Haematological Changes in *Trypanosoma brucei brucei* Infected Wistar Rats Treated with a Flavonoid Mixture and/or Diminazene Aceturate

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**Abstract**

The aim of the study was to evaluate the effect of treatment with DAFLON® 500 mg (DF) and/or diminazene aceturate (DZ) on haematological parameters in rats, infected with *Trypanosoma brucei brucei*. Rats in the control group were administered with distilled water (DW) only (5 mL/kg), while those in other groups were infected with *Trypanosoma brucei brucei* (10⁶ cells/ml), and treated with DF and/or DZ. Packed cell volume (PCV), haemoglobin (Hb) concentration, erythrocyte (RBC) and neutrophil counts were lower in the infected untreated and DF-treated group, than in any other group. Total leucocyte, lymphocyte, platelet counts and mean corpuscular volume (MCV) decreased in the infected untreated group, compared to values obtained in those administered with DF and/or DZ. The MCV reduced in the DZ-treated group, compared to the groups treated with DF and DZ. Haemoglobin concentration (MCHC) was lower in the infected untreated and DZ-treated group than in the DW or DZ group. Rats administered with DZ had higher leucocyte and lymphocyte counts compared to those in DF-treated group. It is concluded that the administration of DF and/or DZ ameliorated the anaemia caused by *Trypanosoma brucei brucei* - infection in Wistar rats.

**Keywords:** Flavonoids; *Trypanosoma brucei brucei*; Haematological parameters

**Introduction**

For several decades, trypanosomiasis has continued to exert adverse effects on the economic and social well-being of sub-Saharan Africans [1,2]. The pathogenesis of African trypanosomiasis is partly due to the generation of reactive oxygen species (ROS) by the parasite, which cause degenerative changes in cells, tissues and organs of infected animals [3,4]. The ROS attack both the membrane polyunsaturated fatty acids and proteins of RBCs, leading to hemolysis and, consequently, anaemia; and depletion of endogenous antioxidant reserves in the blood and other tissues of trypanosome-infected animals [5]. The anaemia is characterized by a rapid decrease in RBC count, haemoglobin (Hb) concentration and packed cell volume (PCV) [6]. In animals, anaemia has been described as either normocytic normochromic, or macrocytic normochromic [7]. However, it has been reported that the anaemia caused by the human infective *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* in rodents ranges between macrocytic normochromic to microcytic hypochromic anaemia [7]. The difference in the types of anaemia may be attributed to many factors, including stage of the disease, pathogenicity of trypanosomes and host species [7]. Trypanosomiasis is fatal if left untreated, and chemotherapy, which remains the main form of control and eradication of the disease in African countries, is associated with toxicity and increasing incidence of resistance among the trypanosomes to the existing drugs [8,9]. Thus, the search for new drugs and formulations, which are safe, affordable and effective against both early and late stages of the disease, has been recommended [9-11]. Flavonoids are effective and common antioxidants, possessing many pharmacological activities [12-15]. They exhibit anti-allergic, anti-inflammatory, antimicrobial and antitumour activities [14]. It is conceivable that the administration of flavonoids, possessing antioxidant activity, may reduce the cellular injury caused by ROS generation in trypanosome infection.

**Materials and Methods**

**Experimental animals**

Fifty (50) adult male Wistar rats, weighing between 210-330g were used for the experiment. The animals were obtained from the animal house of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. They were kept in polypropylene cages under room temperature (24-26°C), with approximately 12-hour light and 12-hour dark cycle, and were allowed to acclimatize for two weeks. They were given free access to rat pellets and water *ad libitum*. All experimental protocols were approved and conducted with strict adherence to guidelines of the Institutional Animal Care and Use Committee of Ahmadu Bello University, Zaria, Nigeria, which are in accordance with the principles of the laboratory animal care [16].

**Trypanosome parasites**

*Trypanosoma brucei brucei* (Federi strain) used for this study was obtained from Nigerian Institute for Trypanosomosis Research, Yom, Nigeria. The parasite was maintained by serial passages in donor rats.

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Parasitaemia was monitored daily by preparing a wet mount, viewed under the light microscope (Olympus® CH23, Germany) at × 400 magnifications [17].

Infection of experimental animals

The infected blood was collected from a donor rat at peak parasitaemia and diluted with physiological saline. The rats were inoculated (1 mL/rat) intra peritoneally with a suspension, containing 3 or 4 trypanosomes per view at × 100 magnification (approximately 10⁶ cells per ml) as described by Adeyemi et al. [8].

