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Research article

Biochemical Evaluation of Fermented Wheat Germ Extract on *Trypanosoma brucei* -infected rats

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ABSTRACT: The biochemical effects of ethylacetate extract of wheat (Avemar) on some serum and liver enzymes were evaluated in *T. brucei* infected rats. The results show significant increase in specific activities of serum aspartate transaminase (AST) in infected untreated rats when compared with infected treated rats. However, there were no significant difference in serum and liver alanine transaminase (ALT). Liver Alkaline phosphatase (ALP) activities were significantly increased in infected untreated when compared with the infected treated rats. Results also show significant increase in specific activities of serum catalase (CAT) and Superoxide dismutase (SOD) in infected treated rats when compared with infected untreated rats. Whereas, there was no significant difference in specific activities of liver catalase, (CAT) and Superoxide dismutase (SOD) in infected treated rats when compared with infected untreated rats. We hereby conclude that ethylacetate extract of wheat has no toxic effect and can ameliorate the effect caused by *T. brucei* infection.

Keywords: Wheat germ, *T. brucei*, sleeping sickness, management

INTRODUCTION

African trypanosomes cause trypanosomiasis, also known as sleeping sickness for which about 300 - 500,000 new cases are reported annually in 36 countries of sub-Sahara Africa. Where about 60 million people are at risk. The disease has been described as one of the most neglected. In Nigeria, trypanosomiasis has a severe impact on livestock and human. Economic losses due to tsetse and trypanosomiasis have never been fully quantified (PAAT, 2006).

The parasites are transmitted in the saliva of blood sucking tsetse flies and it proliferates at the site of the fly's bite, then spread into the lymph and bloodstream. The circulatory stages of the disease are characterized by headache, fever, etc. As the disease progresses, the headache becomes severe, sleep disrupted and mental functions become impaired. Without treatment infection is fatal; damage to the central nervous system ends in coma and death. In the bloodstream, the parasite attacks the host immune system. They get round the host immune system through a process known as antigenic variation due to the presence of a thick surface coat of densely packed glycoproteins, which protects them from attack by the host (WHO, 1990; Wellcome News, 2006).

The fight against the disease has relied heavily on vector control strategies and old chemotherapies that their effectiveness remains unsatisfactory due to toxicity of the drugs and resistance shown by the trypanosomes to the drugs. Therefore, there is urgent need to find new target and drug leads (Pepin and Milford, 1994).

Medicinal plants are widely used worldwide to address a variety of health problems. About 25 to 50% of current pharmaceuticals are derived from plants (Cowan, 1999). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, saponin, flavonoids etc. The increasing

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demand for medicinal plant products has stimulated research in this field. Fermented wheat germ extract called avemer has been reported to control cell growth and proliferation mainly by inhibiting ribonucleotide reductase needed to make new DNA to support replication (Sukkar and Edoardo, 2004). It had also been reported that avemer limit the access to glucose, needed to make the ribose sugar for DNA and RNA for new cancer cells (Boros *et al*., 2002; Boros *et al*., 1997). Therefore, in this study, Wheat germ known to contain excellent nutrient for weak immune systems and antioxidant properties was employed.

MATERIALS AND METHODS

Collection of plant material: Wheat germ (seeds) was 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, Plataeu State of Nigeria. The parasite was maintained through passaging other rats.

Preparation of plant extract: Wheat germ 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.

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.

Administration of crude extract: Infected and uninfected rats were administered intraperitoneally with 0.5ml solution of fermented wheat ethylacetate extract in distilled water containing 300mg/kg body weigh (therapeutic dose) 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 24 hours interval to monitor infection progress. This was done by counting the number of parasite under the light microscope at X100 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 and liver was obtained at the late stage of infection from all the groups and control for this stage was normal uninfected untreated group under the same experimental condition. The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min and collected by pipetting. The animals were thereafter quickly dissected and the liver removed. The liver was suspended in ice-cold0.25 M sucrose solution and homogenized.

