Haemo and Biochemical Changes in the Evaluation of Antimalarial Drugs as Therapeutic Alternatives to Mainstream Trypanosides in Experimentally Induced Trypanosomosis in Wistar Rats

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DOI: https://doi.org/10.51584/IJRIAS.2025.100600142

Received: 16 June 2025; Accepted: 24 June 2025; Published: 24 July 2025

ABSTRACT

This study evaluated the haematological and biochemical effects of antimalarial drugs as therapeutic alternatives to conventional trypanocides in Wistar rats experimentally infected with *Trypanosoma brucei brucei*, *T. congolense*, and *T. vivax*. Although parasite suppression and clinical signs were recorded, they are not included in this manuscript.

Fifty-four adult rats were randomly assigned into three experimental models: (A) infected then treated, (B) treated before infection, and (C) infected but untreated. Each model consisted of six groups, with three replicates per group, each comprising three rats, adhering to the 3Rs principle. Rats were inoculated intraperitoneally with 5×10^4 trypanosomes/mL and sampled at 60, 120, and 180 minutes post-infection.

Haematological parameters (PCV, RBC, Hb, WBC) were measured from heparinized blood, while serum biochemical indices; serum creatinine, AST, ALT, ALP, BUN, bilirubin, total glucose (tGL), total protein, total cholesterol (TCH), and HDL were assessed from non-heparinized blood using automated analyzers.

Significant ($P < 0.05$) reductions in PCV, RBC, Hb, WBC, and neutrophils were observed in groups treated with pyrimethamine-sulphadoxine, regardless of the *Trypanosoma* species used. Artemether-treated groups showed milder or no such haematological suppression. Similarly, significant ($P < 0.05$) elevations in serum creatinine, AST, and tGL were recorded in pyrimethamine-sulphadoxine groups, while BUN, bilirubin, and ALT increased non-significantly. These changes were less pronounced or absent in artemether-treated groups.

The findings suggest a differential impact of antimalarial drugs on blood and biochemical parameters in trypanosome-infected rats, with artemether demonstrating better safety and tolerability. These results support the potential of antimalarial agents, especially artemether, as viable alternatives to conventional trypanocides in managing trypanosomosis.

INTRODUCTION

African Animal Trypanosomiasis (AAT) "nagana" and human African trypanosomosis (HAT) "sleeping sickness" is an infectious arthropod-borne disease prevalent in tropical areas of the African continent, it is a disease complex caused by infection with *T. congolense, T. vivax, T. brucei brucei,* either singly or in combination (Heather and Sellon, 2014). It is transmitted by bites from *Glossina* (Tsetse fly), *Tabanus* and *Stomoxys calcitrans* (Michael *et al.,* 2008). Tsetse flies covers an approximately 80% of the land mass in Nigeria (Anene *et al.,* 2006). HAT affects 8.7 million km^2 of sub-Saharan Africa (SSA) where the climate and environment are suitable niches for the tse-tse fly vector (Baker *et al.,* 2013). Human African trypanosomiasis threatens approximately 70 million people distributed in over 36 countries in sub-Saharan Africa (Carvalho *et al.,* 2015). In East Africa, *T. congolense* is the most prevalent species incriminated in disease while in West

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Africa, T. *congolense* and *T. vivax* are the commonest species affecting cattle, sheep, goats, horses and pigs. Trypanosomosis is documented to have severely repressed the African economy (Muhanguzi *et al.,* 2017; Jolayemi *et al.,* 2021) Human trypanosomosis "sleeping sickness" and AAT are both devastating diseases with significant economic consequences to mostly poor nations of developing countries. The attention of global health organization is drawn to the zoonotic potential and host range of rapidly mutating species of trypanosome. The national and global strategies to control and limit the dispersal of trypanosome, attracted participation of western countries and led to the establishment of surveillance and monitoring programs since the 1980's, one of such resulting programs is the National Institute for Trypanosomosis Research (NITR) tasked with monitoring the evolution of ecology providing niches for both *Glossina* the vector and trypanosome the parasite in Nigeria. Diagnosis of AAT in Nigeria has relied on direct microscopy, having low sensitivity with consequential low prevalence that is misrepresentative (OIE, 2012), it is however, still being used with accuracy in resource limited settings. A better technique that concentrates the parasites by centrifuging is being used along with genetic and antibody based assays for diagnosis.

