UMYU Scientifica, Vol. 4 NO. 4, December 2025, Pp 231 – 237
https://doi.org/10.56919/usci.2544.019
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Ameliorating the Effects of *Trypanosoma Evansi* Infection in Wistar Rats Using Selected Nutraceuticals, Ethidium Bromide and Ethidium Chloride

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ABSTRACT
African Trypanosomiasis is a disease that affects humans and animals, caused by trypanosomes in sub-Saharan Africa, despite decades of vector control and treatments. The disease remains endemic and an impediment to socio-economic development. 45 male Wistar rats were purchased from the animal colony of the Nigerian Institute for Trypanosomiasis Research (NITR) and divided into 9 groups of 5 rats each. Group I, (uninfected, untreated), Group II (Infected, untreated), while Groups III, IV, V, VI, VII, VIII and IX were the groups tested. Each rats were infected with 1×10^5 Trypanosomes and treated with 6.43 mg/kg Glucosamine (Group III), 24.6 mg/kg Omega-H3 (Group IV), 1 mg/kg Ethidium bromide (Group V), 1 mg/kg Ethidium chloride (Group VI), Group VII were treated with Ethidium bromide and Glucosamine, Group VIII were treated with Ethidium chloride and Omega H3, while Group IX was treated with both of the nutraceuticals for 7 days. PCV, body temperature, and body weight were also determined. Results showed that PCV was significantly reduced ($P < 0.05$) in the infected and treated groups compared to group I, and was greater in group II. No significant ($P > 0.05$) difference was observed between the groups treated with the Nutraceuticals and the Trypanocides. Body temperature increased in all groups compared with group I and was highly significant ($P < 0.05$) in group II. No significant ($P < 0.05$) increase in body weight of rats was observed in the groups treated compared to group II, but a decrease in body weight was observed in all groups compared to group I. No difference observed in groups treated with Nutraceuticals and the Trypanocides. Nutraceuticals have ameliorated the effects of *Trypanosoma evansi* infection on PCV, body temperature, and body weight in Wistar rats.

ARTICLE HISTORY
Received July 12, 2025
Accepted December 20, 2025
Published December 30, 2025

KEYWORDS
Trypanosomiasis, Nutraceuticals, Wistar rats, *Trypanosoma evansi,* Ethidium bromide, Ethidium chloride

INTRODUCTION
Trypanosomiasis is a disease of both humans and animals that has analogous causative agents (WHO, 2021). For several decades, it has been a chronically debilitating protozoan disease of livestock, of great economic importance in sub-Saharan Africa, that has continued to adversely affect the economic and social well-being of of Africans (Kumela and Behablom, 2021*)*. The disease is located in some foci in sub-Saharan Africa, with manifestations in 37 countries, affecting nearly 60 million people and 48 million cattle (World Health Organisation, 2022). The protozoan genus *Trypanosoma*, which live and multiply in the blood and tissue fluids of hosts (mammals), is the causative agent of the illness (Kargbo, 2020) and is transmitted through the bite of infected tsetse flies (*Glossina* sp.). The disease has severely affected the economies of the African continent and its cultural development throughout history. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are the species of Trypanosome that infect man in Western, Central, Eastern and Southern Africa, causing 'sleeping sickness' (WHO, 2021). Likewise, *T. congolense, T. vivax, T. evansi* and *T. brucei brucei* are the agents responsible for the disease called 'nagana or sammore' in livestock (Kasozi *et al.*, 2022). As the disease progresses, the infected animals become weak and eventually unable to work or walk. In wild animals, these agents (parasites) cause mild infections; at the same time, in domestic animals, they cause a severe and fatal illness if left untreated. The dipteran vectors (tsetse flies) responsible for the disease are found in rainforest, savannah, and woodland ecological zones, respectively (IAEA, 2021).

