Amelioration of Trypanosome-infection-induced Alterations in Serum Cholesterol, Triglycerides and Proteins by Hydro-ethanolic Extract of Waltheria indica in Rats

A.Y. Bala, T. Adamu and M.J. Ladan

1Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
2Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

Corresponding Author: A.Y. Bala, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
Tel: 08035988924

ABSTRACT

A study was conducted to investigate the amelioration potential of trypanosome infection induced alterations in rat serum cholesterol, triglycerides and proteins by hydro-ethanolic extract of Waltheria indica L. The results from the study showed that infection with Trypanosoma brucei brucei resulted in significant (p<0.05) increases in the concentrations of serum triglycerides (hyperglyceridemia), total protein (hyperproteinaemia) and globulin (hyperglobulinaemia) while the levels of cholesterol and albumin were significantly reduced leading to hypocholesterolaemia and hypoalbuminæmia, respectively. However, treatment with the hydro-ethanolic extract of W. indica significantly (p<0.05) ameliorates these infection induced biochemical changes in fashion comparable to the standard trypanocide Berenil®. The corrective action of the plant may revolve around reduction in parasite load coupled with some hepato-protective properties that need further research to ascertain.

Key words: Waltheria indica, Trypanosoma brucei brucei, triglycerides, cholesterol, protein, globulin, albumin

INTRODUCTION

Trypanosomiasis is a disease caused by infection with trypanosome parasites that live in the blood of the host, commonly transmitted by tsetse flies. It remains a significant health problem in sub-saharan Africa. It affects most species of domestic animals and man, causing alterations in serum biochemistry of the hosts including decrease in blood albumin, increase in globulin levels, hypoglycaemia and increase in triglycerides, have been reported in donkeys, horses, rats and rabbits (Rouzer and Cerami, 1980; Marques et al., 2000; Bala et al., 2011b).

Increased serum lipids in trypanosome infected animals has led to the suggestion that lipolysis is the major mechanism for supplying the high energy demanded by the fever following trypanosome infection (Akinbamijo et al., 1994). Adamu et al. (2009) also reported a significant reduction in serum levels of cholesterol, triglycerides and high density lipoprotein in Trypanosoma brucei brucei infected pigs while Rouzer and Cerami (1980) found a significant increase in triglycerides in T. brucei brucei infected rabbits. Because of the aforementioned and many others (Orhue et al., 2005; Orhue and Nwanze, 2006), it could logically be put forward that trypanosomes can cause serious alterations in hosts’ serum biochemistry.
Waltheria indica L. (sleepy morning) is a popular medicinal plant here in Sokoto State, Nigeria. Phytochemical screening of the plant revealed that it is rich in alkaloids, tannins, flavonoids terpenes, among others and the pharmacological actions of W. indica are believed to be due to the presence of these phytochemicals (Bala et al., 2011a). The present study is designed to assess the amelioration potential of the hydroethanolic extract of W. indica on trypanosome induced changes in serum cholesterol, triglycerides and proteins in albino rats. This is because the ethanol extract of the plant was shown elsewhere (Bala et al., 2011b) to significantly reduced parasitaemia and also improved the hypoglycaemic condition of trypanosome infected and treated rats.

MATERIALS AND METHODS

Collection of the plant: The plant used in this study was collected from Dabagi farm of the Usman Danfodiyo University, Sokoto and transported to the Botany Unit of the Usman Danfodiyo University, Sokoto, for identification with the help of a taxonomist. A voucher specimen of the plant was deposited in the herbarium of the Department where it was identified.

Preparation of plant material: The whole plant was cut into pieces, air-dried (in open air in the laboratory to avoid destruction of the active components) at room temperature and pulverized using mortar and pestle. Forty grams of the coarse powder of the plant part were dissolved in 50 mL of water and ethanol (50% v/v). It was then left to stand for 72 h (3 days) at room temperature after which it was sieved first with cotton wool and then with number 1 Whatmann filter paper. It was then evaporated to dryness under reduced pressure and stored until required (Hostettamnn et al., 1995).

