

## **Efficacy of Diminazene Aceturate with and without Levamisole or Dimethyl Sulfoxide in Reducing Organ Weight and Parasitemia in *T. congolense* Infected Rats**

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**Abstract:** The efficacies of diminazene aceturate alone and in separate combinations with levamisole and Dimethyl Sulfoxide (DMSO) in the treatment of *T. congolense* infection in rats were assessed on day 7 post infection and days 7 and 14 post treatment using changes in the weights and histology of the liver, spleen, heart and brain as well as parasitemia as parameters. Infected rats were treated with 7.0 mg/kg diminazene aceturate on day 7 post infection following which DMSO (0.5, 1.0 and 2.0 g/kg, respectively) and levamisole (10, 20 and 40 mg/kg, respectively) were administered as daily supplements to different groups of rats. *Trypanosoma congolense* only caused significant increase in spleen weight. There were no histopathological lesions in any organ. Infection had no effect on heart weight. Liver and spleen weights were lower in the diminazene group by day 7 Post Treatment (PT), but this situation was reversed by day 14 PT. Increase in the dose of DMSO caused increased liver weight. Diminazene/DMSO combination was more effective at 14 days PT in reducing spleen weight than treatment with diminazene alone. On the contrary, diminazene/levamisole combination was less effective than diminazene alone in reducing spleen weight. Parasites disappeared after diminazene treatment but reappeared only in the diminazene and levamisole groups by day 14 PT. Early relapse and high virulence of the Basa strain of *T. congolense* used may be responsible for the ineffectiveness of the three treatment protocols.

**Keywords:** Diminazene, DMSO, levamisole, liver weight, parasitemia, rat, spleen weight, trypanosome

### **INTRODUCTION**

The involvement of oxidative stress arising from generation of Reactive Oxygen Species (ROS) by trypanosome activity and activated mononuclear phagocytic system has been suggested as the cause of anemia in trypanosomiasis (Igbokwe, 1994). Eze *et al.* (2008) reported increased malondialdehyde levels in the spleen, lung and eye indicative of the presence of free radicals and lipid peroxidation following trypanosome infection.

One of the significant and complicating factors in the pathogenesis of trypanosomiasis is profound immunosuppression (Grosskinsky and Askonas, 1981; Mathewos *et al.*, 2001). Extensive studies in various clinical and experimental models have shown that levamisole normalizes deficient cell-mediated immunity and that it possesses no, or only marginal effects in the immunologically competent animal (Snyman and Sommers, 1998; Del-Rio-Navarro *et al.*, 2007). Thus, a defective immune status, as occurs in trypanosomiasis,

seems to be an important prerequisite for the effectiveness of levamisole.

Dimethyl sulfoxide has been extensively studied but not thoroughly understood. Doctors prescribe it for a variety of ailments, including pain (Haigler, 1983); interstitial cystitis and arthritis (Santos and Tipping, 1994). Dimethyl sulfoxide is reported to be an effective hydroxyl radical scavenger (Panganamala *et al.*, 1976; Del Maestro *et al.*, 1980).

The benefit of administering levamisole as an adjunct to vaccination has been demonstrated in some viral infections such as Infectious Bursal Disease (IBD) (Abraham *et al.*, 2007), complicated viral influenza (Grishchenko *et al.*, 1984), Foot and Mouth Disease in buffaloes (Qureshi *et al.*, 2000) and Blue Tongue in sheep (Stelletta *et al.*, 2004). Similarly, the benefit of combining DMSO with conventional treatment was realised by Dake (1967) who found that cats with overwhelming viral infection treated with either DMSO alone or conventional therapy for viral infections all died. When DMSO was combined with standard antiviral

treatment, the figures were reversed with the majority of the cats surviving.