Animal trypanosomosis has ravaged Africa over the last century with severe epidemics in West Africa, Kenya, Tanzania, Uganda, Nigeria and the Congo basin (Tong *et al.,* 2011). With increasing livestock population and the critical importance it has on sustainable development in Africa, the control of HAT and AAT becomes imperative through the use of newer less toxic drugs for better patient management and prophylaxis. The current options for trypanosomosis treatment are poor, due to scarcity and toxicities of mainstream trypanosides (Nok, 2005). Elarsoprol, pentamidine, diminazene aceturate, isometamidium chloride, and homidium chloride are the main classes of drugs used to treat HAT-sleeping sickness and AAT-nagana (Baker *et al.,* 2013), the latter is carcinogenic and its availability is seldom in most countries. These drugs are mainstream trypanosides used for cure and prophylaxis, with over 5 decades of use. However, there is evidence of rapidly developing resistance amongst species of trypanosome to these drugs (Wastling and Welburn, 2011). With no vaccine available and limited therapeutic alternatives, the emergence of drug resistance is a major threat to both human and livestock population in this regard (Fairlamb, 2003). The high cost and toxicities of the mainstream trypanosides are also secondary issues blocking patient management and prevention of symptomatic diseases in endemic areas.

Repurposing oral drugs with proven efficacy for a wide spectrum of parasites of economic, medical and zoonotic importance could reduce both the time and cost involved in management of clinical trypanosomosis and drug discovery (Carvalho *et al.,* 2015).

It is against this backdrop, that this research is conceived, to see if credible alternatives to mainstream trypanosides can be explored for therapeutics and prophylaxis of HAT and AAT in resource limited settings of developing countries. Central to the objective of this study too was to investigate the side effects of these alternative trypanosides especially hemodynamic and hepatic alterations in models as a safeguard as the drugs are leveraged for general patient management of cases in settings where there is persistent scarcity of the well studied mainstream trypanosides.

MATERIALS AND METHODS

Experimental Design

This research evaluated the efficacy of two antimalarial as alternative trypanosides in models, three commonly reported trypanosome (*Trypanosoma brucei brucei; Trypanosoma congolense; Trypanosoma vivax*) causing disease in animals and humans were used for the investigation. The therapeutic and prophylactic promises of the drugs were investigated in 6 groups with three replicates each. Each replicate consisted of A, B, C which corresponded to Infected+Disease, Prophylactic+Disease and Control (infected and not treated) groups respectively. Replicate A was infected intraperitoneally with titers of parasites then treated subsequently, replicate B was treated before infected to test prophylaxis and C was infected but not treated at all.

The design applied to all groups (Groups 1 to 6). Blood samples were collected at graduated intervals to monitor parasitaemia and abundance in circulation (data on relative abundance of parasites in relation to time in press in another publishing platform), the blood was collected according to methods described by Parasuraman *et al.,* (2017) and was taken at sixty minutes post inoculation (PI); 120minutes PI; 180 minutes PI. For each group with

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

an initial sample at zero minute to serve as control in tandem with known reference values for the adopted model. Known titer of *Trypanosome* was inoculated and abundance determined by counting unit volume sampled at intervals post inoculation. The hematocrit and differential blood cell counts (CBC), liver function test (LFT) was also determined from collected heparinized blood samples as described by Jolayemi *et al.* (2021). The efficacy of two common anti-malarials was evaluated in adopted models using the group design described by Nok (2005).

