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Case Report

# Diminazene aceturate poisoning in a dog (*Canis familiaris*) treated for visceral leishmaniasis: pathological and toxicological evaluation

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**Abstract**

A one-year-old Whippet dog with a prior diagnosis of visceral leishmaniasis was treated with diminazene aceturate with intramuscular and oral routes, developing acute neurological signs, and was necropsied after death. The necropsy revealed marked systemic congestion, multiple hemorrhagic foci, pulmonary edema, and hemorrhage and necrosis in the brainstem. Brain histopathology showed marked neutrophilic encephalitis with vascular fibrinoid necrosis and hemorrhage restricted to the brainstem. Liver and kidney samples were submitted for toxicological evaluation, revealing elevated levels of diminazene aceturate, corroborating with the diagnosis of poisoning. The pathological findings observed in this case are consistent with those described in the literature regarding diminazene aceturate poisoning. A diagnosis should be performed based on the animal's clinical history, pathological findings, and the results of the toxicological examination.

**Keywords:** fibrinoid necrosis, *Leishmania infantum*, encephalitis, toxicology, pathology.

## Introduction

Drug poisoning is a common problem in veterinary medicine. A significant source of poisoning in domestic animals is the improper use of medications, including incorrect dosages and lack of concern about their pharmacokinetics (2). In cases of poisoning, a definitive diagnosis can be challenging. Although necropsy and histopathology are crucial tools for determining the cause of death, there are limitations, in some cases, being impossible to identify the drug involved accurately. Therefore, it is necessary to conduct complementary toxicological tests to reach a definitive diagnosis and determine which toxic substance was involved in the death. However, conducting toxicological tests is not always feasible due to the lack of resources or limited accessibility, which can compromise the diagnostic conclusion.

Diminazene aceturate is an antiprotozoal drug that has been used for decades against parasitic infections in domestic animals, such as cases of trypanosomiasis (8) and babesiosis (11). Recent studies have also explored its use as an antihypertensive and immunomodulatory agent (18). Chemically, diminazene aceturate is composed of an aromatic diamidine with two aminophenyl groups connected by a triazene bridge (9). The toxicity of this compound has been associated with adverse effects on the central nervous system (CNS), with evidence suggesting that its toxicity is related to dosage, indicating that repeated doses may lead to neurotoxicity due to cumulative effects (18).

The recommended dosage of diminazene aceturate varies according to the species and the therapeutic purpose. For the treatment of babesiosis in dogs and horses, the indicated dosage is 3.5 mg/kg administered intramuscularly in two applications with a 24-hour interval (7). For the treatment of *Trypanosoma brucei* in dogs, gazelles, horses, and mules, the dosage is 7.0 mg/kg; for *Trypanosoma evansi* in cats and goats, the recommended dose is 3.5 mg/kg; for *Trypanosoma*

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*congolense* in goats and cattle, the recommended dosage is 7.2 mg/kg; while for *Trypanosoma vivax*, the dose is 3.5 mg/kg in cattle. A single intramuscular application is recommended, and the need for reapplication should be assessed by the veterinarian (18).

An *in vitro* study evaluated the efficacy of diminazene aceturate against *Leishmania donovani*, showing low selectivity and causing cytotoxicity on both the parasite and human mononuclear cells (6). In Brazil, treating dogs with canine visceral leishmaniasis, caused by *Leishmania infantum*, is controversial. The recommended medications showed low efficacy, causing only temporary remission of clinical signs without preventing recurrence. Furthermore, these drugs have a limited effect on the infectivity of phlebotomine sandflies, failing to reduce the role of the dogs as a reservoir, risking the development of drug-resistant parasites, especially those used in human treatment (1). Since 2016, the drug used in the standard treatment for canine leishmaniasis in Brazil is miltefosine. However, the treatment of dogs has shown limited clinical efficacy, and treated animals may continue to have a high parasite load and, consequently, spread the parasite (4).

This study aims to describe the pathological and toxicological aspects of diminazene aceturate poisoning in a dog treated for canine visceral leishmaniasis.