Experimental Design

Fifty experimental rats were randomly divided into five groups (I, II, III, IV and V) of ten animals each. Group I was uninfected control animals, administered with distilled water only. Groups II and IV were inoculated with 10⁶ trypanosomes/ml of blood intraperitoneally. In addition, group IV was treated with a single dose of 3.5 mg/kg body weight DZ intraperitoneally on day 5 post-infection, but group II was left untreated. Groups III and V were first pre-treated with 100 mg/kg body weight DF, once daily for 14 days post-infection, but then infected with 10³ trypanosomes/ml of blood. After infection, treatment with DF continued daily for three weeks. In addition, group V rats were administered with a single dose of DZ at 3.5 mg/kg body weight intraperitoneally on day 5 post-infection.

Blood collection

At the end of the five weeks of experiment, the rats were sacrificed by jugular venisection after light chloroform anaesthesia. Blood (5 ml) was collected from each rat into sample bottles, containing Ethylene diamine-tetra acetic acid (EDTA) as anticoagulant for the evaluation of haematological parameters. Haematological parameters of PCV, Hb concentration, RBC, platelet, absolute and differential leucocyte counts were determined using the automated haematologic analyzer (Sysmex, KX-21, Japan) as described by Dacie and Lewis [20]. Erythrocytic indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the value of PCV, Hb concentration and RBC count as described by Schalm et al. [21].

Statistical Analysis

Values obtained were expressed as mean ± SEM. Data were subjected to one-way analysis of variance (ANOVA); followed by Tukey’s multiple comparison post-hoc test, using Graph Pad Prism version 4.0 for windows (Graph Pad Software, San Diego, California, USA). Values of P<0.05 were considered significant.

Results

Effect of treatments on the level of parasitaemia

Figure 1 shows the effect of treatments on the level of parasitaemia in all the treatment groups. All the infected groups showed presence of parasites 4 days after infection. There was significant (P<0.05) reduction in the level of parasitaemia in groups III (infected treated with DF) and V (infected treated with combination of DF and DZ) compared to groups II (infected control) and IV (infected treated with DZ).

Effect of treatments on RBC

There was significant decrease in RBC count of rats in group II compared to other groups. The RBC count of rats in group III was relatively higher than that of group II rats (Figure 2).

Effect of treatments on packed cell volume

Figure 3 shows the effect of treatments on the PCV. A significant decrease was recorded in the PCV of rats in groups II and III, when compared with the corresponding values obtained in groups I (uninfected control), IV and V (infected treated with the combination of DF and DZ). However, rats in groups IV and V showed no significant difference in PCV values, when respectively compared to that of group I.

Effect of treatments on haemoglobin concentration

Haemoglobin concentration was significantly lower in groups II and III, when compared to all other treatment groups (Figure 4). Although not significant, Hb concentration was relatively higher in group III compared to group II.

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The effect of treatments on erythrocytic indices is shown in Figure 5. The MCV of rats in group II decreased significantly, when compared to the values obtained in groups I, III, IV and V, respectively. There was a significant increase in the MCV of rats in group II, compared to the values obtained in groups I, III, IV and V, respectively. Although absolute leucocyte count obtained in group V was relatively higher than that of group III, the difference in the count was insignificant.

Effect of treatments on total leucocyte count

Total leucocyte count in groups I and IV rats rose significantly, when compared to counts obtained in groups II and III, respectively (Figure 6). There was a significant decrease in the absolute leucocyte count of rats in group II, compared to that of groups III and V, respectively. Although absolute leucocyte count obtained in group V was relatively higher than that of group III, the difference in the count was insignificant.

Effect of treatments on differential leucocyte count

Figure 7 shows the effect of treatments on neutrophil and
lymphocyte counts. Neutrophil count was significantly lower in groups II and III than the counts obtained in groups I, IV and V, respectively. There was a significant decrease in lymphocyte count of rats in group II, compared to the corresponding counts recorded in groups I, III, IV and V. Lymphocyte counts of rats in groups I and IV were significantly higher than the count obtained in group III. There was a relative increase in lymphocyte count in group V rats, compared to that of group III, but the difference in the counts was not significant.

**Effect of treatments on platelet count**

A significant decrease was recorded in the platelet count of rats in group II, when respectively compared to the counts obtained in other groups (Figure 8).

**Discussion**

All infected rats became parasitaemic at day 4 post infection and the major clinical signs observed were; respiratory distress, pale ocular mucous membrane, raised hair coat, anorexia and weight loss. The prepatent period of 4 days observed in this study is consistent with the findings of Umar et al. [3,22] in rats infected with *Trypanosoma brucei brucei*. The administration of DF did not affect the onset of parasitaemia, but significantly reduced the parasite load. Polyphenols like flavonoids have been reported to have the ability to form complex with extra- cellular and soluble proteins and also the parasite cell wall, thereby disrupting the parasite cell membrane [23]. In addition, flavonoids may regenerate other antioxidants with known immune-enhancing activity, such as vitamin E [24] and carotenoids [25]. This may explain why DF was effective in reducing the level of parasitaemia.