Pathogenesis of disease occurs as a result of reactive oxygen species (ROS) generated by the trypanosome, which cause degenerative changes in the cells, organs, and tissues of animals infected (Zhang *et al.*, 2021). The attack

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How to cite: Shuaibu, Y., Abdulfatai, K., & Dadah, A. J. (2025). Ameliorating the Effects of *Trypanosoma Evansi* Infection in Wistar Rats Using Selected Nutraceuticals, Ethidium Bromide and Ethidium Chloride. *UMYU Scientifica*, 4(4), 231 – 237. https://doi.org/10.56919/usci.2544.019

of membrane polyunsaturated fatty acids and proteins of red blood cells (RBCs) by ROS causes haemolysis, anaemia, and depletion of endogenous antioxidant reserves in the blood and other tissues (Abubakar and Dabo, 2023). The redox imbalance is a typical challenge for the host and the parasite during infections with extracellular African trypanosome species. Oxidative pressure, however, plays a crucial role in the initiation of extensive host-associated pathological repair (Abubakar and Dabo, 2023).

Trypanosomiasis control has been difficult because trypanocidal drugs have not improved their efficacy against the parasites due to parasite resistance, drug toxicity to the host, the high cost of existing drugs, and the slow discovery of new drugs (Ajakaiye, 2018). Failure of all control measures to manage the resurgence of the disease presents a major constraint on the development of the African continent (Büscher *et al.*, 2019). These constitute a major threat to attaining food security in several parts of sub-Saharan Africa and Nigeria. Nutraceuticals were described as products detached from foods, which are in most cases sold in medical designs not typically linked with food. The majority of nutraceuticals possess antioxidant activity, with the ability to improve health and neutralise infections (Afam *et al.*, 2021). From the literature consulted, there is a lack of comparative data on nutraceutical-trypanocide synergy in *T. evansi* infection in animals. Therefore, this study aimed to investigate the ameliorating effects of *Trypanosoma evansi* infection in Wistar rats using selected Nutraceuticals, Ethidium bromide and Ethidium chloride, on Packed Cell Volume (PVC), body temperature, and live body weight to address the problems of resistance and toxicity.

MATERIALS AND METHODS

Study Area

The study was conducted at the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITR), Kaduna, Nigeria.

Study Design

Ethical Clearance

Ethical clearance was obtained from the Ministry of Agriculture and Forestry, Kaduna State, before commencement of the experiment.

Research Animals

Forty-five (45) healthy adult male (6-8 weeks of age) Wistar rats of average weight between 120 and 150 g were purchased from the small animal/Laboratory animal colony of the Nigerian Institute for Trypanosomiasis Research (NITR).

Grouping of the Animals

The rats were randomly assigned to 9 groups. Each group contained five rats in well-ventilated plastic cages in the laboratory, de-wormed with albendazole and acclimatised to laboratory conditions for two weeks before the commencement of the experiment. Group I, positive control (uninfected, untreated), group II, negative control (Infected not treated), while III, IV, V, VI, VII, VIII and IX were the groups tested. The animals were fed with pelleted basal diet obtained from a commercial feed outlet and also water was given (*ad-libitum*) to the animals (Muhammad et al., 2024; Salisu et al., 2019; Usman et al., 2025).

Source of the Parasites

The cryopreserved parasite (*Trypanosoma evansi*) in liquid nitrogen was obtained from the Vector and Parasitology Department of the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria.

Inoculation of the Parasites

Trypanosoma evansi was inoculated into a clean, worm-free rat, which served as the donor rat. Infected blood from a donor rat with high parasitaemia was collected by tail pricking and diluted with 0.9% Normal saline. The number of parasites in the diluted blood were determined through the method described by Herbert and Lumsden (1976) and a volume of blood diluted containing approximately 1×10^5 trypanosomes/ml was injected intraperitoneally into each rat in Group II, (positive control), Group III, Group IV, Group V, Group VI, Group VII, Group VIII and Group IX using syringe and needle, while group I remained uninfected (control) (Mohammed and Shuaibu, 2018).

