Serum total protein, albumin and globulin levels in *Trypanosoma brucei*-infected rabbits: Effect of orally administered *Scoparia dulcis*

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The effect of orally administered *Scoparia dulcis* on *Trypanosoma brucei*-induced changes in serum total protein, albumin and globulin were investigated in rabbits over a period of twenty eight days. Results obtained show that infection resulted in hyperproteinaemia, hyperglobulinaemia and hypoalbuminaemia. However these lesions were less severe (p<0.05) in the infected and treated group relative to their untreated counterparts. We speculate that the herb may be involved in modulating the severity of these trypanosome associated lesions by some yet undefined mechanisms.

Key words: *Trypanosoma brucei*, total protein, albumin, globulin, *Scoparia dulcis*.

INTRODUCTION

*Scoparia dulcis* or sweet broom weed is an erect annual herb with serrated leaves, producing white flowers and measuring up to a half meter in height when fully grown. It has for several years enjoyed the patronage of ethnomedicinal practitioners having been reputed for its efficacy in the treatment and management of a wide array of health conditions (Branch and daSilva, 1983; Denis, 1988). More recently, a number of the speculated medicinal values of *S. dulcis* have been validated by scientific research. These include hypoglycaemic activity (Jain, 1985), antitumour promoting activity (Nishino, 1993), and antiviral activity (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; Kawasaki, 1987; Ahmed and Jakupovic, 1990). The present research interest/effort arose from the widely speculated efficacy of *S. dulcis* in the management of sickle cell anaemia in parts of Nigeria. Mrs. Hilda Ogbe has for over two decades employed the herb in the management of sickle cell anaemia with profoundly outstanding results. There were claims of massive boost in haematocrit or packed cell volume (PCV) and haemoglobin (Hb) levels, as well as some degree of amelioration of the frequent crisis associated with the disorder. The lack of animal model for sickle cell disease prompted us to investigate the efficacy of *S. dulcis* using animal models experimentally infected with *Trypanosoma brucei brucei*. Progressive anaemia is widely accepted as a cardinal feature of *T. brucei brucei* infection (Moulton and Sollod, 1976; Suliman and Feldman, 1989). The compelling evidence in favour of the anti anaemic claim prompted us to investigate the effect of *S. dulcis* on selected *T. brucei* induced biochemical and haematological lesions in the rabbit. The present report summarizes our findings on the efficacy of *S. dulcis* in the management of trypanosome induced changes in serum total protein, albumin and globulin.

MATERIALS AND METHOD

Treatment of animals

Fifteen (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 on growers mash (product of Bendel Feeds and flour Mill, Ewu, Edo State, Nigeria) prior to the commencement of experiment. Group 1 served as control while groups II and III were inoculated with T. brucei (infected whole blood: normal saline) preparation, and with each group allowed a 14 days acclimatization on growers mash (product of Bendel Feeds and flour Mill, Ewu, Edo State, Nigeria. The control animals (Group 1) were each given the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The control animals (Group 1) were each given the intraperitoneal injection of 0.5 ml of normal saline in lieu of parasite. All animals were allowed unlimited access to food and clean drinking water throughout the duration of the experiment. In addition the inoculated and treated animals (group II) were given Scoparia dulcis at a daily oral dose of 25 mg/kg body weight. Preparation of S. dulcis involved only air drying and blending of the entire shoot system. The required weight of pulverized S. dulcis was administered as an aqueous suspension in clean drinking water through gavage. Blood samples were collected prior to infection on day 0 and analyzed for baseline data. Subsequent data obtained on days 7, 14, 21 and 28 were compared with these pre infection values. 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 plain sample bottles void of anticoagulant. The resultant serum obtained after centrifugation at 2,500 rpm for 10 min was analyzed within a few hours of sample collection.

Biochemical analysis
Serum total protein and albumin were analyzed using the biuret and bromocresol green methods, respectively. In both cases, commercially available test kits, products of Randox laboratories, U.K. were used and with the manufacturers instructions strictly adhered to. Serum globulin was determined as the difference between serum total protein and albumin. 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-Krammer multiple comparison test. Values of p<0.05 were considered as statistically significant.