## Case description

A one-year-old male Whippet dog, previously diagnosed with canine visceral leishmaniasis (CVL) and treated with diminazene aceturate via intramuscular and oral routes, was submitted for necropsy to the Veterinary Pathology Service of the Veterinary School from Universidade Federal de Minas Gerais (UFMG), after death, which was preceded by acute neurological signs.

According to the owner, the dog was rescued from the street in October 2023 and tested positive for CVL at enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA). For treatment, the veterinarian prescribed an antiparasitic drug but did not provide an official prescription with dosage, concentration, and active ingredient, recommending the administration of a solution he had prepared *in-house* following the dosage of 1 ml orally once a day and 1 ml intramuscularly every ten days. On January 16 and 26, 2024, the animal received intramuscular injections, and on the remaining days, it received the daily oral doses. After the death of the animal, the owner inquired the veterinarian about the medication, and it was identified as diminazene aceturate.

The owner reported that on January 30, 2024, the dog presented with vomiting, sialorrhea, and muscle rigidity and was taken to the veterinarian. Cerebrospinal fluid was collected for cytological and molecular analysis. Cytology showed a predominance of neutrophils, suggesting a suppurative inflammatory process in the CNS. Polymerase chain reaction (PCR) for *Neospora caninum, Ehrlichia* spp., *Blastomyces dermatitidis, Histoplasma capsulatum, Babesia* spp., *Cryptococcus* spp., *Coccidioides* spp., and *Toxoplasma gondii*; and reverse transcriptase PCR (RT-PCR) for canine distemper virus were conducted in a private laboratory on the cerebrospinal fluid, with all results returning negative. Finally, a computed tomography (CT) scan of the CNS was recommended and performed on the same day, showing no abnormalities. However, the animal died at the end of the CT procedure while still under anesthesia and was sent for necropsy.

Grossly, there was severe cyanosis and marked and generalized congestion. In the CNS, there were multifocal to coalescing pinpoint hemorrhages associated with malacia, restricted to the brainstem, particularly in the pons and medulla (Figure 1). Superficial pinpoint hemorrhagic foci were also observed in the left ventricular endocardium and in the serosa of the bladder. The lungs showed intense diffuse hemorrhage and mild diffuse edema; mild multifocal ulcers were observed in the duodenum, and melena was observed in the colon.

During the necropsy, samples of CNS, bone marrow, heart, trachea, lung, liver, spleen, kidneys, intestine, mesenteric lymph node, bladder, and pancreas were collected and fixed in 10% buffered formalin, embedded in paraffin,

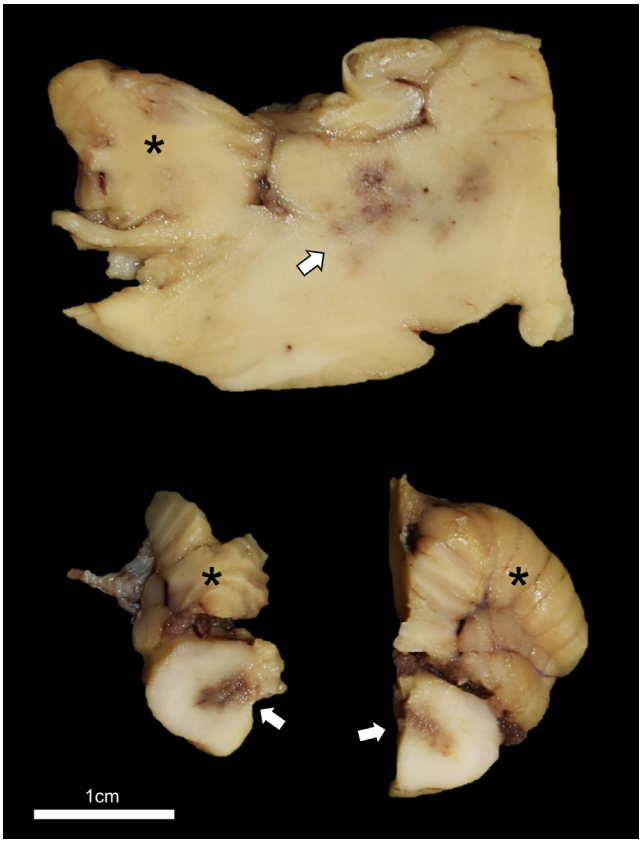


**Figure 1**. Brain stem of a dog poisoned by diminazene aceturate showing millimetric multifocal to coalescent areas of hemorrhage and malacia (arrow). * = cerebellum. Scale bar = 1cm.

