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Full Length Research Article

Effect of Methanol extract of *Musca domestica* larva on some Enzymes and Haematological parameters in *Trypanosoma brucei brucei* - infected rats

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ABSTRACT

This study investigated the effect of methanol extract of *Musca domestica* (400mg/kg body weight) on some biomarker enzymes and haematological parameters in *Trypanosoma brucei brucei* - infected rats. Twenty albino rats were intraperitoneally infected with *Trypanosoma brucei brucei* and were grouped into five (5) groups of four (4) rats each. Group1 was set up as infected not treated (0.2ml normal saline/kg body weight), group 2 was treated with diaminazene aceturate (standard drug), group 3 as prophylactic treated (treatment for 72 hours before inoculation of parasite), group 4 as early treatment with the extract (treatment commenced after the sight of parasite) and group 5 as the control (uninfected untreated) group. Results shows significant ($p<0.05$) decrease in liver AST and ALT activities with concomitant increase in serum activities of the infected untreated rats when compared with the early treated, prophylactic treated, standard treated and normal control. Serum ALP activity of the infected not treated group was significantly ($p<0.05$) higher when compared to the control group and other experimental groups. No significant ($p>0.05$) difference in the liver ALP activities of the extract treated infected groups with standard drug treated group However, serum and liver GGT activities of the uninfected untreated (control) was significantly lower ($p<0.05$) than all the other experimental groups. Haematological studies shows significant decrease ($p<0.05$) in packed cell volume (PCV) , haemoglobin concentration (Hb) and red blood cell count (RBC) of infected not treated when compared to infected prophylactic treated and infected early treated. There was likewise significant increase in white blood cell count (WBC) of infected not treated compared to infected prophylactic treated and infected early treated. Findings from this study showed that methanol extract of *Musca domestica* larva has trypanocidal properties thereby ameliorating the *T. brucei* induced biochemical changes in rats.

Key words: *Musca domestica* larva, Haematology, Trypanosomiasis, Enzymes, Methanol, Extract.

INTRODUCTION

Sleeping sickness, otherwise called Human African Trypanosomiasis (HAT), is one of the neglected tropical diseases of sub-Saharan Africa. It has plagued human health and agricultural development (Aksoy, 2011). The disease is caused by a protozoan parasite known as trypanosomes. It is transmitted to the mammalian host through the bite of an infected tsetse fly (Sara *et al*., 2004). There are three different sub-species of *T. brucei* which cause different variants of trypanosomiasis. *T. brucei gambiense* which gives rise to a chronic disease that mainly affects humans, is common in Central and Western Africa. However, *T. brucei rhodesiense* which causes fast onset acute trypanosomiasis in humans is common in East Africa (Ene *et al*., 2014). African Animal Trypanosomosis (AAT) is caused by *Trypanosoma brucei brucei, T. vivax and T. congolense* (Mergia *et al*., 2014).

Up to date, the fight against this disease relies chiefly on old chemotherapy and chemoprophylaxis, which are expensive, toxic with severe and fatal side effects with problem of crossing the blood brain barrier (Muhd-Haffiz *et al*., 2013). Also, the appearance of drug resistant trypanosomes aggravate the problem, which calls for more research into the development of

alternative, less toxic and efficient drugs (Bashir *et al*., 2015a).

The use of insects and their products constitute essential ingredients in the preparation of drugs in folklore medicine (Fred-Jaiyesimi and Awobajo, 2011). House fly (*Musca domestica*) is the most common of all domestic flies, accounting for about 91% of all flies in human habitation. It is one of the major insect that is globally distributed (Marek *et al*., 2012). According to Teich and Myers, (1986), the larva of *Musca domestica* possess therapeutic effect against Osteomyelitis, ecthyma, decubial necrosis, and lipid. Extractives from house fly has been cited for their antioxidants activities (Shittu *et al*., 2014), antimalarial (Shittu *et al*., 2013a), antibacterial, immuno activator, antiviral and antitumor agents (An *et al*., 2004).