Pathogen Inoculum and Drugs

Standard inoculum of T*rypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax* was obtained through official requisition from the National Institute of Trypanosomiasis Research (NITR), Plateau State, Nigeria. Labeled stock cultures were shipped in standard conditions in sealed specified tubes with hazards labels. Pathogens were assessed upon arrival by a thin blood smear and subsequent microscopy to assess viability and motility. Trypanosomes were seen stained within the Red Blood Cells (RBCs). Antimalarials were sourced from licensed pharmaceuticals within the study area. Two classes of antimalarials: The ACT (Artemisinin Combination Therapy): Atemether 80mg was used as reported by Nok (2005) and sulpha-based antimalarials (Pyrimethamine and sulphadoxine) in a ration 5mg and500mg) as previously used by Jolayemi *et al.* (2021). Both drugs and pathogen inoculum were stored in standard conditions in the Department of Veterinary Medicine, Usmanu Danfodiyo University in Nigeria until required for use in the study.

Source of Animals, Grouping and Ethical Clearance

Wistar rats of various age ranges with mean of 8 months to 12 months ±0.4 was purchased from an institutional vivarium at the Ahmadu Bello University Zaria, in Nigeria. The models were allowed to acclimate for two weeks over glucose at dosage of 500mg daily, industrial pelletized feed (with vegetables added) and drinking water *ad libitum.* Models were kept under 18hours close observation in standard cages with sawdust and wood peeling as beddings as reported by Delwata *et al.* (2018) (industrial feed with vegetables added. Grouping (schematized below) consisted of random allocation of 3rats per replicate with 3 replicates (A, B, C) making up a group with an overall of six groups assembled. Ethical clearance with strict adherence to the principle of 3Rs and other animal welfare requirements as outlined in the Helsinki convention was complied with. The ethical clearance permission was obtained from Institutional Animal Care and Use Committee, Usmanu Danfodiyo University Sokoto (IACUC, UDUS), Nigeria with identification number UDUS/IACUC/2023/AUP-RO08.

Sample Collection and Preservation

Blood sample was collected using the tail vein. In some instances, difficulty was encountered getting adequate volume of blood (2mls) required, an alternative set up to draw from the heart was made available in contingent. This is achieved by anesthetizing the models in a sealed transparent glass container with cotton soaked in chloroform. The rats were dropped for average of 60seconds and when reflexes are lost, the rats were placed on dorsal recumbency and blood drawn by inserting the needle just on the v-shaped furrow below the sternum as adopted in the Standard Operating Protocol (SOP) of Queens University (www.queensu.ca; assessed 2nd June 2025 at 11am). For each volume of blood collected, aliquots of 1ml was made into a heparinized tube and rocked gently before packing in iced styroform boxes, and another into a plain sample bottle to harvest serum. The heparinized blood sample as well as the harvested serum (obtained by centrifuging at 3,000g for 3minutes) by a mobile centrifuge positioned at site of study) were submitted to the Clinical Pathology section of Veterinary Pathology Laboratory, Usmanu Danfodiyo University Sokoto, Nigeria for determination of hematology and clinical biochemical profile.

Pathogen Inoculation and Chemotherapy

A standard inoculum of 5×10^4 Trypanosome per mililitre (already contained in procured standard stock culture from NITR) was injected intraperitoneally by restraining models in dorsal recumbency using a 28guage needle as described by (Wenzler *et al.,* 2013). Infected hosts were transferred to cages for close study of exhibiting clinical signs (compendium of clinical signs relative to time of infection submitted but not part of this manuscript. Fixed dose of the selected antimalarial (artemether 80mg; pyrimethamine and sulphadoxine in 5mg-500mg

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

respectively) was administered by dissolving tablets in distilled water and inoculating drops orally using a Pasteur pipette as previously done by Nok, (2005).

Laboratory Analysis: Haematology and Serum Biochemistry

Packed cell volume (PCV), hemoglobin concentration (Hbc), complete blood count (CBC) with differential and total white blood cell (WBC) counts were all done using an automated hemoanalyser: PEC Medical, Model HB 7021, Tunisia.

