Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2022, Vol 2, Issue 2:143-151

Review

A review on therapeutic activities of diminazene aceturate

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ABSTRACT

Diminazene aceturate is the drug most commonly used for the treatment of trypanosome infections in cattle, sheep and goat. It has been reported to also have anti-babesial, anti-inflammatory, anthelmintic, anti-viral and anti-bacterial activities unlike other trypanocides. The drug is an aromatic diamidine compound popularly marketed as Berenil®. Retention of high concentrations of diminazene aceturate in plasma and tissues can be enhanced by combining it with other drugs such as Oxytetracycline long acting. The enhanced concentrations can lead to prevention of relapse of treated trypanosome infections and it can also act as a prophylactic treatment against trypanosomosis in dogs. The present review discussed current status of knowledge concerning therapeutic spectrum of diminazene, its chemical structure, physical properties, mechanism of actions, contraindications, formulations and combination therapeutic regimens in which diminazene has been administered together with other compounds. Analytical techniques for diminazene, the pharmacokinetics of diminazene, its toxicity, and clinical uses in livestock are also discussed.

Keywords: Anthelmintic; anti-bacterial; anti-inflammation; anti-viral properties; diminazene aceturate

INTRODUCTION

Diminazene aceturate (Berenil®) is anti-protozoan agent, widely used to treat babesiosis (Barcelo *et al.*, 2000) and trypanosomosis (Sykes & Papich, 2014). It is an aromatic diamidine compound discovered in 1944 (Fussganger, 1995), developed from a drug called "Congasin" and other aminoquinolidines. It was found to be active against trypanosomes and some babesias (Hwang *et al*., 2010). It is commercialized as Azidin®, Berenil®, Ganasag® or Pirocide® Veriben, Dimisol among others (Swan, 2002).

Diminazene aceturate is probably the most commonly used therapeutic agent for trypanosomosis in livestock in Sub-Saharan Africa even in Nigeria (Geerts & Holmes, 1998). Diminazene aceturate is also an angiotensin-converting enzyme 2 (ACE2) activator and has strong and potent anti-inflammatory properties (Castardeli *et al*., 2018)

Moreover, there are many contradictory reports regarding the toxicity and clinical benefits of diminazene aceturate thus this review was done to expound other therapeutic activities and off label effects of diminazene aceturate.

CHEMICAL STRUCTURE OF DIMINAZENE ACETURATE

Diminazene aceturate (figure 1) is an N-acetyl glycine compound chemically described as 4-4' (diazoamino) dibenamidine diaceturate; 1, 3–bis (p–amidinophenyl) triazene bis (N–acetylglycinate) diaceturate; 1, 3 bis (4–guanylphenyl) triazene diaceturate; and 4–4'–diamidinodiazoaminobenzene diaceturate, (Martindale *et al*., 1989).

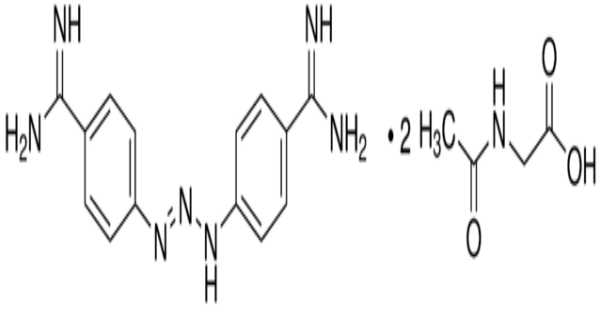

Figure I. Structure of Diminazine aceturate
Peregrine & Mamman. (1993)

http://doi.org/10.54328/covm.josvas.2022.070

PHYSICAL CHARACTERISTICS OF DIMINAZENE ACETURATE

Diminazene aceturate has a molecular weight of 515.5 Da and decomposes at 217 ^{o}C (Castardeli *et al.,* 2018). It is soluble in 14 parts of water at 20 ^{o}C and slightly soluble in alcohol, ether and chloroform (Martindale, 1989). Due to the fact that diminazene aceturate consists of an organic base and an organic acid, once it is dissolved in water, it dissociates and each component has its own characteristics. Hence, it is an amphoteric compound (Martindale, 1989).

