



Effect of Intramuscular Administration of Diminazene di-aceturate, Isometamidium chloride and Homidium chloride on Organ Damage and Packed Cell Volume of Wistar Rats Infected with *Trypanosoma brucei brucei* (Federe strain)

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ABSTRACT

African trypanosomiasis is a menace in sub-Saharan Africa, but more threatening is the avalanche of reports from the field of the case of drug resistance. Therefore, this study was carried out to investigate the effect of intramuscular administration of trypanocides on organ damage and packed cell volume profile of Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain). Twenty-five adult Wistar rats weighing between 200 and 240 g were randomly divided into five groups. Group A uninfected untreated, group B were infected with 1×10^6 of the parasites. Groups C, D, and E were administered the same dose as in group B, and were treated with 3.5 mg/kg b.w. of Diminazene di-aceturate, 1 mg/kg b.w. of Isometamidium chloride, and 1 mg/kg b.w. of Homidium chloride, respectively. There was a highly significant increase in organ weight of group B compared to the other groups. However, the increase in the heart and kidney were not significant in group A compared to the treated groups, whereas for the reticulo-endothelial organs of the liver and spleen, the increment was lowest in the group treated with Isometamidium chloride, followed by the groups treated with Diminazene di-aceturate and Homidium chloride at the same level of significance when compared to group A. The final values of PCV in groups B and E showed a highly significant decrease compared to their final values. Whereas, the same value significantly decreased in group C. However, there was no significant difference in the final value of the PCV in group treated with Isometamidium chloride compared to its initial value. The administration of the trypanocides reduced, and modulated *T. brucei brucei* – induced organ damage and increased the PCV of the rats in the treated groups. In conclusion, we recommend their continued use according to the manufacturer's instructions in order to avoid iatrogenic factors that could reduce their potency and elicit parasite drug resistance.

Key words:

Diminazene di-aceturate,
Isometamidium chloride,
Homidium chloride,
hematocrit, *Trypanosoma brucei brucei* (Federe strain), *Wistar rats*.

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1. INTRODUCTION

The African trypanosomiasis is a parasitic disease caused by *Trypanosoma* spp. which affects both man and animals in 38 countries in the sub-Saharan region of the continent. In Humans, the disease is known as Human African Trypanosomiasis (HAT) or Sleeping

sickness caused by *Trypanosoma b. gambiense* and *Trypanosoma b. rhodensiense*; while Animal African Trypanosomiasis (AAT) or *Nagana/Sammoré* is majorly caused by *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei* (Maré, 2004).

AAT is recognized as both a serious health problem and a severe constraint to Africa's socio-economic development, which every year claims the lives of over 3 million livestock, causing huge economic losses and untold human misery (Dede *et al.*, 2007). It has been documented that Nigeria has an estimated population of 19.5 million cattle, 72.5 million goats, 41.3 million sheep, 7.1 million pigs, 28,000 camels and 974, 499 donkeys (National Agriculture Sample Survey, Nigeria, 2010). However, the majority of this livestock are at risk of AAT because they are located in tsetse-infested regions.

In Animals, the cardinal clinical signs observed in trypanosomiasis are: anemia, undulating fever, edema, loss of condition and abortion may be seen in the females and infertility in males. To curb this trend, two control and treatment strategies were developed, namely: vector and disease control. The latter, which is carried out through chemoprophylaxis and chemotherapy, has been in practice for decades, but the rate at which trypanosomes develop resistance to the trypanocides in use has become a source of worry (Ezeokonkwo *et al.*, 2007; Grace *et al.*, 2009). Several reasons have been advanced for this phenomenon among them are: cross-resistance due to the combined application of Isometamidium and Homidium chlorides, antigenic variation by the invading parasites, iatrogenic factors such as: under/overdosage of drugs during treatment, use of fake/sub-standard drugs, application of drugs by unauthorized persons, and outright ignorance of the knowledge of the drug to use and how to use them (Sinyangwe *et al.*, 2004; Anene *et al.*, 2006; Sonibare *et al.*, 2016). A gold standard in the evaluation of the efficacy of trypanocides is to evaluate the cellular organs of predilection of the parasites in the host- vis-à-vis the reticulo-endothelial system. Similarly, we evaluated the effect of the parasite on the host hematocrit as a generally accepted measure of the health status of animals. Therefore, the objective is to ascertain the efficacy or not of these trypanocides using the PCV and the organs of predilection as a yardstick.