Enzyme and Protein Determination: All enzyme assay kits were products of Randox Laboratories Ltd, United Kingdom. Total protein concentration was determined using Biuret method described by Gornall *et al*; 1949 as modified by Plummer, 1978. Alkaline phosphatase (ALP) was determined based on the method of Wright *et al*; 1972. Aspartate transaminase (AST) and alanine transaminase (ALT) activities was assayed using the method described by Reitman and Frankel; 1957.Catalase (CAT) activity was determined as described by Bock *et al*; 1980. The method employed in the assay of superoxide dismutase (SOD) activities was that of Winterbourn *et al*. (1975) and is based on the ability of superoxide dismutase to inhibit the reductionof nitroblue tetrazolium by superoxide.

Statistical analysis: The group mean $\pm$ 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 comparism tests. Values of $p< 0.05$ were considered as statistically significant (Adamu and Johnson, 1997).

RESULTS

The results of various enzymes studied are presented in figures 1,2,3,4 and 5 respresenting the specific activities of aspartate transaminase (AST), alanine transaninase (ALT), catalase (CAT), alkaline phosphatase (ALP) and superoxide dismutase (SOD) respectively.

Alanine transaninase: The serum AST activities show significant difference in the uninfected treated, infected untreated and infected treated groups when compared with the uninfected not treated (normal) groups (Fig 1). The liver AST activities in the infected untreated,

uninfected treated and infected treated groups were significantly higher than that of the uninfected not treated (normal) group.

Alanine transaminase: The results of the ALT activities in the serum and liver are presented in Fig 2. The serum enzyme activities in the infected un and infected treated treated group are not significantly different when compared with the uninfected not treated group while the uninfected treated group is low when compare with other experimental groups. Also, there was no significant difference in liver enzyme activities of uninfected not treated, infected untreated and infected treated groups.

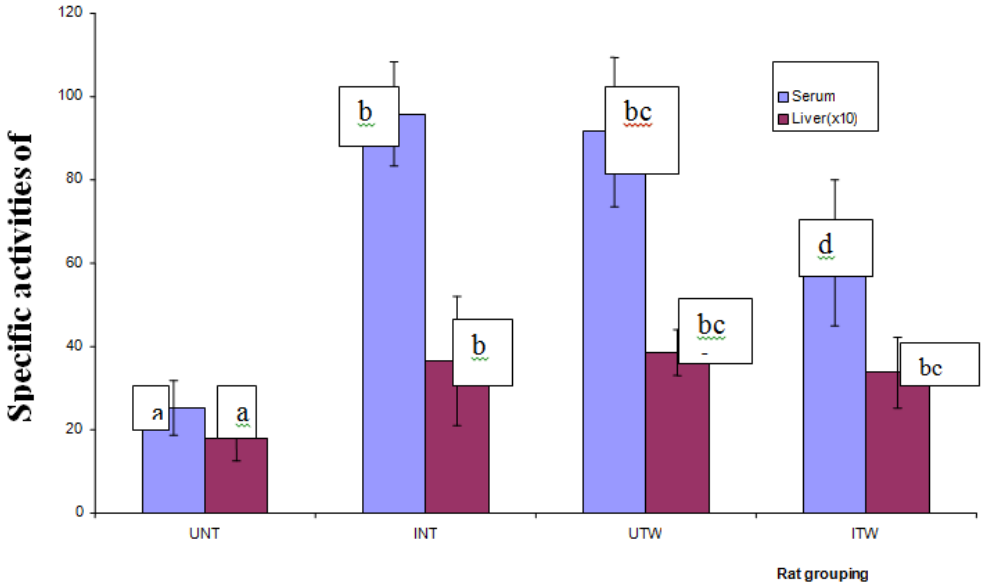

Fig 1: Specific activities of Aspartate Transaminase in the serum and liver of uninfected and untreated rats (UNT), infected and untreated rats (INT), uninfected rats treated with wheat (UTW) and infected rats treated with wheat (ITW) rats. Results are mean of four determinations + S.E.M. Bars carrying different letters are significantly different mean of four determinations + S.E.M. Bars carrying different letters are significantly different at $p<0.05$

