Introduction

One of the few drugs available for chemotherapy of animal infections of the haemo flagellate parasitic protozoa *Trypansoma brucei brucei*, *Trypansoma congolense* and *Trypansoma vivax* is Diminazene aceturate (Berenil: N-1,3 diamidino-phenyl triazene diaceturate, tetrahydrate) whose trypanocidal activity was first reported by (Fussganger and Bauer, 1958). It is used in tropical countries for the treatment of animal trypanosomiasis and babesiosis, usually as an intramuscular injection.

The trypanocidal activity of Diminazene aceturate was discussed in detail by (Fussganger and Bauer, 1958) and the report of these workers showed that a single intramuscular dose of 3.5 mg kg^{-1} to cattle cured *T. congolense* and *T. vivax* infections was followed by an increase in the use of the drug for treatment of bovine trypanosomiasis (Fairclough, 1963).

Treatment of trypanosomiasis relies on the use of Diminazene aceturate which is effective for the treatment of disease in cattle, buffalo, sheep, camels and pigs (Peregrine and Mamman, 1993; Sirivan *et al.*, 1994). However single doses of medicine are not effective for horses, mules and dogs (Tuntasuvan *et al*., 2003, Colpo *et al*., 2005). Ineffectiveness of Diminazene aceturate already had been observed in bovines, horses and mules infected with *T. evansi* and *T. vivax* (Silva *et al*., 2002, Tuntasuvan *et al*., 2003, Da Silva *et al*., 2011), and therefore, new treatment protocols are needed.

Babesiosis or piroplasmosis is a tick-borne intra-erythrocytic disease of domestic and wild mammals caused by protozoan parasites of the genus Babesia (Smith, 2002). Equine babesiosis in horses, donkeys,

mules, and zebras is caused by *Babesia equi* and *Babesia caballi* (Radostits *et al*., 2000).

Diminazene was found to be hepatotoxic to camels; 10 or 40 mg/kg bw given to 3 dromedary camels by the intramuscular route resulted in hyperaesthesia, salivation, intermittent convulsions, frequent urination and defaecation and sweating. At necropsy, the lungs were congested and edematous while the liver was congested and haemorrhagic with evidence of fatty change. Congestion of the brain and urinary bladder was noted along with haemorrhage and congestive changes in the kidneys and heart (Homeida *et al*., 1981).

The sulphonamides are agroup of complex synthetic organic chemical compaunds with chemotheraputic activity, they have a common chemical nucleus which is essential for antibacterial activity. Sulphonamides are broad spectrum antibiotic affecting Gram+ve and Gram-ve bacteria and many protozoal organisms, acting as bacteriostatic rather than bacteriocidal (Brander *et al*., 1991)

With the expanded documented reduced efficacy of Diminazene in treatment of babesia as well as trypanosomes, we thought a Diminazene aceturate potentiated with sulphonamide could be an alternative protocol. Hence, the current work aimed to evaluate the effect of Diminazene diaceturate alone or in combination with Sulphadimidine sodium on some haematological and biochemical parameters of donkeys.

The specific objectives of the study are to:

(1) Determine spectrum of specific effects of repeated administration of Diminazene aceturate on liver and kidney functions.

(2) Investigate the possible haematological changes that may follow co-administration of injectable formulations of Sulphadimidine sodium and Diminazene diaceturate.

(3) Determine the changes, if any that may result following the co -administration of tested drugs to animals subjected to stress.

Chapter One

Literature review

1.1 Diminazene diaceturate

"Remarkable therapeutic success has been obtained during the last years in the treatment of protozoal diseases in domestic animals using a novel drug developed in research laboratories of *Fabwerke Hoechst, A.G.R. Fussganger* wrote this in 1955, about his work on *Diminazene "Berenil*", an aromatic diamidine compound discovered in 1944, (Fussganger, 1995), this aromatic diamidine was developed from a drug called "*Congasin*" and other aminoquinaldines that were found to be active against *Trypanosomes* and other *Babesias*.

Diminazene has subsequently become the most commonly used therapeutic agent for trypanosomiasis in domestic livestock. This has been due to a number of factors, a higher therapeutic index than other trypanocides, in most livestock species (Fairclough, 1963; Williamson, 1970); activity against trypanosomes that are resistant to other trypanocides used in cattle (Williamson, 1970); and the low incidence of resistance to Diminazene that has occurred as a result of using the compound (Williamson, 1976).

Diminazene diaceturate is an aromatic diamidine used extensively as a Veterinary trypanocide, and babesiacide in affected areas of the world (Turnipseed *et al*., 2006; Schad *et al*., 2008). Although not licensed for human use, it has been successfully employed in the

treatment of early stage cases of human African sleeping sickness. However, its efficacy has been shortened by widespread drug resistance (Atsriku *et al.*, 2002).

1.1.1 Identity

Diminazene aceturate is an N-acetylglycine compound chemically described as 4-4 (diazoamino) dibenamindine diaceturate, 1, 3 bis (P-amidinophenyl) triazene bis (N-acetylglycinate) diaceturate; 1, 3 bis (4-gluanylphenyl) triazene diaceturate; and4-4 diamidinodiazoaminobenzne aceturate (Wien, 1943, Martindale, 1989).

1.1.2 Chemical name

4-4(diazoamino) dibenzamidine diaceturate

1, 3-bis (P-amidinophenyl) triazine bis (N-acetyl-glycinate)

4-4diamidino diazoaminobenzene diaceturate

P, P-diguanyl diazoaminobenzene diaceturte.

1.1.3 Synonym

Berenil

1.1.4 Molecular formula

$C_{22}H_{29}N_9O_6 \bullet 4H2O$

1.1.5 Molecular weight

587.6

1.1.6 Appearance and physical characteristics

Yellow solid, Diminazene decomposes at 217 ≯C; it is soluble in 14 part of water at 20 ≯C is slightly soluble in alcohol and only slightly soluble in ether and chloroform (Martindale, 1989).

1.1.7 Doses and administration

The recommended therapeutic dose of Diminazene aceturate is 3.5 mg/kg body weight, administered intramuscularly (Taboada and Mercalant, 1991).

1.1.8 Mechanism of action

Today, over than 50 years after its development, 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 as well as with synthesis of DNA in the parasite (Swan, 1995; Ariyibi *et al*., 2001, Barceló *et al.*, 2001).

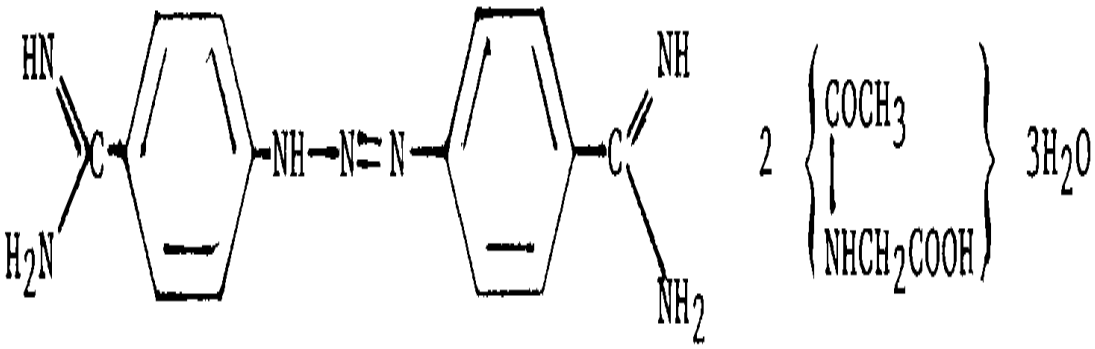

Figure 1.1: Diminazene aceturate structure

Some of the exact actions have recently been elucidated (Pilch *et al*., 1995). Diminazene as anti-trypanosomes agent binds to the AT-rich regions of nucleic acid duplex. Binding occurs via complexation into minor groove of AT-rich domain of the DNA double helices it can bind to DNA as well RNA duplexes. While exhibiting properties characteristic of both intercalation and minor groove binding, this binding unwinds negative super coils in plasmids and has also been found to interfere with the activities of the eukaryotic type II topoisomerase enzymes (Portugal, 1994).

A concentration- dependent inhibition of membrane Ca^{++} -ATPase activity, as well as significant secondary binding of Diminazene aceturate within DNA corresponding to G+ C rich sites have also reported (Ariyibi *et al*., 2001, Barceló *et al*., 2001).

1.1.9 Pharmacokinetics of Diminazene aceturate

1.1.9.1 Goats and Sheep

In a study preformed on the disposition and bioavailability of Diminazene aceturate in dairy goats Aliu *et al.*, (1984) found that 60-90% of the drug was bound to plasma proteins and they reported elimination half –life of 14-30 h.

Mamman and Peregrine (1994) and Mamman and co–Workers, (1996) looked at the pharmacokinetics of Diminazene in the plasma and CSF as well as in the plasma and lymph of goats following a 3.5 mg/kg IM injection of Diminazene. A peak concentration of

4.31ug/m¬ was found in the plasma and the CSF study and the Diminazene concentration in the cerebrospinal fluid was 3-4 times lower than in the plasma. A median peak plasma concentration of 4.30 ⌙g/m¬ was detected in the lymph study, Diminazene could be detected (concentration not given) in the entire plasma sample collected from the goats for 5 weeks (35 days).

After administration of Diminazene to sheep peak plasma concentration of 6.3 -7.57 ⌙g/m¬ at 20-45 minutes was reported *(*Aliu *et al*., 1984). In the same study plasma protein binding of 65-85% was reported. The systemic availability of intramuscular (IM) versus the intravenous (IV) dose was 95.10≒23.21% and mean residence time averaged 14.16≒1.55 h when Diminazene was administration to sheep at 3.5 mg/kg IM the pharmacokinetics of Diminazene administration IV was found to fit a 3- compartmental model.

1.1.9.2 Cattle

Klatt and Hajdu (1976), using colorimetric method of analysis, during investigation of the pharmacokinetics of a combination of Diminazene and rolitetracycline, found a peak concentration of 3.23 ⌙g/m¬ of Diminazene after 30 min and a second phase of elimination of Diminazene with a half–life of 63 h. This long second phase of elimination was considerably shortened when Diminazene was given in combination with rolitetracycline.

Kellner *et al*., (1985), studied the disposition of Diminazene in two calves. Radioactivity was determined in

samples collected after IM injection of radio labelled Diminazene at 3.5 mg/kg intramuscularly. Peak blood concentration of 4.6 and 4.7 ⌙g/m⌝ occurred after 15 and 40 min and the decrease in concentration followed biphasic process with half-live of 2h and 188h.

The pharmacokinetics of Diminazene in cows was described by Aliu *et al*., (1993), Diminazene concentration was determined using HPLC. Non –linear regression analysis of the IV and IM data indicated that the plasma disappearance curves were best described by tri-exponential equations, the IV bolus had a biphasic distribution with half-life of 0.04 h and 0.58 Diminazene was rapidly absorbed following IM administration and peak plasma concentration (C_{max}) 4.68≒1.12 ⌙g/m⌝ was attained in10-15 min, the half-life of the terminal elimination phase was 145.48 h. In vitro after 30min the Diminazene was partitioned 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. After 12h the partition had change to 1.24≒0.08, 1.60≒0.07 and 1.99≒0.44 respectively. This showed that most of the Diminazene was in the plasma 30 min after treatment but that after 12h the majority of Diminazene in the blood was abound to red blood cells. In vitro Diminazene was bound to bovine plasma albumin to extent of 38.01-91.10% (Aliu *et al.*, 1993).

Mamman *et al*., (1993), compared the pharmacokinetics of Diminazene in non infected and *T.*

conglense infected cattle after 3.5mg/kg BW Diminazene injection IM there were few significant pharmacokinetics differences between the cattle. The maximum concentration of the Diminazene in the plasma was significantly higher in animal with acute infection 8.25≒1.72 ⌊g/m⌉ versus animals with chronic infection 5.04≒0.26 ⌊g/m⌉ and the non-infected cattle 4.26≒0.76 ⌊g/m⌉ . Similarly the time to maximum concentration was significantly shorter in the acute infection versus chronic and non infected cases (18.36 and 33.75min).

