International Scholars Journals

African Journal of Food Science Research ISSN 2375-0723 Vol. 7 (7), pp. 001-005, July, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Scoparia dulcis protects against *Trypanosoma brucei*-induced immunosuppression in experimentally infected rabbits

N.E.J. Orhue[1]*, E. A. C. Nwanze[1] and A. Okafor[2]

[1]Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B 1154, Benin City Nigeria.
[2]Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, 405 Hilgard Avenue, P. O. Box 951361, Los Angeles, CA. 900095-1361.

Accepted 10 March, 2019

The present paper summarizes our findings on the effect of *Scoparia dulcis* on the population of immune cells during a 28 day experimental *Trypanosoma brucei* infection in rabbits. The result obtained showed that infection resulted in an initial rise in both total white blood cells (WBC) and the absolute number of circulating lymphocytes followed by a progressive decrease in total WBC and all WBC subtypes namely; lymphocytes, monocytes and granulocytes, although the % lymphocytes (lymphocytes expressed as % of total WBC) remained consistently higher than normal throughout the study period. These changes are consistent with the development of trypanosome-induced immunosuppression in their mammalian host. Treatment with *S. dulcis* at a daily oral dose of 25 mg/Kg body weight significantly reduced the severity of the observed lesions ($p < 0.05$) when compared with untreated infected animals. Thus the herb demonstrates significant potency in protecting against the parasite induced decrease in the population of immunologically active cells.

Key words: *Trypanosoma brucei*, Immunosuppresion, *Scoparia dulcis*, Rabbit.

INTRODUCTION

African trypanosomiasis is a disease complex caused by several species of the parasitic protozoan *Trypanosoma.* The disease can affect both wild and domesticated mammals (Ashcroft et al., 1959). Infection of humans with either *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* results in sleeping sickness a debilitating and often fatal disease (Truc et al., 1992).

Although there is massive immune response mounted by the host secondary to infection, this is often overpo-wered by the ability of the parasite to express variable surface glycoproteins and thus evades the host's immune response (Spriggs, 1985). The result is a profound immunosuppression that lowers the host's resistance, paving way for other infections which further complicates both the clinical and pathological features of the disease. The immunosuppression that develops is the result of both a progressive loss in the population of immunolo-gically active cells as well as the ineffectiveness of the humoral immune response owing to the phenomenon of antigenic variation. The more complex phenomenon of antigenic variation may be viewed as a survival strategy and consist of spontaneous, periodic changes in the molecular architecture of the variant surface glycoprotein that serves as a protective blanket on the parasite cell membrane (Cross, 1975, 1976; Borst and Greaves, 1987; Pays et al., 1989; Borst et al., 1996, 1998; Barry, 1997; Cross et al., 1998; Deitsch et al., 1997; Pays and Nolan, 1998). The severity of the clinical signs as well as the degree of mortality depends on several factors including the dose and virulence of the parasite as well as the specie and breed of affected animals (Moulton and Sollod, 1976; Suliman and Feldman, 1989).

Current research efforts in our laboratory have been focused partly on studying the biochemical lesions associated with experimental African trypanosomiasis, including the possibility of managing the disorder using locally available medicinal plants. The present paper focuses on the amelioration of trypanosome induced loss in the population of immune cells using *Scoparia dulcis* (Vassourinha, Bitterbroom or Sweetbroom weed), a widely

*Corresponding author. E-mail: jerryorhue@yahoo.com.

Table 1. Effect of *Scoparia dulcis* on TrypanosoSme-induced decrease in total white blood cells.

Groups	Total WBC (x 10^{-3}/u/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	8.541^{a}	8.397^{a}	8.613^{a}	8.428^{a}	8.602^{a}
	0.216	0.304	0.195	0.206	0.324
Group II	8.422^{a}	9.860^{b}	9.276^{a}	6.851^{b}	5.030^{b}
	0.304	0.231	0.165	0.202	0.154
Group III	8.643^{a}	9.610^{b}	10.463^{b}	5.820^{c}	3.012^{c}
	0.278	0.164	0.241	0.136	0.102

Values are mean ± S.E.M. Values on the same column, but with different superscripts differ significantly ($p < 0.05$).

Table 2. Effect of *Scoparia dulcis* on Trypanosome-induced decrease in total circulating lymphocytes

Groups	Total Lymphocytes (x 10^{-3}/u/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	5.114^{a}	5.050^{a}	5.340^{a}	5.033^{a}	5.264^{a}
	0.118	0.130	0.124	0.116	0.120
Group II	5.091^{a}	6.920^{b}	6.740^{b}	4.503^{b}	3.420^{b}
	0.130	0.125	0.126	0.100	0.108
Group III	5.066^{a}	7.188^{b}	8.383^{c}	4.653^{b}	2.258^{c}
	0.128	0.132	0.138	0.090	0.112

Values are mean ± S.E.M. Values on the same column, but with different superscripts differ significantly ($p < 0.05$).

distributed herb reputed for numerous ethnomedicinal values (Branch and daSilva, 1983; Denis, 1988). Many 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, 1987, 1990, 1991; Kawasaki, 1987; Ahmed, 1990).

