

Serum and stress marker indices of West African dwarf goats challenged with *trypanosoma evansi* and then treated with artemether-lumefantrine

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Abstract

The study assessed efficacy of Artemether-Lumefantrine in goats infected with Surra using examination of parasitaemia directly and indirectly with animal inoculation using mice as a more sensitive diagnosis, serum biochemistry and stress indices. This antimalarial drug was investigated for its trypanocidal potentials in goat. Twelve male goats approximately weighing 6.94 ± 0.39 kg were divided into 3 groups of 4 animals each as replicates in a completely Randomized Design. The untreated control, Diminazene treatment and Artemether treatment represented groups 1, 2 and 3 respectively. Three (3) male albino mice were used for the animal inoculation technique with the 1st, 2nd and 3rd mouse representing groups 1, 2 and 3 respectively. The AST, ALT, Albumin, Total Protein, MDA, GSH, SOD and parasitaemia of the goats were subjected to one way ANOVA, they were also represented as histogram graphs. Results revealed similar ($P > 0.05$) influence across the groups except GSH which had group 2 value to be significantly higher ($P < 0.05$) than group 1. Parasitaemia of the goats were low (0.03) and most times undetected, the mice of groups 1 and 3 had massive parasitaemia (4.83 and 4.63) and that of group 2 was not detected (0.00) because the goats were treated twice with a double dose Diminazene which may have cleared the parasites from its blood stream. Mice inoculation showed the inability of the anti malaria drug to clear completely the *T. evansi* from the goat's blood. The serum biochemical indices and stress markers revealed higher values at infection of *T. evansi* when compared to their values prior to *T. evansi* inoculation across the three groups with exception of GSH of groups 2 and 3 where the values prior to *T. evansi* inoculation was higher than the values at the 37th day of infection. The study has shown that Diminazene acetate has better potency in clearing the *T. evansi* from the blood stream of the WAD goats than the tested drug Artemether-lumefantrine. Although the test drug ameliorated the effect of the disease on the liver of the goats as revealed in the lower liver enzymes compared to control and diminazene treated groups 1 and 2.

Keywords: Surra, Serum, Stress markers, animal inoculation, parasitaemia.



Indices sériques et marqueurs de stress de chèvres West African Dwarf défiées avec *Trypanosoma Evansi* puis traitées avec de l'artéméther-luméfantrine

Résumé

L'étude a évalué l'efficacité de l'artéméther-luméfantrine chez les chèvres infectées par le Surra en examinant la parasitémie directement et indirectement avec l'inoculation animale en utilisant des souris comme diagnostic plus sensible, la biochimie sérique et les indices de stress. Ce médicament antipaludéen a été étudié pour ses potentiels trypanocides chez la chèvre. Douze boucs pesant environ $6,94 \pm 0,39$ kg ont été divisés en 3 groupes de 4 animaux chacun en tant que répétitions dans une conception entièrement randomisée. Le contrôle non traité, le traitement au diminazène et le traitement à l'artéméther représentaient respectivement les groupes 1, 2 et 3. Trois (3) souris albinos mâles ont été utilisées pour la technique d'inoculation animale avec les 1ère, 2ème et 3ème souris représentant les groupes 1, 2 et 3 respectivement. L'AST, l'ALT, l'albumine, la protéine totale, le MDA, le GSH, la SOD et la parasitémie des chèvres ont été soumises à une ANOVA à un facteur, elles ont également été représentées sous forme de graphiques d'histogramme. Les résultats ont révélé une influence similaire ($P > 0,05$) dans tous les groupes, à l'exception du GSH dont la valeur du groupe 2 était significativement

