Biology and Medicine
ISSN: 0974-8369

Kobo et al., Biol Med J 2014, 6:3
DOI: 10.4172/0974-8369.1000213

Research Article Open Access

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

Kobo PI[1]*, Ayo JO[2], Tagang Aluwong[2], Zezi AU[3] and Maikai VA[4]

[1]*Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[3]*Department of Pharmacy and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Nigeria*

[4]*College of Agriculture and Animal Science, Ahmadu Bello University, Mando-Kaduna, Nigeria*

Abstract

The aim of the study was to evaluate the effect of treatment with DAFLON® 500 mg (DF) and/or diminazene aceturate (DZ) on hematological 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* (10^6 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 the combination of DF and DZ. Mean corpuscular haemoglobin concentration (MCHC) was lower in the infected untreated and DF 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, trypanosomosis has continued to exert adverse effects on the economic and social well-being of sub-Saharan Africans [1,2]. The pathogenesis of African trypanosomosis 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 anemia is characterized by a rapid decrease in RBC count, haemoglobin (Hb) concentration and packed cell volume (PCV) [6]. In animals, anemia 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]. Trypanosomosis 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 anticancer 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, Vom, Nigeria. The parasite was maintained by serial passages in donor rats.

***Corresponding author:** Kobo PI, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, Tel: +234806549004; E-mail: patriciakobo@yahoo.com

Received July 16, 2014; **Accepted** September 29, 2014; **Published** October 06, 2014

Citation: Kobo PI, Ayo JO, Aluwong T, Zezi AU, Maikai VA (2014) Haematological Changes in *Trypanosoma brucei brucei* Infected Wistar Rats Treated with a Flavonoid Mixture and/or Diminazene Aceturate. Biol Med 6: 213. doi: 10.4172/0974-8369.1000213

Copyright: © 2014 Kobo PI, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 10^6 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 10^6 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 [18,19] body weight DF, once daily for 14 days *per os*, and then infected with 10^6 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).

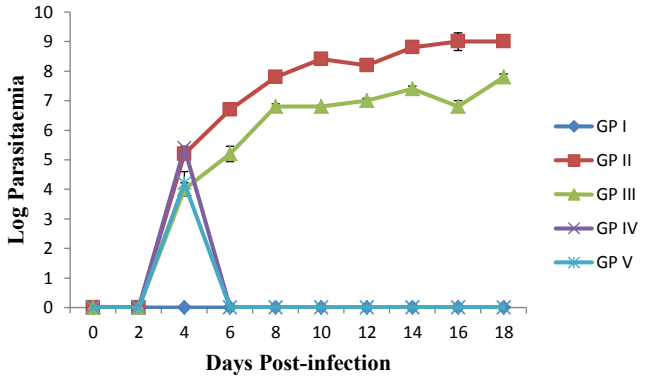

Figure 1: Effect of treatment with Daflon® 500 mg and/or diminazene aceturate in rats experimentally infected with *Trypanosoma brucei brucei*; **Keys:** Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100 mg/kg b.wt (Daflon® 500 mg) (T+DF); Group IV, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate (T+DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg b.wt. (Daflon® 500 mg) (T+DF+DZ).

