

Effects of secnidazole-diminazene aceturate combination therapy on parasitaemia and serum biochemical profile after late treatment in *Trypanosoma brucei brucei* infected dogs

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Abstract: Relapse parasitaemia is a major setback in the chemotherapy of a late stage *Trypanosoma brucei brucei* infection. An aberrant serum biochemical profile resulting from a *T. b. brucei* infection in dogs has been attributed to multiple organ injuries resulting from the invasive nature of the organism. Therapy with diminazene aceturate alone has not been satisfactory. This study evaluated the effects of a secnidazole-diminazene aceturate (SEC-DA) combination therapy on parasitaemia and the serum biochemical profile after the late treatment of a *T. b. brucei* infection in dogs. Eighteen dogs were randomly assigned to 6 groups ($n = 3$) as follows: Group A: uninfected nor treated; group B: infected without treatment; group C: infected and treated with DA (3.5 mg/kg) (DA-monotherapy) intramuscularly (i.m.) once; group D: infected and treated with SEC (100 mg/kg) and DA (3.5 mg/kg); group E: infected and treated with SEC (200 mg/kg) and DA (3.5 mg/kg) and group F: infected and treated with SEC (400 mg/kg) and DA (3.5 mg/kg). Secnidazole was administered orally for 5 days while DA was given i.m. once in groups D–F. The dogs were infected with 5×10^5 trypanosomes intraperitoneally and treatment started 14 days post-infection. The parasitaemia was monitored daily while the serum biochemical indicators were monitored 14, 21, and 28 days post-infection. The total aparasitaemia was achieved in the SEC-DA treated dogs 72 h post-treatment and in 86 h in the DA-monotherapy dogs. A relapse parasitaemia occurred in the DA-monotherapy dogs 17 days post-treatment. The SEC-DA combination therapy caused a significant ($P < 0.05$) decline in the hitherto elevated urea and creatinine concentrations, and the ALP, ALT, AST activities. Also, there was a significant ($P < 0.05$) increase in the previously decreased serum albumin in the SEC-DA treated dogs. In conclusion, secnidazole-diminazene aceturate combination therapy prevented the relapse parasitaemia and ameliorated aberrant serum biochemical profiles of *T. b. brucei* infected dogs after late treatment.

Keywords: relapse parasitaemia; chemotherapy; protozoan disease; tissue invasive; trypanosomosis

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Trypanosomosis is an important tropical protozoan disease in dogs. Untreated and poorly managed cases are usually fatal. The disease is transmitted cyclically through the bite of an infected tsetse fly or mechanically by some biting flies, contaminated syringes or surgical instruments (Akpa et al. 2008). *Trypanosoma brucei brucei* is the most common species affecting dogs in Nigeria, *T. cruzi* and *T. evansi* have been implicated in South America and in Asia, respectively (Eloy and Lucheis 2009). *T. b. brucei* causes a severe acute or subacute disease in dogs and the infection is characterised by undulating parasitaemia, principally because of its tissue invasive nature and antigenic variation. The extensive tissue invasion leads to organ desquamation and necrosis which culminate in organ or system failure. These pathological changes associated with a *T. b. brucei* infection usually manifest as elevated activities of some serum biochemical markers (Allam et al. 2011). Elevated activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were reported in dogs infected with *T. b. brucei* (Ezeokonkwo et al. 2012). Some changes in the lipid profile, creatinine, urea, TP (total protein) and albumin have also been attributed to canine trypanosomosis (Ezeokonkwo et al. 2012; Nwoha et al. 2013).

Therefore, without a prompt and an effective treatment, infected dogs die due to multiple organ or system failure, thus, the need for effective treatment. Isometamidium chloride and diminazene aceturate (DA) are the two most common drugs used for the prophylaxis and curative treatment, respectively, of canine trypanosomosis (Onyeyili and Egwu 1995), but DA is by far the most commonly available. The efficacy of DA has been criticised due to the high frequency of the non-clearance of parasitaemia and relapse parasitaemia even where reinfections were precluded (Onyeyili and Egwu 1995). Relapse parasitaemia is the re-emergence of trypanosomes into the blood stream after the initial clearance even where re-infection was precluded (Onyeyili and Egwu 1995).

