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Daflon® 500 mg alone and its combination with diminazene aceturate inhibits alterations in lipid profile induced by *Trypanosoma brucei brucei* infection in Wistar rats.

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Abstract

The effect of Daflon® 500 mg (DF) and Diminazene aceturate (DZ) on the lipid profile of adult male Wistar rats infected with T. brucei brucei were investigated for their use in trypanosomosis. Fifty adult male Wistar rats were randomly divided into 5 groups of 10 rats each. Group I rats were treated with distilled water (1 mL/kg) per os, while group II rats were intraperitoneally infected with T. brucei brucei (10^6 trypanosomes/mL of blood). Group III rats were infected with T. brucei brucei (10^6 trypanosomes/mL of blood) and intraperitoneally administered with DZ at 3.5 mg/kg once, when infection was established. Groups IV and V were first pretreated with DF at 100 mg/kg per os for three weeks, followed by infection with T. brucei brucei (10^6 trypanosomes/mL of blood) intraperitoneally. Treatment with DF continued for another one week. In addition, group V rats were intraperitoneally administered with DZ at 3.5 mg/kg once, when infection was established prior to continuation with DF for another one week. Pretreatment with DF alone or in combination with DZ decreased parasitaemia, lowered triglyceride level and significantly ($P < 0.05$) decreased cholesterol and low-density lipoprotein concentrations in T. brucei brucei-infected Wistar rats. In conclusion, daflon® 500 mg alone or in combination with diminazene aceturate may be beneficial in the treatment of the infection in Wistar rats.

Keywords: Daflon® 500 mg, Diminazene aceturate, lipid profile, *T. brucei brucei*, Wistar rats.

Daflon® 500 mg seul et son association avec l'acéturate de diminazène inhibent les altérations du profil lipidique induites par l'infection de *Trypanosoma brucei brucei* chez les rats Wistar.

Résumé

L'effet du Daflon® 500 mg (DF) et de l'acéturate de diminazène (DZ) sur le profil lipidique de rats Wistar mâles adultes infectés par T. brucei brucei a été étudié pour leur utilisation dans la trypanosomose. Cinquante rats Wistar mâles adultes ont été répartis au hasard en 5 groupes de 10 rats chacun. Les rats du groupe I ont été traités avec de l'eau distillée (1 mL/kg) per os, tandis que les rats du groupe II ont été infectés par voie intrapéritonéale avec T. brucei brucei (106 trypanosomes/mL de sang). Les rats du groupe III ont été infectés par T. brucei brucei (106 trypanosomes/mL de sang) et administrés par voie intrapéritonéale avec

du DZ à raison de 3,5 mg/kg une fois, lorsque l'infection était établie. Les groupes IV et V ont d'abord été prétraités avec DF à 100 mg/kg per os pendant trois semaines, suivi d'une infection par T. brucei brucei (106 trypanosomes/mL de sang) par voie intrapéritonéale. Le traitement par DF s'est poursuivi pendant encore une semaine. De plus, des rats du groupe V ont reçu une administration intrapéritonéale de DZ à raison de 3,5 mg/kg une fois, lorsque l'infection était établie avant la poursuite du traitement par DF pendant une semaine supplémentaire. Le prétraitement avec DF seul ou en association avec DZ a diminué la parasitémie, abaissé le taux de triglycérides et significativement (P < 0,05) diminué les concentrations de cholestérol et de lipoprotéines de basse densité chez les rats Wistar infectés par T. brucei brucei. En conclusion, le daflon® 500 mg seul ou en association avec l'acéturate de diminazène pourrait être bénéfique dans le traitement de l'infection chez le rat Wistar.