Braz J Vet Pathol, 2024, 17(3), 207-212
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sectioned into 3µm thick slices, and stained with hematoxylin and eosin (H&E) for evaluation under light microscopy. Spleen and bone marrow paraffinized tissue were subjected to immunohistochemistry to detect *Leishmania* spp. antigens. The tissues were rehydrated in crescent sections of xylene and alcohol, endogenous peroxidase was blocked with 3% hydrogen peroxide solution for 40 min, and nonspecific reactions were blocked with 6% powdered milk solution for one hour. The tissues were incubated with a primary antibody (1:500) anti-*Leishmania* spp. (*in-house*, polyclonal) for one hour in a humid dark chamber at room temperature. A secondary antibody conjugated with peroxidase (Envisioflex HRP, Dako) was used for 30 min at room temperature in a dark chamber. The chromogen used was Envision Flex HRP Magenta (Dako) for 5 minutes, followed by counterstaining with hematoxylin and dehydration in increasing alcohol and xylene. A known canine-positive case was used as a positive control.

Histologically, in the brainstem, there were severe multifocal to coalescing hemorrhages associated with a marked neutrophilic inflammatory infiltrate (Figure 2A). The arterioles and venules exhibited an expanded Virchow-Robin space, with occasional transmural neutrophils, deposition of bright eosinophilic fibrillar and amorphous material in the tunica media, and endothelial hypertrophy (vasculitis and fibrinoid necrosis) (Figures 2B). In the neuroparenchyma, a moderate number of shrunken hyper-eosinophilic neurons with karyolysis and increased glial cells (necrosis) were observed, along with a rare formation of glial nodules. Leptomeninges had mild to moderate multifocal hemorrhage. Additionally, there was severe multifocal to coalescing bronchopneumonia associated with intense diffuse hemorrhage, and mild multifocal lymphoplasmacytic interstitial nephritis. In the bone marrow, moderate multifocal histiocytic myelitis was identified, with intra-histiocytic amastigote forms consistent with *Leishmania* spp., confirmed by positive immunohistochemical staining. The spleen showed congestion, with subcapsular macrophages also positive for *Leishmania* spp. on immunohistochemistry (Figure 3).

Due to the presumptive diagnosis of poisoning, kidney and liver samples were also collected during the necropsy, frozen, and sent to the Toxicology Laboratory at Veterinary School, UFMG. The extraction and clean-up process of the samples was performed according to the procedure previously described (12). In a 10 ml Falcon tube, 2 g of the sample (liver and kidney) were homogenized with 4 ml of deionized water, vortexed for one minute, and placed in an ultrasonic bath for 10 minutes. The tube was then centrifuged, and 1 ml of the supernatant was transferred to a C8 cartridge (Chromabond 6ml/500 mg, Macherey-Nagel, Düren, Germany) that had been preconditioned with 5 ml of water, 5 ml of methanol, and 5 ml of water. After washing the cartridge with 2 ml of water, diminazene was eluted with 2x 0.5 ml of a mixture of 30% acetonitrile and 70% 5 mM sodium 1-octanesulfonate in 2% acetic acid aqueous solution. The eluate was collected in a vial for high-performance liquid chromatography (HPLC) analysis.