Chemotherapy is the only method of trypanosomiasis control that is readily available to individual farmers. Since no new trypanocidal drugs are available or likely to appear soon, there has been a widespread call to focus research and development strategy on making clinically established drugs do their therapeutic best. Considering the myriads of potent biologically active substances that have been identified in the tissues and blood of victims of trypanosomiasis and the depletion of systemic antioxidants, combination of a trypanocide with other drugs which could aid the reversal of pathological damage due to trypanosome infection may lead to rapid healing. Hence, this study was carried out to elucidate the efficacy of combining diminazene, a trypanocide, with an immunostimulant such as levamisole which could correct the characteristic immunodepression, and an antioxidant like DMSO which could halt the pathological process of trypanosome infection.

## MATERIALS AND METHODS

**Animals:** Albino rats of Wistar strain, mixed sexes and weighing 80-100 g were used in this study which was conducted at the University of Ibadan, Nigeria in 2011. The animals were housed in standard rat cages (International Biological Laboratories, Haryana, India) with white plastic solid bottom and wire tops. Wood shavings were used as beddings. The cages were accommodated in a well ventilated fly proof house. Animals were humanely cared for in compliance with The Principles of Laboratory Animal Care. Animals were fed *ad libitum* with commercially formulated 8 mm pelletized mouse cubes (Ladokun Feeds, Ibadan). Water was provided *ad libitum* using plastic bottles equipped with sipper tubes. Excess feed and water were removed and replaced with fresh ones daily. The Faculty of Veterinary Medicine's ethics committee approved and monitored the protocol.

**Trypanosomes:** The Basa strain of *Trypanosoma congolense* was used in this study. The parasite was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom where it had been stabilized and maintained in liquid Nitrogen. The parasites were maintained in rats by serial passage after removal from liquid nitrogen.

**Experimental procedures:** Ten (10) uninfected rats were used to generate baseline organ weight and histopathology data. The rats were certified free of the parasite. The data obtained from them represented the uninfected control. Seventy (70) rats were used to evaluate the effects of treatment. The rats were randomly put in seven groups (I to VII) of ten rats per group. They were infected with *T*

*congolense* by intraperitoneal injection of approximately  $1.5 \times 10^6$  trypanosomes obtained from the blood of a rat with heavy parasitemia. Seven days after infection (DOT), all animals were treated intramuscularly with diminazene aceturate at 7.0 mg/kg body weight. In addition to diminazene aceturate treatment, animals in groups II, III and IV received 10, 20 and 40 mg/kg body weight levamisole (Pantex, Holland BV) respectively every day beginning from DOT. Rats in groups V, VI and VII were administered respectively with 0.5, 1.0, and 2.0g/kg body weight DMSO (BDH Laboratory Reagents, England) daily beginning on DOT. Levamisole and DMSO were dispensed daily in small volumes of drinking water. Fresh water was given to the animals after the drug solutions were exhausted. Animals in group I received no supplement.

On days 7 and 14 post diminazene treatment, five rats from each of the seven groups were sacrificed by euthanasia with chloroform (BDH Laboratory Reagents, England) inhalation. The levels of parasitemia in wet smear of tail blood were determined in each rat using the rapid matching method of Herbert and Lumsden (1976) before chloroform euthanasia. The liver, spleen, heart and brain of euthanized rats were removed and weighed immediately. The organs were then processed routinely for histopathology and sections stained with hematoxylin and eosin were examined with the light microscope.

## ANALYSIS OF RESULTS

The weights of liver, spleen, heart and brain of each animal were calculated as percentages of body weight. The differences in the means of all parameters were analyzed statistically with Instat<sup>®</sup> software (GraphPad Inc., USA) using one-way Analysis of Variance (ANOVA) (for  $\geq 3$  means) and two-tailed p value (for 2 means). Statistical estimates were made at confidence interval of 95%. Probability values less or equal to 0.05 ( $p \leq 0.05$ ) were considered significant.