Gummow *et al*., (1994), in the course of bioequivalence and pharmacokinetics evaluation for two Diminazene aceturate formulations given IM in cattle; they found that two compartmental model best described the behaviour of Diminazene in cattle, peak concentration of Diminazene 3.24≒0.16 ⌊g/m⌉ was reached 49.8≒7.6 min after IM injection of 3.5 mg/kg of Diminazene, they found a half–life of absorption ($T_{1/2_{ka}}$) of 1.93≒0.95h, Diminazene was slowly eliminated with a residence time of 13.27 days and a long elimination half-life ($T_{1/2_{\lambda z}}$ of 222h) .

Mdachi *et al.*, (1995), conducted a study where cows were repeatedly infected with *T. congolense* and then treated with a different dose of Diminazene aceturate each time. The results of their study indicated that the level of parasitaemia and the degree of anaemia in the animals at the time of treatment affected the distribution,

disposition and elimination of Diminazene aceturate from animal.

1.1.9.3 Dogs

Bauer (1967) used serum Diminazene concentration to inhibit growth of *Brucella spp.* in culture as compared to control concentration of Diminazene, thus using the bactericidal action of the drugs to biologically determine blood concentration after Diminazene injection. Concentration of Diminazene as low as 1 g/m could be determined in this studies, peak serum concentration of Diminazene occurred at 3h (3 g/m) and all traces of Diminazene were absent by 24 h. He concluded that Diminazene was excreted via the kidney within 24 h.

Onyeyili and Anika (1989, 1991), used a colorimetric method to determine the influence of *T. congolense* on the disposition of Diminazene in the dogs using each dog as its own control. They reported that drug elimination followed a biphasic process, irrespective of infection but that infection significantly shortened $T_{1/2}$ of Diminazene from the 0.17h to 0.12h, although the urinary excretion of the drug remained constant (Onyeyili and Anika, 1991)

They also gave 3.5ml/kg Diminazene IM to both healthy dogs and dogs with trypansomiasis. The dogs were autopsied at 48, 72, 120, 168 and 240h after injection. Mean plasma concentration were reported as 0.2 0.008 g/ml, but no peak concentration were given. No Diminazene was found in the plasma after 48h. Higher plasma concentration levels were present in healthy dogs.

In all tissue samples at 48h the highest concentration of Diminazene was found in the kidneys and liver in both groups and low Diminazene concentration was found in the brain. Diminazene persisted in the tissues for more than 10 days (Onyeyili and Anika, 1989).

Another publication by same authors, regarding the same study, reported $T_{1/2\lambda}$ significantly decreased in dogs after trypanosome infection 0.14 h vs. 0.2 (Onyeyili and Anika, 1991).

1.1.9.4 Blood products

Alvi *et al.,* (1985) incubated Diminazene aceturate with blood products and found that binding to plasma and serum was 50% and 35% respectively. On examination of the red blood cells they found that 70% of Diminazene was bound to purified haemoglobin and that red cell membrane did not show any binding. They concluded that Diminazene bind to number of blood proteins and could cross the red blood cell membrane to bind to haemoglobin.

1.1.10 Use of Diminazene aceturate in animals and human

1.1.10.1 Use of Diminazene aceturate in equine

Back at 1980 Singh *et al.*, reported that Diminazene diaceturate (Berenil, Hoechst) at 12 mg/kg intramuscularly (IM) and repeated after 24 hours controlled the rising parasitaemia of *Babesia equi* infection in four out of five donkeys and they approached that drug was more effective in the early stages of the disease and had a prophylactic effect for at least 30-35 days.

In horses approximately the same efficacy ratio was established (Rashid *et al.*, 2008); they studied the efficacy of two diamidine drugs i.e. Diminazene aceturate and Diminazene diaceturate. Efficacy of the drugs was determined by the reversal of clinical signs and a negative blood smear examination. The efficacy of Diminazene diaceturate was demonstrated to be 80% while Diminazene aceturate was found to have 90% efficacy against babesiosis.

1.1.10.2 Use of Diminazene aceturate in dogs and cats

Diminazene is the antibabesial drug of choice for the treatment of canine babesiosis in South Africa (Anika and Onyeyili, 1989). Differences in the dosage described for Diminazene and the occurrence of mortality at doses equal to or close to the recommended therapeutic dose for the treatment of canine babesiosis have been described (Oppong 1969, Losos and Crockett 1969, Naude *et al*., 1970, Moore, 1979, Stewart 1983).

Howes *et al*., (2011) investigated Diminazene aceturate efficacy on dogs at dose rate of 3.5 mg/kg injected intramuscularly for 5 days at 24h intervals, to clear *Trypansoma evansi* infection. After treatment the dogs showed clinical signs of health improvement, and clinical signs disappeared after the seventh day of treatment. The parasite was not found in blood smears after the third day of treatment and PCR was negative on days 30 and 50 post-treatment.

In a study carried out on cats to investigate the efficacy of Diminazene aceturate in the control of *Trypansoma evansi* it has been reported that, treatment with Diminazene aceturate at the recommended dose 3.5mg/kg intramuscularly for 5 consecutive days had an efficacy of 85.7% (Da Silva *et al*., 2009).

1.1.10. 3 Use of Diminazene aceturate in cattle and sheep

The efficacy of Berenil was earlier reported by Joyner *et al.*, (1963) in doses of 3-5 mg/kg against cattle babesiosis. Niazi and his colleagues (2008), determined the prevalence and chemotherapy of babesiosis in calves, single dose of Diminazene aceturate at 3.5mg/kg BW intramuscularly (IM) was used to investigate the efficacy of the drug, blood samples were collected at day 0, 3, 7 and 10 post medication. The efficacy was determined on the basis of disappearance of babesia in the blood smears which indicated that the efficacy of Diminazene aceturate was increased from 40% at day 3 up to 90% at day 10.

Another study in the same area in 2010 by Rashid and others in sheep included a total of 310 blood smears to investigate the efficacy of Diminazene and imidocarb on babesiosis chemotherapy proved that the efficacy of Diminazene aceturate at the single recommended dose was 30% and increased up to 80% from day 3 to 7 of the treatment.

1.1.10.4 Use of Diminazene aceturate in human

In addition to trypanocidal and babesiocidal activity, Diminazene had also been effectively used as a therapeutic agent for visceral and cutaneous leishmaniasis in man (Rees *et al.*, 1985; Lynen and Van Damme, 1992).

The use of Berenil in man was limited until now to resistant kala-azar in Kenya (Rees *et al.*, 1985) and early stage human trypanosomiasis in East –and West –Africa (Temu, 1975; Abaru and Matovu, 1981) and Zaire (Ruppol and Burke, 1977). Very few side effects were noted and all were reversible (Temu, 1975; Ruppol and Burke, 1977; Abaru and Matovu, 1981).

1.1.11 Toxicity and residues

Berenil was reported to cause mild to severe toxicity in horses and mules after injection, with minimal protective effect of the drug (Tuntasuvan *et al*., 2003). Berenil caused fatal reactions in camels, horses and dogs at doses which are considered to be normal and harmless in cattle (Sirivan *et al.* 1994), in camels, however, a signal dose of 7.0 mg/kg b.w can be highly toxic (Leach, 1961). In 1981 Homeida and his colleagues, investigated the toxicity of Diminazene aceturate in camels at three unusual doses, single intramuscular dose of 40mg/kg which caused death after 4 hours, the clinical signs observed in camels were similar and developed within 15 min of injection of Diminazene. They showed tremors, itching, and frequent urination, frothing at the mouth and sweating.

Diminazene is also relatively toxic in dogs. Experimentally; intramuscular administration at dose 10.0 mg/kg b.w., once or repeatedly, resulted in sever signs of disturbances in gastrointestinal tract, respiratory, musculoskeletal and nervous systems in dogs (Losos and Crockett, 1969), in the other hand cattle can tolerate Diminazene as high as 21 mg/kg without signs of toxicity (Fairclough, 1963).

Although Diminazene has not been formally evaluated for its toxicity in man (Apted, 1980), the manufacturers do not recommend its use in people because of the toxicity observed in some animal species

(Abaru *et al.*, 1984). However, in 17 cases reported by Hutchinson and Watson (1962), no local or systemic toxicity was observed.

Furthermore, it was concluded from a study of 99 cases reported by Abaru *et al.*, (1984) that although various transient side-effects were observed, these were no more serious than those produced by other trypanocidal drugs, such as suramin. It is, however, noteworthy that acute idiopathic polyneuritis (Laudry-Guillain-Barŕe syndrome), developed in a human patient infected with *B. microti* after treatment with Diminazene aceturate (Ruebush *et al.*, 1979).

Diminazene is extensively distributed in the body of treated animals. Residues of the compound may persist for several weeks, principally in the liver and kidneys, and also, to a lesser extent, in the gastrointestinal tract, lungs, muscle, brain and fat (Gilbert, 1983; Kellner *et al.*, 1985; Murilla and Kratzer, 1989; Onyeyili and Anika, 1991).

After treating lactating goats intravenously with 2 mg Diminazene base/kg b.w. the maximum concentration of Diminazene in milk (1.68 ┗ g/ml) was detected at 4 h (Aliu *et al.*, 1984). Milk to plasma ratio of approximately 0.45 was maintained at equilibrium. Trace amounts of Diminazene (0.05 ug/ml) were present for up to 72 h following treatment. Based on pharmacokinetic (Aliu *et al.*, 1984) and residue studies (Kellner *et al.*, 1985), a pre-slaughter withdrawal period of 21-35 days has been

recommended for Diminazene when treated animals are intended for human consumption.

Finally, although Diminazene and related aromatic diamidines interact with DNA (Newton, 1980), it has been concluded 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 has been shown to be mutagenic for *Saccharomyces cerevisiae* (Mahler and Perlman, 1973).

1.2 Suphonamides

The sulphonamides are a group of complex synthetic organic chemical compounds with chemotherapeutic activity, they have a common chemical nucleus which is essential for antibacterial activity. Potentiated sulphonamides are broad-spectrum bactericidal Antimicrobials with a large volume of distribution in horses (Plumb, 1998). They are available as oral formulations and are fairly inexpensive, making them a practical antimicrobial choice in equine medicine (Duijkeren *et al.*, 1994). These drugs are often chosen for prolonged antibiotic therapy because of their perceived safety and low incidence of recognized adverse effects (Wilson *et al.*, 1996). Sulphonamides are widely used for therapeutic and prophylactic purposes in both people (Kim and Park, 1998) and animals (Schwarz and Chaslus-Dancla, 2001).

1.2.1 History

Sulphanilamide was first syntheseized in1908 by Gelmo, but it was not until 1935 when Domagk of I.G Farben Industrie discovered the ability of certain azo-dyes related to sulphanilamide to protect mice against challenges with streptococci, that attentoin became concentration on these products of dye industry. Prontosil and Neo-prontosil, the latter a water soluble red dye which stained everything it contacted, were the first of these azo-compounds to be used therapeutically and with a good effect. It was stated during the same year in Paris that the activity of prontosil and other –dyes was due to their release of sulphanilamide and soon this was followed in 1935 by work in England which showed that sulphanilamide is less toxic than prontosil and yet equal in activity.

Since 1936 a great amount of investigation has produced many sulphonamides but only a few have been of therapeutic value. In recent years the sulphonamides have been largely displaced therapeutically by antibiotics. Nevertheless, interest in the sulphonamides continues especially as synergism and delay in the development of antibiotics (Brander *et al*., 1991).

