Serum biochemical changes in dromedaries experimentally infected with *Trypanosoma evansi* and treated with melarsenoxyde cysteamine hydrochloride

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ABSTRACT

This study was carried out to study the effect of melarsenoxyde cysteamine hydrochloride (Cymelarsan®) in modulating serum biochemical changes in dromedaries experimentally infected with Trypanosoma evansi. A total of twenty dromedaries were used in the study. They were randomly divided into Groups A - D of 5 each. Group A was infected but treated with Cymelarsan® at 0.25 mg/kg body weight. Group B was the infected control; Group C was the uninfected control while Group D was uninfected but treated with Cymelarsan®. Uniform parasitaemia (2.4 ± 0.19) was observed following a pre-patent period of 4 days (Group A and B). Parasitemia increased significantly ($P<0.05$) to 210.2 ± 1.81 (Groups A) and 200.2 ± 1.77 (Group B) at 20 days post-infection (D.P.I.). Following treatment in Group A, parasitemia was not seen at 32 D.P.I. In Group B it attained a peak count of 400.2 ± 2.50 at day 36 D.P.I. On the one hand, alanineaminotransferase, creatinine, total bilirubin concentrations increased significantly ($P<0.05$) in the infected dromedaries while on the other serum glucose, and total protein levels decreased significantly ($P<0.05$). These biochemical changes were however amended to their pre-infection values at 16 D.P.I. in Group A in contrast to Group B. These parameters in Group C and Group D remained fairly constant. In conclusion, the infection caused biochemical changes suggestive of liver and kidney dysfunctions, with muscular wasting, which were amended following treatments with Cymelarsan®.

Key words: Cymelarsan®, biochemical changes, dromedaries, *Trypanosoma evansi*

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Introduction

Tryanosoma evansi is the cause of "surra" in camels, horses, cattle, sheep, goats and pigs (SOULSBY, 1982; LAHA and SASMAL, 2008; MBAYA et al., 2010; WOMACK et al., 2001). It constitutes one of the major veterinary problems worldwide and is endemic in parts of Africa, South-East Asia, Europe and America (ARADIB and MAJID, 2006). Different wildlife species, such as water buffaloes (*Cyncerus natalensis*), tapirs (*Tapirus indicus*), capybaras (*Hydrochoerus hydrochaeris*), foxes (*Vulpis vulpis*), hyaenas (*Crocuta crocuta*), mongooses (*Lemur m. mongoz*) (WOMACK et al., 2001; MACARAEG et al., 2013) bears (*Selanarctos thibetanus*) (MUHAMMAD et al., 2007) and Asian tigers (PARIJA and BHATTACHARYA, 2005) have been infected with *T. evansi*. Most recently, the first human case of *T. evansi* infection was reported in India (JOSHI et al., 2006).

All species of *Glossina* transmit the organism (WOMACK et al., 2001). However, it is best adapted to mechanical transmission by haematophagus arthropod vectors of the family *Stomoxynae* and *Hippoboscidae* in South East Asia (WICHER et al., 2003) and the semi-arid region of northeastern Nigeria (MBAYA et al., 2010). Surra in camels occurs in the acute or chronic phase and causes significant morbidity and mortality (ARADIB and MAJID, 2006). It also causes immunosuppressive effects, infertility, retarded growth and decreased feed conversion efficacy (NJIRU et al., 2004). Although the prevalence of natural "surra" in camels has been reported in various regions of the world (NJIRU et al., 2004; MBAYA et al., 2010), there is a paucity of information regarding the complete biochemical changes in dromedaries experimentally infected with *T. evansi* and its treatment. In view of this, camels were experimentally infected with a field strain of *T. evansi* (CT/29) to study the effect of Cymelarsan® in amending the biochemical changes that might occur in the course of the infection.

