Influence of Secnedazol on the Pharmacokinetics of Diminazene Aceturate in Dogs Infected with Trypanosoma Brucei: A Crucial Study for Enhancing Treatment Efficacy

Azor N.N
Department of Animal Health and Production,
Enugu State Polytechnic Iwollo
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Abstract
This study evaluated the pharmacologic effect of Diminazene aceturate and Secnidazol combination in dogs infected with trypanosome brucei and effect of Secnidazol in pharmacokenitics disposition of DA in infected dogs. Trypanosome brucei stock used was obtained from natural breed of dogs and infected in albino rats in which the Trypanasome is stored and was kept at the department of Veterinary Parasitology and Entomology University of Nigeria Nsukka.18 dogs was used in the Experiment and the dogs were randomly selected from both sexes between the age of 5-10 months into five groups, four of the groups were infected and only one was uninfected.The dogs were experimentally infected by intraperitoneal injection of 5x10 trypanosome in 1 ml of phosphate buffer saline(PBS). Effect of combination of SEC and DA was studied on Parasitemia, heamatology T. brucei infected dogs.Data on parasitemia and Heamatology were analysed with one way analysis of variance. Variant means were separated Post-hoc using the Least Significant Difference (LSD) methods. The level of significance was accepted at $p \leq 0.05$. Pharmacokinetic data for DA alone and DA plus SEC combination were analysed using Independent sample T.test significant value was accepted at p<0.05. The effect on parasitemia in SEC and DA treated group was lower on day 7 than group treated with DA alone and there was no parasitemia in all the groups on day 21. on heamatology /there was significant difference between the group on PCV,RBC and Hb on day7 but no difference on day 14. A mean pick serum concentration of 1.29±0.12ug/ml was achieved at 0.5h after drug administration in dog pretreated with SEC. However as 0.25h in DA treated alone serum concentration observed was 1.23±0.04 ug/ml. The T max for DA plus SEC group was 0.5h against 0.25h for DA group. AUC of dog pretreated with SEC significantly(p<0.05) lower than the AUC of dogs treated with DA alone, there was no significant difference in CPO, Ka, Ke, T1/2Ke, Vd Cl and Cmax, there was no significant difference between the DA concentration.But DA values of CPO, Ka and T1/2Ke were higher than SEC plus DA whereas T1/2 Ka, Vd,Cl Tmax and Cmax was higher in sec pretreated. It can be concluded from the pharmacokinetics study that SEC improved the efficacy of DA by altering the pharmacokinetic constants. There was an improvement in the absorption and elimination of DA in the group that received heterotherapy treatment, hence heterotherapy treatment of SEC and DA is more efficient in the treatment of T.brucei in infected dogs compared to when a monotherapy of DA is used in the treatment of the disease.

Keywords: *Pharmacologic effect. Diminazene aceturate & Secnidazol combination. Dog Trypanosomosis*

1.0 Introduction

Trypanosomosis is a disease complex caused by various species of Trypanosoma, transmitted both cyclically by tsetse flies (Glossina spp.) and mechanically by biting flies such as Tabanids and Stomoxys (Luckins and Dwinger, 2004). These hemoflagellate protozoa inhabit the blood plasma, lymph, and tissues of their hosts, impacting both human and animal health, including cattle, horses, dogs, and laboratory animals. Clinical manifestations of the disease include fever, fluctuating parasitemia, anemia, and immunosuppression (Ikede and Losos, 1972; Anosa, 1983). The tsetse fly serves as the primary vector for the majority of trypanosome species; however, transmission can also occur through contaminated medical instruments (Anene et al., 2001). The repercussions of trypanosomosis on livestock productivity are substantial, leading to increased management costs associated with disease treatment, decreased reproductive success, and diminished meat and milk yields (WHO, 2018). Therefore, there is a pressing need for effective therapeutic interventions to enhance the health and productivity of these animals. At present, there exists no effective vaccine against trypanosomosis, primarily due to the occurrence of antigenic variation among the parasites. Current control strategies include tsetse fly management, chemoprophylaxis, and chemotherapy utilizing compounds such as diminazene aceturate and homidium salts (Eke et al., 2018). Nevertheless, the limited efficacy of existing antitrypanosomal drugs when administered singularly has prompted investigations into drug combinations, including difluoromethylornithine (DFMO) used in conjunction with suramin or diminazene aceturate. The use of these combinations poses challenges, such as potential toxicity and the risk of disease relapse. Notably, the combination of schneidazol and diminazene aceturate has demonstrated enhanced efficacy in canine subjects (Eke et al., 2018). Consequently, ongoing research seeks to identify safe and effective trypanocidal agents for both standalone and combination therapies.

