Safety of simultaneous administration of Diminazene diaceturate and Sulphadimidine sodium in donkeys

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Abstract

A study was conducted to evaluate the safety of repeated administration of Diminazene diaceturate and/or Sulphadimidine sodium in donkeys. For this purpose 18 healthy, male donkeys, 4-10 years of age were allocated into three treatment groups, each of 6 animals. The first group was treated with Diminazene diaceturate at a dose rate of 3.5 mg/kg intramuscularly for three successive days, animals in the second group was subjected to treatment with sulphadimidine sodium at a dose rate of 3 ml/10 kg for three consecutive days, while the third group has been subjected to treatment with combination of the two drugs Diminazene diaceturate and sulphadimidine sodium with the same previous doses for three successive days.

Animals were monitored for two hours following each administration of the drugs. Blood samples were collected before (Baseline), during (1, 2, and 3), and at (5, 7, and 10) days following the first treatment to evaluate some haematological (PCV, RBCs, and HB) and blood biochemical parameters (total protein, albumin, Bilirubin, urea, creatinine, and the effectiveness of liver enzymes (Alanine aminotransferase ALT and Aspartate aminotransferase enzyme AST), calcium, phosphorus, sodium, and potassium). Results obtained showed fluctuation in the parameters tested but still the values were within the normal reference values in donkeys reported in donkeys.

It is to be concluded that administration of diminazene diaceturate, Sulphadimidine sodium alone or in combination for three continuous days is safe in healthy donkeys.

Keywords: Berenil, Sulphonamides, sub acute toxicities, donkeys.

Introduction

Treatment of trypanosomiasis relies on the use of diminazene aceturate "*Berenil*" 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, and Da Silva *et al.*, 2011), and therefore, new treatment protocols are needed.

In Brazil, three doses of 7 mg kg^{-1} at 7-day intervals were sufficient to cure all horses parasitized by *T. evansi* in a farm in the southern region of the country (Da Silva *et al.*, 2009a). The same protocol was used for cats

experimentally infected with *T. evansi*, obtaining 85.7% (6/7) of curative efficacy (Da Silva *et al*., 2009b). The difference in the drug concentration and in the number of doses may have been the cause for the therapeutic success in this case.

Previously, the cure in rats occurred when animals received a dose of 3.5 and 7.0 mg kg-1 during 5 consecutive days (Da Silva *et al*., 2008). No clinical signs of drug toxicity were detected in the felines, as hepatic and renal parameters remained inside normal values. Similar results were observed in rats (Da Silva *et al*., 2008) and equines (Da Silva *et al.*, 2009a) treated with five and three doses of diminazene aceturate, respectively. Alternatively, Tuntasuvan *et al.*, (2003) reported mild to severe toxicity by the use of diminazene aceturate in horses and mules.

Homeida *et al.,* (1981), conducted a study in four one-humped camels (*Camelus dromedaries*) of either sex, 3-5 years of age. Camels were either received 10 mg per kg body weight on days 0 and 3 and 0 and 4 respectively or treated with a single intramuscular dose at the rate of 40 mg/kg.

Camel that received a single intramuscular dose of 40 mg per kg, died 4 h later. Camel which received two intramuscular doses of 10 mg per kg body weight on days 0 and 3, died on the 8th day of the experiment. While, the camel that received 2 intramuscular doses of 10 mg per kg on days 0 and 4, was killed on the 8th day (Homeida *et al.*, 1981).

The clinical signs observed in camels were similar and 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 (Homeida *et al*., 1981). There were no significant changes in the concentration of total protein and bilirubin or in the activity of ALT in serum of any of the camels. In camel 1, the concentration of ammonia and the activity of AST commenced to rise on day 2 and reached peaks on day 8 and 5 respectively. In camel 2, there was increase in the activity of AST and in the concentration of ammonia at the time of slaughter. No significant haematological changes were observed in Berenil-treated camels. The lack of increase in the activity of ALT suggests that damage to muscle was slight and supports the earlier suggestion that increased AST activity in serum may have originated from the liver.

The damage to the liver and kidney was accompanied by increases in the activity of AST and in the concentration of ammonia and by decreases in the concentration of calcium and magnesium in serum (Homeida *et al.*, 1981).

