

Full Length Research Paper

Effects of combination therapy of *Bridelia ferruginea* stem bark and diminazene aceturate on testicular and haematological changes in *Trypanosoma brucei* infected male rats

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The study was done to assess the ameliorative effects of co-administration of methanol extract of *Bridelia ferruginea* stem bark and diminazene aceturate on trypanosomosis induced testicular and haematological changes in infected male rat. Twenty five male rats were used in the study and assigned into five groups (n=5). *Trypanosoma brucei* was inoculated into Groups 1, 2, 3 and 4 rats except Group 5. Diminazene aceturate (7 mg/kg) was administered to group 1. Methanol extract of *B. ferruginea* (20 mg/kg) was administered to Group 2. Diaminazine aceturate (7 mg/kg) and *B. Ferruginea* (20 mg/kg) were administered to Group 3. The infected and untreated rats constituted Group 4. The normal control was Group 5. Parameters assessed were weight of the testicles, testicular sperm and epididymal sperm counts, packed cell volume, haemoglobin concentration and red blood and white blood cell counts. At the end of the experiment, there were significant increases in the testicular sperm (56.40 ± 4.55) and epididymal sperm counts (24.34 ± 0.5) of group 3 compared to Group 2 – testicular sperm reserve (51.34 ± 6.19), epididymal sperm reserve (19.38 ± 4.14) and Group 1 – testicular sperm reserve (54.01 ± 6.92), epididymal sperm reserve (20 ± 5.79). The WBC was significantly higher in infected untreated (13.70 ± 5.15) compared to Groups 1 (10.96 ± 1.21), 2 (10.36 ± 2.09) and 3 (10.55 ± 0.49). The results above showed that co-administration of diminazene aceturate and *B. ferruginea* extract produced optimum ameliorative effect.

Key words: *Bridelia ferruginea*, diminazene aceturate, testicular changes, trypanosomosis.

INTRODUCTION

Trypanosomosis is a clinical disease caused by the protozoa, *Trypanosoma*. *Glossina* species transmit the disease. Trypanosomosis is common in the tropics. Two

groups of trypanosomes affect both man and animals. They are *Trypanosoma congolense* and *Trypanosoma vivax* (haematic group) and *Trypanosoma brucei*,

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Trypanosoma evansi, *Trypanosoma rhodesiense*, *Trypanosoma gambiense* and *Trypanosoma equiperdum* (tissue invading group) (Anosa, 1988).

Over 66 million people in 37 countries of sub-Saharan Africa suffer from sleeping sickness caused by trypanosomes. If not treated, the resultant effect is death. The species commonly affecting livestock are *T. vivax*, *T. congolense* and *T. brucei* causing sleeping sickness in animals (Steverding, 2008). Trypanosomosis in livestock causes drastic weight loss, decrease in milk yield, decrease in carcass quality and inability to work due to poor feed conversion efficiency (Itard, 1989; Abenga et al., 2002).

It has been reported that trypanosomosis reduces reproductive function in male animals. It causes decrease in libido, delayed maturity, degeneration of the testicles leading to production of poor quality spermatozoa in male animals (Sekoni, 1994).

Bridelia ferruginea belongs to the family, Euphorbiaceae. It thrives in savannah areas. In Hausa, Yoruba and Igbo, it is called kizni, iralodan and ola, respectively. It has been reported to have purgative effect (Cimanga et al., 1999), reduce microbial proliferation (Adeoye et al., 1988) and combat inflammation (Olajide et al., 1999). It ameliorates different types of toxicities in the body of animals (Atawodi, 2005). There is lack of information on *B. ferruginea* combating the toxic effect of trypanosomosis on the testis and blood.

Diminazene aceturate is a common trypanocide used in the management of animal trypanosomosis in Africa (Olajide et al., 1999); chemotherapy has been associated with resistance, relapse, cross resistance and toxicity of drugs to animals. Despite the advances in various aspects of trypanosomosis therapy, therapy has not been able to protect the testicular and haematological changes in treated animals; hence, there is the need to develop a therapy regimen that will solve the problems of testicular and haematological disorders associated with trypanosomosis.