Well-preserved sera samples were analyzed using the POINTE reagent kit on an fully automated chemistry analyzer in the laboratory. Biochemical metrics measured included serum creatinine (Scr), blood Urea Nitrogen (BUN), bilirubin, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total glucose (tGL) total protein, total cholesterol (TCH), high density cholesterol (HDL), procedure is as detailed in the kit protocol and as previously reported by Delwata *et al.*, 2018). This was done is stated laboratory using semi-auto chemistry analyzer Model SK 3002B Sinothinker Technology Co, Shenzhen, China.

Animal Grouping and Cohorts

Table 1. Intraperitoneal Infection with *T. brucei brucei* and treatment with yrimethamine+Sulphadoxine and Artemether in Rat Models in Cohort 1

Group 1 *T. brucei*+ Pyr X+ SulX	**Infected Later Treated**	**Treated Before Infected**	**Infected Never Treated**	**Treatment: Drugs Pyrimethamine+Sulphadoxine 5mg+500mg**	**Blood sample 60min**	**Blood sample 120mins**	**Blood sample 180mins**
A (Replicate)	✓	X	X	✓	✓	✓	✓
B (Replicate)	X	✓	X	✓	✓	✓	✓
C (Replicate)	X	X	✓	X	✓	✓	✓
Group 2 *T. brucei*+artemether	**Infected Later Treated**	**Treated Before Infected**	**Infected Never Treated**	**Treatment: Artemether 80mg**	**Blood sample 60mins**	**Blood sample 120mins**	**Blood sample 180mins**
A (Replicate)	√	X	X	√	√	√	√
B (Replicate)	X	√	X	√	√	√	√
C (Replicate)	X	X	√	X	√	√	√

Keys: **Pyr X: Pyrimethamine; Sul X: Sulphadoxine;** X: Nil agent or no action on group; √: administered agent or plus action on group

Table 2: Intraperitoneal Infection with *T. congolense* and treatment with Pyrimethamine + Sulphadoxine and Artemether in Rat Models in Cohort 2

Group 3 *T. congolense* +PyrX+SulX	**Infected Later Treated**	**Treated Before Infected**	**Infected Never Treated**	**Treatment: Drugs Pyrimethamine+Sulphadoxine 5mg+500mg**	**Blood sample 60min**	**Blood sample 120mins**	**Blood sample 180mn**
A (Replicate)	✓	X	X	✓	✓	✓	✓
B (Replicate)	X	✓	X	✓	✓	✓	✓
C (Replicate)	X	X	✓	X	✓	✓	✓

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Group 4 *T. congolense*+ artemether	**Infected Later Treated**	**Treated Before Infected**	**Infected Never Treated**	**Treatment: Artemether 80mg**	**Blood sample 60mins**	**Blood sample 120mins**	**Blood sample 180mins**
A (Replicate)	√	X	X	√	√	√	√
B (Replicate)	X	√	X	√	√	√	√
C (Replicate)	X	X	√	X	√	√	√

Keys: **Pyr X: Pyrimethamine; Sul X: Sulphadoxine;** X: Nil agent or no action on group; √: administered agent or plus action on group

Table 3: Intraperitoneal Infection with *T. vivax* and treatment with Pyrimethamine + Sulphadoxine and Artemether in Rat Models in Cohort 3

Group 5 *T. vivax* +PyrX+SulX	**Infected Later Treated**	**Treated Before Infected**	**Infected Never Treated**	**Treatment: Drugs Pyrimethamine+Sulphadoxine 5mg+500mg**	**Blood sample 60min**	**Blood sample 120mins**	**Blood sample 180mn**
A (Replicate)	✓	X	X	✓	✓	✓	✓
B (Replicate)	X	✓	X	✓	✓	✓	✓
C (Replicate)	X	X	✓	X	✓	✓	✓
Group 6 *T. vivax*+ artemether	**Infected Later Treated**	**Treated Before Infected**	**Infected Never Treated**	**Treatment: Artemether 80mg**	**Blood sample 60mins**	**Blood sample 120mins**	**Blood sample 180mins**
A (Replicate)	√	X	X	√	√	√	√
B (Replicate)	X	√	X	√	√	√	√
C (Replicate)	X	X	√	X	√	√	√

