ERYTHROCYTE AND LEUCOCYTE RESPONSES OF GOATS ADMINISTERED MELATONIN TO DIMINAZENE ACETURATE-INDUCED TOXICITY

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Abstract
The experiment was performed with the aim of evaluating erythrocyte and leucocyte responses of goats administered with melatonin to diminazene aceturate-induced toxicity. Simple random sampling was used to assign 20 goats into four groups, comprising five (5) goats each. Group I goats served as untreated control, without melatonin supplementation; Group II melatonin (0.5 mg/kg *per os*); Group III pre-treated with melatonin for three days and administered with diminazene aceturate (7 mg/kg, deep i.m. once), and melatonin administration was continued for additional 10 consecutive days; Group IV goats were administered with diminazene aceturate alone. Two millilitres of blood were collected from the jugular veins of each goat on days 1, 4, 7 and 10 post-diminazene aceturate administration for determination of erythrocyte and leucocyte counts using standard methods. The erythrocyte counts obtained on days 1 and 4 ($6.76 \pm 1.07 \times 10^6$/l and $6.68 \pm 0.86 \times 10^6$/l, respectively) in melatonin-treated groups were the highest ($P > 0.05$). However, on days 7 and 10 post-administration of diminazene aceturate, the highest ($P < 0.05$) leucocyte counts ($13.56 \pm 1.06 \times 10^9$/l and $13.74 \pm 1.80 \times 10^9$/l, respectively) were recorded in goats pre-treated with melatonin and administered diminazene aceturate. The findings demonstrated that, diminazene aceturate intoxication did not exert significant effect on erythrocyte count, but it decreased leucocyte count in goats. Pre-treatment with melatonin reversed the toxicity by increasing the leucocyte count.
Key words: Goats, melatonin, leucocytes, erythrocytes and diminazene aceturate

Introduction

Diminazene was introduced to the market in 1955 as a trypanocide and babesiacide for livestock, including goats (Ge *et al*., 2018). It is normally curative at a dose of 3.5 mg/kg body weight (Da Silva Oliveira and De Freitas, 2015). The use of high doses of diminazene aceturate (Berenil®) may offer some advantages in the treatment of trypanosomosis (Sudan *et al*., 2017), but the merits of this practice should be carefully balanced in view of the adverse effects observed when animals are administered with the trypanocide (Oguejiofor *et al*., 2010). The mechanisms of action of Berenil® have remained poorly understood. While some earlier reports show that Berenil® possesses trypanolytic and trypanostatic properties, some studies have shown that it may also indirectly suppress the host immune system (Kuriakose and Uzonna, 2014). Due to its higher therapeutic index and low incidence of resistance compared to other compounds, it has become the most commonly used therapeutic agent for trypanosomosis in livestock (Peregrine and Mamman, 1993). Melatonin is a neurohormone produced by the pineal gland, possessing antioxidant activity (Maldonado *et al*., 2012). It crosses all cell membranes, including the blood-brain barrier due to its amphiphilicity (Hardeland and Pandi-Perumal, 2005). There is paucity of information in the available literature on mitigating effects of diminazene aceturate-induced toxicity in goats, particularly on haematology. Effects of melatonin administration in goats subjected to diminazene aceturate treatment are currently lacking in the available literature. The objective of the study was to evaluate erythrocyte and leucocyte responses in goats administered with melatonin to diminazene aceturate-induced toxicity.

Materials and Methods

The experiment was performed in a goat experimental pen in the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria (11° 10′ N, 07° 38′ E), located in the Northern Guinea Savannah zone of Nigeria. Twenty (20) healthy goats were bought from Tudun Sebu market in Soba Local Government Area of Kaduna State for the study. They were screened for both endo- and ecto-parasites. The goats were fed with hay with zero grazing. They were given access to feeds and water *ad libitum*. By simple

random sampling, the 20 goats were assigned into four groups, comprising five (5) goats each. Group I goats served as untreated controls, without melatonin supplementation. Group II goats were treated with melatonin (ISI Brands, Inc. Utah, USA) alone at 0.5 mg/kg *per os*. Melatonin was administered once daily for 13 consecutive days at 17:00 h to goats in groups II and III. Group III goats were pre-treated with melatonin for three days and administered diminazene aceturate (7 mg/kg deep i.m. once). Thereafter, melatonin administration was continued for additional 10 consecutive days. Group IV goats were administered with diminazene aceturate alone. Two (2) millilitres of blood were collected from the jugular vein of each goat on days 1, 4, 7 and 10 post-diminazene aceturate administration into separate sodium ethylenediaminetetraacetic acid bottles. The blood was taken to the Veterinary Clinical Pathology Laboratory, Ahmadu Bello University, Zaria for haematological analysis. Erythrocyte count was determined as described by Dacie and Lewis (1991). Similarly, the total leucocyte count in 1 mm^3 of blood sample was obtained using the method described by Weiss *et al*. (2010). Data were expressed as mean ± standard error of the mean (mean ± SEM). All data obtained were subjected to one-way analysis of variance (ANOVA). Multiple means were compared by Duncan's (1955) multiple range test. Values of $P < 0.05$ were considered significant.

