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Full Length Research Paper

Effects of treatment with ethanol extract of *Gardenia sokotensis* on haematological and biochemical changes in *Trypanosoma brucei brucei* infected rabbits

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This study was carried out to determine the comparative effects of 7-day treatment with the ethanolic crude root extract of *Gardenia sokotensis* (administered orally at dose rate of 60 mg/kg body weight) and diminazen aceturate (Berenil, administered once, intramuscularly, at the dose of 3.5 mg/kg body weight) on *Trypanosoma brucei brucei* infection-induced changes in serum biochemical and haematological parameters in rabbits. The treatments with the two forms of drugs were commenced when parasitaemia was at its peak level on day 41 post-infection. Significant reduction in the level of parasitaemia was observed following the 7-day treatment of the *T. brucei brucei* infected group of rabbits with the root extract of *G. sokotensis*. Concomitantly, amelioration of the anaemia, as reflected by improvement in the packed cell volume, and some of the serum biochemical changes was observed. These latter changes were however not as marked as those observed in the Berenil-treated group. The findings in this study strongly suggest that the root extract of *G. sokotensis* may contain some active substance(s) that exhibit anti-trypanosomal properties.

Key words: *Gardenia sokotensis*, crude root extract, haematological and biochemical changes.

INTRODUCTION

Trypanosomiasis (trypanosomosis) is a disease syndrome caused by one or more of the pathogenic trypanosome species. The disease is characterized by slow, progressive loss of condition accompanied by an array of clinical disorders among which are anaemia, which is the principal and most consistent feature of the disease, central nervous manifestations, weakness, extreme emaciation, coma, infertility and abortion, and death in some cases (Murray, 1979; Monzon et al., 2003; Ngaira et al., 2003; Tuntasuvan et al., 2003; FAO, 2005; Enwezor et al., 2006; Chechet et al., 2010; Jansena et al., 2010).

Despite advances in modern medicine and existence of a large body of research findings on African trypanosomiasis, treatment of the disease is still beset with problems of drug resistance, narrow spectrum of activities and prohibitive costs of some of the drugs used in controlling the disease. For example, one of the drugs developed of recent, eflornithine, is effective for late-stage of the gambiense disease and its cost makes it unaffordable. Moreover, relatively few drugs used in treatment of tropical diseases reach the market (WHO/TDR, 2004). These factors, thus, necessitate the search for herbal remedies. *Gardenia sokotensis*, which is found on dry rocky hills of the drier savanna regions of

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the tropical Africa, is one of the species of plants whose leaves were reported to be widely used in folk medicine (Traore, 1983; Ake-Assi and Guinko, 1991). Indeed, various parts of the plant were reported to have a promising *in vivo* anti-plasmodial activity (Traore et al., 2006). There is however dearth of information on the effects of treatment with the root extract of this plant in animals infected with other protozoan organisms; for example, trypanosome infections in animals. This study was therefore designed to determine the comparative effects of treatments with ethanolic root extract of *G. sokotensis* and diminazen aceturate on some serum biochemical and haematological parameters in rabbits infected with *T. brucei brucei*.

MATERIALS AND METHODS

Plant collection and identification

Plant collection and identification were as previously described (Jodi et al., 2008a, b).

Preparation of the crude extract of the root of *Gardenia sokotensis*

Crude extract of the root was prepared following the procedure described by Kudi et al. (1999) and Samy and Ignacimuthu (2000). Briefly, the procedure involved air drying the roots of *G. sokotensis* in the laboratory at room temperature for 7 days. The air dried roots were then broken into smaller sizes, and grounded to powder using an electric blender. Five grams of the powdered root material were mixed with 50 ml of 80% ethanol at room temperature and left overnight. It was then filtered using Wattman filter paper. The recovered filtrate was concentrated by evaporation and then oven dried. The resultant dark brown crude extract was used in this study. Five hundred milligrams of this concentrated residue were weighed and dissolved in 100 ml distilled water for use on each day of the experiment (Kudi et al., 1999).

Experimental animals

Fifty apparently healthy rabbits of both sexes with ages ranging between 3 to 4 months old and weighing between 1.05 to 1.6 kg were purchased from Sokoto central market. The rabbits were on arrival kept in a highly ventilated animal house of the Usmanu Danfodiyo University, Sokoto, Nigeria and fed on grower's mash (Pfizer Nigeria Plc), fresh green vegetables and water was provided *ad libitum*. The animals were ethically treated as contained in the Animal Use and Care Policy section of the Research Policy of Usmanu Danfodiyo University, Sokoto, Nigeria, which is in conformity with international standards. Before the commencement of the experiment, the animals were confirmed to be free of haemoprotozoan parasites through blood screening using parasitological techniques.

