



Original Article

EFFECT OF EXTRACTS OF FERMENTED WHEAT AND GARLIC BULB ON SOME HAEMATOLOGICAL AND ELECTROLYTE PARAMETERS IN *T. brucei*-INFECTED RATS

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ABSTRACT

Serum haematological and electrolyte concentrations were assessed in rats infected with federe strain of *Trypanosoma brucei* treated with wheat and garlic extracts. The results showed significant increase in haemoglobin (Hb) concentration, packed cell volume (PCV) and red blood cell (RBC) count of infected treated group when compared with infected untreated (control) group. While the white blood cell (WBC) count increase significantly in infected untreated group, results showed a significant increase ($p < 0.05$) during infection in Na^+ concentration of infected untreated group when compared with other experimental groups. While there were no significant ($p < 0.05$) difference in the level of K^+ , Po_4 , and HCO_3^- in all experimental groups, there was significant increase ($p < 0.05$) in Cl^- concentration in all experimental groups when compared with uninfected untreated (control). Results suggest that these plants have greater potential in the management of African trypanosomiasis.

Keywords: Fermented wheat, garlic bulbs, *Trypanosoma brucei*, haematological and electrolytes parameters

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INTRODUCTION

Trypanosomiasis is a disease caused by the parasitic protozoa, trypanosome. They are microscopic elongated unicellular parasites that live and multiply in the blood and other body fluids of their host, causing sleeping sickness in humans and related diseases in domestic animals (IRALD, 1991).

Chemotherapy available for control and eradication of trypanosomiasis is very limited at the moment. Effective trypanocidal drugs available is beset with problems of drug resistance and toxicity (Pepin and Millord, 1994). Underscoring, there is urgent need to develop more effective and safer trypanocidal drugs. Several reports on evaluation of different chemicals/drugs

for trypanocidal activity have appeared just as interesting reports on the anti-trypanosomal effects of plant extracts (Asuzu and Chineme, 1990). Some reports have shown that Fermented wheat and garlic bulb extracts possess anti-trypanosomal activity (Yusuf and Ekanem, 2010).

In this study, our objective was to assess the effect of fermented wheat and garlic bulb which are natural food sources and known to contain excellent nutrient to boost weak immune systems and antioxidant properties on some haematological and electrolyte parameters in trypanosome infected rats.

MATERIALS AND METHODS

Collection of plant material

Wheat seeds and Fresh bulbs of *Allium sativum* L., commonly known as garlic were collected from Minna Central Market in the month of March/April 2008.

Parasite inoculum

Trypanosoma brucei brucei was obtained from the Veterinary and Livestock Studies Department of the Nigerian Institute for Trypanosomiasis Research, (VOM), Plateau State of Nigeria. The parasite was maintained through passaging other rats.

Experimental Animals

Albino rats weighing approximately 200g were obtained from the animal breeding unit of the department of Biochemistry, University of Ilorin, kwara state and fed with animal feed obtained from Bendel Feeds and Flour Mill Ltd, Ewo, Edo state.

Preparation of Plant Extract

Wheat seeds powder of (70g) was fermented using 30g of *Saccharomyces cerevisiae* (baker's yeast) for 48 hours

and the paste was extracted using 250ml ethyl acetate. The filtrate was concentrated using rotary evaporator and stored at room temperature. Garlic bulbs (*Allium sativum*) were opened to reveal the fleshy sections called cloves. The cloves were peeled and blended. One hundred gram of *A. sativum* was soaked using 250ml methanol for 24 hours and filtered. The solvent was removed using rotary evaporator. The crude extract was used in subsequent studies.

Administration of Crude Extract

Infected and uninfected rats were administered intraperitoneally with 0.5ml solution of fermented wheat and garlic bulbs extract in distilled water containing 300mg/kg body weight on the first day of sighting parasite in the blood (normally 3days post infection) of infected rats. Administration of crude extract continued on daily basis for 10days before the rats were sacrificed.

Parasitaemia Determination

Evaluation of parasitaemia was carried out at 24 hours interval to monitor infection progress. This was done by counting the number of parasite under the light microscope at X 100 magnification from thin blood smear freshly obtained from the tip of the tail of infected rats.

