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## Haematological Parameters and Enzyme Studies in *Trypanosoma brucei*-infected Rats Reared on *Nigella sativa* Oil-based Diet

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### ABSTRACT

The effect of *Nigella sativa* (black seed) oil based diet on the management of *Trypanosoma brucei* infected rats was investigated. Parasitaemia was monitored and haematological and enzymatic studies were carried out on liver and blood of infected rats fed with diet formulated with black seed oil (treatment). Comparison was made with untreated infected rats and uninfected rats. The diet formulated with black seed oil extended the life span for 7 and 5 days for prophylactic and early stage feeding, respectively while the late stage feeding extended it by a day. The *T. brucei*-infection significantly ( $p < 0.05$ ) increased liver Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Gamma Glutamyl Transaminase (GGT). Serum GOT and GPT specific activities were also significantly ( $p < 0.05$ ) increased. However serum GGT activity showed no significant ( $p < 0.05$ ) difference in all the rat groups. While haemoglobin concentration, white blood cells, red blood cells, pack cell volume, mean cell haemoglobin concentration and platelet were significantly ( $p < 0.05$ ) reduced, as observed in the control and late stage feeding, by the infection, prophylactic and early stage feeding with diet formulated with *N. sativa* oil significantly ( $p < 0.05$ ) improved the parameters towards those obtained in uninfected rats. We concluded that the *N. sativa* oil used in formulated diet could be involved in the improvement of the pathologic events observed in the *T. brucei* infected rats with prophylactic and early stage feeding.

**Key words:** *Nigella sativa* oil, diet, trypanosomiasis, enzymes, parasitaemia, haematological parameters

### INTRODUCTION

Trypanosomiasis, caused by African trypanosome continues to be a major threat in the Tropical Africa (Abenga and Lawal, 2005; Chreihen and Smoak, 2005). Drug regimen are cumbersome in addition to being expensive (Kioy and Mattock, 2005; Moore, 2005). The search for new drugs and formulations that are safe and effective against both the early and late stages of the diseases is recommended (Jannin and Cattand, 2004; Chibale, 2005; Pink *et al.*, 2005). Formulations and natural products, which boost the host immune system and possibly reduce parasitaemia or completely remove parasite from the host system could contribute extensively to the control or management of the disease (Hoet *et al.*, 2004; Chibale, 2005). We have earlier reported that administration of honey supplemented diet extended the life span of *T. brucei*-infected rats by 6,

5 and 3 days for prophylaxis, early stage and late stage treatments respectively (Ekanem *et al.*, 2006). Honey is a natural product reported to have immune boosting properties (Abuharfeil *et al.*, 1999).

The seed of *Nigella sativa* is known through out the world with different names such as seed of blessing (habbat-ul baraka), black cumin, black caraway, kalonji, etc. *Nigella sativa* has been used for centuries, both as an herb and pressed into oil, by people in Asia, Middle East and Africa for medicinal purposes. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support and for general overall being (Ferdous *et al.*, 1992). In the Unani Tibb System of Medicine, *Nigella sativa* has been regarded as a valuable remedy in a number of diseases. Ibn Sina (980-1037A.D), most famous for his volume called The Canon of Medicine regarded by many as the most famous book in the history of medicine, refers to *Nigella* as the seed that stimulates body's energy and help recover from fatigue and dispiritedness and several therapeutic effects on digestive disorders, gynecological diseases and respiratory system were ascribed to the oil. It is also included in the list of natural drugs of ebb e nabwi or prophetic medicine according to the tradition hold on to the use of black seed for in it is healing for all diseases except death (Sahih Bukhari). Recent reports however suggest that thymoquinone present in the oil might be the active component (Tekeoglu *et al.*, 2006; Khattah and Nagi, 2007).

In this report, we assessed the immuno-regulatory potential of a diet formulated with black seed oil and its effects on some liver and serum enzymes in *T. brucei*-infected rats in order to assess its possible relevance in the control or management of African Sleeping Sickness.