2. MATERIALS AND METHODS

2.1. Experimental area

The study was conducted at the Nigerian Institute for Trypanosomiasis and (Onchocerciasis) Research (NITR). The Institute is located in Kaduna North Local Government Area of Kaduna State. Kaduna State is located at latitude 10° 30' 00'' N and longitude 7° 25' 50'' E of Nigeria.

2.2. Experimental animals

The study was conducted under protocols in the guidelines established by the "Guide for the Care and use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Science, Washington D.C. 1996). Twenty-five adult Wistar rats weighing between 200 and 240 g were obtained from the rat colony of NITR. Before the commencement of the experiment, the animals were screened for the presence of blood parasites using standard techniques (Schalm *et al.*, 1975). After two weeks of acclimatization, the animals were randomly divided into five groups of five rats each, and kept in well ventilated standard plastic cages 15×22×10 m³ as follows:

Group A: Uninfected, untreated.

Group B: Infected, but not treated.

Group C: Infected, and treated with Trypadim® (Diminazene di-aceturate).

Group D: Infected, and treated with Trypamidium® (Isometamidium chloride).

Group E: Infected, and treated with Novidium® (Homidium chloride).

The animals were fed with a pelleted basal diet obtained from a commercial feed outlet (Vital Feeds Plc., Kaduna, Nigeria), and the bromatological content of the feed was made up of 54% carbohydrate, 20% protein, 2% minerals, 10% fiber, 1% vitamin, and 13% fat. Water was given *ad libitum*.

2.3. Trypanosome infection Inoculation of rats with the parasite

The parasite *T. brucei brucei* (Federe strain) was obtained from the cryogen kept in the Vector and Parasitology Research Department of NITR, Kaduna, Nigeria. The parasite was inoculated into a clean rat which served as donor rat. Infected blood from a donor rat at peak parasitemia that is 4 days post-infection (DPI) was collected by means of tail pricking and diluted with cold physiological saline. The number of parasite in the diluted blood was determined through the method described by Herbert and Lumsden (1976), and a volume containing approximately 1×10⁶ parasites was injected intraperitoneally into each rat in the infected groups.

2.4. Drugs administered

All drugs used were obtained from a commercial outfit in Kaduna, Nigeria. The drugs were products of the same Company (Merial, 29, Avenue Tony Garnier, 69007 Lyon, France) which were all administered as a single dose from the onset of parasitemia. Briefly, the

drugs were dissolved and reconstituted in distilled water according to manufacturer's instruction, and given intramuscularly in the following concentrations: Diminazene di-aceturate at 3.5 mg/kg/body weight (bw); Isometamidium chloride at 1.0 mg/kg/bw and Homidium chloride at 1.0 mg/kg/bw, respectively.

2.5. Blood sample and organ collection

Tail blood was collected daily for monitoring of parasitemia as described by Herbert and Lumsden (1976). The micro-hematocrit centrifugation technique (HCT) was used in determining the PCV from blood samples collected with heparinized tubes. At 28 days post-infection, the rats were sacrificed by humane decapitation prior anesthesia with sterile cotton impregnated with chloroform, and organs were harvested and preserved in 10 % buffered formalin for further analysis

2.6. Statistical analysis

All the data obtained for the organ weights are presented as mean \pm SEM. Data were analyzed by the one-way analysis of variance (ANOVA) and the significance of differences between mean values computed for particular levels of experimental factors was determined by Duncan (1955) *post-hoc* test, and means that differ at $p < 0.05$ were considered significant. PCV was analyzed using the paired *t-test*. All data were analyzed with the SPSS statistical package version 19.