MATERIALS and METHODS

Study location and housing:

The experiment was conducted at the college farm, College of Veterinary Medicine (SUST), located in Khartoum North, Hillat Kuku, Sudan. Animals were housed in pens, the enclosures made of iron and wood; with 3X7

meters dimension. Experimental animals were supplemented with *Abu sabeen* and water ad lib.

Experimental drugs:

Diminazene diaceturate: TRYPONIL (*Interchemi, Holland*) and *Sulphadimidine sodium:* SULFA 333 (*Interchemi, Holland*) were used as experimental drugs in this study.

Animals and treatments

For the purpose of this study, a total of 18 male donkeys, clinically healthy, 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.

Donkeys were divided into three groups each of six donkeys. Animals in the first group (DM) were treated with Diminazene diaceturate once daily for three successive days at the manufacturer recommended dose 3.5 mg/kg b.w. Animals in the second group (S) 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 (DMS) were treated with both Diminazene diaceturate and Sulphadimidine sodium once daily for three successive days at the manufacturers recommended dose. Animals were monitored for two hours following the administration of each dose.

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 ≍C until analyzed.

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

Sampling schedule:

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

Haematological methods:

The following haematological indices were determined using routine laboratory methods. Packed cell volume (PCV) was determined by the micro haematocrit method described by Dacie and Lewis (1984) and Schalm *et al*., (1975). Erythrocytes (RBC) were counted using the improved Neubauer haemocytometer (Dacie and Lewis, 1984). Haemoglobin concentration (Hb) was determined by method described by Jain (1986).

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 level.

The following blood biochemical parameters were tested using standard methods: Total protein (King and Wooton, 1956), albumin (Doumas *et al*., 1971), bilirubin (Heinemann and Vogt, 1988), Aspartate and alanine aminotransferase (AST & ALT) activities were determined according to the method of (Reitman and Frankel, 1957), Urea (Fawcett and Scott, 1960), Creatinine (Bartels *et al.,* 1972), Calcium (Barnett *et al*., 1973), Inorganic phosphorus (Goldenburg and Fernandez, (1966), and Sodium and Potassium (Wootton, 1974).

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.

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.

RESULTS and DISCUSSION

The current study was conducted to evaluate some pharmacotoxic aspects of repeated administration of Diminazene diaceturate and/or Sulphadimidine sodium IN donkeys. In this study three groups of donkeys each of six animals were either treated with Diminazene diaceturate injection formulation at the recommended dose (DM) or with Sulphadimidine sodium at the recommended dose (S) or with a combination of diminazene and Sulphadimidine (DMS) for three successive days. The effect of medication was evaluated with special emphasis on liver and kidney functions as well as some haematological indices.

Post- Treatment Reactions

Following Diminazene diaceturate administration, donkeys immediately and up to two hours monitoring period showed some sort of irritation as well as oedema at the site of injection. Signs of toxicity observed in treatment group (DM) following administration of the drug are in accordance with the results of Homeida and his colleagues (1981) who reported that camels treated with 10 and 40 mg per 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 3 developed hyperaesthesia, frequent urination and defaecation, frothing at the mouth, convulsions, sweating, recumbency and regurgitation of ruminal contents.

Haematological results:

The PCV values were significantly (P<0.05) decreased in the second 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 (Table 1)

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.*, (2009b) 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 diaceturate 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.

Here the fluctuation in haematological indices observed following repeated administration of experimental drugs was within the normal reference values reported previously for Hb (Normal range of Hb 10.43 ± 1.45 g/100ml) and PCV(31.59 ± 3.80) (Seri *et al.*, 2006a) and RBCs (4.56 – 8.74 x10^6/ˉˡ l) reported by (.....) .