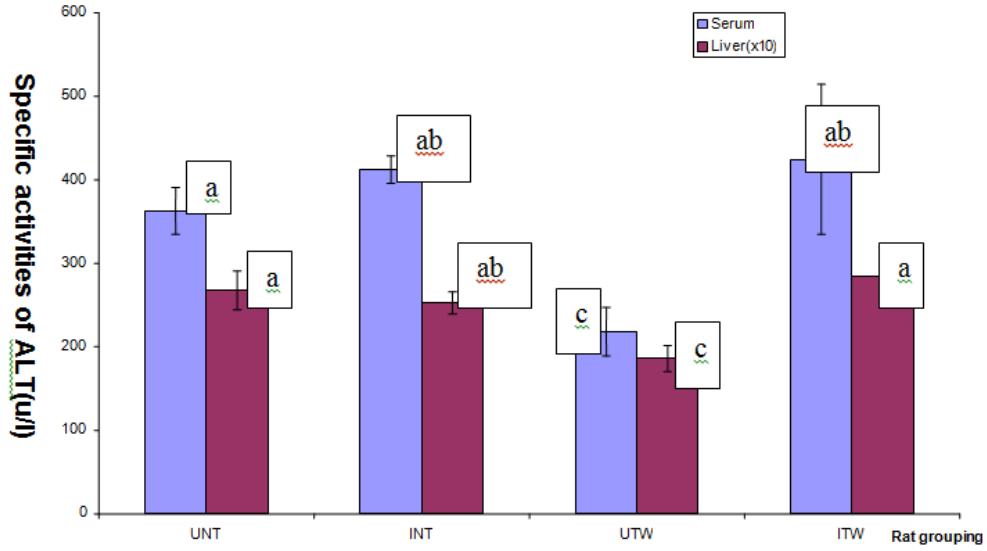

Fig 2: Specific activities of Alanine Transaminase in the serum and liver of uninfected and untreated rats (UNT), infected and untreated rats (INT), uninfected rats treated with wheat (UTW) and infected rats treated with wheat (ITW) rats. Results are mean of four determinations + S.E.M. Bars carrying different letters are significantly different mean of four determinations + S.E.M. Bars carrying different letters are significantly different at $p<0.05$

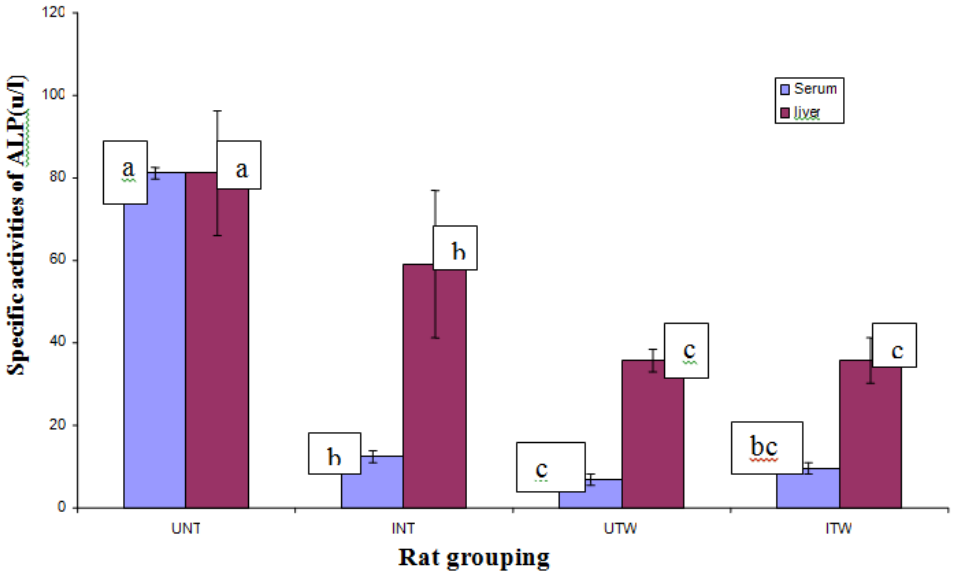

Fig 3: Specific activities of Alkaline Phosphatase in the serum and liver of uninfected and untreated rats (UNT), infected and untreated rats (INT), uninfected rats treated with wheat (UTW) and infected rats treated with wheat (ITW) rats. Results are mean of four determinations + S.E.M. Bars carrying different letters are significantly different mean of four determinations + S.E.M. Bars carrying different letters are significantly different at $p<0.05$

