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Original Article

BIOCHEMICAL EVALUATION OF FERMENTED GARLIC BULB EXTRACT ON *T. brucei*-INFECTED RATS

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ABSTRACT

The biochemical effect of garlic extract (*Allium sativum* L.) on some serum and liver enzymes in rats infected with *Trypanosoma brucei* was evaluated. The results show significant increase in specific activities of serum and liver aspartate transaminase (AST) of the infected untreated when compared with the normal (control) and infected treated group. There was significant decrease in liver alanine transaminase (ALT), with slightly increase in serum activities when compared to normal control and infected treated group. Serum Alkaline phosphatase (ALP) had a significant decrease when compared with the control and infected treated group with no change in liver Alkaline phosphatase (ALP). There were no significant differences ($p>0.005$) in Serum and liver catalase activities (CAT), while serum and liver superoxide dismutase (SOD) activities of the infected untreated were significantly lower when compared with the control and infected treated group. This study scientifically established that fermented garlic methanolic extract has ameliorative effect on damage caused by *T. brucei* infection, thereby making it a possible agent for management of African sleeping sickness.

Keywords: Garlic bulb, *T. brucei*, sleeping sickness, Management

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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 (Welcome, 2005). 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, and as the disease progresses, the headache becomes severe, sleep disrupted and mental functions become

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 (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 and resistance shown by the trypanosomes to the drugs. Therefore, there is urgent need to find new target drug (Pepin, 1994). Medicinal plants are widely used world wide to address a variety of health problems. About 25 to 50% of current pharmaceuticals are derived from plants (Raskin *et al.*, 2002). This is because plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, saponin, flavonoids etc. The increasing demand for medicinal plant products has stimulated research in this field. Therefore, in this study, Garlic bulbs which are natural food source and known to contain excellent nutrient for weak immune systems and antioxidant properties would be employed.

MATERIALS AND METHODS

Collection of Plant Material

Fresh bulbs of *Allium sativum* L. commonly known as garlic were purchased from Minna Central Market in the months 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.

Preparation of Plant Extract

Garlic bulbs *(Allium sativium)* were opened to reveal its fleshy sections called cloves. The cloves were peeled and blended. *A. sativium* (100g) was soaked in 250ml methanol for 24 hours and filtered. The solvent was removed using rotary evaporator. The crude extract was used in subsequent studies.

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 Nigeria.

Administration of Crude Extract

Infected and uninfected rats were administered intraperitoneally with 0.5ml solution of fermented garlic methanolic extract in distilled water, containing 300mg/kg body weigh 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 progress of infection. This was done by counting the number of parasite under the light microscope at X40

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. The liver was suspended in ice-cold 0.25 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 of by Gornall *et al* (1949) as described by Plummer, (1978). Alkaline phosphatase was determined based on the method of Wright *et al* (1972). Glutamate oxaloacetate and glutamate pyruvate transaminase activities were assayed using the method described by Reitman and Frankel, (1957). Catalase activity was determined as described by Bock *et al*. (1980). The method employed in the assay of superoxide dismutase (SOD) activity was that of Winterbourne *et al.* (1975) and is based on the ability of superoxide dismutase to inhibit the reduction of nitroblue tetrazolium by superoxide.

Statistical Analysis

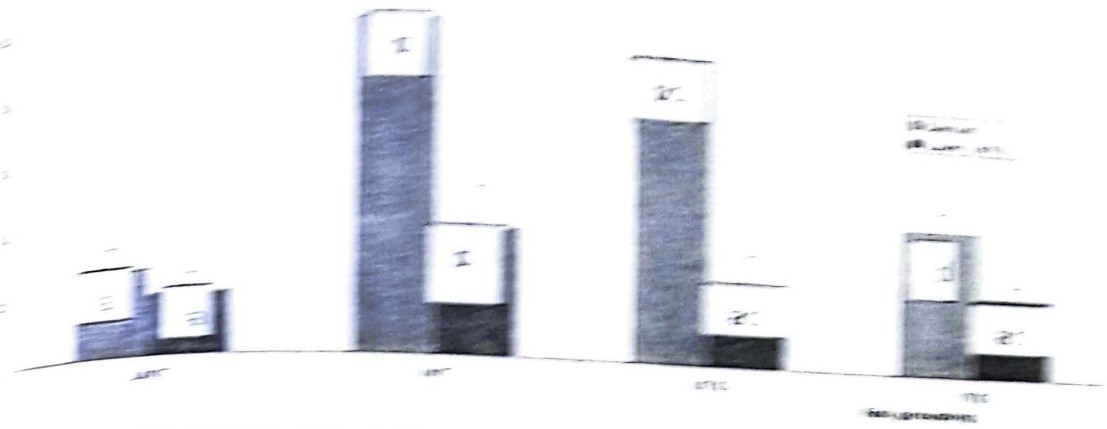
The groups mean $\pm$S.E.M was calculated for each analyst and significant difference between means evaluated by analysis of variance (ANOVA). Post test analysis was done using the Duncan multiple comparism tests.