Mots-clés: Daflon® 500 mg, acéturate de diminazène, profil lipidique, T. brucei brucei, rats Wistar

Introduction

Trypanosomosis is a disease affecting almost all vertebrates, particularly humans and livestock. African trypanosomosis is caused by a protozoan parasite belonging to the genus *Trypanosoma*, and is transmitted by tsetse flies to the final host. The disease is characterized by high morbidity and mortality of infected livestock, leading to loss in revenue in the livestock industry in many parts of Africa (Onyeyili and Egwu, 1995; Abdullahi *et al*., 2019). In Africa, about 70 million people inhabiting over 1.55 million km^2 (Simarro *et al*., 2012), and 250 million animals distributed over approximately 25 million km^2 are at risk of the disease (WHO, 2006). The rate of new infections and mortality due to the disease show no sign of decline (Aksoy and Rio, 2005).

Infection with trypanosomes results in the production of large amounts of reactive oxygen species (ROS) which are cytotoxic agents, damaging vital components of the cell, including proteins and lipids (Mishra *et al*., 2017; Ojo *et al*., 2021). The ROS generated attack the membrane lipids, resulting in their destruction and exhaustion of endogenous antioxidants that provide protection for cellular macromolecules (Kobo *et al*., 2014a). Trypanosomosis also results in immunosuppression due to the ability of the parasite to weaken the host immune system. This in turn adversely affects the host ability to eliminate the parasite (Stijlemans *et al*., 2017; Akazue *et al*., 2019).

To date, there is no effective vaccine against animal trypanosomosis (Donnelson, 2003; Magez *et al*., 2021). The control of the disease relies mainly on chemotherapy and chemoprophylaxis (Chekwube *et al*., 2014). Unfortunately, the existing drugs for trypanosomosis are toxic and/or expensive (Ajagbonna *et al*., 1995; Alayande *et al*., 2011). In addition, the unavailability of the drugs in rural areas, incidence of therapeutic failure and relapses from treatments occur frequently (Alayande *et al*., 2011; Giordani *et al*., 2016). Therefore, there is need to search for alternative drugs that are cheap, readily available and effective in the treatment of trypanosomosis. Daflon® 500 mg, whose active principle is a micronized purified flavonoid mixture (consisting of 90% diosmin and 10% hesperidin) with antioxidant properties may boost the host immune system. It may scavenge the ROS produced during trypanosomosis infection, thereby protecting vital components of the cell against oxidative damage.

Materials and methods

Experimental animals

Fifty (50) male adult Wistar rats weighing between 100 – 120 g were obtained from the Animal House of the Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The animals were housed in metal cages with wood shavings as beddings. They were allowed to acclimatize for a period of 14 days, while feed and water were provided *ad libitum*.

Trypanosome parasite

Trypanosoma brucei brucei used for this study was obtained from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria. The parasite was maintained by serial passages in donor rats. Parasitaemia was monitored daily by preparing a wet mount and viewed under the light microscope (Olympus® CH23, Germany) at × 400 magnifications (Herbert and Lumsden, 1976).

Inoculation of rats

Infected blood was collected from a donor rat at peak parasitaemia and diluted with physiological saline. The rats were inoculated (1 mL/rat) intraperitoneally 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*. (2012).

Experimental design

The fifty (50) male adult Wistar rats were divided at random into five groups of ten rats each and treated as follows:

Group I: Rats in this group were treated with distilled water only at 1mL/kg *per os*.

Group II: Rats in this group were intraperitoneally infected with 10^6 trypanosomes/mL of blood only.

Group III: Rats in this group were intraperitoneally infected with 10^6 trypanosomes/mL of blood and then treated once with DZ at 3.5 mg/kg when parasite load was 3 or 4 per field.

Group IV: Rats in this group were pretreated daily with **Daflon®** 500 mg at 100 mg/kg (Meyer, 1994; Inan *et al*., 2006) orally for 3weeks and then intraperitoneally infected with 10^6 trypanosomes/mL of blood. Treatment with **Daflon® 500 mg** continued till the termination of the experiment.

Group V: Rats in this group were pretreated with **Daflon® 500 mg** daily at 100 mg/kg (Meyer, 1994: Inan *et al*., 2006) for 3 weeks, after which they were intraperitoneally infected with 10^6 trypanosomes/ml of blood. Diminazene aceturate (DZ) was administered once, at 3.5 mg/kg intraperitoneally when parasitaemia load was 3 or 4 per field. Treatment with **Daflon® 500 mg** continued till the termination of the study.