MATERIALS AND METHODS

Animal treatment

A total of 15 healthy rabbits of the New Zealand white breed (initial 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 and with all groups of animals allowed a 2 week orienttation prior to the commencement of experiment. Animals in groups 1 served as controls while those in groups 2 and 3 where inoculated with *T. brucei.* Inoculation was by intraperitoneal injecttion of 0.5 ml of a 1:1 (Infected whole blood: normal saline) preparation and with each inoculum containing about 2×10^{6} of the parasite. Parasite estimation was by the rapid "matching" method (Herbert and Lumbsden, 1976) the original stock of *T. brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research

Table 3. Effect of *Scoparia dulcis* on Trypanosome-induced decrea-se in total monocytes.

Groups	Total monocytes (x 10^{-3}/u/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	1.707^{a}	1.770^{a}	1.760^{a}	1.622^{a}	1.726^{a}
	0.082	0.078	0.102	0.124	0.095
Group II	1.773^{a}	1.540^{a}	1.394^{a}	1.198^{b}	0.889^{b}
	0.120	0.116	0.110	0.104	0.076
Group III	1.913^{a}	0.984^{b}	0.852^{b}	0.638^{c}	0.465^{c}
	0.115	0.087	0.096	0.010	0.102

Values are mean ± S.E.M. Values on the same column, but with different superscripts differ significantly ($p < 0$.

Vom, Plateau State, Nigeria, group 1 animals (control) were each given intraperitoneal injection of 0.5 ml of normal saline instead of parasite. All animals were allowed unlimited access to food (growers pellets, product of Bendel feeds and flours mill, Ewu, Edo State, Nigeria) and clean drinking water. In addition, animals in group 2 were given *Scoparia dulcis* at a daily oral dose of 25 mg/Kg body weight throughout the 28 day study period. The required weight of the air-dried and pulverized herb was administered as an aqueous suspension by gavage. Blood samples were collected prior to infection on day 0 and analyzed for baseline values. Subsequent data obtained on day 7, day 14, day 21 and day 28 were compared with these pre infection values within group in order to ascertain the pattern of observed changes. In addition, comparisons were also made across groups to evaluate the extent of trypanosome induced changes as well as the degree of *S. dulcis*-mediated amelioration. For the avoidance of doubt, blood samples were collected in EDTA bottles and the subsequent analysis carried out within a few hours of sample collection.

Haematological analysis

All haematological parameters were auto analyzed by means of an automated Beckam coulter autoanalyzer.

Statistical analysis

Statistical analyses were done using the analysis of variance (ANOVA) and post test multiple comparisons were by the Turkey-Krammer method. In all instances p values < 0.05 were considered statistically significant.

RESULTS

The results obtained from this study are presented in Tables 1 - 5. The data are supportive of previous findings that *T. brucei* is immunosuppressive. Whereas the number of circulating monocytes and granulocytes decreased on day 7 post-infection, and continue to do so steadily for the rest of the study period, total white blood cell count (WBC) and lymphocyte patterns showed an initial progressive increase on day 7 and day 14 and thereafter followed a drastic downward slide throughout the rest of the study period. By day 21and beyond, all parameters investigated had reached dangerously low levels, falling to

Table 4. Effect of *Scoparia dulcis* on Trypanosome-induced decrea-se in total granulocytes.

Groups	Total granulocytes (x 10^{-3}/u/L)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	1.635^{a}	1.577_{a}	1.510^{a}	1.773^{a}	1.512^{a}
	0.058	0.032	0.092	0.063	0.052
Group II	1.556^{a}	1.410^{a}	1.146^{b}	1.149^{b}	0.712^{b}
	0.069	0.054	0.072	0.045	0.021
Group III	1.661^{a}	1.438^{a}	1.228^{b}	0.629^{c}	0.257^{c}
	0.072	0.047	0.081	0.026	0.034

Values are mean ± S.E.M. Values on the same column, but with different superscripts differ significantly ($p < 0.05$).

Table 5. Effect of *Scoparia dulcis* on Trypanosome-induced decrease in % lymphocytes.