3. RESULTS

3.1. Organ-weight ratio

Table 1 shows the result of organ-body weight ratio at the end of the experimental period. One notable feature is that there was a significant increase ($p < 0.05$) of the reticulo-endothelial organs and the heart in group B compared to all the other experimental groups. A common trend of significant increase was recorded for the heart and kidney on the one hand, while a similar trend occurred in the case of the spleen and the liver on the other hand. The heart and the kidney did not show any significant ($p > 0.05$) difference between treated groups, but the converse was the case for the values recorded for the spleen and the liver as there was a significant decrease ($p < 0.05$) in the values recorded for these organs in the Isometamidium chloride treated group compared to the other trypanocides.

3.2. Packed cell volume (PCV)

The result of the initial and final PCV of the experimental animals is as shown in Table 2. There was a significant decrease ($p < 0.05$) in the final value of the PCV in group C when compared to its initial counterparts. Whereas, groups B and E shows a highly significant ($p < 0.01$) decrease in the final values of the hematocrit compared to their initial values, respectively. However, no significant difference ($p > 0.05$) was observed in the initial values of this parameter in groups A and E when compared to their final values.

Table (1): Organ-body weight ratio of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with Trypanocides (Means \pm SEM, n = 5).

Group	Uninfected untreated	Infected not treated	Infected and treated with Trypadim	Infected and treated with Trypamidium	Infected and treated with Novidium
Heart	1.56 \pm 0.05 ^b	3.44 \pm 0.22 ^a	1.87 \pm 0.08 ^b	1.66 \pm 0.04 ^b	1.78 \pm 0.07 ^b
Liver	3.15 \pm 0.04 ^c	5.10 \pm 0.10 ^a	3.38 \pm 0.07 ^b	3.18 \pm 0.03 ^c	3.54 \pm 0.08 ^b
Spleen	0.63 \pm 0.03 ^d	2.28 \pm 0.08 ^a	1.65 \pm 0.11 ^b	1.33 \pm 0.12 ^c	1.85 \pm 0.02 ^b
Kidney	0.77 \pm 0.01 ^b	1.27 \pm 0.05 ^a	0.77 \pm 0.03 ^b	0.77 \pm 0.02 ^b	0.81 \pm 0.02 ^b

Mean values with different superscripts in the same row are significantly different ($p < 0.05$) with measurement ratio at ($\times 10^{-2}$)

Table (2): Initial and Final PCV of Wistar rats infected with *T. brucei brucei* (Federe strain) and treated with Diminazene di-aceturate, Isometamidium chloride, and Homidium chloride during the Experimental period.

Groups	Initial PCV	Final PCV	<i>t</i>	p-value
A	48.80 ± 1.38	49.10 ± 0.78	-0.79	0.862
B	49.00 ± 1.00	28.80 ± 0.84	22.647	< 0.001**
C	48.90 ± 0.89	45.70 ± 1.27	2.709	0.024*
D	49.10 ± 1.08	47.50 ± 1.54	0.787	0.452
E	48.80 ± 1.05	41.90 ± 1.12	3.455	0.007**

* = significant difference exists at $p \leq 0.05$; ** = significant difference exists at $p \leq 0.01$

4. DISCUSSION

The increase in the size of the organs observed in this experiment and documented in Table 1 has consistently been reported as an emblematic sign of trypanosome infection in animals. Similar reports were documented by Morrison (1978) and Umar *et al.* (2007) which noted hypertrophy and hyperplasia of the reticulo-endothelial organs of the liver, spleen, and kidney. The microorganism of the *T. brucei brucei* subgroup, unlike other trypanosomes, is found in the body organs (Losos and Ikede 1972; Anosa, 1983). It is this tissue localization and the subsequent multiplication at the heart, kidney, liver, and the spleen of the infected groups that leads to inflammatory cell infiltration and the subsequent tissue damage. This enlargement is presumably due to membrane damage caused by the huge number of free radicals and other oxidative species being generated by the *Trypanosoma brucei brucei* infection through the activation of the host Ab and the invading parasite Ag reaction during infection (Morrison *et al.*, 1978). It is well documented that during normal body metabolism, free radicals also known as "reactive oxygen species", which are injurious to the body are generated, but they are quickly neutralized through the internal defense mechanism by the endogenous supply of anti-oxidants to avert the raging danger. However, during stress or disease invasion, the number of free radicals generated far outweighs the number of endogenous anti-oxidants produced resulting in somatic necrosis which could only be averted through the exogenous supply of the anti-oxidants or elimination of the etiology of the invading disease-causing organism (Umar *et al.*, 1999,