Parasitaemia was monitored in all the infected rats every other day using the rapid matching method of Herbert and Lumsden (1976).

At the end of the four weeks of the experiment, the rats were sacrificed by jugular venesection after light chloroform anaesthesia. Blood (5 mL) was collected from each rat into plain test tubes, and allowed to clot. They were centrifuged at 1000 *g* for 10 minutes. The serum harvested was used for evaluation of lipid profile.

Determination of serum lipid profile

The determination of serum level of cholesterol was carried out using colorimetric enzymatic end point method. Serum triglycerides were analyzed using colorimetric method after enzymatic hydrolysis with lipases. High-density lipoprotein-cholesterol (HDL-cholesterol) was determined using precipitant method. All the analyses were carried out using standard commercial test kits (RANDOX Laboratories Ltd., Ardmore, Diamond

Road, Crumlin, Co., Antrim, UK), following the instructions provided by the manufacturers. Low-density lipoprotein-cholesterol (LDL-cholesterol) was calculated from the values of total cholesterol, triglycerides and HDL-cholesterol using the formula described by Friedewald *et al*. (1972):
LDL-Chol = Total CHOL– TRIGS/2.2 – HDL-Chol

Data analyses
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 5.0 for windows (Graph Pad Software, San Diego, California, USA). Values of P < 0.05 were considered significant.

Results

Effect of treatment with Daflon® 500 mg and diminazene aceturate on level of parasitaemia

The effect of treatment on the level of parasitaemia is shown in Figure 1. At days 2 and 4 post-infection, the level of parasitaemia was significantly higher (P < 0.05) in groups II and III, when compared to groups IV and V. Following the administration of DZ, the level of parasitaemia in groups III and V dropped to zero at day 6 post-infection. Group II rats had higher ($P < 0.05$) level of parasitaemia, when compared to group IV at days 6 and 8 post-infection, respectively.

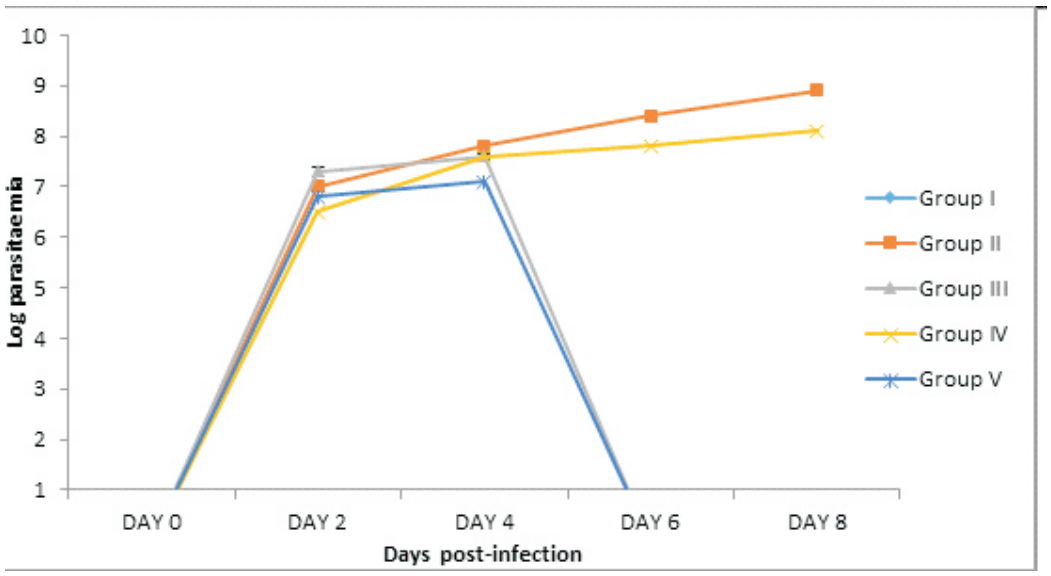

Figure 1: Effect of treatment with Daflon® 500 mg and Diminazene aceturate on the level of parasitaemia in rats infected with *Trypanosoma brucei brucei*

KEY**:** Group I, uninfected untreated; Group II, infected untreated; Group III, infected and treated with Diminazene aceturate (3.5 mg/kg b. wt); Group IV, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks and infected with *T. brucei brucei*; Group V, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks, infected with *T. brucei brucei* and DZ (3.5 mg/kg b. wt.).