Anemia is a consistent feature of trypanosome infections caused by, amongst other factors, oxidative damage to erythrocyte membrane components. Reactive oxygen radicals generated during infections such as trypanosomosis can attack erythrocyte membrane, induce its oxidation and thus trigger haemolysis [23]. The administration of DF was shown in the present study to ameliorate the anaemia induced by the trypanosomases which is in consistent with other findings [3,26-29] in rats infected with *Trypanosoma brucei brucei* and treated with antioxidant vitamins. This study has demonstrated that DF possesses an in vivo ability to protect erythrocytes from haemolysis probably due its antioxidant activity; scavenging the ROS produced during the infection thus, causing reduction in the susceptibility of erythrocytes membrane to destruction.

Erythrocytic indices are used to determine the types of anaemia [30]. In this study, the significant decrease in MCV and MCHC obtained in the infected untreated group agrees with the findings of Kagira et al. [7] in Trypanosomabrucei-infected vervet monkeys. However, the result of the present study disagree with the findings of Abenga et al. [31] and Omer et al. [32] in rats, infected with *Trypanosoma brucei*, who observed only an increase in MCV. The type of anaemia observed in this study was microcytic hypochromic anaemia, associated with iron deficiency. It is possible that during *Trypanosoma brucei* infection, failure of incorporation of iron into RBC precursors, even in the presence of adequate iron storage, may precipitate the occurrence of this type of anaemia [7]. Inefficient recovery of iron from phagocytosed RBCs may also lead to iron deficiency in the body [7]. Igboke [33] reported that dyserythropoiesis is associated with animal trypanosomosis, and this may be the reason for the decreased MCV that was observed in the infected untreated group. The higher MCV obtained in DF-treated group was suggestive of the antioxidant activity of DF, which protected the RBCs from oxidative damage and trypanosome-induced dyserythropoiesis.

The significant decrease in total leucocyte count observed in the infected untreated group was in agreement with the findings of Abubakar et al. [34] in *Trypanosoma brucei* infected rats. In contrast, Omer et al. [32] and Adeyemi et al. [35] recorded a significant increase in total leucocyte count. Leucopenia in animal trypanosomosis has been attributed to factors such as trypanosomal antigen coating of leucocytes and depression of leucocyte production [7]. The decrease in neutrophil count recorded in the infected untreated group agrees with the findings of Kagira et al. [7] and Allam et al. [36]; but disagrees with that of Chaudhary and Iqbal [37], who observed an increase in neutrophil count in camels infected with *T. evansi*. The decrease in neutrophil counts observed in the present study may be as a result of overwhelming secondary bacterial infection due to immunosuppression in the infected untreated group [7,36]. The protection against external pathogens offered by the immune system is a potential source of ROS.
[36], necessary for the microbiocidal activity; but the immune cells are also sensitive to external ROS, due to the high polyunsaturated fatty acid content of their cytomembranes [38,39]. Therefore, in *T. brucei* infection with increased ROS production, the immune cells are vulnerable to oxidative damage, which may be responsible, among other factors, for the leucopenia observed in the infected untreated group. The relative increase in leucocyte count in the group treated with DF is an evidence of protection against trypanosome-induced leucopenia, probably due to DF antioxidant effect and the relatively low parasitaemia caused by the flavonoid mixture. The protection from leucopenia may facilitate the immunogenic response in combating the trypanosome infection.

The major function of platelets is to activate the clotting mechanism and prevent loss of blood from blood vessels through coagulation [40]. In this study, the low platelet count observed in the infected untreated group may indicate destruction of platelets by toxic products, emanating from the trypanosomes [41]. Low platelet counts may also be due to other factors, including: pooling of blood in the spleen, removal of platelets by mononuclear phagocytic system and increased consumption of platelets by disseminated intravascular coagulation reaction in trypanosome infection [7]. Furthermore, the thrombocytopenia recorded in the infected untreated group may be due to oxidative damage to the platelet membranes, provoked by the trypanosome parasite. This may result in the formation of lipid peroxides within the platelet membranes, thereby causing cellular lysis [42]. Treatment with DF was shown in the present study to increase significantly the thrombocyte count, which may partly be due to DF antioxidant effect. This finding is in consonance with the results obtained by Yabku et al. [43], who reported a significant increase in platelet counts in rats, administered with an aqueous extract of Fodogia agrestis stem, containing flavonoid.

In conclusion, this study has provided evidence that the administration of DAFLON® 500 mg has the potential to reduce the state of anaemia in rats, infected with *Trypanosoma brucei brucei*. 

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