2.0 Methodology

Experimental animals

Eighteen (18) clinically healthy local dogs of either sex, between ages of 5-10 months were used for the study. They dogs were kept in screened dog kennels and in separate cages. They were feed with standard pelletized dry dog food (Wagg Dog food for puppies Pet food from England) and given drinking water ad libitum. Prior to the study the dogs were screened for trypanosomes and hemoprotozoan parasites using Giemsa stained blood smear. The dogs were also physically examined and de-wormed with Prazisam ® (fenbendazole, praziquantal and pyrental pamoate). They were all acclimatized for four weeks before the study commenced.

Trypanosome Stock

Trypanosoma b. brucei (FedereStrain) used in the study was obtained from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The experimental animals were infected intraperitoneally with 5 x 105 trypanosomes in 1 ml of phosphate buffered saline (PBS). Trypanosomes were quantified using the method of Herbert and Lumsden (1976)

Experimental Design

Eighteen (18) dogs were randomly assigned to five groups of 4 dogs (A-C) and 3 dogs in groups D and E. As stated above, they were infected with trypanosomes. They were treated as follows:

Groups A- They were infected and treated with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular route and 100 mg/kg of secnidazole *per os* for five days.

Group B – The dogs were infected and treated with 3.5 mg diminazene accturate alone i.m.
Group C – The dogs were infected and treated with 100 mg/kg secnidazole alone (*po*) for five days.
Group D – The dogs were infected and untreated (Negative control).
Group E – The dogs were uninfected and untreated (Normal control).
Treatment was initiated on the 14th day postinfection (d.p.i.), when parasitaemia was well established on assessment of wet blood films (Jennings, Whitelaw, Urquhart, 1977; Onyeyili,Anika, 1989). The choice of doses used in this study was based on the findings of Eke et al., (2017b).

Parameters for Assessment

Level of Parasitemia: The level of parasitemia was determined by collection of blood from cephalic vein which was used in preparing wet mounts. They were examined under light microscope for trypanosomes. The level of parasitemia was quantified using the rapid matching technique of Herbert and Lumsden (1976). Parasitemia was monitored every two days post treatment (PT) till 21 days and to determine relapse of infection.

Rectal Temperature: Rectal temperature of the dogs was determined before and after infection every two days with the aid of digital thermometer. After treatment, rectal temperature were determined every day for 7 days post-treatment and then every 2 days for 14 days post – treatment.
Body weight: Body weight of the dogs was determined daily before infection and then every two days for 14 days post infection.
Packed cell volume (PCV): The method used to determine the PCV was the microhaematocrit method. Capillary tubes were filled to about 75% of its length by capillary action with anticoagulated blood and one end was sealed with plasticine. The filled tubes were loaded into a microhaematocrit centrifuge (Hawksley, England) and centrifuged at 10,000 rpm for 5 minutes. The PCV was read using a microhaematocrit reader and expressed in percentage (Thrall & Weiser, 2002).

Haemoglobin Concentration (Hb): The haemoglobin concentration was determined by the cyanomethaemoblobin method. 20 microlitres of anticoagulated blood and 20 microlitres of standard were added to a test tube labeled sample and standard respectively each containing 5ml of Drabkin's reagent. The solution was mixed thoroughly and allowed to react for 20 minutes at room temperature. The absorbance of sample was read against a blank of the Drabkin's reagent at a wavelength of 540nm using a spectrophotometer (Higgins et al., 2008[b])

Red blood cell (RBC) count: The RBC count was done using a haemocytometer. 20 microlitres of anticoagulated blood was added in a test tube containing 4ml of RBC diluting fluid to give a dilution ratio of 1:200. The solution was mixed and drops were charged onto the Neubauer counting chamber and allowed to settle for 1-2 minutes. The Neubauer counting chamber was mounted on a light microscope and the RBC was counted at high dry magnification. 5 central squares were counted and multiplied by 10,000 to obtain the RBC count per microlitre of blood (Coles, 1986)

Statistical Analysis

The results were analyzed using one-way analysis of variance (ANOVA) using statistical packages version 15.0 computer software. Variant means were separated Post-hoc using the Least Significant Difference (LSD) methods. The level of significance was accepted at $p \leq 0.05$. Summary of the data from all groups were presented as mean ± SEM in tables.

3.0 Results

Pharmacokinetics

The data presented in Table 1 and 2 indicated that the intramuscular administration of DA at the dose of 3.5mg/kg bodyweight in dogs pre-treated orally with SEC and those not pre-treated with SEC resulted in a measurable blood level for 72h. Generally, there was an overall decrease in serum DA concentration in dogs treated with SEC/DA combination and dogs treated singly with DA. For dogs treated with SEC/DA combination, the peak serum DA concentration (1.29±0.12) was observed at 0.5 h after administration. This was however different in the case of dogs treated with DA alone as the peak serum DA concentration (1.23±0.04) was observed at 0.25 h after administration. No significant ($p>0.05$) differences were observed in the serum DA concentration between SEC/DA and DA alone across the 72-h period.