1.2.2 Chemistry

In chemistry, the sulphonamide functional group (also spelt sulfonamide) is -S $(=O)_2$-NH_2, a sulphonyl group connected to an amine group. A sulphonamide (compound) is a compound that contains this group. The general formula is RSO_2NH_2, where R is some organic group. For example, "methane sulphonamide" is $CH_3SO_2NH_2$. Any

sulphonamide can be considered as derived from a sulphonic acid by replacing a hydroxyl group with an amine group.

1.2.3 Chemical name

4-Amino-*N*-(4,6-dimethyl-2-pyrimidinyl) benzene sulphonamide

1.2.4 Synonym

2-(4-Aminobenzenesulfonamido)-4,6-dimethylpyrimidine; 2-(*para*aminobenzenesulfonamido)- 4,6-dimethylpyrimidine; 4-amino-*N*-(2,6-dimethyl-4-pyrimidinyl) benzenesulfonamide; 4,6-dimethyl-2-sulfanilamidopyrimidine; sulfadimethylpyridine; 2-sulfanilamido-4,6-dimethylpyrimidine; sulphadimidine; sulphamidine; sulphadimethylpyrimidine;

1.2.5 Molecular formula

$C_{10}H_{11}N_3O_3S$

1.2.6 Molecular mass

253.279 g/mol

1.2.7 Chemical structure

The chemical structure of sulphonamides and sulphadiazine is present in figures 1.2 and 1.3 below.

1.2.8 Appearance

Sulphonamides are pale-yellow crystals. Slightly soluble in water (1.5 g/L at 29 °C), acids and alkali; solubility increases rapidly with an increase in pH (Lide and Milne, 1996; Budavari, 2000). Melting-point: 198.5 °C (Lide and Milne, 1996)

1.2.9 Mechanism of action

Bacteriostatic Sulphonamides interfere with the biosynthesis of folic acid in bacterial cells; they compete with para-aminobenzoic acid (PABA) for incorporation in

the folic acid molecule. By replacing the PABA molecule and preventing the folic acid formation required for DNA synthesis, the sulphonamides prevent multiplication of the bacterial cell. Only organisms that synthesize their own folic acid are susceptible; mammalian cells use preformed folic acid and, therefore, are not susceptible.

Cells that produce excess PABA or environments with PABA, such as necrotic tissues, allow for resistance by competition with the sulphonamide (Vree *et al.*, 1986).

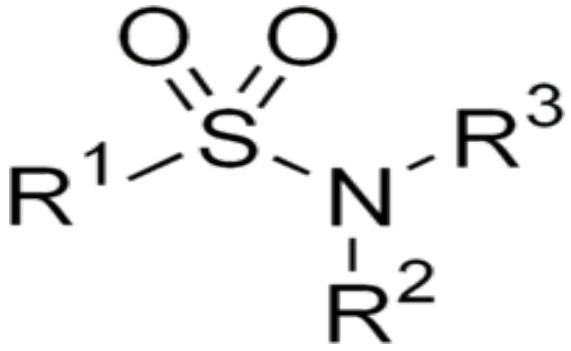

Figure 1.2: Sulphonamide structure

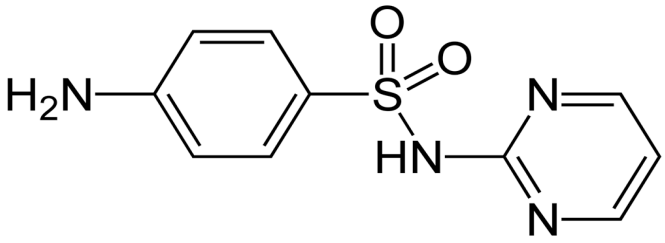

Figure 1.3: Sulphadimidine structure

Bacterial resistance to sulphonamides is caused by mutations in the enzymes involved in folic acid synthesis that prevent the drug from binding to it.

1.2.10 Toxicity

Idiosyncratic toxic reactions to sulphonamides have been reported in dogs and humans (Werner and Bright, 1982; Mandell and Sande, 1985). Because idiosyncratic toxicoses appear clinically to include an immunological component, they are often referred to as hypersensitivity reactions, drug allergies, or drug-induced immune mediated disease (Anderson and Adkinson, 1987). There have been 28 cases of idiosyncratic reactions to sulphonamides in dogs reported in the literature (Grondalen, 1987; Harvey, 1987; Taksda, 1987; Wuhr, 1987; Thornburg, 1988).

Sulphonamide idiosyncratic toxicities in dogs can be divided into three categories, based on the primary clinical abnormality, polyarthritis fever, cutaneous drug eruption and hepatitis. The relationship between these clinical syndromes is unclear, as the occurrence of multi-organ toxicity is not uncommon (Giger *et al.*, 1985).

In humans, the clinical manifestations of idiosyncratic toxicities are broad and virtually any combination of toxicities can occur in a given patient (Shear *et al.*, 1986); therefore a division based on clinical abnormalities, though convenient, may be somewhat artificial. The adverse effects reported in horses include diarrhoea, fever, nephrotoxicity, transient pruritis, cardiovascular

collapse and death, blood dyscrasias, bone marrow suppression, teratogenesis and anaemia (Thomas and Livesey 1998, Wilson *et al*., 1996).

In humans the toxic side-effects of sulphamethazine are expected to be similar to those of other sulphonamides, which include disorders of the haematopoietic system and hypersensitivity reactions. Heinonen *et al*., (1977) reported no increase in malformation rates in the offspring of 47 women treated with sulphamethazine during the first four lunar months of pregnancy.

Groups of 12 male and female B6C3F1 mice and Fischer 344 rats were fed either a control diet or a diet containing 300, 600, 1200, 2400 or 3600 mg/kg sulphamethazine for 90 days. In the mice, no treatment-related lesions were seen grossly or by light microscopy. Thyroid gland enlargement was seen in one of 24 rats fed the diet containing 2400 mg/kg and in 12 of 24 rats at the highest dietary concentration. Thyroid gland hyperplasia was evident in all treated rats but was more pronounced and occurred at a higher incidence in rats at the higher concentrations (Heath and Littlefield, 1984a,b).

In B6C3F1 mice that received diets containing sulphamethazine at a concentration of 0, 300, 600, 1200, 2400 or 4800 mg/kg for 24 months, non-neoplastic dose-related lesions were observed in both males and females, including follicular-cell hyperplasia (diffuse and focal) of the thyroid gland (Littlefield *et al.*, 1989).

Fischer 344 rats received diets containing sulphamethazine at a concentration of 0, 10, 40, 600, 1200 or 2400 mg/kg for 24 months, and interim sacrifices were carried out after 3, 12 and 18 months. The incidences of non-neoplastic lesions of the thyroid gland were significantly higher among treated animals than among controls and included follicular-cell hyperplasia, follicular cellular change and multilocular cysts (Littlefield *et al.*, 1990).

There were no significant effects at low concentrations, but a sharp, relatively linear rise was seen at higher concentrations: thyroid weights increased and serum thyroxine and triiodothyronine concentrations decreased at ≥ 3300 mg/kg of diet, and the serum TSH concentration increased at ≥ 1600 mg/kg of diet. All the morphological changes seen in the thyroid gland were reversible after withdrawal of sulphamethazine treatment. Supplemental dietary administration of thyroid hormone completely inhibited the functional and morphological changes observed with sulphamethazine at concentrations that normalized but did not suppress TSH. Further, no detectable effects on the thyroid gland were observed in hypophysectomized rats treated with sulphamethazine. *In vitro*, sulphamethazine did not increase cell proliferation in FRTL-5 cells in the absence of TSH. No effect on thyroid gland function was observed in cynomolgus monkeys (*Macaca fascicularis*) at doses of up to 300 mg/kg bw per day for 13 weeks (McClain, 1995).

The effect of sulphamethazine on fertility was assessed in three groups of 20 male and 20 female Swiss CD-1 mice given diets containing the drug at 0.25, 0.5 or 1.0% (equivalent to 0, 313, 625 and 1250 mg/kg bw per day) and compared with a control group of 38 males and 38 females. The mice were exposed to sulphamethazine continuously during the 7-day pre-mating and 98-day co-habitation periods. At the conclusion of this phase of the study, cross-over matings were performed with the parental mice, consisting of control male × control female; high-dose (1% sulphamethazine) male × control female; control male × high-dose female. The effects observed in the F0 group receiving 1% sulphamethazine included significant decreases in the number of litters produced and in the number of live pups per litter and a significant increase in the proportion of live male pups per total live pups per litter. No significant difference was found in the percentage of motile sperm, sperm concentration or percentage of abnormal sperm in the cauda epididymis in the group fed 1% sulphamethazine versus the control group. The cross-over part of the study showed that fertility was affected in animals of each sex, the average number of live pups per litter being significantly decreased. No treatment-related histopathological effects were observed in the pituitary or reproductive organs of male or female mice in the group fed 1% sulphamethazine. Exposure of mice to 0.25 or 0.5% sulphamethazine in the diet during the continuous

breeding phase of the study had no effect on fertility or reproductive performance (Reel *et al*., 1992).

Intraperitoneal administration of various doses of sulphamethazine to adult male Wistar rats for 3 or 5 days and to Hubbard chickens for 3 days significantly increased the electron transport components (rats only) and the activities of aminopyrine *N*-demethylase and aniline hydroxylase at a dose of 150 mg/kg bw. A dose of 300 mg/kg bw produced a significant decrease in cytochrome P450 content and in the activity of aminopyrine *N*-demethylase in the rats and of aniline hydroxylase in the chickens. Administration of sulphamethazine to young male rats resulted in significant induction of electron transport components and drug-metabolizing enzymes at both 150 and 300 mg/kg bw. However, treatment of old rats produced significant decreases in electron transport components and aminopyrine *N*-demethylase activity at both doses. A significant increase in electron transport components was observed with 150 mg/kg bw sulphamethazine in female rats. These studies suggest that sulphamethazine is a substrate of the mixed-function oxidase system, and induction is dependent on the dose and on the age and sex of the animals. Intraperitoneal administration of a single dose of 300 mg/kg bw sulphamethazine to rats pretreated with intraperitoneal doses of 80 mg/kg bw per day phenobarbital for 3 days decreased microsomal protein, electron transport components and drug-metabolizing enzyme activities to a

greater extent than phenobarbital alone (Kodam and Govindwar, 1995; Kodam *et al*., 1996; Kodam and Govindwar, 1997).

Sulphamethazine did not induce mutations in *Salmonella typhimurium* and it did not induce unscheduled DNA synthesis in human fibroblasts in culture. The Working Group was aware of data from three different laboratories, showing that sulfamethazine (*a*) did not induce gene mutation at the *Hprt* locus (at concentrations up to 7 µg/mL) or chromosomal aberrations (at up to 5000 µg/mL) in Chinese hamster ovary cells in the absence or presence of exogenous metabolic activation, (*b*) gave rise to sister chromatid exchange in these cells (at concentrations up to 1500 µg/mL) in the absence but not in the presence of an exogenous metabolic activation systems, and (*c*) did not induce chromosomal aberrations in bone-marrow cells of rats treated with a single oral dose of 3000 mg/kg bw (WHO, 1994).

1.2.12 Residues

Sulphonamides are sometimes being used as additives in animal feed because prolonged ingestion of sulphonamides may have a growth-promoting effect (Long *et al.*, 1990). If the proper withdrawal periods are not observed before slaughtering or milking of the medicated animals, meat and milk from these animals may be contaminated with residual sulphonamides (Saschenbrecker and Fish 1980, Franco *et al.*, 1990, McEvoy *et al.*, 1999).