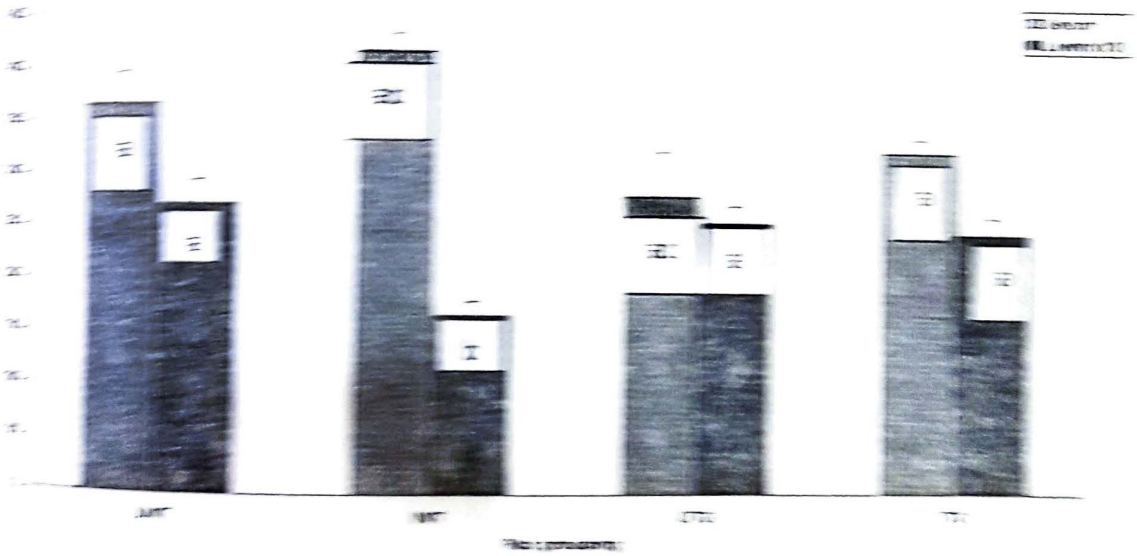
RESULTS

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.

Aspartate Transaminase (AST)

The serum aspartate transamimase show significant increases in infected untreated and uninfected treated with garlic when compared with uninfected not treated (normal) rats, which are not significantly different from infected treated group. The liver aspartate transaminase activities in infected untreated rats were slightly higher when compared with uninfected not treated (normal), uninfected treated and infected treated with garlic (fig 1).