plus élevée ($P < 0,05$) que celle du groupe 1. La parasitémie des chèvres était faible (0,03) et la plupart dutemps non détectée, les souris des groupes 1 et 3 avaient une parasitémie massive (4,83 et 4,63) et celle du groupe 2 n'a pas été détectée (0,00) car les chèvres ont été traitées deux fois avec une double dose de Diminazène qui peut avoir éliminé les parasites de son sang. L'inoculation aux souris a montré l'incapacité du médicament antipaludique à éliminer complètement le *T. evansi* du sang de la chèvre. Les indices biochimiques sériques et les marqueurs de stress ont révélé des valeurs plus élevées lors de l'infection de *T. evansi* par rapport à leurs valeurs avant l'inoculation de *T. evansi* dans les trois groupes à l'exception du GSH des groupes 2 et 3 où les valeurs avant l'inoculation de *T. evansi* étaient supérieures aux valeurs au 37ème jour d'infection. L'étude a montré que l'acéturate de diminazène a une meilleure efficacité pour éliminer le *T. evansi* du flux sanguin des chèvres WAD que le médicament testé Artemether-lumefantrine. Bien que le médicament testé ait amélioré l'effet de la maladie sur le foie des chèvres, comme cela a été révélé dans les enzymes hépatiques inférieures par rapport aux groupes témoins et traités au diminazène 1 et 2.

Mots-clés : Surra, Sérum, Marqueurs de stress, inoculation animale, parasitémie.

Introduction

Surra is a serious tropical or subtropical haemo-parasitic protozoan disease caused by *Trypanosoma evansi*. It is transmitted mechanically by sucking and biting insects, basically tabanids and stomoxys, vampire bats (Desquennes *et al.*, 2013) and by contamination of wound with infected blood (Ereqat *et al.*, 2020). Also known as *Mal de caderas* in Brazil (Aregawi *et al.*, 2019), it has a wide distribution due to the parasite's wide host range. Surra though most prevalent in camels, may also be found in goats (Desquennes *et al.*, 2013). Susceptibility to this disease is dependent on the host (Desquennes *et al.*, 2013 and Tewari *et al.*, 2015) and geographical area (Desquennes *et al.*, 2013). Animals of all ages and sexes are vulnerable without any form of immunity post infection (Ereqat *et al.*, 2020). Successful *Trypanosoma evansi* infections occur with high parasitaemia, about 10 to power 6 trypanosomes/mL of blood (Desquennes *et al.*, 2013) and short blood sucking intervals between infected and non-infected animals (Aregawi *et al.*, 2019).

Oxidative stress sets in with trypanosomosis (Sharma *et al.*, 2022) and is characterized by an imbalance in the production as well as accumulation of reactive oxygen species in cells and tissues. The system therefore becomes toxic due to the presence of excessive free radicals and lipid peroxidation (Pizzino *et al.*, 2017). The presence of liver mitochondrial enzymes in the plasma serve as markers of a malfunctioning liver (Contreras-Zentella *et al.*, 2016).

Enzymes such as aspartate amino transferase (AST) and alanine amino transferase (ALT) levels are raised (Sharma *et al.*, 2022). Oxidative stress and alteration of antioxidant enzymes have shown some level of correlation to diseases such as trypanosomiasis (Akpa *et al.*, 2021). The quantification of serum concentrations of oxidative stress markers such as Malondialdehyde (MDA), Glutathione-S-Transferase (GSH) and Super Oxide Dismutase (SOD) and antioxidant enzymes also facilitates detection of diseases in mammals (Winter *et al.*, 2009). In acute infections, a high mortality rate is recorded and in chronic infections clinical symptoms include oedema, fever, lethargy, weight loss, abortion, nasal and ocular bleeding, limbs stiffness, immune suppression, neuropathy, anaemia leading to death (Aregawi *et al.*, 2019). Surra is therefore, a serious limitation to food safety, security and economy (Mamman *et al.*, 2021) as losses are from yields, market value, and deaths of animals in their thousands (Desquennes *et al.*, 2013, Ereqat *et al.*, 2020, and Mamman *et al.*, 2021) yet many countries fail to report Surra to the World Organization for Animal Health (OIE). It suffers severe neglect despite its health and economic effects (Aregawi *et al.*, 2019).

Diminazene aceturate, a standard drug administered at 3.5 mg/kg body weight, is gradually becoming less potent as a trypanocide (Aregawi *et al.*, 2021). This may probably result from the emergence of chemo resistant strains due to under dosing (Desquennes *et al.*, 2013). Aregawi *et al.* (2021) reported a relapse with the

standard treatment dose. However, a doubled dose of 7mg/kg body weight was found to kill all the parasites (Aregawi *et al.*, 2021). Vaccines to tackle *Surra* are nonexistent as reported by Centre for Agriculture and Bioscience International (CABI) and those against other diseases fail to protect when administered to already infected goats due to immunosuppression (Birhanu *et al.*, 2015).

