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Full Length Research Paper

Anti anaemic properties of *Scoparia dulcis* in *Trypanosoma brucei* infected rabbits

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A major feature of infection with trypanosomes is the development of anaemia. In this study, the effect of *Scoparia dulcis (*Atiotiousha in Akoko-Edo) on *Trypanosoma brucei* induced anaemia was investigated in fifteen rabbits divided into three groups of n = 5 over a period of twenty eight days. Changes in Packed cell volume (PCV), Haemoglobin (Hb) concentration, Red blood cell count (RBC), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration, (MCHC) and Mean cell volume (MCV) were monitored over the period. The results obtained indicate that infection with T. brucei results in a significant decrease ($p<0.05$) in PCV, Hb concentration and RBC. No significant ($p>0.05$) changes were observed in MCH, MCHC and MCV. However the severity of observed anaemia was significantly less pronounced ($p<0.05$) in the infected rabbits that were treated with *S. dulcis* when compared with their infected but untreated counterparts. It is concluded that *S. dulcis* therapy may prove useful in the management of *T. brucei* anaemia, and possibly other forms of anaemia, although the precise mechanism by which the preparation enhances the haematological indices investigated remains to be fully understood.

Key words: *Scoparia dulcis*, *Trypanosoma brucei*, Rabbits, PCV, Haemoglobin.

INTRODUCTION

Scoparia dulcis (Atiotiousha in Akoko-Edo) is an erect annual herb with serrated leaves, producing white flowers and measuring up to a half meter in height when fully grown. Its ethnomedicinal uses amongst various indigenous tribes in the rain-forest zone is well-documented (Branch and daSilva, 1983). Some aspects of the several speculated pharmacological properties of *S. dulcis* have been validated by scientific research and includes the hypoglycaemic, anti tumour promoting and anti viral activities (Jain, 1985; Nashino, 1993; Hayashi, 1990). Phytochemical screening of the herb revealed that it is rich in flavonoids and terpenes and the pharmacological actions of *S. dulcis* are believed to be due to the presence of these phytochemicals (Hayashi, 1990; Kawasaki, 1987; Hayashi, 1987; Hayashi, 1991; Ahmed, 1990).

In Nigeria, the plant has been used in the management of sickle cell anaemia for over two decades (Hilda Ogbe, personal communication). The widespread claims of massive boost in haematocrit or packed cell volume (PCV) and haemoglobin (Hb) levels in these patients and the apparent amelioration of the frequent and severe crisis associated with the disease prompted this study. Progressive anaemia is widely accepted as a cardinal feature of *Trypanosoma brucei* infection (Moulton and Sollod, 1976; Suliman and Feldman, 1989).The present paper summarizes our report on the efficacy of *S. dulcis* in the management of trypanosome induced anaemia in the rabbit.

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MATERIALS AND METHOD

Experimental design

A total of 15 New Zealand white rabbits (average weight= 1.50 kg) obtained from a private farm in Benin City were used for the experiment. These were randomly divided into 3 groups of n = 5 with each group allowed a 14 days acclimatization prior to the commencement of experiment. The rabbits were fed on growers mash (Bendel Feeds and flour Mill Ewu, Edo State, Nigeria) and water *ad libitum*. Group 1 served as un-infected control while group II and III were inoculated with *T. brucei*. Inoculation was by intraperitoneal injection of 0.5 ml of 1:1 (infected whole blood: normal saline) preparation, and with each inoculum containing about 2 x 10^6 of the parasite. Parasite estimation was by the rapid "matching" method of Herbert and Lumbsden, (1976). The original stock of *T. brucei* was obtained from the Nigerian Institute for Trypanosomiasis

Table 1. Effect of *S. dulcis* on Packed Cell Volume (PCV) in *T. brucei* infected rabbits.

Groups	PCV (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (I)	35.32±1.47 [a]	36.10±1.03 [a]	34.58±2.46 [a]	36.14[a]±2.04 [a]	35.62 ±1.08 [a]
Inoculated treated (II)	36.42±1.64 [a]	35.63±1.83 [a]	31.19±2.04 [a]	29.40± 2.11[a]	27.43 ±1.24 [a]
Inoculated untreated (III)	36.60±1.05 [a]	32.72±0.63 [a]	21.16± 2.22 [b]	22.12±1.37 [c]	19.23 ±2.00 [c]

Values are Mean ± S.E.M. Values on the same day with different superscript differ significantly ($p<0.05$).