Relapse parasitaemia occurs as a result of the sequestration of trypanosomes in drug inaccessible sites, like the brain where the accumulation of trypanocide is below the therapeutic levels, late treatment, drug resistance or under-dosage (Onyeyili and Egwu 1995). Diminazene aceturate is a polar drug with very limited accumulation in the brain and this is a big setback in the efficacy of DA.

Also, the *T. b. brucei* organism is less susceptible to the trypanocidal effects of DA (Onyeyili and Egwu 1995). The standard dose of DA is 3–7 mg/kg intramuscularly. Consecutive repeat treatments with DA are toxic and increasing the dose of DA is not a viable option for the treatment in dogs due to their sensitivity to DA, so there is need for the development of an effective treatment regimen devoid of toxicity and capable of eliminating relapse parasitaemia in dogs. Unfortunately, no new trypanocide has been introduced into the market over the last 40 years.

Therefore, a combination therapy seems to be the most viable option. Some drug combinations have been previously evaluated in dogs, but toxicity concerns have precluded their clinical use (Onyeyili and Egwu 1995). The *in vitro* and *in vivo* antitrypanosomal effects of secnidazole have been previously reported (Eke et al. 2017a). Furthermore, the efficacy of a SEC-DA therapy against parasitaemia, rectal temperature and haematological indices of *T. b. brucei* infected dogs had recently been reported (Eke et al. 2020).

The authors therefore, hypothesised that a SEC-DA combination therapy will be effective in treating *T. b. brucei* parasitaemia and the aberrant serum biochemical profile of infected dogs after late treatment. This study evaluated the impact of a secnidazole-diminazene aceturate (SEC-DA) combination therapy on the parasitaemia and some serum biochemical markers of infected dogs as one of the yardsticks for assessing the efficacy and safety of the treatment regimen.

MATERIAL AND METHODS

Experimental animals

Eighteen Nigerian indigenous dog breeds aged 8–10 months were used for the study. Both male and female dogs were used in the study. Only healthy dogs shown to be free of any haemoprotzoan parasites were selected. They were housed in a screened dog kennel in separate cages and fed with standard commercial pelletised dry dog food. Water was provided *ad libitum*. Prior to the commencement of the study, the dogs were physically examined, and dewormed with Prazisam® (Vetoquinol India Animal Health PVT Ltd., Mumbai, India). They were screened for the presence of trypano-

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somes and other haemoprotozoan parasites using a Giemsa-stained blood smear and a haematocrit buffy coat technique.

They were vaccinated with a distemper, hepatitis, leptospirosis, parainfluenza and parvo-virus polyvalent vaccine (DHLPP) and an anti-rabies vaccine (ARV) (Bioveta a.s., Ivanovice na Hané, Czech Republic). They were acclimatised for 3 weeks.

Ethical statement

The animal experimental protocol was approved by the Experimental Animal Ethics Committee of our institution (Approval No. UNFVM/08/15/4) and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

Trypanosome stock

The *Trypanosoma b. brucei* (Federe strain) 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 5×10^5 trypanosomes in 1 ml of phosphate buffered saline (PBS). The trypanosomes were quantified using a standard method (Herbert and Lumsden 1976).

Drugs

Secnidazole (Secwid® May and Baker Nig. Ltd, Lagos, Nigeria).

Diminazene aceturate (Veriben® Ceva Sante Animale, Libourne Cedex, France).

Experimental groups

The dogs were randomly assigned to six groups ($n = 3$). They were treated as follows:

- Group A: normal, uninfected, untreated.
- Group B: infected without treatment.
- Group C: infected and treated with a single dose of DA (3.5 mg/kg) i.m.; standard drug (DA-monotherapy).

- Group D: infected and treated with SEC (100 mg/kg) orally for 5 days and a single dose of DA (3.5 mg/kg) i.m., (SEC-DA 100/3.5).
- Group E: infected and treated with SEC (200 mg/kg) orally for 5 days and a single dose of DA (3.5 mg/kg) i.m., (SEC-DA 200/3.5).
- Group F: infected and treated with SEC (400 mg/kg) orally for 5 days and a single dose of DA (3.5 mg/kg) i.m., (SEC-DA 400/3.5).

The treatment was initiated on day 14 post-infection (late treatment) (Jennings et al. 1977; Onyeyili and Anika 1989), when the parasitaemia was well established on the assessment of wet blood films. The choice of doses used in this study was based on previous studies in rats (Eke et al. 2017a). The parasitaemia was monitored daily to determine the prepatent period.

