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Increasing doses of diminazene aceturate: adverse reproductive effects in female Wistar rats

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ABSTRACT

Objective: To investigate the effects of comparatively high doses of diminazene aceturate on the reproductive performance of female rats in the early stage of pregnancy. **Methods:** After oestrus synchronisation and successful mating, 20 pregnant female rats were randomly divided into four groups (A–D). Group A rats served as the control and were given single intraperitoneal injection of 0.5 mL sterile water (vehicle only) while groups B, C and D rats were given single intraperitoneal doses of 7, 14 and 21 mg/kg body weight diminazene aceturate respectively, on day 7 of pregnancy. The gestation length, litter size and weight at birth, and areas of foetal resorption in the uterus were determined post partum. The post-implantation survival index (%) and the gestation index (group %) were also evaluated for rats in all the groups. **Results:** There was a graded increase in the number of observed resorbed foetuses as the dose of diminazene aceturate was increased, although only groups C (14 mg/kg) and D (21 mg/kg) revealed a significant decrease ($P<0.01$, ANOVA) in the post implantation survival index of rat embryos. There was also a significant decrease ($P<0.05$) in the litter weights of rats in groups C and D. **Conclusions:** Although the pregnant rats showed no overt signs of systemic toxicity even at the highest dose of 21 mg/kg body weight diminazene aceturate in this study, it was concluded that the use of high doses of diminazene aceturate in an effort to combat resistant trypanosomes could have adverse reproductive effects on female animals in the early period of pregnancy.

1. Introduction

Trypanosomosis has been described as a complex debilitating and often fatal disease caused by infection with one or more of the pathogenic tsetse-transmitted protozoan parasite of the genus *Trypanosoma*[1]. In the 37 African countries with endemic animal trypanosomosis, trypanocides play a key role in the control of the disease[2]. Diminazene was introduced onto the market as a trypanocide and babesiacide for domestic livestock in 1955[3], and is normally curative at a dose of 3.5 mg/kg body weight (b.w.)[4].

Unfortunately, diminazene and most other trypanocides have been in use for over half a century with development of resistance of trypanosomes to the drugs; some field isolates of trypanosomes requiring up to 45 mg/kg b.w. diminazene aceturate as minimum required dose to achieve cure[1]. The emergence of drug resistance in the field may be consistent with indiscriminate usage by unqualified personnel and farmers in Africa[1]. Trypanocidal drugs are probably the most commonly used veterinary products in Sub-Saharan Africa (SSA); they are often the first drugs tried by farmers when their cattle develop (any) symptoms of disease because they are affordable[2].

As a result of the unavailability of new trypanocidal drugs for use in domestic animals, several strategies relying on the use of the old existing drugs such as the use of sanative pairs, use of high dose and repeated treatment regime, and combination therapy have been in use for combating drug resistance[1].

Some studies have suggested that high dose treatment offer good opportunities for eliminating infections with trypanosomes which express high degree of resistance to diminazene aceturate[5,6].

However, in spite of the potential merit offered by the high dose therapy, concerns have been raised about the increased risk of toxicity from the use of large doses[1,7,8]. Diminazene aceturate toxicity has been reported in mice by Muller (Hoechst AG; 1988); and in rats[9].Clinical and pathological manifestations of diminazene aceturate toxicity have also been reported in other species[10,11]. There are no data on the effects of increase in doses of the trypanocide on pregnancy metrics in female reproduction. Thus the objective of this study was to examine the effects of administration of increasing doses of diminazene aceturate on the reproductive performance of rats in the early stage of

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gestation.

2. Materials and methods

2.1. Animals

Thirty eight mature healthy outbred Wistar rats (*Rattus norvegicus*) comprising 30 females (12–14 weeks old) and eight males (15 weeks old) were used for the study. All the animals weighed 140–160 g at the beginning of the study. The rats were housed in cages at room temperature 28–32 ℃ in the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria Nsukka. Water and commercial feed (Vital® feed, GCOML, Jos, Nigeria) containing 14.5% crude protein were provided *ad libitum*.

