African Journal of Biotechnology Vol. 5 (10), pp. 883-887, 16 May 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Scoparia dulcis reduces the severity of *Trypanosoma brucei*-induced hyperlipidaemia in the rabbit

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Accepted 31 March, 2006

We investigated the effect of oral administration of the herb, *Scoparia dulcis*, on *Trypanosoma brucei*-induced changes in plasma lipid profile in rabbits over a period of twenty eight days. Results obtained show that infection with *T. brucei* resulted in significant increases in plasma total cholesterol, trriacylglycerol, and low density lipoprotein (LDL)-cholesterol, while the level of high density lipoprotein (HDL)-cholesterol was also significantly reduced. Further comparative analysis of data revealed that these lesions were significantly less severe ($p<0.05$), in the infected and treated group relative to their untreated counterparts. However, the precise mechanism underlying the plasma lipid modulating effects of the herb is still a matter of speculations.

Key words: *Scoparia dulcis*, *Trypanosoma brucei*, cholesterol, triacylglycerol lipoproteins.

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. In view of its high reputation and wide acceptance in ethnomedicine, this plant has attracted not only wide publicity but also intensified research efforts by researchers (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 hypoglycemic activity (Jain, 1985; Latha et al., 2004a, 2004b; Pari and Venkateswaran, 2002; Pari and Latha., 2005), antitumour promoting activity (Nishino, 1993), and antiviral activity (Hayashi, 1990). Ratnasooriya et al. (2003) has also demonstrated a significant analgesic and antihyperalgesic activity for *S. dulcis* decoction. 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 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 associated dyslipidaemia, characterized by elevated plasma cholesterol, elevated

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triacylglycerol, elevated LDL cholesterol and depressed HDL cholesterol level.

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 brucei*. Inoculation was by intraperitoneal injection of 0.5 ml of a 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 (Herbert and Lumbsden, 1976). The original stock of *T. brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The control animals (Group 1) were each given 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 *S. 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, comparisms 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 after an overnight fast, and in heparinized sample bottles. The resultant plasma obtained after centrifugation at 2,500 rpm for 10 min was analyzed within a few hours of sample collection.

Biochemical analysis

The determination of plasma lipids namely, total cholesterol, triacylglycerol, and the lipoproteins (HDL and LDL cholesterol) were carried out using previously described protocols, and by means of commercially available test kits (products of Randox laboratories, U.K). In all instances, the manufacturer's instructions were strictly adhered to.

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 comparism tests. Values of $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The results obtained in this study are presented in Tables 1 to 4. Values are expressed as mean ± S.E.M. There were significant and progressive increases ($p<0.05$) in plasma triacylglycerol, total cholesterol and LDL cholesterol in infected animals when compared with controls. HDL cholesterol levels were however significantly lower ($p<0.05$) in infected animals relative to controls.

African trypanosomes are lipid auxotrophs that live in the blood stream of their mammalian host, and exogenous lipids play essential roles in the parasite's cell structure and metabolism. Their requirement for lipoproteins as an essential growth factor is now well established. However, lipoproteins that are devoid of their lipid components are ineffective in maintaining the growth of trypanosomes. These parasites find ready sources of lipids namely cholesterol esters, cholesterol and phospholipids in the plasma lipoproteins, LDL and HDL (Coppens et al., 1987; Gillet and Owen, 1992). Trypanosomes lack ability for the *de novo* synthesis of fatty acids (myristate being an exception) and yet require lipid for the biosynthesis of glycosylated phosphatidyl-inositol anchors (Morita et al., 2000; Paul et al., 2001). The parasite induced hyperlipidaemia is believed to be somewhat related to its compulsory requirement for lipids. There is now compelling evidence for the existence of a trypanosome lipoprotein scavenger receptor that is believed to facilitate the endocytosis of both native and modified lipoproteins, including HDL and LDL (Green et al., 2003). There are also indications that altered plasma levels of these lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerol) may raise the risk of cardiovascular complications in the mammalian host particularly during chronic infection.

It is known that aberrations in the levels of plasma lipids and lipoprotein contribute significantly to the aetiology of arteriosclerosis and associated cardiovascular disorders (Hajjar and Nicholson, 1995; Olson, 1998; Mayne, 1994; Wallach, 1996). Atherosclerosis could be considered as the distortion and obstruction of the artery resulting from the calcification and ulceration of atheromatous plaques. The lesion is often accompanied with the proliferation of intima smooth muscle cells, massive accumulation of macrophages and formation of large amount of connective tissues by the proliferated cells. Accumulation of lipids, notably free and esterified cholesterol within the cell, and in the surrounding connective tissues is also a cardinal feature of the disorder (Ross and Glomset, 1973, 1976; Ross and Harker, 1976; Wisserler et al., 1976).

There is a positive correlation between the risk of developing ischaemic heart disease and raised levels of total and esterified cholesterol in plasma. Several studies have revealed that the lesions of arteriosclerosis are secondary to endothelial dysfunction occasioned by such factors as elevated and modified LDL, hypertension, diabetes mellitus, cigarette smoking amongst others (Ross and Glomset, 1976; Ross, 1981, 1986, 1993). Chronically elevated levels of LDL cholesterol lead to an increase in the number of cholesterol molecules in the

Table 1. Effect of *Scoparia dulcis* on *Trypanosomia brucei*-induced hypercholesterolaemia.

Group	Plasma total cholesterol concentration (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	114.76 ± 2.47[a]	116.87 ± 3.14[a]	120.67 ± 2.82[a]	118.46± 3.135[a]	120.19 ±4.01 [a]
Inoculated treated	112.48 ± 1.98[a]	120.52 ± 2.11[a]	130.72 ± 2.63[b]	128.98 ± 2.12[b]	142.68 ± 4.39 [b]
Inoculated untreated	116.12 ± 2.09[a a]	115.24 ± 2.06[a]	138.75 ± 3.61[c]	143.60 ± 3.25[c]	176.38 ± 8.162 [c]

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

Table 2. Effect of *Scoparia dulcis* on *Trypanosomia brucei*-induced hypertriglyceridaemia.