Keys: **Pyr X: Pyrimethamine; Sul X: Sulphadoxine;** X: Nil agent or no action on group; √: administered agent or plus action on group

Statistical Analysis

Data generated in the study were carefully recorded and presented as mean±standard error of mean (SEM) and analyzed using two way ANOVA contain Graph-pad Prism version 8 for windows, San Diego California, USA. Values of $P<0.05$ was considered significant for tested research variables

RESULTS

Table 4: Haemobiochemical Indices in Models after intraperitonel infection With *T. brucei* and Treatment with Binary of Pyrimethamine-Sulphadoxine and Artemether Separately

			PCV (%)	**Hb (g/dl)**	**RBC (10^6/ mm^3)**	**WBC (10^3× 10^3/mm^3)**	**N (×10^3/ mm^3)**	**L (×10^3 / mm^3)**	**M (×10^3/ mm^3)**	**E (×10^3/mm^3)**	**B (×10^3/ mm^3)**
PYRIMETHAMINE **Cohort One**	**Group 1**	**0M** **A** **B** **C**	34±0.15 23±0.30 26±0.04* 22±0.03*	12±0.30 10±0.20 09±0.30	5.34±0.27 3.51±0.34 2.18±0.12	10.20±0.10 15.71±0.43 16.78±0.21 46.59±0.27	20.17±0.15 13.24±0.40* 16.80±0.06	69.12±0.80 56.78±0.30 61.43±0.10	0.32±0.06 0.48±0.40 4.25±0.20 1.19±0.07	3.12±0.30 2.73±0.06 1.31±0.00 1.11±0.23	0.00 0.00 0.00 0.00

