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Antitrypanosomal Activities of Ethyl Acetate Extracts of Honey Bee (*Apis mellifera*) and Its Effect on Haematological Parameters of *Trypanosoma brucei brucei* Infected Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OKS designed the study, wrote the protocol, and final editing of the manuscript. Authors UE and BBM carried out the study. Author UE and author BL did the literature search and wrote the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Ethyl acetate extract of honey bee *Apis mellifera* (600 mg/kg/bw) was investigated for its effect on parasitemia and some haematological parameters in *Trypanosoma brucei brucei* infected rats.
Methodology: Five groups comprising of four mice per group were used in the study. Group 1, 2 and 3 were infected prophylactic treated, infected early treated and infected standard drug (berenil) treated (3.5 mg/kg/day) respectively. Group 4 and 5 serve as negative control (infected not treated) and normal control (uninfected not treated) respectively. The crude extract was partially purified

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using column chromatography to give fraction 1-3.
Results: The administration of the crude extract shows reduced parasitaemia and extension of life span from 5 days infected not treated (control) groups to 14 and 15days for infected prophylactic and infected early treated groups respectively. Also, the partially purified fraction 1 and 2 shows low parasitemia with survival of 6 days while that of fraction 3 is 3 days compared with infected untreated group which survive for 5 days. There were significant increase ($p<0.05$) in the haemoglobin (HB) concentration, packed cell volume (PCV), red blood cells (RBC) counts and white blood cells (WBC) counts of infected treated groups when compared with infected not treated group. Whereas, there was no significant difference ($p<0.05$) in the RBC and WBC counts of infected early treated group when compare with infected untreated group.
Conclusion: It can be deduced that methanol extract of *Apis mellifera* possessed antitrypanosomal activities with ameliorative effect against haematological symptoms of Africa trypanosomiasis.

Keywords: Honey bee; Apis mellifera; Trypanosomiasis; haematological; Parasitemia.

1. INTRODUCTION

African trypanosomes cause trypanosomosis or sleeping sickness in man and nagana in cattle's, for which about 300,000 new cases are reported annually in some 36 developing African countries of the Sahara. Where about 60 million people in 200 locations are exposed to the risk of infection [1].

The protozoan parasites that cause trypanosomiasis have been subdivided into two groups, the haematinic group (*Trypanosoma congolense*, *T. vivax*) which remains in the plasma and the tissue invading group (*T. brucei*, *T. evansi*, *T. gambiense*, *T. rhodesiense and T. equiperdum*) found in extra and intra vascular spaces [2].

Trypanosoma brucei infection precipitate increased red blood cell destruction which results in anaemia as well as tissue damage [3]. These changes together with the need by the host to destroy the parasite are presumably responsible for the symptoms of African sleeping sickness. Vector control strategies and chemotherapeutic agents are the means of eradicating this dangerous parasite. However, these drugs are expensive, limited in availability and poorly distributed in rural areas [4]. The antigenic variation exhibited by the parasites coupled with unsatisfactory effect of the existing drugs such as toxicity, drug resistance increase the need for the development of drug agents that is inexpensive, delay, suppress the growth, completely prevent the growth or kill the parasites or have the potentials to ameliorate anemia, a hall mark sign of trypanosomiasis in mammals [5]. Studies have shown that insects and their product possess several medicinal properties [6,7,8]. More so, several semi-synthetic and synthetic drug derivatives were originally isolated from natural compounds [9]. These suggest the need for exploring more medicinal insect for efficient and cheaper trypanocides.

Bees are flying insects closely related to wasps and ants, and are known for their role in pollination and for producing honey and beeswax.

Honey has been reported to acts as an antibacterial and antifungal agent. It has also been used as disinfect and speed the healing process in wound, crapes and burns [10;11]. However, the information on the uses of honey bee in the treatment of trypanosomiasis is inadequate. Therefore, this work evaluates the therapeutic potentials (*in vivo*) of ethylacetate extracts of honey bees (*Apis mellifera)* on *T. brucei brucei* and its effect on haematological parameters of the infected rat.