## MATERIALS AND METHODS

This study was carried out from March to July, 2007. Federe strain of *T. brucei* was obtained from the Veterinary and Livestock Studies Department, Vom, Plateau state, Nigeria and was used to infect albino rats from which parasite infested blood was collected for subsequent infection of the experimented rats. The black seed oil used for this work was extracted using soxhlet method (Soxhlet, 1879). Assay kits for glutamate pyruvate and glutamate oxaloacetate transaminases were products of Randox laboratory Ltd., United Kingdom and that of gamma glutamyl transaminase was a product of Biolab. SA, Maizy, France.

**Feed formulation:** The feeds used for feeding the infected and normal rats were compounded using corn starch and soy bean as the sole sources of carbohydrate and protein respectively while soy bean oil and black seed oil were used as the sources of lipids in control diet and treatment diet, respectively (Table 1).

**Inoculation of rats:** Parasite infested blood was obtained from the tail of infected rat at high parasitaemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated (about 0.3 mL) into the peritoneal cavity of uninfected rats. The suspension contained three or four trypanosomes per view at x100 magnification (approximately  $10^6$  cells mL<sup>-1</sup>).

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

Table 1: Composition of the formulated diets (g kg<sup>-1</sup>)

Feed components	Weight
Corn starch	476.0
Soy bean	250.0
<i>N. sativa</i> oil (soybean oil for control)	80.0
Rice husk	40.0
Vitamin/mineral mix	50.0
D-L-methionine	4.0
Sucrose	100.0

Vitamin/mineral mix (per kg of diet): Thiamin hydrochloride, 6 mg; Pyridoxin hydrochloride, 7 mg; Nicotinic acid, 2 mg; Calcium pantothenate, 16 mg; Biotin, 0.2 mg; Cyanocobalamin, 0.01 mg; Retinal palmitate, 4000 IU; Cholecalciferol, 1000 IU; Tocopherol acetate, 50 IU; Menadione, 0.05 mg; Choline chloride, 2 g; CoCl<sub>2</sub>.6H<sub>2</sub>O, 0.001 g; CuSO<sub>4</sub>.5H<sub>2</sub>O, 0.079 g; MnSO<sub>4</sub> 0.178 g; KI, 0.032 g; KH<sub>2</sub>PO<sub>4</sub> 5.559 g; CaSO<sub>4</sub>, 5.25 g; NaCl, 5.573 g; ZnCO<sub>3</sub>, 1.6 g; FeSO<sub>4</sub>.7H<sub>2</sub>O, 1.078 g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 2.292 g

**Anaesthetization of animals and collection of tissues:** Rats were anaesthetized using cotton wool soaked in chloroform. Blood samples were collected by cardiac puncture using needle and syringe from where they were transferred into centrifuge tubes.

The blood was allowed to clot for 10 min at room temperature and then centrifuged using laboratory centrifuge (SM800B, Surgified Medicals, England) at 3000 rpm for 5 min. The serum was then separated with a clean Pasteur pipette and stored frozen until required for use (Ogbu and Okechukwu, 2001).

**Experimental design:** A total of forty male albino rats (*Rattus norvegicus*) with average weight of 250±5 g were obtained from the animal holding of the Department of Biochemistry, University of Ilorin, Nigeria. Four groups of rats with 5 in each group were infected with *T-brucei brucei*. The control group was infected with the parasite and reared on diet formulated with soybean based oil. The prophylactic group was maintained on black seed oil formulated diet 72 h before infection, the early group was infected and the feeding with black seed oil formulated diet commenced immediately the parasite was sighted in the blood of the infected rats. The late group was infected with the parasite and maintained on feed with soybean-based oil until 72 h prior to their expected deaths as confirmed from the control experiment before the feed was substituted with black seed oil formulated diet. Parasitaemia was monitored until all the rats died. In the repeat of the experiments the rats were sacrificed 24 h before the expected death of the control animals and haematological parameters such as Haemoglobin concentration (Hb), Pack Cell Volume (PCV), Red Blood Cell count (RBC), White Blood Cell (WBC), Mean Cell Haemoglobin Concentration (MCHC) and platelet count were determined on the blood samples collected from the rats using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the method described by Dacie and Lewis (1984). Protein concentration was determined using the method of Gornall *et al.* (1949) as described by Plummer (1978). Serum and liver specific activities of GPT, GOT and GGT were also carried out on liver and blood of uninfected rats, infected rats fed with the diet formulated with soybean oil as well as those fed with diet formulated with black seed based oil.

