



TRYPANOCIDAL ACTIVITY AND HEAMATOLOGICAL CHANGES IN *T. BRUCEI* INFECTED RATS TREATED WITH METHANOLIC LEAF EXTRACT OF *THYMUS VULGARIS*

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ABSTRACT

Drugs for African trypanosomiasis are beset with different problems including cost, toxicity and increasing resistance. 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. In this study, the efficacy of locally used medicinal spice *T. vulgaris* in the treatment of trypanosomiasis was investigated. Two different stages of infection (prophylactic and early) were investigated using a dose of 500mg/kg body weight. The two groups were observed to show low rate of replication of parasite and extension of surviving days (8 days) than that of the infected not treated (6 days). Also, some haematological changes in *T. brucei* infected rats treated with methanolic extract of *thymus vulgaris* leave were investigated. The result show significant decrease ($P < 0.05$) in packed cell volume (PCV) of infected not treated compared to infected prophylactic treated and infected early treated. There was also significant decrease haemoglobin (Hb) concentration and red blood cell (RBC) count of infected not treated compared to infected prophylactic treated (72 hrs before inoculation) and infected early treated (After sighting of parasite). Whereas, there was no significant change in white blood cell (WBC) count of infected not treated group compared to uninfected untreated (normal) group. Therefore, this investigation showed that *T. vulgaris* has antitrypanosomal potentials by ameliorating effect caused by *T. brucei* infection on some haematologicals parameters.

Keywords: *Thymus vulgaris*, Antitrypanosomal, haematological parameters.

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INTRODUCTION

Trypanosomiasis, a disease of major importance in human and animals has continued to threaten human health and economical development of the affected areas (WHO, 2009). World health organization report that 66 million people in 36 African countries are affected and animal trypanosomiasis causes the death of 3 million cattle each year (WHO, 1998; Truc, 2002). The control of this disease relies principally on old chemotherapy and chemoprophylaxis. (Anene *et al.*, 2001) However, these are beset by numerous limitations including toxicity and resistance by the parasite (Moore, 2005). These limitations prompted the need for more research into the development of a less toxic drug with little side effects. It has been reported that the parasitaemia correlates with the severity of infection and also further complicated by anaemia, thrombocytopaenia and leucopaenia (Anosa, 1988; Davis, 1982; Suliman and Feldman, 1989; Biryomumaisho and Katunguka-Rwakishaya, 2007) all or some of which may be related observable pathological consequences of infection. It is estimated that some 20,000 species of higher plants are used medicinally throughout the world (Tagboto and Townson, 2001). *Thymus vulgaris* is a species of the mint Family LAMIACEAE. It is a valuable medicinal plant used for antiseptic skin tones and in several traditional medicines for treating coughs and cold. It has been shown to have antimicrobial activity against fungi, viruses, helminths, gram positive bacteria (including botulinum) and gram negative bacteria. The medicinal effect has been attributed to its oil components, thymol and carvacrol (Samaiya *et al.*, 2011). However, we have earlier reported that the administration of methanolic leaf extract *thymus vulgaris* at 500 mg/kgbody weight to *Trypanosoma brucei*-infected rats shows changes in the serum and liver specific

activities of some biomarker enzymes (Shittu *et al.*, 2013)

In this study, the effect of the methanolic leaf extract of *thymus vulgaris* on parasitaemia levels and some haematological parameters were evaluated in *T. brucei brucei* infected rats.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *T. vulgaris* were purchased from Minna Central Market, Niger State, Nigeria in the months of May/ June 2012 and authentication was carried out at Department of Biological Science, Federal University of Technology, Minna, Niger state.

Plant extraction

Fresh leaves of the plant were dried at room temperature and pulverized to powder using an electric blender, 200g of the powder was percolated in 1600ml of absolute methanol and kept in shade for 48 hours after which it was filtered (Adebayo *et al.*, 2003). The solvent was removed from the filtrate using rotary evaporator.

Trypanomes

Trypanosome brucei brucei infected blood was obtained from the tail of infected rats at high parasitemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritoneal cavity of uninfected rat weighing approximately 250 g. The suspension as described earlier (Ekanem and Yusuf, 2005; Ekanem *et al.*, 2006) contained 3 or 4 trypanosomes per view at x100 magnification (approximately 106 cells per ml).

Parasite Count

Parasite count in infected rats was carried out on daily basis to monitor the progress of infection until the animals died. Parasitaemia

was determined by counting the number of trypanosomes per view under the light microscope at x100 magnification from thin blood smear freshly obtained from the tip of the tail of infected rats.

Experimental Design

The experiments were carried out in two stages. Each group of experiments in the two stages contained five rats. In the first stage, infected rats were administered 500mg/ kg body weight of *thymus vulgaris* methanolic leaf extract on daily basis 72 hours before inoculation (prophylactic) and also from the day parasite was first sighted in the blood (early) until the rats died. The control group for this stage was infected not treated. In the second stage, whole blood was obtained from late stage infected rats (Day 6 post infection) as well as from the uninfected- not treated and uninfected extract treated rats. The control group for second stage was uninfected- not treated (normal).

Haematological indices

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), mean cell

haemoglobin concentration (MCHC) and platelet count were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (1991).

