NSAP 47th Annual Conference (JOS 2022) CONFERENCE PROCEEDINGS
THEME: SECURING ANIMAL AGRICULTURE AMIDST GLOBAL CHALLENGES

# EFFECTS OF DAFLON® 500 mg AND DIMINAZINE ACETURATE ON THE SERUM PROTEIN CONCENTRATION OF *TRYPANOSOMA BRUCEI BRUCEI* - INFECTED WISTAR RATS.

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## ABSTRACT
The study was carried out to determine the effects of Daflon® 500 mg (DF) and Diminazine aceturate (DZ) on serum total protein concentration of *Trypanosoma brucei brucei*-infected Wistar rats. Fifty male adult Wistar rats weighing between 100 – 120g were randomly assigned into five groups (I-V) of 10 rats each. Group I (uninfected untreated) rats were treated with distilled water (1 ml/kg) *per os* while group II (infected untreated) rats were intraperitoneally infected with *Trypanosoma brucei brucei* ($10^6$ trypanosomes/ml of blood). Group III rats were infected with *Trypanosoma brucei brucei* ($10^6$ trypanosomes/ml of blood) and intraperitoneally administered with diminazene aceturate at 3.5 mg/kg once, when infection was established. Groups IV and V were first pretreated with daflon® 500 mg at 100 mg/kg *per os* for three weeks, followed by infection with *Trypanosoma brucei brucei* ($10^6$ trypanosomes/ml of blood) intraperitoneally. Treatment with daflon® 500 mg continued for another one week. In addition, group V rats were intraperitoneally administered with diminazene aceturate at 3.5 mg/kg once, when infection was established prior to continuation with daflon® 500 mg for another one week. Pretreatment with Daflon® 500 mg alone or in combination with diminazene aceturate resulted in a significant ($P < 0.05$) increase in the level of serum total protein and albumin, and an insignificant ($P > 0.05$) increase in the level of globulins. Thus, suggesting that such combination therapy may be useful in the management of African trypanosomosis.
**Keywords:** Trypanosomosis, Daflon® 500 mg, Diminazine aceturate, Protein concentration, Wistar rats.

## INTRODUCTION
Trypanosomosis is a disease caused by a flagellated heamo-protozoan parasite belonging to the genus *Trypanosoma.* The parasite causes disease in domestic and wild animals as well as humans (Egbe-Nwiyi *et al.,* 2020). In animals, the disease is called *Nagana* and is caused by *Trypanosoma congolense, T. vivax* and *T. brucei brucei* (Steverding, 2008) while in human, the disease is known as "sleeping sickness" and cause by two subspecies of protozoan parasites, *Trypanosoma brucei rhodensiense*, and *T. brucei gambiense* (CDC. 2019).
Serum biochemical and hematological changes are characteristics of trypanosome infection, the severity of which are often determine by species and strain of the infecting trypanosome and the host defense (Addisu *et al*., 2017). The disease affects wide range of body organs of infected animal host which leads to alterations in serum biochemical parameters like liver enzymes, total proteins, glucose, lipids, albumin, globulin, urea, creatinine and minerals. Oxidative stress imposed by reactive oxygen species (ROS) generated during trypanosome infections have been incriminated in trypanosome-induced cellular injury (Saleh *et al.,* 2009). Chemotherapy and chemoprophylaxis are the major approaches usually employed in the control of animal trypanosomosis. However, the available Veterinary chemotherapeutic agents (trypanocides) are associated with narrow therapeutic indices and low level resistant which restrict their use (Federica *et al.,* 2016). Thus, the search for new drugs and formulations which are non-toxic, affordable and effective against trypanosome parasites is recommended. Flavonoids are effective and commonly use antioxidants, possessing many pharmacological activities. Daflon®500 mg is a flavonoid mixture consisting of diosmin 450 mg and hesperidin 50 mg. It is a powerful scavenger of reactive oxygen species (Atakan *et al.,* 2011) and has been reported to ameliorate the anemia and lipoperoxidative changes induced by *Trypanosoma brucei brucei* infection in Wistar rats (Kobo *et al*., 2014a,b). Therefore, the administration of Daflon® 500 mg with antioxidant activity may help scavenge the ROS generated during trypanosome infection there by ameliorating the damage caused to tissues and organs during trypanosomosis.