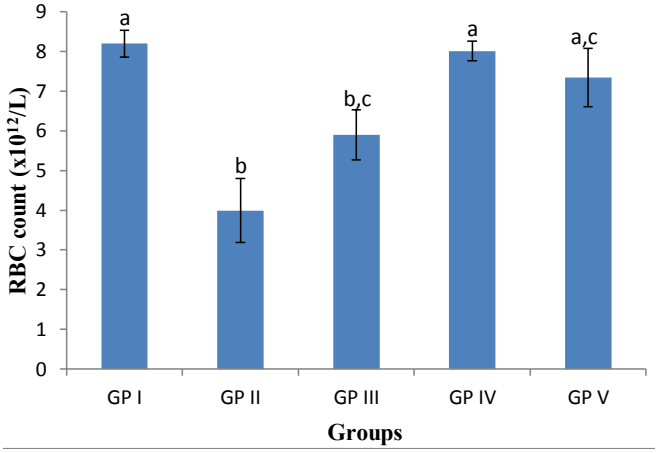

Figure 2: Effect of treatments with daflon and/or diminazene aceturate on red blood cell count of rats experimentally infected with ***Trypanosoma brucei brucei***; [a,b,c] = Means with different superscript letters are significantly ($P<0.05$) different from one another; Keys: GP = Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100 mg/kg bwt (Daflon® 500 mg) (T+DF); Group IV, infected treated with 3.5 mg/kg b.wt. Diminazeneaceturate(T+DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg bwt. (Daflon® 500 mg) (T+DF+DZ).

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 haemohlobin 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.

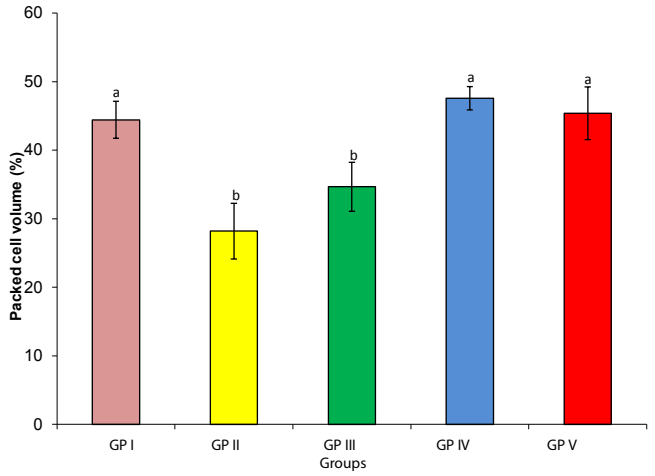

Figure 3: Effect of treatments with Daflon® and/or diminazeneaceturate on packed cell volume of rats experimentally infected with *Trypanosoma brucei brucei*; [a,b] = Means with different superscript letters are significantly (P<0.05) different from one another; **Keys:** GP = Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100 mg/kg bwt (Daflon® 500 mg) (T+DF); Group IV, infected treated with 3.5 mg/kg b.wt. Diminazeneaceturate(T+DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg bwt. (Daflon® 500 mg) (T+DF+DZ).

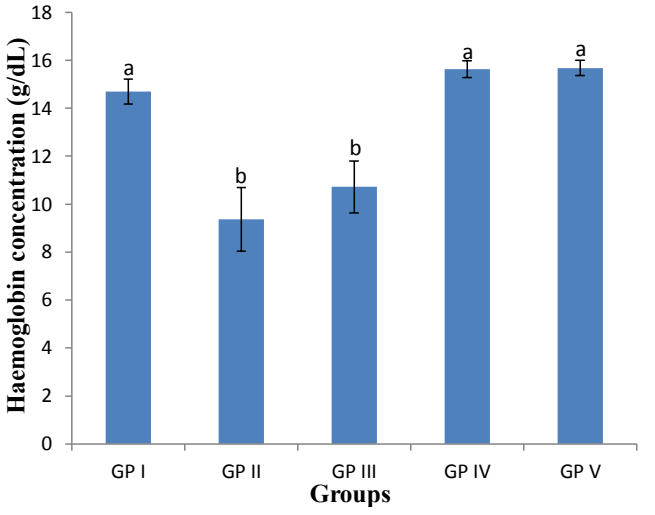

Figure 4: Effect of treatments with daflon and/or diminazene aceturate on haemoglobin concentration of ratsexperimentally infected with *Trypanosoma brucei brucei*; [a,b]= Means with different superscript letters are significantly (P<0.05) different from one another; **Keys:** GP = Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with100 mg/kg b.wt (Daflon®500 mg) (T+DF); Group IV, infected treated with 3.5 mg/kg bwt. Diminazene aceturate(T + DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg b.wt. (Daflon®500 mg) (T+DF+DZ).