Effect of treatment with Daflon® 500 mg and diminazene aceturate on low-density lipoprotein

There was a significant ($P < 0.05$) difference in the level of LDL of rats in group I, when compared to groups II, III and IV. The level of LDL in group II rats did not differ from those of groups III and IV; however, the value recorded in group IV was relatively higher than those of groups II and III. Rats in group V had relatively, but insignificantly higher level of LDL in comparison to group IV (Figure 2).

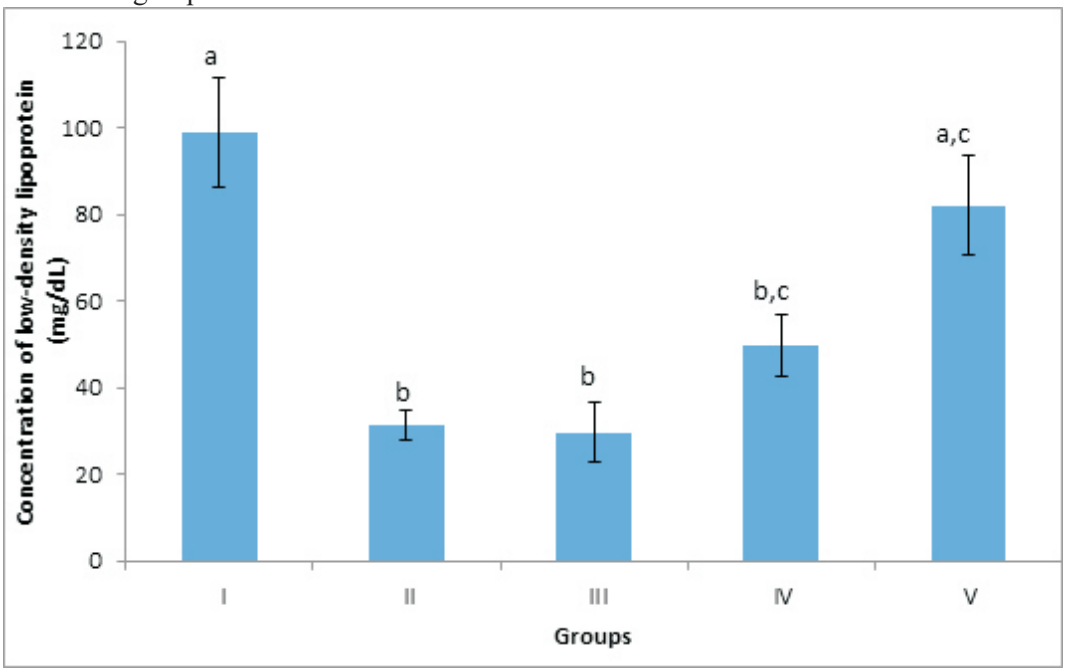

Figure 2: Effect of treatment with Daflon® 500 mg and Diminazene aceturate on the level of low-density lipoprotein in rats experimentally infected with *Trypanosoma brucei brucei*

[a, b, c] = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.

KEY**:** Group I, uninfected untreated; Group II, infected untreated; Group III, infected and treated with Diminazene aceturate (3.5 mg/kg b. wt); Group IV, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks and infected with *T. brucei brucei*; Group V, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks, infected with *T. brucei brucei* and DZ (3.5 mg/kg b. wt.).

Effect of treatment with Daflon® 500 mg and diminazene aceturate on high-density lipoprotein

The effect of treatment on HDL is shown in Figure 3. The level of HDL did not differ in all the treatment groups.

