Effects of Allium sativum Bulb Extract, Diminazene Aceturate and Their Combination on Parasitaemia, and Biochemical Indices in Rats Experimentally Infected with Trypanosoma Brucei Brucei

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ABSTRACT

Thirty five clinically healthy albino rats of both sexes weighing between 100 – 120 grams were used to study the effects of Allium sativum bulb extract in combination with diminazene aceturate on parasitemia and biochemical indices in trypanosoma brucei brucei infection. The rats were divided into seven groups (A-G) of five rats each. All the infected rats developed Parasitemia five days post infection. Weakness, increase respiratory rate, rough hair coat, Biochemical changes at interval and possible deaths were the major parameters which were carefully observed. All the treatments commenced at the onset of parasitemia by day five post infection. Sub therapeutic dose of Allium sativum at 20mg/kg/bw in combination with 1.7mg/kg/bw of diminazene aceturate (Group C), diminazene aceturate at single standard dose of 3.5mg/kg/bw (Group B) caused a significant reduction (P<0.05) in parasitemia. The liver function enzymes ALT and AST level in rats infected and not treated showed significant increase liver function enzymes (Group A) while those treated with standard and sub therapeutic dose (Group BCD) respectively. Had their liver function enzymes towards normal, compare with control (Group G). Its trypanocidal activity was assessed through daily examination of blood parasite. Sub therapeutic doses of Allium sativum bulb extracts and its combination appear to be more effective in reducing severity of trypanosoma brucei brucei infection and provide alternative in reducing the toxicity of existing trypanocide.

Keywords: Allium sativum, Diminazene aceturate, Trypanosoma brucei brucei, Rats, Serum Liver enzymes.

INTRODUCTION

Trypanosomosis is one of the most important diseases of livestock and humans in sub-Saharan Africa (Dina, 2002; Ajagbonna et al, 2003). It is caused by several species of trypanosomes which are unicellular parasites transmitted by the bite of tsetse fly and the causative agent of sleeping sickness in humans and related diseases in animals (Warren 1988; Kuzoe, 1993). The disease causes anemia, weight loss, infertility, decrease milk yield, abortion and mortality in affected animals (Onyeyili and Egwu 1995; Ajagbonna et al., 2002).
The search for vaccine against this disease remains elusive and effective treatment is beset with problems of drug resistance and toxicity (Onyeyili and Egwu, 1995; Ajagbonna et al., 2003). Chemotherapy and chemoprophylaxis still remains the most effective single method of controlling the disease. The compound used clinically for control of trypanosome infections were introduced in the country about 30 years ago, but considerable toxicity and resistance of trypanosomes to the existing drugs have developed (Onyeyili et al., 1991; Ajagbonna et al., 1993; Onyeyili and Egwu, 1995). Many investigators are of opinion that the use of combination therapy may be very effective (Ajagbonna and Olaniyi, 1999). This is due to mild to moderate toxicity and enhancement of the potency and of efficacy of drugs recorded in their studies (Ajagbonna et al. 2005, Biobaku et al. 2008).

These considerations demand a local strategy for the management of trypanosomiasis apart from the optional use of relatively old existing drug such as diminazene aceturate. Recently the bulb of Allium sativum has been demonstrated to posses some trypanocidal activity both invitro and invivo in rabbits infected with trypanosome brucei (Ajagbonna et al., 2002, 2003). Relapses occur with Allium sativum therapy in rabbits infected with Trypanosoma brucei at 40mg/kg/bw (Mikhail et al., 2002). In view of these limitation this study is therefore aimed at determining the therapeutic activity of Allium sativum bulb extract and diminazene aceturate or their combination in rats infected with Trypanosomes brucei brucei and to also evaluate the safety of the agents through toxicity studies.

MATERIALS AND METHODS

Source Of Plant Material

The fresh bulb of Allium sativum were purchased at Sokoto Central Mark in Sokoto state north western Nigeria in November 2010, the fresh bulb of Allium sativum was identified by a Botanist in the Biological Sciences Department, Usman Danfodiyo University, Sokoto Nigeria. A voucher specimen was retained in its herbarium.

Preparation Of Extract

500g of air dried at room temperature of allium sativum bulbs were cut in to pieces and pulverized bulbs were soaked in to 1500ml of distilled water and heated to boiling point. The mixture was filtered using what man filter paper No.1 by inserting it in to a funnel. The filtrate obtained was further concentrated in an oven (Gallen Kamp oven BS sized three) at 50°C. The concentrate then was preserved in a refrigerator for further use in the study. The percentage yield of the extract was calculated to be 47 %. The percentage yield of the plant extract was estimated as a ratio of the weight of the oven dried crude extract to the weight of the powder extract multiply by one hundred.