2. MATERIALS AND METHODS

2.1 Collection of Insects (Honey bees)

The insects were collected from apiculturist at Sanyo, Ibadan, Oyo state in the month of June 2013. The Honey bees were sun dried and pulverized and stored in an airtight container at room temperature till further use.

2.2 Laboratory Animals

Male albino rats weighing between 150-250 g were used for the experiment and they were obtained at Animal Facility Centre (AFC) of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The

experiment was conducted according to the Guide for the Care and the Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, USA [12].

2.2.1 Trypanosome

Trypanosoma brucei brucei was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Jos, Nigeria and maintained in the Laboratory of Biochemistry, Federal University of Technology Minnaby serial passage into other albino rats.

2.2.2 Extract preparation

Two hundred gram (200 g) of the insect powder was percolated in 1600 mL of absolute methanol and ethyl acetate room temperature for 72 hrs with constant agitation. The extract was filtered with muslin cloth afterwards and the filtrate was collected in a beaker, exposed to air and allowed to evaporate at room temperature to yield the extract concentrate [13,14].

2.3 Inoculation of Donor Rat and Infection of Animals

Blood from a highly infected rat was obtained by cardiac puncture and appropriately diluted with physiological saline to serve as inoculum. Healthy rat of weight range 150-250 g were infected intraperitoneally with 0.1 ml of the inoculum containing about 10^3 trypanosomes.

2.4 Experimental Design

The experiments were carried out in two stages as described by Shittu et al. [8], with slight modification. A total of 20 rats were used for this study. The rats were randomly divided into five (5) groups of four (4) rats each. Group 1 were infected with the parasite and administered 600 mg/kg body weight ethyl acetate extract of honey bee 72 hours before inoculation (prophylactic), group 2 were administered 600 mg/kg body weight ethyl acetate extract of honey from the day parasite was first sighted in the blood (early) and group 3 were given standard drug of berenil (3.5 mg/kg/day). Group 4 and 5 serve as negative control (infected not treated) and normal control (uninfected not treated) respectively. The extract was given once daily for 14 days. The parasitaemia levels were determined on a daily basis by microscopic examination of wet blood film. This was carried out using blood obtained from the tail on a grease free microscope slide. The crude extract was partially purified by column chromatography using Hexane, ethyl acetate and methanol to give fraction 1-3 respectively.

2.5 Haematological Studies

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), were determined according to method described by Dacie and Lewis, [15], using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan.

2.5.1 Ethical clearance

Ethical clearance was given by Federal University of Technology, Minna/Nigeria ethical review board (CUERB) in accordance with international standard on the care and use of experimental animals.

2.6 Statistical Analysis

Values were analyze using statistical package for social science (SPSS) and presented as means ± SE of the mean. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) the level of significance was set at $P < 0.05$[16].

3. RESULTS

3.1 Parasitaemia

Fig. 1 shows the parasitemia profile of *T. brucei brucei* infected rats treated with ethyl acetate honey bee extract. The administration of the crude extract shows reduced parasitaemia and extension of life span from 5days infected not treated (control) groups to 14 and 15 days for infected prophylactic and infected early treated groups respectively while Fig. 2 shows parasitaemia counts of *T. brucei brucei* infected rats treated with fractions of partially purified ethyl acetate crude honey bee extract for early treatment studies. The partially purified fraction 1-3 shows low parasite reduction and survive for 6, 6 and 3 days for fractions 1-3 respectively comparable with infected untreated group which survive for 5days.

3.2 Haematological Studies

There were significant increase ($p<0.05$) in the haemoglobin (HB) concentration, packed cell volume(PCV), red blood cells(RBC) counts and white blood cells (WBC) counts of infected treated groups when compared with infected not treated group. Whereas, there was no significant difference ($p<0.05$) in the RBCand WBC counts of infected early treated group when compare with infected untreated group (Table 1).