Table 1: Change in PCV (%), RBCs (x10^{12}/litter), and Hb (g/dl) following administration of Diminazene, Sulphadimidine sodium, and Diminazene+ Sulphadimidine sodium in donkeys for three successive days

Days	PCV (%)			RBCs (x10^{12}/litter)			Hb (g/dl)		
	DM	S	DMS	DM	S	DMS	DM	S	DMS
0	34.50±1.46	35.20+1.15	29.20+1.01	5.12≒0.47	4.46≒0.37	4.92≒0.25	12.00≒0.21	12.16≒0.39	11.86≒0.31
1	36.16±3.55	34.20+1.46* (0.01)	29.80+1.39	5.06≒0.51	4.14≒0.36	4.69≒0.24	11.78≒0.32	12.20≒0.08	11.92≒0.31
2	37.50±2.86	30.40+1.43* (0.01)	30.00+0.63	5.43≒0.42	4.59≒0.37	4.34≒0.30	11.88≒0.15	11.96≒0.25	11.78≒0.26
3	31.00+1.26	28.80+1.52	30.20+0.66	5.73≒0.44	4.31≒0.18	4.36≒0.18	12.01≒0.15	12.00≒0.27	11.84≒0.29
5	29.33+1.33	33.40+0.68	29.40+0.68	5.05≒0.23	4.41≒0.35	4.28≒0.24	12.05≒0.23	11.96≒0.24	11.78≒0.33
7	30.33+0.76	31.20+1.31	28.80+1.24	4.45≒0.41	4.54≒0.30	4.62≒0.20	11.65≒0.23	11.84≒0.21	11.88≒0.31
10	29.33+1.20	33.40+0.93	28.80+0.97	4.70≒0.39	4.67≒0.28	4.51≒0.20	11.73≒0.15	11.72≒0.21	11.82≒0.38

Values in the columns are mean≒s.e.m

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

Blood biochemical constituents

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 diaceturate treated group total serum protein decreased significantly (P<0.05) following two days of treatment. While in the group

that was 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 2).

Homeida *et al.*, (1981) observed no significant changes in the concentration of total protein in serum of any of the camels treated with Diminazene aceturate.

The significant increase in albumin concentration following the second dose in the group that received Diminazene diaceturate (Table2) 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 diaceturate 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 (Table 2). 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 Normal value of serum total protein (60.0 -75.2) (.....), while that of albumin is Normal value of serum albumin (17.5 - 36.1)

Table 2: Change in serum total protein (g/l), albumin (g/l), and bilirubin (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Total proteins (g/l)			Albumin (g/l)			Bilirubin (mg/dl)		
	DM	S	DMS	DM	S	DM	DM	S	DMS
0	63.97+2.87	70.07+2.31	71.55+1.36	8.23+0.10	22.16+1.08	22.36+1.54	0.62+0.04	0.14+0.05	0.07+0.02
1	63.75+5.18	69.45+2.12	71.32+1.24	7.18+0.49	21.84+1.32	23.04+1.46	0.67+0.06	0.23+0.13	0.26+0.09
2	46.43+3.79* (0.01)	68.71+2.32	68.12+0.55	12.41+1.40* (0.03)	23.40+1.71	21.02+1.27	0.80+0.02*(0.01)	0.21+0.06	0.15+0.05
3	45.09+7.14	64.62+4.41	66.90+0.68* (0.04)	13.39+1.91* (0.04)	22.06+2.22	20.30+1.16	0.87+0.04*(0.01)	0.38+0.11	0.16+0.05*(0.04)
5	56.47+4.21	67.62+4.51	67.64+1.09	15.89+0.60* (0.00)	21.98+2.06	20.64+1.31	0.86+0.03*(0.01)	0.46+0.16	0.20+0.05*(0.02)
7	55.47+3.63	68.14+5.23	69.20+1.73	15.55+1.16* (0.00)	21.72+2.02	21.34+1.07	0.74+0.02*(0.03)	0.09+0.04	0.17+0.05*(0.04)
10	64.56+4.	68.88+2.	74.94+2.	15.81+1.47*(0	22.09+2.	21.86+1.	0.72+0.03	0.07+0.	0.16+0.06

61	74	89	.00)	18	28	02

Means in the columns are mean + s.e.m
*Means with asterisk in the same column are significantly (P<0.05) different with day zero

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

AST level was increased significantly (P<0.05) from the 2nd day of treatment in the combination group and the 1st day in Diminazene and Sulphadimidine sodium groups and remained increased up to the end of the study (Table 3).

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. 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).