Groups	Lymphocytes (% of total white blood cells)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	59.874^{a}	60.138^{a}	62.041^{a}	59.720^{a}	61.200_{a}
	1.906	2.104	2.241	2.018	2.120
Group II	60.460^{a}	70.136^{b}	72.632^{b}	65.731^{a}	68.172^{a}
	2.015	2.302	2.612	1.782	2.008
Group III	58.631^{a}	74.800_{b}	80.124^{b}	78.603^{b}	75.272^{b}
	2.112	3.062	3.182	2.560	2.141

Values are mean ± S.E.M. Values on the same column, but with different superscripts differ significantly ($p < 0.05$).

as low as 34.84% for total WBC, 44.57% (lymphocytes), 64.59% (monocytes) and 31.73% for granulocytes on day 28 when compared with their respective pre- infection values. The only exception to this trend was % lymphocytes (lymphocytes expressed as % of total WBC) which remained persistently above normal and may be explained on the basis of sustained or thriving infection.

DISCUSSION

As stated earlier, there was an initial increase in lymphocyte count and % lymphocyte (lymphocyte as % of total WBC). These changes became significant as early as day 7 ($p < 0.05$). This is certainly indicative of the animal's swift immunological response to infection. Although the % lymphocyte remained persistently higher than the pre-infection levels throughout the 28 day study period, the absolute number of lymphocytes began to decrease on day 21 and continued progressively so throughout the experiment. Other WBC parameters investigated such as % monocyte and % granulocyte also progressively decreased to varying degrees. The pattern obtained is assertive of progressive immunosuppressoin characteristic of *T. brucei* infection in mammals.

Several other studies have shown that *T. brucei* is capable of evading the host immune response by the process of antigenic variation, by which process the parasite switches the identity and specificity of antigenic determinants expressed on its cell surface. This phenomenon, made possible by means of trypanosomal glycolsylated phosphatidyl inositol (GPI)-anchored variable surface glycoproteins thus puts the parasite way ahead of the host immune response. This subject has been widely researched and reviewed (Agur et al., 1989; Askonas, 1985; Barry and Turner, 1991; Blum et al., 1993; Bulow and Overath, 1985, 1986; Bulow et al., 1989b).

The results obtained from this study show that within 7 days of infection with the parasite, the population of immunologically active cells began to decrease and indeed continued to do so for as long as infection persisted. The complexity here is that, not only is the host' immune system rendered ineffective occasioned by rapid switching of antigenic surface glycoproteins, but even the means of doing so progressively decreases as the number of immunologically active cells (notably lymphocytes and macrophages) decreases. The persistently higher than normal levels ($p < 0.05$) of % lymphocytes observed in this study should not be misconstrued as a sparring effect on this class of WBC, but rather a possible preferential production of lymphocytes relative to other classes of WBC. This is readily understood when it is recalled that the absolute number of lymphocyte continued to decrease throughout the study.

It is probable that the aetiology of trypanosome-induced leucopenia in the rabbit may be similar to the case with trypanosome-induced anaemia. 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 et al., 1989; Igbokwe et al., 1994).

It is note worthy that there was no significant difference ($p > 0.05$) observed in all parameters investigated in control animals. It is also interesting to note that these deleterious changes were much less severe in the *S. dulcis* treated infected animals relative to those that were not treated. The possible mechanisms by which the herb carry out this role remain a subject of great speculations. Several possible mechanism working separately or in concert may account for the observed effect. The possibility that *S. dulcis* or certain components of the herb may

help stabilize the membrane of blood cells cannot be out rightly dismissed. Specifically, it is not out of place to suggest that the anti-oxidant or free radical scavenging properties of *S. dulcis* previously reported by other workers (Babincova and Sourivong, 2001; Sourivong et al., 2007; Babincova et al., 2008), may play vital roles in this regard especially against the backdrop of the role of free radicals in the pathogenesis of *T. brucei* infection It is also probable that increased production of blood cells helps in replenishing of these cells. In the absence of any evidence of possible trypanocidal activity for the herb, it does not seem an attractive option to speculate that the higher level of immunological cells in treated animals could be due to the destruction of the parasite by agents native to the plant. Be that as it may, the need to screen the plant for possible trypanocidal activity cannot be over emphasized. Whatever the possible mechanism may be, the present study under scores the fact that *S. dulcis* provides a measure of immunological boost during experimental *T. brucei* infection in rabbits.

ACKNOWLEDGEMENTS

This project was funded by the University of Benin (UNIBEN), Benin City, Nigeria and executed under the UNIBEN-MIRT (Minoruty in research training), U.S.A. exchange programme. The herb (*Scoparia dulcis*) was freely made available by Dr (Mrs.) Hilda Ogbe who has employed the plant in the management of sickle cell disorder for over 2 decades.

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