Materials and methods

Experimental animals. Twenty dromedaries (*C. dromedarius*) of both sexes, aged between 1 to 3 years, and weighing between 160 and 400 kg were used in this experiment. All animals were screened for blood, intestinal and external arthropod parasites and were kept for a 60 day acclimatization period before the experiment began, in concrete floored and fly-proof stalls, and fed on groundnut husks, wheat bran, leaves, chopped cucumbers, water melons, concentrates, and water was provided *ad-libitum* throughout the experiment. The experiment was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. All handling and experimental procedures were in accordance with the international guidelines for the use of animals for biomedical research (BROOM and JOHNSON, 1993).

Source of trypanosomes. The *Trypanosoma evansi* (CT/29) field strain used for the study was isolated by the authors from a natural infections of "surra" in camels at

the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. It was identified on the basis of morphology and negative blood inhibition and an infectivity test (BIIT), stabilised by 6 passages in Wistar rats, and sent to the Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Vom, Nigeria, where it was authenticated as the *T. evansi* strain (CT/29) by DNA sequencing using polymerase Chain Reaction (PCR). Approximately 1.0×10^3 'tryps'/mL was inoculated intraperitonealy into Wistar rats to expand the inoculums when the parasitaemia reached approximately 5.0×10^3 'tryps'/mL of blood, and the infected blood was obtained from the rats by cardiac puncture and serially diluted with phosphate buffered glucose saline (PBSG, Ph 7.2) until 1.0×10^3 trypanosomes/0.5 mL was obtained. Each dromedary was inoculated intravenously via the lateral abdominal vein with 0.5 mL of the infected blood.

Experimental design. The camels were randomly separated into four groups (A-D) of five each. They were weighed using a Tru-Test® multi purpose digital livestock scale (Algen Scale Corporation Bohemia, New York) model XHD with extra heavy load bars and an aluminium alloy weight platform placed inside a crush. Group A was infected but treated via the intramuscular route with a single dose of melarsenoxyde cysteamine hydrochloride (Cymelarsan®, Rhone Merieux, Lyon France) at a dose rate of 0.25 mg/kg in a single dose, at 20 days post infection (D.P.I.). Group B was infected but not treated, while Group C remained uninfected. Group D was uninfected but treated with Cymelarsan®.

Parasitemia detection. The dromedaries were examined every 4 days for parasitemia detection using wet mount and haematocrit buffy coat microscopy (SOULSBY, 1982) for 36 D.P.I., after which they were monitored for a period of two months for the possibility of relapse parasitemia. The degree of parasitemia was estimated by the rapid matching technique (HERBERT and LUMSDEN, 1976).

Biochemical analysis. Every 4 days, 3 mL blood samples were obtained from the lateral abdominal vein using a vacutained system, without anticoagulant, for a period of 36 days. All sera samples were stored at 4 °C until used. Liver enzyme (alanine) aminotransferase was estimated by commercial kits (Randox Laboratories, UK) as described by AFONJA (1997). The glucose level was determined by the oxidase procedure of Folin-Wu (COLES, 1986). Total protein was determined by the burette reaction method (AFONJA, 1997). Serum creatinine was measured by the Jaffe reaction method of SEATON and ALLI (1984) while total bilirubin was estimated by the Vandenberg reaction method (MICHAELSON, 1991). The values obtained were read with a spectrometer (Boehringer 4010, Germany) at appropriated wavelengths and the values calculated using standard formulae (COLES, 1986).

Data analysis. The data obtained from the study were summarized as means ± standard deviation and the differences between the means determined at 5% level of significance using the analysis of variance ANOVA (GRAPH PAD INSTAT 2000).

Results

The infected dromedaries in groups A and B showed signs of anorexia, weakness, anemia and cachexia. These symptoms started from 4 D.P.I., when parasitemia became apparent, and were most severe at 20 D.P.I. However, following treatment with Cymelarsan® (Group A) at 20 D.P.I., these symptoms were alleviated at 36 D.P.I. having 0% mortality in contrast to the infected control (Group B), where the symptoms progressed, leading to vaginal prolapse, prepucial edema, orchitis, coma and death. Similarly, no symptoms or mortality were encountered in Group C or D.