Assessments of biochemical parameters have been reported to be a useful marker for assessment of tissue damage (Lawal *et al*., 2016a). Deficiencies in the activities of these markers mirror the degree of level of infection and toxicity of test compounds (Yakubu *et al*., 2006). It has been established that trypanosomal infection causes alteration in biomarker enzymes (Ekanem and Yusuf, 2005). This study therefore gives the impact of methanol extract of *Musca domestica* on some enzymes and haematological parameters in *T. brucei* infected rats.

MATERIALS AND METHODS

Collection of *Musca domestica*

Larva stage of *Musca domestica* (House fly) was obtained from poultry dung, in Bosso, Minna, Niger state, Nigeria. It is located on Latitude 09 31N and Longitude 07 58E and about 20 km north of Abuja the Federal Capital of Nigeria. The larva were identified and authenticated by an entomologist in the Department of Biological Science, Federal University of Technology, Minna., Nigeria.

Experimental Animals

Healthy albino male rats of average weight 150-180g were obtained from Animal House, of the University of Ibadan, Oyo State Nigeria. The rats were housed in plastic cages and maintained under standard laboratory conditions in Department of Biochemistry, Federal University of Technology Minna. They had free access to rat pellets and tap water *ad-libitum*. Ethical Clearance was given by Federal University of Technology, Minna/Nigerian Ethical Review Board (CUERB) in accordance with International standard on care and use of experimental animals.

Preparation *Musca domestica* extract

Matured House flies were allowed to lay on the poultry dung and later developed to fourth instar (larva) stage. These were collected, washed in salt water (10 %) and dried in the shade at room temperature for two weeks and thereafter pulverized to powder using an electric blender. A 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 allowed to evaporate for 2 weeks at room temperature to yield the extract concentrate (Shittu *et al*., 2013).

Inoculation of rats with parasite

Trypanosoma brucei brucei was obtained from Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Kaduna state, Nigeria. Parasite infected blood was obtained from the tail of infected rats at high parasitaemia and was suspended in 0.90% saline solution until a solution of 3 to 4 parasites per field was achieved. This was used to inoculate the healthy rats through the peritoneal cavity.

Parasitaemia Counts

Parasitaemia was monitored on daily basis as described by Adeyemi *et al*., (2009). A thin film of blood was obtained from animal tail and viewed under light microscope at x100 magnification.

Treatment of Animals

The animals were divided into groups and treated according to their body weight as follows:
Group 1 – was inoculated with *T. brucei brucei* parasite and received no treatment
Group 2 – was inoculated with *T. brucei brucei* parasite and treated with 400 mg/kg of diminazene aceturate (berenil),
Group 3 –was inoculated with *T. brucei brucei* parasite and treated with 400 mg/kg extract prophylactic.
Group 4 –were inoculated with *T. brucei brucei* parasite and early treated with extract dose of 400 mg/kg body weight
Group 5 – Uninfected and received 0.2 ml of distilled water (positive control)
The prophylactic treatment started 3 days before inoculation of *T. brucei brucei* parasite and lasted for 7 days while other treatment started first day the parasite was sighted in the blood and lasted for 7 days.

Serum and Liver Collection and Preparation

Blood sample was collected as described previously (Akanji *et al*., 2013). Briefly, the animals were anaesthesized with chloroform. Blood was collected by cutting the jugular vein and carefully drained into heparinised and EDTA bottles. This was followed by centrifugation at 3000 rpm for plasma preparation. The plasma samples were collected and kept in a freezer (-20°C) until needed for biochemical analyses. The blood samples collected into EDTA bottles were used immediately for haematological analyses. The liver were excised and transferred into ice-cold 0.25 M sucrose solution. This was later homogenized in ice-cold 0.25 M sucrose solution [1:5w/v] and the supernatant was used for biochemical analysis

Serum and Liver Enzyme Assays

The enzymes: alkaline phosphatase (ALP) (EC 3.1.3.1), aspartate aminotransferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2) and gamma glutamyl transferase activities were assayed using diagnostic enzyme assay kits protocol of Randox Laboratories Limited (Reitman and Frankel, 1957). All measurements were done using UV Spectrophotometer (Shimadzu model uv 1800).

