks of final dose.

Chronic weight loss is a debilitating symptom of a wide range of diseases including those resulting from infections with bacteria, parasites and viruses (Wiley, 2020). The insignificant change in weight of mice after introducing haemolymph to the mice before infection could be as a result of the effect of treatment with sampled arthropod haemolymph, this supports the work of Fred and Awobajo (2011), the authors reported that haemolymph of *Achachatina marginata* exhibited a weight loss effect in normal rat but Bashir et al.(2015) in another study reported a significant increase in the body weight gain of albino rats when treated with haemolymph of *Achachatina marginata.*

The effect of arthropod haemolymph on the haematological parameter was studied. Using Packed Cell Volume (PCV) as an indicator of trypanosomiasis infection in mice, there was a general decrease in the mean PCV values of mice pre-treated with 100% concentration and 50% concentration of arthropod haemolymph before infection with *T. b. brucei*. At day 12, a gradual increase in the mean PCV was recorded in the mice of all prophylactic treatment groups. A similar observation was also made in the control group1(mice infected and treated with standard drug) but death of all mice was recorded in the control group 2 (mice infected without pre-treatment).There was a reduction in the mean values of PCV in all the haemolymph-treatment groups. From the 12th day of observation to the last day, a gradual increase in mean PCV value was later observed. Statistical analysis of the PCV values at all concentrations showed no significant difference at ($P<0.05$).The reduction in mean PCV value observed in this study is similar to the work of Shittu *et al*. (2017) who reported a decrease in PCV value of rats infected and not treated when compared to infected prophylactic treated and infected early treated. It is also in support of Wellde *et al*. (1974) who reported that there was a decrease in PCV during the first week of infection of cattle with *T. congolense*. Magez *et al*. (2011) and Naessens *et al*. (2005) also reported anaemia and cachexia as pathology associated with African trypanosome.

CONCLUSION

Mice administered prophylactic treatment with haemolymph of crab, cockroach and grasshopper eliminated trypanosome parasites in the blood of experimental mice, although, these arthropods could not satisfy conditions for a good vaccine. From this study, arthropods with prophylactic properties could be harnessed for their curative abilities against *T. b. brucei* infection.

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