Preparation of the Nutraceuticals and the Trypanocides for treatment

The Nutraceuticals (Omega H3 and Glucosamine) and the trypanocides (Ethidium bromide and Ethidium chloride) were obtained from a commercial outlet in Kaduna town. One capsule of 250 mg of Glucosamine was dissolved in 10 ml of distilled water, and the same procedure was followed to prepare one capsule of Omega H3, which contains 33 vital elements at different concentrations. The solutions were administered orally. While 1 tablet of 250 mg of Ethidium bromide was dissolved in 10 ml of hot water, and 1 tablet of 250 mg of Ethidium chloride was dissolved in 10 ml of sterile distilled water according to the manufacturer's instruction and both were administered intramuscularly at the onset of parasitaemia (3 days post-infection).

Treatment of the Infected Groups

Group I: uninfected, untreated; Group II: infected, untreated; Group III rats were treated with Glucosamine at 6.43mg/animal; Group IV rats were treated with Omega H3 at 24.6mg/animal. The treatment was given daily for 7 days using feeding tubes. Group V rats were treated with Ethidium bromide at 1 mg/Animal; Group VI rats were treated with Ethidium chloride at 1 mg/Animal once using a syringe and needle. Group VII rats were synergistically treated with Ethidium bromide and Glucosamine; Group VIII rats were synergistically treated with Ethidium chloride and Omega H3; and Group IX rats were treated with both Omega H3 and

Glucosamine combined. The treatment commenced three days post-infection using the method adopted by Ajakaiye et al. (2018) and Mohammed and Shuaibu (2018).

Collection and processing/Examination of Blood Samples

Using the rapid-matching method to estimate the host's parasitemia, parasitemia in the infected groups was monitored daily. A drop of blood was collected from the tail of each rat to assess parasitaemia by the wet-mount method as described by Herbert and Lumsden (1976). The blood was placed on a clean glass slide, covered with a cover slip, and observed under the x40 objective lens of a binocular microscope. The average parasitemia in 10 fields was used to evaluate the degree of parasitemia. Likewise, blood samples were also collected using Capillary tubes to check packed cell volume (PCV) using the micro-haematocrit centrifuge technique (HCT) through the method described by Woo (1970), in which the capillary tubes were arranged in a rotor and spun at a maximum speed of 12,000 revolutions per minute, then readings were taken using a micro-haematocrit reader.

Determination of Body Temperature

The body temperature of animals in all the groups was measured daily. Each animal was gently held, and a digital thermometer was inserted 3cm into the wall of the colon-rectum. At the sound of a beep, the thermometer was withdrawn, and the values obtained were recorded immediately, as demonstrated by Mohammed and Shuaibu (2018).

Measurement of Live Body Weight

The live body weight of the rats in all groups was measured twice per week throughout the experimental period using a top-loading balance, as described by Mohammed and Shuaibu (2018).

Data Analysis

All data obtained from this research were presented as mean ± standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA), and mean values that differed at $p < 0.05$ were considered significant, as demonstrated by Duncan (1955).

RESULTS

The results of Packed Cell Volume (PCV) of all the Wistar rats' groups obtained in this experiment are presented in Figure 1. Packed Cell Volume reduced significantly ($P < 0.05$) in all the groups infected at 9 (Days Post infection), 12DPI and 15DPI compared to group I. The level of significance is more pronounced in group II, which is the infected, untreated group at 12DPI and 15DPI. However, no significant ($P > 0.05$) difference was observed in all groups infected and treated with the Nutraceuticals and the Trypanocides compared to the group infected and untreated at 3DPI, 6DPI, and 9DPI. But a significant ($P < 0.05$) difference was observed between the group II and the group I at 9DPI, 12DPI, and 15DPI, respectively.