PHARMACOLOGICAL PROPERTIES AND OFF-LABEL EFFECTS OF DIMINAZENE ACETURATE

MECHANISM OF ACTION

The exact mechanism of action and *in vivo* behaviour of diminazene aceturate is still poorly understood. Its effect on the *Babesia* parasite appears to relate to interference with aerobic glycolysis, and synthesis of deoxy-ribonucleic acid (DNA) in the parasite (Ariyibi *et al.,* 2001). Diminazene aceturate binds to the AT-rich regions of nucleic acid duplexes. Binding occurs via complexation into the minor grooves of AT-rich domains of the DNA double helices. It can also bind to DNA as well as RNA duplexes causing intercalation and minor groove binding. The binding unwinds negative supercoils in plasmids and interfere with the activities of the eukaryotic type II topo-isomerases enzymes (Gutierrez *et al*., 2013). A concentration-dependent inhibition of membrane Ca^{++}-ATPase activity, and significant secondary binding of diminazene aceturate within DNA corresponding to G+C rich sites have also been reported (Barcelo *et al.,* 2000).

ANTI-INFLAMMATORY MECHANISM OF ACTION OF DIMINAZENE ACETURATE

Diminazene aceturate performs this function by acting as an angiotensin-converting enzyme 2 (ACE2) activator and this made it to have strong and potent anti-inflammatory properties (Kuriakose *et al.,* 2012). Treatment with Berenil reduces pro-inflammatory cytokine (IL-6, IL-12 and TNF) production in macrophages *in vivo* and *in vitro* following stimulation with *Trypanosoma congolense*, lipopolysaccharide (LPS), unmethylated bacterial CpG motifs (Kuriakose *et al.,* 2014). This global effect was not due to downregulation of Toll-like receptor (TLR) expression on innate immune cells. Instead, Berenil significantly reduced phosphorylation of mitogen activated protein kinases (Mitogen-activated protein kinase MAPKs, including Extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 MAP kinase (p38), signal transducer and activator of transcription (STAT) proteins (including STAT1 and STAT3) and nuclear factor-kappaB (NFκB p65) subunit, key signaling molecules and transcription factors involved in the production of proinflammatory cytokines (Castardeli, 2018). The ability of Berenil to reduce major intracellular signaling pathways that lead to proinflammatory cytokine production suggests that it could be used to treat conditions caused by excessive production of inflammatory cytokines.

It reduces liver injury by causing massive decrease in AST and ALT enzymes in schistosomiasis infected rats (De Brito *et al*., 2020).

ANTI-BACTERIAL MECHANISM OF ACTION OF DIMINAZENE ACETURATE

A study investigated the bacteriostatic and bactericidal effects of diminazene aceturate (DA) against five strains of pathogenic bacteria (*Escherichia coli (E.coli),* O157:H7) and two strains of non-pathogenic bacteria (commensal *E. coli)* (Wu *et al*., 2016). The results showed the ability of DA to inhibit or suppress the growth of *E. coli* O157:H7 via reactive oxygen species (ROS) accumulation (Wu *et al*., 2016). Increase in intracellular ROS levels decrease extracellular peroxidase activity making the host bacteria more susceptible to external ROS attack. Elevated ROS levels will reduce membrane integrity of the bacteria making lysis/death easier (Martinez-Castillo and Munies, 2014). In addition, the study revealed that DA is an effective uncoupler of the proton motive force (PMF) in more than one strain of *E. coli* O157:H7 and that the underlying mechanism does not induce the production of (Vertoxins) VTs. Therefore, it is suggested that DA is a suitable antibacterial agent for *E. coli* O157, because it can inhibit and kill different strains of Shiga-toxin-producing *E. coli* (STEC) at various stages of the fission cycle (Martinez-Castillo and Munies, 2014). Diminazene aceturate is reported to selectively inhibit the growth of commensal *E. coli* by binding to the ribosomal subunits (Bielawski *et al.,* 2008).