Effect of treatments on erythrocytic indices

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 groups III and V, compared to that of group I or IV. The MCHC of rats in groups II and III was significantly lower than that of group I or IV.

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

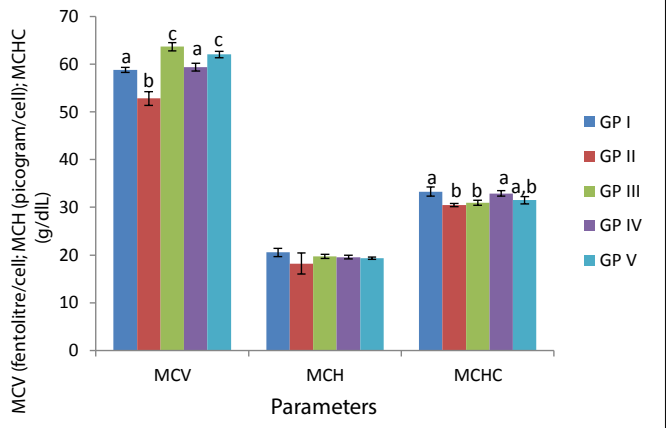

Figure 5: Effect of treatments with Daflon® and/or diminazene aceturate on erythrocytic indices of rats experimentally infected with *Trypanosoma brucei brucei*; [a,b,c]= Means with different superscript letters are significantly (P<0.05) different; **Keys:** GP = Group, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration. Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100 mg/kg bwt (Daflon®500 mg) (T + DF); Group IV, infected treated with 3.5 mg/kg bwt. Diminazeneaceturate (T + DZ); Group V, infected treated with 3.5 mg/kg bwt. Diminazeneaceturate and 100 mg/kg b.wt. (Daflon®500 mg) (T+ DF+DZ).

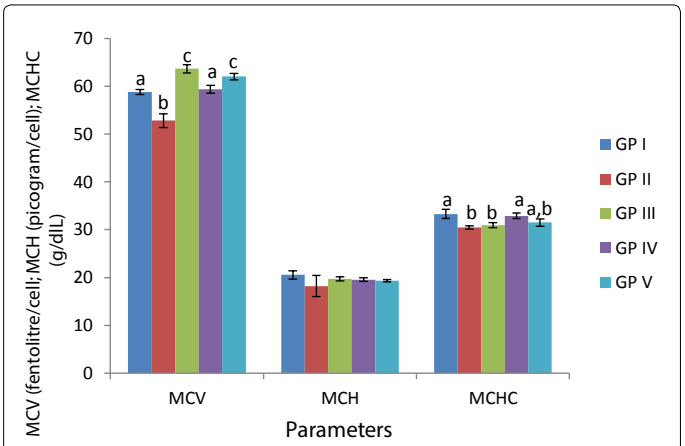

Figure 6: Effect of treatments with Daflon® and/or Diminazeneaceturate on absolute leucocyte (WBC) count of rats experimentally infected with *Trypanosoma brucei brucei*; [a,b,c]= Means with different superscript letters are significantly (P<0.05) different from one another; **Key:** Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100 mg/kg bwt (Daflon®500 mg) (T + DF); Group IV, infected treated with 3.5 mg/kg bwt. Diminazene aceturate (T +DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg bwt. (Daflon®500 mg) (T + DF + DZ).

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 extracellular 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.

