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Effects of Methanol Extract of *Musca domestica* Larvae on Antioxidants Enzymes in *T. Brucei* Infected Rats

Shittu, O. K., Bashir, L. and Isaac, O. O.

Tropical Disease Research Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 65, Minna, Nigeria

Abstract: The anti-trypanosomal potential of crude methanol extract of *Musca domestica* was investigated in *T. brucei* infected rats. Twenty (20) rats were intraperitoneally infected with the parasite and grouped into 4 (A-D) of 5 animals each. Group A rats received 1 mL of distilled water, groups B and C received 400mg/kg body weight of the extract, in prophylactic and suppressive model respectively, while group D received 3.5mg/kg of diminazene aceturate (Berenil®). A control group containing 5 rats was also set up. Results obtained showed that the extracts extend the lifespan from day 5 of the rats that received distilled water to 7 and 11 days and reduced the level of parasite replication for prophylactic and early treated (26% and 49% respectively) on the last day. There were significant ($p<0.05$) decrease in serum catalase (CAT) activities of control (normal) group when compare with other experimental groups but not significantly ($p>0.05$) difference with prophylactic treated rats. Serum superoxide dismutase (SOD) activities were significantly ($p<0.05$) higher in rats that received distilled water when compare with other experimental group but not significantly difference when compare with early treated group. The results suggest that methanol extract of *Musca domestica* probably has trypanocidal properties as well as the ability to reduce parasitaemia and the severity of the disease. The extract also possesses some antioxidant properties that could serve as a source for new drugs lead for the treatment of trypanosomiasis.

KEYWORDS: *Musca domestica,* Anti-trypanosomal, Antioxidant enzymes, Parasitemia

1.0 Introduction

Trypanosomiasis is a complex debilitating and fatal disease caused by one or more of the pathogenic tsetse transmitted protozoan parasites of the genus *Trypanosoma* (Abenga *et al.*, 2004).

Upon invasion trypanosomes proliferate rapidly to establish its population in infected host (Pentreath and Kennedy, 2004) and release toxin (Ekanem *et al*., 1994; Shittu *et al*., 2013a). The antibodies produced by the host against the parasite are not effective because the parasite have the ability to produce a large repertoire of antigens. In the process, organs are invaded by trypanosomes including the central nervous system (Sternberg, 2004: Gloria, 2012)

The chemotherapy of African trypanosomiasis remains unsatisfactory and besieged with numerous problems such as toxicity, lengthy periods of administration, lack of efficacy and unaffordable for most of the patients (Legros *et al*., 2002). Therefore, the search for new drug lead and formulations that are safe, affordable and effective against both early and late stages of the disease is highly recommended. Insects and their products are recently considered alternatives to conventional therapy as they constitute a rich source of bioactive chemicals against number of parasite (Fred-Jaiyesimi and Awobajo, 2011).

Housefly (*Musca domestica*) is a major domestical medical and vertinary pest of the family musidae, Class Insecta and Order Diptera. It is the most common of all domestic flies accounting for about 91% of all flies in human habitation it is considered has a pest and can carry serious disease like typhoid fever and cholera. However, biological studies have reported that larvae of housefly have anti microbial (Teich and Myers, 1986),

***Corresponding Author**
Tel.: +234 8033883658
E-mail: toscueyusuf@gmail.com

immunoactive antiviral and antitumor (An *et al.*, 2004), antibacterial (Park *et al*., 2010) and antimalaria activity (Shittu *et al.*, 2013b).

However reports are lacking on the antitrypanosomal activities of this insect. This study therefore aimed to evaluate the efficacy of the larvae of *musca domestica* on prophylactic and early trypanosome infection with a view of bridging the gap in knowledge and providing baseline information for the use of zootherapy against trypanosomiasis. It is hope that this insect extracts will provide alternative mechanism with little or no toxic effects as in the case of some plants.

2.0 Materials and Methods

2.1 Collection of maggots

Musca domestica (House fly) maggots were collected in the month of February, 2014, from Bosso poultry dung in Minna, Niger state, Nigeria.

2.2 Parasite strain

Trypanosomal brucei brucei was as obtained from the Parasitology Section of Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. The parasite was maintained in other rats by repeated passaging.