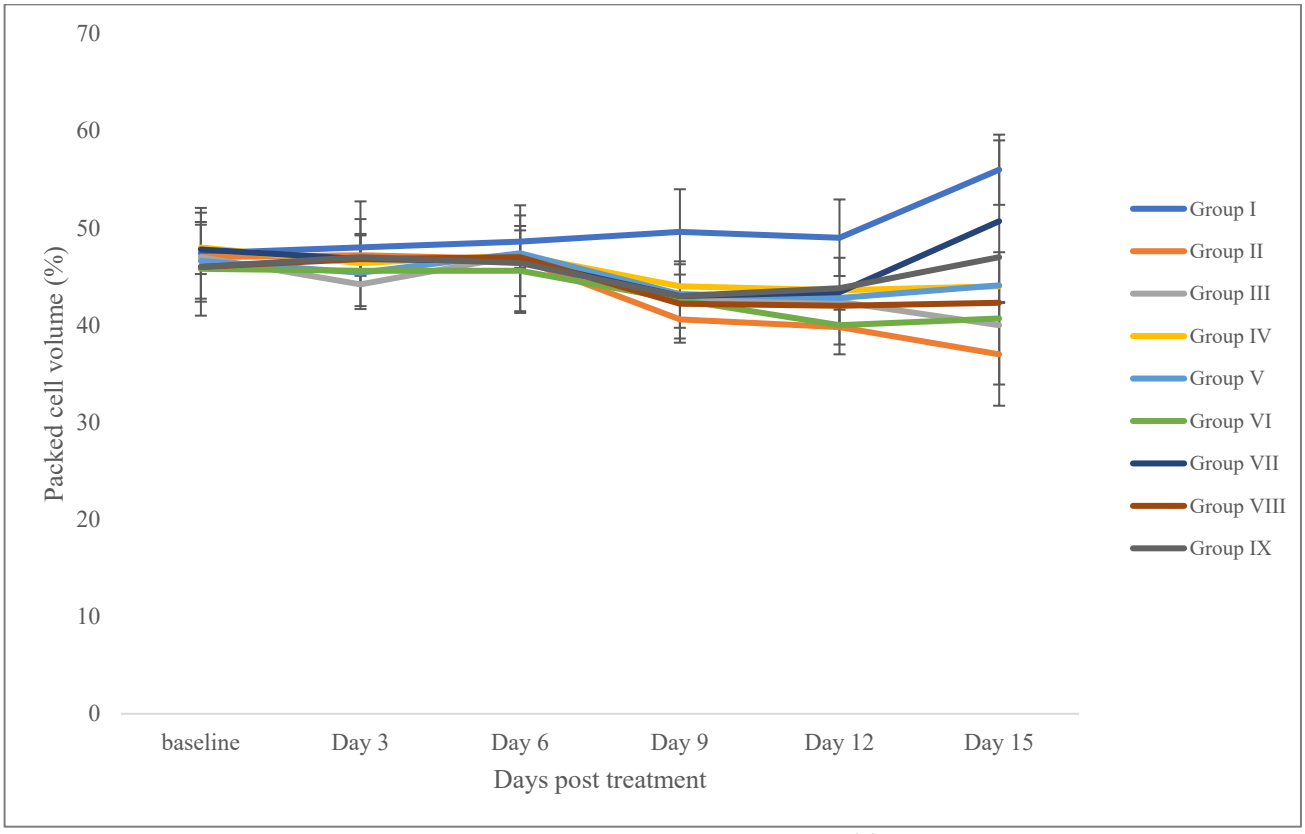

Figure 1: Packed Cell Volume (PCV) Changes in Wistar Rats Following Treatment

However, a significant ($P < 0.05$) difference in the percentage of PCV was also observed in group VII, which was synergistically treated with Ethidium bromide and Glucosamine at 15DPI, compared with other groups

infected and treated. This may be a result of the treatment administered. Furthermore, no significant ($P > 0.05$) increase was observed in any of the Wistar rat groups at 3DPI and 6DPI compared with the initial PCV percentage.

The body temperature results obtained in this study across all groups are presented in Figure 2. The body temperature increased in all infected groups compared with group I. This increase is so significant ($P < 0.05$) in group II at 9DPI, 12DPI, and 15DPI, compared with groups treated with the Nutraceuticals and Trypanocides. However, no significant increase ($P > 0.05$) was observed in the values recorded across all groups at 3DPI compared with the initial values (group I). Likewise, no significant ($P > 0.05$) increase in body temperature was observed in any of the groups treated with the Nutraceuticals compared to the groups treated with the Trypanocides at 12DPI and 15DPI, except in group VII, where body temperature declined at 12DPI compared to the groups infected and treated. In addition, there is little decline in body temperature observed in the groups synergistically treated with Nutraceuticals and Trypanocides, compared to groups treated only with Nutraceuticals or Trypanocides throughout the experiment.