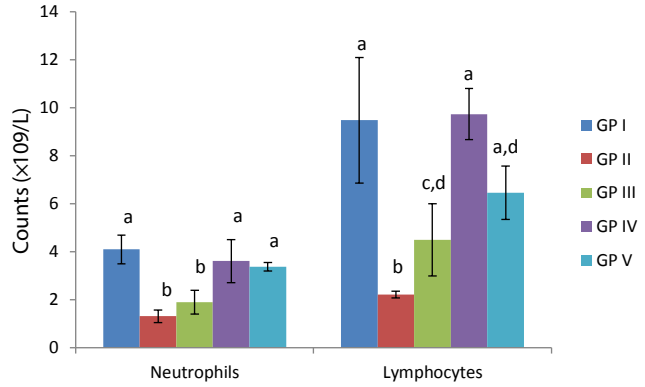

Figure 7: Effect of treatments with Daflon® and/or diminazene aceturate on neutrophil and lymphocyte counts of rats experimentally infected with *Trypanosoma brucei brucei*; [a,b,c,d]= Means with different superscript letters are significantly ($P < 0.05$) different from one another; **Key:** Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100mg/kg bwt (Daflon®500 mg) (T+DF); Group IV, infected treated with 3.5 mg/kg bwt. Diminazene aceturate (T+DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg b.wt. (Daflon®500 mg) (T+DF+DZ).

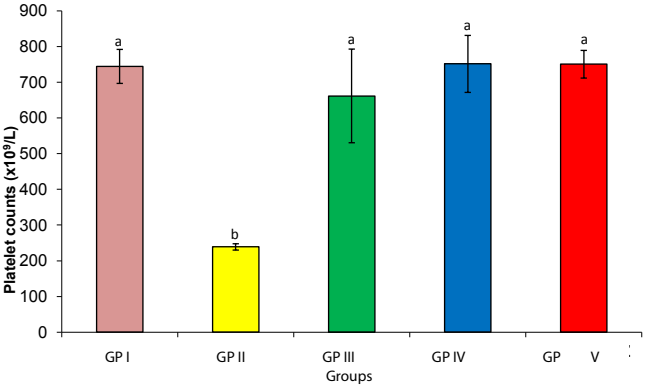

Figure 8: Effect of treatments with Daflon® and/or diminazene aceturate on the platelet count of rats experimentally infected with *Trypanosoma brucei brucei*; [a, b]= Means with different superscript letters are significantly ($P < 0.05$) different from one another; **Key:** Group I, uninfected untreated (DW); Group II, infected untreated (T); Group III, infected treated with 100mg/kg bwt (Daflon®500 mg) (T+DF); Group IV, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate (T+DZ); Group V, infected treated with 3.5 mg/kg b.wt. Diminazene aceturate and 100 mg/kg b.wt (Daflon® 500 mg) (T+DF+DZ).

Anaemia 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 trypanosomes 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 *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 disagrees 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 phagocytized RBCs may also lead to iron deficiency in the body [7]. Igbokwe [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 microbicidal 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 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 Yakubu et al. [43], who reported a significant increase in platelet counts in rats, administered with an aqueous extract of Fadogia 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*.