## RESULTS

**Infection and treatment effects on parasitemia and mortality:** The parasite produced apparent parasitemia by day 4 Post Infection (PI). The first death of untreated rats was recorded on day 8 PI. By day 14 PI, all infected and untreated animals had died. Parasites were cleared from the blood in all treatment groups by day 7 Post Treatment (PT) but parasites reappeared only in the diminazene and diminazene/levamisole groups by day 14 PT (Fig. 1) and by 21 days PT more than 50% of treated animals had died. Hence, the effects of treatment beyond 14 days PT could not be presented.

**Response of liver to infection and treatment:** The increase in mean liver weight caused by infection was statistically insignificant ( $p > 0.05$ ). On gross examination,

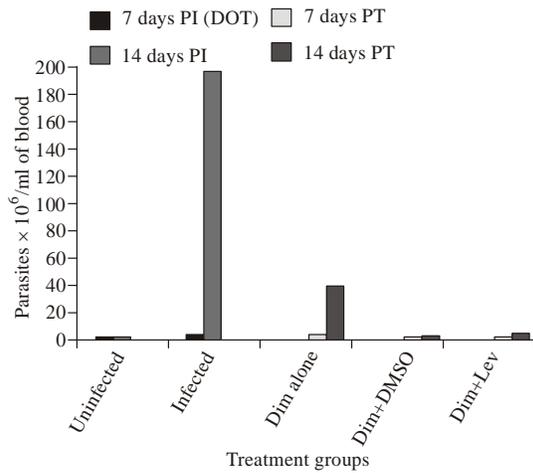


Fig. 1: Effects of diminazene treatment with and without daily DMSO and levamisole supplementations on parasitemia in *T. congolense* infected rats

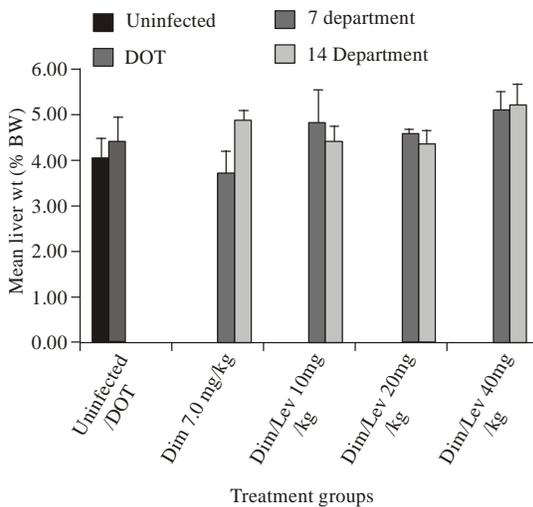


Fig. 2: Effects of diminazene treatment with and without daily DMSO supplementation on liver weight in *T. congolense* infected rats

the livers appeared normal as there were no discernible hemorrhages or other gross pathological signs. At histopathology, no lesions were seen in the livers.

Treatment with diminazene acetate alone reduced the mean liver weight from the DOT value of  $4.32 \pm 0.57\%$  Body Weight (BW) to  $3.65 \pm 0.50\%$  BW by day 7 Post Treatment (PT). This reduction was statistically insignificant ( $p > 0.05$ ). Treatment with diminazene in combinations with the three doses of DMSO resulted in statistically insignificant ( $p > 0.05$ ) increases in mean liver weight from the DOT value. However, the means of liver weight in the diminazene acetate/DMSO groups were significantly ( $p < 0.05$ ) higher than the value recorded in the diminazene acetate group ( $p < 0.05$ ) (Fig. 2).