2.2. Diminazene aceturate

Diminazene diaceturate (Diminaveto®; VMD, Belgium) was reconstituted appropriately in sterile water and given as single intraperitoneal injection at doses of 7, 14 and 21mg/kg b.w. to the groups of rats concerned. Drug administration was done on day 7 of pregnancy; as implantation occurs between days 4 and 5 of pregnancy in rats[12].

2.3. Synchronisation of oestrus

30 female rats were caged as dense groups and kept in a separate room from the 8 males for 3 weeks so as to induce anoestrus; hence Whitten effect to ensure that oestrus synchronisation occurred within a few days of introducing the males[13].

2.4. Determination of successful mating

The vaginal plug method of Bennett and Vickery[14], modified by Ochiogu et al[13] was used to determine successful mating in the female rats. Following introduction of the female rats to males of proven fertility, vaginal wet smears were grossly examined every 12 hours for presence of protein coagulates (remnants of copulatory plug) as evidence of successful mating. The day of mating was considered as day 0 of pregnancy.

2.5. Experimental design

The 20 pregnant rats used for the study were randomly divided into 4 groups of 5 rats each, identified with letters A to D. Group A rats served as the control and were given single intraperitoneal injections of 0.5 mL sterile water (vehicle only). Groups B, C and D were given single intraperitoneal doses of 7, 14 and 21 mg/kg b.w. diminazene aceturate respectively.

The following parameters were monitored: gestation length, litter size and weight at birth, and areas of foetal resorption (determined by sacrifice after parturition). The post-implantation survival index (PISI) and the gestation index were calculated as follows:

$$\text{Post-implantation Survival Index (\%)} = \frac{\text{Total number of offspring born}}{\text{Total number of implantation sites}} \times 100$$

$$\text{Gestation index (group \%)} = \frac{\text{Number of live foetuses born/group}}{\text{Number of animals pregnant/group}} \times 100$$

2.6. Statistical analysis

Statistical analysis was performed using SPSS software® (Version 15.0 for Windows, SPSS Inc., Chicago, USA). Experimental data were subjected to One-way Analysis of Variance (ANOVA) and the variant means separated by using Least Significance Difference (LSD) test. The results are presented as the mean ± standard error of the mean (SEM).

3. Results

3.1. Effect of increasing doses of diminazene aceturate on foetal resorption

As shown in Figure 1, there was a graded increase in the number of observed foetal resorption as the dose of diminazene aceturate was increased. However, only groups C (14 mg/kg) and D (21 mg/kg) had significantly higher (P<0.01) number of resorbed foetuses.

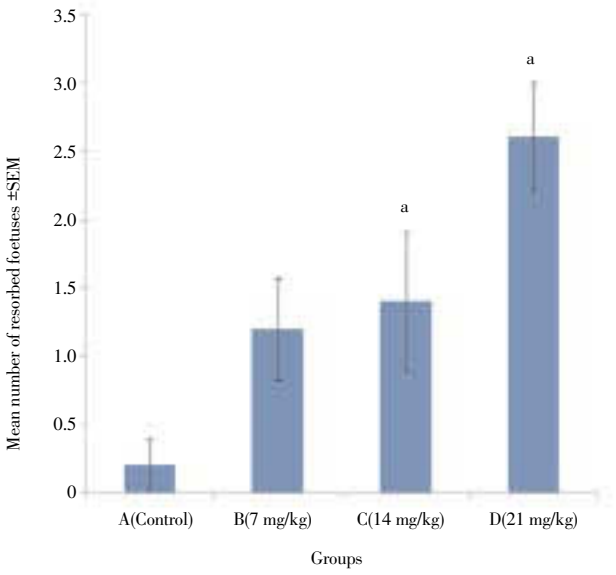

Figure 1. Effect of increasing doses of diminazene aceturate on foetal resorption in female rats.
a represents P<0.01 (ANOVA).