Activities of serum enzymes like AST and ALT represent the functional status of the liver (Cremer and Seville, 1982). 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 1, the activity of AST commenced to rise on day 2 and reached peaks on day 5. In camel 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.

Table 3: Change in serum ALT (U/l) and AST (U/l) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Day	ALT (U/l)			AST (U/l)		
s	DM	S	DMS	DM	S	DMS
0	11.56+.441	18.96+1.81	36.08+2.45	131.26+4.79	263.68+11.97	301.98+38.05
1	11.91+.527	22.12+1.26	46.99+7.39	239.86+25.28*(0.01)	394.12+48.73*(0.03)	403.94+51.56
2	12.53+1.00	27.12+4.76	37.50+4.73	268.77+11.72*(0.00)	429.68+48.56*(0.02)	475.54+12.85*(0.01)
3	14.65+1.06*(0.03)	27.40+5.43	33.68+3.48	244.89+10.28*(0.00)	370.90+19.48*(0.01)	448.76+15.68*(0.02)
5	13.97+.485*(0.00)	24.22+1.77	32.86+3.49	312.24+12.85*(0.00)	357.56+24.27*(0.01)	444.94+20.46*(0.03)
7	13.12+.361	24.42+1.47	30.22+3.00*(0.04)	253.70+15.01*(0.00)	372.98+24.77*(0.00)	494.08+19.47*(0.02)
10	12.69+.561	26.98+1.45*(0.00)	20.12+3.08*(0.01)	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 level of serum ALT 18 ± 32 (0-83) Zinkl *et al.*, (1990)
Normal volume ratio of serum AST 487 ± 119 (248-725) U/litter Zinkl *et al.*, (1990)
*Means with asterisk in the same column are significantly (P<0.05) different with day zero

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 continued days. By the

end of the observation the level of urea decreased significantly (P<0.05) below that of day zero as shown in Table (4).

The significant increase in urea blood level observed in the current study was also observed by Homeida and his colleagues (1981), in camel 1, the concentration of ammonia commenced to rise on day 2 and reached peaks on day 8. In camel 2, there was increase in the concentration of ammonia at the time of slaughter.

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 4).

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).

Table 4: Change in serum Urea (mg/dl) and Creatinine (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Urea (mg/dl)			Creatinine (mg/dl)		
	DM	S	DMS	DM	S	DMS
0	25.62+2.55	25.64+1.84	21.78+1.22	1.18+0.08	0.91+0.07	1.04+0.05
1	21.90+1.41	25.16+2.30	30.08+1.80*(0.01)	1.10+0.13	1.00+0.08	0.95+0.06*(0.04)
2	31.09+2.64	23.06+2.54	31.64+1.88*(0.00)	0.74+0.09*(0.01)	1.07+0.14	0.93+0.06*(0.01)
3	34.43+4.91	22.30+2.37	29.10+2.04*(0.01)	0.59+0.06*(0.00)	1.02+0.09	0.89+0.04*(0.00)
5	28.59+3.55	20.62+3.98	24.56+2.44	0.97+0.07*(0.02)	0.84+0.14	0.86+0.03*(0.01)
7	36.94+4.04	21.40+3.13	19.50+1.57	0.87+0.05*(0.00)	0.74+0.08	0.84+0.02*(0.02)
10	21.90+1.41	22.56+2.90	18.18+1.24*(0.04)	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 urea 16.0-56.8 mg/dL
Normal volume ratio of serum creatinine 0.49-1.56 mg/dL
*Means with asterisk in the same column are significantly (P<0.05) different with day zero.

Calcium level decreased significantly (P<0.05) in the donkeys received Diminazene for three successive days from the first day up to the 7th day, while in day 10 the level returned to almost the same level as in day zero (Table, 5). Serum phosphorus level increased once in the second group (day 5) and decreased at three time points in the third group (3rd, 4th and the 5th day) (Table, 5).

The concentration of serum magnesium and calcium were reduced terminally camel that was treated with 10 mg/kg body weight (Homeida *et al.*, 1981). 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.