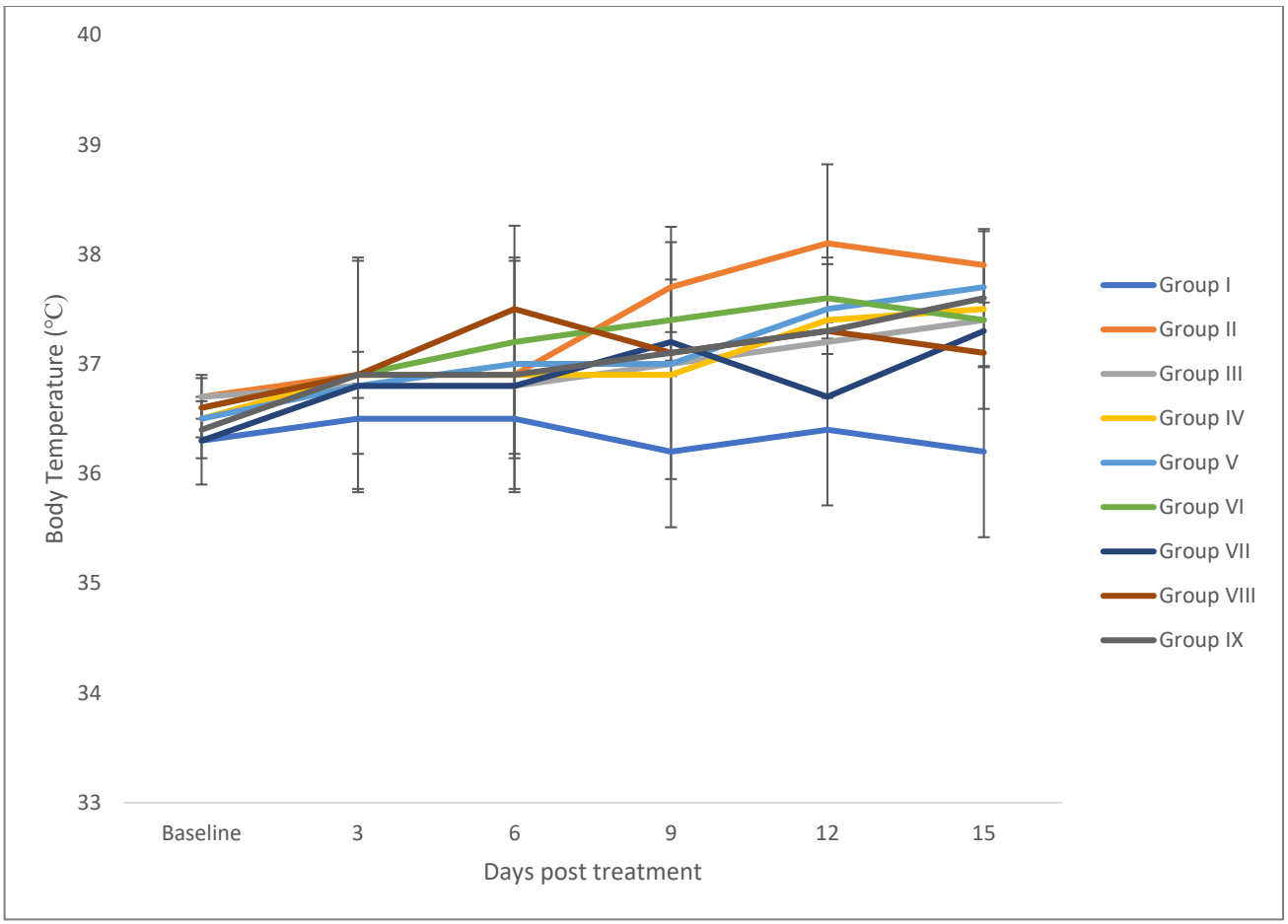

Figure 2: Changes in Body Temperature During Infection and Treatment

The results of live body weight recorded in all groups during the period of this study are shown in Figure 3. A decrease in live body weight is observed in all groups compared with the initial values (baseline). However, there is no significant ($P < 0.05$) increase in the live body weight of the rats observed in all the groups tested (infected and treated) in comparison to group II at 6DPI, 9DPI, 12DPI and 15DPI. A significant ($P < 0.05$) increase in live body weight is observed in the rats in group I (uninfected untreated) at 9DPI, 12DPI, and 15DPI, compared to group II (infected untreated). Moreover, an increase in the live body weight of rats in the groups treated with Nutraceuticals and the Trypanocides is not observed during this experimental period.

DISCUSSION

The packed cell volume (PCV) results for all Wistar rat groups infected in this experiment showed decreased values. This could probably be due to trypanosome infection in the animals. The PCV of individual animals were useful indicator of anaemia, and in trypanosome-endemic areas, endemic areas were the most typical signs of nagana in domestic animals as reported by Latif *et al.* (2019). Anaemia was recognized as the most important clinical manifestation of both animal and human Trypanosomiasis. The mechanism of anaemia due to Trypanosomiasis is complex and multifactorial, and the rate at which anaemia developed was influenced by energy intake and protein gain (Kumela and Behablom, 2021). The interplay of several factors acting either individually

or synergistically contributes to the development of haemolytic anaemia in human and animal trypanosomiasis. Most common among these factors were increased intravascular red blood cell destruction caused by the whipping action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation, and malnutrition (Kumela and Behablom, 2021)