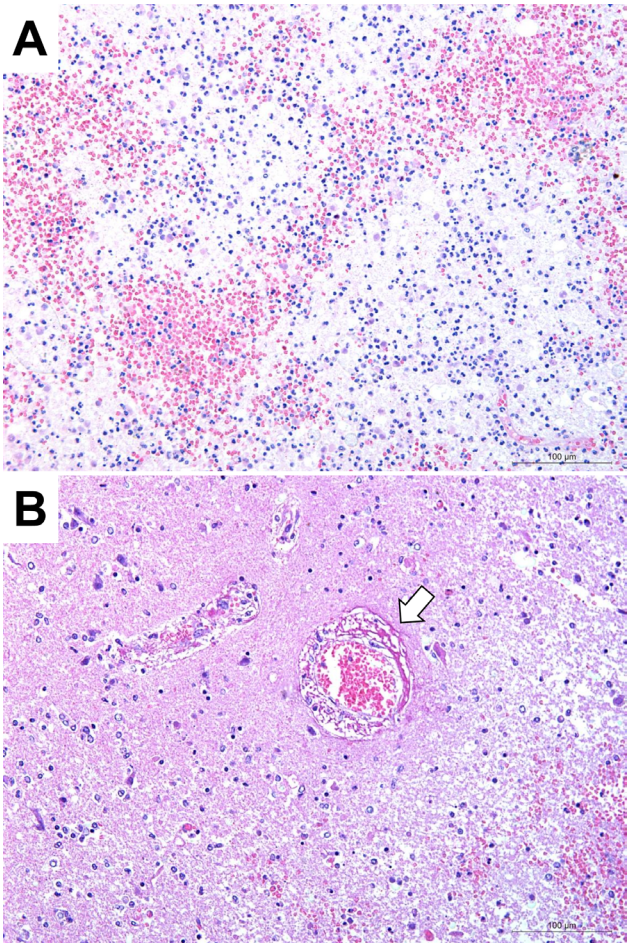


**Figure 2**. Brain stem of a dog poisoned by diminazene aceturate. A- Focal, extensively intense hemorrhage associated with marked neutrophilic inflammatory infiltrate (H&E, 200X). B- Arterioles and venules with expanded Virchow-Robin spaces, occasional transmural neutrophilic inflammatory infiltrate, deposition of bright eosinophilic fibrillar and amorphous material below the endothelium (H&E, 200X).

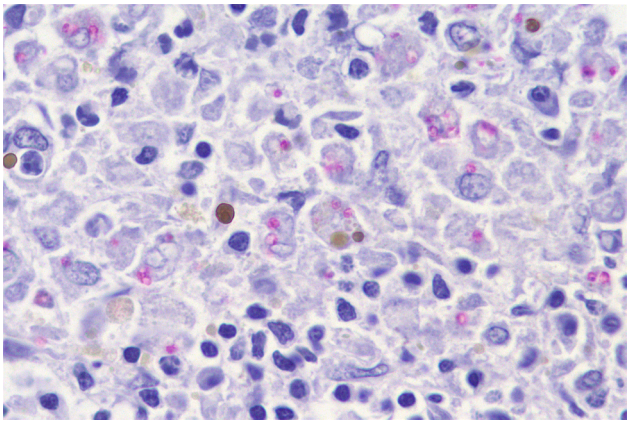

**Figure 3**. Spleen of a dog poisoned by diminazene aceturate showing subcapsular macrophages with intracytoplasmic amastigotes immunolabelled in magenta (Magenta chromogen, 1000X).

Braz J Vet Pathol, 2024, 17(3), 207-212
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Chromatographic analyses were performed according to the methodology previously described (13). An HPLC system (Shimadzu Prominence LC-20A) equipped with a diode array detector (SPD-M20A) and a C18 column (Welch Welchrom, 4.6 x 100 mm, 5 µm) with a pre-column of the same material was used. The mobile phase consisted of acetonitrile and 5 mM sodium 1-octanesulfonate in 1% acetic acid aqueous solution (30:70) at a flow rate of 1.0 ml/min. Detection was carried out at 370 nm, with UV absorbance spectra recorded in the 190 to 600 nm range. The limit of detection was 0.5 µg/g, and the limit of quantification was 2.0 µg/g. The concentrations of diminazene found in the liver and kidney samples were 123.7 µg/g and 36.1 µg/g, respectively (Figure 4). Furthermore, the clinical, pathological, and toxicological findings observed in this case support the diagnosis of diminazene aceturate poisoning.

## Discussion

This study reports a case of diminazene aceturate poisoning in a dog, providing contributions with clinical, pathological, and toxicological characterization, and enhancing the understanding of this relatively unknown poisoning in veterinary medicine.