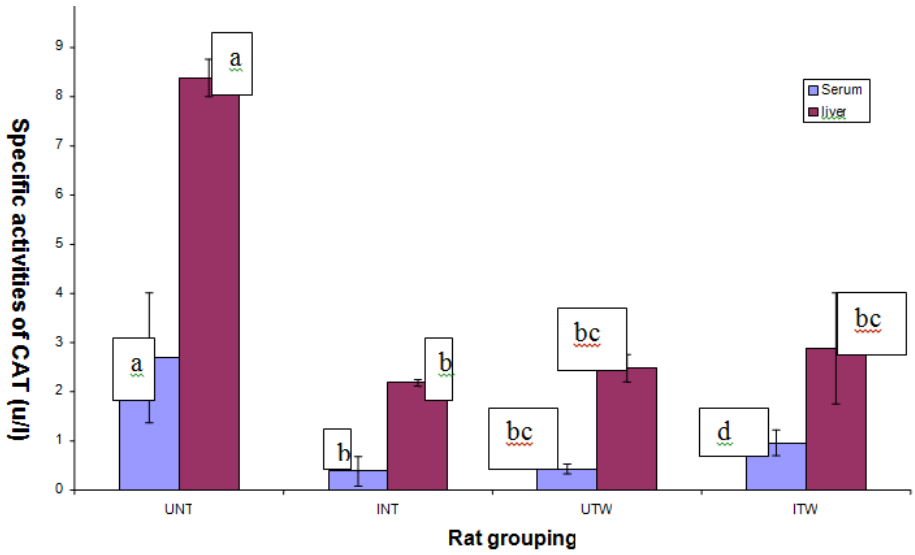

Fig 4: Specific activities of Catalase in the serum and liver of uninfected and untreated rats (UNT), infected and untreated rats (INT), uninfected rats treated with wheat (UTW) and infected rats treated with wheat (ITW) rats. Results are mean of four determinations + S.E.M. Bars carrying different letters are significantly different mean of four determinations + S.E.M. Bars carrying different letters are significantly different at $p<0.05$

Alkaline phosphatase : Results of serum and liver ALP assays are shown in Fig 3. At $p<0.05$, serum ALP activities were significantly lower in infected-untreated, uninfected treated and infected treated groups when compared with uninfected not treated (normal) group. There was significant decrease in the liver enzyme activities infected untreated, uninfected treated group and infected treated group when compare with uninfected not treated (normal) group. Whereas of infected un treated shows a significant decrease when compare with uninfected treated and infected treated groups.

Catalase: The specific activities of catalase in serum and liver are shown in Figure 4. Serum and liver catalase activities of uninfected not treated (normal) were significantly higher than the other experimental groups.

