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Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats

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ABSTRACT

The effect of black seed oil (*Nigella sativa* oil) on parasitaemia, some serum and liver enzymes as well as some haematological parameters in *Trypanosoma brucei*-infected rats was investigated. The results show there was low parasitaemia and extension of life span of rats from 12 days of the infected untreated (control) rats to 22 days for the infected black seed oil-treated rats. Results also show significant increases in activities of serum alkaline phosphatase as well as glutamate oxaloacetate and glutamate pyruvate transaminases with decreases in the liver enzyme activities. Protein concentrations show significant decreases in the serum and increases in the liver. There were also significant increases in the haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and platelet counts of infected oil-treated rats when compared with the infected untreated. We suggest that black seed oil has trypanocidal properties and probably stimulate the host immune system to control parasite proliferation thereby making it a possible agent for managing African sleeping sickness despite possible gradual damage to host organs as shown by increases in some serum enzymes.

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Key Words; Black seed oil, *T. brucei*, sleeping sickness, management

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INTRODUCTION

African trypanosomes cause trypanosomiasis, also known as sleeping sickness for which about 300,000 new cases are reported annually in some 36 developing African countries south of the Sahara where about 60 million people in some 200 locations are exposed to the risk of infection (Chretien and Smoak, 2005). Trypanosomiasis is fatal if left untreated and chemotherapy which forms the most important and major aspect of control and eradication of the disease in African countries is beset with problems of toxicity and increasing incidence of resistance among the trypanosomes to the existing drugs (Kioy & Mattock, 2005; Moore, 2005). The search for new drugs and formulations that are safe, affordable and effective against both early and late stages of the disease is recommended (Jannin and Cattand, 2004; Chibale, 2005; Pink *et al.*, 2005).

Parasitaemia correlates with the severity of infection (Anosa, 1988). The disease is further complicated by anaemia, thrombocytopaenia and leucopaenia (Davis, 1982; Suliman and Feldman, 1989; Biryomumaisho and Katunguka-Rwakishaya, 2007) all or some of which may be related to breakdown of the immune system and the observable pathological consequences of infection. Formulations or natural products which boost the host immune system and possibly reduce parasitaemia or completely remove parasites 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 the administration of honey to *Trypanosoma brucei*-infected rats was able to reduce the parasitaemia and extensively extend the life span of infected rats when compared with infected non-treated rats (Ekanem and Yusuf, 2005), even when included as part of the diet (Ekanem *et al.*, 2006). Honey is a natural product reported to have immune-boosting properties (Abuharfeil, 1999).

The seed of black seed plant (*Nigella sativa*) is known throughout the world with different names such as Seed of blessing (*habbat-ul baraka*), black cumin, black caraway, Kalonji etc. The black seed oil is reported to be beneficial due to its content of over a hundred components such

as aromatic oils, trace elements, and vitamins (Ali & Blunden, 2003). It is a phytotherapeutic known to reduce the risk to illness and disease by strengthening immune system and protecting the body (Hajhashemi *et al.*, 2004; Tekeoglu *et al.*, 2006). Recent reports however suggest that thymoquinone present in the oil might be the active component (Tekeoglu *et al.*, 2006; Khattab and Nagi, 2007).

In this report, we assessed the trypanocidal properties of black seed oil, its potential as an immunoregulator in *T. brucei*-infected rats as well as its effect on some liver and serum enzymes as a way of determining its possible use in the control or management of African sleeping sickness.

MATERIALS AND METHODS

Federe strain of *T. brucei* was obtained from the Veterinary and Livestock Studies Department, Nigerian Institute for Trypanosomiasis Research, Vom, Plateau state, Nigeria. Black seed oil used for this work was a product of Iman International, United Kingdom purchased from around the University of Ilorin Central mosque, Ilorin, Nigeria.

Assay kits for glutamate oxaloacetate and glutamate pyruvate transaminases were products of Randox laboratories Ltd, United Kingdom and that of alkaline phosphatase was a product of Teco Ltd, United Kingdom.

Inoculation of rats

Parasite infested 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 250g. The suspension as described earlier (Ekanem and Yusuf, 2005; Ekanem *et al.*, 2006) contained 3 or 4 trypanosomes per view at x100 magnification (approximately 10^6 cells per ml).

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.