2.3 Experimental animals

Healthy albino rats of average weight 120-150 g were purchased from Animal House, University of Ibadan, Oyo State Nigeria. (ILAS,1997).The rats were kept in clean plastic cages and maintained under standard laboratory conditions (temperature: 22±3ºC; photoperiod: 12 h natural light and 12 h dark; humidity: 40-45%).The animals were maintained on standard animal feeds (Bendel Feeds and Flour Mills, Edo State, Nigeria) and clean tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (ILAS,1997) were duly observed.

2.4 Preparation of extract

Matured House flies were allowed to lay on the poultry dung and developed to fouth instar stage and later collected. They were washed and killed in salt water. These were dried in the shade at room temperature until constant weight was attained and pulverized to powder using an electric blender. 200 g of the powder was percolated in 1600ml of absolute methanol and kept in the shade for 48 hours after which it was filtered. The filtrate was collected in a beaker, exposed to air and concentrated using rotary evaporator **(**Adebayo *et al*., 2003)

2.5 Parasite inoculation and treatment of animals

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. The suspension was as described by Ekanem *et al* (2006) contained 3 or 4 trypanosome per view at x100 magnification.

The animals were divided into the following groups and were treated accordingly:

Group 1-Negative Control were inoculated with *T. brucei* parasite and received no treatment

Group 2-were inoculated with *T. brucei* parasite and received 400mg/kg of extract. The extract administration started 3 days before the inoculation of the parasite (prophylactic).

Group 3-were inoculated with *T. brucei* parasite and treated with 400mg/kg of extract. The treatment started on the first day the parasite was sighted in the blood (early treated)

Group 4-were inoculated with *T. brucei* parasite and treated with 0.2 ml containing 3.5mg/kg of diminazene aceturate (Berenil®)

Group 5-Positive Control received 0.2 ml of distilled water (vehicle for drug administration)

2.6 Parasite count

Parasitaemia was monitored and checked daily according to the method of Herbert and

Lumsden (1976). The percentage parasitaemia was determined by counting the number of trypanosomes per view under the light microscope at X100 magnification and percentage parasite reduction was calculated using the following expression:

$$= \frac{\text{Infected Untreated-Infected Extract Treated} \times 100}{\text{Infected Untreated}}$$

(Ekanem *et al*., 2008)

2.7 Preparation of serum

Collection of blood sample for biochemical analyses was as described previously (Yakubu et al., 201). Rats were anaesthetized in slight chloroform and blood samples collected into clean, dry centrifuge tubes by cardiac puncture. Blood samples which were processed individually were allowed to stand for 10 minutes at room temperature and then centrifuged at 1000 rpm for 15 minutes on a laboratory centrifuge. The supernatant (serum) was carefully removed with Pasteur pipette, and stored frozen until needed for further analysis.

2.8 Determination of total protein concentration and enzyme activity

The serum total protein concentration was estimated by biuret method as described by Gornall *et al*., 1949). Catalase activities was determine as described by Johansson and Bors, (1988) while superoxide dismutase (SOD) activities was determined adopting the procedure described by Woollian *et al* (1983).

2.9 Statistical analysis

Values were analyzed using statistical package for social science (SPSS) version 16 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 $P = 0.05$ (Adamu and Johnson, 1997)

3.0 Results

The parasitaemia count of the infected rats that received distilled water increase infinitely while infected rats treated with *Musca domestica* maggot extract shows a decrease in proliferation (figure 1). The graphs shows low replication of parasite before relapse occur and extension of surviving days in prophylactic infected treated (PRO) and infected early treated group (IET) from 5 days of control (infected untreated) to 7 days and 11 days respectively at the dose of 400mg/kg body weight.

The percentage parasite reduction of 38%, 71%, 61% and 54% for day 2 to day 5 respectively in the prophylactic treated group. The highest percentage parasite reduction of 71% was recorded on day 3 of the experiment for prophylatic while the highest percentage parasite reduction in the infected early treated group was 89% on the third day of experiment (Table 1).

Results of specific superoxide dismutase activities are presented in Figure 2.The specific superoxide dismutase activities was significantly ($p<0.05$) raised in the infected rat receiving distilled water only when compare with positive control rats and other experimental groups but not significantly($p>0.05$) difference when compare with early treated group.

Results of specific catalase activities are presented in Figure 3. The specific catalase activities were significantly ($p<0.05$) higher in all the experimental group when compare with the positive control values. However no significant ($p>0.05$) difference in catalase activities of prophylactic treated rats when compare with negative control.