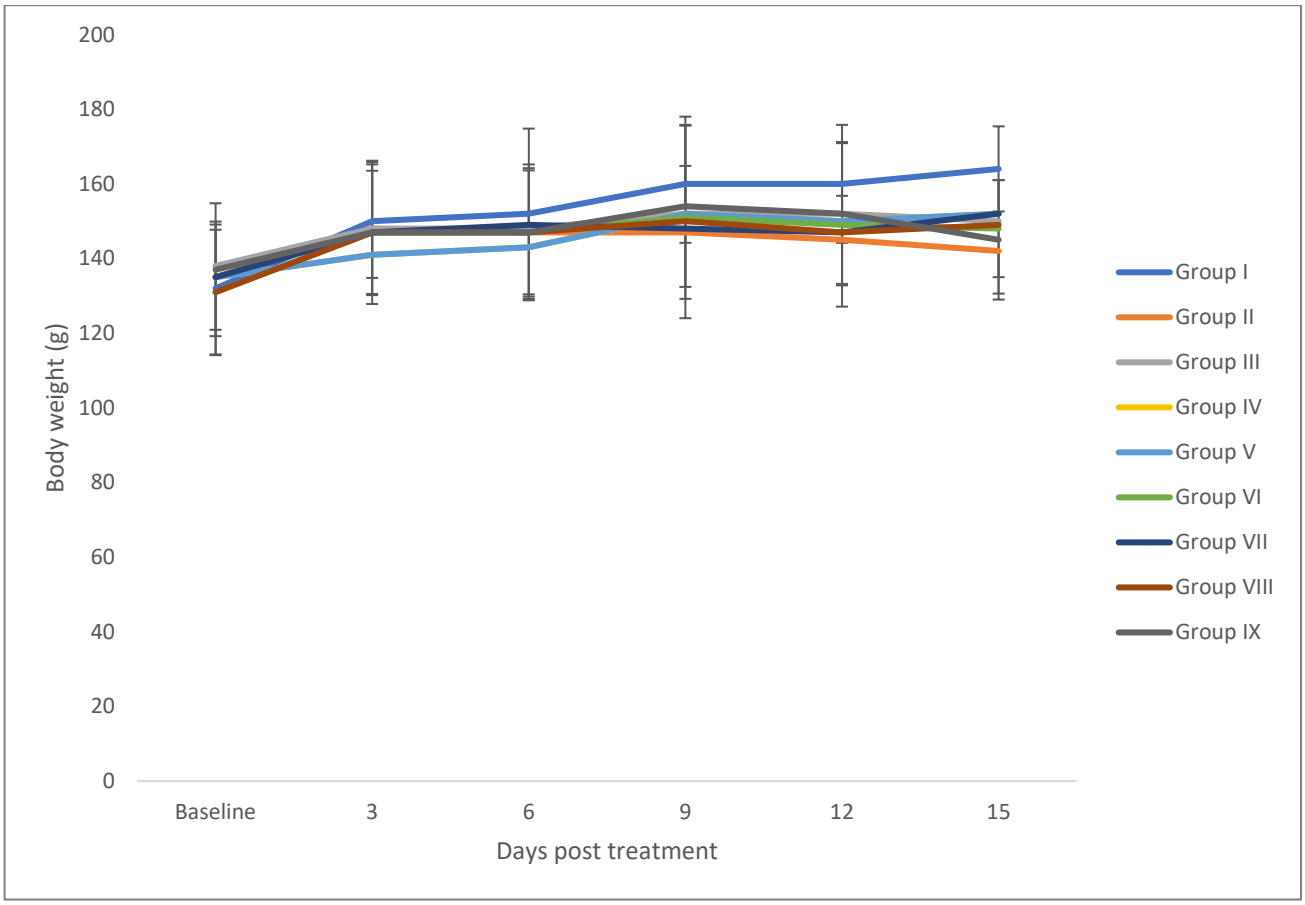

Figure 3: Live Body Weight Changes Across Treatment Groups

In this experiment, the packed cell volume observed decreased significantly ($P < 0.05$) in group II compared with group I and the treated groups, especially at 12DPI and 15DPI. The significant increase in PCV values obtained in this study, particularly in group VII at 15DPI, could be due to the synergistic effects of the treatment, in which the Trypanocide and the Nutraceutical exert their action against T. evansi, leading to a boost in PCV. The lower PCV values observed in the infected groups, especially in group II, could be due to acute haemolysis caused by growing trypanosome infection. This statement agrees with the findings reported by Meharenet and Tsegaye (2020), which revealed the significant difference in the prevalence of anaemia due to the increasing rate of Trypanosomiasis in animals.

Unlike the untreated group, the groups treated with nutraceuticals and trypanocides, as well as the groups synergistically treated, showed a significant ($P < 0.05$) increase in PCV levels compared to group II. It's suggested that the nutraceuticals and the trypanocides might have enhanced the effect of *Trypanosoma evansi* infection on PCV in Wistar rats due to the treatment administered.

Experimental murine models using gene-specific-deficient animals have been crucial for unravelling the mechanisms underlying trypanosomiasis-associated pathogenicity, particularly anaemia (Stijlemans *et al.*, 2018). In general, anaemia occurs during all stages of a typical African trypanosome infection and is divided into distinct phases. The phases were (i) an early or acute stage whereby following or coinciding peak parasitaemia clearance, there is occurrence of a drastic drop in red blood cells (RBCs) numbers (i.e., acute or consumptive anaemia) which was followed by a recovery phase and (ii) a more late or chronic phase coinciding with progressive anaemia development (Stijlemans *et al.*, 2018). Different factors might account for the occurrence of acute anaemia in the murine trypanosome infection model. Hence, the acute stage of anaemia could be due to a "natural" reaction of the host following infection as well as to parasite-derived factors resulting in a rapid drop in RBC numbers due to the developmental process of trypanosomes infection confined to intravascular blood and caused haemolysis of red blood cells (Meharenet and Tsegaye (2020). A study of the antioxidant status of *T. evansi* infection in rats reported by Abubakar and Dabo (2023) indicated that all rats developed fulminating parasitemia.