Haematological parameters

Automated haematologic analyzer SYSMEX KX21, (SYSMEX Corporation, Japan) was used to determine the haematological indices including the packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and Haemoglobin concentration (Hb).

Statistical analysis

Data were presented as mean ± SEM. The data obtained were subjected to Analysis of Variance (ANOVA) using SAS statistical package. Means were separated using Duncan's Multiple Range Test (DMRT) at 95% ($p < 0.05$) level of significance

RESULTS

Aspartate Transaminase Acitivity

Elevated levels ($p<0.05$) of liver AST activities was observed for all experimental groups except for the infected not treated group (Group 1) as presented in Figure 1. However, serum AST was significantly low in group treated with standard drug, prophylactic treated and early treated groups compared to uninfected untreated (normal) group (Figure 1).

Alanine Transaminase Activity

The liver ALT activities were significantly increased in uninfected untreated, early treated, prophylactic treated and standard treated (diaminazene aceturate) groups when compared to the infected untreated group. But there was significant ($p<0.05$) decrease in the serum ALT of standard treated, prophylactic treated and early treated groups when compared to infected untreated group (Figure 2).

Results are mean of four determinations ± S.E.M. Bars carrying different letters are significantly different at $p<0.05$. INT: infected not treated STD: infected treated with standards PROPH: infected prophylactic treated ET: infected early treated, UUT: uninfected untreated

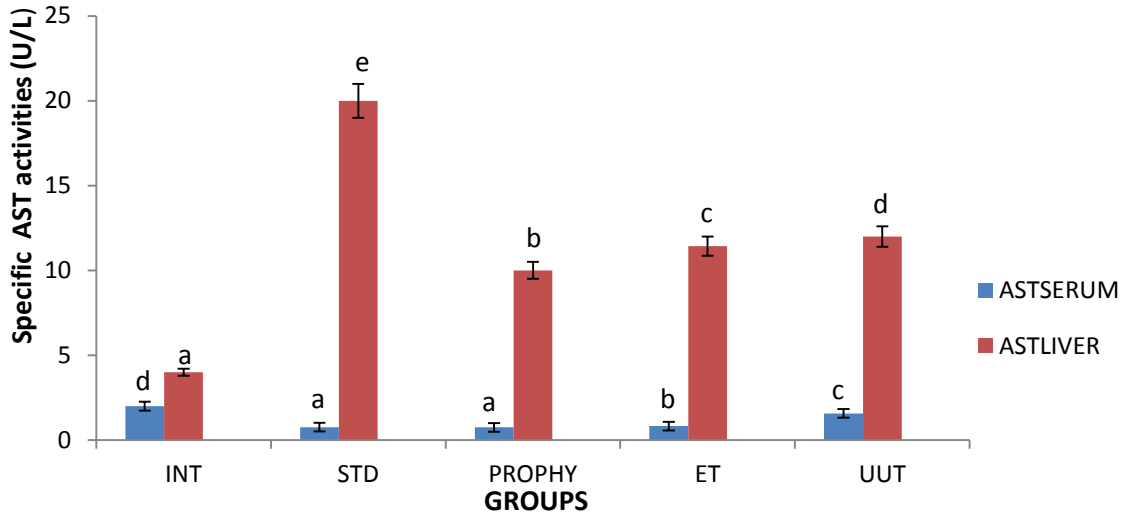

Figure 1: Specific activities of Aspartate transaminase in serum and liver of *T. brucei brucei* infected rats treated with methanolic extract of *Musca domestica* larva.

Results are mean of four determinations ± S.E.M. Bars carrying different letters are significantly different at $p<0.05$. INT: infected not treated STD: infected treated with standards PROPH: infected prophylactic treated ET: infected early treated, UUT: uninfected untreated.

Alkaline Phosphatase Acitivty

The result of alkaline phosphate activity as presented in Figure 3 shows that there was no significant difference ($p<0.05$) in the liver ALP activities of the infected treated with standard group when compared to the control group, whereas other groups showed a significant increase. Also, serum ALP activity of the infected not treated group was significantly higher than the others when compared to the control group (Figure 3).