The mean parasite counts (1.0 x10³ 'tryps'/mL) and alanine aminotransferase activity of the dromedaries infected with *T. evansi* and treated with Cymelarsan® and controls are presented in Fig. 1. For parasitemia, a uniform pre-patent period of 4 days was encountered in the infected Groups A and B. In Group A, parasitemia reached a peak count of 210.20 ± 1.81 at 20 D.P.I. Following treatment with Cymelarsan® at the peak of parasitaemia at 20 D.P.I. parasitemia declined to 10.00 ± 0.40 and was not seen at 32 D.P.I. All the dromedaries in the group survived. In Group B, parasitemia fluctuated and attained a peak count of 400.20 ± 2.50 at 36 D.P.I. One dromedary died in group B at 28 D.P.I. when parasitemia was 228.00 ± 1.89 while the remaining 4 dromedaries died at 36 D.P.I.
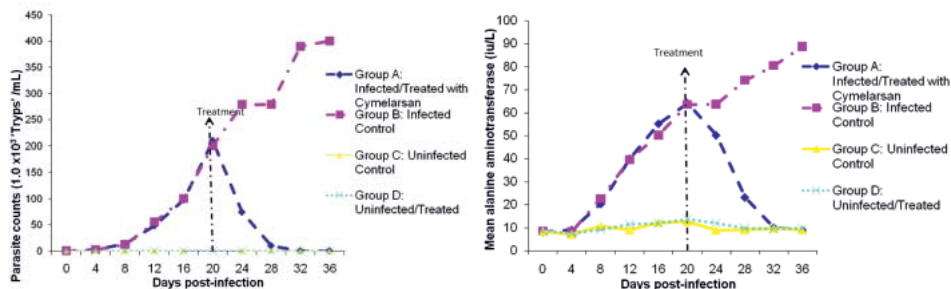

Fig. 1. Mean parasite counts (1.0×10³ 'Tryps'/mL) and mean alanine aminotransferase (iμ/L) of dromedaries (*C. dromedarius*) experimentally infected with *Trypanosoma evansi* and treated with melarsenoxyde cysteamine hydrochloride (Cymelarsan®) and controls

For alanine aminotransferase in Group A, the pre infection value of 8.20 ± 0.66 increased significantly (P<0.05) to 64.20 ± 1.00 from day 4 D.P.I. Following treatment with Cymelarsan® at 20 D.P.I., it declined significantly (P<0.05) to pre-infection values at 36 D.P.I. In Group B, the pre infection value of 8.40 ± 0.36 increased significantly

(P<0.05) to a peak count of 88.90 ± 1.18 at 36 D.P.I. Meanwhile in Groups C and D their pre-infection values remained constant (P>0.05). The mean serum glucose and total protein levels of the experimentally infected dromedaries and controls are presented in Fig. 2. For glucose in Group A, the pre-infection value of 80.20 ± 1.12 declined significantly (P<0.05) to 54.20 ± 0.92 at 20 D.P.I. Following treatment with Cymelarsan® at 20 D.P.I. the pre-infection value was attained at 36 D.P.I. In Group B, the pre infection value of 80.10 ± 1.12 declined significantly (P<0.05) to 2.10 ± 0.18 at 36 D.P.I. Meanwhile in Groups C and D the values remained constant throughout the study (P>0.05).
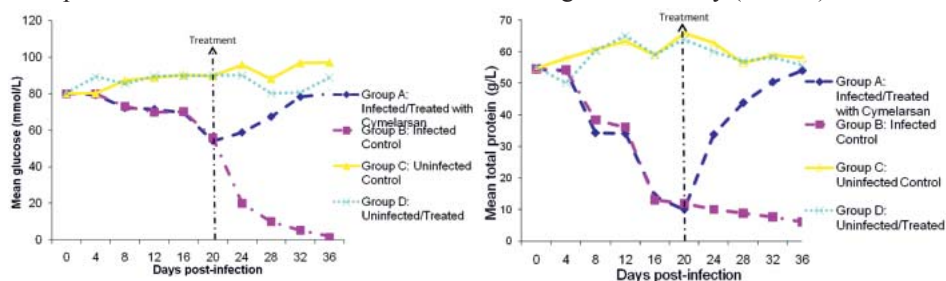