The parasite (*Trypanosoma brucei brucei*)

The *T. brucei brucei* used in this study was obtained from the parasitology division of Nigerian Institute for Trypanosomiasis Research (N. I. T. R), Vom, Jos, Plateau State, Nigeria. The stabilate of the parasite was inoculated, intraperitoneally, into 4 donor rabbits, which were then transported to Usmanu Danfodiyo University, Sokoto. The infected rabbits were kept in highly ventilated animal house, well fed, and monitored for appearance of the parasites in their blood (parasitaemia) through daily collection and examination of blood from the rabbits (Woo, 1969). Following the development of parasitaemia in the rabbits, the *T. brucei brucei* organisms were maintained in the laboratory by serial passages in other rabbits.

Animal allocation, Infection with *T. brucei brucei* organisms and treatment with the crude root extract and diminazen aceturate

A total of thirty rabbits of both sexes were selected at random and allocated into five groups (leveled groups A, B, C, D and E) of 6 rabbits, each. When parasitaemia was at swarming degree in the donor rabbit, the infected blood samples were collected and every 0.5 ml of the blood was diluted with 5 ml of normal saline. Each of the rabbits in the infected group was infected with the trypanosomes through intraperitoneal inoculation of 1 ml of the diluted blood containing an estimated number, as determined using method described by Mutayoba et al. (1994), of 5×10^5 *T. brucei brucei*. Following infection of the experimental animals with the trypanosomes, parasitaemia was determined daily as previously described. The treatments with the two forms of root extract and diminazen aceturate were commenced when the mean parasitaemia was at its peak level on day 41 post-infection. Diminazen aceturate was administered intramuscularly at a dose rate of 3.5 mg/kg body weight. The crude root extract preparation, in a concentration of 5 mg/ml, was orally administered to the rabbits (Kudi et al., 1999). The various experimental animal groups were as follows: Group A comprised rabbits that were infected with *T. brucei brucei* and treated once daily, after the establishment of the peak parasitaemia level, through oral administration of the root extract of *G. sokotensis* at a dose rate of 60 mg/kg body weight for 7 days. Group B rabbits were infected with *T. brucei brucei* and treated once, after the establishment of the peak parasitaemia level, through intramuscular injection of diminazene aceturate (berenil®) at a dose rate of 3.5 mg/kg. Group C comprised rabbits that were infected with *T. brucei brucei* but did not receive any treatment. Group D consisted of rabbits that were uninfected with the *T. brucei brucei* but were treated orally with the root extract of *G. sokotensis* at a dose rate of 60 mg/kg for 7days. Group E rabbits were uninfected with the *T. brucei brucei* and did not receive any treatment and were therefore termed the pure control group.

Collection of blood samples for parasitological, haematological and biochemical analyses

Beginning from day 1 post-infection about 6 ml of blood were collected from the ear vein of each of the animals in the experimental groups and continued at intervals of 2 days up to day 41 post-infection (day of commencement of treatments with root extract of *G. sokotensis* and diminazen aceturate) after which the blood sampling was carried out daily until the experiment was terminated on day 50 post-infection. About 0.5 ml of the blood was dispensed into a vacitainer containing ethylene diaminetetraacetic acid (anticoagulant) and was used to determine parasitaemia level (Herbert and Lumsden, 1978) and packed cell volume using standard microcapillary technique. The remaining 4.5 ml was allowed to clot and serum was harvested and used for serum chemistry and biochemical analyses. Total serum protein, albumin, globulin, total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, sodium and potassium concentrations were determined pre- and post-infection of the rabbits with *T. brucei brucei* organisms.

Table 1. Levels of parasitaemia (trypanosomes/per field) in *T. brucei brucei* infected rabbits before treatment with either *Gardenia* or Berenil.