Post-treatment, the parasitaemia was monitored daily for the first 6 days, then every 3 days for 40 days post-treatment to determine the point of the relapse parasitaemia. The haematocrit buffy coat technique was used to confirm the total clearance of parasitaemia (OIE 2008).

The data collection from each group was terminated as soon as mortality or relapse parasitaemia was recorded in that group and the animals in such a group were removed for proper medication. The serum levels of the alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST), total cholesterol (Tchol), triglycerides (TG), albumin, total protein (TP), urea and creatinine were evaluated using the applicable Randox® diagnostic reagents (Randox Laboratories Ltd, London, UK) according to the manufacturer's directions.

These serum biochemical parameters were assayed on days 14, 21 and 28 post-infection.

Statistical analyses

The statistical analysis was conducted using SPSS v15 (IBM, Armonk, NY, USA) for Windows. The general linear model (GLM) repeated measures method was used to analyse the data generated from the study. The variant means were separated using the least significant difference (LSD). The significance was accepted at $P < 0.05$. The results were presented as the mean \pm SEM in the figures.

RESULTS

Effects of SEC-DA combination therapy on parasitaemia due to *T. b. brucei* infection in the dogs

The parasitaemia was detected in most of the infected dogs on day 5 post-infection and by day 14 there was full blown parasitaemia in all the infected dogs. Following treatment on day 14, there was a steady decline in the parasitaemia in all the treated dog. However, the total clearance of the parasite was observed in all the SEC-DA treated dogs 72 h post-treatment while this was observed 86 h post-treatment in the DA-monotherapy dogs. The relapse parasitaemia was detected in the DA-monotherapy dogs on day 31 post-infection (17 days

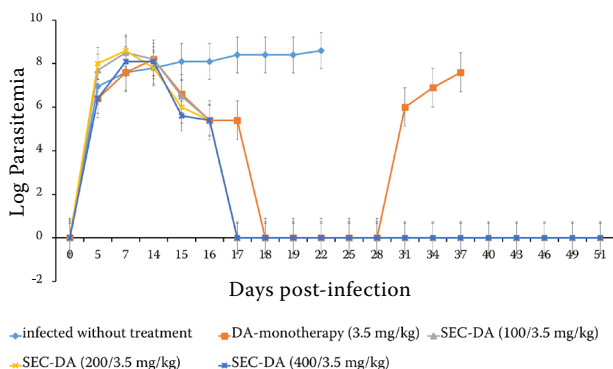


Figure 1. Effects of the secnidazole-diminazine aceturate (SEC-DA) combination therapy on the parasitaemia due to the *T. b. brucei* infection in the dogs. Mortality was recorded in group B before day 28

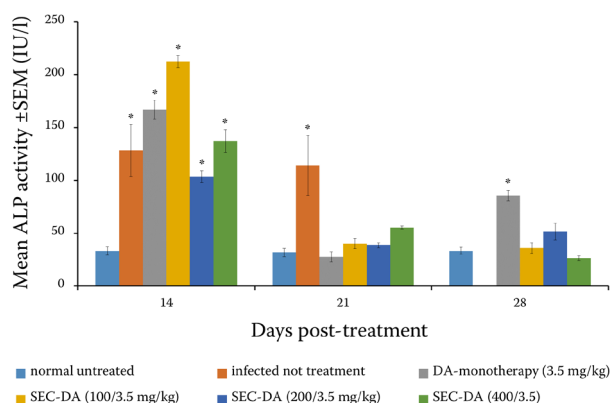


Figure 2. Effect of the SEC-DA combination therapy on the serum alkaline phosphatase (ALP) activity of the *T. b. brucei* infected dogs

*Significant ($P < 0.05$)

Mortality was recorded in group B before day 28

post-treatment). Nevertheless, no relapse parasitaemia occurred in any of the SEC-DA treated dogs till day 40 post-treatment when the experiment was terminated (Figure 1).

Alkaline phosphatase activities in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

On day 14 post-infection, there was a sharp increase ($P < 0.05$) in the ALP activities in all the infected dogs over those of the normal untreated dogs. Interestingly, following the treatment, there was a significant ($P < 0.05$) decline in groups C, D, E and F compared to group B.

However, on day 28 post-infection there was a significant ($P < 0.05$) rise in the ALP activities of the dogs in group C compared to those in groups A, D, E and F (Figure 2).