Table 1: Diminazene aceturate concentration in serum following intramuscular administration of DA (3.5mg/kg) and DA plus SEC (100mg/kg).

Time (h)	SEC/DA (DA conc ug/ml)	DA (DA conc ug/ml)
0.25	1.23±0.35	1.23±0.04
0.5	1.29±0.12	1.14±0.07
1	1.15±0.04	1.20±0.11
2	1.11±0.06	1.01±0.06
3	1.04±0.09	1.09±0.06
6	1.02±0.12	1.02±0.05
12	1.07±0.06	1.07±0.10
24	1.05±0.08	1.13±0.05
36	0.95±0.11	1.02±0.03
48	1.01±0.08	1.1±0.09
60	1.01±0.09	1.15±0.03
72	1.14±0.05	1.07±0.09

Values are means ± standard deviation. Means with different superscripts within the same row are significantly different (P<0.05)

Diminazine aceturate (3.5mg/kg) was injected 15minutes after collection of sample begins.

Pre-treatment with SEC increased the Kevalue when compared with DA alone but this increase was statistically insignificant ($p>0.05$).There was a significant ($p<0.05$) variation in AUC between the two groups of dogs, with the AUC of dogs pre-treated with SEC significantly ($p<0.05$) lower than the AUC of dogs treated with DA alone. For the other pharmacokinetics parameters (CPO, Ka,Ke, t½Ke, Vd, Cl, and Cmax) there were no significant differences between the DA concentration.Even though no significant differences were observed, the DA values of CPO, Ka, and t½Ke were higher for dogs administered with DA alone compared to dogs treated with the combination of Sec/DA. On the other hand, the t½Ka, Ke, t½Ke, Vd, Cl, Cmax, and Tmax were higher in dogs pre-treated with SEC.

Table 2: Pharmacokinetic parameters and constants for DA in dogs following pretreatment with SEC and DA alone

Pharmacokinetic parameters	Sec/Da (DA conc µg/ml)	Da (DA conc µg/ml)
CPO	1.12±0.04	1.143±0.017
Absorption rate constant (Ka) (h^{-1})	2.40±0.26	3.493±1.832

Absorption half-life (t½Ka) (h)	0.29±0.03	0.230±0.082
Elimination rate constant (Ke)(h^{-1})	0.006±0.002	0.005±0.001
Elimination half-life (t½Ke) (h^{-1})	129.25±38.99	136.20±26.82
Volume of distribution (Vd)(L/kg)	3.130±0.110	3.065±0.048
Total body clearance (Cl) (L/h)	0.018±0.004	0.016±0.002
AUC (h.µg/ml)	207.533^{a}±56.262	222.833^{d}±35.959
Cmax	1.380±0.220	1.245±0.066
Tmax	0.333±0.144	0.313±0.125

Values are means ± standard deviation. Means with different superscripts within the same row are significantly different (P<0.05)

Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on the level of parasitemia of dogs experimentally infected with *Trypanosoma brucei brucei*

Results for parasitemia are presented in Table 3. Parasitemia was not observed in any of the groups on day 0. On day 7 post infection and treatment, Groups A, B, C, and D had no significant ($p<0.05$) differences in mean parasitemia values. There was no significant ($p<0.05$) difference between Groups A, B, and D with the normal control. On day 14, mean parasitemia values in Groups A, B, C, and D showed no significant ($p>0.05$) differences but were significantly ($p<0.05$) higher than Group E. However, on the 21st day post treatment, there were no significant ($p>0.05$) differences in parasitemia values between Groups A, B and E. Groups C and D had no significant ($p>0.05$) difference in their mean values but were significantly higher than the normal control and Groups A and B.

Table 3: Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on mean body weight gain in dogs experimentally infected with *Trypanosoma brucei brucei*

	treatment	**Days- Post treatment**			
S/N	Group	**0**	**7**	**14**	**21**
1	A	6.38^{a} ±0.86	6.05^{a} ±0.80	6.28^{a} ±0.81	6.10^{a} ±0.79
2	B	6.23^{a} ±0.84	6.28^{a} ±0.82	6.53^{a} ±0.94	6.40^{a} ±0.92
3	C	7.67^{a} ±0.82	7.67^{a} ±0.82	7.60^{a} ±0.95	6.73^{a} ±0.63
4	D	7.63^{a} ±0.80	5.73^{a} ±0.80	5.50^{a} ±0.81	5.13^{a} ±0.59
5	E	6.80^{a}±0.28	6.36^{a}±0.34	6.42^{a}±0.37	6.20^{a}±0.33

Values are means ± standard deviation. Means with different superscripts within the same row are significantly (P<0.05) different while means with the same superscript are statistically ($p>0.05$) insignificant. Groups A- Infected dogs + treatment with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular (IM) route and 100 mg/kg of secnidazole *per os* for five days.Group B –Infected dogs + treatment with 3.5 mg diminazene aceturate alone IM. Group C – Infected dogs + treatment with 100 mg/kg secnidazole alone (*po*) for five days. Group D –Infected dogs+ untreated (Negative control). Group E –Uninfected dogs + untreated (Normal control).

Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on body temperature of dogs experimentally infected with *Trypanosoma brucei brucei*

The mean temperature values are presented in Table 4. There was no significant ($p>0.05$). differences in mean values on day 0 On the 7th day, Groups A, B, and D were significantly ($P<0.05$) higher than Group E. There was no significant ($p>0.05$) difference between Group C and the other groups. On the 14th day, Group E was significantly ($p<0.05$) lower than all the groups. On the other hand, Group A showed no significant ($p>0.05$) difference in temperature between Groups B, C, and D while group B was significantly ($p<0.05$) different from Group C. On day 21, there was no significant ($p>0.05$) difference between Groups A and E. Group D that was infected and untreated recorded the highest value in terms of temperature with a level of significance ($p<0.05$) different from other groups.

Table 4: Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on body temperature changes in dogs experimentally infected with *Trypanosoma brucei brucei*

	Treatment	**Days post treatment**			
S/N	Group	**0**	**7**	**14**	**21**
1	A	$38.35^{a}\pm0.36$	$38.65^{b}\pm0.29$	$39.03^{bc}\pm0.26$	$36.93^{a}\pm0.75$
2	B	$37.90^{a}\pm0.28$	$38.65^{b}\pm0.24$	$40.13^{c}\pm0.50$	$37.65^{b}\pm0.14$
3	C	$38.23^{a}\pm0.68$	$38.43^{ab}\pm0.45$	$39.20^{b}\pm0.15$	$38.83^{c}\pm0.27$
4	D	$37.40^{a}\pm0.67$	$38.57^{b}\pm0.67$	$39.20^{bc}\pm0.15$	$39.53^{d}\pm0.27$
5	E	$38.23^{a}\pm0.86$	$37.60^{a}\pm0.31$	$37.47^{a}\pm0.38$	$37.43^{ab}\pm0.21$

Values are means ± standard deviation. Means with different superscripts within the same row are significantly ($P<0.05$) different while means with the same superscript are statistically ($p>0.05$) insignificant. Groups A- Infected dogs + treatment with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular (IM) route and 100 mg/kg of secnidazole per os for five days.Group B –Infected dogs + treatment with 3.5 mg diminazene aceturate alone IM. Group C – Infected dogs + treatment with 100 mg/kg secnidazole alone (po) for five days. Group D –Infected dogs+ untreated (Negative control). Group E –Uninfected dogs + untreated (Normal control).

Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on body weight of dogs experimentally infected with *Trypanosoma brucei brucei*

Results of weight gain in dogs infected with *T. b. brucei* and treated with SEC-DA are presented in Table 5. On day 0 before infection and treatment there was no significant ($p>0.05$) differences in weight between the groups. On day 7, 14, and 21, there were no significant ($p>0.05$) changes in weight of the dogs between the groups. Only group D had a slight decrease in weight through day 0 to day 21.

Table 5: Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on mean body weight gain in dogs experimentally infected with *Trypanosoma brucei brucei*

	treatment	**Days- Post treatment**			
S/N	Group	**0**	**7**	**14**	**21**
1	A	$6.38^{a}\pm0.86$	$6.05^{a}\pm0.80$	$6.28^{a}\pm0.81$	$6.10^{a}\pm0.79$
2	B	$6.23^{a}\pm0.84$	$6.28^{a}\pm0.82$	$6.53^{a}\pm0.94$	$6.40^{a}\pm0.92$
3	C	$7.67^{a}\pm0.82$	$7.67^{a}\pm0.82$	$7.60^{a}\pm0.95$	$6.73^{a}\pm0.63$
4	D	$7.63^{a}\pm0.80$	$5.73^{a}\pm0.80$	$5.50^{a}\pm0.81$	$5.13^{a}\pm0.59$

5	E	6.80^{a}±0.28	6.36^{a}±0.34	6.42^{a}±0.37	6.20^{a}±0.33

Values are means ± standard deviation. Means with different superscripts within the same row are significantly (P<0.05) different while means with the same superscript are statistically (p>0.05) insignificant. Groups A- Infected dogs + treatment with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular (IM) route and 100 mg/kg of secnidazole *per os* for five days.Group B –Infected dogs + treatment with 3.5 mg diminazene aceturate alone IM. Group C – Infected dogs + treatment with 100 mg/kg secnidazole alone (*po*) for five days. Group D –Infected dogs+ untreated (Negative control). Group E –Uninfected dogs + untreated (Normal control).

Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on the haematology of dogs experimentally infected with *Trypanosoma brucei brucei*

Pack cell volume (PCV)

The results of the pack cell volume in dogs treated with SEC-DA for 21 days are presented in Table 6. On day 0 post-treatment, there were no significant (p>0.05) differences in mean values for the PCV between the groups. On the 7th day, a significant difference (p<0.05) was observed in dogs in group A compared to other groups. Group C dogs had a significant (p<0.05) higher mean value compared to groups B, D, and E. On the 14th day post-treatment, mean values for group A dogs were significantly (p<0.05) different from the mean values of the other groups. Group C recorded the highest value of PCV that was significantly (p<0.05) different from groups B and D, with the lowest mean value was recorded in D. Again, by day 21, Group D recorded the lowest mean value for PCV which was significantly (p<0.05) different from the other groups with group A this time having the highest mean value. Dogs in groups B, C, and E showed no significant (p>0.05) differences in their mean values but were significantly different from groups A and C.

Table 6: Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on the Pack Cell Volume (PCV) in dogs experimentally infected with *Trypanosoma brucei brucei*

	Treatment	**Days post-treatment**			
S/N	Group	**0**	**7**	**14**	**21**
1	A	52.25^{a}± 1.03	42.50^{ab}± 3.28	42.50^{bc}±3.28	43.00^{b}±4.80
2	B	52.25^{a}±0.85	39.50^{a}±0.85	39.50^{b}±2.36	39.00^{ab}±1.35
3	C	53.67^{a}±0.67	49.00^{b}±3.22	49.00^{c}±3.22	41.33^{ab}±2.73
4	D	50.67^{a}±0.67	39.33^{a}±7.45	31.00^{a}±0.55	32.00^{a}±1.16
5	E	54.67^{a}±0.89	40.00^{a}±1.00	41.00^{bc}±0.58	40.67^{ab}±0.33

Values are means ± standard deviation.Means with different superscripts within the same row are significantly different (P<0.05) while means with the same superscript are statistically insignificant (p>0.05). Groups A- Infected dogs + treatment with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular (IM) route and 100 mg/kg of secnidazole per os for five days. Group B –Infected dogs + treatment with 3.5 mg diminazene aceturate alone IM. Group C –Infected dogs + treatment with 100 mg/kg secnidazole only (po) for five days. Group D –Infected dogs + untreated (Negative control). Group E –Uninfected dogs + untreated (Normal control).

Red blood count (RBC)

Table 7 showed the results for the RBC in dogs infected with *T. b. brucei* and treated with SEC-DA combinations. There were no significant (p>0.05) differences between the groups post-

treatment on day 0. On day 7, group A was significantly ($p<0.05$) higher than Groups D and E with mean differences statistically similar to other groups. Group B was significantly ($p<0.05$) lower than lower than Groups C, D, and E. On day 14, there was no significant ($p>0.05$) difference between groups B, C, and E but significantly ($p<0.05$) lower than Groups A and D. On the 21st day, there was no significant ($p>0.05$) difference between groups B, C, and E but significantly ($p<0.05$) lower than Groups A and D. There was no significant ($p>0.05$) difference between Group D and A.

Table 7: Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on Red blood Count (RBC) in dogs experimentally infected with *Trypanosoma brucei brucei*

	treatment	**Days post-treatment (x10^{x}?)**			
S/N	Group	**0**	**7**	**14**	**21**
1	A	$5.76^{a}\pm0.57$	$3.27^{ab}\pm0.06$	$5.33^{b}\pm0.19$	$4.90^{b}\pm0.06$
2	B	$5.95^{a}\pm0.21$	$2.68^{a}\pm0.07$	$2.35^{a}\pm0.27$	$3.17^{a}\pm0.28$
3	C	$6.00^{a}\pm0.36$	$3.93^{b}\pm0.54$	$3.00^{a}\pm0.55$	$3.42^{a}\pm0.19$
4	D	$5.76^{a}\pm0.57$	$4.90^{c}\pm0.06$	$5.33^{b}\pm0.19$	$4.90^{b}\pm0.06$
5	E	$5.59^{a}\pm0.37$	$5.21^{c}\pm0.38$	$2.80^{a}\pm0.29$	$3.61^{a}\pm0.22$

Values are means ± standard deviation.Means with different superscripts within the same row are significantly (P<0.05) different while means with the same superscript are statistically insignificant (p>0.05). Groups A- Infected dogs + treatment with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular (IM) route and 100 mg/kg of secnidazole per os for five days. Group B –Infected dogs + treatment with 3.5 mg diminazene aceturate alone IM. Group C –Infected dogs + treatment with 100 mg/kg secnidazole only (po) for five days. Group D –Infected dogs+ untreated (Negative control). Group E –Uninfected dogs + untreated (Normal control).