Days	Parasitaemia				
	Group A	Group B	Group C	Group D	Group E
1	0	0	0	0	0
3	1* (10^3)	1* (10^3)	1* (10^3)	0	0
5	7 (10^5)	3 (10^4)	3 (10^4)	0	0
7	3 (10^4)	4 (10^4)	5 (10^5)	0	0
9	2 (10^4)	5 (5×10^5)	13 (5×10^5)	0	0
11	2 (10^4)	9 (10^5)	9 (10^5)	0	0
13	2 (10^4)	5 (10^5)	2 (10^4)	0	0
15	15 (5×10^5)	7 (10^5)	14 (5×10^5)	0	0
17	3 (10^4)	10 (10^5)	10 (10^5)	0	0
19	5 (10^5)	12 (5×10^5)	12 (5×10^5)	0	0
21	8 (10^5)	13 (5×10^5)	2 (10^4)	0	0
23	2 (10^4)	10 (10^5)	6 (10^5)	0	0
25	10 (10^5)	14 (5×10^5)	5 (10^5)	0	0
27	9 (10^5)	7 (10^5)	4 (10^4)	0	0
29	6 (10^5)	4 (10^4)	7 (10^5)	0	0
31	7 (10^5)	11 (5×10^5)	12 (5×10^5)	0	0
33	14 (5×10^5)	18 (5×10^5)	13 (5×10^5)	0	0
35	21 (5×10^5)	20 (5×10^5)	17 (5×10^5)	0	0
37	29 (5×10^5)	27 (5×10^5)	27 (5×10^5)	0	0
39	47 (5×10^5)	43 (5×10^5)	39 (5×10^5)	0	0
41	44 (5×10^5)	46 (5×10^5)	38 (5×10^5)	0	0

A = Infected and treated with *Gardenia sokotensis,* B = infected and treated with Berenil®, C = infected but untreated (control of treatments), D = uninfected but treated with *Gardenia sokotensis,* E = uninfected and untreated group (control of the experiments). Each value is the average number of trypanosomes per microscopic fields in a group. *Values represent number of trypanosomes per preparation, figures in parentheses represent the corresponding average number of trypanosomes per millilitre of blood.

Determination of serum total proteins, albumin and globulins

Total serum protein and albumin were determined using the Biuret (Weigsebaum, 1965), while the differences between the total serum protein and corresponding albumin concentrations represent the concentrations of globulins.

Determination of serum cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol

The serum levels of cholesterol, triglycerides and high-density lipoprotein cholesterol, measured in mmol/L, were determined using standard commercial test kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom) as described elsewhere (Adamu et al., 2008). Instructions provided by the manufacturers were strictly followed.

Low-density lipoprotein cholesterol was calculated from the values total serum cholesterol, triglycerides and high-density lipoprotein using the formula described by Friedewald et al. (1972).

Measurement of serum sodium and potassium concentrations

Sodium and potassium concentrations were determined using the flame photometer method (Cheesbrough, 1998).

Statistical analysis

All the results obtained were presented as means ± standard deviations. Levels of significance between means in the various experimental groups were determined using Student t-test (Steel and Torie, 1980).

RESULTS

Parasitaemia

The parasites, *T. brucei brucei,* were detected in, at least, some of the animals in all the infected groups on day 3 post-infection and by day 5, all the animals infected with the trypanosome were showing parasitaemia. The mean parasitaemia levels in the different experimental animal groups were shown in Table 1. The parasitaemia level was fluctuating in individual animals of the infected groups. Mean parasitaemia levels rose gradually as the infection aged and peaked on day 39 in groups A (5×10^5 trypanosomes/ml) and C (5×10^5 trypanosomes/ml), and on day 41 post-infection in group B (5×10^5 trypanosomes/ml). The attainment of the peak

Table 2. Serum sodium (mmol/L), potassium (mmol/L), total protein (T-Protein in g/dl), albumin (g/dl) and globulins (g/dl) concentrations in rabbits infected with *Trypanosoma brucei brucei* and treated with the extract of *Gardenia sokotensis* or Berenil (*Diminazine aceturate*).