The West African Dwarf goats found in the humid and sub humid zones are known to adapt to less favourable environments. They are among livestock that play important role in the economic and cultural livelihood of rural populace in Africa (Aziz, 2010). They possess lower metabolic activities, have good feeding efficiency and relatively better tolerance to diseases (Daramola *et al.*, 2009) than other breeds of goats. Studies related to oxidative stress and serum biochemistry with ruminants infected with *Surra* are scanty (Rashdi *et al.*, 2017) and none have shown the ameliorative effect of Artemether-Lumefantrine on oxidative stress induced in caprine trypanosomiasis. New trypanocidal drugs have not been discovered in decades. Artemether has been extensively tested in vitro and in vivo studies using laboratory animals that showed potent antitrypanosomal activities (Golenser *et al.*, 2006, Mbaya *et al.*, 2018, Salifu *et al.*, 2020, Salifu *et al.*, 2021).

This study was conducted to assess the efficacy of Artemether Lumefantrine (a conventional anti malaria drug) in the treatment of *Surra* in these goats by examining their daily parasitaemia, serum biochemical and oxidative stress markers indices pre inoculation and at termination stages post treatment.

Materials and methods

The experiment was conducted at a housing facility in Livestock Division located at Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, between May and September; 2021. *Trypanosoma evansi* used for the study was obtained from Parasitological Division where it is currently being preserved. Parasitized blood was collected via the tail vein of a donor rat with massive parasitaemia using a 5 mL syringe containing normal saline which was used to dilute the blood until 32 parasites per field was observed under a $\times 40$ objective.

This represents $12.9 \times 10^6 \text{ mL}^{-1}$ of parasitized blood using the methods of Herberts and Lumsden (1976). Twelve male WAD goats with mean weight of $6.94 \pm 0.39 \text{ kg}$ were procured from Vom market. They were quarantined for two weeks. Feed and potable water were supplied to the animals on a daily basis. The animals at time of purchase were vaccinated against Pest de Pest Ruminants (PPR) at 0.14 mL/Kg body weight, dewormed with Albendazole and treated against ecto parasites with Ivermectin at 1 mL/50Kg body weight respectively.

A broad Long Acting (L.A) antibiotic spectrum (Oxytetracycline) was administered to the animals at 0.14 mL/Kg body weight. Routine washing and disinfection of the pens, feeding and watering troughs was adhered. The feed ingredients comprised of yam peels, molasses, groundnut cake, palm kernel meal, wheat offal, cowpea husk, rice offal, table salt, vitamins and mineral premix. The goats were allotted to three treatments of 1 (Control), 2 (Diminazene diacetate treatment) and 3 (Artemether-lumefantrine treatment) comprising four animals per group in a Completely Randomized Design. All the goats were inoculated intravenously with 0.5 mL of *Trypanosoma evansi* blood collected from the donor mouse and treatment commenced at prepatency. Blood samples of 2 mL were collected twice (at the beginning and termination of goat experiment) using new 5ml syringes and 18 inches gauge needles via the jugular vein of each goat and taken to the biochemistry laboratory of National Veterinary Research Institute (NVRI) Vom for Serum biochemistry (AST, ALT, Albumin and Total protein) and oxidative stress biomarkers (MDA, GSH and SOD) analysis. The oxidative stress biomarkers were determined using the methods as described by Nazifi *et al.* (2009). Serum biochemistry parameters were determined using the method of Shin *et al.* (2009). At termination of the post infection period, blood was collected from a goat from each group and inoculated into 3 mice, with each mouse representing groups 1, 2 and 3 respectively where parasitaemia was observed for another sixty days. The mice used were bred in the small laboratory animal house at NITR, Vom. The fixed combination of 14% artemether and 86% lumefantrine (Artelumex®),

anti malaria powdered form was procured from a pharmaceutical store at Vom, Plateau State. The dose administered to the experimental animals was 5 mL given orally per goat for 3 days. The trypanosome parasites were monitored daily at morning hours via drop of blood collected from the marginal ear vein of each goat using the wet film parasitological method. The absolute numbers of parasites viewed were estimated using the Herbert and Lumsden (1976) method and presented as Logarithm Equivalent Values. Same method was used for the animal inoculation with blood collected from the tail vein of each mouse.