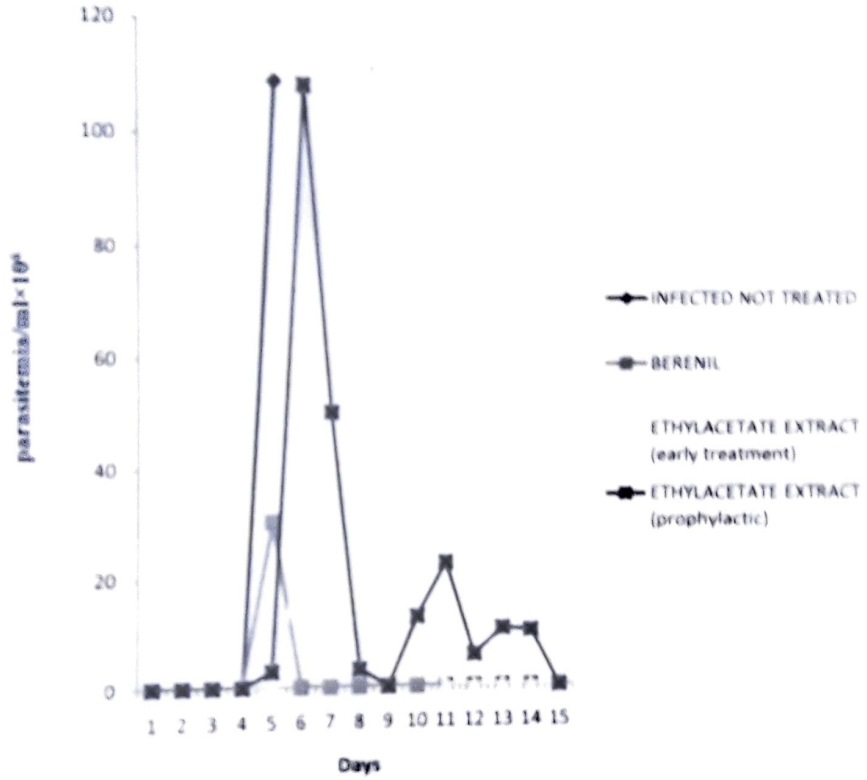

Fig. 1. Parasitaemia profile of *Trypanosoma brucei brucei* infected rat treated with ethyl acetate extract of honey bee

Table 1. Effects of daily administration of honey bee ethylacetate extracts on heamatological parameters for 14 days post *T. brucei brucei* infection

	PCV(%)	Hb(g/dL)	RBC(x10⁶ cells/μL)	WBC(x10³ cells/μL)
Infected not treated	35.5±1.50^a	11.83±0.50^a	3.45±0.55^a	3.00±0.20^a
Infected treated with berenil	53.67±0.33^b	17.89±0.11^b	4.80±0.76^b	6.53±0.74
Infected treated (early treatment) with ethyl acetate extract	53.5±0.50^b	17.83±0.17^b	3.20±0.00^a	3.50±0.00^a
Infected treated (prophylactic) with ethyl acetate extract	49.67±2.85^b	16.56±0.95^b	4.15± 0.05^b	4.33±0.89^a

Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4). Values with different superscript are significantly different ($p<0.05$)