Antibacterial effect of diminazene aceturate has been utilised to inhibit the growth of *Brucella specie* in culture (Marangoci *et al*., 2019).

ANTHELMINTHIC PROPERTIES

De Brito *et al*., (2020) reported that diminazene aceturate has *in vitro* anthelmintic properties against different stages of intravascular parasitic *Schistosoma mansoni* in a time- and concentration-dependent manner. Morphological studies revealed substantial tegumental alterations in adult parasites (De Brito *et al*., 2020). In vivo, it markedly reduced worm and egg burdens in mice harboring chronic *Schistosoma mansoni* infections by their ability to interfere with the blood-feeding parasite's ability to detoxify heme and by their cytotoxicity effects. Since the liver carries the main burden of *Schistosoma mansoni* infection, we found that diminazene aceturate achieved significant reductions in hepatosplenomegaly and lowered ALT and AST levels, suggesting that the drug reduces liver injury (De Brito *et al*.,

2020). Diminazene aceturate has moderate, but significant, antischistosomal properties in early S. mansoni infection (De Brito *et al*., 2020).

ANTI- VIRAL MECHANISM OF ACTION OF DIMINAZENE ACETURATE

Diminazene aceturate has been reported to improve the clinical outcome of COVID-19 virus also known as coronavirus (SARS COV-2) due to its: (1) Angiotensin converting enzyme 2 (ACE2) activation, (2) anti-inflammatory profile, and (3) known tolerance in humans (Oliveira and Frietas, 2015). Wang and Cheng (2020) reported that COVID-19 causes depletion of ACE2 in lung tissue, a process that could accelerate their replication and spread. It was noted that diminution in ACE2 levels increased the level of Ang II, a pro-inflammatory peptide when it binds to AT1 receptors (Kuba *et al.*, 2005). This explains the painful breathing and respiratory distress syndrome observed in COVID-19 infection. Diminazene aceturate ameliorates these effect by activating ACE2, which stimulates the protective axis of the renin-angiotensin system (RAS), leading to the cleavage of Ang II. ACE2 metabolizes Ang II to Ang-(1–7) and thus counter regulates the deleterious effects of Ang II (Kulemina & Ostrov 2011). Fang and coworkers have demonstrated the beneficial effects of DA in pulmonary disorders in animal models by regulating NF-κB and Nrf2 gene expression routes which encode proinflammatory cytokines that compromise lung function (Fang *et al*., 2019).

CONTRAINDICATIONS OF DIMINAZENE ACETURATE

Diminazene aceturate is contraindicated in animals with known hypersensitivity to diminazene and/or phenazone (Homeida *et al*., 1981). It should not be used in animals with an impaired renal and/or hepatic function and in camels (Homeida *et al*., 1981).

FORMULATIONS OF DIMINAZENE ACETURATE

Diminazene aceturate (DA) is composed of 45 % m/m diminazene diaceturate and 55 % m/m antipyrine (Ahmed, 2015). Aqueous solutions of pH 7 were prepared by adding sterile water to a calibrated container containing 2.36 g of berenil® granules (which contains 1.05 g of the active component) up to the 25 ml mark. Therefore the 25 ml berenil® solution contained 1.05g of diminazene aceturate. This resulted in a 42 mg/ml of diminazene aceturate. The solution was administered at a dose rate of 4.2 mg/kg (Ahmed, 2015). That means 1.05g of DA in 25ml solution = 1050 mg of DA in 25ml. Therefore concentration of the drug solution will be 1050mg/25ml = 42mg/ml.