Diminazene aceturate is known to cause encephalic damage in dogs, although the pathophysiology of this process is not well known. Besides its use as an antiparasitic drug, diminazene aceturate has also been studied for its ability to modulate the activity of angiotensin-converting enzyme 2 (ACE2). It features a small molecule with a chemical structure similar to ACE2 activator known as xanthone (XNT 18, 19) and promotes the production of a vasodilatory peptide (16). ACE2 is a crucial enzyme in the renin-angiotensin system (RAS), which regulates blood pressure, fluid balance, and cardiovascular function. ACE2 counterbalances the vasoconstrictive and pro-inflammatory effects of angiotensin-converting enzyme (ACE) and angiotensin II. ACE2 converts angiotensin II, a potent vasoconstrictor, into angiotensin (1-7), which has vasodilatory and anti-inflammatory effects. This process is essential for maintaining hemodynamic balance and protecting blood vessels from damage (9,15,16,19). When ACE2 activity is modulated by diminazene aceturate, it potentially increases the production of angiotensin 1-7 (16), and this effect could lead to a reduction in blood pressure and destabilize hemodynamic balance. Excessive vasodilation could result in significant hypotension, leading to endothelial injury and tissue hypoperfusion. Additionally, angiotensin 1-7 exerts biological effects by activating the Mas receptor. Activation of the Mas receptor is known to induce the production of nitric oxide (NO) in endothelial cells (15). NO, in turn, is an important modulator of the inflammatory cascade (10). Therefore, excessive activation of ACE2, angiotensin 1-7, and the Mas receptor could lead to endothelial injury and inflammation.

Some studies correlate the toxicity of diminazene aceturate to the central nervous system (CNS) with its dosage, indicating that repeated doses may lead to CNS toxicity due to cumulative effects. Research shows that diminazene aceturate binds to plasma and is widely distributed throughout the body, but it remains uncertain how this affects tissue concentrations in the brain or influences transport mechanisms at the blood-brain barrier (11). After intramuscular administration, the compound is rapidly distributed and stored in the liver, followed by a slower terminal phase, during which the drug is redistributed to peripheral tissues and/or excreted by the kidneys. The excretion from the body has a generally quick half-life of 5.31 hours. However, the rate of distribution and