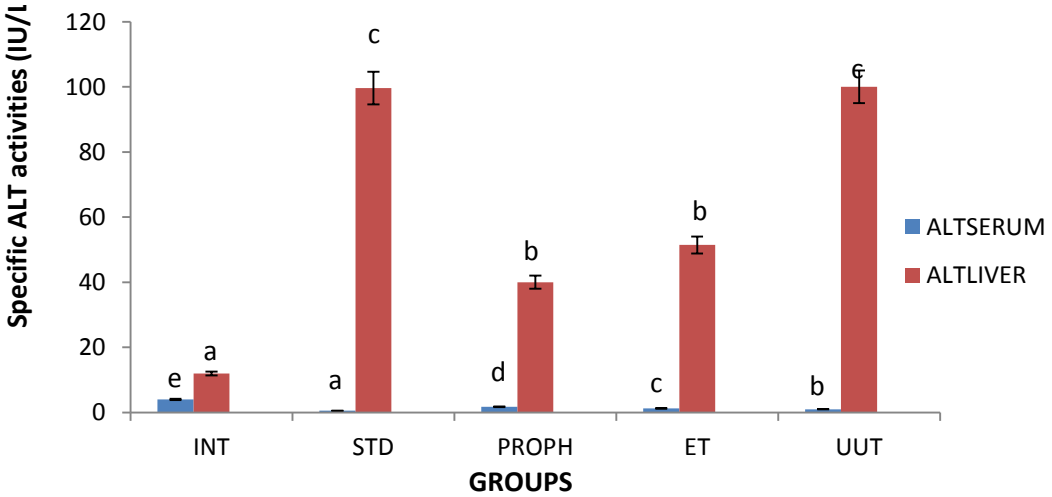

FIGURE 2: Specific activities of alanine transaminase in serum and liver of *T. brucei brucei* infected rats treated with methanolic extract *Musca domestica* larva.

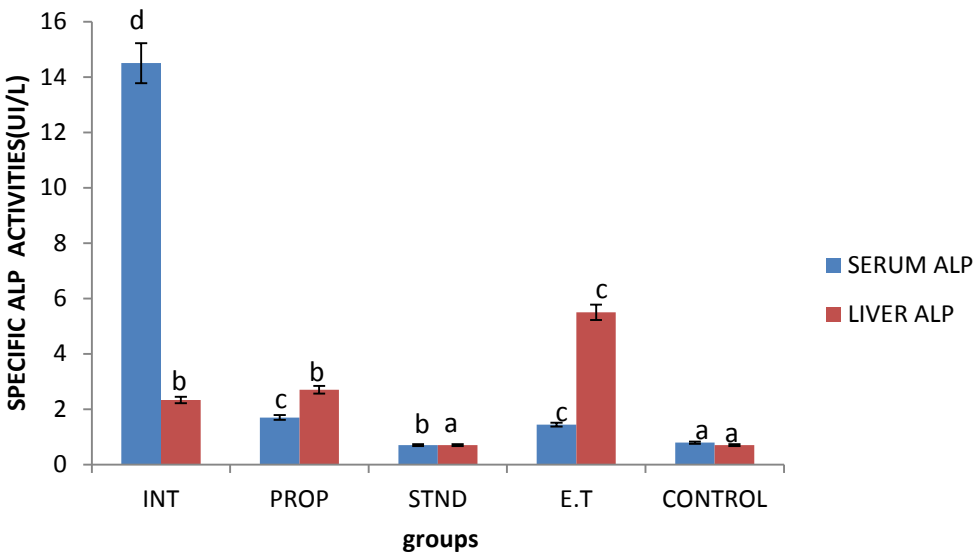

Figure 3: Specific activities of alkaline phosphatase in serum and liver of *T. brucei brucei* infected rats treated with methanolic extract of *Musca domestica* larva

Results are mean of four determinations ± SEM bars carrying different letters are significantly different at P<0.05.
INT: infected not treated STD: infected treated with standards PROPH: infected prophylactic treated ET: infected early treated, UUT: uninfected untreated

Gamma Glutamyl Transferase

The liver GGT activities of the uninfected untreated (control) was significantly lower (p<0.05) than all the other experimental groups. Likewise, the activities of serum GGT increased significantly in all the groups when compared to the control group. Although, the activities in the infected prophylactic treated with extract and infected treated with standard drug was significantly higher than the infected not treated and early treated groups (Figure 4).