2000). In this experiment, the administration of trypanocides served the purpose of the latter.

Furthermore, we observed generalized pallor of the organs measured in all test groups, and in particular, there was an enlargement of adrenal glands contiguous to the kidney, congestion of the liver, and the spleen was swollen, frail and purple in group B; accompanied by general body weight loss. Similar findings have been documented in the studies carried out by Igbokwe and Nwosu (1997); Umar *et al.* (2007, 2010); Ibrahim *et al.* (2010) and Kobo *et al.* (2014). However, the heart was seen to be thickened and swollen, suggesting accommodation due to reduced volemia as a result of the parasite hematophagous activity causing anemia. The infected and treated groups showed that all the drugs tested in the study demonstrated different levels in effectively reducing the degree of heart, kidney, spleen, and liver invasion by the trypanosomes, notable report in this direction was documented in the work of Ogunbanwo *et al.* (2001) which noted that anti-protozoal drugs could reduce the degree of tissue invasion and inflammatory reactions associated with trypanosomes.

The result of the initial and final hematocrit is as shown in Table 2. PCV is an indirect indication of the existence or not of anemia, and it has been established that the measurement of anemia gives a reliable indication of the disease status and productive performance of trypanosome-infected animals (Ekanem *et al.*, 2005; 2006). In this study, a significant decrease was observed in the PCV of group C, whereas, a highly significant decrease was recorded in groups B and E when the final PCV were compared to their initial values. A similar outcome was reported in the

experiment involving trypanosomal challenge in rats carried out by Igbokwe and Nwosu (1997) which suggested that observed anemia may be as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host.

Trypanosoma brucei brucei is a tissue parasite which induces anemia in infected rabbits as previously reported by Mwangi *et al.* (1995), resulting in the significant reduction in PCV and hemoglobin concentrations in their work. The low PCV observed in groups C, B and E may be as a result of acute hemolysis due to growing infection. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione and cleavage of the sialic acid on the surface of the red blood cell (Igbokwe *et al.*, 1994; 1996; Taiwo *et al.*, 2003; Akanji *et al.*, 2009). The severity of anemia usually reflects the intensity and duration of parasitemia. Several reports (Ogunsanmi and Taiwo, 2001; Umar *et al.*, 2007; Ekanem *et al.*, 2008; Saleh *et al.*, 2009) have also ascribed acute anemia in trypanosomiasis to proliferating parasites.

However, the significant, highly significant and non-significant decreases observed in the PCV values recorded in treated groups C, E and D, respectively is a pointer to the different pharmacokinetics of the trypanocides used, and the mechanism of action/response of the invading parasite to the drugs given rise to the recorded output. Results showed that the trypanocides improved blood components possibly by depletion of proliferating parasites, as a result, there was a positive influence on the PCV and the state of anemia in the infected treated groups, a similar report was documented by Orhue *et al.* (2005) and Ekanem and Yusuf (2008). This assertion is evidenced by the superior efficacy of isometamidium over the other trypanocides in the present study.

5. Conclusion

In conclusion, our result suggests the possibility of an evading mechanism of the trypanocides particularly, Homidium and Diminazene di-aceturate by the invading parasites which could probably be through the intractable antigenic variation pathway. Therefore, we recommend the combined therapy of Isometamidium chloride and Diminazene di-aeturate or Isometamidium and Homidium chloride in order to make available their synergistic potential in combating the menace of African trypanosomiasis and tackling the

intractable and evasive mechanism of the invading parasites.

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