Administration of black seed oil

Infected and uninfected rats were administered intraperitoneally with 0.5 ml solution of black seed oil in distilled water containing 3.0mg/kg body weight on the first day of sighting parasite in the blood (normally 3 days post infection) of infected rats. Administration of the black seed oil continued on daily basis until the infected rats died. Infected untreated rats were considered as the control against the infected oil-treated ones.

Biochemical and haematological indices

The biochemical and haematological determinations were carried out on the rats when the infections in the infected rats had progressed to the late stage of the disease (11 days). Serum and liver collection as well as alkaline phosphatase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase assays were carried out as earlier described (Ekanem and Yusuf, 2005; Ekanem *et al*, 2006).

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.

Protein concentrations were determined using biuret method (Gornall *et al*, 1949) as described by Plummer, 1978.

Statistical analysis

Data were statistically analyzed and differences compared using the Student's 't' test (Student, 1908) as described by Adamu and Johnson, 1997 while the level of statistical significance was taken at 5% confidence.

RESULTS

The parasitaemia of infected untreated group increased infinitely while infected oil-treated group shows a decrease in the proliferation (Fig 1). The treatment commenced the first day of sighting parasite in the bloodstream. The graph shows low replication of parasite and extension of life span of rats treated black seed oil from 12 days of the control (infected untreated) to 22 days for the infected-black seed oil treated rats.

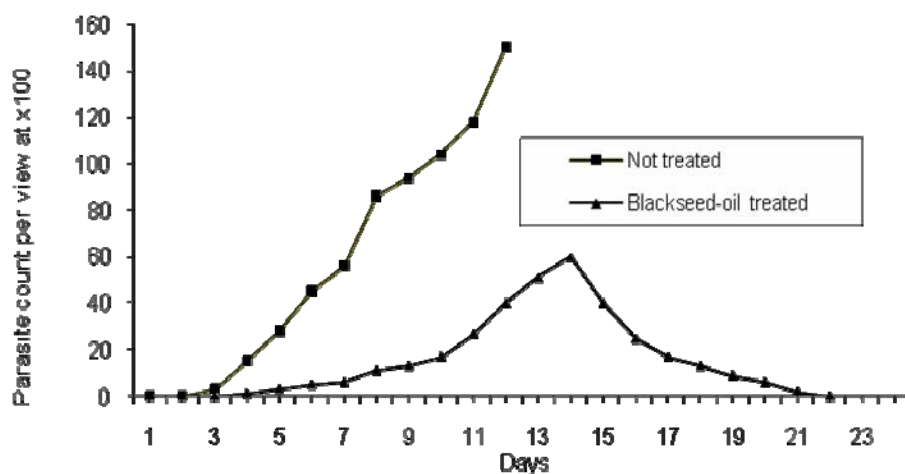


FIG 1: Parasite count of *T. brucei*-infected rats. Rats treated with honey and blackseed oil were inoculated at 3.0mg/kg body weight once daily until the animals died. Each point is an average count from five infected rats

Protein concentrations

There was significant decrease in serum protein concentration in the uninfected oil-treated and infected oil-treated rats in comparison with the infected untreated and uninfected (normal) rats. There was no significant difference in serum protein concentration in infected oil-treated, infected untreated and uninfected oil-treated rats when compared together. Significant increase was also observed when liver protein concentration in infected oil-treated rats were compared with infected untreated as well as with uninfected oil-treated rats (Table 1)

Alkaline phosphatase activities

Results of serum and liver alkaline phosphatase assays are shown in Table 2. At $p < 0.05$, serum alkaline phosphatase activities were significantly higher in infected untreated, uninfected oil-treated and infected oil-treated rats when compared with uninfected normal rats. The infected oil-treated rats also showed significantly high alkaline phosphatase activity in serum when compared with the serum activity in infected untreated rats. There was significant decrease in the liver enzyme activities in infected untreated, uninfected oil-treated and infected oil-treated rats when compared with the uninfected (normal) rats. The infected oil-treated values were also significantly lower than those of infected untreated rats.

Glutamate oxaloacetate transaminase activities

The serum Glutamate oxaloacetate transaminase activities show significant increases in the uninfected oil-treated, infected untreated and infected oil-treated rats when compared with the uninfected (normal) rats (Table 3). The liver glutamate oxaloacetate transaminase activities in the uninfected oil-treated, and infected oil-treated were significantly lower than that of the uninfected normal rats as well as infected untreated. The serum enzyme activity in the infected oil-treated was significantly higher than that of infected untreated rats while it was significantly lower in the liver.