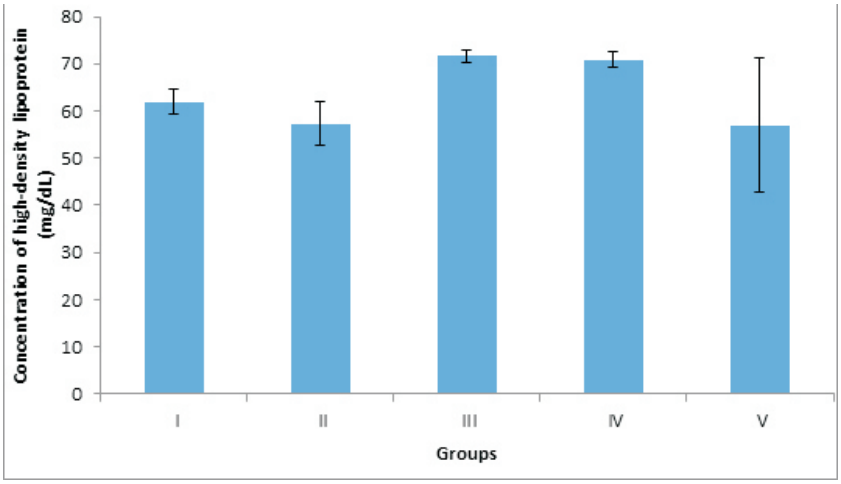

Figure 3: Effect of treatment with Daflon® 500 mg and Diminazene aceturate on the level of high-density lipoprotein in rats experimentally infected with *Trypanosoma brucei brucei*

Values are mean ± SEM of 5 animals per group.

KEY**:** Group I, uninfected untreated; Group II, infected untreated; Group III, infected and treated with Diminazene aceturate (3.5 mg/kg b. wt); Group IV, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks and infected with *T. brucei brucei*; Group V, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks, infected with *T. brucei brucei* and DZ (3.5 mg/kg b. wt.).

Effect of treatment with Daflon® 500 mg and dimnazene aceturate on the level triglyceride

Rats in group II and IV had higher ($P < 0.05$) level of triglyceride in comparison to the values obtained in groups I, III and V (Figure 4). Although not significant ($P < 0.05$), the level of triglyceride was relatively higher in group II rats, when compared to those of group IV. Similarly, rats in group III had relatively higher level of triglyceride compared to those of group V.

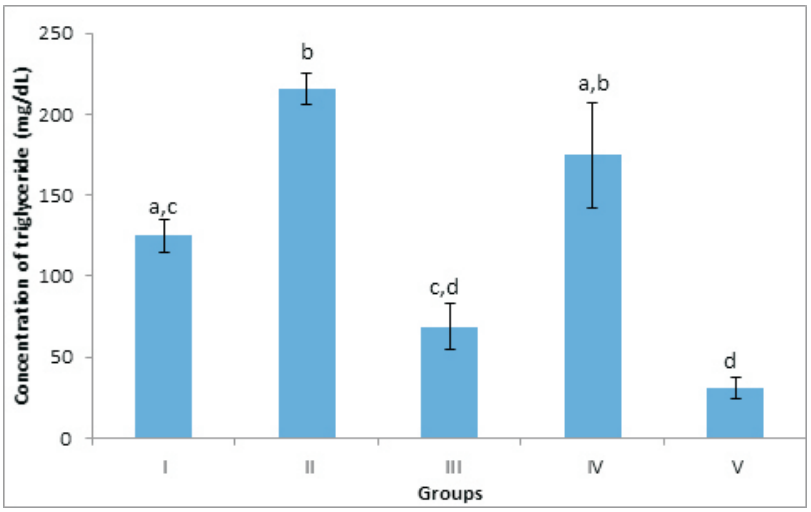

Figure 4: Effect of treatment with Daflon® 500 mg and Diminazene aceturate on the level of triglyceride in rats experimentally infected with *Trypanosoma brucei brucei*

[a, b, c,d] = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.
KEY**:** Group I, uninfected untreated; Group II, infected untreated; Group III, infected and treated with Diminazene aceturate (3.5 mg/kg b. wt); Group IV, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks and infected with *T. brucei brucei*; Group V, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks, infected with *T. brucei brucei* and DZ (3.5 mg/kg b. wt.).