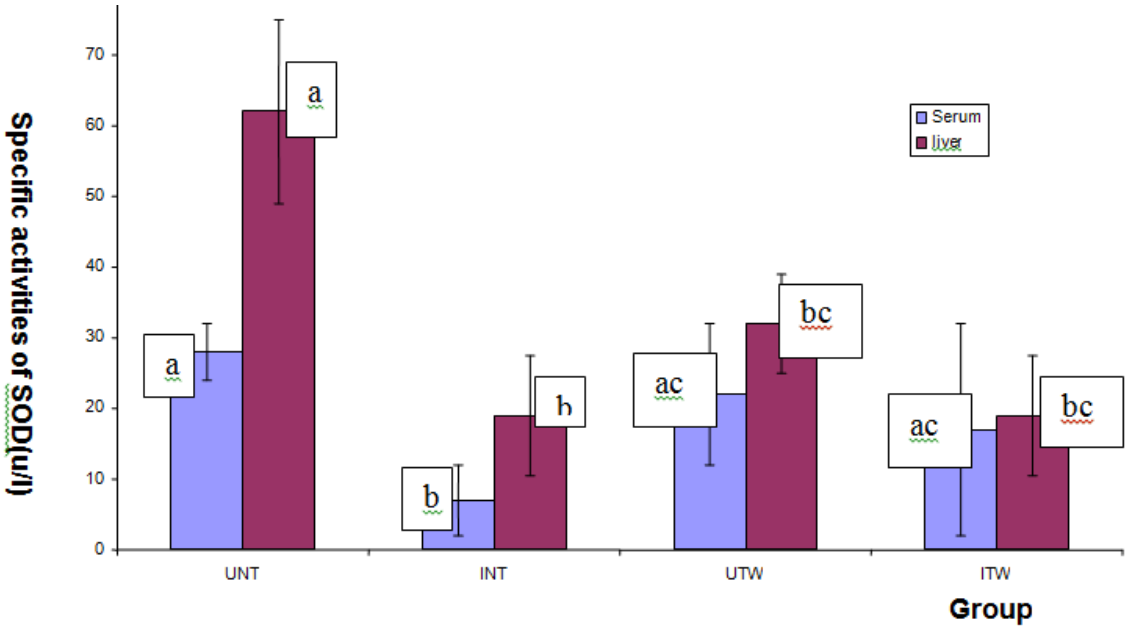

Fig 5: Specific activities of Superoxide dismutase in the serum and liver of uninfected and untreated rats (UNT), infected and untreated rats (INT), uninfected rats treated with wheat (UTW) and infected rats treated with wheat (ITW) rats. Results are mean of four determinations + S.E.M. Bars carrying different letters are significantly different mean of four determinations + S.E.M. Bars carrying different letters are significantly different at $p<0.05$

Superoxide Dismutase: The results of serum and liver SOD activities are presented in Figure 5. The serum SOD activities of infected untreated were significantly lower when compared with uninfected treated, infected treated and uninfected not treated (normal) groups. The liver SOD activities of infected untreated and infected treated groups was significantly lower when compared with uninfected treated group which was significantly lower than the uninfected not treated control (normal) group.

DISCUSSION

Because of their beneficial nutritional values, wheat germ and wheat bran are frequently used in human food supplements, breakfast cereals, nutri-bars and various fibers drink mixtures, and therefore they are part of the regular Western diet.

Many studies have been carried out in recent years on the pharmacological effects of crude extract of wheat (Suttle *et al*, 2000). This fermented wheat extract has a clear and definitive anti-proliferative action that target nucleic acid synthesis enzymes and induces cell cycle arrest and apoptosis through a cascade based mechanism (Tian *et al*, 1991). The extract also has analgesic, antimicrobial, anti-inflammatory and immunological effects (Tsen, 1985). The wheat extract are also characterized by no known toxicities and evidence of no any indication of adverse effects been identified.

Many enzymes are present in plasma (or serum) and their activity can be easily assayed in serum with diagnostic reagents. Elevation or depression of the levels of activity of specific enzymes may indicate the presence of a disease or damage to a specific tissue (Nelson and Cox, 2005). Analysis of serum enzymes have been reported to be of value and are early warning signs for certain diseased conditions. Wilkinson (1962) reported also that changes in enzymes levels are a good marker of soft tissue damage, he also noted that damage to body Cells result in the alteration of membrane permeability and consequent release of enzymes into the extracellular fluid (ECF). Elevated enzyme levels may also result form effect of trypanosomes lyses resulting from effect of the host defense mechanism (Kennedy, 2004).

The significant increase in serum Aspartate transaminase activities of infected untreated groups when compared with infected treated group could probably confirms earlier results that infection could gradually affect enzyme level (Kennedy, 2004). Treatment with ethylacetate wheat extract however reduces the increase caused by infection. However there was no significant difference in serum and liver ALT of the infected treated group in comparison with

the infected untreated group as well as uninfected untreated (normal).

Alkaline phosphatase (ALP) is known as orthophosphoric monoester phosphohychoxylase. It is a marker enzyme for endoplasmic reticulum and palsma memberane. It is present in most tissues and organs. ALP levels were significantly reduced in the liver and serum of infected untreated, uninfected treated and infected treated groups when compared with uninfected untreated group.

Antioxidant systems are normally put in place in living aerobic organisms to counter the effect of oxidative stress (Elstner and Osswald, 1994).The specific activities of catalase, the peroxisomal marker enzyme, was only reduced in infected untreated groups when compared infected treated group. This may be as a result of induction of enzyme.

The result of this work showed that serum SOD activity increased significantly in the uninfected treated and infected treated groups when compared with infected untreated group (fig 5).The increase in SOD activity in the uninfected treated and infected treated groups may be attributed to an induction of the enzyme protein in the presence of reactive metabolite from infection or the drug metabolism.

In conclusion, the results of this study indicate that the fermented ethylacetate wheat extract has significant effect against *Trypanosoma brucei* in vivo with safe dose level of 300 mg/kg body weight with low toxicity and better therapeutic index.

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