Parasites and animals: T. brucei brucei were obtained from stabilities maintained at the Nigerian Institute of Trypanosomiasis and Onchocerciasis Research (NITOR) Vom, Plateau State. The parasites were maintained in the laboratory by continuous passage in rats until required. Passage was considered necessary when parasitaemia reaches a range of 16-32 parasites per field (usually 3-5 days post infection). In passaging, 1x10^6 parasites in 0.1-0.2 mL blood/PBS solution was introduced intraperitoneally into clean rats acclimatized under laboratory condition for one week. Thirty adult Wistar albino rats of the same age group, weighing between 200-260 g, were obtained from the Animal House of the Department of Biological Sciences, Usman Danfodiyo University Sokoto. They were housed in metal cages in the Parasitology Laboratory of the same Department. They were allowed to acclimatize for two weeks and were fed growers mash (Pfizer Nigeria PLC) and given drinking water ad libitum. The rats were sorted and grouped into 5 groups of 6 rats each as follows:

- **Group A** (n = 6) Uninfected untreated rats as positive control
- **Group B** (n = 6) Infected but untreated rats as negative control
- **Group C** (n = 6) Infected treated with 300 mg kg^{-1} body weight concentration of plant extract for 5 days
- **Group D** (n = 6) Infected and treated with 300 mg kg^{-1} b. wt. plant extract for 10 days
- **Group E** (n = 6) Infected and treated with full dosage of Berenil® (3.5 mg kg^{-1}) once intraperitoneally.
The animals were subjected to the same physical conditions. A clean environment was maintained throughout the course of the experiment. They were fed on grower's mash (Pfizer Nigeria PLC) and water given ad libitum, throughout the duration of the study.

**Animal inoculation:** At the end of the acclimatization period, the experimental animals were inoculated with *T. brucei brucei* parasites, using needle and syringe (Onyezili et al. (1994). One millilitre (1 mL) of infected blood was taken from the donor rat with fulminating parasitaemia and was diluted with 9 mL phosphate buffered saline (pH 8.0). The trypanosomes were counted and thereafter 0.5 mL of the diluted blood containing approximately 2.0×10⁶ trypanosomes per mL of blood was inoculated into the rats in groups B, C, D and E. Inoculation was done intraperitoneally as described by Onyezili et al. (1994) and was preceded by cleaning the area to be inoculated with cotton wool soaked in 70% alcohol. The same process (cleaning) was repeated after the injection, to prevent secondary infection by microorganism.

**Preparation and administration of the treatments:** Twenty percent concentration of the extract was prepared. The rats in groups C and D received 300 mg kg⁻¹ body weight for 5 and 10 days, respectively as indicated above. Administration was done orally with the aid of oral cannula. The rats in group E received full dosage of Berenil®. The indicated dose of Berenil® is 3.5 mg kg⁻¹ body weight as recommended by the manufacturer. Administration was carried out intramuscularly with the help of a syringe and needle. Administration of all treatments began day 5 after inoculation (peak of parasitaemia).

**Serum collection (separation of plasma from blood):** Three milliliters (3 mL) of blood was collected from the rats in plain sample bottles. Plasma was obtained from blood by centrifugation at 2000 rpm for 10 min. Blood samples were analyzed within a few hours of sample collection by enzymatic colorimetric methods using the appropriate commercial kits according to established techniques.

**Biochemical analysis:** Determination of biochemical parameters were carried out using previously described protocols. The total cholesterol was determined by the Enzymatic Method of Allain et al. (1974). Triglycerides were determined by Enzymatic Hydrolysis with Lipases as described by Tietz (1990). Serum total protein and albumin were analyzed using the Biuret and Bromocresol Green methods, respectively. Serum globulin was determined as the difference between serum total protein and albumin concentrations (Harshmohan, 2002). In both cases, commercially available test kits, products of Randox Laboratories, U.K. were used and with the manufacturer’s instructions strictly adhered to.