Effect of treatment with Daflon® 500 mg and diminazene aeturate on the level cholesterol

The level of cholesterol was significantly ($P < 0.05$) lower in groups II and III rats when compared to groups I, IV and V. There was a significant ($P < 0.05$) increase in the level of cholesterol in group II rats in comparison to the value obtained in group III rats (Figure 5).

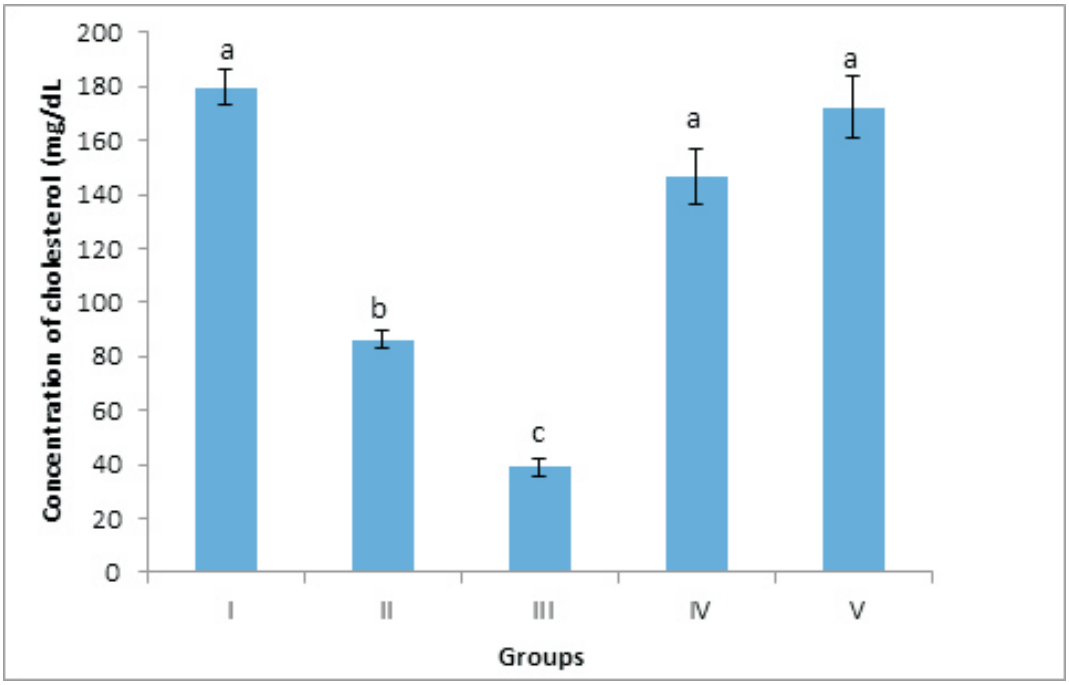

Figure 5: Effect of treatment with Daflon® 500 mg and Diminazene aceturate on the level of cholesterol rats experimentally infected with *Trypanosoma brucei brucei*

[a, b, c] = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.
KEY**:** Group I, uninfected untreated; Group II, infected untreated; Group III, infected and treated with Diminazene aceturate (3.5 mg/kg b. wt); Group IV, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks and infected with *T. brucei brucei*; Group V, pretreated with Daflon® 500 mg (100 mg/kg b. wt.) for three weeks, infected with *T. brucei brucei* and DZ (3.5 mg/kg b. wt.).

Discussion

The pre-patent period of 2 days observed in this study agrees with the result obtained by Kobo *et al*. (2014b) in mice, but disagrees

with the report of Umar *et al*. (2007, 2008) in rats. The pre-patent period of 2 days observed in this study may be associated with variation in the strain of the parasite inoculated, individual infectivity or host susceptibility to infection. The significant ($P < 0.05$) decrease observed in the level of parasitaemia in the group treated with daflon alone (group IV) or in combination with diminazene aceturate (group V) agrees with the report of Umar *et al.* (2008) in rats infected with *Trypanosoma brucei brucei* and treated with antioxidant vitamins. The decrease observed may be attributed to the antioxidant activity and the immune-stimulating effect of DF that boosted the host immune response, thereby resulting in a decrease in parasite multiplication.