1.2.13 Use of sulphonamide in animals and human

Sulphamethazine is a sulphonamide used to treat a variety of bacterial diseases in humans and other species. It has been used since the late 1950s to treat respiratory disease and promote growth in food-producing animals (cattle, sheep, pigs and poultry). It is a short-acting sulphonamide drug with similar properties to those of sulphamethoxazole. There is currently no single-entity dosage form of sulphamethazine, and it is used only in combinations. It has been used with trimethoprim and with other sulpha drugs, particularly sulfadiazine and sulphamerazine. The sulphamethazine sodium salt may be given orally (2 g initial dose followed by 0.1–1.0 g every 6–8 h) or parenterally. The usual adult dose of a combination with equal amounts of sulphadiazine and sulphamerazine (trisulphapyrimidines) has been 6–8 g/day (Gennaro, 1995; WHO, 1995; American Hospital Formulary Service, 1997; Food and Drug Administration, 1988; Royal Pharmaceutical Society of Great Britain, 2000).

Potentiated sulphonamides are broad-spectrum bactericidal antimicrobials with a large volume of distribution in horses (Plumb, 1998). They are available as oral formulations and are fairly inexpensive, making them a practical antimicrobial choice in equine medicine (Duijkeren *et al.*, 1994). These drugs are often chosen for prolonged antibiotic therapy because of their perceived safety and low incidence of recognized adverse effects (Wilson *et al*., 1996).

1.3 Babesiosis infection and drug resistance

Babesiosis is a parasitic infection caused by haemotropic protozoa of the genus Babesia, family Babesiidae, and order Piroplasmida.

This malaria-like protozoan parasitizes the erythrocytes of wild and domestic animals. It is a well-recognized disease of Veterinary importance in cattle, horses and dogs, which has gained increasing attention as an emerging zoonotic disease problem. This organism may cause a malaria-like syndrome including fever, haemolysis and hemoglobinuria. Babesia infections have probably been complicating the lives of humans since antiquity, primarily through infections of domestic livestock.

The first recorded reference to babesiosis is probably in the biblical book Exodus 9:3, which described a plague of the cattle of the Egyptians Pharaoh Ramses II that could have been red water fever of cattle (caused by *Babesia bovis*) and could have included haemoglobinuria as a prevalent sign. However, the genus was not formally recognized until the work of Babes in 1888, who described an intraerythrocytic pathogen (suspected to be a bacterium) causing a febrile haemoglobinuria in Rumanian cattle (Babes, 1888).

Shortly thereafter, the agent of Texas Cattle Fever, initially called Pyrosoma, was discovered by Smith and Kilbourne in 1893 (Smith, 1983) and later identified as *Babesia bigemina*. The first evidence that humans, could be infected with Babesia parasites was reported by Wilson and Chowning (1904), who described piriform, intra-erythrocytic inclusions like those described by Smith and Kilbourne, in the blood of patients with Rocky Mountain

spotted fever in the western US. They called this agent *Pyroplasma hominis.*

Equine babesiosis is one of the most common infectious tick-borne diseases in southern Africa (Heerden 1996; de Waal and Van Heerden, 2004).

The parasites *Theileria equi* and *Babesia caballi* cause acute, sub acute or chronic diseases in Equidae (Phipps, 1996; Ribeiro *et al.,* 1999; de Waal and Van Heerden, 2004) or neonatal babesiosis or abortion in mares (Heerden 1996; de Waal and Van Heerden, 2004). The disease leads to haemolytic anaemia (Schein, 1988; Phipps, 1996; de Waal *et al.* 2004) and death can occur (de Waal and Van Heerden, 2004).

For many years, three babesiacides, quinuronium sulphate (Ludobal1, Bayer Ltd.), amicarbalide isothionate and Diminazene aceturate were available in most European countries for the treatment of bovine babesiosis.

Drug resistance is a broad problem in the fight against infectious diseases. After the long exposure to drugs, parasites can frequently develop resistance towards chemotherapeutics. For examples, the *Pfmdr1* gene in *P. falciparum* (Foote *et al.*, 1989), the *LmpgpA* gene in *L. major* (Callahan and Beverley, 1991) and the *TbmrpA* gene in *T. brucei* (Shahi *et al.,* 2002) is involved in drug resistance by the alteration of drug accumulation into parasites. Other mechanisms of drug resistance, such as mutation with mitochondrial genes in *B. gibsoni* (Matsuu

et al., 2006; Sakuma *et al.*, 2009), are continuously studied.

In *B. gibsoni*, the unsuccessful efficacy of Diminazene aceturate has become a serious problem for the treatment of canine babesiosis since it is one of the most popular antibabesial drugs for the *B. gibsoni* infection. Furthermore, a veterinary practice survey in South Africa indicated that relapse of *B. canis* after Diminazene aceturate treatment was observed, although the genetic differences was not detected by molecular biological methods to certify the resistance (Collett, 2000).

1.4 Combination regimens of Trypanocidal and Babesiocidal drugs

Many of the trypanocidal compounds used for human trypanosomiasis have a low therapeutic index. Drug toxicity is therefore often observed. In an attempt to reduce drug doses, and therefore the incidence of toxicity, Diminazene has been combined with a variety of compounds to determine if this has an additive or synergistic effect.

Combination with various nitroimidazoles was shown to have at least an additive effect against *T. b. brucei* infections in mice (Jennings *et al.*, 1980; Jennings, 1993) and against *T. simiae* infections in pigs (Zweygarth and RÖttcher, 1987) that appear to have accessed the central nervous system (CNS).

Similar effects have also been demonstrated when Diminazene has been combined with either

difluoromethylornithine (Jennings, 1992) or suramin (Williamson *et al.*, 1982) for the treatment of *T. brucei sp.* CNS infections.

The molecular bases for these observations have not been elucidated but may be due to inhibition of different biochemical pathways. Lastly, combination with either mepyramine maleate or piroxicam has been shown to enhance the therapeutic activity of Diminazene 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 may increase the bioavailability of the trypanocide.

Chapter two

Materials and Methods

2.1 Materials

2.1.1 Study location and housing

The experiments were conducted at the college farm, College of Veterinary Medicine (SUST), located in Khartoum North, Hillat Kuku, Sudan (Figure 2.1). Animals were housed in pens, the enclosures made of iron and wood; with 3X7 meters dimension (Figure 2.2). Experimental animals were supplemented with *Abu sabeen* and water ad lib.

2.1.2 Experimental drugs

Diminazene aceturate: TRYPONIL (*Interchemi, Holland*) intramuscular injection was used according to the manufacturers recommended dose i.e. 3.5 mg/kg b.w.

Sulphadimidine sodium: SULFA 333 (*Interchemi, Holland*) intramuscular injection was used according to the manufacturers recommended dose i.e. 3 ml/10 kg b.w.

2.1.3 Experimental animals

For the purpose of this study, a total of 42 male donkeys, 4-10 years for age, weighing 90-150 kg, was used. The animals were purchased from local market *Alkriab*. Upon their arrival animals were clinically examined and treated with albendazole and Penicillin.

2.2 Methods

2.2.1 Experimental design

Two experiments were conducted to investigate the probable toxicities of Sulphadimidine sodium, Diminazene

diaceturate administered individually or simultaneously to donkeys.

2.2.1.1 First experiment

In this experiment, a total of 18 donkeys were divided into three groups of each of six donkeys. Animals in the first group were subjected

Figure 2.1: Location of Khartoum, in the centre of Sudan

Figure 2.2: Experimental animals housed in pens in the college farm

to treatment with Diminazene aceturate once daily for three successive days at the manufacturer recommended dose i.e. 3.5 mg/kg b.w.

Animals in the second group were treated with Sulphadimidine sodium once daily for three successive days at the manufacturer recommended dose of 3 ml/10kg. Animals in the third group were treated with a combination of Diminazene aceturate and Sulphadimidine sodium once daily for three successive days at the same dosage regimen described above. Animals were monitored for two hours following the administration of each dose.

2.2.1.2 Second experiment

Another 24 donkeys were divided into four groups each of six animals. In this experiment animals were subjected to fasting for 48 hours before the start of the experiment. The first group remained without treatment as control group and the other three groups were treated with the same dosage regimen described in the first experiment. Animals were observed for two hours after each injection to record any physical changes or abnormal behaviour.

2.2.2 Blood samples collection

Blood samples were collected directly from the jugular vein of the animals using 10 ml syringes. Blood samples were transferred immediately into two containers; the first ones were plain vacutianer tubes and they were allowed to clot, the clotted blood samples were

centrifuged and sera were separated and sorted at -20 ⁰C until analyzed.

The other one was kept in vacutianer containing heparin as anti coagulant, these samples were immediately used to evaluate the haematological parameters.

2.2.3 Sampling schedule

Blood samples were collected at the following time points: day zero (before treatment), 1, 2, 3, 5, 7, and 10. Where:

1. Day zero before injection (baseline value).
2. Days 1, 2 and 3 are three continuous days during the treatment period
3. Days 5, 7 and 10 following the first injection.

This schedule was the same in the second experiment except that day zero was before the 48 hours fasting.

2.2.4 Haematological methods

2.2.4.1 Red blood cells count (RBCs)

Red blood corpuscles were counted in an improved Neubauer haemocytometer (Neubauer-improved -Germany) using hymen’s solution as diluents. Consisting of 0.5g mercuric chloride 0.5g sodium sulphate and 1.0g sodium chloride, made up to 200 ml with distilled water as described by (Dacie and Lewis, 1984).

2.2.4.2 Haemoglobin concentration (Hb)

Haemoglobin was determined by acid haematin method (Sahli technique) using Sahli haemoglobinometer.

Sahli method depends up on conversion of haemoglobin to acid haematin. The red cells are lacked in dilute hydrochloric acid (HCL) to form acid haematin which is brown in colour and is matched with standard colour following the method described by Jain (1986).

2.2.4.3 Packed cell volume (PCV)

Blood samples were drawn into heparinised capillary tubes sealed at one end by cristaseal and centrifuged at 1200 rpm for five minutes using microhaematocrit centrifuge (Hettich-ZENTRIFUGEN –Germany). The PCV% was read in microhaematocrit reader following the method described by Dacie and Lewis (1984) and Schalm *et al*., (1975)

2.2.5 Biochemical methods

Serum samples were subjected to 11 biochemical tests to assess the effect of treatment, if any, on the liver and kidney functions, and effect on minerals profile.

The following blood biochemical parameters were tested using standard methods: Total protein, albumin, bilirubin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Urea, Creatinine, Calcium, Inorganic phosphorus, Sodium and Potassium.

Analyses were conducted using A15-BioSystem (Barcelona, Spain) biochemistry analyzer, random access full automated machine (150 samples /hr, and designed to estimate 60 parameters).

Serum sodium and potassium were measured by (EASYLIGHT-Ione elective electrons analyzer-Germany), random access full automated machine.

2.2.6 Statistical analysis

The difference between mean values of data collected were tested by the t-test, the comparisons were made between mean treatment values and baseline values within the same group to eliminate individual variation, differences were considered significant at $P<0.05$ level.

Chapter Three

Results

3.1 First experiment

3.1.1 Post- Treatment Reactions

Following Diminazene aceturate administration, donkeys immediately and up to two hours monitoring period showed some sort of irritation as well as oedema at the site of injection (Figure 1.1).