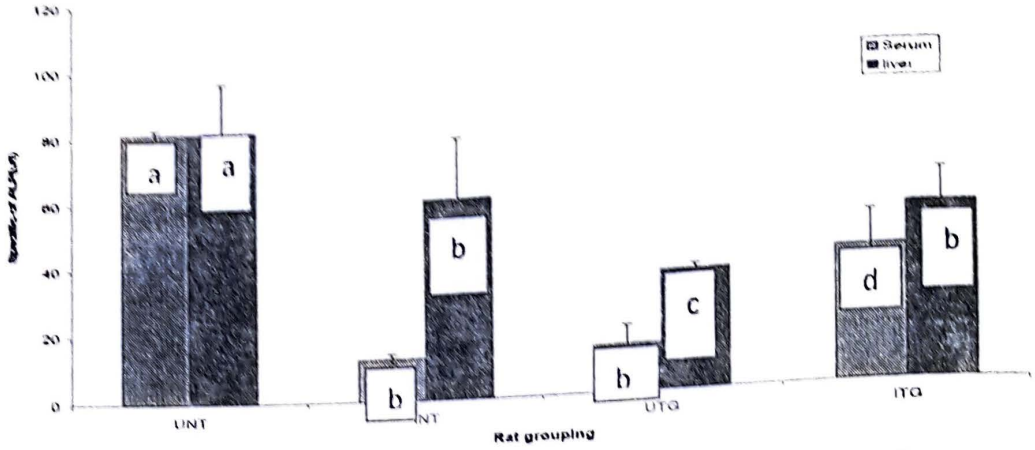


Fig 3: Specific activities of Alkaline Phosphatase in the serum and liver of rats. Result are mean of four determinations + S.E.M. Bars carrying different letters are significantly different at p<0.05

UNT: Uninfected not treated [a] INT: Infected not treated [b] UTG: Uninfected treated with garlic [c] ITG: Infected treated with garlic [d]

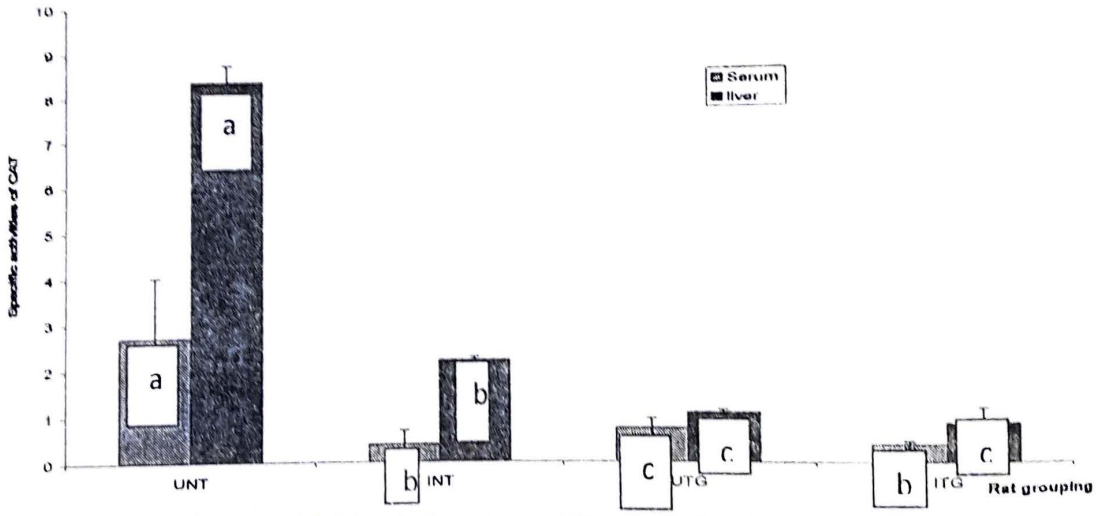


Fig 4: Specific activities of Catalase in the serum and liver of rats. Result are mean of four determinations + S.E.M. Bars carrying different letters are significantly different at p<0.05

UNT: Uninfected not treated [a] INT: Infected not treated [b] UTG: Uninfected treated with garlic [c] ITG: Infected treated with garlic [d]