Alanine transaminase activities in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

The *Trypanosoma b. brucei* infection led to a significant ($P < 0.05$) rise in the serum ALT activities in all the infected dogs on day 14 post-infection. Nevertheless, following treatment, there was a significant ($P < 0.05$) decline in groups C, D, E and F

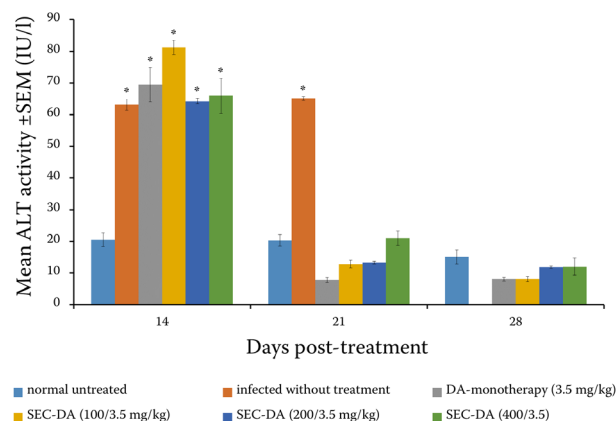


Figure 3. Effect of the SEC-DA combination therapy on the serum alanine transaminase (ALT) activity of the *T. b. brucei* infected dogs

*Significant ($P < 0.05$)

Mortality was recorded in group B before day 28

on day 21 post-infection compared to group B. There was no significant variation between groups C, D, E and F on day 21 and 28 (Figure 3).

Aspartate transaminase activities in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

The infection with *T. b. brucei* led to a significant ($P < 0.05$) rise in the serum AST activity in all the infected dogs on day 14 post-infection compared to group A.

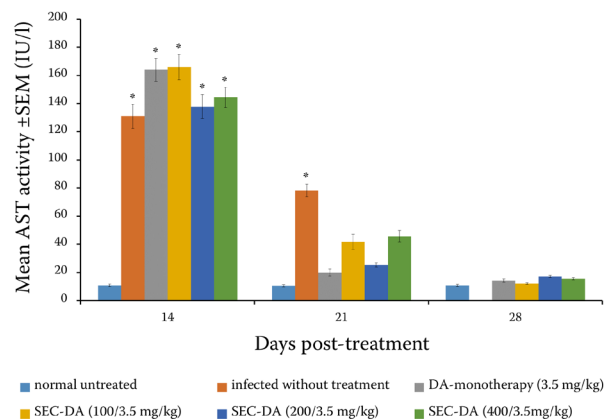


Figure 4. Effect of the secnidazole-diminazine aceturate (SEC-DA) combination therapy on the serum aspartate transaminase (AST) activity of the *T. b. brucei* infected dogs

*Significant ($P < 0.05$)

Mortality was recorded in group B before day 28

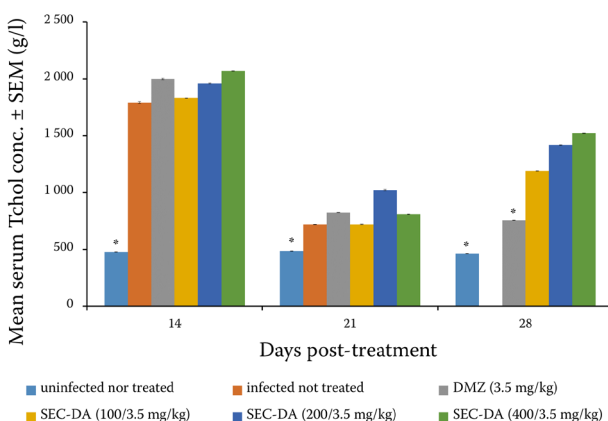


Figure 5. Effect of the SEC-DA combination therapy on the serum total cholesterol concentration of the *T. b. brucei* infected dogs

*Significant ($P < 0.05$)

Mortality was recorded in group B before day 28

Conversely, following the treatment, there was significant ($P < 0.05$) decline in groups C, D, E and F over those in group B on day 21. No significant variation existed between the treatment groups on day 21 and 28 (Figure 4).

Effect of SEC-DA combination therapy on the serum total cholesterol concentration in the *T. b. brucei* infected dogs

There was significant increase in the serum total cholesterol concentration in the *T. b. brucei* infected dogs on day 14 post-infection. However, the Tchol concentration declined from day 21 through day 28, though still significantly ($P < 0.05$) higher than that of the normal uninfected dogs (Figure 5).