Group	Plasma triacylglycerol concentration (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	122.85 ± 5.06 [a]	126.43 ± 3.12[a]	131.33 ± 4.39[a]	128.16 ± 3.40[a]	130.49 ±3.81[a]
Inoculated treated	126.08 ± 3.64[a]	132.41 ± 3.41[a]	204.19 ± 6.02[b]	215.60 ± 7.13[b]	242.65 ± 6.83[b]
Inoculated untreated	127.54 ± 4.14 [a]	131.42 ± 3.82[a]	236.15 ± 6.86[c]	284.07± 8.82[c]	308.112 ± 7.71[c]

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

Table 3. Effect of *Scoparia dulcis* on *Trypanosomia brucei*-induced decrease in HDL cholesterol concentration.

Group	Plasma HDL-cholesterol concentration (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	60.15 ± 3.41[a]	56.34 ± 3.87[a]	63.37 ± 4.19[a]	61.43 ± 3.44[a]	64.22 ±2.87[a]
Inoculated treated	58.62 ± 3.71[a]	63.42 ± 2.27[a]	52.74 ± 2.700 [a, c]	48.85± 3.5 [b, c]	56.14 ± 2.21[a]
Inoculated untreated	62.39 ± 4.00 [a]	60.18 ± 3.61[a]	49.78 ± 3.12 [b, c]	43.46 ± 2.46[c]	40.36 ± 3.72 [b]

Values are Mean ± S.E.M and values on the same column with different superscript differ significantly ($p<0.05$). Significant difference between treated and untreated animals was recorded only on day 28 post infection.

Table 4. Effect of *Scoparia dulcis* on *Trypanosomia brucei*-induced elevation in LDL cholesterol concentration.

Group	Plasma HDL-cholesterol concentration (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	30.09 ± 1.64[a]	35.24 ± 1.98[a]	31.04 ± 2.11[a]	31.43 ± 2.02[a]	29.87 ±2.11[a]
Inoculated treated	28.64 ± 2.20[a]	30.62 ± 1.05[a]	37.15 ± 2.12[a, c]	37.01± 3.5 [a, c]	38.01 ± 2.11[a]
Inoculated untreated	28.23 ±1.85[a]	32.78 ± 1.63[a]	41.73 ± 2.01[b, c]	43.46 ± 2.63 [b, c]	74.40 ± 3.81[b]

Values are Mean ± S.E.M and values on the same column with different superscript differ significantly ($p<0.05$). Significant difference between treated and untreated animals was recorded only on day 28 post infection.

plasma membrane. The result is an increase in the cholesterol: phospholipid ratio of the membrane. The increased viscosity and thus decreased malleability of the endothelial membrane has been speculated to be the primary potentiating factor in the aetiology of atherosclerosis (Jackson and Gotto, 1976). It has been suggested that oxidized LDL is injurious to endothelium and smooth muscle *in vitro* (Boyd et al., 1976; Steingerg, 1997). Once trapped in the artery, LDL particles undergo oxidation and subsequent receptor mediated internalization by macrophages. The result is the formation of lipid peroxides, accumulation of cholesteryl esters and formation of foam cells (Khoo et al., 1988, 1992; Morel et al., 1983; Griendling and Alexander, 1997; Han et al., 1997). Thus a rational approach to preventing the incidence of atherosclerosis should include reduction in LDL levels, prevention of LDL oxidation or both.

Several epidemiological and clinical studies have suggested a link between elevated triacylglycerol and increased cardiovascular risk (Austin, 1988; Carlson and Bottiger, 1985). It has been suggested also that the link between elevated triacylglycerol and cardiovas-cular complications may be secondary to other diseases (Kaplan, 1989). A number of conditions that are associated with elevated plasma triacylglycerol such as diabetes mellitus and renal failure are known to be asso-

ciated with increased risk of cardiovascular complications.

HDL particles are responsible for the reverse transport of free cholesterol from peripheral tissues by way of a putative HDL receptor (Mayne, 1994). This receptor mediated reverse transport may explain why patients with elevated HDL concentration are less prone to coronary artery disease (Krieger, 1999). Rational management of hypercholesterolaemia are geared towards reducing plasma total cholesterol, LDL cholesterol and triacylglycerol, while increasing the proportion of HDL cholesterol (Olson, 1998; Kane, 1986; Spady, 1993).

As mentioned earlier, infection with *Trypanosoma brucei* is accompanied by a mixed type hyperlipidaemia characterized by abnormal plasma lipid and lipoprotein levels. Total cholesterol, LDL cholesterol and triacylglycerol are highly elevated while the HDL: total cholesterol and HDL: LDL cholesterol ratios are depressed in infected animals relative to control. The ability of *S. dulcis* to mitigate against these plasma lipid anomalies is underscored in the present study The level of total cholesterol, LDL cholesterol and triacylglycerol in treated animals were significantly lower ($p<0.05$) relative to the infected but untreated group. Furthermore, the parasite-induced decrease in HDL cholesterol was also significantly resisted in the treated group, thus enhancing the HDL: total cholesterol and the HDL: LDL ratios. This phenomenon no doubt favours a reduction in cardiovascular risk. While the exact mechanism by which *S. dulcis* achieves this feat is not immediately apparent, it seems logical to speculate that the putative mechanism may revolve around reduction in parasite load or the control of the induced dyslipidaemia by means that are independent of any possible trypanocidal activity of *S. dulcis*. This and many more aspects require further investigation.

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