Results and Discussion

The erythrocyte counts obtained in the goats are shown in Table 1. The values of 6.76 ± 1.07 x 10^6/l and 6.68 ± 0.86 x 10^6/l obtained on days 1 and 4, respectively in melatonin-treated groups were the highest. The values were not significantly ($P > 0.05$) different from those recorded in other groups on the same day (Table 1). Similarly, the values of 5.86 ± 0.60 x 10^6/l and 6.30 ± 0.70 x 10^6/l obtained in diminazene aceturate-treated goats recorded on post-diminazene aceturate administration, days 7 and 10, respectively were the highest. However, the values did not differ significantly from those obtained in the other groups of goats (Table 1). The overall erythrocyte counts did not differ significantly ($P > 0.05$) across the groups. The leucocyte counts obtained in the goats are shown in Table 2. The highest ($P > 0.05$) leucocyte count (11.66 ± 1.29 x 10^9/l) was obtained on day 1 post-administration of diminazene aceturate in goats administered with melatonin only (Table 2). On day 4, the lowest leucocyte count (11.00 ± 1.39 x 10^9/l) recorded in the control group did not differ significantly from the counts obtained in other groups. At days 7 and 10 post-administration of diminazene aceturate, the highest ($P < 0.05$) leucocyte counts (13.56 ± 1.06 x 10^9/l and 13.74 ± 1.80 x 10^9/l, respectively) were recorded in goats pre-treated with melatonin and administered with diminazene aceturate (Table 2). Overall, the highest ($P < 0.05$) leucocyte count (13.36 ± 0.94 x 10^9/l) was recorded in goats pre-treated with melatonin and administered with diminazene aceturate (Table 2). The findings of the study demonstrated, for the first time, that diminazene aceturate intoxication did not affect erythrocyte count. Hence, diminazene aceturate-induced toxicity at 7 mg/kg in goats exerted no adverse effects on the erythrocyte counts. The results showed that diminazene aceturate administration decreased leucocyte counts in goats. Interestingly, pre-treatment of goats with melatonin before diminazene aceturate administration increased the leucocyte counts. The results suggest that diminazene aceturate exerted an undesirable depressant effect on the bone marrow, alleviated by melatonin. The finding is consistent with the report of Anwar

Table 1: Red blood cells count (x 10^6/l) of Kano Brown goats co-administered with diminazene aceturate (DA) and melatonin.

Groups	Day post-administration of diminazene aceturate				
	1	4	7	10	Overall
Control	6.26 ± 0.57	6.38 ± 0.96	4.76 ± 0.46	4.88 ± 0.60	5.57 ± 0.35
Melatonin	6.76 ± 1.03	6.68 ± 0.86	5.70 ± 0.87	5.48 ± 0.87	6.16 ± 0.44
Melatonin + DA	5.44 ± 0.54	5.38 ± 0.64	4.72 ± 0.52	5.26 ± 0.69	5.20 ± 0.28
DA	6.74 ± 0.55	5.90 ± 0.53	5.86 ± 0.60	6.30 ± 0.70	6.20 ± 0.29

Table 2: White blood cells count (x 10^9/l) of Kano Brown goats co-administered with diminazene aceturate (DA) and melatonin.

Groups	Day post-administration of diminazene aceturate				
	1	4	7	10	Overall
Control	10.30 ± 0.59	11.00 ± 1.39	11.38 ± 1.35^{ab}	12.90 ± 1.44	11.62 ± 0.61^{ab}
Melatonin	11.66 ± 1.29	13.50 ± 1.86	10.66 ± 2.04^{ab}	12.04 ± 1.39	11.97 ± 0.80^{ab}
Melatonin + DA	11.02 ± 1.49	15.12 ± 2.82	13.56 ± 1.06^{b}	13.74 ± 1.80	13.36 ± 0.94^{b}
DA	11.24 ± 1.78	11.02 ± 1.43	8.20 ± 0.52^{a}	10.42 ± 1.38	10.22 ± 0.68^{a}

$^{a, b}$: Means within the same column belonging to different groups and having different superscript letters are significantly ($P < 0.05$) different.

et al. (1998) that melatonin exerts protective and stimulatory effects on bone marrow, exposed to damage by chemical substances. The result shows that immunosuppression occurring in trypanosomosis (Bassi *et al*., 2018) may be aggravated in diminazene aceturate-treated goats by reduction in the leucocyte counts. This may be responsible for the death of trypanosomosis-infected animals administered with diminazene aceturate at high dose of 7 mg/kg. However in the present study, melatonin enhanced immune responses by increasing the leucocyte counts in treated goats. The findings agreed with the report of Nickovic *et al*. (2018) that melatonin is an immunostimulant agent, and may be beneficial in developing novel adjuvant therapies against diminazene aceturate-induced toxicity.

Conclusion and Recommendation

Diminazene aceturate decreased total leucocyte count (10.22 ± 0.68 x 10^9/l) in goats, while melatonin increased the count in diminazene aceturate-treated goats (13.36 ± 0.94 x 10^9/l). Clinicians should co-administer melatonin to trypanosomosis-infected goats treated with diminazene aceturate in order to increase their leucocyte counts.

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