In most formulations, antipyrine is added to diminazene acturate at a concentration of 55 % m/v as a stabiliser, since diminazene aceturate is unstable in water. This represents a dose of 5.24 mg/kg of antipyrine when diminazene aceturate is administered at its recommended dose (Ahmed, 2015). This is 2-4 folds lower than the dose of antipyrine (10 - 20 mg/kg) administered intravenously (Boothe, 1994). Antipyrine is one of the most extensively used compounds in testing of the oxidative drug metabolising systems of the liver (Janus and Antoszek, 1999). It is metabolized by cytochrome P-450 linked monooxygenase. Antipyrine is negligibly bound to tissue and plasma proteins (Janus and Antoszek, 1999).

COMBINATION REGIMENS

Since most of trypanocidal compounds used for human trypanosomiasis have a low therapeutic index, drug toxicity is often observed (Jennings, 1993). With the interest to reduce drug doses, and the incidence of toxicity, diminazene aceturate has been combined with a variety of compounds to ascertain if such combination would have an additive or synergistic effect. Combination with various nitroimidazoles was shown to have an additive effect against *T. b. brucei* infections in mice (Jennings *et a*l., 1980) and against *T. simiae* infections in pigs that appear to have accessed the central nervous system (CNS). Similar effects have been demonstrated with diminazene aceturate in combination with either difluoromethylornithine (Jennings, 1992) or Suramin (Williamson et al., 1982) for the treatment of T.brucei species caused central nervous system infection. The molecular bases for these observations have not been clarified but it could be due to inhibition of different biochemical pathways. Furthermore, combination with either mepyramine maleate or piroxicam has been shown to enhance the therapeutic activity of diminazene aceturate against *T. vivax* infections in sheep (Joshua and Babalola, 1983) and *T. b. brucei* infections in mice (Abatan, 1991), respectively. Since both mepyramine and piroxicam are anti-inflammatory compounds, their co-administration with diminazene aceturate may increase the bioavailability of the trypanocide.

Diminazene has been co-administered with oxytetracycline long acting in healthy dogs and it produced a synergistic effects that brought about increased concentrations of diminazene aceturate in plasma (Onyeachonam *et al.,* 2022) and sampled tissues (Skeletal muscle, brain, kidney, heart and liver) for up to 20 days post drug administration (Onyeachonam *et al.,* 2021).

Eke *et al*. (2017) observed greater efficacy with the combination therapy of secnidazole and diminazene aceturate in the treatment of rats and dogs experimentally infected with *Trypanosoma brucei.*

ANALYSIS OF DIMINAZENE ACETURATE

Multiple analytical methods have been used to quantify concentrations of diminazene aceturate in plasma, blood and

tissues of animals. A colorimetric method with a relatively low level of sensitivity of 0.5 lag/ml (Raether *et al*., 1972) was used to investigate the pharmacokinetics of diminazene in rats (Raether *et al*., 1972), monkeys (Raether *et al*., 1974), cattle (Klatt & Hajdu, 1976) and dogs (Anika & Onyeyili, 1989; Onyeyili & Anika, 1991). Using a bioassay method, disposition of diminazene aceturate was studied in cattle (Cunningham *et al.,* 1964) and rabbits (Goodwin & Tierney, 1977). In order to increase the sensitivity of diminazene aceturate assays, radiometric methods were described by Gilbert (1983) and Kellner *et al*. (1985). Although these assays can detect diminazene aceturate in concentrations as low as 0.028 I~g/ml of plasma, or 0.18 ~g/g of tissues, they are deficient in specificity/accuracy, since they measure total radioactivity and do not distinguish intact from modified drug (Aliu & Odegaard, 1983). Another method of examining diminazene aceturate is Gas chromatography mass spectrometry by (Fouda, 1978), high-performance liquid-chromatography (Aliu & Odegaard 1983; Onyeachonam *et al.,* 2021) and thin-layer chromatography (Gluth *et al.,* 1986) have proven the most sensitive and specific methods for extraction and detection of diminazene.