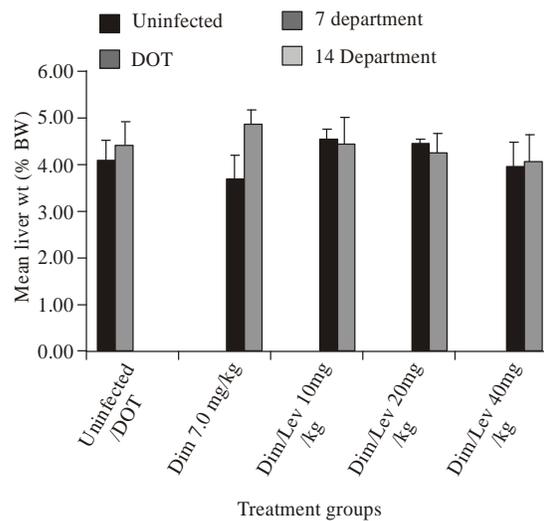


Fig. 3: Changes in liver weight following treatment with diminazene with or without daily levamisole supplementation in *T. congolense* infection

At 14 days PT with diminazene acetate alone, the mean liver weight of  $4.86 \pm 0.15\%$  BW was not significantly ( $p > 0.05$ ) different from the value recorded on DOT but was significantly ( $p < 0.05$ ) higher than the value recorded on day 7 PT. The mean liver weight of  $4.34 \pm 0.34\%$  BW on day 14 PT in the diminazene/DMSO (0.5 g/kg) group was significantly ( $p < 0.05$ ) lower than the mean liver weight recorded with diminazene acetate treatment alone. Dimethyl sulfoxide supplementation at 1.0 g/kg also resulted in significantly ( $p < 0.01$ ) lower liver weights than diminazene acetate treatment. Increase in the dose of DMSO exacerbated the increase in liver weight. The mean liver weight on day 14 PT in the diminazene acetate/DMSO 2 g/kg group was not significantly ( $p > 0.05$ ) higher than the mean liver weight in the diminazene acetate group but was significantly ( $p < 0.05$ ) higher than the means of liver weight in the DMSO 0.5- and 1.0- g/kg groups, respectively on DOT and in uninfected group (Fig. 2).

On day 7 PT with diminazene acetate/levamisole (10 mg/kg) combination, the mean liver weight rose beyond the DOT value to  $4.53 \pm 0.09\%$  BW by day 7 PT. This increase was statistically insignificant ( $p > 0.05$ ) compared to the value on DOT but was significantly ( $p < 0.05$ ) higher than the mean liver weight of  $3.99 \pm 0.44\%$  BW recorded in uninfected rats. On day 7 PT, the means of liver weight of  $4.53 \pm 0.09\%$  BW and  $4.42 \pm 0.07$  respectively in the 10 and 20 mg/kg levamisole supplemented groups were significantly ( $p < 0.05$ ) higher than the value recorded in the diminazene treated group. Increase in the dose of levamisole resulted in smaller liver weights.

There were further but insignificant ( $p > 0.5$ ) reductions in mean liver weight in the levamisole groups by day 14 PT differing from result in the diminazene

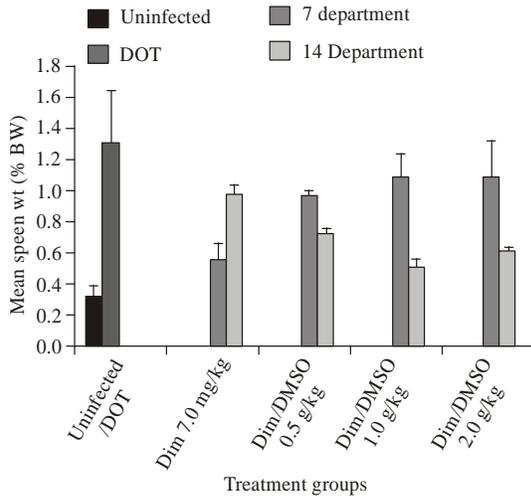


Fig. 4: Effects of diminazene treatment with and without daily DMSO supplementation on spleen weight in *T. congolense* infected rats

group (Fig. 3). The mean liver weight in the 40 mg/kg levamisole group on day 14 PT was significantly ( $p < 0.05$ ) lower than the value recorded in the diminazene treated group but was not significantly ( $p > 0.05$ ) different from the value recorded in the uninfected group.