## MATERIALS AND METHODS

Fifty (50) male Wistar rats were divided at random into 5 groups of 10 animals each and treated as follows:
**Group I:** Distilled water only at 1mL/kg *per os*.
**Group II:** infected with $10^6$ trypanosomes/ml of blood only, intraperitoneally.
**Group III:** infected with $10^6$ trypanosomes/ml of blood, and administered Diminanazene aceturate (DZ) at 3.5mg/kg when parasite load was 3 or 4 per field.
**Group IV:** pretreated daily with Daflon® 500 mg at 100 mg/kg (Inan *et al*., 2006) orally for 3weeks, then infected with $10^6$ trypanosomes/ml of blood. Treatment with Daflon® 500 mg continued till the termination of the experiment.
**Group V:** pretreated with Daflon® 500 mg daily at 100 mg/kg for 3 weeks, after which they were infected with $10^6$ trypanosomes/ml of blood. DZ was administered once, at 3.5mg/kg intraperitoneally when parasite load was 3 or 4 per field. Treatment with Daflon® 500 mg continued till the termination of the study.
At the end of the four weeks of experiment, the rats were sacrificed, blood (5 ml) was collected into plain test tubes, allowed to clot, centrifuged at 1000 *g* for 10 minutes. The serum harvested was used for evaluation of serum total protein, albumin and globulin.

**Determination of Serum Protein profile**

Total serum protein concentration was determined by the biuret method as described by Weichselbaum (1946) and the albumin fraction was determined by the bromocresol method as described by Doumas *et al*. (1971) using the standard Randox® diagnostic kit (Randox Laboratories LED, U.K.), spectrophotometrically. Globulin fraction was calculated by subtracting the albumin fraction from the total serum protein.

**Statistical Analysis**

Data obtained were expressed as mean ± SEM and 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 AND DISCUSSION

Infection of rats resulted in a significant ($P < 0.05$) decrease in the level of serum total protein and albumin, and relative decrease in the level of serum globulins in group II rats when compared to group V. The level of total protein, globulin and albumin did not differ in all the treatment groups (III, IV and V), however, the group pretreated with DF and administered with DZ (V) had relatively higher values.

The significant decrease observed in the level of serum total protein and albumin in group II agrees with the work of Pandya *et a*l. (2018) in *Trypanosoma evansi* infected cattle and Orhue *et al.* (2005) in *Trypanosoma brucei* infected rabbits, respectively. The decrease could be associated with decreased protein synthesis arising from damage liver or as a result of excessive protein breakdown arising from reduced feed intake due to pyrexia (Eze *et al*., 2013). The hypoalbuminaemia observed may also be due to increased utilization of albumin by the trypanosome parasite. It has been reported that trypanosome parasites utilize albumin for their growth and multiplication (Addisu *et al.,* 2017).