PHARMACOKINETICS OF DIMINAZENE ACETURATE IN VARIOUS SPECIES OF ANIMALS

RABBITS AND RATS

A biphasic pharmacokinetics with maximum blood and interstitial fluid concentrations after 15 min in rabbits following an intramuscular injection of 3.5 mg/kg was reported (Gilbert, 1983). The concentration of diminazene aceturate in *Trypanosoma brucei brucei* infected rats after an intramuscular injection of 3.1 mg/kg was significantly higher in the organs of infected compared to non-infected rats (Gilbert, 1983). Concurrent administration of lithium chloride with diminazene aceturate significantly increased the concentration of diminazene aceturate in the brain tissue of the rats (Odika *et al*., 1995).

GOATS AND SHEEP

In dairy goats 60-90% of diminazene aceturate was bound to plasma proteins, having an elimination half-life of 14 – 30 h. After administration of diminazene aceturate to sheep, peak plasma concentrations of 6.3 –7.57 µg/ml at 20 – 45 min were reported (Aliu & Olegaard, 1984). Malgwi (2021) reported that the intramuscular treatment of diminazene aceturate alone and its combination with oxytetracycline long acting produced peak concentration of 6.91±0.34 and 7.55± 0.26 µg/ml, respectively in goats, with Mean residence time (MRT) of 19.70±2.53 and 25.11±1.81 h, respectively. The pharmacokinetic evaluation of the drug showed that the data fitted a two compartment open model (Malgwi, 2021).

CATTLE

Pharmacokinetics of a combination of diminazene aceturate and rolitetracycline, gave a peak concentration of 3.23 µg/ml of diminazene aceturate after 30 min and a second phase of elimination of diminazene aceturate with a half-life of 63 h, respectively. The elimination half-life was considerably shortened by diminazene aceturate (Klatt and Hadju, 1976). Radiolabeled intramuscular diminazene aceturate at 3.5 mg/kg gave peak blood concentrations of 4.6 and 4.7 µg/ml after 15 and 40 min and half-lives of 2 h and 188 h, respectively (Kellner *et al*., 1985).

The intravenous bolus showed a biphasic distribution with half-lives of 0.04 h (intramuscular) and 0.58 h (intravenous), respectively. Diminazene aceturate was rapidly absorbed following i.m. administration and peak plasma concentrations (Cmax) of 4.68 ± 1.12 µg/ml was attained in 10-15 min (Aliu *et al*., 1993). The half–life of elimination phase was 145.48 h. The diminazene aceturate was partitioned 30 minutes after administration between plasma, whole blood and red blood cells at a ratio of 6.65 ± 0.06; 5.02 ±0.27 and 1.93 ±0.87, respectively. The partition changed after 12 h to 1.24 ± 0.08; 1.60 ±0.07 and 1.99 ± 0.44, respectively. This showed that most of the diminazene aceturate was in the plasma 30 min after treatment, but 12 h after large quantity of diminazene aceturate in the blood was bound to red blood cells. Diminazene aceturate was bound to bovine plasma albumin *in vitro* to the extent of 38.01–91.10% (Aliu *et al*., 1993).

The pharmacokinetics of diminazene aceturate (3.5 mg/kg) was compared between non-infected and *T. congolense* infected cattle. The maximum concentration (8.25 ± 1.72 µg/ml) of the diminazene aceturate in plasma was significantly higher in animals with acute infection as compared to animals with chronic infection (5.04 ± 0.26 µg/ml) and the non-infected cattle (4.76 ± 0.76 µg/ml), respectively. Similarly, the maximum concentration time (Tmax) was significantly shorter in the acute infection (18 min) as compared to chronic (36 min) and non-infected (33.75 min), respectively, (Mamman *et al*., 1993).

Two diminazene aceturate (3.5 mg/kg) formulations administered i.m. in cattle followed a two-compartmental model. Peak concentration of diminazene aceturate (3.24 ± 0.16 µg/ml) was reached at 49.8 ± 7.6 min, having absorbtion half-life of 1.93 ± 0.95 hours. Diminazene aceturate was slowly eliminated with a residence time of 13.27 days and a long elimination half-life of 222 h, respectively (Gummow *et al*., 1994).