Table 2. Effect of *S. dulcis* on haemoglobin concentration in *T. brucei*-infected rabbits.

Groups	Haemoglobin concentration (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (I)	12.14 ±0.21 [a]	12.37±0.19 [a]	1.890.24 [a]	12.42±0.26 [a]	11.94±0.33 [a]
Inoculated treated (II)	12.52 ±0.20 [a]	12.25±0.20 [a]	10.72±0.23 [a]	9.78±0.32 [b]	8.28 ±0.43 [b]
Inoculated untreated (III)	12.58±0.33 [a]	11.01±0.28 [a]	6.68±0.72 [c]	6.64± 0.84 [c]	5.78 ±0.62 [c]

Values are Mean ± S.E.M. Values on the same day with different superscript differ significantly ($p<0.05$).

Table 3. Effect of *S. dulcis* on Red Blood Cell (RBC) count in *T. brucei*-infected rabbits.

Groups	RBC (10^6/µL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (I)	5.62±0.19 [a]	5.48±0.14 [a]	5.58±0.19 [a]	5.434±0.18 [a]	5.27 ±0.15 [a]
Inoculated treated (II)	5.53±0.19 [a]	5.40±0.17 [a]	4.82±0.20 [a]	4.32±0.23 [a]	4.13 ±0.33 [b]
Inoculated untreated (III)	5.55±0.17 [a]	4.99±0.09 [a]	2.89±0.36 [b]	2.69±0.45 [b]	2.35 ±0.34 [c]

Values are Mean ± S.E.M. Values on the same day with different superscript differ significantly ($p<0.05$)

Research (NITRE). The control animals (Group 1) were given intraperitoneal injection of 0.5 ml of normal saline instead of parasite. In addition the inoculated and treated animals (group II) were given *S. dulcis* at a daily oral dose of 25 mg/kg body weight. The dose used was calculated on the basis of ethnomedicinal practice and the duration of study chosen to allow enough time for *T. brucei*-anaemia to develop.

Preparation of S. *dulcis* involved only rinsing with water to remove dust, air drying and blending of the entire shoot system. The plant which was obtained from a private farm belonging to Mrs. Hilda Ogbe was identified at the Department of Botany, University of Benin. The required weight of pulverized herb was administered as an aqueous suspension through gavage.

Blood samples were collected from the ear vein and put in EDTA-containing bottles. The blood samples were first collected prior to infection on day 0 and analyzed for baseline haematological values. Subsequent data obtained on days 7, 14, 21 and day 28 were compared with these pre-infection values within group in order to ascertain the pattern of observed changes. In addition, comparesons were also made across groups to evaluate the extent of trypanosome induced changes as well as the degree of *S. dulcis* mediated amelioration.

Haematological analysis

The haematological parameters, Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell Count, (RBC), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and Mean Cell Volume (MCV) were analyzed by means of an automated Beckman Coulter differential analyzer

Statistical analysis

The group Mean ± S.E.M. was calculated for each analyte and significant difference between means evaluated by analysis of variance (ANOVA). Post test analysis was done using the Tukey-Kramer multiple comparism tests. P values < 0.05 were considered as statistically significant

RESULT

The results obtained from this study are presented in Tables 1-6. There was significant decrease ($p<0.05$) in PCV, Hb and RBC in infected animals relative to control. These changes were however remarkably minimal in the infected *S. dulcis* treated animals. The values obtained for these parameters were significantly higher ($p<0.05$) for group II (inoculated and treated) when compared with group III (inoculated untreated). See Tables 1-3. Specifically, significant differences ($p<0.05$) between the treated and untreated groups were observed on days 14, 21 and 28. There were however no significant changes ($p>0.05$) in MCH, MCHC and MCV across the three groups (Tables 4-6)

DISCUSSION

The present study evaluated the possible anti-anaemic properties of *S. dulcis* in trypanosome-infected rabbits.