Sample preparation for Biochemical Evaluation

The rats were sacrificed and blood was collected from the rats by cardiac puncture. Serum was obtained at the late stage of infection from all the groups and control group was uninfected untreated group. The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min and collected by pipetting.

Haematological and Electrolyte Indices

The haematological and electrolytes determination were carried out on the rats when infection in the infected rats had progressed to late stage of the disease (10 days). Haematocrit (PCV) and haemoglobin concentration were determined using the method of Catwright (1968). While red blood cell and White blood cell count were determined by method described by Dacie and Lewis (1991). Sodium and potassium ions were determined by flame emission spectrophotometer by Kaplan (1988). Chloride and bicarbonate level were determined by

titration method described by Varly (1962). Inorganic phosphate concentration was determined by colorimetric method.

Statistical Analysis

The group means \pm S.E.M was calculated for each analyst and significant difference between means evaluated by analysis of variance (ANOVA). Post test analysis was done using the Duncan multiple comparison tests. Values of $p < 0.05$ were considered as statistically significant (Adamu and Johnson, 1997).

Table 1: Changes in Haematological levels of *T. brucei* infected rats after treatment with fermented wheat and garlic bulbs extracts for 10 days post infection.

Rat Grouping	PCV (%)	Hb (g/dl)	RBC ($\times 10^{12}/l$)	WBC ($\times 10^{10}/l$)	MCHC (%)
Grp1	53.33 \pm 0.00 ^a	17.76 \pm 0.00 ^a	4.95 \pm 0.38 ^a	2.44 \pm 0.06 ^a	33.31 \pm 0.00 ^a
Grp2	34.50 \pm 4.68 ^b	11.50 \pm 1.57 ^b	2.99 \pm 0.45 ^b	8.19 \pm 0.38 ^b	33.39 \pm 0.07 ^a
Grp3	57.50 \pm 5.83 ^{ac}	18.83 \pm 1.95 ^{ac}	6.75 \pm 0.63 ^c	2.57 \pm 0.22 ^{ac}	33.39 \pm 0.43 ^a
Grp4	56.50 \pm 6.83 ^{ac}	18.85 \pm 1.51 ^{ac}	6.15 \pm 0.44 ^c	2.72 \pm 0.40 ^{ac}	33.36 \pm 0.05 ^a
Grp5	45.00 \pm 8.33 ^a	15.00 \pm 3.25 ^{ac}	5.08 \pm 0.44 ^{ac}	4.12 \pm 0.16 ^{acd}	33.33 \pm 0.04 ^a
Grp6	47.50 \pm 10.20 ^a	15.85 \pm 3.39 ^{ac}	5.17 \pm 0.48 ^{ac}	4.59 \pm 1.81 ^{acd}	33.38 \pm 0.61 ^a

Means along the same column with different superscript are significantly different in comparison with ^a control, ^b infected-untreated and ^c uninfected treated ($P < 0.05$), values are means of four determinations \pm S.E.M

Group 1: Uninfected not treated (control) ^a

Group 2: Infected not treated ^b

Group 3: Uninfected treated with wheat ^c

Group 4: Uninfected treated with Garlic

Group 5: Infected treated with Garlic ^c

Group 6: Infected treated with wheat

RESULTS

Haematological Indices

The results of the haematological studies are presented in Table 1. Within the period of 10 days post infection, there were significant ($p < 0.05$) difference in the values of PCV, Hb, RBC and WBC counts of the infected

untreated when compared with the other experimental groups.

Electrolyte Indices

The effects of the extract on some serum electrolyte are present in Table 2. Increase in Na^+ was observed in the infected untreated group at ($p < 0.05$) when compared with the uninfected untreated (Normal), uninfected treated

& infected groups (Table 2). Also, Cl⁻ (p<0.05) concentration was elevated in all other experimental groups when compared with uninfected untreated (normal) group (Table 2). Whereas

there was no significant difference in all other electrolyte K, PO₄, HCO₃ parameters monitor compared to the uninfected untreated (normal) group.

Table 2: Serum electrolyte profile of *T. brucei* infected rats after 10 days of treatment with fermented wheat and garlic bulbs extracts.