3.1.2 Haematological results

The PCV values were significantly ($P<0.05$) decreased in the second group (S) where animals received Sulphadimidine sodium for three successive days (Table 3.1), while RBCs count and haemoglobin level showed no significant ($P<0.05$) fluctuation during the study period in the three treated groups (Tables 3.2 and 3.3)

Figure 3.1: Oedema at the site of Diminazene aceturate injection

Table 3.1: Change in Packed cell volume (PCV) (%) following administration of Diminazene aceturate, Sulphadimidine sodium, and a combination of Diminazene+ Sulphadimidine in donkeys for three successive days

Days	Diminazene	Sulphadimidine	Combination
0	34.50±1.46	35.20±1.15	29.20±1.01
1	36.16±3.55	34.20±1.46* (0.01)	29.80±1.39
2	37.50±2.86	30.40±1.43* (0.01)	30.00±0.63
3	31.00±1.26	28.80±1.52	30.20±0.66
5	29.33±1.33	33.40±0.68	29.40±0.68
7	30.33±0.76	31.20±1.31	28.80±1.24
10	29.33±1.20	33.40±0.93	28.80±0.97

Values in the columns are mean ⇌ s.e.m

Normal PCV (%) 31.59 ± 3.80 (Seri *et al.*, 2006a)

*Values in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.2: Change in Red blood cells count (RBCs) ($x10^{12}$/litter) following administration of Diminazene aceturate, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	5.12 ≒ 0.47	4.46 ≒ 0.37	4.92 ≒ 0.25
1	5.06 ≒ 0.51	4.14 ≒ 0.36	4.69 ≒ 0.24
2	5.43 ≒ 0.42	4.59 ≒ 0.37	4.34 ≒ 0.30
3	5.73 ≒ 0.44	4.31 ≒ 0.18	4.36 ≒ 0.18
5	5.05 ≒ 0.23	4.41 ≒ 0.35	4.28 ≒ 0.24
7	4.45 ≒ 0.41	4.54 ≒ 0.30	4.62 ≒ 0.20
10	4.70 ≒ 0.39	4.67 ≒ 28	4.51 ≒ 0.20

Values in the columns are mean ≒ s.e.m

Normal range of RBC 4.56 – 8.74 $x10^6$/˩ l (Zinkl *et al.,* 1990)

*Values in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.3: Haeamoglobin concentration (Hb) (g/100ml) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	12.00 ⩭ 0.21	12.16 ⩭ 0.39	11.86 ⩭ 0.31
1	11.78 ⩭ 0.32	12.20 ⩭ 0.08	11.92 ⩭ 0.31
2	11.88 ⩭ 0.15	11.96 ⩭ 0.25	11.78 ⩭ 0.26
3	12.01 ⩭ 0.15	12.00 ⩭ 0.27	11.84 ⩭ 0.29
5	12.05 ⩭ 0.23	11.96 ⩭ 0.24	11.78 ⩭ 0.33
7	11.65 ⩭ 0.23	11.84 ⩭ 0.21	11.88 ⩭ 0.31
10	11.73 ⩭ 0.15	11.72 ⩭ 0.21	11.82 ⩭ 0.38

Values in the columns are mean ⩭ s.e.m

Normal range of Hb 10.43 ± 1.45 g/100ml (Seri *et al.*, 2006a)

3.1.3 Blood biochemical constituents

In the Diminazene aceturate treated group total serum protein decreased significantly ($P<0.05$) following two days of treatment. While in the group that treated with the combination the total protein concentration decreased significantly ($P<0.05$) following three continued days of treatment. At the end of the study total protein level returned to pre-treatment level with no significant difference (Table 3.4). Albumin increased significantly ($P<0.05$) following the second dose and continued to be like that up to the end of the study period in the group that received Diminazene aceturate (Table 3.5).

Serum bilirubin increased significantly ($P<0.05$) in the group that received Diminazene aceturate from the second day up to the 7^{th} day of the study. Another increase was also monitored in the group that received the combination from the 3^{rd} up to the 7^{th} day of treatment (Table, 3.6). While, administration of Sulphadimidine sodium induced no significant ($P>0.05$) increase in bilirubin concentration.

As we could observe in Table (3.7) the level of ALT showed significant ($P<0.05$) increase in the first two treated groups in the 3^{rd} and 5^{th} days (Diminazene aceturate), 10^{th} day (Sulphadimidine sodium), and significant decrease ($P<0.05$) the 7^{th} and 10^{th} days in the combination group.

AST level was increased significantly ($P<0.05$) from the 2^{nd} day of treatment in the combination group and the 1^{st} day in Diminazene and Sulphadimidine sodium groups

and remained increased up to the end of the study (Table 3.8).

Significant increase ($P<0.05$) in urea level was observed in first three days following treatment in the third group where donkeys received a combination of Diminazene and Sulphadimidine sodium for three successive days. By the end of the observation period the level of urea decreased significantly ($P<0.05$) below that of day zero as shown in Table (3.9).

Administration of Diminazene alone or in combination with Sulphadimidine sodium to donkeys for three successive days resulted in significant ($P<0.05$) decrease in creatinine level (Table 3.10).

Calcium level decreased significantly ($P<0.05$) in the donkeys received Diminazene for three successive days from the first day up to the 7^{th} day, while in day 10 the level returned to almost the same level as in day zero (Table, 3.11).

Serum phosphorus level increased once in the second group (day 5) and decreased at three time points in the third group (3^{rd}, 4^{th} and the 5^{th} day) (Table, 3.12).

Donkeys in the three treated groups exhibited significant ($P<0.05$) increase in sodium level during the study period (Table, 3.13). Potassium level increased significantly ($P<0.05$) in the first two treatment groups that received Diminazene and Sulphadimidine sodium, respectively (Table 3.14).

Table 3.4: Change in serum Total Protein (g/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	63.97≒2.87	70.07≒2.31	71.55≒1.36
1	63.75≒5.18	69.45≒2.12	71.32≒1.24
2	46.43≒3.79* (0.01)	68.71≒2.32	68.12≒0.55
3	45.09≒7.14	64.62≒4.41	66.90≒0.68* (0.04)
5	56.47≒4.21	67.62≒4.51	67.64≒1.09
7	55.47≒3.63	68.14≒5.23	69.20≒1.73
10	64.56≒4.61	68.88≒2.74	74.94≒2.89

Means in the columns are mean + s.e.m

Normal value of serum total protein (44.20 - 66.90) Seri *et al.*, (2006b)

*Means in the same column with asterisk are significantly ($P<0.05$) different with day zero

Table 3.5: Change in serum Albumin (g/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	8.23≒0.10	22.16≒1.08	22.36≒1.54
1	7.18≒0.49	21.84≒1.32	23.04≒1.46
2	12.41≒1.40* (0.03)	23.40≒1.71	21.02≒1.27
3	13.39≒1.91* (0.04)	22.06≒2.22	20.30≒1.16
5	15.89≒0.60* (0.00)	21.98≒2.06	20.64≒1.31
7	15.55≒1.16* (0.00)	21.72≒2.02	21.34≒1.07
10	15.81≒1.47*(0.00)	22.09≒2.18	21.86≒1.28

Values in the table are mean + s.e.m.

Normal value of serum albumin (20.62 - 36.00) Seri *et al.*, (2006b)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.6: Change in serum Bilirubin (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	0.62 ≒ 0.04	0.14 ≒ 0.05	0.07 ≒ 0.02
1	0.67 ≒ 0.06	0.23 ≒ 0.13	0.26 ≒ 0.09
2	0.80 ≒ 0.02*(0.01)	0.21 ≒ 0.06	0.15 ≒ 0.05
3	0.87 ≒ 0.04*(0.01)	0.38 ≒ 0.11	0.16 ≒ 0.05*(0.04)
5	0.86 ≒ 0.03*(0.01)	0.46 ≒ 0.16	0.20 ≒ 0.05*(0.02)
7	0.74 ≒ 0.02*(0.03)	0.09 ≒ 0.04	0.17 ≒ 0.05*(0.04)
10	0.72 ≒ 0.03	0.07 ≒ 0.02	0.16 ≒ 0.06

Values in the table are mean + s.e.m.

Normal value of serum bilirubin $0-0.4$ mg/dl Zinkl *et al*., (1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.7: Change in serum ALT (IU/L) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	11.56 ≒ 0.44	18.96 ≒ 1.81	36.08 ≒ 2.45
1	11.91 ≒ 0.53	22.12 ≒ 1.26	46.99 ≒ 7.39
2	12.53 ≒ 1.00	27.12 ≒ 4.76	37.50 ≒ 4.73
3	14.65 ≒ 1.06*(0.03)	27.40 ≒ 5.43	33.68 ≒ 3.48
5	13.97 ≒ 0.49*(0.00)	24.22 ≒ 1.77	32.86 ≒ 3.49
7	13.12 ≒ 0.36	24.42 ≒ 1.47	30.22 ≒ 3.00*(0.04)
10	12.69 ≒ 0.56	26.98 ≒ 1.45*(0.00)	20.12 ≒ 3.08*(0.01)

Values in the table are mean + s.e.m.

Normal level of serum ALT 18 ± 32 (0-83) Zinkl *et al.*, (1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.8: Change in serum AST (IU/L) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	131.26 ≒ 4.79	263.68 ≒ 11.97	301.98 ≒ 38.05
1	239.86 ≒ 25.28* (0.01)	394.12 ≒ 48.73*(0.03)	403.94 ≒ 51.56
2	268.77 ≒ 11.72* (0.00)	429.68 ≒ 48.56*(0.02)	475.54 ≒ 12.85* (0.01)
3	244.89 ≒ 10.28*(0.00)	370.90 ≒ 19.48*(0.01)	448.76 ≒ 15.68*(0.02)
5	312.24 ≒ 12.85*(0.00)	357.56 ≒ 24.27*(0.01)	444.94 ≒ 20.46*(0.03)
7	253.70 ≒ 15.01*(0.00)	372.98 ≒ 24.77*(0.00)	494.08 ≒ 19.47*(0.02)
10	260.76 ≒ 19.12*(0.00)	401.62 ≒ 13.72*(0.00)	476.64 ≒ 19.38*(0.02)

Values in the table are mean + s.e.m.

Normal volume ratio of serum AST 487 ± 119 (248-725) IU/litter Zinkl *et al.*, (1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.9: Change in serum Urea (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	25.62 ⩭ 2.55	25.64 ⩭ 1.84	21.78 ⩭ 1.22
1	21.90 ⩭ 1.41	25.16 ⩭ 2.30	30.08 ⩭ 1.80* (0.01)
2	31.09 ⩭ 2.64	23.06 ⩭ 2.54	31.64 ⩭ 1.88*(0.00)
3	34.43 ⩭ 4.91	22.30 ⩭ 2.37	29.10 ⩭ 2.04*(0.01)
5	28.59 ⩭ 3.55	20.62 ⩭ 3.98	24.56 ⩭ 2.44
7	36.94 ⩭ 4.04	21.40 ⩭ 3.13	19.50 ⩭ 1.57
10	21.90 ⩭ 1.41	22.56 ⩭ 2.90	18.18 ⩭ 1.24*(0.04)

Values in the table are mean + s.e.m.

Normal volume ratio of serum urea 16.0-56.8 mg/dl (Jordana *et al.*, 1998)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.10: Change in serum Creatinine (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	1.18≒0.08	0.91≒0.07	1.04≒0.05
1	1.10≒0.13	1.00≒0.08	0.95≒0.06* (0.04)
2	0.74≒0.09*(0.01)	1.07≒0.14	0.93≒0.06*(0.01)
3	0.59≒0.06*(0.00)	1.02≒0.09	0.89≒0.04*(0.00)
5	0.97≒0.07*(0.02)	0.84≒0.14	0.86≒0.03*(0.01)
7	0.87≒0.05*(0.00)	0.74≒0.08	0.84≒0.02*(0.02)
10	1.56≒0.18	0.79≒0.08	0.87≒0.04*(0.04)

Values in the table are mean + s.e.m.

Normal volume ratio of serum creatinine 0.49-1.56 mg/d (Jordana *et al.*, 1998)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero.

Table 3.11: Change in serum Calcium (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	7.83 ≒ 0.21	5.93 ≒ 1.81	8.43 ≒ 0.81
1	7.33 ≒ 0.23* (0.00)	6.02 ≒ 1.86	8.45 ≒ 0.85
2	5.92 ≒ 0.49* (0.01)	6.62 ≒ 1.92	8.89 ≒ 0.88
3	5.36 ≒ 0.77* (0.03)	5.98 ≒ 1.64	8.86 ≒ 0.86
5	6.41 ≒ 0.51* (0.01)	5.57 ≒ 1.69	9.10 ≒ 0.79
7	6.32 ≒ 0.49* (0.03)	5.99 ≒ 1.64	10.10 ≒ 0.33
10	7.65 ≒ 0.54	5.96 ≒ 1.60	10.47 ≒ 0.36

Values in the table are mean + s.e.m.