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				09±0.40	3.29±0.47		41.00±0.08	62.35±0.50			
Cohort Two	**Group 3**	**0M** **A** **B** **C**	36±0.04 22±0.23 19±0.05 28±0.10	14±.20 10±0.05 09±0.53 12±0.20	4.49±0.40 2.08±0.31 2.82±0.20 3.49±0.23	20.10±0.20 19.90±0.37 13.10±0.26* 19.23±0.47	27.46±0.10 17.08±0.60 8.35±0.30* 28.18±0.10	76.2±0.30 63.4±0.34 44.06±0.40 54.98±0.50	1.41±0.30 0.22±0.70 0.13±0.25 0.37±0.30	3.60±0.20 1.15±0.11 1.13±0.31 1.00±0.21	0.00 0.00 0.00 0.00
Cohort Three	**Group 5**	**0M** **A** **B** **C**	39±0.53 24±0.31 22±1.04* 25±0.55	13±0.10 8±0.02 8±0.70 8±0.20	5.93±0.30 3.12±0.32 2.26±0.30 3.16±0.62	21.90±0.20 17.60±0.60 13.70±0.25 20.25±0.34	34.40±0.20 11.62±0.10 17.14±0.20* 4.71±0.10	50.44±0.20 47.5±0.40 45.7±0.43 31.81±0.30	0.88±0.70 0.88±0.30 0.69±0.20 0.36±0.60	7.00±0.13 2.00±0.02 0.23±0.20 3.10±0.05	0.00 0.00 0.00 0.00
ARTEMETHER Cohort One	**Group 2**	**0M** **A** **B** **C**	33±0.07 30±0.24 30±0.06 28±0.08	11±0.10 10±0.10 10±0.40 9±0.60	4.65±0.80 4.18±0.30 4.73±0.10 4.70±0.20	13.85±0.01 27.93±0.30 12.50±0.06 2.95±0.30	9.97±0.20 20.95±0.10 7.75±0.40 1.06±0.30	27.05±0.50 24.47±0.20 19.25±0.20 31.53±0.60	0.14±0.40 0.84±0.05 0.75±0.70 0.18±0.30	0.14±0.50 0.00±0.00 0.00±0.00 0.00±0.00	0.00 0.00 0.00 0.00
Cohort One	**Group 4**	**0M** **A** **B** **C**	38±0.21 34±0.05 35±0.05 34±0.10	13±0.01* 11±0.03* 12±0.10 11±0.07	5.30±0.10 5.14±0.40 4.29±0.05 5.31±0.01	8.88±0.10 1.30±0.40 18.75±0.30 15.85±0.09	3.37±0.30 0.30±0.80 9.38±0.10 9.83±0.10	44.21±0.30 30.16±0.30 26.25±0.31 24.12±0.34	0.27±0.60 0.22±0.40 0.56±0.60 0.48±0.80	0.00±0.00 0.00±0.00 0.19±0.50 0.48±0.60	0.00 0.00 0.00 0.00
Cohort Three	**Group 6**	**0M** **A** **B** **C**	34±0.10 34±0.10 34±0.10 34±0.10 34±0.10	11±0.07 11±0.07 11±0.07 11±0.07 11±0.07	5.31±0.01 5.31±0.01 5.31±0.01 5.31±0.01 5.31±0.01	15.85±0.09 15.85±0.09 15.85±0.09 15.85±0.09 15.85±0.09	9.83±0.10 9.83±0.10 9.83±0.10 9.83±0.10 9.83±0.10	44.12±0.41 24.12±0.30 21.12±0.20 24.13±0.30	0.5±0.27 0.80±0.31 0.45±0.23 0.25±0.31	0.4±0.44 0.75±0.34 0.44±0.31 0.50±0.35	0.00 0.00 0.00 0.00
	Ref. Values		**30-47**	**10-16**	**3.8-6.7**	**3.6-14.0**	**13-61**	**55-86**	**0.0-4.0**	**0.0-8.0**	**0.0-2.0**

KEY: PCV- Packed cell volume, **Hb-** Haemoglobin concentration, **RBC-** Red blood cells, **WBC-** White blood cells, **N-** Neutrophils, **L-** Lymphocytes, **M-** Monocytes, **E-** Eosinophils, * Statistically significant. **A-** infected+treated, **B-** treated later infected, **C-** Infected never treated, **OM**: at start before any infection is done Reference (Delwata *et al.*, 2018)

Table 5: Haemobiochemical Indices in Models after intraperitonel infection With *T. congolense* and Treatment with Binary of Pyrimethamine-Sulphadoxine and Artemether Combination Separately

			PCV (%)	**Hb (g/dl)**	**RBC (10^6/mm^3)**	**WBC ($10^3\times10^3$/mm^3)**	**N ($\times10^3$/mm^3)**	**L($\times10^3$/mm^3)**	**M ($\times10^3$/mm^3)**	**E ($\times10^3$/mm^3)**	**B ($\times10^3$/mm^3)**
PYRIMETHAMINE	**Group 1**	**0M** **A**	40±0.27	16±0.15	6.34±0.17	45.91±0.03	21.39±0.22	54.31±0.43	0.32±0.31	4.31±0.32	0.00 0.00