Serum triglyceride concentration in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

Following the infection with *T. b. brucei*, there was a significant ($P < 0.05$) rise in the serum triglyceride concentrations in all the infected dogs compared to group A on day 14 post-infection. However, after treatment, there was a significant ($P < 0.05$) decline in the triglyceride concentration in groups C, D, E and F compared to group B 7 days later.

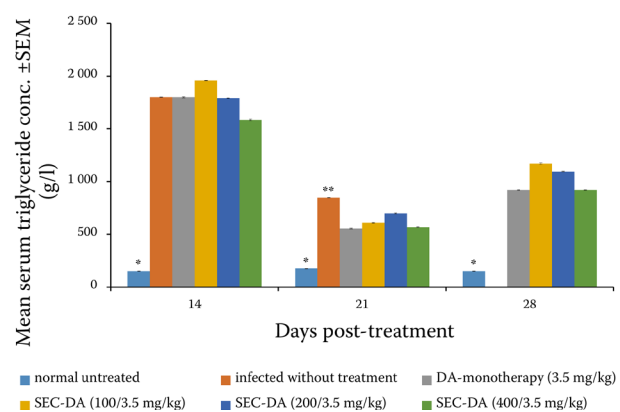


Figure 6. Effect of the SEC-DA combination therapy on the serum triglyceride concentration of the *T. b. brucei* infected dogs

*Significantly ($P < 0.05$) lower than the other experimental groups; **Significantly ($P < 0.05$) higher than the other experimental groups

Mortality was recorded in group B before day 28

There was no significant ($P > 0.05$) variation in the triglyceride concentration between the treatment groups on day 28 post-infection (Figure 6).

Serum albumin concentration in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

The infection with *T. b. brucei* led to a significant ($P < 0.05$) decline in the serum levels of albumin in all the infected dogs on day 14 post-infection compared to group A. Meanwhile, on day 21 post-treatment, there was a rise in the serum levels of albumin in groups D, E and F over those of groups B and C.

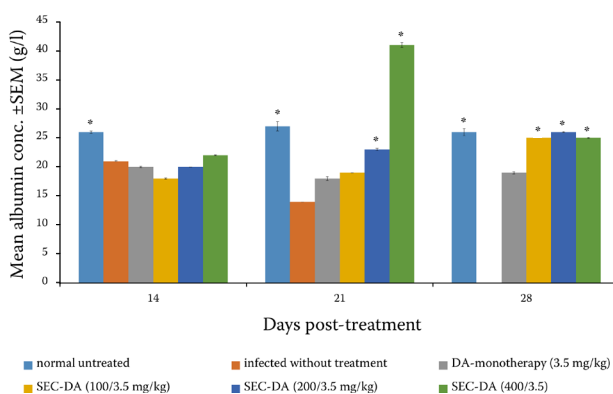


Figure 7. Effect of the secnidazole-diminazine aceturate (SEC-DA) combination therapy on the serum albumin concentration of the *T. b. brucei* infected dogs

*Significant ($P < 0.05$)

Mortality was recorded in group B before day 28

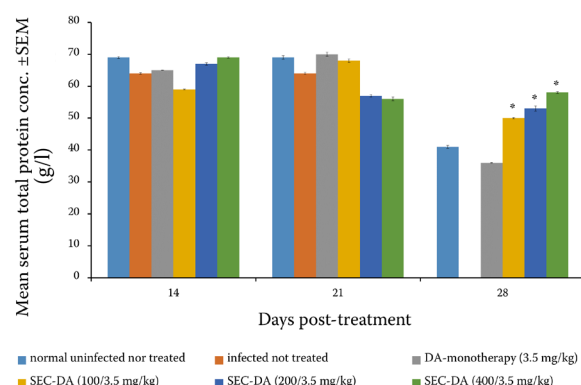


Figure 8. Effect of the SEC-DA combination therapy on the serum total protein concentration of the *T. b. brucei* infected dogs

*Significant ($P < 0.05$)

Mortality was recorded in group B before day 28

However, the albumin concentration in the dogs in group F was significantly ($P < 0.05$) higher than those in the other experimental groups. This increase in the albumin levels in groups D, E and F continued up to day 28 post-infection and was significant ($P < 0.05$) compared to group C (Figure 7).