**Response of spleen to infection and treatment:** *Trypanosoma congolense* infection caused significant increase in mean spleen weight ( $p < 0.05$ ). The spleens of infected rats were grossly enlarged.

Seven days after treatment with diminazene aceturate alone, the mean spleen weight of  $0.57 \pm 0.09\%$  BW recorded in treated rats was significantly ( $p < 0.05$ ) lower than the value of  $1.30 \pm 0.35\%$  BW on DOT. However, by day 14 post treatment with diminazene alone, the mean spleen weight of  $0.98 \pm 0.06\%$  BW was significantly higher ( $p < 0.05$ ) than the value on day 7 PT of the same group. There was no significant ( $p < 0.05$ ) difference in the means of spleen weight in the diminazene group on day 14 PT and DOT.

Treatment with diminazene aceturate plus DMSO supplementation also reduced the increased spleen weight arising from *T. congolense* infection. By 7 and 14 days PT, the means of spleen weight were  $0.98 \pm 0.01\%$  BW and  $0.74 \pm 0.13\%$  BW respectively in the Diminazene/DMSO (0.5 g/kg) group. These values were significantly lower ( $p < 0.005$ ) than the DOT value. Diminazene aceturate treatment resulted in significantly ( $p < 0.05$ ) lower spleen weight on day 7 PT than diminazene plus DMSO supplementation. This situation was reversed on day 14 PT when the mean spleen weight in the diminazene aceturate treated group was significantly ( $p < 0.05$ ) higher than the value in the DMSO supplemented groups (Fig. 4).

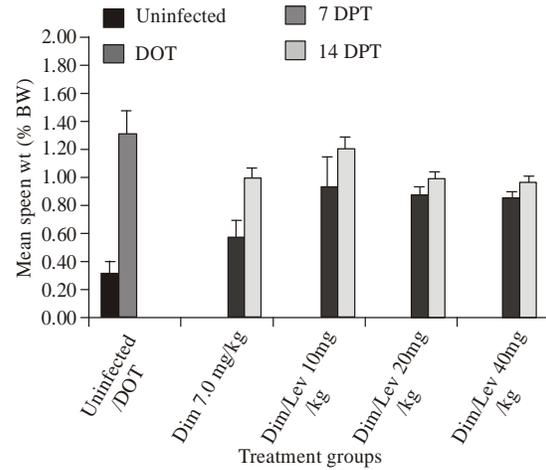


Fig. 5: Changes in spleen weight following treatment with diminazene with or without daily levamisole supplementation in *T. congolense* infection

Treatment with diminazene aceturate in combination with higher doses (20 and 40 mg/kg, respectively) of levamisole also caused significant reduction ( $p < 0.05$ ) in spleen weight from the value on DOT (Fig. 5). Even so, the mean spleen weight values of  $0.92 \pm 0.21$ ,  $0.86 \pm 0.40$  and  $0.84 \pm 0.02\%$  BW, respectively in the 10-, 20- and 40-mg/kg groups, respectively were significantly ( $p < 0.05$ ) higher than the value recorded in the diminazene treated group on day 7 PT.

On day 14 PT, no significant ( $p > 0.05$ ) changes in spleen weights between days 7 and 14 PT were observed in the levamisole supplemented groups but increase in the dose of levamisole gave corresponding lower spleen weights by day 14 PT. The mean spleen weight of  $0.98 \pm 0.06\%$  BW in the 10 mg/kg levamisole group was significantly ( $p < 0.05$ ) higher than the values in the 20 and 40 mg/kg levamisole groups and on DOT (Fig. 5). There were no significant ( $p > 0.05$ ) differences in the means of spleen weight in the diminazene group and the 20- and 40- mg/kg levamisole group on day 14 PT, respectively.

**Responses of heart and brain to infection:** There were no gross changes resulting from infection in the heart and brain. Similarly, no visible lesions were seen at histopathology.