**Statistical analysis:** Results were expressed as mean ± standard error of the mean of 6 animals. The efficacy of treatments between treated and control groups and between treated groups was compared using one way ANOVA and correlation analysis. Post test analysis was done using the Duncan’s multiple range test. Values of p<0.05 were considered as statistically significant. In all cases data was entered into computer and analyzed using SPSS (Version 11.0) statistical package.

**RESULTS**

Results from this study showed that at the peak of parasitaemia all the infected animals developed hypocholesterolaemia. The level of cholesterol of the untreated rats continued to decline
culminating in the death of animals in that group. However, the cholesterol level increased in the treated animals. Percentage restoration of cholesterol was 29.03, 53.18 and 30.85% for groups C, D and E, respectively. There was no statistical difference (p>0.05) between the standard drug Berenil® and W. indica in their normocholesterol restoration potential in the treated animals (Fig. 1).

On day 5 post inoculation (peak of parasitaemia) the infected animals showed an elevated concentration of triglycerides which is almost 3-fold its initial value. The triglycerides concentration continued to increase in the untreated rats, resulting in the death of animals in that group. However, following treatment, the triglycerides concentration dropped significantly (p<0.05). Percentage drop in triglycerides concentration was in the order 52.99, 56.45 and 57.91% for groups C, D and E, respectively (Fig. 2). There was no significant difference (p>0.05) between Berenil® and W. indica in their amelioration effects on triglycerides levels in the treated animals.
The results of protein analysis indicated an increase in total protein concentration (hyperproteinaemia) in the infected animals when compared to the normal control. The protein concentration of the untreated animals continued to increase resulting in the death of animals in that group. Treatment with Berenil® (W. indica) resulted in reduction in the concentration of the proteins. Percentage restoration being 8.51, 15.34 and 16.64% for animals in groups C, D and E, respectively (Fig. 3). There was no significant difference (p>0.05) between the Berenil® and W. indica in their restoration effects on total protein levels of the treated animals. Evaluation of serum albumin showed that there was a significant (p<0.05) decrease in values obtained for infected animals relative to the uninfected control. In the untreated animals, the infection-associated hypoalbuminaemia continued to manifest. Following treatment with Berenil® and ethanol extract of W. indica, there was a significant (p<0.05) amelioration of the infection-associated hypoalbuminaemia in the treated animals. Percentage amelioration of the albumin levels in the treated animals was in the order 24.39, 33.33 and 33.11% for groups C, D and E, respectively (Fig. 4). There was no significant difference (p>0.05) between
Fig. 5: Levels of plasma globulin concentration in trypanosome infected rats before and after treatment

Berenil® and the ethanol fraction of *W. indica* on their effect on correcting hypoalbuminaemia of the treated animals.

The levels of plasma globulin showed a 2-fold increase above the pre-infection values, leading to hyperglobulinaemia. This increase was significant (p<0.05) when compared with the uninfected control. The hyperglobulinaemic condition of the untreated animals continued to manifest, culminating in death of the animals in that group. However, treatment with Berenil® *W. indica* resulted in significant reduction in the parasite-induced hyperglobulinaemia. Percentage restoration being in the order 27.67, 42.25 and 46.77% for groups C, D and E, respectively (Fig. 5). No significant difference (p>0.05) was found between Berenil® and ethanol extract of *W. indica* in reducing the parasite associated hyperglobulinaemia.

**DISCUSSION**

Biochemical evaluation of the body fluids gives an indication of the functional state of the various body organs and biochemical changes in body fluids that result from infections depend on the species of the parasite and its virulence (Anosa, 1988). It has been observed that protozoan parasites including trypanosomes depend on their hosts for energy and nutrients required for their growth, motility and reproduction. There is evidence (Gillett and Owen, 1992) suggesting that parasites can take up the lipids and cholesterol they need from lipoproteins present in the host body which may lead to alterations in serum concentrations of such metabolites. The role of lipids in pathogenesis of trypanosomiasis had been reported (Tizard *et al.*, 1978). Hypocholesterolaemia during trypanosome infection has been shown to occur in sheep (Katunguka-Rwakishava *et al.*, 1992), pigs (Adamu *et al.*, 2009) and in humans (Awobode, 2006), as also recorded in this study.