The increase in body temperature (fever) is a typical symptom of Trypanosomiasis, which reflects the response to successive waves of parasitaemia (Ajakaiye *et al.*, 2018). The consistent increase in body temperature, as it was

observed mostly in all the groups infected and treated with nutraceuticals and the trypanocides at 9DPI, 12DPI and 15DPI and also the infected untreated group, was higher than the rest at 12DPI and 15DPI when compared to group I (uninfected untreated), may be as a result of infection due to proliferating trypanosomes that leads to pyrexia. A study conducted by Jia-Yi *et al.* (2025) indicated that trypanosomes exhibited high thermo-sensitivity to elevated temperatures, accompanied by apoptosis-like events, mitochondrial damage and oxidative stress. However, infection with *T. evansi*, like that of many other pathogenic trypanosomes, elicits febrile responses in animals. A few experiments mimicking fever have been carried out on trypanosomes using heat shock, revealing a thermosuppressive effect associated with fever (Ooi *et al.*, 2020).

The thermal increment observed in the infected untreated group, particularly at 12DPI and 15DPI, may be due to the temperature setting point in the hypothalamus, which changes under the influence of pyrogenic stimuli released during infection. It has been reported that elevated temperatures can induce oxidative stress within cells, manifested by increased production of reactive oxygen species (ROS), which may disrupt intracellular homeostasis (Wargnies *et al.*, 2021). An increase in body temperature leads to pyrexia, one of the major clinical signs of Trypanosomiasis in mammals. In contrast, pyrexia was reduced in the infected animals treated with nutraceuticals and the trypanocides. This suggested that administered Trypanocides and Nutraceuticals suppressed the pyrexia-triggering mechanism of proliferating parasites. Though resistance and toxicity of Ethidium Compounds in animals have been reported by Kasozi et al. (2022), strategic treatment produced attractive results, with cattle protected for up to 6 months, as reported by Latif *et al.* (2019). The majority of the functions of Nutraceutcals were similar to those of Antioxidants. A report by Afam *et al.* (2021) indicated that antioxidants delay the onset of free radical formation by donating a hydrogen atom (electron/proton) or by chelating metals involved in ROS formation. A report by Muhammed and Shuaibu (2018) indicated that the administration of zinc and selenium thwarted the reduction of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in the liver and kidney in animals infected with trypanosomes.

The results of live body weight obtained from this study show a significant increase ($P < 0.05$) in all groups: infected/treated, uninfected untreated, and infected untreated, as seen from the Baseline (initial values recorded). This might occur as a result of good nutrition given to the animals during the experimental period. However, a significant reduction in the live body weight of animals in group II was also observed at 12 and 15 days post-infection (12DPI and 15DPI) compared to group I. The highly significant decrease in live body weight observed in the infected, untreated group (II) indicated the severity of the infection, as reported by Muhammed and Shuaibu (2018). This phenomenon may be a result of parasite-induced anorexia. However, weight loss recorded in the groups of animalss treated with nutraceuticals and trypanocides, as well as in the group synergistically treated with nutraceuticals and trypanocides, was not significant ($P > 0.05$) compared to the uninfected, untreated group. This could probably be due to nutraceuticals and Ethidium exerting their potential to prevent loss of live body weight. This statement agrees with the report by Ajakaiye et al. (2018), which stated that the potential use of antioxidants in combination is possibly more efficient and crucial than single antioxidant treatments.

CONCLUSION

The Nurecticals and Ethidium compounds used in this study have contributed to suppressing the effects of *Trypanosoma evansi* infection on Wistar rats' packed cell volume (PCV), body temperature, and live body weight, as seen in the results obtained. Both the Nuraceutcals and Ethidium compounds prevented the diminution of live body weight and suppressed anaemia and high body temperature, respectively. Therefore, from the outcomes of this study, it is concluded that the Nutraceuticals (Omega H3 and Glucosamine) have demonstrated the ability to ameliorate the effects of Trypanosoma evansi infection in experimental Wistar rats, as evidenced by packed cell volume (PCV), body temperature, and live bodyweight.

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