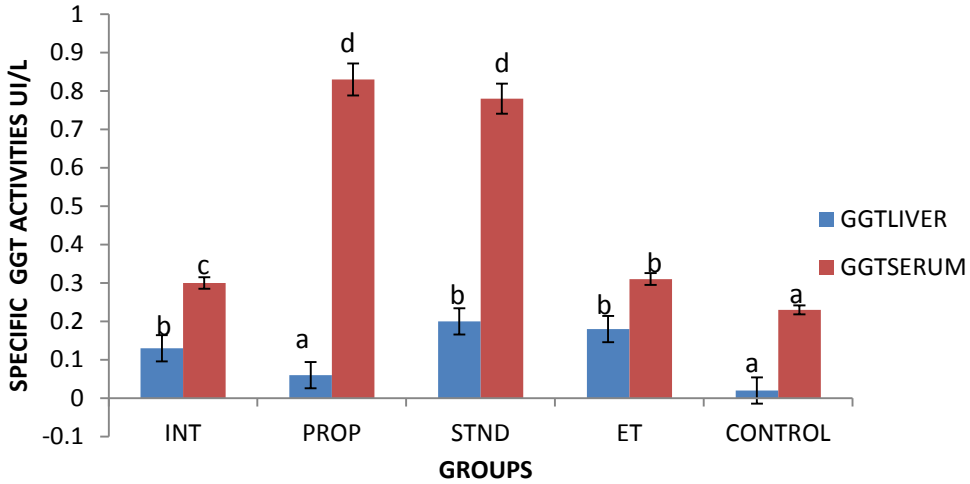

Figure 4: Specific activities of gamma glutamyl transferase (GGT) in liver of rats infected with *T. brucei brucei*.

Results are mean of four determinations ± SEM. Bars carrying different letters are significantly different at p<0.05. INT: infected not treated STD: infected treated with standards PROP: infected prophylactic treated ET: infected early treated, UUT: uninfected untreated

Haematological studies

The results of haematological parameters studied are as presented in Table 1. There was significant decrease (p<0.05) in the values of Hb (3.40±0.10 g/dl), PCV (10.20±0.25 %), and RBC counts (0.50±0.15 $\times10^{12}$/L) of infected not treated group in comparison with the normal control, infected early treated and prophylactic treated groups. However, the white blood cell count (4.75±0.25 $\times10^{9}$/L) of infected not treated group were significantly higher (p<0.05) in comparison with the control and other experimental groups.

Table 1: Haematological studies of T. *brucei brucei* infected rats treated with methanolic extract of *Musca domestica* larva

	RBC ($\times10^{12}$/L)	WBC($X10^{9}$ /L)	PCV (%)	HB(g/dl)
IUT	0.50 ± 0.15^{a}	4.75 ± 0.25^{c}	10.20 ± 0.25^{a}	3.40 ± 0.10^{a}
STND	2.15 ± 0.25^{c}	2.25 ± 1.25^{a}	12.20 ± 0.25^{ab}	4.08 ± 0.08^{ab}
PROP	1.60 ± 0.20^{b}	3.75 ± 0.25^{b}	17.20 ± 0.25^{bc}	5.74 ± 0.08^{b}
ET	1.70 ± 0.10^{b}	5.25 ± 0.50^{c}	22.00 ± 3.00^{c}	7.38 ± 1.00^{c}
Control	2.79 ± 0.32^{c}	2.03 ± 0.43^{a}	15.30 ± 0.42^{b}	5.25 ± 0.45^{b}

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

DISCUSSION

The practice of using larvae (maggots) of *Musca domestica* as therapy against certain pathogenic and bacterial disease infections have been around for many years. In earlier studies, the anti-inflammatory and anti-sclerotic functions of housefly maggots have been found (Bexfield *et al*., 2004). They have also been found to regulate endothelial cell dysfunction through decreasing cell proliferations and migration (Hou *et al*., 2007).