**Statistical analysis:** Data are presented as mean of five replicates ±standard error of mean (SEM). Analysis of variance was carried out. Level of statistical significance was taken at p<0.05 (Adamu and Johnson, 1997).

**RESULTS**

Prophylactic feeding extended the lifespan of the infected rats by 7 days while early and late stage treatments extended it for 5 days, respectively. At the peak parasitaemia early treatment showed lowered parasitaemia when compared with the control (Fig. 1). Figure 2 presented a significant increase ( $p < 0.05$ ) of the Glutamate Pyruvate Transaminase (GPT) specific activities in the control and late stage treatment compared with the normal while early stage and prophylactic treatments showed significant decrease ( $p < 0.05$ ) when compared with the control group. The control and late stage treatments showed a significant increased ( $p < 0.05$ ) in the specific activity of AST when compared with the normal while prophylactic and early stage treatments compared favorably with the normal value (Fig. 3).

Figure 4 showed no significant difference ( $p < 0.05$ ) in the serum GGT activities among the normal, control and infected treated rats. However, liver GGT activities of the control and the late

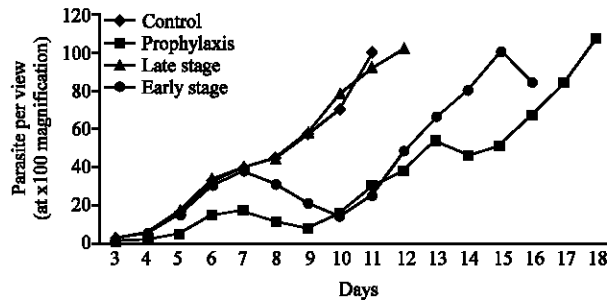


Fig. 1: Levels of parasitaemia in *T. brucei*-infected rats reared on diets formulated with the extracted black seed oil and soy bean containing diet

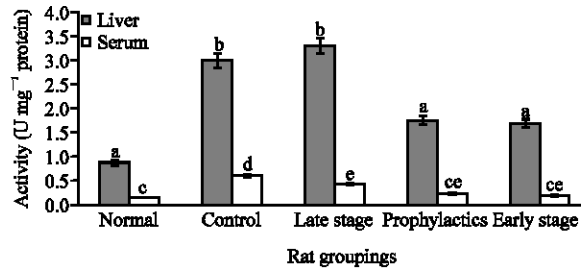


Fig. 2: ALT activities in experimented rats. Bars with different letters are significantly ( $p < 0.05$ ) different. Each value is a mean of 5 determinations  $\pm$ SEM

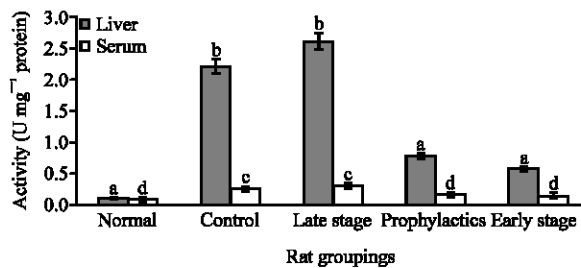


Fig. 3: AST activities in experimented rats. Bars with different letters are significantly ( $p < 0.05$ ) different. Each value is a mean of 5 determinations  $\pm$ SEM

stage groups showed a significant increase ( $p < 0.05$ ) when compared with the normal while those of early stage and prophylactic treatments compared favorably with the normal values.