4.0 Discussion

The used of insect and insect product in treatment of various ailment is increasing around the globe. During trypanosome infections the parasite are sequestered in several organs including the liver (Akpa *et al*., 2008), leading to gradual tissue damage (Ekanem and Yusuf, 2008). This effect has been reported to be directly related to the severity of anaemia and levels of parasitaemia (Anosa, 1988).

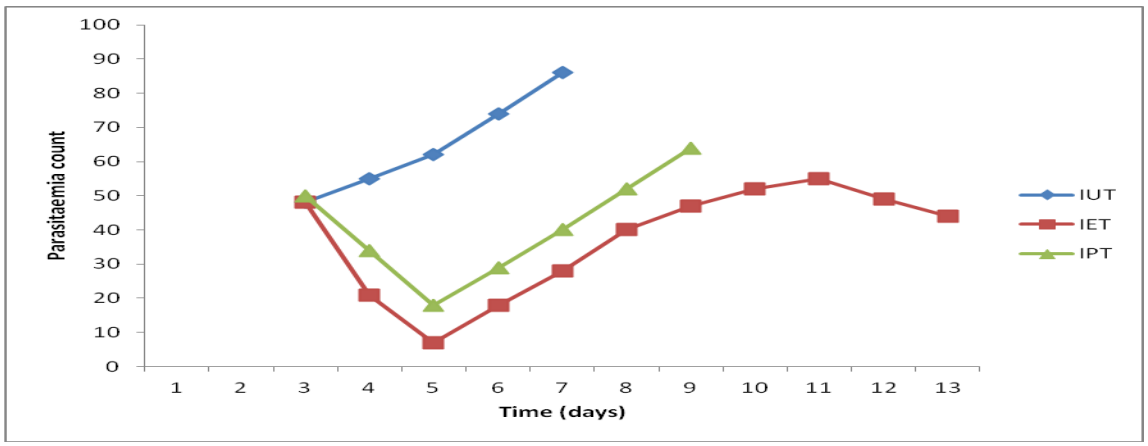

Figure 1: Parasitaemia count of *trypanosome brucei* infected rats treated with methanolic extract of *Musca domestica* maggot

IUT- infected not treated; IPT- infected prophylactic treated; IET-infected early treated

Table 1: percentage parasite reduction of *T. brucei* infected rat treated with methanolic extract of *Musca domestica*

Days	Prophylactic		Early treated	
	Mean parasitaemia	Parasite reduction (%)	Mean parasitaemia	Parasite reduction (%)
1	50.00±3.21	-	48.10±3.01	0
2	34.00±2.04	38	21.56±2.21	62
3	18.97±3.11	71	7.06±3.09	89
4	29.09±1.22	61	18.29±0.99	76
5	40.23±3.07	54	28.03±1.21	67

Data are Mean ± SEM of four determinations

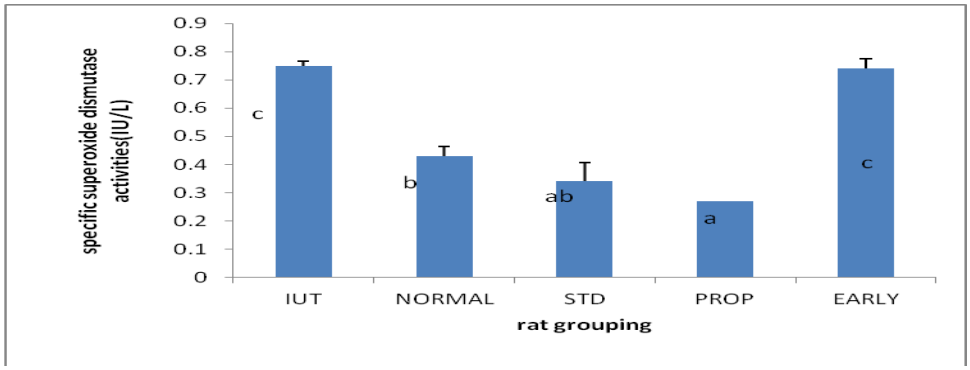

Figure 2 . Specific activities of superoxide dismutase in the serum of rat infected with *T. brucei*. Bars carrying different letters are significantly different at $p<0.05$. IUT-infected not treated; IST-infected treated with standard; PROP-infected prophylactic treated; EARLY-infected early treated; Normal-Control