Normal value of serum calcium 10.2 – 13.3 mg/dl (Zinkl *et al*., 1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero.

Table 3.12: Change in serum Phosphorus (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	2.41≒0.24	2.87≒0.12	2.79≒0.13
1	2.60≒0.28	2.84≒0.10	2.76≒0.14
2	2.71≒0.22	3.15≒0.18	2.59≒0.11
3	2.76≒0.26	3.10+0.34	2.56≒0.01*(0.00)
5	2.29≒0.28	2.20≒0.23*(0.01)	2.48≒0.07*(0.01)
7	2.81≒0.21	2.69≒0.22	2.51≒0.13*(0.04)
10	2.97≒0.26	2.67≒0.19	2.51≒0.17

Values in the table are mean + s.e.m.

Normal volume ratio of serum inorganic phosphorus 2.21 – 5.90 mg/dl (Jordana *et al.*, 1998)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero.

Table 3.13: Change in serum Sodium (mEq/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	122.11+0.96	129.12+1.97	126.82+1.99
1	120.75+1.96	138.24+2.40*(0.01)	129.72+1.20
2	124.16+2.58	137.92+2.65*(0.02)	128.64+2.48
3	127.28+2.35	135.46+2.56*(0.01)	128.72+1.49
5	132.45+1.46*(0.00)	138.26+1.20*(0.01)	129.50+0.82
7	140.50+4.03*(0.00)	130.24+2.15	133.08+2.02
10	122.15+5.34	130.60+1.33	135.86+0.99*(0.03)

Values in the table are mean + s.e.m.

Normal volume ratio of serum sodium 132 – 149 mEq/l (Zinkl *et al*., 1990)

*means with asterisk in the same column are significantly (P<0.05) different with days zero.

Table 3.14: Change in serum Potassium (mEq/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Diminazene	Sulphadimidine	Combination
0	2.46≒0.13	3.73≒0.09	4.20≒0.19
1	2.57≒0.06	4.39≒0.14* (0.00)	4.75≒0.13
2	2.52≒0.10	4.65≒0.07* (0.00)	4.62≒0.23
3	3.15≒0.16*(0.00)	4.69≒0.21* (0.01)	3.94≒0.15
5	3.13≒0.15*(0.02)	4.89≒0.15*(0.00)	4.04≒0.14
7	4.08≒0.24*(0.00)	4.33≒0.31	4.59≒0.08
10	3.15≒0.13*(0.00)	4.40≒0.24*(0.02)	4.51≒0.20

Values in the table are mean + s.e.m.

Normal volume ratio of serum potassium 3.0 – 5.4 mEq/l Zinkl *et al*., (1990)

*Means with asterisk in the same column are significantly (P<0.05) different with days zero.

3.2 Second experiment

3.2.1 Side and/or adverse effects

The same as in the first experiment animals showed irritation and oedema at injections site

3.2.2 Haematological parameters

In the control group immediately following fasting significant ($P<0.05$) decrease in PCV (%) was observed in the third and fifth day and continued with non significant decrease up to the end of the experiment period. Significant decrease in PCV (%) was also observed following injection of Diminazene up to the fifth day of treatment, while in the group treated with Sulphadimidine sodium a simultaneous significant decrease was observed following fasting and remained with no significant difference during the treatment period. The combination of Diminazene and Sulphadimidine sodium resulted in non significant change in PCV (%) (Table 3.15).

A subsequent decrease in RBCs count was observed in the different treatment groups as well as in the control group immediately following injection of the first dose, while Sulphadimidine sodium and the control groups exhibited another significant decrease at days 2 and 3 respectively (Table 3.16). By the end of the experiment at day 10 almost all the groups showed no significant difference with pre-treatment values.

A significant increase in Hb concentration was observed in the control and Sulphadimidine sodium treated group. While, the other two treatment groups

exhibited no significant fluctuation in Hb concentration when compared with pre-treatment level as shown in Table (3.17).

Table 3.15: Change in Packed Cell Volume (%) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	34.20 ≒ 1.68	40.60 ≒ 1.69	36.40 ≒ 1.20	35.20 ≒ 2.47
1	30.00 ≒ 1.58*	37.60 ≒ 1.46	33.20 ≒ 0.97*	32.20 ≒ 1.98
	(.002)		(.008)	
2	26.20 ≒ 1.85	36.60 ≒ 1.69*	30.60 ≒ 1.16	32.20 ≒ 2.26
		(.037)		
3	27.00 ≒ 1.48*	35.40 ≒ 1.40*	33.00 ≒ 1.44	36.80 ≒ 2.31
	(.033)	(.025)		
5	30.40 ≒ 1.36*	35.20 ≒ 1.82*	34.20 ≒ 1.15	32.60 ≒ 1.07
	(.009)	(.020)		
7	30.40 ≒ 1.02	38.40 ≒ 1.46	39.60 ≒ 1.46	34.00 ≒ 2.25
10	31.40 ≒ 0.98	38.60 ≒ 1.56	40.40 ≒ 1.02*	33.20 ≒ 1.62
			(.014)	

Values in the columns are mean ≒ s.e.m

Normal PCV (%) 31.59 ± 3.80 (Seri *et al.*, 2006a)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.16: Change in Red Blood Cells count (x10^{12}/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	4.98 ⩭ 0.09	5.00 ⩭ 0.17	5.06 ⩭ 0.25	4.04 ⩭ 0.25
1	4.11 ⩭ 0.24* (.03)	4.76 ⩭ 0.18* (.03)	4.85 ⩭ 0.28* (.04)	3.67 ⩭ 0.19* (.01)
2	4.51 ⩭ 0.30	4.66 ⩭ 0.23	4.00 ⩭ 0.15* (.02)	3.49 ⩭ 0.16
3	4.41 ⩭ 0.14* (.02)	4.63 ⩭ 0.22	4.31 ⩭ 0.20	3.66 ⩭ 0.14
5	4.72 ⩭ 0.07	4.64 ⩭ 0.24	4.59 ⩭ 0.10	3.81 ⩭ 0.14
7	4.84 ⩭ 0.06	4.80 ⩭ 0.20	4.70 ⩭ 0.09	4.07 ⩭ 0.18
10	4.89 ⩭ 0.08	5.13 ⩭ 0.09	4.68 ⩭ 0.15	4.02 ⩭ 0.18

Values in the columns are mean ⩭ s.e.m

Normal range of RBC 4.56 – 8.74 x10^{6}/ˡ⁻ l (Zinkl *et al.,* 1990)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.17: Change in Hb concentration (g/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadiazine	Combination
0	11.84^ 0.21	12.34^ 0.21	12.08^ 0.15	12.08^ 0.10
1	12.28^ 0.21* (.03)	12.56^ 0.16	12.56^ 0.16*(.00)	12.26^ 0.13
2	12.52^ 0.17*(.03)	12.86^ 0.11	12.90^ 0.10* (.02)	12.36^ 0.13
3	12.36^ 0.19*(.03)	12.52^ 0.16	12.72^ 0.10*(.01)	12.10^ 0.20
5	12.24^ 0.19*(.04)	12.44^ 0.07	12.36^ 0.12	12.12^ 0.10
7	12.10^ 0.22	12.48^ 0.06	12.32^ 0.11	12.06^ 0.10
10	11.90^ 0.11	12.20^ 0.11	12.04^ 0.12	12.04^ 0.13

Values in the columns are mean ⎓ s.e.m

Normal range of Hb 10.43 ± 1.45 g/100ml (Seri *et al.*, 2006a)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

3.2.3 Blood biochemical parameters

A significant increase in total protein concentration was observed in the groups treated with Diminazene and the combination, respectively. While the group treated with Sulphadimidine sodium showed significant decrease following the 3rd injection (Table 3.18).

Significant decrease in albumin concentration was observed in the control group and Sulphadimidine sodium treated group, while Diminazene treated group exhibited significant increase during fasting period and continued with no significant increased level up to the end of the experiment (Table 3.19).

Although a significant ($P<0.05$) decrease in bilirubin concentration was observed in the control group, a significant ($P<0.05$) increase in bilirubin concentration was observed in the first two days following treatment in the groups treated with Diminazene and Sulphadimidine sodium. While in the combination group there was no significant change (Table 3.20).

There was significant increase in ALT activity in the groups treated with Diminazene and the combination while in the group treated with Sulphadimidine sodium there was no significant increase (Table 3.21). There was significant increase in AST activity in all treatment groups when compared with pre-treatment level following administration of drugs (Table 3.22).

Urea level increased significantly following treatment with Diminazene in the 1st and 2nd day of treatment and a

no significant increase in urea level was also observed in the other two treated groups, by the end of the experiment the urea level was almost the same or below the level of day zero (Table 3.23).

Slight significant increase in creatinine concentration was observed in the first two days of treatment in the groups treated with Diminazene and Sulphadimidine sodium, while the increase continued up to the end of the experiment in the combination treatment group (Table 3.24).

A significant ($P<0.05$) increase in calcium concentration was observed in the groups treated with Sulphadimidine sodium and the combination (Table 3.25).

A significant increase in phosphorus concentration was observed only in the group treated with Sulphadimidine sodium while, in the other two treatment groups there was significant decrease (Table 3.26).

Although there was significant decrease in the sodium level in the control group, there was only simultaneous decrease in the group treat with Diminazene (Table 3.27). A prominent significant increase in potassium level was observed in the groups treated with Diminazene and Sulphadimidine sodium respectively (Table 3.28).

Table 3.18: Change in serum Total Protein (g/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	sulphadimidine	Combination
0	73.31^ 2.80	72.58^ 2.16	71.34^ 3.04	72.49^ 1.46
1	73.66^ 1.97	75.40^ 1.67* (.02)	72.83^ 2.84	72.91^ 1.76
2	72.54^ 2.01	77.36^ 1.95	72.99^ 2.16	77.25^ 2.23* (.04)
3	68.75^ 0.78	74.42^ 1.38	67.79^ 2.47* (.02)	75.65^ 1.45
5	70.77^ 2.21	72.83^ 1.28	68.97^ 1.89	77.55^ 0.58* (.01)
7	72.18^ 2.19	73.47^ 2.48	72.54^ 4.82	71.52^ 1.23
10	73.26^ 2.25	72.13^ 343	72.99^ 2.92	73.38^ 1.53

Means in the columns are mean + s.e.m

Normal value of serum total protein (44.20 - 66.90) Seri *et al.*, (2006b)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.19: Change in serum Albumin (g/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	24.08^ 1.89	26.78^ 1.34	26.62^ 1.73	26.20^ 1.69
1	23.70^ 1.61	28.26^ 1.77* (.04)	26.80^ 1.74	24.98^ 1.71
2	23.60^ 1.42	28.28^ 1.50	27.22^ 1.83	27.96^ 1.66
3	20.24^ 1.68* (.01)	28.27^ 1.36	24.78^ 1.58* (.03)	26.94^ 1.41
5	21.52^ 1.48* (.03)	26.72^ 1.34	25.12^ 1.68* (.01)	26.64^ 1.75
7	22.26^ 1.54* (.02)	27.40^ 1.86	26.08^ 1.55	24.82^ 1.60
10	23.06^ 1.87* (.01)	27.92^ 1.70	26.70^ 1.36	24.62^ 1.79

Values in the table are mean + s.e.m.