Data generated were subjected to one way analysis of Variance at 5% significant difference and descriptive statistics using the Statistical Package for Social Science (SPSS) version 24 and histogram graphs plotted using excel to illustrate the trends observed. Ethical approval was obtained from faculty of pharmaceutical sciences, University of Jos, Nigeria with reference number UJ/FPS/F17-00379.

Results and discussion

The results of the serum biochemistry of WAD goats infected with *T. evansi* and treated with Artemether are presented in Table 1. All the liver enzymes parameters (AST, ALT, Albumin) and Total protein were similar ($P>0.05$). The Artemether treated group 3 continually had the least values for all the serum parameters evaluated. The Artemether-Lumefantrine drug administered to group 3 showed some level of antitrypanosomal potentials against *Surra* infection in the goats as observed in the lower values for the liver enzymes and total protein which signify better functioning of the liver. Biochemical indices have been reported to help determine the degree of damage to hosts organs caused by trypanosomiasis and other related diseases (Otesile *et al.*, 1991, Contreras-Zentella *et al.*, 2016) which corroborates with the findings of the current study of the ability of the artemether drug having the effect of lowering the serum biochemical values more than the other groups. Other workers also reported higher AST and ALT in cattle naturally infected with *Surra* than the non-infected cattle (Rashmi *et al.*, 2017) which agrees with figures 1 and 2 of the current study that indicated a

lower AST and ALT before inoculation of *T. evansi* into the WAD goats. Figures 3 and 4 also showed similar trend for albumin and total protein.

Table 1. Serum biochemical parameters of West African Dwarf goats experimentally infected with *Trypanosoma evansi* and treated with Artemether-Lumefantrine.

Parameters	Treatment Groups			P-value	SEM
	1 (Untreated control)	2 (Diminazene treated)	3 (Artemether treated)		
AST(U/L)	21.04	18.18	14.23	0.43	2
ALT (U/L)	4.83	4.83	4.5	0.8	0.21
Albumin (U/L)	2.81	2.93	2.38	0.91	0.13
Total protein (g/dl)	5.01	5.21	4.63	0.65	0.23

$P>0.05$ are not significant, SEM Standard Error of Mean, AST Aspartate amino Transferase, ALT Alanine amino Transferase.

The oxidative stress markers of WAD goats infected with *Surra* are presented in table 2. Malondialdehyde, and superoxide dismutase values were similar ($P>0.05$), while Gluthione-S Transferase (GSH) of Diminazene treated group 2 was significantly ($P<0.05$) higher than only untreated control group 1. This finding disagrees with the report of Akpa *et al.* (2021) that worked on dogs infected with *T. brucei* which belongs to the same sub genus (trypanozoon) as *T. evansi* used in infecting WAD goats of the current study. The distant relationship between the goat and dog may have altered the non-similarity of the oxidative stress markers with exception of the MDA. The SOD reported for raining season of semi extensive system of WAD goat management had slightly higher values (39.03-43.81 units/Mol) (Yusuf *et al.*, 2017) than SOD values (35.42-38.04 μ MM/Min) of the current study.

Table 2. Oxidative stress markers of West African Dwarf goats experimentally infected with *Trypanosoma evansi* and treated with Artemether-Lumefantrine.

Parameters	Treatment Groups			P-value	SEM
	1 (Untreated control)	2 (Diminazene treated)	3 (Artemether treated)		
MDA (μ Mol/mg)	2.39	2.48	2.35	0.96	0.17
GSH (μ g/L)	9.16 ^b	12.83 ^a	11.38 ^{ab}	0.05	0.68
SOD (μ MM/Min)	35.42	38.04	35.44	0.19	0.67

Different a, b superscripts are significant at $P\leq 0.05$, SEM Standard Error of Mean, MDA Malondialdehyde, GSH Gluthione-S Transferase, SOD Super oxide Dismutase.