Hemoglobin concentration (HB)

The mean values for haemoglobin concentration of dogs experimentally infected with *T. brucei brucei* treated with the combination of diminazene aceturate and secnidazole are presented in Table 8. On day 0 before infection, the HB concentration had no significant ($p>0.05$) differences between the groups. On the 7th day post treatment, there was a significant ($p<0.05$) difference between group C and the other groups. On the other hand, the mean differences between groups A, B, D, and E were statistically ($p>0.05$) insignificant. On day 14, there was no significant ($p>0.05$) differences between group A and the other groups while groups D and B were significantly ($p<0.05$) lower than Groups C and E. On day 21 post treatment, only Group D was significantly ($p<0.05$) lower than Group E. There were no significant ($p>0.05$) differences between the other groups.

Table 8: Effects of the combination of diminazene aceturate (3.5 mg/Kg) and Secnidazole (100 mg/Kg) on haemoglobin concentrationin dogs experimentally infected with *Trypanosoma brucei brucei*

		Days post infection (unit)			
S/N	**Group**	**0**	**7**	**14**	**21**
1	A	$15.04^{a}\pm1.61$	$11.82^{ab}\pm1.14$	$11.49^{ab}\pm0.61$	$12.32^{ab}\pm1.50$
2	B	$13.81^{a}\pm0.88$	$10.73^{a}\pm0.72$	$9.79^{a}\pm0.27$	$11.11^{ab}\pm0.25$
3	C	$16.04^{a}\pm0.48$	$14.01^{c}\pm0.92$	$12.68^{b}\pm1.39$	$11.84^{ab}\pm0.94$
4	D	$12.71^{a}\pm0.42$	$12.48^{ab}\pm0.35$	$9.47^{a}\pm0.15$	$9.67^{a}\pm0.29$
5	E	$13.11^{a}\pm0.41$	$12.96^{ab}\pm0.55$	$12.90^{b}\pm0.38$	$13.03^{b}\pm0.27$

Values are means ± standard deviation. Means with different superscripts within the same row are significantly (P<0.05) different while means with the same superscript are statistically (p>0.05) insignificant. Groups A- Infected dogs + treatment with single dose of 3.5 mg/kg of diminazene aceturate (DA) by intramuscular (IM) route and 100 mg/kg of secnidazole per os for five days.Group B –Infected dogs + treatment with 3.5 mg diminazene aceturate alone IM. Group C – Infected dogs + treatment with 100 mg/kg secnidazole alone (po) for five days. Group D –Infected dogs+ untreated (Negative control). Group E –Uninfected dogs + untreated (Normal control).

4.0 Discussion and Conclusion

The peak serum concentration (1.29±0.12conc µg/ml DA) at 0.5 h for dogs pre-treated with SEC compared to the peak serum concentration (1.23±0.04conc µg/ml DA) could be attributed to the prolonged elimination phase of DA since at peak serum concentration, the rate of absorption is equal to the rate of elimination (Le, 2014). This could also be attributed to the lower absorption rate of DA in dogs pre-treated with SEC. Generally, the slower the absorption rate, the later the peak time. These results obtained revealed a typical IM route of absorption. A similar observation was made by Murilla et al.(1996) in cattle after pre-treatment with ^{14}C homidium. At the end of the 72-h period of the pharmacokinetic studies, even though there was no significant (p>0.05) difference between the two groups, the DA concentration in dogs pre-treated with SEC was higher than that the DA concentration of dogs treated with DA alone.

How much the drug is absorbed from the site of administration per unit time is described by the absorption rate constant (Ka). Hence, the higher the absorption rate constant, the faster the absorption rate of the drug, otherwise, the slower the absorption rate. Even though there was no significant (p>0.05) difference in the absorption rate constant between the group pre-treated with Sec (2.403 h^{-1}) and the group treated with DA alone (3.493 h^{-1}), the higher absorption rate constant of the later indicated how DA could easily be adsorbed and eliminated. This study however contradicted the findings of Eke (2016) who reported a higher absorption rate for dogs pre-treated with SCE.The absorption half-life in the group pre-treated with SEC was 0.29 h^{-1} compared to 0.23 h^{-1} in the group treated with DA alone. The absorption half-life can be used to predict the time (Tmax) of peak concentration of the drug (Holford, 2016). The higher value of absorption half-life in the group pre-treated with SEC could therefore be justifying the high Tmax and the Cmax values for the same group compared the group of dogs that were treated with a single dose of DA. There was no significant (p>0.05) difference in the elimination rate constant for both groups of dogs treated with the hetero- and monotherapies. The elimination rate constant describes the fraction of drug eliminated per unit of time or the rate at which plasma concentrations will decline during the elimination phase(Bourne, 2002).The lower elimination half-life constant (129.250 h^{-1}) for dogs pre-treated with SEC against the elimination half-life constant (136.203 h^{-1}) for dogs treated with DA alone was an indication that pre-treatment with SEC could reduce the toxicity of the DA in the body. This could also be indicating thelower rate of accumulation of DA in the dogs when pre-treated with SEC. A similar observation was made by Eke (2016) for pharmacokinetics studies in dogs pre-treated with SEC.From the results, the volume of distribution (Vd) in dogs that received the heterotherapy was 3.130 L/kg compared to a value of 3.065 L/kg in dogs that received the monotherapy of DA. There was no significant (p>0.05) difference in Vd in the two groups. The volume of distribution indicates how a drug is distributed into the body fluids and tissues(Anon, 2018). Vd is a proportionality constant that relates the total amount of drug in the body to the plasma concentration of the drug at a given time(Smith et al., 2015).A drug with a high Vd has a propensity to leave the plasma and enter the extravascular compartments of the body, meaning that a higher dose of a drug is required to achieve a given plasma concentration. On the other hand, if Vd is low, the drug will have a propensity to remain in the plasma and lower doses of