3.2. Effect of increasing doses of diminazene aceturate on litter size and litter weight

As shown in Table 1, only group C (14 mg/kg) had significantly lower (P<0.05) litter size. The litter weights of rats in groups C (14 mg/kg) and D (21 mg/kg) were significantly lower (P<0.05) than that of the control.

3.3. Effect of increasing doses of diminazene aceturate on gestation length

A graded increase in the length of gestation was observed with increase in dose of diminazene (Table 1), although only rats in group D (21 mg/kg) had a significantly longer (P<0.05) gestation length.

3.4. Effect of increasing doses of diminazene aceturate on other reproductive indices

From the results presented in Table 1, there was a

Table 1
Mean (±SEM) effect of increasing doses of diminazene aceturate on some reproductive indices of female rats.

Reproductive Indices	Groups			
	A (Control)	B (7 mg/kg)	C (14 mg/kg)	D (21 mg/kg)
Litter size	7.20 ± 0.37	6.00 ± 0.71	4.80 ± 0.86[a]	5.60 ± 0.24
Litter weight (g)	39.88 ± 2.94	31.00 ± 3.72	26.00 ± 4.75[a]	27.84 ± 1.14[a]
Gestation length (days)	21.00 ± 0.32	21.20 ± 0.20	21.60 ± 0.40	22.00 ± 0.32[a]
PISI (%)	97.50 ± 2.50	82.79 ± 5.08[a]	71.84 ± 5.00[b]	68.61 ± 4.06[b]
Gestation index (group %)	760	600	480	560

Values are expressed as mean ± SEM of five observations (n=5). a represents significant value at P<0.05 and b significant values at P<0.01 (ANOVA). PISI: Post-implantation survival index.

significant reduction (P<0.01) in the Post-implantation Survival Index of rat embryos in groups C and D. The Gestation Index (group %) of all the groups (B, C and D) given diminazene were also low when compared with the control group (A).

4. Discussion

The present study indicated that administration of increasing doses of diminazene aceturate to female albino rats in the early stage of pregnancy had negative effects on their reproductive performance.

The significant increase (P<0.01) in foetal resorption and decrease (P<0.05) in litter weight observed in this study are similar to the findings of Yoshimura[9], although higher doses of 100, 250, 500 or 1000 mg/kg b.w. diminazene aceturate were given orally to female rats in that toxicity study.

Although the pregnant rats were apparently healthy with no overt signs of systemic toxicity even at the highest dose of 21 mg/kg diminazene aceturate, this study revealed a significant reduction (P<0.01) in the post-implantation survival of rat embryos in the diminazene-treated females.

The mechanism by which diminazene produces the adverse effect on reproductive function of the experimental rats is not clear but may involve some sort of toxicant-induced cellular dysregulation and alterations in cellular maintenance[15].

The significantly longer (P<0.05) gestation length seen in the rats given the highest dose of 21 mg/kg diminazene (group D) is suspected to be a consequence of the significantly low (P<0.05) litter weight of the rats in this group.

Schonefeld et al[5] reported that three different field isolates *of Trypanosoma* vivax from cattle in three districts of Kenya which were resistant to treatment with 3.5 mg/kg diminazene aceturate were all cured when infected cattle were treated with 7 mg/kg b.w. of the same drug. A more recent study reported that in experimental rats infected with *Trypanosoma brucei*, there were relapse infection in the groups treated with 3.5, 7.0 and 14 mg/kg b.w. diminazene aceturate, but only the rats that received the highest dose of 21 mg/kg b.w. diminazene were successfully treated for trypanosomosis without relapse infection[6]. However, a similar dose of 21 mg/kg diminazene produced the highest level of reproductive adversity observed in this study.

Use of high doses of diminazene aceturate may offer some advantage in the treatment of trypanosomosis, but the merits of this practice should be carefully balanced in view of the adverse reproductive effects observed when female rats in the early pregnancy are administered with the trypanocide.

Conflict of interest statement

We declare that we have no conflict of interest.

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