The 3-fold increase in triglycerides seen in this study contrasted the work of Awobode (2006) who reported a reduced triglycerides concentration in humans infected with *Trypanosoma brucei gambiensae*. However, the work of McGowan *et al.* (1983) who showed that the level of parasitaemia is directly proportional to the triglyceride concentration in *Trypanosoma cruzi* infected murine animals (*Calomys callosus*). The increase in the serum triglycerides concentrations observed in this study may be due to reduced serum concentration of albumin. Albumin (reduced plasma concentration of which lead to reduced total serum proteins)
is required to bind to neutral fats (triglycerides and cholesterol) for these lipids to be transported in the plasma (because the lipids are hydrophobic in nature and therefore, require some forms of hydrophilic adaptation in the form of lipoproteins). However, infection can lead to a fall in serum albumin due to decreased synthesis of albumin and or increased catabolism of albumin, consequently causes reduction in the binding capacity and leading to increased plasma free concentration of these analytes. Treatment with the ethanol extract of W. indica significantly improved the levels of serum cholesterol and triglycerides of the treated animals possibly by increasing the synthesis of albumin which now binds and transports the lipids. The plant extract may also have helped the treated animals to degrade and remove the triglycerides efficiently, as Rouzer and Cerami (1980) attributed trypanosome-induced hyperglyceridemia in rabbits to be due to defective triglyceride removal by the host. The amelioration of the cholesterol and triglycerides levels of the treated animals by W. indica was comparable to that of the standard trypanocide Berenil.

Hyperproteinaemia, hyperglobulinaemia coupled with hypoalbuminaemia in the infected animals, as evident in this study, is similar to observations found in other mammalian hosts parasitized by trypanosome species (Singh et al., 1988). Hyperproteinaemia due to trypanosome infection may be due to lysis of the parasites by the host immune system which leads to accumulation of the parasites proteins in the body of the host. Hypoalbuminaemia during infections could be due to malnutrition, hepatic damage, gastrointestinal malabsorption and or increased protein need following infection while the increase in globulin levels in the infected animals may be caused by damage to the liver by trypanosomes and or antibody production by the host against the trypanosomes. In this study, there was a statistically significant increase in total protein and globulin and a drop in serum albumin 5 days post infection. Worthy of note however, is the ability of W. indica to effectively control or resist these changes. This is because treatment with the plant resulted in significant reduction in parasite-induced hyperproteinaemia and hyperglobulinaemia as well as a significant amelioration of infection associated hypoalbuminaemia which favourably compared to the standard trypanocide.

The mechanism by which W. indica achieves its action is not immediately apparent, it is logical to speculate that the mechanism may revolve around the reduction in parasite load in the treated animals or the plant may have phytochemicals that have hepatoprotective activity. As put forward by Pagana and Pagana (2010), albumin is used to test how well the liver and kidneys are working. Damaged liver cells lose their ability to make proteins. The liver’s ability to make proteins may be used to predict the course of certain liver disease. Therefore, the fact that the plant extract was able to ameliorate the hyperproteinaemia and correct the hypoalbuminaemia is suggesting some hepatoprotective potential of the extract but this needs further studies on liver enzymes, as well as the effect of the extract on the excretory and synthetic functions of the liver.

It could be concluded that the trypanosome infection induces serious alterations in the host biochemistry and treatment with the ethanol extract of W. indica has significant amelioration effect comparable to the standard trypanocide, Berenil, coupled with suggestive hepatoprotective property which needs further studies to ascertain. This is part of an ongoing research.

REFERENCES