the drug will be required to achieve a given plasma concentration.Large volumes of distribution usually indicate that the fraction of the drug in plasma is very small (Murilla et al., 1996). Hence, lower Vd value in the group of dogs that received the monotherapy was indicating a high fraction DA in the plasma and lower doses are required to achieve a given plasma concentration compared to the group that received the heterotherapy. High level of DAin the plasma could also be attributed to the low elimination rate of DA in the group that was treated only with DA. From Table 2 the elimination half-life of Sec and DA was1 2 9 . 2 5 ± 3 8 . 9 9 which is lower than DA alone which is 1 3 6 . 2 0 ± 2 6 . 8 2 this shows that the higher value of elimination half-life indicates increase in Serum concentration of DA over time which is an indication of low efficacy. Bourne (2002) stated that in active Tryponosomosis, DA is usually very high but does not translate to killing of Trypanosome. Here we report that Sec and DA combination causes a shorter elimination half-life of DA compared to DA alone thereby indicating that Sec and DA combination is more efficacious in treatment of *Trypanosoma brucei* in dogs. The AUC for the group pre-treated with SEC was significantly lower than the AUC for the group treated with DA alone. The AUC is useful in determining the bioavailability of a drug and it is a constant directly proportional to the total amount of unchanged drug that reaches systemic circulationor the total amount that is absorbed(Le, 2014). It is inversely proportional to clearance with an increase in clearance leading to a decrease in the AUC. A low value of AUC for the group of dogs pre-treated with SEC indicated the shorter exposure of the drug in the body but when there is higher elimination half-life, AUC increases which indicates drug accumulation in the system and may relate directly to the safety of the drug as in the case of DA alone. Therefore Sec and DA combination is safer than DA alone (Le, 2014). The Packed Cell Volume (PCV) is a measure of the percentage of red blood cells in circulating blood as a screening test for anemia(Al-Ani, 2011; Stoltz & Donner, 1991). A dog is generally considered anemic if the PCV is below 35% and normal if the value fall between 35% and 55% (Llera, 2018). Looking at the results presented in Table 1, day 0 showed no significant differences ($p<0.05$) in PCV between the groups but these values decreased from day 7 to day 21 in all the groups. Group D, which was infected and untreated (Negative control) showed significantly ($p<0.05$) lower values in the values of PCV. This could be an indication of the development of anemia in this group as a result of the breakdown of red blood cells. The other groups where within the range values for normal PCV in dogs as they fall within 35% to 55%. At day 21, there was a significant difference in PCV between group A and B, C, and E. This indicated that treatment with SEC-DA combination (group A) or treatment with either DA (group B) or SEC (group C) normalized PCV with the positive control (group E, uninfected, untreated), with SEC-DA combination more effective than the single treatment with SEC or DA. A similar observation was made by Eke et al.(2017, 2020) for PCV values on *T. b. brucei* infected rats and dogs, respectively, and treated with SEC-DA combination.

Anemia has been reported to be a feature of *T. b. brucei* infection in dogs (Aneke et al., 2016; Chekwube et al., 2014). This condition is caused due to a decrease in the number of red blood cells, which implies that the blood caries less oxygen resulting to fatigue and weakness of the animals (Cotter, 2017). This is also due to a depletion of glutathione on the surface of the red blood cells resulting to decrease in red blood cells (Akanji et al., 2009; Aneke et al., 2016). A slight decrease in RBC values in Groups A, B, C, and D on day 7 could be an indication of anemia in due to infection with parasite. However, by day 14, Group A that was treated with SEC-DA combination had no significant difference in mean RBC count ($p<0.05$) while groups A and C had significantly lower values. By day 21, the monotherapies of either SEC or DA and the combination of SEC-DA normalized the anemic condition with the normal control. Only the infected untreated group (Group D) had lower values to indicate the effectiveness of the therapies given to the infected dogs. Chekwube et al., (2014) and Wulari et al, (2009) had a

similar observation and reported that the severity of anemia in dogs depends on the level and duration of parasitemia.