Fig. 2. Mean serum glucose (mmol/L) and mean total protein (g/L) of dromedaries (*C. dromedarius*) experimentally infected with *Trypanosoma evansi* and treated with melarsenoxyde cysteamine hydrochloride (Cymelarsan®) and controls.
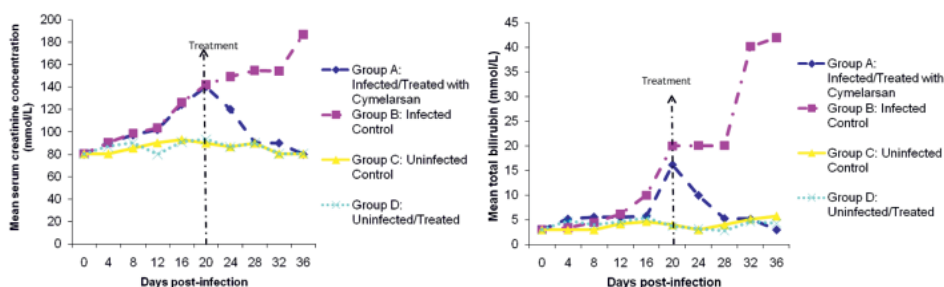

Fig. 3. Mean serum creatinine and total bilirubin concentrations (mmol/L) of dromedaries (*C. dromedarius*) experimentally infected with *Trypanosoma evansi* and treated with melarsenoxyde cysteamine hydrochloride (Cymelarsan®) and controls.

For total protein in Group A, the pre-infection value of 54.40 ± 0.92 declined significantly (P<0.05) to 10.80 ± 0.41 at 20 D.P.I. Following treatment with Cymelarsan® at 20 D.P.I., it increased significantly (P<0.05) to its pre infection value at 36 D.P.I. In Group B, the pre infection value of 54.50 ± 0.92 declined significantly (P<0.05) to 6.00 ±

0.31 at 36 D.P.I. Meanwhile in Groups C and D the values remained constant throughout the period of the study (P>0.05).

The mean creatinine and total bilirubin concentrations of the experimentally infected dromedaries and controls are presented in Fig. 3. For creatinine Group A, the pre infection value of 80.10 ± 1.12 increased significantly (P<0.05) to 140.30 ± 1.48 at 20 D.P.I. Following treatment with Cymelarsan® at 20 D.P.I. the values declined significantly (P<0.05) to their pre infection values at 36 D.P.I. Similarly, the values in Groups C and D remained constant (P>0.05).

In Group A, the pre infection value of 3.00 ± 0.22 for total bilirubin increased significantly (P<0.05) to 12.60 ± 0.44 at 20 D.P.I. Following treatment with Cymelarsan® at 20 D.P.I. it decreased significantly (P<0.05) to its pre-infection value at 36 D.P.I. For Group B, their pre-infection value of 3.00 ± 0.22 increased significantly (P<0.05) to 42.00 ± 0.79 at day 36 D.P.I. However, for the Groups C and D the total bilirubin concentration remained constant throughout the study (P>0.05).

Discussion

The results of this study showed that from day 4 D.P.I. the dromedaries from groups A and B experienced increased alaninaminotransferase, creatinine and total bilirubin sera concentrations. While on the other hand, they experienced a decrease in concomitant serum glucose and total protein levels. These biochemical changes were however modulated in the infected animals treated with Cymelarsan® (Group A).

The elevations of alaninaminotransferase and total bilirubins were suggestive of liver damage. This was observed in the control animals (Group B) where these increased as the infection progressed without abating. The infection was also accompanied with hypoproteinemia. Hypoproteinemia in trypanosomosis in camels has been linked with hepatic damage due to tissue invasion by the organism (RAISINGHANI and LODHA, 1980). Similar findings have been reported in *T. evansi* infection of dogs (AQUINO et al., 2002), donkeys (CADIOLI et al., 2006), *T. brucei* infection of gazelles (MBAYA et al., 2008) and in *T. brucei gambiense* infection of baboons (*Papio anubis*) (MBAYA et al., 2009).