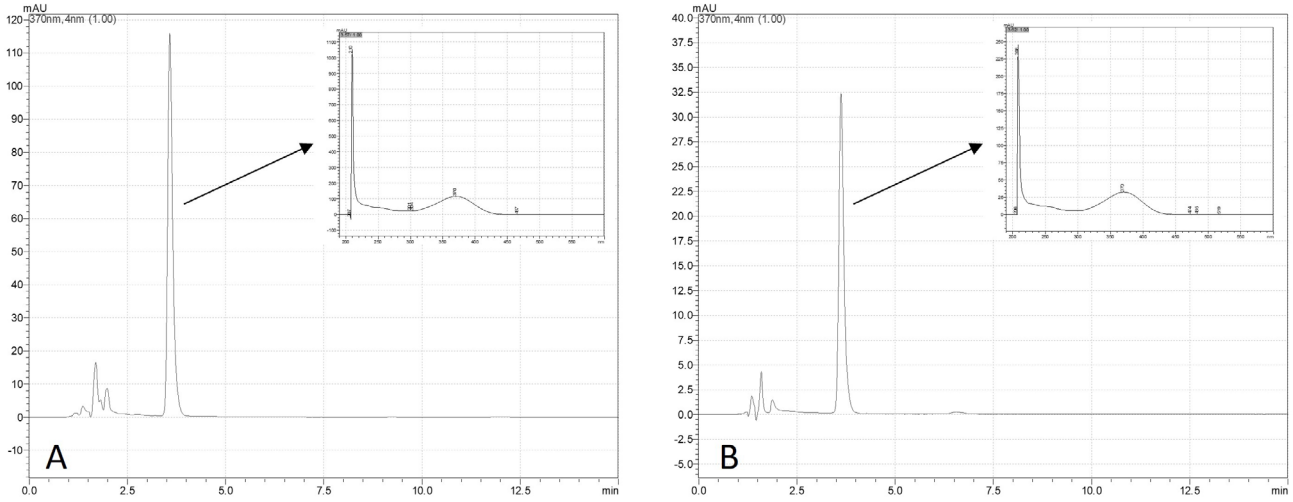


**Figure 4**. A- Liver: chromatogram showing the main peak corresponding to diminazene aceturate with a retention time of 2.65 minutes and a concentration of 123.7 µg/g. B- Kidney: chromatogram showing the diminazene peak with a retention time of 2.65 minutes and a concentration of 36.1 µg/g. The upper right corner of each graph displays a UV-Vis absorbance spectrum (190-600 nm) with a maximum absorbance near 370 nm, characteristic of diminazene, confirming the compound's identity.

Braz J Vet Pathol, 2024, 17(3), 207-212
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excretion may vary among dogs, and concurrent diseases should be evaluated. Due to this distribution and excretion profile, the intramuscular administration of 4.2 mg/kg should not be repeated within a 21-day interval to avoid cumulative effects and CNS damage (11). The animal in this case received daily oral doses of the medication, in addition to two intramuscular doses with interval of 10 days, which probably favored the poisoning. Unfortunately, we do not know the concentration of the preparation used. The values of diminazene aceturate observed in the liver and kidney of the dog of this study indicate an abnormally high concentration of diminazene, which is consistent with reported cases of poisoning. Previous studies have shown that significantly lower concentrations are observed in the liver (12.91 ± 2.71 µg/g) and kidneys (12.91 ± 2.71 µg/g) 240 hours after treatment with the recommended therapeutic dose of 3.5 mg/kg (14).

Poisoning by diminazene aceturate has been previously documented in different species, including camelids (17), mice (8), and dogs (3,5). Consistent with observations in other studies, the animal in this report exhibited neurological clinical signs, hemorrhage, and fibrinoid necrosis of the brainstem vessels, pulmonary edema and hemorrhage, congestive lymphadenomegaly, and hemorrhages in other organs (2,3,5,17). In Brazil, diminazene aceturate is sold freely and widely used in veterinary medicine, frequently found in agricultural and veterinary establishments. In contrast, in the United States, the use of diminazene aceturate is prohibited by the FDA (Food and Drug Administration) (3). The literature does not report a specific antidote for diminazene poisoning. Therefore, treatment should focus on symptomatic management of adverse effects. The approach should be individualized, with an emphasis on stabilizing the patient and alleviating clinical signs.

The pathological findings observed in this case are consistent with those described in the literature regarding diminazene aceturate poisoning. A diagnosis should be performed considering the animal's clinical history, pathological findings, and the results of the toxicological examination. This work expands the literature and knowledge on poisoning by this drug, while also highlighting the need for toxicological evaluation for a definitive diagnosis.

## Conflict of Interest

The authors declare no competing interests.