RESULT AND DISCUSSION
The changes in total protein, albumin and globulin are shown in Tables 1 to 3. There were highly significant

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**Table 1.** Effect of *Scoparia dulcis* on Trypanosome-Induced increase in Serum total protein

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Total protein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Group I</td>
<td>5.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>Group II</td>
<td>5.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Group III</td>
<td>5.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
</tr>
</tbody>
</table>

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 Serum Albumin protein

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Albumin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Group I</td>
<td>3.44 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>3.47 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>3.36 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
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Values are Mean ±S.E.M. Values on the same column, but with different superscripts differ significantly (p<0.05).

**Table 3.** Effect of *Scoparia dulcis* on trypanosome induced increase in serum globulin levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum globulin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Group I</td>
<td>2.32 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>2.50 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>2.53 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M. Values on the same column, but with different superscripts differ significantly (p<0.05).
increases in total protein and globulin (p<0.05) in infected animals when compared with controls. The increase in serum total protein may have been due to increased release of tissue specific enzymes and other intracellular proteins secondary to parasite-induced cell membrane disruption. We have earlier reported a marked elevation in the serum levels of alkaline phosphatase (ALP), glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) in Trypanosoma brucei infected rabbits (Orhue and Nwanze, 2004). Elevations in ALP, SGOT and SGPT are usually secondary to tissue damage. This is because such damage results in the leakage of these enzymes from their intracellular stores into plasma. SGPT is most prevalent in the liver whereas SGOT may also be found in heart, skeletal muscle and liver to nearly the same extent. Significant increases in the transaminases commonly accompany such liver diseases as toxic hepatitis, acute liver necrosis or hepatic cirrhosis. Increases in SGOT are often seen in hemolytic anaemia, myocardial infarction and cholestatic diseases of the liver (Mayne, 1994; Wallach, 1996).

Since anaemia is a cardinal feature of T. brucei infection in mammals, (Igbokwe and Mohamed, 1992; Igbokwe et al., 1994, 1998; Anosa and Kaneko, 1983; Omotainse and Anosa, 1995; Egbe-Nwiyi and Antia, 1983; Anosa, 1988; January et al., 1991), release of erythrocytes-derived enzymes and proteins may be one possible source of plasma protein. Multiple factors have been proposed to account for trypanosome-induced disruption of the cell membrane (Anosa and Kaneko, 1983; Banks, 1979, 1980; Huan et al., 1975; Tizard et al., 1978; Pereira, 1983; Knowles et al., 1989; Esievo et al., 1982; Aminoff, 1988; Olaniyi et al., 2001). It is also likely that the increase in total protein may have been due to 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 due to enhanced antibody secretion in response to infection would no doubt have contributed immensely to the observed hyperproteinaemia.

Evaluation of serum albumin showed that there was a highly significant decrease in values obtained for infected animals relative to uninfected controls. Albumin is produced entirely in the liver and is of great importance in highly significant decrease in values obtained for infected animals relative to uninfected controls. 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 course of liver diseases (Halsted and Halsted, 1981; Cheesbrough, 1998). In this study, there was a statistically significant dip in serum albumin 14 days post infection. This may imply a combination of impaired synthesis and loss via the gut, kidney or both. The low level of albumin in infected animals may have contributed significantly to the observed oedema since albumin is important in the maintenance of plasma colloid osmotic pressure (Cheesbrough, 1998). These changes in serum proteins accompanying infection with trypanosomes have been reported and reviewed by several workers (Igbokwe and Mohamed, 1992; January et al., 1991; Ogunsanmi et al., 1994; Frommel et al., 1988). The changes in trypanosome infected animals observed in this study, no doubt may have been complicated by multi system involvement (Nyakundi et al., 2002a, b; Taylor et al., 2001; Girard et al., 2003; Agu and Egbuji, 2002). What is most remarkable, however, is the ability of S. dulcis to effectively control or resist these changes.

Treatment with S. dulcis resulted in significant reduction in parasite induced hyperproteinaemia and hyperglobulinaemia as well as a significant amelioration of infection associated hypoalbuminaemia. Careful analysis of the data shows that the lesions of hyperproteinemia, hyperglobulinaemia and hypoalbuminaemia were significantly less severe (p<0.05) in the infected but treated group (group II) when compared with their untreated counterparts (group III). 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, it does not appear clearly, the exact mechanism(s) by which S. dulcis exerts its effect nor can this activity be readily ascribed to any one of the many biologically active compounds present in the plant. This is part of an ongoing research effort in our laboratory.

ACKNOWLEDGEMENTS

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REFERENCES