Table 4. Effect of *S. dulcis* on Mean Cell Haemoglobin (MCH) in *T. brucei*-infected rabbits.

Groups	MCH (pg)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (I)	22.48±0.31 [a]	22.01±0.22 [a]	23.04±0.31 [a]	22.57±0.19 [a]	22.59 ±0.23 [a]
Inoculated treated (II)	22.36±0.26 [a]	22.14± 0.22 [a]	22.07±0.36 [a]	23.26±1.08 [a]	22.89 ±1.60 [a]
Inoculated untreated (III)	22.72±0.38 [a]	22.30±0.29 [a]	23.60±0.56 [a]	24.68±1.66 [a]	24.82 ±1.543 [a]

Values are Mean ± S.E.M. Values on the same day with different superscript differ significantly (p<0.05).

Table 5. Effect of *S. dulcis* on Mean Cell Haemoglobin Concentration (MCHC) in *T. brucei* infected rabbits.

Groups	MCHC (g/d/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (I)	34.02 ±0.25 [a]	33.86±0.30 [a]	33.75±0.22 [a]	34.13±0.34 [a]	33.44 ±0.16 [a]
Inoculated treated (II)	33.78 ±0.35 [a]	33.44±0.22 [a]	32.86±0.42 [a]	32.08±0.32 [a]	30.19 ±1.19 [a]
Inoculated untreated (III)	34.28±0.27 [a]	33.94±0.24 [a]	31.46±0.54 [a]	29.97±2.1 6 [a]	31.94 ±1.25 [a]

Values are Mean ± S.E.M. Values on the same day with different superscript differ significantly (p<0.05).

Table 6. Effect of *S. dulcis* on Mean Cell Volume (MCV) in *T. brucei*-infected rabbits.

Groups	MCV (fl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control (I)	66.42 ±0.54 [a]	65.37±0.93 [a]	67.21±1.04 [a]	68.14±0.95 [a]	67.98±0.82 [a]
Inoculated treated (II)	65.27 ±0.68 [a]	66.31±0.84 [a]	68.84±3.01 [a]	74.12±4.12 [a]	75.85±6.78 [a]
Inoculated untreated (III)	67.78 ±0.71 [a]	65.56±0.68 [a]	75.30±2.98 [a]	82.28±5.57 [a]	80.60±7.93 [a]

Values are Mean ± S.E.M. Values on the same day with different superscript differ significantly (p<0.05).

The results obtained indicate that infection with *T. brucei* resulted in a significant decrease (P<0.05) in haematocrit or packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count. (RBC) These findings are consistent with earlier reports (Moulton and Sollod, 1976; Suliman and Feldman, 1989).

The observed decrease was progressive and became persistently severe reaching dangerously low levels on day 28. For instance, the values for PCV, Hb and RBC were reduced to as low as 52.526, 45.958 and 42.363 % respectively when compared with their pre-infection values. Igbokwe and Mohamed (1992) had earlier reported similar trends in goats experimentally infected with *T. brucei*, and the work of several others also indicate that progressive anaemia is a cardinal feature of trypanosome infection in rats and mice (Igbokwe et al., 1994, 1998; Anosa and Kaneko, 1983). Other mammalian species including dogs (Omotainse and Anosa, 1992, 1995; Egbe-Nwiyi and Antia, 1993) and humans (Anosa, 1988) are also known to be susceptible to *T. brucei* anaemia. There are reports that *T. congolense* induces anaemia in both livestock and experimental animals (Wellde et al., 1989). One earlier study (January et al., 1991) showed that *Trypanosomai brucei* infection is associated with anaemia and increased circulating nucleated red blood cells in the rabbit.

There are striking indications that the onset of anaemia in African trypanosomiasis may be strongly related to disruption of erythrocyte membrane caused directly by parasite attack on red cells. (Banks, 1979, 1980; Anosa and Kaneko, 1983) It has also been suggested that products secreted by the parasite may play a significant role in the disruption of red cell membrane (Huan et al., 1975; Tizard et al., 1978; Pereira, 1983; Knowles et al., 1989). Reduction in red cell membrane sialoglycoprotein secondary to elevated activity of plasma sialidases promotes the rapid destruction of erythrocytes, (Esievo et al., 1982; Aminoff, 1988; Olaniyi et al., 2001). A role for parasite and macrophage derived free radicals and proteases in the pathogenesis of trypanosome induced anaemia has also been postulated, (Knowles, 1989; Igbokwe et al., 1994).