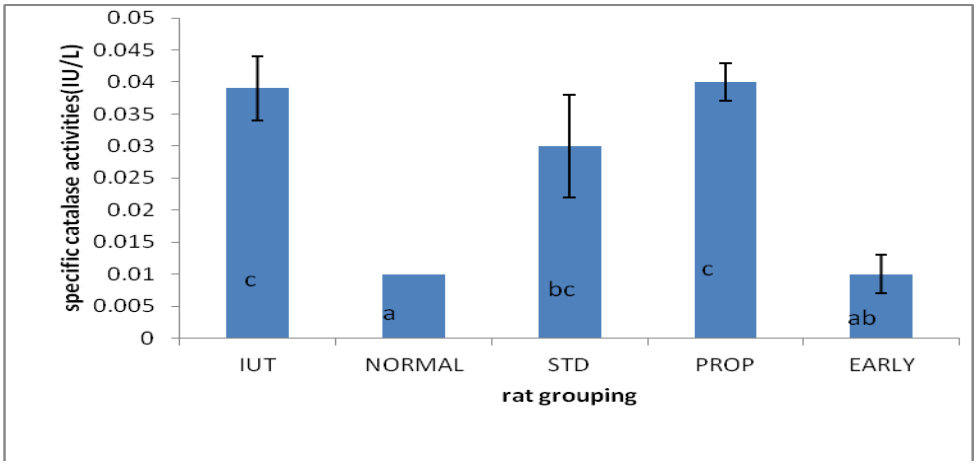

Figure 3. Specific activities of Catalase in the serum of rats infected with *T. brucei*. Bars with different letters are significantly different at $p<0.05$. IUT- infected not treated; IST- infected treated with standard; PROP- infected prophylactic treated; EARLY- infected early treated; Normal- Control.

Removal of the parasite from the system and simultaneously boosting the host immune system could be very relevant in the control of African sleeping sickness (Hoet *et al.*, 2004). A compound with reduction in parasitemia ≥ 30% is considered active (Carvalho, et al., 1991). *Musca domestica* has trypanocidal properties by the ability to lower the parasite replication of prophylactic and early treated rats, as well as the ability to extend the life span of *T. brucei*-infected rats (Figure 1). Despite the removal of the parasites from the blood, the infected rats still died by Day 7 and 11 for prophylactic and early treated rats suggesting the involvement of agents that are not necessarily life parasites. Factors extracellularly derived from the parasites (Ekanem *et al*., 1996) could be responsible for the death.

Oxidative stress play important etiologic role in the pathogenesis of African trypanosomiasis (Ogunsami and Taiwo, 2007). Alteration in enzyme level may result from the effect of the trypanosome lyses resulting from effect of the host defense mechanism (Pentreath and Kennedy, 2004). Antioxidant system are normally put in place in living aerobic organism to counter the effect of oxidative stress (Elstner and Oswald, 1994).The functions of antioxidant system in the body is very important for the removal of free radicals. Superoxide dismutases reduce the concentration of highly reactive superoxide radicals by converting it to H_2O_2 whereas catalase convert H_2O_2 into H_2O and O_2 and protect the tissue from a highly reactive hydroxyl radical (Chance and Greenstein, 1992).

The results of the effect of the extract on endogenous antioxidants show that the infected control had higher catalase than normal control. This may be because their antioxidant defense system, which included catalase was mobilized to fight the presence of the parasites. It appeared that the antioxidant defense system of these animals was not yet suppressed or exhausted at the very early stage of infection. . Importantly, the extract was able to decreased catalase levels of early treated rats compared to infected negative control rats. Perhaps the extract was able to provide some antioxidants components and spared the use of endogenous catalase, to fight trypanosomes-generated free radicals (Yusuf *et al*., 2012).

The significant increase in superoxide dismutase activities of all the experimental group when compare with the positive control (normal) probably validate early findings that infection could gradually alter enzyme level (Kennedy, 2004). Ataley *et al* (2000) also reported that under condition of oxidative stress, activities of antioxidant enzymes such as SOD, increases as seen in the present work the extract caused increased SOD activity of early treated rats and decreased SOD activity of prophylactic

treated rats compared to normal control. The decrease observed in prophylactic treated rats may reflect the activities of accumulated antioxidant components of the extracts, however increased SOD activity of early treated rats indicate the mobilization of endogenous SOD

In conclusion, the available results from this study suggest that methanol extract of *Musca domestica* probably has trypanocidal properties as well as the ability to reduce parasitaemia and the severity of the disease. The extract also possesses some antioxidant properties that could serve as a source for new drugs lead for the treatment of trypanosomiasis.

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