The results of parasitaemia represented in its Logarithm Equivalent Value (LEV) from *T.*

evansi infected WAD goats and the mice are presented on Table 3. The parasitaemia for the WAD goats were relatively scanty and sometimes undetected for all groups. Only the mouse inoculated with blood from group 2 survived beyond the 60 days of parasitaemia observation with no detection of the parasite, while mice inoculated with blood from groups 1 and 3 died at days 13 and 17 respectively. Parasitaemia in group 2 mouse was not detected because the goats were treated twice with double dose Diminazene after relapse occurred which may have cleared the parasites from its blood stream.

The finding of direct wet film detection being low and undetected concurs with the report of Behnke *et al.* (2010) for *T. evansi* screening in WAD goats. However, this study revealed highersensitivity to the detection of *T. evansi* in WAD goats using the mice inoculation technique.

Table 3. Parasitaemia of WAD goats infected with *Trypanosoma evansi* and the animal inoculation using male albino mice.

Parameters	Groups			P-value	SEM
	1 (Untreated control)	2 (Diminazene treatment)	3 (Artemether treatment)		
Parasitaemia (LEV of WAD goats)	0.03	0.03	0.31	0.13	0.07
*Parasitaemia (LEV of Sub inoculated Mice)	4.83	0.00	4.63		

*Not subjected to ANOVA, P>0.05 are not significant, SEM Standard Error of Mean and LEV Logarithm Equivalent Value.

The trend of the serum biochemical and oxidative stress markers shows that the healthy goats before inoculation were lower than the infected goats at day 37 for all the groups 1, 2 and 3 (Figures 1, 2, 3, 4, 5 and 7). Except for GSH (Figure 6) where groups 2 and 3 healthy goats before *T. evansi* inoculation had higher values than the infected goats at day 37. This trend is contrary to the work of Akpa *et al.* (2021) for SOD and GSH, while only MDA at day 35 of *T. brucei* infection of the dogs had a similar trend with the current study. The reversal of the increasing GSH by the Artemether and Diminazene drugs at day 37 of infection of the goats with *Surra* was also not observed in the report of Akpa *et al.* (2021) that used Diminazene and Isomethamedium Chloride in

the treatment of *T. brucei* infected canine. The zero mortality reported for the Artemether treated group (Salifu *et al.*, 2021) for caprine *surra* infection may have suggested the ability of the antimalaria drug to having the ability in lowering the AST, ALT, Albumin, Total protein and GSH when compared to Diminazene treated group that had mortality due to relapse. The lower or similar values for some biochemical (ALT) and oxidative stress markers (MDA, GSH and SOD) as observed in figures 2, 5, 6 and 7 respectively in the untreated control group when compared to the Artemether treated group may signify some level of stress due to the drug intake, although not fatal, instead having a neutralizing effect on the disease, since the untreated control group was also reported to have recorded mortality (Salifu *et al.*, 2021).

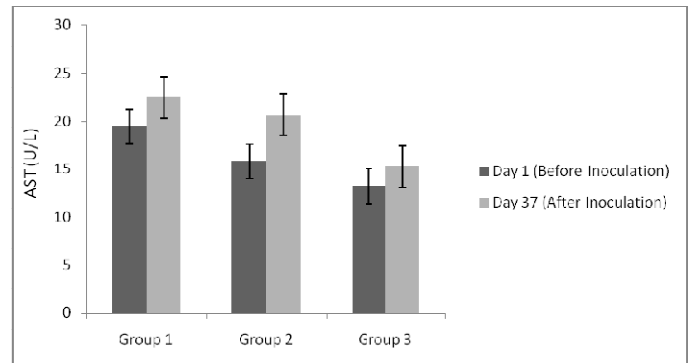


Figure 1: Aspartate amino Transferase (AST) indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2 (Diminazene treated), group 3 (Artemether-Lumefantrine treated).

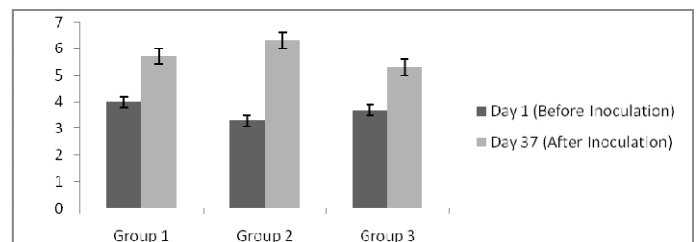


Figure 2: Alanine amino Transferase (ALT) indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2

(Diminazene treated), group 3 (Artemether-Lumefantrine treated).