Table 1: Protein concentrations in *T. brucei* infected rats at Day 11 post infection

Rat groupings	Protein concentration (mg/ml)	
	Serum	Liver
Uninfected (normal)	2.00±0.20	0.400±0.00
Infected Untreated	1.07±0.47 ^a	0.467±0.18 ^a
Uninfected oil-treated	1.20±0.44 ^a	0.488±0.17 ^a
Infected oil-treated	1.15±0.51 ^{ab}	0.850±0.20 ^{ab}

Each protein value is an average of five determinations \pm SEM. Values are significantly different in comparison with ^auninfected (normal) rats and ^binfected untreated rats at $p < 0.05$.

Table 2:

Specific activities of alkaline phosphatase at Day 11 post infection

Rat groupings	Enzyme activity (IU/L)	
	Serum	Liver
Uninfected (normal)	31.95±2.00	32.50±5.00
Infected Untreated	55.63±2.00 ^a	16.10± 2.79 ^a
Uninfected oil-treated	54.65±9.38 ^a	20.11± 5.26 ^a
Infected oil-treated	63.98±13.15 ^{ab}	9.47± 2.82 ^{ab}

Each specific enzyme activity is an average of five determinations \pm SEM. Values are significantly different in comparison with ^auninfected (normal) rats and ^binfected untreated rats at $p < 0.05$.

Table 3:

Specific activities of glutamate oxaloacetate transaminase at Day 11 post infection

Rat groupings	Enzyme activity (IU/L)	
	Serum	Liver
Uninfected (normal)	18.31± 4.40	90.00±10.50
Infected Untreated	27.35± 5.77 ^a	86.67±13.90
Uninfected oil-treated	39.28± 5.16 ^a	58.75±13.19 ^{ab}
Infected oil-treated	37.09±4.89 ^{ab}	23.42± 15.23 ^{ab}

Each specific enzyme activity is an average of five determinations \pm SEM. Values are significantly different in comparison with ^auninfected (normal) rats and ^binfected untreated rats at $p < 0.05$.

Table 4: Specific activities of glutamate pyruvate transaminase at Day 11 post infection

Rat groupings	Enzyme activity (IU/L)	
	Serum	Liver
Uninfected (normal)	20.39± 1.30	130.00±17.50
Infected Untreated	52.24± 5.03 ^a	74.43± 10.27 ^a
Uninfected oil-treated	23.42± 4.29	150.00± 17.25
Infected oil-treated	51.41±6.47 ^{ab}	43.45± 11.01 ^{ab}

Each specific enzyme activity is an average of five determinations \pm SEM. Values are significantly different in comparison with ^auninfected (normal) rats and ^binfected untreated rats at $p < 0.05$.

Glutamate pyruvate transaminase activities

The results of the glutamate pyruvate transaminase activities in the serum and liver are presented in Table 4. The serum enzyme activities in the infected untreated and infected oil-treated rats are significantly higher than that of the uninfected normal and uninfected oil-treated rats. There was no significant difference in infected untreated and infected oil-treated rats. The liver glutamate pyruvate transaminase activity in the infected oil-treated rats was significantly lower than those of the normal and uninfected oil-treated rats, which were themselves comparable with each other.

Haematological Indices

The results of haematological studies are presented in Table 5. There were significant increases ($p < 0.05$) in the values of Hb, MCHC, PCV, RBC, WBC and platelet counts of the infected oil-treated rats in comparison with the infected untreated rats

Table 5: Haematological indices of *T. brucei* - infected rats at 11 days post infection.

Rat Groupings	Hb (g/dl)	MCHC (g/dl)	PCV (%)	RBC ($\times 10^{12}/L$)	WBC ($\times 10^9/L$)	Platelet ($\times 10^9$)
Normal rats uninfected	12.95±1.45	33.00±1.00	39.50±3.50	6.63±0.18	16.80±3.20	899.50 ±10.50
Infected untreated	7.20±0.00 ^a	24.00±0.50 ^a	30.00±0.50 ^a	3.99±0.70 ^a	7.20±1.14 ^a	319.67±89.59 ^a
Uninfected oil-treated	12.35±0.95	34.50±0.50	36.00±0.00	6.08±0.41 ^a	16.55±3.95	691.50±50.50 ^{ab}
Infected oil-treated	10.80±0.40 ^{ab}	30.66±1.20 ^b	38.67±2.60 ^b	5.69±0.39 ^b	12.07±1.55 ^{ab}	701.50±10.50 ^{ab}

Each value is an average of five determinations \pm SEM. Values are significantly different in comparison with ^acontrol and ^binfected-untreated ($p < 0.05$).