Effect of SEC-DA combination therapy on the serum total protein concentration in the *T. b. brucei* infected dogs

The *T. b. brucei* infection did not cause any significant change in the serum TP concentration in infected dogs on days 14 and 21. However, on day 28, there was a significant ($P < 0.05$) decline in the serum TP concentration in the dogs in group C (Figure 8).

Serum urea concentration in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

A significant ($P < 0.05$) rise in the serum urea concentration was observed in all the infected dogs on day 21 post-infection, though the dogs in groups D, E and F had significantly lower levels of urea compared to those in groups B and C. However, there was no significant difference between the groups on day 28 (Figure 9).

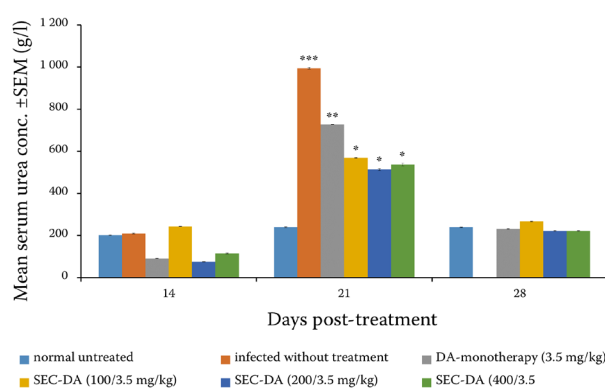


Figure 9. Effect of the SEC-DA combination therapy on the serum urea concentration of the *T. b. brucei* infected dogs

*Significantly ($P < 0.05$) higher than only group A; **Significantly ($P < 0.05$) higher than groups A, D, E and F; ***Significantly ($P < 0.05$) higher than the other experimental groups

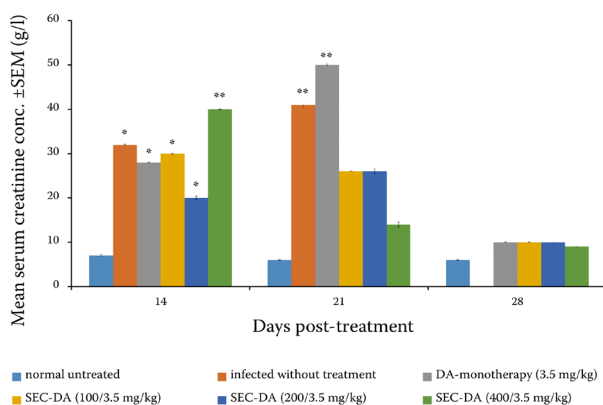


Figure 10. Effect of the SEC-DA combination therapy on the serum creatinine concentration of the *T. b. brucei* infected dogs

*Significantly ($P < 0.05$) higher than group A; **Significantly higher than other experimental groups

Mortality was recorded in group B before day 28

Serum creatinine concentration in the dogs infected with *T. b. brucei* and treated with either the SEC-DA combination therapy or DA-monotherapy

Following the *T. b. brucei* infection, there was a significant ($P < 0.05$) increase in the serum levels of creatinine in all the infected dogs on day 14 post-infection. Though, following the treatment, there was a significant ($P < 0.05$) decline in groups D, E and F compared to groups B and C on day 21 post-infection. On day 28, there was no significant difference among the treatment groups (Figure 10).

DISCUSSION

The SEC-DA combination therapy did not cause any significant alteration in the serum biochemical parameters assayed, rather the treatments ameliorated the altered serum biochemical changes caused by the *T. b. brucei* infection in the dogs. The SEC-DA combination therapy caused the earlier total clearance of the parasitaemia and prevented the relapse parasitaemia in the treated dogs unlike the DA-monotherapy where the relapse parasitaemia was detected on day 17 post-treatment.

Parasitaemia was detected in most of the infected dogs 5 days post-infection, thus, the incubation period of *T. b. brucei* in this study was 5 days which is consistent with reports of previous researchers (Anene et al. 1989). The fluctuations in parasitaemia

observed between day 5 and 14 was typical of *T. b. brucei* (Nwosu and Ikeme 1992). The antiparasitic effects of SEC-DA on day 15 (day 2 of the treatment) was dose-dependent. However, from day 16 (day 3 of the treatment) there was no observable difference. The earlier clearance of the parasitaemia and the subsequent inhibition of the relapse parasitaemia could be attributed to the combined antitrypanosomal effects of SEC and DA.