Experimental Animals

Thirty five clinically healthy rats of both sexes weighing between 100-120grammes were randomly assigned to seven groups of five animals. The rats were sourced from the Department of Pharmacology, University of Jos, Nigeria. The animals were maintained on commercially prepared feed and housed in metallic disinfected cages; clean water was provided ad libitum for fourteen days. The animals were acclimatized for two weeks prior to the commencement of the experiments. The rats were screened for the presence of haemoprotozoans parasites and were all confirmed negative. The animals were subjected to different doses of plant extract, Group A infected not treated as negative control. Group B infected and treated with a single standard dose (3.5mg/kg/bw) diminazene aceturate intra peritoneal. Group C infected treated with sub therapeutic dose (1.75mg/kg/bw) diminazene aceturate and sub therapeutic dose (20mg/kg/bw) Allium sativum bulb extract orally for three days consecutively.

Group D: - Infected treated with quarter dose(0.875mg/kg/bw) Diminazene aceturate and quarter dose(10mg /kg/bw) Allium sativum bulb extract orally for three days consecutively.

Group E: - Pre-treated for three days with subtherapeutic dose 20mg/kg/bw Allium sativum bulb extract orally and subtherapeutic dose(1.75mg/kg/bw) Diminazene aceturate

Group F: - Infected treated with standard dose( 40mg/kg/bw) Allium sativum bulb extract for three days orally

Group G: - The uninfected control.

PHYTOCHEMICAL SCREENING OF THE EXPERIMENTAL PLANT

The phytochemical test that were carried out include, qualitative screening to identify saponins, alkaloids, glycosides, flavonoids, anthroquinones, tannins, volatile oils and triterpenoids in the extract residue by using standard method as described by El-Olemmy et al., (1994) was carried out.

Assessment Of Therapeutic Activity

The criteria used in the assessment of the trypanocidal effect of the various agents include, the degree of parasitemia by examination of blood specimen daily for parasite, clinical changes during treatment and after treatment, possible death, and biochemical changes before and after treatment and frequency of relapse.

Blood Collection And Serum Analysis

A 21 guage needle with stringe was used to carry out cardiac puncture in anaestized rats using 1% chloroform. 2ml of blood was collected in a sterile bottles total serum protein was determined using the Biuret method. Serum alanine aminotransferasphosphatase were determined through spectrophotometer using commercially available kits( Randox laboratories ltd, crumlin, uk).

Statistical Analysis

Results are presented as means± standard deviation, the means were compare by Analysis of varians (ANOVA) and probability level at( P<0.05) was considered significant.
trypanosome in the five test groups (ABCDF) combinations on biochemical iturate (1.75mg/kg/bw) + ne as against combination (Group different groups of rats with T. brucei in inoculation on day five (peak parasitemia) showed mild to moderate reduction. Furthermore, in Group A infected but not treated exhibited progressive parasitemia that resulted in the death of all the rats by day 16 of observation (p<0.05) while non infected control (Group G) showed no signs of trypanosomosis or other infection which is in agreement with (Ajagbonna 2005., Dina et al., 2002). In the pre treatment (Group E) with a combination of half doses of Allium sativum bulb extract conferred a complete protection against T. brucei infection in rats throughout 60 days period of observation(Table2). Similarly relapse parasitemia was detected in rats treated with 40mg/kg/bw Allium sativum bulb extract alone as against combination (Group
C), (Milkail2002, Peni et al 2009). The biochemical indices presented in (Table 3) showed that the serum protein level of the test groups were significantly different at pre and post treatment following parasitemia.

There was rise in the plasma total protein Group A 92±1.54 vs. 71±6.33 in the un infected( Group G). The various treatment regimen significantly reduced (p<0.05) Total protein value to 71.0 ± 1.73,. 77.0 ± 3.60, and 71.7±6.33 in the combination groups( CDE) respectively as against 82 ± 4.26 from the control group (A). The increase in ALT, AST and ALP in the infected not treated group (A) was compared significantly different than the tested groups (BCEDFG). The increase in ALT, AST was as a result of hepatocyte damage (Nybom et al 2004 and 2006). Similarly, the significant increase in the ALT,AST and ALP in the infected not treated group (A) is a reflection of moderate to mild hepatic damage which is in agreement with earlier findings (Omotainse et al 1994, Ajagbonna et al 2003). However, the decrease levels of these enzymes infected treated groups (C D) as against the infected but not treated group (A) reflect mild to moderate synergistic and ameliorative effects of sub therapeutic dose of allium stvm bulb extract in combination diminazene aceturate. Robert,1984 demonstrated infection with T. brucei resulted in hypocholesrolaemia, this is the case in this study but the treatment with a combination group (C) corrected the hypocholesterolemia. It is also known that antiparasitic activity of most aromatic dimidine including diminazene aceturate may be related to their interference with aerobic glycolysis and are known to cause hypoglycemia in treated animals which was observed in all the tested groups (ABCDEF) as against positive control group(G). The phytochemical result of Allium sativum bulb extract indicate the presence of 2.3g%w/w of saponin, this substance may be attributed to its trypanocidal activity and synergistic effect in this study, (El olemy,et al,1994). In conclusion sub therapeutic doses of Allium sativum bulb extract and its combination with diminazene aceturate appears to be more effective in chemotherapeutic and prophylactic treatment and reducing the severity of Trypanosoma brucei infection as evidence of effective alternative, to already existing toxic and costly trypanocide.

REFERENCES


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