References

1. Adeyemi SO, Akanji MA, Oguntoye S (2009) Ethanolic leaf extract of Psidiumguajava: phytochemical and trypanocidal activity in rats infected with Trypanosoma brucei brucei, J. Med Plants Res 3: 420-423.
2. Adenike SF, Stephen AO (2010) Changes in haematological indices and protein concentrations in Trypanosomabrucei-infected rats treated with homidium chloride and diminazeneaceturate. EXCLI Journal 9: 39-45.
3. Umar IA, Ogenyi E, Okodaso D, Kimeng E, Stancheva GI, et al. (2007) Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamins A and C to Typanosomabruceibrucei-infected rats. Afr J Biotechnol 6: 2083-2086.
4. Ogunsanmi AO, Taiwo VO (2001) Pathobiochemical mechanisms involved in the control of the disease caused by Trypanosomacongolense in African grey duiker (Sylvicapragrimmia). Vet Parasitol 96: 51-63.
5. Igbokwe IO, Lafon JY, Umar IA, Hamidu LJ (1998) Erythrocyte and hepatic glutathione concentrations in acute T. brucei infection of rats. Trop Vet 16: 81-83.
6. Oyewusi JA, Saba AB, Oridupa OA (2010) The course of trypanosomosis in laboratory rabbits following experimental infection, treatment and re-infection: haematological study. Eur J Sci Res 42: 411-419.
7. Kagira JM, Thuita JK, Ngotho M, Mdachi R, Mwangangi DM, et al. (2006) Haematology of experimental Trypanosomabruceirhodesiense infection in vervet monkeys. Afr J Health Sci 13: 59-65.
8. Adeyemi OS, Akanji MA, Ekanem JT (2012) Ethanolic extract of Psidiumguajava influences protein and bilirubin levels in Trypanosoma brucei brucei-infected rats. J BiolSci 12(2): 111-116.
9. Abimbola AM, Baba IA, Yenusa EZ, Omanibe SJ, Oladimeji IH (2013) Anti-trypanosomal effect of Peristrophebicalyculata extract on Trypanosoma brucei brucei-infected rats. Asian Pac J Trop Biomed 3: 523-531.
10. Pink R, Hudson A, Mouriès MA, Bendig M (2005) Opportunities and challenges in antiparasitic drug discovery. Nat Rev Drug Discov 4: 727-740.
11. Ekanem JT, Yusuf OK (2008) Some biochemical and haematological effects of black seed (Nigella sativa) oil on Trypanosomabrucei-infected rats. Afr J Biomed Res 11: 79-85.
12. Ayo JO, Oladele SB (1996) Natural antioxidants and their potential uses in prophylaxis and therapy of disease conditions. West Afr J Pharmacol Drug Res 12: 69-76.
13. Garg A, Garg S, Zaneveld LJ, Singla AK (2001) Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. Phytother Res 15: 655-669.
14. Kim JY, Jung KJ, Choi JS, Chung HY (2004) Hesperetin: a potent antioxidant against peroxynitrite. Free Radic Res 38: 761-769.
15. Maikai VA, Kobo PI, Maikai BVO (2010) Antioxidant properties of Ximenia americana. Afr J Biotechnol 9: 7744-7746.
16. Canadian Council on Animal Care Guide (CACC) (1993), 2nd Edn.
17. Herbert WJ, Lumsden WH (1976) Trypanosoma brucei: a rapid "matching" method for estimating the host's parasitemia. ExpParasitol 40: 427-431.
18. Meyer OC (1994) Safety and security of Daflon 500 mg in venous insufficiency and in hemorrhoidal disease. Angiology 45: 579-584.
19. Inan A, Sen M, Koca C, Akpinar A, Dener C (2006) The effect of purified micronized flavonoid fraction on the healing of anastomoses in the colon in rats. Surg Today 36: 818-822.
20. Dacie JV, Lewis SM (1991) Practical Haematology, 7th Edn, Churchill Livingston, London, UK.
21. Schalm OW, Jain NC, Carroll EJ (1975) Veterinary Haematology, 3rd Edn, Philadelphia. Pa: Lea and Febige, USA.
22. Umar IA, Rumah BL, Bulus SL, Kamla AA, Jobin A et al. (2008) Effects of intraperitoneal administration of vitamin C and E or A and E combinations on the severity of Trypanosoma brucei brucei infection in rats. Afri J Biochem Res 2(3): 88-91.
23. Ngure RM, Ongeri B, Karori SM, Wachira W, Maathai RG et al. (2009) Antitrypanosomal effects of Azadiractaindica (neem) extract on Trypanosomabruceirhodesiense-infected mice. Eastern J Med 14: 2-9.
24. Zhu QY, Huang Y, Chen ZY (2000) Interaction between flavonoids and alpha-tocopherol in human low density lipoprotein. J Nutr Biochem 11: 14-21.
25. Pietta P, Simonetti P (1998) Dietary flavonoids and interaction with endogenous antioxidants. Biochem. Mol. Biol. Int., 44: 1069-1074.
26. Ajakaiye JJ, Mazadu RM, Benjamin MS, Bizi LR, Shuaibu Y et al. (2013) Effects of dietary vitamins C and E oral administration on body temperature, body weight and haematological parameters in Wistar rats infected with Trypanosoma brucei brucei (Federi strain) during the hot rainy season. Int Res J Pharm Pharmacol 3: 105-111.
27. Umar IA, Toh ZA, Igbalajobi FI, Igbokwe IO, Gidado A (1999) The effect of orally administered vitamins C and E on severity of anaemia in T. brucei-infected rats. Trop Vet 18: 71-77.
28. Umar IA, Toh ZA, Igbalajobi FI, Gidado A, Buratai LB (2000) The role of vitamin C administration in alleviation of organ damage in rats infected with T. brucei. J Clin BiochemNutr 28: 1-7.
29. Umar IA, Igbalajobi FI, Toh ZA, Gidado A, Shugaba A, et al. (2001) Effect of repeated daily doses of vitamin E (alpha-tocopherol) on some biochemical indices of rats infected with T. brucei (Basa strain). West Afr J BiolSci 12: 1-7.
30. Neiger R, Hadley J, Pfeiffer DU (2002) Differentiation of dogs with regenerative and non-regenerative anaemia on the basis of their red cell distribution width and mean corpuscular volume. Vet Rec 150: 431-434.
31. Abenga JN, Ezebuiro CO, David K, Fajinmi AO, Samdi S (2005) Studies on anaemia in Nigerian local puppies infected with Trypanosoma congolense. Vet Arhiv 75: 165- 174.
32. Omer OH, Mousa HM, Al-Wabel N (2007) Study on the antioxidant status of rats experimentally infected with Trypanosoma evansi. Vet Parasitol 145: 142-145.