Infected cows with *T. congolense* treated with different doses of diminazene aceturate indicated that, level of parasitaemia and degree of anaemia affected distribution, disposition and elimination of diminazene aceturate (Mdachi *et al*., 1995). The pharmacokinetics of diminazene aceturate in the plasma, cerebro-spinal fluid and lymph of goats following a 3.5

mg/kg i.m. injection of diminazene aceturate showed peak concentration of 4.31µg/ml in the plasma. The diminazene concentration in the cerebro-spinal fluid was 3-4 times lower than in the plasma. A median peak plasma concentration of 4.30 µg/ml was detected in the lymph (Mamman *et al*., 1996).

DOGS

Effect of *T. congolense* on disposition kinetics of diminazene aceturate followed a biphasic process. The infection significantly shortened the absorption half-life (T½α) of diminazene aceturate from 0.17 h to 0.12 h, although the urinary excretion of the drug remained constant (Onyeyili & Anika, 1991). Intramuscular diminazene aceturate (3.5 mg/kg) to both healthy dogs and dogs with trypanosomosis gave plasma concentrations of 0.2 ± 0.008 µg/ml. No diminazene aceturate was found in the plasma after 48 hours (Onyeyili & Anika, 1989). Higher plasma concentrations were found in dogs infected with trypanosomes, and higher tissue concentrations were present in healthy dogs. In all tissues sampled at 48 h, the highest concentrations of diminazene aceturate were found in the kidneys and liver, and low concentrations were found in the brain. Diminazene aceturate persisted in the tissues for more than 10 days, (Onyeyili & Anika, 1989). Elimination half-life of 9.87 h in healthy dogs and 12.51 h, in *Trypanosome brucei brucei* infected dogs were reported. The T½α was significantly decreased from 0.2 h to 0.14 h, in dogs after trypanosome infection (Onyeyili & Anika, 1991).

Onyeachonam *et al.* (2022) reported that intravenous treatment of diminazene aceturate alone and its combination with oxytetracycline long acting intramuscularly to healthy dogs gave peak plasma concentration of 20.74±.61 µg/ml and 20.10±.46 µg/ml, respectively with MRT of 9.81±0.75 h and 9.98±0.43 h respectively. Also, No diminazene was found in the plasma after 48 hours (Onyeachonam *et al*., 2022). In all tissues sampled from 240 hours to 480 hours, the highest concentrations of diminazene aceturate were found in the brain, followed by the skeletal muscles and then the liver of dogs administered diminazene combined with oxytetracycline long acting while lower concentrations of diminazene were found in the heart, followed by kidney and lowest concentration in the brain of dogs administered diminazene aceturate alone. High concentrations of diminazene aceturate persisted in sampled tissues for more than 20 days post drug administration, with elimination half-lives of 2.15±0.25 and 3.15±0.13 for diminazene alone and its combination with oxyteracycline long acting, respectively (Onyeachonam *et al*., 2021). The pharmacokinetic evaluation of the drug showed that the data fitted a two compartment open model (Onyeachonam *et al*., 2022).

CLINICAL USES OF DIMINAZENE ACETURATE

(1) Diminazene aceturate co-administered with oxytetratracycline long acting can be used to treat relapse of of treated trypanosomes infection. In a study done by Onyeachonam *et al*., 2022 with apparently healthy dogs administered diminazene aceturate plus oxytetracycline long acting increased the Cp^o of diminazene aceturate to 34.79± 1.74 µg/ml as compared to 26.25± 1.81 µg/ml for diminazene aceturate treated dogs. Oxytetracycline long acting enhances penetration of diminazene aceturate through the blood brain barrier (Onyeachonam *et al*., 2021). This explained the cause of reported toxicity signs often observe when the two drugs are concurrently used but this is also beneficial in the sense that the drug combination can be used to treat relapse of trypanosomes infection.