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Cohort One		**B** **C**	27±0.19 20±0.06	10±0.17 04±0.23*	4.16±0.13 3.12±0.23	31.41±0.23 21.10±0.02	17.13±0.34 18.30±0.18	98.32±0.13 77.21±0.30	0.23±0.33 3.13±0.17	2.45±0.01 1.27±0.12	0.00 0.00
			22±0.023	12±0.27	3.20±0.37	44.32±0.22	53.00±0.27	52.35±0.27	2.01±0.13	2.31±0.12	
Cohort Two	**Group 3**	**0M** **A** **B** **C**	43±1.40 22±1.24 19±0.05 28±0.10	14±.1.00 11±0.15 10±0.51 12±1.10	4.59±0.30 2.11±0.16 2.23±0.03 3.31±0.21	24.30±0.17 21.80±0.13 17.50±0.21 17.23±0.06	24.36±0.30 16.05±1.07 11.21±0.40 27.21±0.34	61.34±0.33 88.3±0.42 97.17±0.38 55.14±0.42	2.34±0.12 1.21±0.15 1.23±0.31 0.21±0.14	4.77±0.12 1.21±0.21 1.22±0.22 1.02±0.11	0.00 0.00 0.00 0.00
Cohort Three	**Group 5**	**0M** **A** **B** **C**	39±0.53 24±0.31 21±1.04* 25±0.52	13±0.10 11±0.01 09±0.73 13±0.28	5.79±0.10 2.12±0.12 2.36±0.20 3.16±0.42	20.42±0.01 10.10±0.11* 17.40±0.71 23.23±0.16	25.67±0.36 13.56±0.10 11.26±0.17* 13.31±0.27	51.46±0.11 31.5±0.32 21.4±0.35 33.23±0.21	0.25±0.23 0.35±0.36 0.35±0.32 0.38±0.19	3.00±0.21 1.35±0.01 0.23±0.31 2.32±0.07	0.00 0.00 0.00 0.00
ARTEMETHER Cohort One	**Group 2**	**0M** **A** **B** **C**	37±0.13 29±0.31 31±0.02 32±0.17	13±0.16 11±0.13 09±0.40 11±0.24	5.13±0.18 4.12±0.32 5.23±0.17 4.32±0.10	53.25±0.31 27.93±0.10 27.87±0.12 11.22±0.11	11.31±0.20 19.34±0.11 14.21±0.02 11.01±0.31	25.05±0.21 23.21±0.12 21.24±0.02 23.53±0.23	0.11±0.04 0.49±0.01 0.57±0.14 0.49±0.27	0.21±0.01 0.00±0.00 0.00±0.00 0.00±0.00	0.00 0.00 0.00 0.00
Cohort One **Cohort Three**	**Group 4** **Group 6**	**0M** **A** **B** **C** **0M** **A** **B**	41±0.22 33±0.06 36±0.01 36±0.11 44±0.17	18±0.16 14±0.04 11±0.21 11±0.07 12±0.21	4.30±0.21 4.17±0.23 4.29±0.04 4.24±0.31 4.21±0.22	25.73±0.23 47.20±0.11 18.75±0.17 22.85±0.06 25.45±0.09	13.33±0.01 0.30±0.06 16.38±0.01 11.21±0.21 12.31±0.12	39.12±0.20 34.11±0.13 28.26±0.03 24.12±0.14 44.12±0.01	0.45±0.03 0.36±0.12 0.68±0.06 0.73±0.18 0.5±0.27 0.80±0.31	0.00±0.00 0.00±0.00 0.21±0.50 0.00±0.00 0.4±0.44 0.75±0.34	0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00

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		C	32±0.12 31±0.11 36±0.17	11±0.03 12±0.01 10±0.11	6.23±0.31 4.60±0.23 4.71±0.02	25.38±0.19 15.94±0.30 23.85±0.01	12.91±0.13 9.12±0.13 10.58±0.51	24.12±0.12 21.12±0.16 24.13±0.30	0.45±0.23 0.25±0.31	0.44±0.31 0.50±0.35	
	Ref. Values		**30-47**	**10-16**	**3.8-6.7**	**3.6-14.0**	**13-61**	**55-86**	**0.0-4.0**	**0.0-8.0**	**0.0-2.0**

KEY: PCV- Packed cell volume, **Hb**- Haemoglobin concentration, **RBC**- Red blood cells, **WBC**- White blood cells, **N**- Neutrophils, **L**- Lymphocytes, **M**- Monocytes, **E**- Eosinophils, **B**- Basophils, * Statistically significant. **A**- infected + treated, **B**- treated later infected, **C**- Infected never treated, **OM**: at start before any infection is done; Reference normal values (Delwata *et al.*, 2018)