DISCUSSION

Many studies have been carried out in recent years on the pharmacological effects of black seed oil (Hajhashemi *et al*, 2004; Tekeoglu *et al*, 2006). The oil has analgesic, antimicrobial, anti neoplastic, anti-inflammatory and immunological effects (Haq *et al*, 1999; Hajhashemi *et al*, 2004; Salem, 2005; Tekeoglu *et al*, 2006). The seed and its oil are characterized by a very low degree of toxicity and have been shown not to induce significant adverse effects on liver functions (Ali and Blunden 2003). The oil of the closely related plant *Nigella segetalis* have been reported to increase haematocrit level but reduced MCHC and mean corpuscular volume (MCV) concentration (Kökdil, 2006).

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 mammalian system (Nwagwu *et al*, 1987; Boutignon *et al*, 1990; Ekanem, 1989; Ekanem *et al*, 1994, 1996). The antibodies produced by the host against the parasite are not effective because of the ability of the parasite to produce a large repertoire of antigens. The host defense mechanism is only partially specific and often lagging behind the progress of the disease in terms of antigen-antibody interaction (Sternberg, 2004).

Eventually there is a breakdown of the host immune system coupled with parasite invasion of the central nervous system leading to coma and death.

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; Chibale, 2005).

Black seed oil has trypanocidal properties as well as the ability to extend the life span of *T. brucei*-infected rats (Fig 1). Despite the removal of the parasites from the blood, the infected rats still died by Day 23 suggesting the involvement of agents that are not necessarily life parasites. Factors extracellularly derived from the parasites (Nwagwu *et al*, 1987; Ekanem, 1989; Boutignon *et al*, 1990; Ekanem *et al*, 1994, 1996) could be responsible for the death.

The increase in protein concentration in the infected treated rats when compared with the infected untreated rats (Table 1) is an indication of increase in protein synthesis that may include those of antibodies and enzymes probably as a result of oil treatment as also observed in the uninfected oil treated rats in comparison with the uninfected (normal) untreated rats.

The activities of serum alkaline phosphatase, Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase ordinarily are increased in the infected untreated rats when compared with those of the normal untreated rats (Tables 2, 3, and 4). This suggests and probably confirms earlier results (Ekanem and Yusuf, 2005; Ekanem *et al*, 2006) that infection could lead to gradual tissue especially liver destruction as decreases in the liver enzyme activities are also observed. Treatment with black seed oil however enhanced the situation as observed in the further increases in the activities of alkaline phosphatase and Glutamate oxaloacetate transaminase (Tables 2 and 3). There however was no significant difference in serum glutamate pyruvate transaminase activity (Table 4) in the infected oil treated rats in comparison with the infected untreated despite the decreases in the liver enzymes. The reasons for this discrepancy cannot be immediately determined but suppression of the glutamate pyruvate transaminase activity could be considered.

Measurement of anaemia gives an indication of severity of the disease (Poltera, 1985; Anosa,

1988; Suliman and Feldman, 1989; Pentreath and Kenedy, 2004). The increases in PCV and MCHC (Table 5) observed for infected oil treated rats in comparison with infected untreated suggest that black seed oil reduces the severity of *T. brucei* infection in rats. The observed increases in Hb and RBC concentrations are probably as a result of reduced severity of the infection and possible increase in the synthesis of the protein in line with observations in Table 1. The increased WBC and platelet counts (Table 5) are also indicative of the increased host action in the presence of black seed oil against the infection as these will contribute to the development of phagocytes and antibodies against the recognizable antigens of parasite origin.

The results suggest that black seed oil probably has trypanocidal properties as well as the ability to reduce parasitaemia and the severity of the disease. Immunodulation is probably one of the ways by which the oil achieves the results (Salem, 2005). Thymoquinone, the recently implicated active component of black seed oil (Tekeoglu *et al*, 2006; Khattab and Nagi, 2007) might be the cytotoxic constituent conferring trypanocidal properties on the oil. We are currently exploring this possibility along with the possibility of inactivating possible toxins extracellularly released by trypanosomes. We however suggest at this point that black seed oil could be a useful cheap agent for the management of African sleeping sickness as its benefits outweigh any possible side effects it may possess.

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