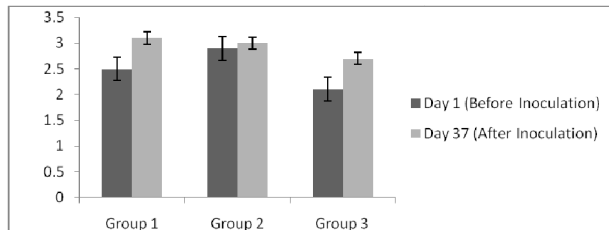


Figure 3: Albumin indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2 (Diminazene treated), group 3 (Artemether-Lumefantrine treated).

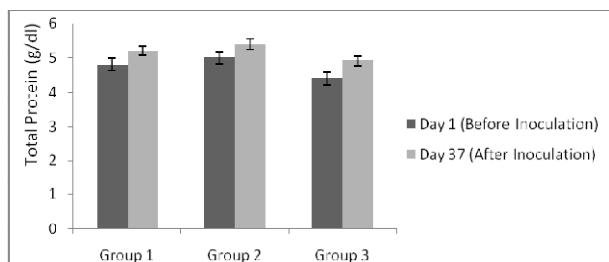


Figure 4: Total Protein indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2 (Diminazene treated), group 3 (Artemether-Lumefantrine treated).

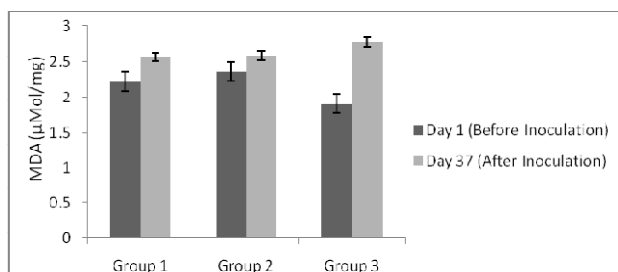


Figure 5: Malondialdehyde (MDA) indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2 (Diminazene treated), group 3 (Artemether-Lumefantrine treated).

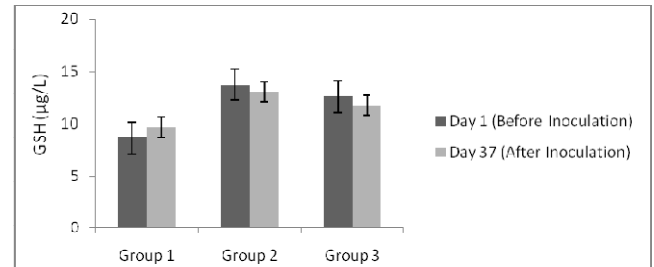


Figure 6: Glutathione-S-Transferase indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2 (Diminazene treated), group 3 (Artemether-Lumefantrine treated).

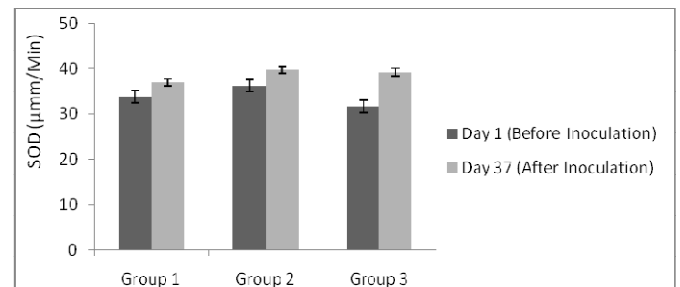


Figure 7: Super Oxide Dismutase (SOD) indices before inoculation and at 37 days after inoculation of *Trypanosoma evansi* in WAD goats. Group (1 untreated control), group 2 (Diminazene treated), group 3 (Artemether-Lumefantrine treated).

Conclusion and recommendation

The use of Artemether-Lumefantrine at 5 mL dose was found to be effective in the reduction of GSH and liver enzymes in the management of *Surra* infected WAD goats. Mice inoculation showed the inability of the antimalaria drug to clear completely the *T. evansi* from the blood system of the goats. Higher doses and other routes of the drug administration could be tried in further studies to test its trypanocidal potentials.

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