## References

1. BRASIL MDS. Manual de vigilância e controle da leishmaniose visceral. Brasília: Ministério da Saúde; 2006. 120 p.
2. Brolo D, Bisso A, Krauspenha C, Alves DA. Intoxicação por aceturato de diminazeno em cães: O que é preciso saber? Rev Cienc Tecnol. 2015;1(1):29-39.
3. Flores MM, Pereira PR, Mazzanti A, Kommers GD, Fighera RA. Aspectos epidemiológicos, clínicos e anatomopatológicos da intoxicação por aceturato de diminazeno em cães. Pesq Vet Bras. 2014;34(7):667-74. doi: 10.1590/S0100-736X2014000700011.
4. Gonçalves G, Campos MP de, Gonçalves AS, Figueiredo FB. Therapeutic success and failure in using miltefosine to treat dogs naturally infected with Leishmania infantum. Braz J Vet Parasitol. 2024;33(1): e015023. doi: 10.1590/S1984-29612024012.
5. Han D, Yoon WK, Hyun C. Cerebellar encephalopathy from diminazene aceturate (Beneril) toxicity in a dog. Korean J Vet Res. 2014;54(3):193-6. doi: 10.14405/kjvr.2014.54.3.193.
6. Jean-Moreno V, Rojas R, Goyeneche D, Coombs GH, Walker J. *Leishmania donovani*: Differential activities of classical topoisomerase inhibitors and antileishmanials against parasite and host cells at the level of DNA topoisomerase I and in cytotoxicity assays. Exp Parasitol. 2006;112(1):21-30. doi: 10.1016/j.exppara.2005.08.014.
7. Khristianovsky P, Belimenko V. Technology for the use of reduced doses of diminazene aceturate for the treatment of pet babesiosis. In: International Research Conference on Challenges and Advances in Farming, Food Manufacturing, Agricultural Research and Education. KnE Life Sciences. 2021;240-6. doi: 10.18502/kls.v0i0.8953.
8. Kuriakose S, Muleme HM, Onyilagha C, Singh R, Jia P, Uzonna JE. Diminazene aceturate (Berenil) modulates the host cellular and inflammatory responses to *Trypanosoma congolense* infection. PLoS One. 2012;7(11):e48696. doi: 10.1371/journal.pone.0048696.
9. Kuriakose S, Uzonna JE. Diminazene aceturate (Berenil), a new use for an old compound? Int Immunopharmacol. 2014;21:342-5. doi: 10.1016/j.intimp.2014.05.027.
10. Laroux FS, Pavlick KP, Hines IN, Kawachi S, Harada H, Bharwani S, Hoffman JM, Grisham MB. Role of nitric oxide in inflammation. Acta Physiol Scand. 2001;173:113-8. doi: 10.1046/j.1365-201X.2001.00891.x.
11. Miller DM, Swan GE, Lobetti RG, Jacobson LS. The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs. J S Afr Vet Assoc. 2005;76(3):146-50. doi: 10.4102/jsava.v76i3.416
12. Mutua F, Kihara A, Rogena J, Ngwili N, Aboge G, Wabacha J, Bett B. Piloting a livestock identification and traceability system in the northern Tanzania–Narok–Nairobi trade route. Trop Anim Health Prod. 2018;50:299-308. doi: 10.1007/s11250-017-1431-4.
13. Naidoo V, Mulders MSG, Swan GE. The intravenous pharmacokinetics of diminazene in healthy dogs. J S Afr Vet Assoc. 2009;80(4):215-9. doi: 10.4102/jsava.v80i4.210.

14. Onyeachonam FO, Onyeyili PA, Saganuwan AS, Agbo OJ. Diminazene aceturate concentrations in tissues of dogs co-treated with oxtetracycline long acting. J Sustain Vet Allied Sci. 2021;1(1):41-6. doi: 10.54328/covm.josvas.2021.010a.
15. Phillips MI, de Oliveira EM. Brain renin angiotensin in disease. J Mol Med. 2008;86:715-22. doi: 10.1007/s00109-008-0331-5.
16. Qaradakhi T, Gadanec LK, McSweeney KR, Tacey A, Apostolopoulos V, Levinger I, Rimarova K, Egom EE, Rodrigo L, Kruzliak P, Kubatka P, Zulli A. The potential actions of angiotensin-converting enzyme II (ACE2) activator diminazene aceturate (DIZE) in various diseases. Clin Exp Pharmacol Physiol. 2020;47:751-8. doi: 10.1111/1440-1681.13251.
17. Seixas JN, Orlando DR, Wouters F, Wouters ATB, Varaschin MS, Raymundo DL. Aspectos patológicos da intoxicação por aceturato de diminazeno em camelídeos sul-americanos. Pesq Vet Bras. 2017;37(12):1509-13. doi: 10.1590/S0100-736X2017001200024.
18. Oliveira GL, Freitas RM. Diminazene aceturate - An antiparasitic drug of antiquity: advances in pharmacology & therapeutics. Pharmacol Res. 2015;102:138-57. doi: 10.1016/j.phrs.2015.10.005.
19. Xu P, Sriramula S, Lazartigues E. ACE2/ANG-(1–7)/Mas pathway in the brain: the axis of good. Am J Physiol Regul Integr Comp Physiol. 2011;300(4):R804-17. doi: 10.1152/ajpregu.00222.2010.