RESULTS

Parasitaemia count

The parasitaemia result showed a reduction in the replication of parasite and extension of life span for prophylactic infected treated compared to infected not treated which died after 6 days. Also, there was extension in the life span of infected early treated. (Fig 1)

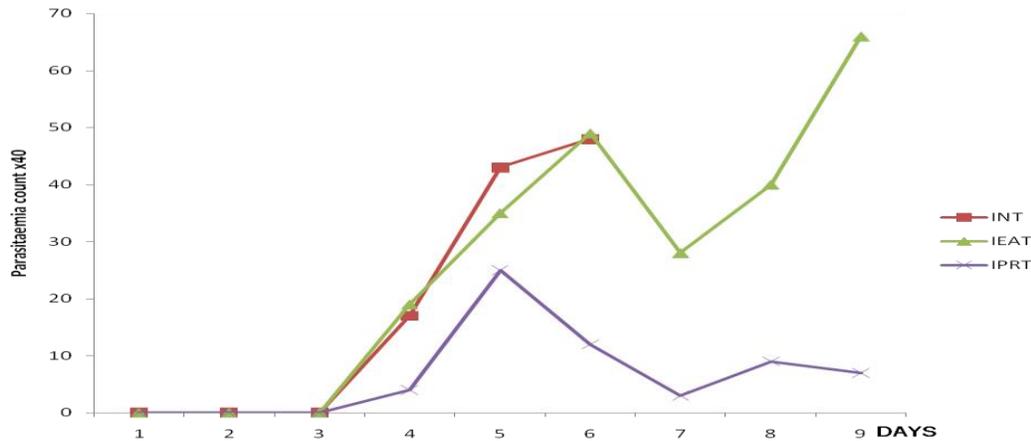


Fig1 : Parasitaemia count of rats infected with *T. brucei* and treated with methanolic leaf extract of *Thymus vulgaris*

INT: Infected Not Treated
 IPROT: Prophylactic Infected Treated
 IEAT: Infected Early Treated

Haematological studies

The results of haematological studies are presented in Table 2. There were significant

decreases ($p < 0.05$) in the value of Hb, PCV and RBC of infected not treated group in comparison with infected treated groups and uninfected untreated group (Table 1).

Table 1: Haematological studies of *T.brucei* infected rats for 6 days post infection.

| Grouping | PCV (%) | Hb (g/dl) | RBC ($\times 10^{12}/l$) | WBC ($\times 10^{10}/l$) |
|-------------------------------|------------------------------------|--------------------------------|-------------------------------|--------------------------------|
| Uninfected untreated | 42.50 \pm 0.00 ^a | 14.16 \pm 0.000 ^a | 2.200 \pm 0.00 ^a | 1.425 \pm 0.000 ^a |
| Infected Treated | Not 23.50 \pm 0.005 ^b | 7.83 \pm 0.005 ^b | 0.320 \pm 0.01 ^b | 1.325 \pm 0.990 ^a |
| Infected Prophylactic Treated | 32.50 \pm 0.068 ^c | 10.83 \pm 0.067 ^c | 2.040 \pm 8.12 ^a | 2.375 \pm 0.720 ^b |
| Early Treatment | 33.00 \pm 0.081 ^c | 11.00 \pm 0.081 ^c | 0.815 \pm 0.02 ^c | 0.875 \pm 0.320 ^a |
| Uninfected extract Treated | 31.00 \pm 0.041 ^c | 10.34 \pm 0.041 ^c | 0.850 \pm 0.03 ^c | 6.250 \pm 0.000 ^c |

Means along the same column with different superscript are significantly different at ($P < 0.05$), values are means of four determinants \pm S.E.M.

DISCUSSION

Upon invasion of the mammalian system trypanosomes proliferate rapidly to establish its population in infected host (Poltera, 1985; Pentreath and Kennedy, 2004). Toxins are released into the host (Boutignon *et al.*, 1990; Ekanem, 1989; Ekanem *et al.*, 1994, 1996). 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, all organs are invaded by trypanosomes including the central nervous system (Sternberg, 2004). Previous studies have shown that many tropical plants contain constituents that are clinically efficacious against many protozoal diseases (Gbile and Adesina, 1987; Le Grand, 1989). The trypanocidal activities of certain plant extracts have been reported to be due to the alkaloids and other constituents present (Oliver-Bever, 1986; Taurus *et al.*, 2002). Many studies have been carried out

on nutritional beneficial (Smith *et al.*, 2006) and therapeutic effect of (Judd *et al.*, 2003)

thymus vulgaris. It has been reported that the oil components: thymol and carvacrol has analgesic, antibacterial and antifungal properties (Samaiya *et al.*, 2011).

Hepatomegaly that occurs in *T. brucei* infection has been reported to be directly related to the severity of anaemia and levels of parasitaemia (Anosa, 1988). Therefore, the result of the parasitaemia shows that Methanolic extract of *Thymus vulgaris* has trypanocidal properties by the ability to extend the life span of *T. brucei* infected rats as well as lower the replication of prophylactic treated rats (Fig 1).

The increase in haematocrit or packed cell volume (PCV), observed for infected treated prophylactic infected early and uninfected extract treated compared to infected untreated suggested that the extract reduces the severity of *T. brucei* infected in rats. Also, the observed increases in Haemoglobin (Hb) and Red blood cell (RBC) concentrations are probably as a result of reduced severity of infection. The increased White Blood Cell (WBC) Prophylactic and uninfected treated extract are also indicative of the increased host action in the presence of the extract against the infection.

Therefore, it can be suggested that methanolic leaf extract of *thymus vulgaris* could be useful agent for management of Africa sleeping sickness.

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