Analyses of serum enzymes have proved to be very important. Many diseased states are related to alter of enzyme activity and measurement of their activities maybe diagnostic for many diseases. These disease conditions can result in elevation of an enzyme activity (Nelson and Cox, 2005). Alkaline phosphatase (ALP) is a protein found in body tissues such as liver, bile ducts and bone. Serum alkaline phosphatase activity is a very useful serum biochemical indicator of liver disease (Lawal *et al*., 2016). The significant increase in serum ALP activities in untreated rats when compare with those treated with the extract confirm the earlier report that infection could gradually affect enzyme level (Kennedy, 2004) by increase activation of the enzyme molecule in situ and release of membrane component (including alkaline phosphatase) into the extracellular fluids (Yakubu *et al*., 2005). However, rat treated with methanol extract of *M. domestica* (early treated group and prophylactic treated group) shows ameliorative effect as it cause significant decrease in the serum activities of the elevated ALP.

Gamma glutamyl transferase (GGT) is an enzyme present in the cell membrane of many tissues including the kidneys, bile duct, pancreas, heart and spleen (Mayne, 2005). It is derived from endoplasmic reticulum of the cells of the hepatobiliary tract (Shittu et al., 2013b). As this reticulum proliferates, for example in response to drugs, synthesis of the enzyme is induced and plasma GGT activity increase.

Therefore a rise in plasma activity recorded in this study does not necessarily indicate hepatocellular damage. this study agrees with the previous works of Abdulhazeez *et al*., (2013) and Shittu et al., (2013b) who also reported increase serum GGT activities in *T. brucei* infected treated rats when compared with the control.

AST and ALT are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage; the ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST (Bashir *et al*., 2015b). In the present study, infection of the rats with *T. brucei* caused significant ($p<0.05$) decreases to the levels of liver AST and ALT activities with concomitant increase in serum activities of the infected untreated rats when compared with the normal control. These observations confirms the previous report that parasitic infectious disease gradually affect enzyme activities by causing elevation of serum activities of AST, ALT and ALP which indicate liver damage (Uraku, 2016). However, treatment with methanol extract of *Musca domestica* larvae (400 mg/kg b.wt) during early treatment and prophylactic treatment regime significantly restored the serum and liver activities of AST, ALT towards the reference value (Figure 1 and 2). Previous study have reported that methanol extract of *Musca domestica* larvae significantly decrease *T. brucei* replication and extend the lifespan of the animals compared to the untreated control (Shittu et al., 2014). This is an indication of the effectiveness of the extract in ameliorating the effect of *T. brucei* infection.

African trypanosomiasis is characterized by haematological changes, which drastically influence the pathogenesis of the disease (John *et al*., 2006). This finding is supported by the decline in red blood cell (RBC) counts, haemoglobin (Hb) concentration and packed cell volume (PCV) observed in the infected untreated group. These results confirmed that anaemia is a critical feature in the pathogenesis of African trypanosomosis (Ekanem and Yusuf, 2005). However, the increase in packed cell volume (PCV), Haemoglobin (Hb) and Red blood cell (RBC) concentrations observed in the infected prophylactic treated and infected early treated groups compared to infected untreated group suggested that the extract reduces the anaemic effect of *T. brucei* in rats. White blood cells are responsible for defending the body against infections or any foreign bodies (Berinyuy *et al*., 2015). The significant increase in WBC of infected untreated rats may indicate the stimulation of immune system against *T. brucei* infection in the rats.

CONCLUSIONS

It is concluded that methanol extract of *Musca domestica* larva has trypanocidal properties. This was demonstrated by ameliorative effects on *T. brucei* induced biochemical and hematological changes. Thus, *Musca domestica* extract may be exploited as good candidate in the development of trypanocidal drugs.

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