Elevations of creatinine during the experimental infection of dromedaries infected with *T. evansi* may be related to severe kidney dysfunction (AROWOLO et al., 1989; MBAYA et al., 2008). The retention of creatinine in the body showed that the kidneys were severely affected, failing to excrete these catabolic products. Similarly, high levels of creatinine, particularly in the infected controls, might also be attributed to muscle wasting shown by the experimentally infected dromedaries during the course of the infection.

Hypoglycaemia observed in the infected controls (Group B) continued without abating as the infection progressed. Sequel to this, they experienced profound weakness.

This might probably be associated with the fact that the high energy demand in the camels during high parasitaemia impaired glucose release from the gluconeogenic pathways, as well as the fact that trypanosomes during high parasiteamia consumed a large quantity of host glucose (IGBOKWE, 1994). Trypanosomes have been reported to consume blood glucose during aerobic glycolysis (IGBOKWE, 1994). *T. evansi* metabolizes glucose to produce 4-hydroxyl-4-methyl α-ketogluterate, which is inhibitory to the tricarboxylic acid cycle (TCA) in the mitochondria, leading to severe energy deficit in the host (ASHMAN and SEED, 1973; IGBOKWE, 1994; MBAYA et al., 2008). This therefore suggests that the TCA cycle and oxidative phosphorylation might have been inhibited, leading to its total failure to generate energy from energy rich compounds (IGBOKWE, 1994). Ninety percent of the energy available in glucose is released when pyruvate is oxidised to Co_2 and H_2O through the TCA cycle and electron transport chain (CONN and STOMPF, 1976). In conclusion, the experimental *T. evansi* infection caused various clinical and biochemical changes suggestive of severe liver and kidney dysfunctions, and muscular wasting. However, Cymelarsan® at 0.25 mg/kg from 20 D.P.I. eliminated *T. evansi*, which allowed the organs, in the absence of continuous injury by this protozoan, to retrieve their normal functions as the serum enzymes returned to their normal levels. It is therefore recommended that treatment with Cymelarsan® at 0.25 mg/kg should start early in order to modulate the biochemical alterations that follow *T. evansi* infection in dromedaries.

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SAŽETAK

Istraživanje je provedeno da bi se odredio učinak melarsenoksid-cisteamin-hidroklorida (Cymelarsan®) na biokemijska obilježja u serumu dvogrbih deva pokusno invadiranih protozoonom T*rypanosoma evansi*. Pokus je proveden na 20 dvogrbih deva razvrstanih u skupine od A do D. Svaka skupina sadržavala je pet deva. Skupina A bila je invadirana i liječena pripravkom Cymelarsan® u dozi od 0,25 mg/kg tjelesne mase. Skupina B bila invadirana kontrola. Skupina C je bila neinvadirana kontrola dok je skupina D bila neinvadirana, ali je dobivala Cymelarsan®. Jednaka parazitemija (2,4 ± 0,19) bila je ustanovljena nakon prepatentnog razdoblja od svega 4 dana (skupina A i B). Jačina parazitemije značajno je porasla dvadesetog dana nakon invazije (P<0,05), do 210,2 ± 1,81 (skupina A) i 200,2 ± 1,77 (skupina B). U životinja skupine A nije zabilježena parazitemija 32. dana nakon invazije. U skupini B parazitemija je 36. dana poslije invazije iznosila 400,2 ± 2,50. U serumu invadiranih životinja značajno su porasle (P<0,05) vrijednosti alaninaminotransferaze, kreatinina i ukupnog bilirubina dok su se vrijednosti glukoze i ukupnih proteina značajno smanjile (P<0,05). Sve vrijednosti bile su različite u odnosu na 16. dan poslije invazije u životinja skupine A što nije bio slučaj u životinja skupine B. Vrijednosti u serumu životinja skupina C i D ostale su nepromijenjene. Zaključuje se da ustanovljene bikokemijske promjene upućuju na poremećenu funkciju jetre i bubrega te na iscrpljenost mišića što se značajno popravilo nakon primjene Cymelarsana®.

Ključne riječi: Cymelarsan®, biokemijske promjene, dvogrbe deve, *Trypanosoma evansi*