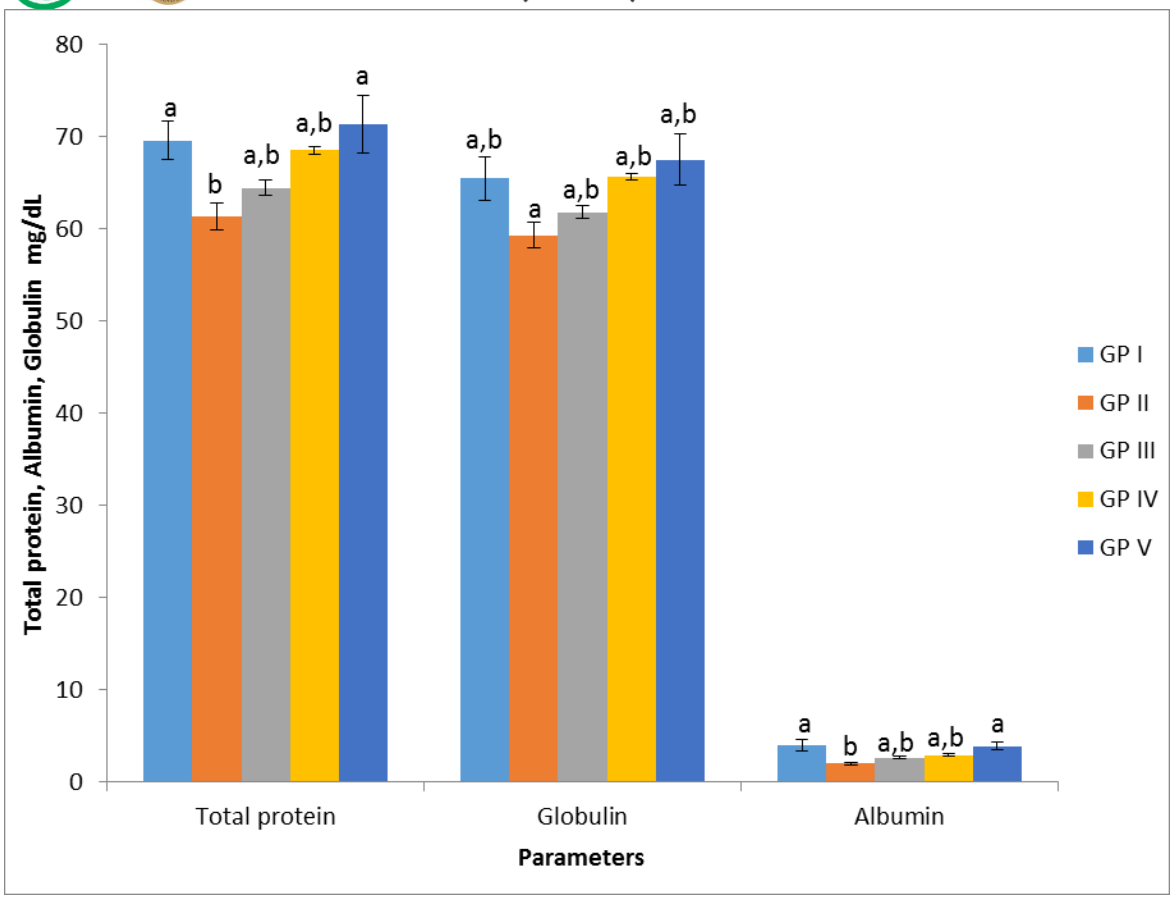


**Figure1:** Effect of treatment with Daflon® 500 mg and diminazene aceturate on the level of serum total protein, albumin and globulin in rats experimentally infected with *Trypanosoma brucei brucei*

[a, b] = 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 diminazene aceturate (3.5 mg/kg b. wt.).

The decrease in serum globulin observed in group II rats disagrees with the report of Orhue *et al.* (2005) who reported an increase in serum globulin in *Trypanosoma brucei* infected rabbits. The relative decrease may be attributed to immunosuppression or hepatic damage which occurs as a result of trypanosome infection. Immunosuppresion is a frequent occurrence in African trypanosome infections (Addisu *et al.,* 2017). The administration of DF and/or DZ resulted in a significant ($P < 0.05$) increase in the level of total protein and albumin, and relative increase in globulin level. This may be attributed to the antioxidant effect of DF which scavenged the ROS produced as a result of trypanosome infection thereby reducing the level of damage caused to the liver by ROS thus increasing protein synthesis. The relative increase recorded in the level of serum globulin may be attributed to the immune enhancing effect of DF that boosted the immune system of the infected rats.

## CONCLUSION

Pretreatment with DF alone or in combination with DZ caused increase in total protein, albumin and globulin in *Trypanosoma brucei brucei* infected rats. However, the group pretreated with DF and treated with DZ was better at improving the alterations in these parameters induced by ROS production in trypanosome infection.

## RECOMMENDATION

Post-treatment of *Trypanosoma brucei brucei* infected Wistar rats with Daflon® 500 mg alone or in combination with diminazene aceturate should be carried out.

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