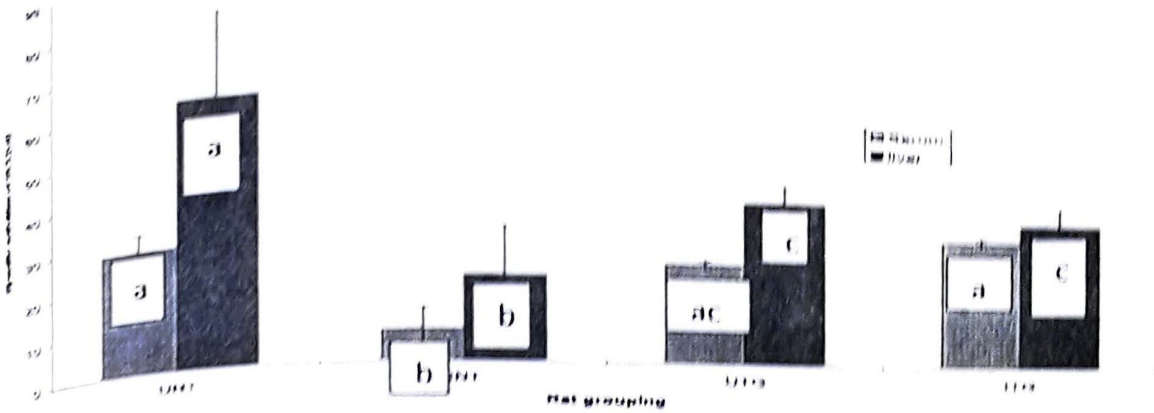


Fig 5: Specific activities of Superoxide Dismutase in the serum and liver of rats. Result are mean of four determinations ± S.E.M. Bars carrying different letters are significantly different at p<0.05

UNT: Uninfected not treated [a] INT: Infected not treated [b] UTG: Uninfected treated with garlic [c] ITG: Infected treated with garlic [d]

Alanine Transaminase (ALT)

The specific activities of alanine transaminase (fig. 2) in serum of infected untreated rats was slightly significantly higher when compared with infected treated and control (normal). Liver ALT activity in infected untreated shows significant difference decrease when compared with uninfected untreated (normal) and infected treated group.

Alkaline Phosphatase (ALP)

Results of serum and liver alkaline phosphatase assay are shown in fig 3. At $P < 0.05$, serum alkaline phosphatase activities were significantly lower in infected untreated and uninfected treated groups when compared with normal uninfected not treated rats. The liver ALP activities in infected not treated and infected garlic treated as well as infected untreated group were significantly higher than that of uninfected treated group

Catalase (CAT)

The specific activities of catalase in serum and liver assays are shown in fig. 4. Liver and serum catalase activities of uninfected not treated (normal) are significantly higher than the other experimental groups.

Superoxide Dismutase (SOD)

The specific activities of superoxide dismutase (SOD) (fig. 5) in the serum of infected untreated group were significantly lower than that of uninfected not treated (normal), uninfected treated and the infected treated group. The liver SOD activities of infected untreated group was significantly lower than the other group while uninfected treated and infected treated groups show no significant difference.

DISCUSSION

Many studies have been carried out in recent years on the pharmacological

effects of crude extract of garlic. Garlic has been claimed to help prevent heart disease including atherosclerosis, high cholesterol, high blood pressure and cancer (Block, 1992). It had been reported that changes in enzymes levels are a good marker of soft tissue damage; it was also noted that damage to body cells result in the alteration of membrane permeability and consequent release of enzymes into the extracellular fluid (ECF) (Nelson and Cox, 2005). Elevated enzyme levels have been reported (Kennedy, 2004). Therefore in this study, the activities of serum Aspartate and Alanine transaminase of infected untreated groups significantly increased when compared with those of infected treated group. This probably confirms earlier results that infection could gradually affect enzyme level. Treatment with methanolic garlic bulb extract however reduces the effect caused by infection. However, the ALP levels decreased in the serum of infected untreated compared to uninfected untreated and infected treated group.This maybe as a result of increase in utilization of the enzyme by the host in order to cope with the infection.

Antioxidant systems are normally put in place in living aerobic organisms to counter the effect of oxidative stress (Elstner and Osswald, 1994). Catalase, the peroxisomal marker enzyme is found in blood, bone marrow, mucous membrane, kidney and liver. It functions assumed to be the destruction of hydrogen peroxide. The result showed reduction in the enzyme in all other experimental groups when compared to the control group (fig 4). This could be an indication of its continuous utilization as a scavenger.

Superoxide dismutase protects blood cells from oxidative stress and damage. It is stimulated by contact with micro organism and neutrophil exhibit respiratory burst. The result of this work showed that serum SOD activity increased significantly in the uninfected treated and infected treated groups when compared with the control 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 the drug metabolism and disease condition.

CONCLUSION

The result generated from this study suggested that the fermented methanolic garlic extract has found to produce a significant effect against *Trypanosoma brucei in vivo*, with ameliorative effect on damage caused by *T. brucei* infection, thereby making it a possible agent for management of trypanosomiasis (sleeping sickness).

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