Normal value of serum albumin (20.62 - 36.00) Seri *et al.*, (2006b)

*Means in the same column with asterisk are significantly ($P<0.05$) different with day zero

Table 3.20: Change in serum Bilirubin (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	0.40 ≒ 0.09	0.42 ≒ 0.12	0.37 ≒ 0.07	0.53 ≒ 0.07
1	0.44 ≒ 0.09	0.59 ≒ 0.13*(.03)	1.03 ≒ 0.17*(.02)	0.45 ≒ 0.10
2	0.39 ≒ 0.09	0.99 ≒ 0.13*(.04)	1.09 ≒ 0.25*(.04)	0.61 ≒ 0.12
3	0.32 ≒ 0.11	0.51 ≒ 0.17	0.38 ≒ 0.07	0.77 ≒ 0.11
5	0.18 ≒ 0.05	0.35 ≒ 0.16	0.45 ≒ 0.09	0.58 ≒ 0.12
7	0.13 ≒ 0.03*(.04)	0.51 ≒ 0.14	0.46 ≒ 0.10	0.66 ≒ 0.09
10	0.16 ≒ 0.03*(.04)	0.64 ≒ 0.29	0.32 ≒ 0.08	0.48 ≒ 0.08

Values in the table are mean + s.e.m.

Normal value of serum bilirubin $0-0.4$ mg/dl Zinkl *et al*., (1990)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.21: Change in serum ALT (IU/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	17.10≒1.43	13.68≒2.02	23.38≒3.57	20.04≒5.66
1	18.24≒0.95	16.80≒2.39*(.01)	31.76≒6.27	30.68≒5.27*(.02)
2	22.14≒2.81	21.56≒3.75	38.42≒8.55	36.02≒6.96*(.02)
3	19.72≒2.01	30.18+9.76	41.42≒9.10	27.28≒3.04
5	16.84≒1.42	27.34≒6.26*(.04)	29.50≒6.26	39.58≒9.29*(.01)
7	16.74≒0.88	22.54≒5.93	26.96≒6.76	25.96≒4.03
10	16.96≒1.00	15.66≒1.09	18.30≒2.70*(.048)	24.84≒2.33

Values in the table are mean + s.e.m.

Normal level of serum ALT 18 ± 32 (0-83) Zinkl *et al.*, (1990)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.22: Change in serum AST (IU/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	307.96≒25.4	291.28≒20.77	289.46≒24.04	288.58≒35.92

1	8 329.92±15.8	236.70±33.03	352.76±26.36*(.03)	391.00±34.43*(.01)
2	6 384.30±29.2	361.12±50.94*(.03)	358.08±25.01*(.047)	484.58±55.56*(.01)
3	6 363.32±21.1	351.26±60.05	359.62±25.72*(.03)	503.26±63.03*(.01)
5	4 315.98±17.5	345.02±51.02	347.22±20.03*(.02)	404.68±42.75
7	6 309.28±16.8	321.14±47.08	381.74±56.96	380.10±33.61
10	9 297.08±18.6	291.42±37.57	303.70±15.47	365.32±28.26
	2			

Values in the table are mean + s.e.m.

Normal volume ratio of serum AST 487 ± 119 (248-725) IU/litter Zinkl *et al.*, (1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.23: Change in serum Urea (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	25.92 ≒ 3.33	21.78 ≒ 1.51	35.20 ≒ 4.31	28.64 ≒ 1.77
1	30.50 ≒ 2.04	37.06 ≒ 3.90*(.01)	37.10 ≒ 3.76	30.50 ≒ 3.28
2	33.04 ≒ 4.77	37.26 ≒ 4.65*(.01)	43.88 ≒ 4.17	29.86 ≒ 3.54
3	39.22 ≒ 4.66*(.03)	28.34 ≒ 3.83	40.44 ≒ 3.31	28.00 ≒ 3.04
5	27.42 ≒ 4.05	24.40 ≒ 4.07	35.12 ≒ 2.27	25.24 ≒ 2.39
7	27.08 ≒ 3.67	19.94 ≒ 3.86	33.10 ≒ 1.87	24.70 ≒ 2.17
10	26.08 ≒ 3.56	18.06 ≒ 2.45*(.02)	31.60 ≒ 3.15	25.02 ≒ 6.34

Values in the table are mean + s.e.m.

Normal volume ratio of serum urea 16.0-56.8 mg/dl (Jordana *et al.*, 1998)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.24: Change in serum Creatinine (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	0.99≒0.10	0.77≒0.06	0.87≒0.07	0.84≒0.06
1	1.05≒0.07	0.87≒0.06*(.03)	0.97≒0.06*(.04)	0.93≒0.04*(.01)
2	1.09≒0.06	0.96≒0.06*(.01)	0.96≒0.10	1.05≒0.09*(.01)
3	0.97≒0.08	0.87≒0.07	0.92≒0.04	1.00≒0.08*(.04)
5	0.97≒0.07	0.80≒0.06	0.83≒0.04	1.16≒0.09*(.01)
7	0.95≒0.07	0.88≒0.08	0.74≒0.03	1.13≒0.08*(.00)
10	0.94v0.05	0.83≒0.06	0.82≒0.08*(.02)	1.17≒0.09*(.01)

Values in the table are mean + s.e.m.

Normal volume ratio of serum creatinine 0.49-1.56 mg/dl (Jordana *et al.*, 1998)

*Means in the same column with asterisk are significantly (P<0.05) different with day zero

Table 3.25: Change in serum Calcium (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	9.37≒1.29	6.99≒0.28	6.79≒0.29	10.78≒0.29
1	10.66≒1.13*(.02)	6.95≒0.85	6.11≒0.65	10.82≒0.32
2	11.62≒0.90	6.01≒1.3	6.17≒1.01	11.03≒0.18
3	10.07≒0.166	6.40≒0.63	9.89≒0.79*(.01)	11.17≒0.17
5	9.54≒1.22	4.58≒1.26	10.17≒0.16*(.00)	12.28≒0.24*(.01)
7	9.83≒1.17	4.55≒1.02	10.98≒0.40*(.00)	11.28≒0.40
10	9.64≒1.21	6.29≒0.19	11.42≒0.28*(.00)	10.80≒0.21

Values in the table are mean + s.e.m.

Normal value of serum calcium 10.2 – 13.3 mg/dl (Zinkl *et al*., 1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.26: Change in serum Phosphorus (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	2.05+.41	3.42+.20	2.86+.37	3.35+.22
1	2.65 ⎓ 0.28*(.03)	3.14 ⎓ 0.14	4.31 ⎓ 0.29*(.03)	2.57 ⎓ 0.26*(.03)
2	3.11 ⎓ 0.21*(.02)	3.25 ⎓ 0.39	3.80 ⎓ 0.178*(.048)I	2.60 ⎓ 0.25*(.01)
3	2.58 ⎓ 0.25	2.51 ⎓ 0.48	2.43 ⎓ 0.35	3.01 ⎓ 0.491
5	1.34 ⎓ 0.07	2.09 ⎓ 0.19*(.02)	2.27 ⎓ 0.38	2.49 ⎓ 0.242
7	1.87 ⎓ 0.14	2.58 ⎓ 0.20	2.94 ⎓ 0.21	2.57 ⎓ 0.22
10	2.11 ⎓ 0.18	2.48 ⎓ 0.15*(.01)	3.30 ⎓ 0.36	2.38 ⎓ 0.25*(.01)

Values in the table are mean + s.e.m.

Normal volume ratio of serum inorganic phosphorus 2.21 – 5.90 mg/dl (Jordana *et al.*, 1998)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.27: Change in serum sodium (mEq/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	139.26≒2.10	131.56≒1.84	130.36≒1.70	132.94≒0.90
1	134.94≒1.51*(.01)	130.24≒1.73	130.36≒1.17	133.08≒1.32
2	130.56≒1.38*(.01)	125.50≒2.09*(.02)	128.78≒1.27	134.02≒1.52
3	132.56≒1.36*(.01)	131.04≒1.50	130.28≒1.23	132.06≒1.00
5	127.14≒0.67*(.01)	126.82≒2.10	131.96≒1.20	130.38≒2.41
7	129.96≒0.69*(.01)	122.52≒1.16*(.03)	132.84≒0.91	128.36≒1.80
10	133.80≒0.94	125.94≒1.26	131.56≒1.74	130.02≒2.12

Values in the table are mean + s.e.m.

Normal volume ratio of serum sodium 132 – 149 mEq/l (Zinkl *et al*., 1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Table 3.28: Change in serum Potassium (mEq/L) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium in fasted donkeys

Days	Control	Diminazene	Sulphadimidine	Combination
0	5.02 ≒ 0.14	6.33 ≒ 1.87	4.65 ≒ 0.17	3.67 ≒ 0.29
1	4.88 ≒ 0.12*(.04)	9.85 ≒ 2.53	4.08 ≒ 2.05	3.93 ≒ 0.20
2	4.79 ≒ 0.15	13.47 ≒ 2.58	11.07 ≒ 2.08*(.03)	3.93 ≒ 0.23
3	3.89 ≒ 0.14*(.01)	8.66 ≒ 1.38	10.26 ≒ 1.01*(.047)	3.48 ≒ 0.08
5	3.89 ≒ 0.21*(.03)	9.72 ≒ 1.29	5.53 ≒ 0.02	3.66 ≒ 0.16
7	3.99 ≒ 0.30	18.81 ≒ 1.79*(.00)	4.21 ≒ 0.15	4.79 ≒ 0.99
10	4.23 ≒ 0.28	15.27 ≒ 0.65*(.01)	4.35 ≒ 0.21	5.22 ≒ 1.25

Values in the table are mean + s.e.m.

Normal volume ratio of serum potassium 3.0 – 5.4 mEq/l Zinkl *et al*., (1990)

*Means with asterisk in the same column are significantly (P<0.05) different with day zero

Chapter four

Discussion

The current study was conducted to evaluate some pharmacological aspects of repeated administration of Diminazene aceturate and/or Sulphadimidine sodium to donkeys. It is also aimed to study the combined effect of induced stress and simultaneous administration of the two drugs on some haematological and biochemical parameters of donkeys.

4.1 Experiment I

In this study three groups of donkeys each of six animals were either treated with Diminazene aceturate injection formulation at the recommended dose or with Sulphadimidine sodium at the recommended dose or with a combination of Diminazene and Sulphadimidine for three successive days. The effect of medication was evaluated with special emphasis on liver and kidney functions as well as some haematological indices.

Following Diminazene aceturate administration, donkeys immediately and up to two hours monitoring period showed some sort of irritation as well as oedema at the injection site. Signs of toxicity observed in the group that received Diminazene are in accordance with the results of Homeida and his colleagues (1981) who reported that camels treated with 10 and 40 mg/kg body weight exhibited clinical signs that developed within 15 minutes of injection with Berenil. The camel defaecated and became hyperaesthetic 5 min later. They showed

tremors, itching, frequent urination, frothing at the mouth and sweating. When the second dose of Berenil was given on days 3 or 4 the animals showed uneasiness, colonic convulsions, grinding of the teeth, frequent urination, sweating, dyspnoea, salivation, recumbency and paddling of limbs. Within 10 minutes of the injection camel No. 3 developed hyperaesthesia, frequent urination and defaecation, frothing at the mouth, convulsions, sweating, recumbency and regurgitation of ruminal contents.

The PCV values were significantly ($P<0.05$) decreased in the group where animals received Sulphadimidine sodium for three successive days, while RBCs count and haemoglobin level showed no significant ($P<0.05$) fluctuation during the study period in the three treated groups.

The no significant change in Hb concentration and total RBCs count observed in the current study following administration of Diminazene is in agreement with the observations of Homeida *et al.*, (1981) in camels and Da Silva *et al.*, (2009) in cats.

Trypanosomiasis was reported to reduce the PCV and RBC counts significantly ($p<0.05$) (Horst, 1996). However, Omoja and his colleagues (2012) reported that treatment with Diminazene aceturate at 7 mg/kg body weight increased both PCV and RBC counts in rats infected with *T. brucei brucei*. They postulated that treatment with Berenil was able to ameliorate the anaemia caused by

trypanosomosis; here this result partially supports results obtained in the current study.

