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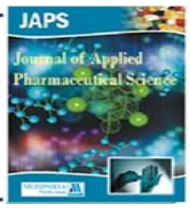

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Effects of *Allium sativum* Bulb Extract, Diminazene Aceturate and Their Combination on Parasitaemia, and Biochemical Indices in Rats Experimentally Infected with *Trypanosoma Brucei Brucei*

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ABSTRACT

Thirty five clinically healthy albino rats of both sexes weighing between 100 – 120grams were used to study the effects of *Allium sativum* bulb extract in combination with diminazene aceturate on parsitemia and biochemical indices in *trypanosome brucei brucei i*nfection. The rats were divided in to 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 treatment 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 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 *trypanosome brucei brucei* infection and provide alternative in reducing the toxicity of existing trypanocide.

Keywords: A*llium 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-Sahara 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 trypanosomosis 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 40m/ 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, Usmanu Danfodiyo University, Sokoto Nigeria. A voucher specimen was retained in its herbarium.

Preparation Of Extract

500g of air dried at room temperature of allium satvum 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 50^0c. 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 *adlibitum* 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.

Table. 1: Phytochemical constituents of the extract of *Allium sativum* bulb extract

S/No	Constituents	Percentage yield
A	*Alkaloids*	*+(4.28)*
B.	*Saponin*	*+ (2.28)*
C	*Tannins*	-
D	*Glycoside*	-
E	*Cardiac Glycosides*	-
F	*Cynogenic glycosides*	-
C.	*Digitalic glycoside*	-
D.	*Anthroquinoles*	-
E.	*Free Anthroquinol*	-
F.	*Flavonoids*	+
G.	*Salkowski steroid*	+

Key:
+ Represent presence of the chemical constituent in plant
- Represent absence of the chemical constituent in plantReducing sugars which are present in moderate to high concentration..

Table. 2: Parasitemia per day of observation in different groups of rats with T. brucei infection.

Treatment	*0*	*1*	*3*	*5*	*7*	*9*	*11*	*13*	*15*	*17*	*19*	*21*	*25*	*30*	*35*	*40*	*45*	*50*	*55*	*60*
Groups (rats)																				
A	0/5	0/5	*3/5*	*5/5*	*5/5*	*5/5*	4/4	*2/2*	*1/1*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*	*%*
B	0/5	0/5	2/5	5/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
C	0/5	0/5	2/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
D	0/5	0/5	3/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
E	0	0	0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F	0/5	0/5	3/5	5/5	5/5	3/5	2/5	2/5	2/5	2/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key
A = Infected not treated
B =Diminazene aceturate (3.5mg/kg/bw) single standard dose i/p.
C = Diminazene aceturate (1.75mg/kg/bw) + *Allium sativum* bulb extract (20mg/kg) treated sub therapeutic dose.
D = Diminazene aceturate (0.875mg/kg/bw) + *Allium sativum* bulb extract (10mg/kg/bw) treated sub therapeutic dose.
E = Pre treated Diminazene aceturate (1.75mg/kg/bw) + *Allium sativum* bulb extract (20mg/kg/bw).
F = *Allium sativum* bulb extract (40mg/kg/bw) standard dose orally.
G = Un infected not treated control

Table. 3: Effects of *Allium sativum bulb extract*, Diminazene aceturate and their combinations on biochemical indices.

	A	**B**	**C**	**E**	**D**	**F**	**G**
Total protein (g/l)	92.0±1.54*	82.3±4.26*	71.0±1.73*	77.0±3.60*	71.7±6.33*	84.7±3.10	71.7±6.33*
Glucose (mg/dl)	4.3±03	4.46±0.59	4.43±0.1	4.40±0.1	4.86±0.77	4.1±0.0	7.3±0.04*
ALP (lu/L)	12.66±0.66	26.0±4.62*	20±0.3.32*	23.33±2.60*	18.3±3.76*	19.0±1.73*	23.0±0.04*
AST (luL)	4.66±0.33	2.66±0.66*	2.0±0.58*	2.67±0.66*	3.33±0.33*	3.33±0.66*	2.33±0.33*
ALT (lu/L)	4±0	1±0	1.3±0.33*	1.3±0.33*	2.3±0.33*	1.7±0.66*	1.7±0*
Cholesterol (g/dl)	2.72±0.06*	4.02±0.12*	3.99±0.26*	3.40±0.39*	4.31±0.21*	3.79±0.26*	4.33±0.06*

Key
A = Infected not treated
B =Diminazene aceturate (3.5mg/kg/bw) single standard dose i/p.
C = Diminazene aceturate (1.75mg/kg/bw) + *Allium sativum* bulb extract(20mg/kg) treated sub therapeutic dose.
D = Diminazene aceturate (0.875mg/kg/bw) + *Allium sativum* bulb extract (10mg/kg/bw) treated sub therapeutic dose).
E =Pre treated Diminazene aceturate (1.75mg/kg/bw) + *Allium sativum* bulb extract (20mg/kg/bw).
F = *Allium sativum* bulb extract (40mg/kg/bw) standard dose orally.
G = Uninfected not treated control
i/p = intra peritoneal
*P<0.05 when compared with the controls values on the same row with different Superscript differ significantly (P<0.05).

DISCUSSION

The result of the study presented in table two showed progressive development of parasitemia within four to five days of inoculation of trypanosome in the five test groups(ABCDF) compared to the control group(G) Ameh *et al* 2006, Anene *et al* 2006. However groups treated with different dosage level employed in this study on day five (peak parasitemia) showed mild to moderate reduction. Total clearance of parasite was observed(p<0.05) in the combination (Group C) treated with sub dose 1.75mg/kg/bw of diminazene aceturate and 20mg/kg/bw of *Allium sativum* within two days of observation and remain parasite free throughout 60 days of observation, also was the (Group B) treated with 3.5mg/ kg/bw diminazene aceturate alone. 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 diminazene aceturate group alone, (Okochi *et al*, 1999). The levels of ALT and AST in the infected and not treated group (A) was compared significantly different than the tested group(BCEDFG). The increase in ALT, AST was as a result of hepatocyte damage (Nyblom *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 stvum 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.

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