Therapeutic efficacy of isometamidium chloride in the treatment of *Trypanosoma congolense* infection in Sokoto Red bucks

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**ABSTRACT**

**Introduction:** Treatment of animal trypanosomiasis using isometamidium chloride (ISM) is largely done with 1% solution however, 2% solution has been found to be more effective. In this study, therapeutic efficacy of ISM drug concentrations was studied in *Trypanosoma congolense* infected Sokoto Red Bucks (SRB). The aim of the study was to determine the efficacy of the curative (1%) and prophylactic (2%) concentrations of ISM in the treatment of experimentally infected SRB with *Trypanosoma congolense*.  

**Methods:** Twelve SRB were divided into three groups of four animals each: Group I (1% ISM treated), Group II (2% ISM treated) at the dose rate of 0.5 mg/kg body weight intramuscularly and Group III (uninfected and untreated-Control). Groups I and II bucks were each inoculated intravenously with approximately $1 \times 10^6$ *T. congolense*. Clinical signs, rectal temperature, body weight, packed cell volume (PCV), total white blood cell count (TWBCC) were monitored. Wet-mount and micro haematocrit centrifugation technique (HCT) were used to monitor the parasitaemia post-infection and post-treatment. Seven days post-treatment the blood from the treated groups were sub-inoculated into mice.  

**Results:** Group I had relapse of the infection two weeks post-treatment while no relapse of the infection was observed in the Group II till five weeks post-treatment. Significant ($p < 0.05$) changes in the PCV, WBCC and body weight were properly recorded as well as the clinical signs and the body weight.  

**Significance:** The 2% ISM confers better and longer cure than the 1% ISM in treating trypanosomosis in goat.

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**Introduction**

Trypanosomiasis is a highly debilitating and haem-parasitic disease of both animals and humans, caused by protozoan parasites of the genus *Trypanosoma*. They are mainly cyclically transmitted by *Glossina* spp which are found worldwide (Kuzoe and Schofield, 2004). In Nigeria, animal trypanosomiasis still constitutes a major obstacle against food security in spite of previous attempts towards chemotherapeutic and tsetse control (Abenga et al., 2004). Isometamidium chloride is the main drug that is used for curative and prophylaxis against trypanosome infections in livestock in Africa. The drug is usually administered to trypanosome infected animals by deep intramuscular (IM) injection as 1% or 2% solution of the drug for curative and prophylactic rates, respectively at the dose rate of 0.25-0.5 mg/kg (Mamman, 1993). Recently, treatment of both *T. brucei* and *T. congolense* single and mixed experimental infections in Red Sokoto bucks using the recommended dosage of 0.5 mg/kg (however, with an unspecified concentration) resulted in reappearance of the parasites two weeks after treatment (Karaye, 2012). This study is aimed at determining the efficacy of therapeutic and prophylactic drug concentrations of isometamidium chloride using 1% and 2% in *T. congolense* infected Sokoto Red bucks (SRB) at a dose rate of 0.5 mg/kg.

**Materials and Methods**

**Parasite acquisition**

*Trypanosoma congolense* used in this study was obtained from the Centre of Biotechnology Research and Training (CBRT) Ahmadu Bello University, Zaria, Kaduna state, Nigeria.

**Donor rats**

Four donor albino rats were inoculated with the *T. congolense* stabiles intraperitoneally in order to multiply and harvest the parasites in sufficient numbers. The donor rats were sacrificed at a $++$ ($1 \times 10^3$ parasites/ml) parasitaemia and their bloods collected into clean ethylene diamine tetra-acetate (EDTA) containing sample bottles.

**Experimental animals (acquisition and management)**

Twelve Sokoto Red Bucks (SRB) aged between 10 and 12 months old were purchased from an open market in Maiadua,
Kaduna state, Nigeria.

Donor rats

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Experimental animals (acquisition and management)

Twelve Sokoto Red Bucks (SRB) aged between 10 and 12 months old were purchased from an open market in Maiadua, Katsina State, Nigeria. The bucks were confined in a fly-proof pen in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and were fed a balanced ration. Water was provided *ad libitum*.

Screening and treatment of experimental animals

The animals were screened for both gastrointestinal parasites (using simple floatation technique) and haemoparasites (using thin blood smear and buffy coat examination) by using their faecal and blood samples respectively. They were also examined for ectoparasites.

The animals were subsequently dewormed with Albendazole (WormKiller®-essential animal care, China) at a dosage of 7.5 mg/kg Per Os (PO) and cypermethrin pour-on (Inothrine® 5 % pour on-Laprovet, France) against ectoparasites. They were then acclimatized for three weeks after the routine treatments before the commencement of the research.

Experimental design: Grouping and infection of the experimental animals

The experimental animals were divided randomly into three groups (I, II and III) of four goats each after proper identification. Groups I and II served as the infected Groups, Group III served as the uninfected control group. Blood from the infected donor rats was pooled and the parasitaemia checked using wet mount. Parasitaemia of 1x10^6 parasites determined as described by Herbert and Lumsden (1976) were inoculated into each of the experimental animals intravenously via the jugular vein.

Post infection monitoring of experimental animals

Temperature

The rectal temperature of each animal in each group was taken and recorded using a digital thermometer twice daily (morning and evening) throughout the study.

Parasitaemia

Following the inoculation of the parasites, parasitaemia was monitored and estimated using wet mount and micro Haematocrit Centrifugation Technique (HCT) of the jugular blood two days post-inoculation then once every day until the disease was established. The effect of the two different concentrations of the drug on the level of parasitaemia was determined daily using wet mount and HCT as described by Woo (1971) for seven days post-treatment and then twice weekly for another three weeks, making a total of four weeks of parasitaemia monitoring.