Cholesterol is the building block for cell membranes and it is essential for formation of bile (which aids in the digestion of fats), vitamin D, other steroids and hormones such as progesterone, testosterone and oestrogen (Adamu *et al*., 2008). The LDL cholesterol transports cholesterol from the liver to organs and tissues of the body; the HDL cholesterol on the other hand, carries cholesterol from various organs and tissues to the liver for recycling or degradation (Adamu *et al*., 2008). Consequently, any significant alteration in the serum levels of these molecules could result in lipid disorders in affected animals. The decrease in the serum HDL, LDL and cholesterol levels observed in the infected untreated group is in agreement with the report of Abdulazeez *et al*. (2013) and Ojo *et al*. (2021) in *T. brucei brucei* infected rats. Many pathophysiological mechanisms may have been involved in the lowering of serum levels of LDL and cholesterol, and relative lowering of HDL observed in the infected untreated rats. It has been shown that trypanosomes depend on serum LDH and HDL for their lipoprotein requirement in order to multiply under axenic culture (Adamu *et al*., 2008; Samuel *et al*., 2018). This finding could partly explain the decrease in serum HDL and LDL levels observed in this study. Blood-stream form of trypanosomes, which are unable to synthesize cholesterol, are also known to require cholesterol along with phospholipids and total lipids for synthesis of their membranes and growth (Green *et al*., 2003; Nok *et al*., 2003; Adamu *et al*., 2008; Abdulazeez *et al*., 2013; Samuel *et al*., 2018). The continuous removal and utilization, from the blood-stream, of these molecules by the parasites could partly be responsible for the lowered serum levels of lipids and total cholesterol observed in the present study (Abdulazeez *et al*., 2013). In this study, the infected untreated group had a significant increase in the serum level of triglyceride, when compared to other treatment groups. This increase is in conformity with the findings of Ngure *et al*. (2008) and Gaithuma *et al*. (2011) in vervet monkeys infected with *T. b. rhodesiense,* but disagrees with the report of Eze *et al*. (2015) in single and mixed infection of *T. brucei* and *T. congolense* in pigs. The alteration in serum triglyceride could be due to acute phase responses during early infection (Khovidhunkit *et al*., 2004). Tumour necrosis factor-alpha produced during acute infection has been shown to inhibit adipose tissue enzyme lipoprotein lipase (Beutler and Cerami, 1988; Gaithuma *et al*., 2011) that is responsible for clearing lipids from plasma. Furthermore, increased production of ROS due to trypanosome infection may have resulted in an imbalance between radical-generating and radical scavenging activity of the host, which can destroy lipids (Saleh *et al*., 2009; Mishra *et al*., 2017); thus, the alterations in the recorded lipid profile. Pretreatment with Daflon 500 mg® and/or diminazene aceturate was able to ameliorate the alteration in the serum LDL, HDL, cholesterol and triglyceride levels induced by *T. brucei brucei* infection. This may be due to the antioxidant effect of

daflon that may have promoted the host ROS-scavenging activity, thereby resulting in the degradation of the ROS responsible for lipid peroxidation. Thus, daflon antioxidant effect protects vital components of the cell, including lipids from oxidative damage. Diminazene aceturate being a trypanocidal agent eliminates the parasites from systemic circulation thereby protecting lipids from parasite-induced oxidative damage. Treatment with the combination of daflon and dimizene aceturate was better, when compared with daflon or diminazene azeceturate alone. This could be due to the combined antioxidant effects of DF and the trypanocidal effects of diminazene aceturate.

Conclusion

In conclusion, the current study revealed that **Daflon® 500 mg** could be used in combination with Diminazene aceturate to alleviate the negative effect of *Trypanosoma brucei brucei* on the lipid profile in Wistar rats.

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