Period	Groups	Parameters				
		Sodium	Potassium	T- Protein	Albumin	Globulin
B.I.	A	137.0±13.6	4.9±1.0	3.5±0.3	2.6±0.2	0.9±0.2
	B	138.0±6.5	6.1±1.5	3.3±0.3	2.6±0.3	0.7±0.2
	C	138.7±6.2	6.4±0.6	3.2±0.2	2.5±0.2	0.7±0.3
	D	138.3±8.1	5.5±1.4	3.9±1.6	2.8±0.6	1.1±1.1
	E	137.7±5.8	5.8±1.0	3.2±0.3	2.5±0.2	0.7±0.3
B.T.	A	149.6±1.1	6.9±1.0	10.5±1.4	1.9±0.1	8.4±1.2
	B	149.0±5.4	6.3±0.7	11.7±1.1	2.0±0.2	9.7±1.1
	C	143.0±8.0	7.2±1.7	9.9± 1.8	1.9±0.2	8.0±1.7
	D	138.1±8.2	5.5±1.4	3.9±1.6	2.8±0.6	1.2±1.1
	E	137.7±5.0	5.8±1.0	3.2±0.3	2.4±0.2	0.8±0.1
A.T.	A	122.4±10.3	4.8±0.2	9.9±1.6	2.0±0.2	7.9±1.6
	B	138.6±8.0	5.9±0.9	8.8±1.7	2.1±0.3	5.6±1.3
	C	144.5±8.7	7.7±1.5	11.4±2.0	1.7±0.2	9.7±1.9
	D	144.0±6.2	6.3±0.3	3.9±1.6	2.6±0.2	1.3±1.6
	E	137.5±4.7	5.8±1.0	7.9±11.4	2.5±0.2	0.7±0.3

Data are mean ± SD with ($P<0.05$). A = Infected and treated with *Gardenia sokotensis,* B = infected and treated with Berenil, C = infected but untreated (control of treatments), D = uninfected but treated with *Gardenia sokotensis,* E = uninfected and untreated group (control of the experiments), B.I = before infection, B.T = before treatments, A.T = after treatments.

parasitaemia levels were followed by a period of rapid decline, which was glaring especially in group C (infected but untreated). The pooled mean post-treatment parasitaemia level in group A (infected and treated with *G. sokotensis*) was 1×10^4, while that in group C was 1×10^5. The difference between the pooled means of parasitaemia in groups A and C were significant ($P < 0.05$).

Biochemical analyses

Results of biochemical analyses (Table 2) showed that serum sodium, potassium, total protein, globulins, triglyceride and LDL concentrations increased with rise in parasitaemia level but decreased following the treatment of the animals with either *Gardenia* or Berenil. On the other hand, serum values of albumin and HDL decreased as parasitaemia increased but increased post-treatment with either *Gardenia* or Berenil (Tables 2 and 3).

DISCUSSION

The chronic and fluctuating parasitaemia observed in this study was a finding typical of trypanosome infections as reported by Barry and Turner (1992). The explanation for this pattern of infection and the failure of the immune response to successfully control the parasitaemia, lies in the ability of parasite populations to express a sequence of antigenically different surface glycoprotein molecules, the variant specific glycoproteins (VSGs) and this exposes the host to a sequence of variant antigen types (or VATs). Each subpopulation expresses one such VSG against which the host mounts a protective antibody response. This response results in the destruction of the VAT concerned, but this merely allows another subpopulation of a different VAT to develop (Logan-Henfrey et al., 1992). The level of parasitaemia decreased rapidly to zero level in the group treated with Berenil resulting in total cure for all the animals in this group. However, only a slight decrease in parasitaemia level in the group treated with the ethanol extract of *G. sokotensis* was observed. This finding, nevertheless, suggests that, the plant *G. sokotensis* has some medicinal potentials in treatment of trypanososis. The mechanisms of action of the ethanol crude root extract were not investigated in this study, thus, indicating the need for a more detailed phytochemical analysis on this plant with the view to elucidating its active ingredients and their possible mechanisms of action. Mansfield (1990) working with *T. brucei brucei* in mice has shown that under normal conditions, the host is continually exposed to novel VATs and is never able to achieve parasite elimination.

The increases in total protein and globulin ($p<0.05$) in infected animals when compared with controls might

Table 3. Serum cholestrol (Cho), tryglyceride (Tri), High Defnsity Lipoprotein (HDL) and Low Defnsity Lipoprotein (LDL) concentrations in mmol/L in rabbits infected with *Trypanosoma brucei brucei* and treated with the extract of *Gardenia sokotensis* or Berenil (Diminazine aceturate).