(2) Diminazene aceturate when combined with oxytetracycline long acting can be used as a prophylactic drug against trypanosomosis. Onyeachonam *et al*., 2021 observed that high concentrations of diminazene aceturate persited in the sampled tissues of apparently dogs administered diminazene aceturate with oxytetracycline long acting for up to 20 days post treatment. Thus suggesting that the two drugs combination can be given to healthy dogs as prophylaxis to protect them from trypanosomosis (Onyeachonam *et al*., 2021).

(3) Diminazene aceturate is indicated for the therapeutic treatment of trypanosomosis, babesiosis, mixed haemoprotozoan infections and theileriosis in cattle, buffalos, sheep, goats, horses and dogs (Lee *et al*., 2009).

(4) It is also used in the treatment of cytauzoonosis (Kuriakose *et al*., 2012).

(5) It is useful in the treatment of many diseases such as coronary and cerebral infarction, vascular clotting, arthritis, tumor cell invasion, and pancreatitis (Kuriakose and Uzonna, 2014).

(6) It acts as an anti-inflammatory drug. Recent studies have demonstrated the beneficial effect of diminazene aceturate in liver diseases (Rajapaksha *et al* 2018), and this drug could provide therapeutic benefits for the treatment and control of several chronic inflammatory diseases (Malek and Nematbakhsh, 2014).

(7) Diminazene aceturate as an angiotensin-converting enzyme 2 can be used to treat COVID-19 and other related viral diseases (Kuba *et al*., 2005).

(8) It can be used to treat blood fluke or schistomiasis (De brito *et al*., 2020)

(9) Diminazene aceturate has antibacterial properties against *Escherichia coli* (Wu *et al*., 2016).

TOXICITY AND SIDE EFFECTS

Pharmacological studies have shown that diminazene aceturate lowers blood pressure for few minutes when given