Clinical signs and determination of body weights

The clinical manifestations of the disease observed in the goats were recorded. The body weights of the animals were taken on weekly basis throughout the period of the study (eight weeks) using bath room weighing balance.

Evaluation of haematological indices

Two (2) ml of blood was collected from each goat in all the groups via jugular venepuncture using 5 ml syringe and 21 gauge needle. The blood was dispensed into labelled sample bottles containing EDTA. The procedure was carried out as follows; Pre-infection twice (at ten days interval), post-infection three days after infection and then on the fourth and fifth day, and post-treatment. The blood samples were analysed to determine the packed cell volumes (PCV) using haematocrit reader. The differential WBC counts was estimated using the thin blood smear method (examined under the light microscope) while total WBC counts was estimated using haemocytometer technique according to the method of Schalm *et al.* (1975).

Preparation and administration of experimental drug

The 1 % isometamidium chloride solution was prepared by dissolving 1 g (Trypamidium-Samorin®- Merial) in 100 ml of sterile water for injection, while the 2 % was prepared by dissolving 1 g powder of the drug in 50 ml of sterile water for injection.

Following the establishment of infection and development of clinical disease in all the infected goats in Groups I and II, chemotherapy was instituted using isometamidium chloride at concentrations of 1 % and 2 % for Groups I and II respectively using the same dosage of 0.5 mg/kg deep intramuscular while Group III which served as uninfected control did not receive any treatment.

Mice inoculation

Following an aparasitaemic phase observed a week post-treatment in the test groups; the bloods were sub-inoculated intraperitoneally into mice (0.2ml/mouse) to rule out the possibility of occult infection. Three mice were sub-inoculated with the blood from each of the treated SRB making a total of twenty four mice. The mice were monitored twice weekly (at three days interval) for five weeks following the sub-inoculation. Both wet mount and HCT were used for the screening of the blood.

Data analysis

The data generated were expressed as mean ± SEM (Standard Error of Mean) in form of tables which were later converted into line graphs. Analysis of variance (ANOVA) with Turkey’s multiple comparison post-hoc test using Graph Pad Prism® version 5.0 for Windows® was used to compare the level of significance among the test groups. P-values < 0.05 were considered significant at 95 % confidence interval.
Results and discussion

Pale mucous membranes, depression, purulent nasal discharges, epiphora, rough hair coats, pyrexia and pre-scapular lymph node enlargement were noticed in all the infected experimental animals. This is in agreement with the findings of Karaye (2012) and Bissalla et al. (2009) who found similar clinical signs in bucks and sheep infected with *T. congolense* respectively.

In this study, the pre-patent period in majority of the animals within the test groups was four days with few animals remaining a parasitaemic until the fifth day. Figures 1 and 2 showed the parasitaemia post infection and post treatment.

![Figure 1](image1.png)

Figure 1. Mean parasitaemia score on wet-mount post-infection and post-treatment of *T. congolense* infected Sokoto Red bucks treated with 1% and 2% isometamidium chloride. Key: a – post-infection, b – post-treatment, 1% ISMTX – 1% Isometamidium chloride treated group (Group I), 2% ISMTX – 1% Isometamidium chloride treated group (Group II).

![Figure 2](image2.png)

Figure 2. Mean parasitaemia score on HCT post-treatment of *T. congolense* infected Sokoto Red bucks treated with 1%, 2% isometamidium chloride. Key: a – post-infection, b – post-treatment, 1% ISMTX – 1% Isometamidium chloride treated group (Group I), 2% ISMTX – 1% Isometamidium chloride treated group (Group II).
The 2% ISMTX group had an aparasitaemic phase on both wet-mount and HCT earlier than the 1% group. The 1% ISMTX group showed a relapse of the infection two weeks post-treatment. There was a significant (p < 0.05) increase in mean rectal temperatures post-infection in all the test groups. A significant (p < 0.05) decrease in the rectal temperature was observed following treatment as shown in figure 3. Two weeks post-treatment, the rectal temperature in 1% ISMTX group increased as a result of re-appearance of the parasites in the blood. A similar observation was made by Karaye (2012) who reported an increase in temperature due to relapse in bucks following treatment with isometamidium chloride. This was attributable to the re-appearance of the parasites in the blood following treatment. There was a significant (p < 0.05) decrease in the mean PCV in both the two test groups (p < 0.05) when compared to the control group both at post-infection and post-treatment. The work of Abenga and Lawal (2005) on T. congolense and T. brucei suggested that trypanosomes were responsible for the progressive development of anaemia. A significant leucopenia (p < 0.05) post-infection and post-treatment was observed in all the test groups when compared to the control group. The leucopenia may be attributed to lymphopenia. The decrease in the TWBCC may be due to immunosuppression as a result of the overwhelming and invasive nature of the disease. This result was in agreement with a study conducted by Adah et al. (1993) on T. congolense infected Sokoto Red goats who reported a similar finding of leucopenia characterized by neutrophilia and lymphopenia. The absolute neutrophil count, eosinophil, monocyte and basophil count were not significantly different (p > 0.05) from that of the control group. The mice sub-inoculated with the blood from the treated groups tested negative of the parasite even at five weeks post sub-inoculation. It was thus evident that the treatment instituted was effective in clearing the parasite from blood.

Conclusion

The 1% isometamidium chloride that is supposedly curative was only able to eliminate the parasites temporarily from circulation, however, two weeks post-treatment a relapse was found despite the institution of treatment early enough. This implies that the 1% isometamidium chloride at 0.5 mg/kg body weight was only effective but not curative. The duration of chemoprophylaxis for 2% isometamidium chloride based on our findings when used at a dosage of 0.5 mg/kg body weight was five weeks.

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Conflict of interests

No conflicts of interest among the authors.