The normal HB level for healthy uninfected dogs ranges from 11.9×10 g/L–18.9×10 g/L(Fielder, 2015). All the groups had normal HB level on day 0 and after infection and treatment of the animals, on the day 7, Group B that was treated only with a monotherapy of DA had the lowest HB level which was significantly ($p<0.05$) lower than the other groups. On day 14 the HB level of the infected-untreated group was below the normal HB level. However, on day 21, all the treatments had normalized the HB levels in all the groups but for the infected-untreated group with a HB level of 9.67x10 g/L. A similar observation was reported by Eke et al.(2020) and Mwangi et al. 2019).

A linear relationship has been developed between PCV and HB concentration in cattle blood samples (Turkson & Ganyo, 2015). And increase in PCV will therefore lead to an increase in HB concentration. Group D that had lower values for HB level could be due to the fact that the animals developed anemia due to the breakdown of red blood cell in this group. This could also be seen from the low values of PCV in this group. Treatment of the animals with SEC-DA combination was more effective in maintaining the HB level at a normal state since there were no significant differences between group A and E across the treatment period. The monotherapy of DA could only stabilize the HB level after day 14 post infection. This was in agreement with the findings of Eke et al. (2020) who reported on a better efficacy of SEC-DA combination in the treatment of dogs infected with *T. b. brucei.*

No significant differences in body temperature was an indication that the condition of trypanosomiasis was not yet induced in the animals on day 0. Significant differences in body temperature on the 7th day was a clear indication of parasitemia in the different groups. This could be seen from significant difference ($p>0.05$) in mean rectal temperature between the uninfected-untreated normal control (Group E) and the other groups. A similar observation was reported by Ngozika et al.(2016) on rabbits infected with *T. b. brucei* and treated with DA and Isomethamidium chloride. A slight increase in body temperature post treatment could be due to an inflammation enhanced by the innate immune system of the dogs to allow leukocytes to kill the parasite (LumenLearning, 2016). Group A that was treated with SEC-DA combination having no significant difference with Group E (normal control) showed the efficacy of SEC-DA combination in the treatment of *T. b. brucei* by day 21. Group B and C that received the monotherapies of diminazene aceturate and secnidazole, respectively, had significant differences to show the ineffectiveness of the single monotherapy treatment. This observation was in agreement with the findings of Eke et al. 2017, 2020; and Ngozika et al.(2016).

No significant change in body weight in any of the groups was in accordance with the work of Akpa el al.(2008) who compared the effects of pentamidine isethionate and diminazene aceturate in the treatment of *T. b. brucei* in dogs. However, the results were contrary to those reported by Mwangi et al.(2019) who reported on significant differences in weight of dogs infected *T. Congolense* and *T. b. brucei* and treated with parvoviral vaccine. Weight decrease in infected untreated group could be attributed to anorexia and dullness observed with the infection. No significant changes in body weight associated with the infected and treated groups (A, B, and C) and uninfected untreated group (Group E) showed the efficacy of the SEC-DA combination in the treatment of *T. b. brucei* infected dogs.

From the study, it can be concluded that the combination of a fixed dose of 3.5 mg/kg of Diminazene aceturate and100mg/kg dose of Secnidazol in treatment of T.*brucei* infected dogs was more effective compared to the monotherapies of either SEC or DA. This was evident from the fact that Group C that was infected and treatment with 100 mg/kg secnidazole alone for five days was having significantly ($p<0.05$) higher level of parasites than the other groups on the 7th day post treatment and Group A was having no significant difference with the positive control. Even though by day 21,Group A that was treated with Sec-DA combination and Group

B that received the monotherapy of DA showed no statistical significance difference ($p<0.05$) with the normal control, however on day 14, Group A showed a high potential as the number of parasites in this group was lower than that in Group B.

In general, SEC-DA combination was more effective in the recovery of altered haematological parameters like PCV, HBC and RBC.Again,the combination treatment was more effective in normalizing thetemperature, ALP, AST, ALP, creatinine with the normal control. This is evident from the fact that there were no significant differences in these set of values with the normal control at the end of the experiment (day 21). Hence, the combination treatment of a fixed dose of 3.5 mg/kg of Diminazene aceturate and100mg/kg dose of Secnidazol in treatment of T.*brucei* infected dogs was more effective on haematology, body temperature, liver function makers, and kidney function makers in *T. brucei* infected dogs.

It can thus be recommended that a combination therapy of Diminazene aceturate andSecnidazol should be used in the treatment of trypanosomiases is dogs as it has been proven in this study to be more effective in parasitemia, hematology, body temperature, liver function makers, and kidney function makers compared to the monotherapies of either Diminazene aceturate or Secnidazol.

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