Table 5: Change in serum Calcium (mg/dl) and phosphorus (mg/dl) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Calcium			Phosphorus		
	DM	S	DMS	DM	S	DMS
0	7.83+0.21	5.93+1.81	8.43+0.81	2.41+0.24	2.87+0.12	2.79+0.13
1	7.33+0.23* (0.00)	6.02+1.86	8.45+0.85	2.60+0.28	2.84+0.10	2.76+0.14
2	5.92+0.49* (0.01)	6.62+1.92	8.89+0.88	2.71+0.22	3.15+0.18	2.59+0.11
3	5.36+0.77* (0.03)	5.98+1.64	8.86+0.86	2.76+0.26	3.10+0.34	2.56+0.01*(0.00)
5	6.41+0.51* (0.01)	5.57+1.69	9.10+0.79	2.29+0.28	2.20+0.23*(0.01)	2.48+0.07*(0.01)
7	6.32+0.49* (0.03)	5.99+1.64	10.10+0.33	2.81+0.21	2.69+0.22	2.51+0.13*(0.04)
10	7.65+0.54	5.96+1.60	10.47+0.36	2.97+0.26	2.67+0.19	2.51+0.17

Values in the table are mean + s.e.m.
Normal value of serum calcium 8.19 – 8.90 mg/dL
Normal volume ratio of serum inorganic phosphorus 1.99 – 3.97 mg/dL.
*Means in the same column with asterisk are significantly (P<0.05) different with day zero.

Donkeys in the three treated groups exhibited significant (P<0.05) increase in sodium level during the study period (Table, 6). Potassium level increased significantly (P<0.05) in the first two treatment groups that received Diminazene and Sulphadimidine sodium, respectively (Table 6). 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.

Normal volume ratio of serum sodium 116.00 – 132.00 mEq/dL .
Normal volume ratio of serum potassium 2.80 – 4.40 mEq/dL

Table 6: Change in serum Sodium (mEq/L) following administration of Diminazene, Sulphadimidine sodium and Diminazene and Sulphadimidine sodium for three successive days in donkeys

Days	Sodium			Potassium		
	DM	S	DMS	DM	S	DMS
0	122.11+0.96	129.12+1.97	126.82+1.99	2.46+0.13	3.73+0.09	4.20+0.19
1	120.75+1.96	138.24+2.40* (0.01)	129.72+1.20	2.57+0.06	4.39+0.14* (0.00)	4.75+0.13
2	124.16+2.58	137.92+2.65*(0.02)	128.64+2.48	2.52+0.10	4.65+0.07* (0.00)	4.62+0.23
3	127.28+2.35	135.46+2.56*(0.01)	128.72+1.49	3.15+0.16*(0.00)	4.69+0.21* (0.01)	3.94+0.15
5	132.45+1.46*(0.00)	138.26+1.20*(0.01)	129.50+0.82	3.13+0.15*(0.02)	4.89+0.15*(0.00)	4.04+0.14

7	140.50+4.03*(0.00)	130.24+2.15	133.08+2.02	4.08+0.24*(0.00)	4.33+0.31	4.59+0.08
10	122.15+5.34	130.60+1.33	135.86+0.99*(0.03)	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.
*Means with asterisk in the same column are significantly (P<0.05) different with days zero.

During the treatment 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 hematological parameters after 10 days of treatment.

A relevant aspect to be considered is the absence of toxic effects of the treatment to the donkeys. 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.*, 2009b). We believe that our three –repeated dose protocol obtained higher safety because it provided greater passage of drug molecules through blood-brain barrier, which could eliminate the parasite from brain.

By the end of the experiment period, all biochemical and hematological parameters returned to normal levels, allowing us to conclude that this new protocol tested was safe to be used in donkeys.

Conclusion

In this work, we used a preparation of diminazene, which belongs to the group of aromatic diamidines. This compound acts on the causative agents of blood protozoan diseases produced by both flagellated protozoa (*Trypanosoma*) and members of the class Piroplasmida (*Babesia*, *Theileria*, and *Cytauxzoon*) in various domestic and wild animals, and it is widely used in veterinary medicine.

We believe that our three –repeated dose protocol obtained higher safety and may provide greater passage of drug molecules through blood-brain barrier, which could eliminate the parasites from brain.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.

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