Although progressive anaemia was observed in this study as a hall mark of *Trypanosoma brucei* infection in the rabbit, the decreases in PCV, RBC and Hb were however not accompanied with significant changes in the mean cell volume,(MCV), mean cell haemoglobin,(MCH) and mean cell haemoglobin concentration,(MCHC). The latter findings are suggestive of normocytic normochromic anaemia.

It is worthy of note that no significant changes were observed in the control group. Of even greater importance

importance is the observation that infected animals that were put on daily oral administration of *S. dulcis* had PCV, RBC and Hb levels that were significantly higher ($p<0.05$) than those of their untreated counterparts. These findings present *S. dulcis* as a plant with enormous therapeutic potentials, a possible natural and viable alternative in the management of anaemia.

The study shows that the herb exhibited remarkable potency in protecting against the haematological lesions accompanying *T. brucei* infection in rabbits. These findings open several doors of speculations and raise multiple questions, the answers to which may help unravel the precise mechanism(s) by which this feat is accomplished by *S. dulcis.* Till date, the exact mechanism(s) by which *S. dulcis* exerts its effect is/are not well established, nor can this activity be readily ascribed to any one of the many biologically active compounds present in the plant. While the exact mechanism remains much a matter of speculations, it seems logical to suggest that the putative mechanism may revolve around some or all of the following: Firstly. It is possible that *S. dulcis* may possess the ability to preserve and conserve the structural and functional capacity of the red cell membrane or erythropoietic tissues to varying degrees in the face of an offending parasitic infection. Secondly, since *T. brucei* infection is associated with massive generation of free radicals (Igbokwe et al., 1994), the antioxidant activity of *S. dulcis* severally reported by researchers (Babincova and Sourivong, 2001; Pari and Latha, 2005; Ratnasooriya et al., 2005) may contribute significantly to the overall effect of the herb observed in this study. Alternatively, the herb may possess a measure of trypanocidal activity or immuno-stimulating properties that help put the parasite in check and thus also control the deleterious effect of uncontrolled parasite proliferation. Although the antimicrobial activity of *S. dulcis* has been reported previously (Riel et al., 2002; Hayashi et al., 1988; Hayashi et al., 1990), the absence of any evidence of possible trypanocidal activity for the herb does not make this speculation an attractive option. This study nevertheless underscores the need to screen *S. dulcis* for possible trypanocidal activity. These and many more aspects of this subject certainly require further investigations.

The potentials of managing other forms of anaemia using *S. dulcis* are under scored by these findings. Anaemia (defined as haemoglobin concentration below normal for age) is the commonest red cell disorder the world over. In tropical and developing countries, 50% or more of pre-school children and pregnant women are moderately or severely anaemic (Cheesbrough, 2000). The disorder may be the result of malnutrition, infection, obstetrical complications resulting in abnormal blood loss or inherited disorders such as haemoglobinopathies or glucose 6- phosphate dehydrogenase deficiency (Cheesbrough, 2000). Manifestations of these causative factors are normally in the form of inadequate red blood cell production, excessive red cell destruction or increased loss of blood (Williams and Beulter, 1990). Usual therapy depends depends upon the nature and type of anaemia and revolves around the use of haematopoietic growth factors, minerals or vitamins (vitamin B_{12} and folate) supplementation (Hillman, 2001). The use of erythropoietin (Eschbach et al., 1987, 1989; Kaufman et al., 1998; Besarab et al., 1998) and Iron or Iron salt (Callender, 1974; Bothwell et al., 1979; Kernoff et al.,1975; Besarab et al., 1999) have been particularly well researched. In extreme cases especially under life threatening situations, blood transfusion becomes the preferred option.

With the problem of Iron overload (haemochromatosis) and poising coupled with the increasing number of blood transmissible diseases, the need for safe and viable alternatives remain ever valid. The possibility of using herbs or herbal preparations in the management of anaemia has been reported (Omoregie and Osagie, 2002).

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