It could also be attributed to some of the physicochemical properties of SEC which allows it to be extensively distributed into tissues and organs including the brain in a therapeutic concentration. A previous report has also shown that the pre-treatment of dogs with SEC led to an increased concentration of DA in the brain of treated dogs (Eke et al. 2017b). Therefore, the inhibition of the relapse parasitaemia by the SEC-DA combination therapy could be attributed to the additive antitrypanosomal effects of both agents and the increased accumulation of both SEC and DA in the brain tissues of the treated dogs, thereby clearing the sequestered trypanosomes in the brain, being the main source of trypanosomes that will later re-emerge into the blood stream in cases of relapse parasitaemia.

Pathological changes in the liver are characterised by an elevation of some liver enzyme markers (Ezeokonkwo et al. 2012). In the current study by day 14 post-infection, there were elevated ALP, ALT, and AST activities in the infected dogs. This is as a result of the tissue invasive nature of *T. b. brucei* and leads to the massive destruction of the hepatic microarchitecture and subsequent leakage and accumulation of these enzyme markers in the serum. These findings were in line with the findings of Ezeokonkwo et al. (2012). Alkaline phosphatase is usually found in high concentrations in the liver, kidney, bone, intestine and placenta. The observed elevated ALP activity could be due to the liver and kidney damage by trypanosomes. However, treatment with either the SEC-DA or DA-mono-therapy caused a decline in the ALP activity in the treated dogs, while the ALP activity in the infected untreated dogs remained high on day 21 post-infection. Nonetheless, a rise in the ALP activity was observed in the DA-monotherapy dogs, while those of SEC-DA declined further on day 28 post-infection. The subsequent mortality in the infected untreated dog group could be related to the impaired hepatic and or renal functions. Furthermore, the second phase rise in the ALP activity in the DA-

monotherapy dogs on day 28 post-infection may be attributed to the increased multiplication of the trypanosomes in the liver which later manifested as relapse parasitaemia on day 31 post-infection. Alanine transaminase and aspartate transaminase activities are usually used as biomarkers for liver health. Higher ALT and AST activity levels were observed in the infected dogs on day 14 post-infection when compared to the normal untreated dogs. The treatments with either the SEC-DA or DA-monotherapy significantly decreased the levels of these enzymes when compared to that of the infected dogs without the treatment on day 21 post-infection. The significant decline in the ALP, ALT and AST activities after 7 days of treatment underscores the deleterious effects of *T. b. brucei* on the hepatocytes and other affected organs. However, the fact that there was no significant difference in the levels of the activities of these enzyme markers between the SEC-DA combination therapy and the uninfected nor treated dogs after treatment showed that the therapy may not have any adverse effects on the liver and other organs of the treated dogs. It is important to note the higher ALP, ALT and AST activities observed on day 21 in the SEC-DA 400/3.5 mg/kg treated dogs, though they were not significantly different from the SE-DA 100/3.5 and 200/3.5 mg/kg treated dogs. Some changes in the lipid profile in some domestic animals have been attributed to trypanosomosis. Adamu et al. (2009) reported a decline in Tchol, TG and HDL in pigs infected with *T. b. brucei*. An elevated Tchol level was reported in rabbits experimentally infected with *T. congolense* (Takeet and Fagbemi 2009) whereas Abenga and Anosa (2007) reported an elevated Tchol level in monkeys infected with *T. b. gambiense*. Reports on the effects of trypanosomosis on the lipid profile of dogs are scant. The work of Nwoha and Anene (2016) showed a decline in the Tchol levels in dogs. In this study, we assayed the Tchol and TG concentrations. Our findings showed elevated Tchol and TG concentrations in all the infected dogs on day 14 post-infection with a general decline in both parameters on day 21 irrespective of the treatment statuses, then a slight increase again on day 28. This agrees with the report of Rouzer and Cerami (1980) who showed that an increased TG concentration in *T. b. brucei* infected rabbits was a result of the decreased lipolytic activity causing a build-up of TG in the serum. The increased Tchol and TG levels could be at-