MATERIALS AND METHODS

Animals

Twenty-five male albino rats aged between 12-14 weeks and weighing between 180- 220 g were used for this study. The animals were obtained from the laboratory of animal unit of Veterinary Physiology and Pharmacology Department, University of Nigeria, Nsukka. Feed and water were given to them *ad-libitum*. The rats were acclimatized for two weeks before the commencement of the study. The ethical guidelines on handling of animals for experiments as directed by Ward and Elsea (1997) and Zimmermann (1983) were followed.

Ethical approval

Rules guiding the use of animals for experiments were in

accordance with the university's animal welfare policies and were approved (ref no. 20170819) by the ethical committee of University of Nigeria, Nsukka.

TRYPANOSOMA ISOLATES

T. brucei used in this study was obtained from the Department of Veterinary Parasitology, University of Nigeria, Nsukka. The parasite was first inoculated in swine and subsequently maintained by serial passage in laboratory mice.

Experimental design

Twenty five male rats weighing 180-220 g were assigned into five groups of five rats each. Groups 1, 2, 3 and 4 were experimentally infected with trypanosomosis, while Group 5 was the normal control. The treatment was carried out as follows:

Group 1: This group was infected and diaminazine aceturate was administered at the dose of 7 mg/kg for 72 h post infection and two weeks after.

Group 2: This group was infected and methanol extract of *B. ferruginea* was administered at the dose of 20 mg/kg once a day for two weeks.

Group 3: This group was infected and diaminazine aceturate + *B. ferruginea* were administered at the doses of 7 and 20 mg/kg, respectively. Diaminazine aceturate was administered on day 3 post infection and repeated after two weeks while *B. ferruginea* was administered once daily for two weeks.

Group 4: This group was infected and not treated.

Group 5: This group was the normal control.

Plant extraction

The fresh plant was obtained from Department of Zoology, University of Nigeria, Nsukka. *B. ferruginea* was spread under a shade for drying. The dried *B. ferruginea* was ground using a grinding machine into powdery form. Cold extraction method with 80% methanol was used to extract the *B. ferruginea* material. The extract was filtered using Watt man no 1 filter paper. The filtrate was concentrated in a hot air oven at 36°C and stored at 4°C before it was finally used.

Evaluation of the weight of the testicles

On day 28 of the study, the rats were euthanized and weighing balance was used to weigh their testicles.

Evaluation of testicular and epididymal sperm reserves

The testicles and tails of the two epididymis were crushed separately in a ceramic mortar, mixed with normal saline (10 ml) and poured into a sieve. The filtrate (0.1 ml) was made up to 1 ml with 0.9 ml of white blood cells diluting fluid. This was then used to charge the improved Neubauer chamber and examined with a microscope at X40 magnification. Sperm cells were counted in 169 squares. This was then used to estimate the number of sperm cells in the original 10 ml of sperm solution (Amann, 1986).

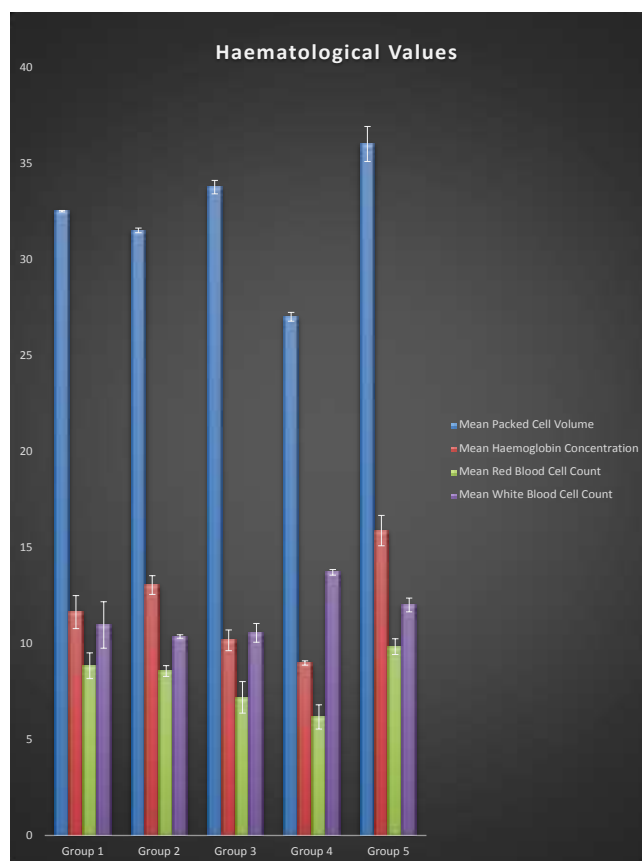


Figure 1. Mean heamatological values.