33. Igbokwe IO (1989) Dyserythropoiesis in animal trypanosomosis. Rev Elev Med Vet Pays Trop 42: 423-429.

34. Abubakar A, Iliyasu B, Yusuf AB, Igweh AC, Onyekwelu NA, et al. (2005) Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wistar rats. Biokemistri 17: 95-99.

35. Adeyemi OS, Akanji MA, Ekanem JT (2010) Anti-anaemic properties of the ethanolic extracts of Psidiumguajava in Trypanosoma brucei brucei-infected rats. Res J Pharmacol 4: 74-77.

36. Allam L, Ogwu D, Agbede RIS, Sackey AKB (2011) Haematological and serum biochemical changes in gilts experimentally infected with Trypanosomabrucei. Vet Arhiv 81: 597-609.

37. Chaudhary ZI, Iqbal J (2000) Incidence, biochemical and haematological alterations induced by natural trypanosomosis in racing dromedary camels. Acta Trop 77: 209-213.

38. Fialkow L, Wang Y, Downey GP (2007) Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic Biol Med 42: 153-164.

39. Itou T, Iida T, Kawatsu H (1996) Kinetics of oxygen metabolism during respiratory burst in Japanese eel neutrophils. Dev Comp Immunol 20: 323-330.

40. Ekanem JT, Kolawole OM, Abbah OC (2008) Trypanocidal potential of methanolic extract of Bridelia ferruginea benth bark in Rattusnovergicus. Afr J Biochem Res 2: 45-50.

41. Dow RB (1994). The clinical and laboratory utility of platelet volume parameters. Austr J Med Sci 15: 1-8.

42. Ohyashiki T, Kobayashi M, Matsui K (1991) Oxygen-radical-mediated lipid peroxidation and inhibition of ADP-induced platelet aggregation. Arch Biochem Biophys 288: 282-286.

43. Yakubu MT, Akanji MA, Oladiji AT (2007) Haematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogia agrestis stem. Pharmacog Mag 3: 34-38.