There was an increase in the serum protein concentration of the prophylactic and early stage treatments when compared with control and late treatment (Table 2). The increase was only significant ( $p < 0.05$ ) in the early stage treatment. Liver protein concentration showed a significant decrease ( $p < 0.05$ ) in the protein level of the control and late stage treatments when compared with normal value, prophylactic and early stage treatments. Normal liver protein concentration was however significantly ( $p < 0.05$ ) higher than those of prophylactic and early treatments.

Prophylactic and early stage treatments showed a significant increase ( $p < 0.05$ ) in WBC, MCHC and platelet when compared with the control and late stage treatments. Prophylactic treatment showed significant increase ( $p < 0.05$ ) in Hb, PCV and RBC when compared with the control, late stage treatment and early stage treatment which were not significantly ( $p < 0.05$ ) different (Table 3).

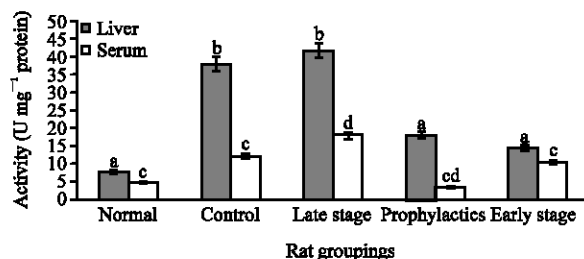


Fig. 4: GGT activities in experimented rats. Bars with different letters are significantly ( $p < 0.05$ ) different. Each value is a mean of 5 determinations  $\pm$ SEM

Table 2: Protein concentrations in *T. brucei* infected rats and normal rats

Rat grouping	Protein concentration (mg m <sup>-1</sup> )	
	Serum	Liver
Control (infected, not treated)	0.121 $\pm$ 0.06 <sup>a</sup>	0.023 $\pm$ 0.04 <sup>b</sup>
Normal (uninfected, not treated)	0.149 $\pm$ 0.02 <sup>ab</sup>	0.061 $\pm$ 0.04 <sup>a</sup>
Prophylactic treatment	0.131 $\pm$ 0.03 <sup>a</sup>	0.032 $\pm$ 0.04 <sup>c</sup>
Early stage treatment	0.179 $\pm$ 0.10 <sup>b</sup>	0.030 $\pm$ 0.03 <sup>bc</sup>
Late stage treatment	0.122 $\pm$ 0.05 <sup>a</sup>	0.025 $\pm$ 0.03 <sup>b</sup>

Each value is a mean of five determination  $\pm$ SEM. Values with different letters are significantly ( $p < 0.05$ ) different

Table 3: Hematological Indices in infected rats and normal rats

Rat grouping	Normal rats	Control rats	Prophylaxis	Early stage	Late stage
Hb (g/vl)	12.43 $\pm$ 0.29 <sup>d</sup>	10.28 $\pm$ 0.55 <sup>ab</sup>	12.23 $\pm$ 0.27 <sup>cd</sup>	11.10 $\pm$ 0.32 <sup>bc</sup>	9.43 $\pm$ 0.43 <sup>a</sup>
PCV (%)	46.00 $\pm$ 0.94 <sup>b</sup>	36.50 $\pm$ 0.76 <sup>a</sup>	43.75 $\pm$ 0.46	36.50 $\pm$ 0.64 <sup>a</sup>	36.00 $\pm$ 0.85 <sup>a</sup>
RBC $\times 10^{12}$ L <sup>-1</sup>	6.93 $\pm$ 0.14 <sup>b</sup>	4.94 $\pm$ 0.37 <sup>a</sup>	6.78 $\pm$ 0.27 <sup>b</sup>	5.43 $\pm$ 0.31 <sup>a</sup>	5.49 $\pm$ 0.28 <sup>a</sup>
WBC $\times 10^4$ L <sup>-1</sup>	17.00 $\pm$ 0.51 <sup>b</sup>	8.70 $\pm$ 0.42 <sup>a</sup>	17.80 $\pm$ 1.1 <sup>b</sup>	17.50 $\pm$ 1.1 <sup>b</sup>	15.80 $\pm$ 1.1 <sup>b</sup>
MCHC x (%)	32.00 $\pm$ 0.63 <sup>c</sup>	24.00 $\pm$ 0.88 <sup>a</sup>	28.30 $\pm$ 0.52 <sup>b</sup>	28.50 $\pm$ 0.35 <sup>b</sup>	26.50 $\pm$ 0.61 <sup>ab</sup>