Evaluation of packed cell volume, red blood and white blood cells

Microhaematocrit method (Coles, 1986) was used to evaluate packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC).

Evaluation of haemoglobin concentration

The cyanomethaemoglobin method (Tiez, 1976) was used to evaluate haemoglobin concentration

Statistical analysis

Data of the study were statistically analysed using one way analysis of variance (ANOVA). The variant means were separated using Duncan Multiple Range Test. Level of significant was accepted at $p < 0.05$.

RESULTS

The clinical signs observed in the infected groups include pale mucous membrane, dullness, inappetence, and rough hair coat. The parasites were detected in the blood of all the infected albino male rats 3 days post infection,

hence 100% parasitaemia. This showed that trypanosomosis was successfully induced. The testicular weight of Groups 1, 3, 4 and 5 increased significantly ($p < 0.05$) compared to Group 2 and did not differ from one another. The testicular and epididymal sperm reserves of Groups 3 and 5 increased significantly compared to Groups 1, 2 and 4 and did not differ from each other. The packed cell volume (PCV) of Group 3 increased significantly compared to Groups 1, 2 and 4. The haemoglobin concentration of Group 2 increased significantly compared to Groups 1, 3 and 4. The RBC of Groups 1 and 2 increased significantly compared to Groups 3 and 4 but did not differ from each other. The WBC of Groups 1, 2 and 3 decreased significantly compared to Group 4 but did not differ from one another (Table 1 and Figure 1).

DISCUSSION

The observed decreases in testicular sperm reserve and epididymal sperm reserve in group 4 agreed with earlier reports (Moulton and Sollod, 1976). The significant higher sperm counts of co-administration of diminazene

Table 1. Epididymal sperm reserve, testicular sperm reserve and testicular weight (Mean $\pm$ SEM).

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
ESR (10^6)	20.51 $\pm$ 5.79 ^a	19.38 $\pm$ 4.14 ^a	24.34 $\pm$ 0.55 ^b	13.59 $\pm$ 2.66 ^a	26 $\pm$ 1.82 ^b
TSR (10^6)	54.01 $\pm$ 6.92 ^a	51.34 $\pm$ 6.19 ^a	56.40 $\pm$ 4.55 ^b	49.54 $\pm$ 4.61 ^a	60.48 $\pm$ 1.47 ^b
TW (grams)	2.80 $\pm$ 0.00 ^b	2.60 $\pm$ 0.00 ^a	2.80 $\pm$ 0.00 ^b	2.80 $\pm$ 0.00 ^b	2.80 $\pm$ 0.00 ^b

aceturate and extract may be due to synergistic drug-extract interaction as a result of the antioxidant activity of *B. ferruginea* extract (Borek, 2001).

Oxidative stress and lipid peroxidation is known to play a major role in the etiology of the defective reduction of sperm count in trypanosome infected males rats (Boonsorn et al., 2010). *B. ferruginea* contains antioxidant which scavenges reactive oxygen species and inhibits lipid peroxides in the body (Borek, 2001; Ekanem et al., 2008). The significant decrease in PCV and Hb of Group 4 may be due to the destruction of RBC membrane proteins (Oyedemi et al., 2011). Following extract administration, the level of RBC was appreciably improved especially in group 3. This gives an indication that *B. ferruginea* stem bark extract stimulates the formation or secretion of erythropoietin in the stem cells of the animals. It may also be attributed to the ability of *B. ferruginea* to lower lipid peroxidation level that causes hemolysis of erythrocytes (Ashafa et al., 2009). The significant increase in WBC of infected control compared to Groups 1, 2 and 3 may be due to sustained oxidative stress in the infected group throughout the study period. Trypanosomosis suppresses the immune system by damaging WBC due to sustained oxidative stress (Oyedemi et al., 2012).

Conclusion

In conclusion, the combined therapy of *B. ferruginea* extract and diminazene aceturate produced optimum protective potentials on testicular and hematological changes in trypanosome infected male rats. Supplementation of *B. ferruginea* in feed of animals in addition to diminazene aceturate will be an effective way to reduce the toxic effects of trypanosomes on the reproductive system and hematology of males, hence improving fertility in infected male animals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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