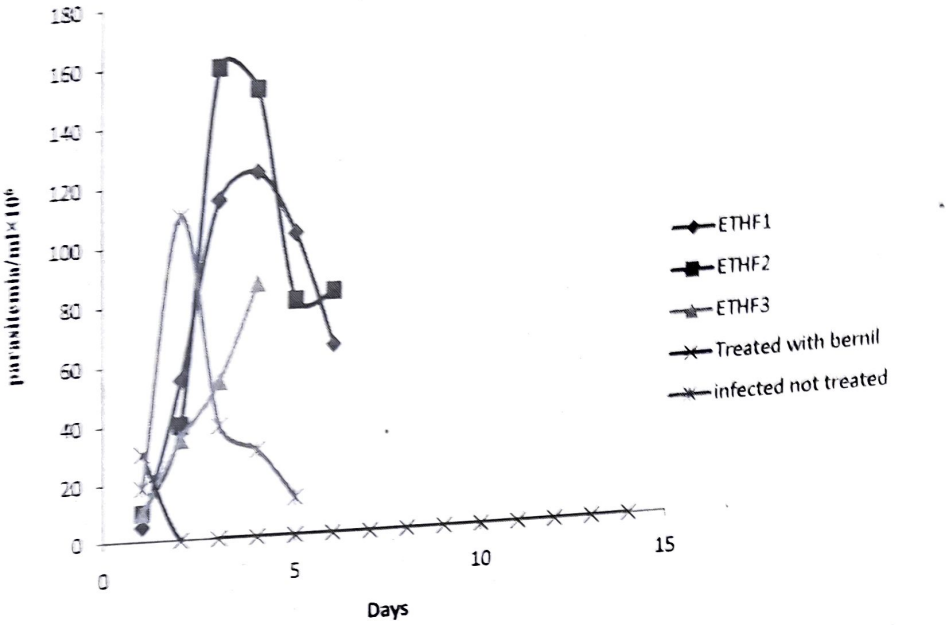

Fig. 2. Parasitaemia profile of *Trypanosoma brucei brucei* infected rat treated with ethyl acetate fractions of honey bee

Key: ETHF: Ethyacetate fractions

4. DISCUSSION

Many studies have been carried out in recent years on the pharmacological effect of bee products. The bee products have been reported to have analgesic, antimicrobial, anti-inflammatory and immunological benefits [17]. This study is the first attempt to demonstrate the in vivo activities of honey bee on Trypanosomiasis.

The administration of ethyl acetate honey bee extract to *T. brucei brucei* infected rats was able to reduce the parasitemia level of infected prophylactic and early treated group which survived for 15 days when compared with infected not treated group where the parasitic load increased infinitelyand survive only for five days. This is in agreement with pervious work of Exarem and Yusuf [6].

The composition and physiochemical properties of honey bee may be responsible for its therapeutic properties [18]. Although; the mechanism by which these honey bee extract elicit it trypanocidal action was not determined. It has however been documented that many natural product exhibit their trypanocidal activity through interference with the redox balance of the cellular defense against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to alterations in redox balance [19].

Administration of partially purified and ethylacetate extracts of honey bee on *T. brucei* infected rat could not survive beyond the 6 days as compared to the crude extracts that survive for 15 days. Therefore, this implies that partitioning of the crude extract resulted in loss of activity and that the crude extract has component that are acting synergistically to elicits the antitrypanosomal activity. This finding is in agreement with the work of Park et al. [20], who find out that the basic therapeutic mechanism of insect is a synergy involving several compounds. Also similar cases of loss of activity due to fractionation have been reported in a study conducted by Noedl et al. [21], where fractionation of the methanolic extracts of *Eucalyptus camaldulensis* (leaf) resulted in decrease of antiplasmodial activity.

Haematologial indices such as packed cell volume (PCV) were studied to assess the toxic

effect of the parasite on blood component. Anaemia is one of the established major pathological features of African *Trypanosomiasis* [22]. Therefore, the presence and severity of anaemia are good indicators of disease status. The significant decrease level of PCV and RBCs of the infected untreated rats observed in this study is an indication of anaemic condition caused by the parasite. Similar findings have been reported by Shittu et al. [8]. In addition to this, the parasite also stimulates certain cells to produce R.O.S thereby resulting in haemoglobin degradation [23]. The increases in PCV observed for infected ethyl acetate honey bee crude extracts treated rats in comparison with infected not treated group suggests that the extracts reduce the severity of *T. brucei brucei* infection in rats. This phenomenon is normal due to the effect of the extracts on the cells in the blood especially the red blood cells (RBCs). Also the observed increases in Hb and RBC concentrations are probably as a result of reduced severity of the infection. The increased WBC is also indicative of the increased host action in the presence of honey bee crude extracts against the infection, as this will contribute to the development of phagocytes and antibodies against the recognizable antigens of parasite origin.

5. CONCLUSION

It can be concluded from this study that ethyl acetate extracts of honey bee contain some bioactive compounds that have *antitrypanosomal* property disturbances. Therefore, honey bee extracts can be further exploited for the developmentof new drug for African Trypanosomiasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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