## DISCUSSION

Treatment with diminazene alone caused reduction in liver and spleen weights by day 7 PT. However, these reliefs were not sustained since by day 14 PT liver and spleen weights increased beyond the values recorded on day 7 PT and DOT. This may have been due to relapse of infection. In this study, parasitemia was re-established by day 14 PT with diminazene alone. The resurgence of

trypanosomes apparently resumed the circle of pathological events which normally follow trypanosome infection leading to reversal of the gains in tissue healing.

Analysis of these results showed that the slight increase in mean liver weight induced by *T. congolense* did not respond favorably to treatment with diminazene alone or in combination with DMSO. Treatment with diminazene plus levamisole appears to have more prospects of reducing liver weight but for the early relapse of infection. Treatment with diminazene plus daily supplements with DMSO resulted in significantly higher mean liver weight than the value obtained in the group treated with diminazene alone.

Increase in the dose of DMSO exacerbated the increased liver weight. This observation may have been due to toxicity of DMSO on the liver since the spleen did not respond by increase in weight to increased dose of DMSO. In adult humans, DMSO doses above 1 g/kg body weight have been associated with liver, kidney and intestinal damages (Willhite and Katz, 1984; Swanson, 1985).

Diminazene/DMSO combination was more effective at 14 days PT in reducing spleen weight than treatment with diminazene alone. Although spleen weights were lower in the diminazene group by day 7 PT, this situation was reversed by day 14 PT apparently due to relapse of infection. DMSO delayed relapse beyond 14 days. On the contrary, diminazene/levamisole combination was not more effective than diminazene alone in reducing the increase in spleen weight. Levamisole also did not delay relapse beyond 14 days. The action of levamisole in this study is not as encouraging as those observed in viral infection such as IBD (Abraham *et al.*, 2007) which, like trypanosomosis, also causes immunosuppression (Grosskinsky and Askonas, 1981; Mathew *et al.*, 2001). The discrepancy between the effectiveness of levamisole in viral and trypanosome infections may be due to differences in the nature of the diseases and the rather short period during which levamisole was supplemented in this study. Morrow (2000) observed that it requires 16 weeks of vitamin E supplementation at doses as high as 3200 IU/day to maximally suppress F2-isoprostane formation, a biomarker for oxidative stress.

In this study heart weights in normal, infected, and treated animals were not significantly different. There were also no histopathological changes in the hearts and brains of infected rats. However, Kaikabo and Salako (2006) reported enlarged hearts in *T. brucei* infection. Similarly, Taylor and Authie (2004) reported histopathological changes in the hearts of infected livestock. The discrepancy between these earlier reports and the result presented here may be explained by differences in the species of host and parasite as well as the course of infection. Taylor and Authie (2004) reported that the lesions associated with the heart were found particularly in terminal cases. In this study, the animals

were sacrificed between days 7 and 14 post infection, usually following diminazene treatment with or without adjuvant drug supplementation. Masumu *et al.* (2006) also observed that the effect of *T. congolense* on the health of susceptible livestock is strain dependent.

The results obtained from this study do not show that levamisole and DMSO in separate combinations with diminazene have outstanding effect over diminazene treatment alone in *T. congolense* infection. This is contrary to the effects of ascorbic acid in *T. brucei* infection in which combination of ascorbic acid and diminazene aceturate led to better remission of the disease (Eghianruwa *et al.*, 2009). The lackluster effects of DMSO and levamisole may be due to the acute and resistant nature of the infection. The Basa strain of *Trypanosoma congolense* used in this study can be adequately classified as highly virulent using the criteria of Masumu *et al.* (2006). The disease it produced was acute and characterized by high mortality and early relapse, notwithstanding the high dose of diminazene administered. The efficacy of any of the three treatment regimen in this study may have been more outstanding if infection was induced with a diminazene sensitive strain that would have excluded the possibility of early relapse. In conclusion, the combinations of diminazene with DMSO and levamisole have no therapeutic advantage over diminazene treatment of *T. congolense* infection especially when the infecting organism is highly virulent and resistant to diminazene.

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