Rat Grouping	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	P (mmol/L)	HCO ₃ ⁻ (mmol/L)	Cl ⁻ (mmol/L)
Grp1	50.00±0.00 ^a	0.55±0.00 ^a	48.67±0.38 ^a	22.62±0.06 ^a	18.00±0.00 ^a
Grp2	91.67±16.67 ^b	0.83±0.28 ^a	46.67±10.69 ^a	25.00±0.33 ^a	24.33±1.76 ^b
Grp3	62.5±19.09 ^{ac}	0.83±0.14 ^a	59.00±13.47 ^a	24.50±1.04 ^a	25.00±1.73 ^{bc}
Grp4	75.00±14.43 ^{ac}	0.83±0.14 ^a	65.00±13.47 ^a	24.50±0.60 ^a	26.00±1.73 ^{bc}
Grp5	75.00±14.43 ^{ac}	0.62±0.25 ^a	47.00±27.23 ^a	24.00±2.03 ^a	28.00±1.73 ^{bc}
Grp6	50.00±28.87 ^a	0.41±0.14 ^a	64.00±8.74 ^a	22.00±1.05 ^a	28.50±1.80 ^{bc}

Means along the same column with different superscript are significantly different in comparison with a control, b infected-untreated and c uninfected treated (P<0.05), values are means of four determinations ± S.E.M

Group1: Uninfected not treated (control)

Group2: Infected untreated

Group 3: Uninfected treated with wheat

Group 4: Uninfected treated with garlic

Group 5: Infected treated with garlic

Group 6: Infected treated with wheat

DISCUSSION

African trypanosomiasis is characterised by haematological changes, which drastically influence the pathogenesis of the disease (Stephen, 1986; Neiger *et al.*, 2002). The disease is associated with decline in the red blood cell (RBC) counts, haemoglobin (Hb) concentration, and haematocrit or packed cell volume (PCV) in the infected hosts, confirming that anaemia is a critical feature in the pathogenesis of African trypanosomiasis (Murray *et al.*, 1982).

The increase in PCV observed in infected treated group when compared with the values observed for infected treated group compared with infected untreated is an indication that the extracts is capable of preventing anaemic condition as well as reduction in severity of *T. brucei* infection. This is because anaemia gives an indication of the severity of the disease (Anosa, 1988; Suliman and Feldman, 1989). Hb and RBC also increased with treatment, this is an added proof to the general appreciation in haematological parameters associated with improved treatment.

While increased WBC observed in infected untreated indicates the action of host immune system against the infection. White blood cells are of two types, granular and a granular. Neutrophil is an example of granular and lymphocytes, agranular. Neutrophils respond to antigen by ingesting and destroying the parasite, thereby protecting the body against infection. The result observed in infected untreated group maybe as a result of phagocytosis mechanism of neutrophil (Davis, 1982). Electrolytes are substance which when either in molten state or in solution conduct electricity and in the process decomposed (Harold, 1980). Electrolyte in the clinical laboratory is taken to refer to only the inorganic ions, but in practice, a request for electrolytes determination usually means the determination of sodium, calcium, potassium, bicarbonate and chloride ions (Bectel, 1970). Changes in electro-neutrality affect the whole body physiology especially conduction of nerve impulse (Datta, 1979).

Na⁺ is essential to the electrical activity of cellular membranes. Their concentration is mainly maintained by the fact that the excessive ingestion of these ions in the diet is eliminated by the kidney or the intestine (Carlson, 1989), bearing in mind that hydro-electrolytic disorders could be associated to food deprivation due to sleepiness and brain injury that affect the central nervous system. The findings in this study show significant increase in Na⁺ concentration of infected untreated group compared with infected treated groups which may be as a result of food deprivation due to sleep disorder and damage to central nervous system (WHO, 1998; WHO, 2001). There was no significant difference in other electrolyte parameters (K⁺, Cl⁻, HCO₃⁻, P) monitor, which was in agreement with the earlier

reports of normal values recorded in experimental *T. brucei* infections in mice (Moun, 1968; Awobode, 2006).

CONCLUSION

The findings in this study support that the fermented wheat and garlic bulbs extracts have ameliorative effect against *T. brucei* infection.

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