A variety of biochemical parameters are measured in toxicity studies, in attempts to evaluate a broad range of physiological and metabolic functions affecting target organ identification and tissue injury assessment (Akhtar *et al*. 2012). Some common biochemical parameters provide better information from pattern recognition, e.g. enzymes like ALT and AST for hepatotoxicity, and urea and creatinine for glomerular function (Evans, 1996).

In the Diminazene aceturate treated group total serum protein decreased significantly ($P<0.05$) following two days of treatment. While in the group that treated with the combination the total protein concentration decreased significantly ($P<0.05$) following three successive days of treatment. At the end of the study total protein level returned to pre-treatment level with no significant difference. Homeida *et al.*, (1981) observed no significant changes in the concentration of total protein in serum of any of the camels treated with Diminazene at 10mg/kg body weight.

The significant increase in albumin concentration following the second dose in the group that received Diminazene aceturate may be attributed to the improvement in animal health following medication and regular feeding.

Serum bilirubin increased significantly ($P<0.05$) in the group that received Diminazene aceturate from the

second day up to the 7th day of the study. Another increase was also monitored in the third group that received the combination from the 3rd up to the 7th day of treatment. While, administration of Sulphadimidine sodium induced no significant (P>0.05) increase in bilirubin concentration. Kaneko *et al.,* (1997) stated that the normal reference level of bilirubin in horses was 1-2 mg/dl, while Zinkl and his colleagues (1990) reported a range of 0-0.4 mg/dl in American donkeys.

Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucouronic acid to form a soluble compound. This conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. An un-conjugated, albumin bound form is also present in the circulation. It is insoluble and does not normally pass through the kidneys into the urine.

Here the prominent rise in bilirubin in the group received Diminazene may be attributed to the low concentration of albumin that minimize ability of unconjugated bilirubin to bind to albumin and hence an increase in bilirubin concentration. However, as above stated the bilirubin level is still within normal level stated by above mentioned authors.

The Significant increase (P <0.05) in ALT and AST activity observed in the current study may be attributed to the increase in activity of the liver following administration of the drugs. Activities of serum enzymes like AST and ALT

represent the functional status of the liver (Cremer and Seville, 1982), as certain hepatic damage is considered pathologically irreversible (Helling *et al*., 1995). Aspartate aminotransferase is an important indicator of liver damage in clinical studies.

There were no significant changes in the activity of ALT in serum of any of the camels treated with Diminazene (Homeida *et al.*, 1981). In camel No. 1, the activity of AST commenced to rise on day 2 and reached peaks on day 5. In camel No. 2, there was increase in the activity of AST at the time of slaughter; a result that was in agreement with the results obtained in the current study. Here it is worth to mention that the level of the two enzymes is still within the normal range suggested by (Zinkl *et al.*, 1990, and Kaneko *et al.*, 1997).

The significant increase in urea blood level observed in the third group where donkeys received a combination of Diminazene and Sulphadimidine sodium for three continued days was also observed by Homeida and his colleagues (1981), in camels where the concentration of ammonia commenced to rise on day 2 and reached peaks on day 8. By the end of the observation the level of urea decreased significantly ($P<0.05$) below that of day zero.

Administration of Diminazene alone or in combination with Sulphadimidine sodium to donkeys for three successive days resulted in significant ($P<0.05$) decrease in creatinine level. There were no significant differences in creatinine levels between serum collection dates in horses

and mules naturally infected with *T. evansi* and treated with Diminazene aceturate (Tuntasuvan *et al.*, 2003).

Calcium significant ($P<0.05$) decrease in calcium level observed in donkeys received Diminazene for three successive days was in agreement with observations of Homeida *et al.*, (1981) who reported significant decrease in magnesium and calcium concentration in camels treated repeatedly with Diminazene at 10 mg/kg body weight. Serum phosphorus level increased once in the second group (day 5) and decreased at three time points in the third group (3^{rd}, 4^{th} and the 5^{th} day). Cornelius and Kaneko (1963) suggested that renal lesions lead to retention of phosphate which in turn reduces the absorption of calcium from the alimentary tract and causes a fall in the concentration of calcium in the serum.

Donkeys in the three treated groups exhibited significant ($P<0.05$) increase in sodium level during the study period. Potassium level increased significantly ($P<0.05$) in the first two treatment groups that received Diminazene and Sulphadimidine sodium, respectively.

The significant increase in sodium and potassium level at the end of current study may be attributed to kidneys dysfunction following administration of the drugs for three successive days as shown in elevated concentration of urea.

4.2 Experiment II

In the second experiment, animals were subjected to fasting for 48 hours and then either kept without

treatment or treated with repeated intramuscular doses of Diminazene aceturate, Sulphadimidine sodium, and combination for three successive days as in experiment I.

In the control group immediately following fasting significant ($P<0.05$) decrease in PCV (%) was observed in the 3rd and 5th day and continued with non significant decrease up to the end of the experiment period. Significant decrease in PCV (%) was observed following injection of Diminazene up to the 5th day of treatment, while in the group treated with Sulphadimidine sodium a simultaneous significant decrease was observed following fasting and remained with no significant difference during the treatment period. The combination of Diminazene and Sulphadimidine sodium resulted in non significant change in PCV (%).

A subsequent decrease in RBCs count was observed in the different treatment groups as well as in the control group immediately following injection of the first dose, while Sulphadimidine sodium and the control groups exhibited another significant decrease at days 2 and 3 respectively. By the end of the experiment at day 10 almost all the groups showed no significant difference with pre-treatment values.

A significant increase in Hb concentration was observed in the control and Sulphadimidine sodium treated group, while, the other two treatment groups exhibited no significant fluctuation in Hb concentration when compared with pre-treatment level.

The gradual development of a mild or moderate degree of anemia is not uncommon during the administration of sulphonamides; it is usually of relatively minor significance and does not necessitate stopping the use of the drug. The abrupt appearance of a severe anemia of hemolytic type has been generally recognized as an occasional complication of sulfonamide therapy since the condition was first reported by Harvey and Janeway (1937). Sulfadiazine has been regarded as relatively safe in this respect, but several cases of such anemia have been reported. We have found reports of cases in which acute hemolytic anemia appeared in patients during or shortly after the administration of sulphadiazine and in which the drug may have caused or participated in the production of anaemia (Donald and Wunsch, 1944; Ross and Paegel, 1946).

The minor changes observed in haematological indices were still within normal range suggested by other researchers (Zinkl *et al.*, 1990, Kaneko *et al.*, 1997, and Seri *et al.*,, 2006a).

Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Balistreri and Shaw, 1987). Therefore, liver can be used as an index for the toxicity of xenobiotics. Hence, the activities of some enzymes and levels of certain biochemical parameters representing liver function, i.e.

AST, ALT, total protein, and albumin were determined in treated donkeys.

A significant increase in total protein concentration was observed in the groups treated with Diminazene and the combination, respectively. While the group treated with Sulphadimidine sodium showed significant decrease following the 3rd injection. Significant decrease in albumin concentration was observed in the control group and Sulphadimidine sodium treated group, while Diminazene treated group exhibited significant increase during fasting period and continued with no significant increased level up to the end of the experiment. The significant decrease in albumin concentration was considered as a sequence to the decrease observed already in total protein concentration.

A significant ($P<0.05$) decrease in bilirubin concentration was observed in the control group, however, a significant ($P<0.05$) increase in bilirubin concentration was observed in the first two days following treatment in the groups treated with Diminazene and Sulphadimidine sodium. While in the combination group there was no significant change.

Here still bilirubin level is within the normal level reported by Zinkl *et al.,* (1990) and Kaneko *et al.,* (1997)

There was significant increase in ALT activity in the groups treated with Diminazene and the combination while in the group treated with Sulphadimidine sodium there was no significant increase. There was significant

increase in AST activity in all treatment groups when compared with pre-treatment level following administration of drugs.

The liver plays a role in the detoxification of metabolic by-products and xenobiotics. In the present study, the increased levels of AST and ALT could be due to hepatotoxicity causing permeability alterations and leakage of lysosomal enzymes enhancing the release of enzymes (Shrivastava *et al*., 1989, and Choudhary *et al*., 2003). The elevation of ALT and AST levels in this study suggests an increase in liver function following administration of experimental drugs.

Many reports had elucidated that hepatocellular damage could be correlated with the disturbed enzymes activities. In this respect, liver tissues which were famous for their rich contents of aminotransferases (AST and ALT) suffer markedly from their loss under many pathological conditions (Rodwell, 1983).

Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes (Plaa and Hewitt, 1982) and is used to detect liver damage (Klaassen and Eaton, 1991). The alteration in serum levels of alanine aminotransferase (ALT) may be indicative of internal organs damage especially in liver (Kaneko *et al.,* 1997).

Urea level increased significantly following treatment with Diminazene and a no significant increase in urea level

was also observed in the other two treated groups.Urea is useful in early deduction of nephrotoxicity induced by exogenous compounds. This parameter is used as index of renal damage in living organisms (Coles, 1986). Elevation of urea concentration in plasma of treated male donkeys may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules (Hayes, 1989; Walmsley and White, 1994).

The increase of urea concentration is a demonstration of impaired kidney function since the organ primarily excretes urea in the urine. The increase in urea due to drugs administration may be attributed to the same pathway of urea excretion. The high serum AST and ammonia levels may be due to liver malfunction, but renal lesions could have contributed to the raised ammonia levels (Homeida *et al.*, 1981).

Hyperammonaemia has been reported to cause the development of nervous signs in domesticated animals (Ahmed and Adam, 1979). The findings show that Berenil (or a metabolite) has a direct effect on the renal tissue, especially the convoluted tubules. The enzyme histochemical changes in the liver and kidney of Berenil-treated camels are evidence of damage to these organs (Homeida *et al*., 1981). By the end of the experiment the urea level was almost the same or below the level of day zero.

Slight significant increase in creatinine concentration was observed in the first two days of treatment in the

groups treated with Diminazene and Sulphadimidine sodium, while the increase continued up to the end of the experiment in the combination treatment group. There were no significant differences in creatinine levels between serum collection dates in horses and mules naturally infected with *T. evansi* and treated with Diminazene aceturate (Tuntasuvan *et al.*, 2003). Here haemconcentration following fasting may cause this transient increase in creatinine.

A significant ($P<0.05$) increase in calcium concentration was observed in the groups treated with Sulphadimidine sodium and the combination. A significant increase in phosphorus concentration was only observed only in the group treated with Sulphadimidine sodium. A result that is partially agrees with that Homeida *et al.,* (1981).

A significant decrease in the sodium level was observed in the control group, a simultaneous decrease was also observed in the group treat with Diminazene. A prominent significant increase in potassium level was observed in the groups treated with Diminazene and Sulphadimidine sodium respectively.

The hepatic and renal functions remained normal during therapy, similar results were observed in a study with cats treated with five doses of Diminazene aceturate (Da Silva *et al.*, 2009). Therefore, nothing prevents the use of this drug in treatment of donkeys infected with *T.*

evansi, though it is advisable to have a close monitoring of the animal during the therapy, as was done in this study.

At the end of the experiment period, all biochemical and haematological parameters returned to normal levels, allowing us to conclude that this new protocol tested was safe to be used for the cure of trypamosomiasis and/or babesiosis in donkeys.

Chapter five

Conclusion and recommendations

5.1 Conclusion

It is to be concluded that, the three –repeated dose protocol is safe to be used in donkeys for the treatment of trypanosomiasis and/ or babesiosis.

Signs of drug intoxication were not observed, as well as hepatic and renal functions were not affected, since hepatic enzymes, urea and creatinine remained within normal limits. The animal showed normal biochemical and haematological parameters after 3 days of repeated administration of Diminazene and/or potentiating with Sulphadimidine sodium.

5.2 Recommendations

It is recommended that

1. Detailed study should be conducted to evaluate therapeutic efficacy of the protocol in infected animals
2. Manipulation of the dosage regimen could also be attempted.

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