Periods	Groups	Parameters			
		Cholesterol	Triglyceride	HDL	LDL
B.I.	A	86.3±12.7	154.3±53.3	22.3±8.7	33.3±16.9
	B	94.3±26.0	122.2±62.4	22.1±12.7	49.5±23.7
	C	67.8±14.3	76.0±16.0	23.5±11.5	29.2±14.2
	D	94.3±48.6	93.2±14.1	19.0±7.7	55.5±43.5
	E	81.7±15.2	85.2±23.8	19.5±7.7	45.2±21.0
B.T.	A	99.4±13.9	178.0±5.3	12.6±5.1	54.8±28.2
	B	106.2±32.0	143.6±38.8	15.3±9.8	63.9±25.3
	C	91.2±17.0	132.3±22.4	16.0±8.7	48.7±25.0
	D	103.5±29.2	84.0±24.5	19.2±7.1	67.5±26.3
	E	82.7±14.5	84.0±20.2	20.9±6.8	49.5±23.2
A.T.	A	123.2±31.1	153.2±43.7	14.3±6.4	77.3±28.5
	B	98.6±38.4	107.6±55.5	22.4±6.6	54.7±32.9
	C	150.7±40.6	146.2±12.8	13.4±4.4	108.0±40.7
	D	100.0±35.3	85.2±21.2	24.7±12.5	58.1±37.2
	E	82.2±13.4	84.0±19.7	20.8±6.8	44.6±2

Key: Data are mean ± SD with ($P<0.05$). A = Infected and treated with *Gardenia sokotensis,* B = infected and treated with Berenil, C = infected but untreated (control of treatments), D = uninfected but treated with *Gardenia sokotensis,* E = uninfected and untreated group (control of the experiments), B.I = before infection, B.T = before treatments, A.T = after treatments.

have been due to increased release of tissue specific enzymes and other intracellular proteins secondary to parasite-induced cell membrane disruption just as was earlier reported in *T. brucei* infected rabbits (Onyelili et al., 2005). It is also possible that the increase in total protein may have been the result of increased mass of parasite proteins as a result of growing infection or possibly increases in parasite-derived intracellular enzymes and proteins as the parasites are lysed by the host immune system. Elevation in globulin could possibly be due to enhanced antibody production in response to infection and would no doubt might have contributed immensely to the observed hyperproteinaemia in the trypanosome-infected animals.

Evaluation of serum albumin showed that there was a decrease in values obtained for infected animals relative to uninfected (controls). Albumin is produced entirely in the liver and is of great importance in regulating the flow of water between the plasma and tissue fluids via its effect on colloid osmotic pressure. Consequently, any hepatic pathology could result in impairment of its production. A drop in serum albumin level could also follow increased protein loss through the gut or the kidney. Other possible cause of decrease in albumin may include malabsorption and increased protein need secondary to infection. It is also possible that the body could be using the albumin to synthesize the vast amounts of antibodies and hence the decrease in the albumin value in the infected animals. The normal half life of albumin is an average of 21 days, and therefore a decrease in serum albumin is usually not apparent early in the cause of liver diseases (Halsted and Halsted 1991; Cheesbrough, 1998).

Oedema is one of the consequences of low plasma albumin level since albumin is essential in the maintenance of plasma colloid osmotic pressure (Cheesbrough, 1998). These changes in serum proteins, which accompanied the trypanosome infection as observed in this study were earlier reported and reviewed by several workers (Igbokwe and Mohamed, 1992; January et al., 1991; Ogunsanmi et al., 1994). The changes observed in the trypanosome infected animals in this study, no doubt, may have been the consequences of multi-system involvement (Nyakundi et al., 2002; Taylor et al., 2001, Agu, 2002; Girard et al., 2003). Treatment with *G. sokotensis* resulted in significant reduction in the level of the parasite-induced hyperproteinaemia and hyperglobulinaemia as well as a significant amelioration of infection associated hyporalbuminaemia.

There was an increase in the cholesterol level in the entire infected group. This may be so since increase in serum cholesterol (Hypercholesterolaemia) can be seen in patient with odema (Chawla, 1999; Varley et al., 1980) and there was an oedema of the face in the infected rabbits. Likewise, sodium in infected group increased significantly and decreased after treatment, while it

continued to increase in untreated. Both the sodium and potassium tend to increase with infection according to Chawla (1999). The metabolism of sodium and potassium is closely linked to the maintenance of fluid balance and to the regulation of acid base status. An increase in one of them may lead to increase of the other. The increase of sodium and potassium in this study may be due to renal impairment or due to the increase in potassium or as a result of the infection itself. Potassium increase (Hyperkalaemia) may be due to trypanosomal infection.

In conclusion, this study has demonstrated the potential of the anti-trypanosomal property of *G. sokotensis* root extract, although there was no complete elimination of the parasites as it was the case with Berenil. All the same, the fact that there was reduction in the parasitaemia level suggests that the extract could improve the state of anemia and, thus, increased survival time of the infected animals. Moreso, since the extent of alterations in haematological and biochemical parameters in trypanosome-infected animals is a function of the level of parasitaemia (Logan-Henfrey et al., 1992).

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