intravenously to dogs and cats, by causing peripheral vasodilation (Doogne and Polasek, 2003). However, such decreased blood pressure was not observed when diminazene aceturate was administered intramuscularly (Onyeyili & Egwu, 1995). Parenteral administration of diminazene aceturate occasionally resulted in vomiting and diarrhoea (Naude *et al.,* 1970). Mild erythema was observed after 24 hr and disappeared within 8 days (Gilbert, 1983). Diminazene aceturate at 3.5 mg/kg caused mild transient swelling at the injection site (Baylis & Stevenson, 1998), whereas Losos and Crockett (1969) reported mild intramuscular oedema at the site of injection 1-3 days after intramuscular diminazene aceturate administration to dogs that eventually died of babesiosis or trypanosomosis. Bleeding and malacia in the mesencephalon and diencephalon were observed (Crockett 1969). Treatment of healthy dogs with 15 mg/kg of intramuscular diminazene aceturate was observed to induce brain lesions which were similar to those of dogs naturally infected with babesiosis (Losos and Crockett, 1969). The highest tolerated dose of diminazene aceturate in healthy dogs was 20 mg/kg intramuscularly and 12.5 mg/kg intravenously (Baylis and Stevenson, 1998). Tremor, nystagmus and ataxia were observed at lower doses, while higher doses resulted in spasms, uncoordinated movements, vomiting and eventually death in dogs 2–3 days after a dose of 30–35 mg/kg of diminazene intramuscularly. Diminazene aceturate administered at 50 mg/kg intramuscular daily for 5 days at intervals 11, 12, 18, 19, 20, was well tolerated (Zanger & Schwab, 2013) and healthy dogs administered ten doses of diminazene within 25 days at 50 mg/kg intramuscularly did not show toxicity signs (Hawking, 1958). However, diminazene aceturate was reported to have a low therapeutic index, with the highest tolerated dose in dogs being 20 mg/kg i.m. and 12.5 mg/kg i.v. (Swan, 1995). The toxic dose appear to vary with individuals, and single dose at 4.2 mg/kg was reported to cause clinical signs of mid-brain toxicity which is rarely observed in clinical setting (Oppong, 1969). The observed histopathological changes in the brain due to diminazene aceturate toxicity cannot be differentiated from mid-brain lesions caused by cerebral babesiosis (Naude *et al*., 1970). Parasympathomimetic effect was hypothesised to have been responsible for acetylcholineesterase inhibition following intravenous diminazene administration (Sakai, 2009). Milner (1997) reported that pseudocholineesterase levels were not significantly changed 15 min after intramuscular diminazene aceturate administration. There are few acute toxicity data available with diminazene aceturate in the usual laboratory species. An oral dose of 1500 mg/kg body weight to 3 males and 3 females resulted in death of a female mouse (Hwang *et al*., 2010). Signs of toxicity were increased spontaneous activity, tactile hyperesthesia and uncoordinated gait (Hwang *et al*., 2010). Brain damage has been reported in asses and dogs given the diminazene aceturate (Abaru *et al.,* 1984). In the dog, spastic paralysis, opisthotonos and nystagmus with involuntary running movements were noted in dogs treated 24-72 hours earlier with diminazene aceturate. Intramuscular haemorrhage and diffuse intramuscular edema were noted at the injection site (Losos & Crockett, 1969). Similar signs accompanied by vomiting and death were reported in dogs given 30-35 mg/kg body weight of the drug (Malek and Nematbakhsh, 2014). In a study, groups of 2 dogs of unspecified strain were given single intramuscular doses of 10, 15, 20 or 60 mg/kg body weight of diminazene aceturate; the animals given 20-60mg/kg of the drug died within 36-54 hours after administration. The signs observed were the same as those reported for dogs treated with the drug, and included extensive haemorrhagic malacia of the brain stem involving the mesencephalon and diencephalon (Losos and Crockett, 1969). Intramuscular injections of 8 mg/kg body weight were well tolerated in various species of animals, except in the buffalo cow where "quivering" and restlessness that followed dosing. The animal recovered after an intravenous dose of dextrose (Zhang *et al*., 2015). Buffalo calves tolerated intramuscular doses of 20 mg/kg body weight of diminazene aceturate. No acute effects occurred in cattle when given 6 times the recommended dose (21 mg/kg body weight) (Velkoska *et al*., 2015). Camels also tolerated diminazene aceturate given intramuscularly at the recommended therapeutic dose of 3.5 mg/kg body weight (Perilo *et al*., 2010). From a total of 154 donkeys usually given 0.5 mg/kg body weight of diminazene aceturate every 3 months to protect against trypanosome infection, 31 became infected with *Trypanosoma brucei* and were treated with 7 mg/kg body weight of diminazene aceturate. About 48 h later, 4 of the animals became weak and displayed signs of staggering and ataxia, and by 96 h, 29 donkeys had developed CNS effects and 6 died (Oppong, 1969). Hepatoxicity in dogs have also been reported after single doses of 3.5 mg/kg body weight, however, pre-existing liver disease could not be excluded as a contributing factor (Oppong, 1969). Intramuscular diminazene aceturate was also hepatotoxic to camels administered 10 or 40 mg/kg body weight. The animals showed hyperaesthesia, salivation, intermittent convulsions, frequent urination, defecation and sweating (Homeida *et al.,* 1981). At necropsy, the lungs were congested and edematous, while the liver was congested and hemorrhagic with evidence of fatty change. Congestion of the brain and urinary bladder was noted along with hemorrhage and congestive changes in the kidneys and heart (Homeida *et al.,* 1981).

Although diminazene aceturate interact with DNA (Newton, 1980), it has been observed that they are not teratogenic (Yoshimura, 1990). Furthermore, although such compounds

do not appear to be mutagenic for *Salmonella typhimurium* (Stauffert *et al*., 1990), diminazene aceturate has been shown to be mutagenic for *Saccharomyces cerevisiae* (Mahler and Perlman, 1973) and produced phenotypic tegumental changes in *Schistosoma mansoni* (De Brito *et al.*, 2020).

CONCLUSION

From this review, therapeutic usefulness or properties/activities of diminazene aceturate surpassed its toxicity effects. It is therefore recommended that diminazene aceturate be used with utmost caution either alone or in combination with other drugs.

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