tributed to the inhibition of the lipase activity, especially the hepatic lipase due to the extensive invasion of the liver cells by the trypanosomes. Since the hepatic lipase is responsible for the hydrolysis of TG, thereby maintaining its level in the body, impairment of the liver by the trypanosomes will ultimately lead to a build-up of serum lipids. Nevertheless, it appears that the clearance of the parasitaemia did not lead to the immediate restoration of the lipase activity because the treatment statuses of the groups did not significantly affect the serum Tchol and TG concentration of the infected animals in any particular pattern, suggesting that the treatment does not immediately restore the lipase activity in the *T. b. brucei* invaded liver. The TP and albumen measurement can be used to assess the liver and kidney functions (American Liver Foundation 2017). Reports on the effect of trypanosomosis on the TP and albumin concentrations in different animal species are variable. While some authors reported an elevation in the TP, others reported a decline. Nwoha and Anene (2016) reported an elevation in the TP and a decline in the albumin in dogs, Rode et al. (2009) reported hypoproteinaemia and hypoalbuminemia, while our findings showed hypoalbuminemia. Hypoalbuminemia was very apparent in the infected dogs on day 14 post-infection and this continued unabated in both the infected dogs without treatment and the DA-monotherapy dogs. However, a significant increase in the albumin was observed after the treatment in all the SEC-DA treated dogs. The effect of SEC-DA was dose-dependent on day 21 with SEC-DA 400/3.5 mg/kg showing the highest recovery in the albumin concentration. On day 28 post-infection, there was no significant difference in the serum albumin concentrations of the SEC-DA treated dogs and those of the normal uninfected nor treated dogs. The TP concentration did not change significantly among the groups up to day 21 post-infection, but there was a significant decline in the TP concentration in the DA-monotherapy dogs on day 28, which coincided with the relapse parasitaemia recorded on day 31 in this group. The effect of SEC-DA again was dose-dependent with TP concentration increasing with an increase in the SEC dose. Thus, while changes in the albumin concentration manifested earlier in the infection process, those of the TP tend to manifest later in the infection process. The hypoproteinaemia and hypoalbuminemia could be

due to increased utilisation of the proteins and albumin by the trypanosomes (Biryomumaisho et al. 2003). The restoration of the albumin concentration to normalcy and maintenance of the TP concentration by SEC-DA could be attributed to the efficient clearance of the trypanosomes from the blood and tissues of the treated dogs. Measurements of urea and creatinine concentration have been used extensively as markers for kidney function. Elevated serum urea concentrations in dogs has been reported earlier (Ezeokonkwo et al. 2012). The elevation in the urea concentration of the infected dogs in our study became apparent on day 21 post-infection, irrespective of the treatment with either SEC-DA or DA-monotherapy. However, the greater elevation in the urea concentration was observed in the infected dogs without the treatment and the DA-monotherapy dogs on day 21 post-infection. The serum urea concentration returned to normal by day 28 post-infection in treated dogs. The rise in the serum urea concentration in the infected dogs could be as a result of the damage to the renal tubules by the parasites, thereby impairing the tubular excretion of the urea and its subsequent accumulation. It could also be as a result of a massive tissue breakdown and anorexia which are regular findings in *T. b. brucei* infections (Nwosu and Ikeme 1992). However, the higher and faster decline in the urea concentration in the SEC-DA treated dogs suggest a more efficient parasite clearance and restoration of the renal integrity. An elevated serum creatinine has been reported in *T. b. brucei* infected dogs (Ezeokonkwo et al. 2012; Nwoha and Anene 2016). This could be due to the tissue invasive nature of *T. b. brucei* and the subsequent kidney obstruction. It could also be due to dehydration and certain medications (Samra and Abca 2012). The elevated serum creatinine concentration became apparent in the present study on day 14 post-infection and continued unabated in the infected dogs without treatment and the DA-monotherapy dogs up till day 21 post-infection. However, the treatment with SEC-DA caused a significant decline in the serum creatinine concentration from day 21 post-infection. However, SEC-DA 400/3.5 mg/kg caused the largest decline in the serum creatinine concentration on day 21. This effect could be attributed to the drug efficacy in relation to the parasite clearance, suggesting that higher doses of SEC are more effective than lower doses.

In conclusion, the SEC-DA therapy ameliorated the aberrant serum biochemical profile of the *T. b. brucei* infected dogs. It also caused the earlier clearance of the parasitaemia and prevented the relapse parasitaemia in the treated dogs. Varying the doses of SEC did not adversely affect the serum biochemical parameters assayed in the study, rather, the higher doses appear to be more beneficial. Secnidazole-diminazene aceturate combination therapy is, therefore, suggested as the treatment of choice in canine *T. b. brucei* infections.

Conflict of interest

The authors declare no conflict of interest.

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