Each value is a mean of five determinations  $\pm$ standard error of mean (SEM) values in the same row with different letters are significantly ( $p < 0.05$ ) different

## DISCUSSION

The extension of the life span of the infected rats in this report for prophylactic and early stage feeding with diet formulated with black seed oil suggests that the diet could be a useful and cheap formulation in the management of African trypanosomiasis. The (7 days) extension of the life span observed with prophylactic feeding suggests that routing consumption of diet formulated with black seed oil in the trypanosomiasis endemic area where administration of toxic and expensive drugs is a problem could have some preventive and control implication for African trypanosomiasis. The activities of enzymes such as GOT, GPT and GGT could be important in the diagnosis of diseases as well as in the investigation and thorough assessment of feed, drugs and extracts used in the treatment as these could give indications of progressive toxicity long before the actual manifestation of the toxic effects (Hanley *et al.*, 1986). The patterns of these enzymes (GOT, GPT and GGT) in the serum and liver showed that as they increased significantly ( $p < 0.05$ ) due to infection (as observed with the control and late stage treatments) in the serum, the increase was also observed in the liver. This suggests that uncontrolled proliferation in the hepatocytes might be the cause for the pattern observed. The increase in the specific activities of the enzymes GOT in the serum might also be due to erythrocytes breakdown. The plasma GGT activities in all the groups were not significantly different ( $p < 0.05$ ) whereas, there was significantly increase ( $p < 0.05$ ) in the liver GGT activity of control and late stage treatment when compared with the normal. This suggests that trypanosomiasis may be a predisposing factor to increased hepatocyte proliferation. The prophylactic and early stage feeding with black seed based oil diet improved significantly this condition towards the normal value. The significant increase ( $p < 0.05$ ) in the protein concentration of the prophylactic and early stage treatment as observed in the liver when compared with the control (Table 1) is an indication of increase in protein synthesis that may include those of antibodies and enzymes probably as a result of feeding with black seed based oil diet. The significant ( $p < 0.05$ ) increase in PCV, MCHC towards normal in comparison with the control and late stage feeding as observed with prophylactic feeding was an indication of improvement due to black seed oil diet. The evaluation of anaemia gives an indication of severity of the disease (Poltera, 1985; Anosa, 1988; Suliman and Feldman, 1989; Pentreath and Kennedy, 2004). The observed increase in Hb and RBC concentration in prophylactic feeding in comparison with control were probably as a result of reduced severity of the infection. WBC and platelet are involved in the defense mechanism and health. During infection WBC contributes to the development phagocytes and antibodies against the recognizable antigens of parasite origin. The significant ( $p < 0.05$ ) increase in WBC and platelet compared with the control was an indication of increased host action. Shigeki *et al.* (2001) showed that volatile oil of black seed oil has inhibitory effects on arachidonic acid induced platelet aggregation. The results suggest that black seed oil supplemented diet has the ability to reduce severity of trypanosomiasis. Salem (2005) showed that immunochilation is probably one of the ways by which the oil achieves its protective role. We therefore suggest that diets similar to the one used in this study could be useful and cheap formulations for the management of African trypanosomiasis.

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