Table 6: Haemobiochemical Indices in Models after intraperitonel infection With *T. vivax* and Treatment with Binary of Pyrimethamine-Sulphadoxine and Artemether Separately

			PCV (%)	**Hb (g/dl)**	**RBC ($(10^6/mm^3)$)**	**WBC ($10^3\times10^3/mm^3$)**	**N ($\times10^3/mm^3$)**	**L ($\times10^3/mm^3$)**	**M ($\times10^3/mm^3$)**	**E ($\times10^3/mm^3$)**	**B ($\times10^3/mm^3$)**
PYRIMETHAMINE **Cohort One**	**Group 1**	**0M** **A** **B** **C**	34±0.20 30±0.30 28±0.06 31±0.08	12±0.80 10±0.20 9±0.03* 10±0.70	5.34±0.20 4.51±0.40 3.18±0.20* 4.89±0.10	10.50±0.20 15.95±0.80 60.78±0.40 10.85±0.30	7.98±0.07 13.24±0.40 46.80±0.06 4.12±0.08	1.89±0.80 1.60±0.30 2.43±0.10 4.56±0.50	0.32±0.06 0.48±0.40 4.25±0.20 1.19±0.07	0.32±0.30 0.00±0.00 0.00±0.00 0.11±0.40	0.00 0.00 0.00 0.00
Cohort Two	**Group 3**	**0M** **A** **B** **C**	37±0.06 22±0.23 33±0.07 33±0.10	12±.20 7±0.05* 11±0.06 11±0.20	4.49±0.40 5.08±0.70 2.82±0.20 4.29±0.10	20.10±0.20 21.90±0.70 13.10±0.40 9.23±0.80	12.46±0.30 17.08±0.60 8.65±0.30 3.88±0.20	4.62±0.30 3.94±0.40 4.06±0.40 4.98±0.50	1.41±0.30 0.22±0.70 0.13±0.25 0.37±0.30	0.60±0.60 0.00±0.00 0.13±0.80 0.00±0.00	0.00 0.00 0.00 0.00
Cohort Three	**Group 5**	**0M** **A** **B** **C**	39±0.55 24±0.40 23±1.06 25±0.06	13±0.10 8±0.06 8±0.70 8±0.20	5.93±0.30 3.79±0.20 3.36±0.30 4.18±0.30	21.90±0.20 17.60±0.60 22.85±0.50 7.25±0.40	18.40±0.20 11.62±0.10 17.14±0.20 4.71±0.10	0.44±0.20 4.75±0.40 4.57±0.70 1.81±0.30	0.88±0.70 0.88±0.30 0.69±0.20 0.36±0.60	0.00±0.00 0.00±0.00 0.23±0.20 0.00±0.05	0.00 0.00 0.00 0.00

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ARTEMETHER Cohort One	**Group 2**	**0M** **A** **B** **C**	33±0.07 30±0.24 30±0.06 28±0.08	11±0.10 10±0.10 10±0.40 9±0.60	4.65±0.80 4.18±0.30 4.73±0.10 4.70±0.20	13.85±0.01 27.93±0.30 12.50±0.06 2.95±0.30	9.97±0.20 20.95±0.10 7.75±0.40 1.06±0.30	3.05±0.50 4.47±0.20 1.25±0.40 1.53±0.60	0.14±0.40 0.84±0.05 0.75±0.70 0.18±0.30	0.14±0.50 0.00±0.00 0.00±0.00 0.00±0.00	0.00 0.00 0.00 0.00
Cohort One **Cohort Three**	**Group 4** **Group 6**	**0M** **A** **B** **C** **0M** **A** **B** **C**	38±0.21 34±0.05 35±0.05 34±0.10 32±0.11 36±0.14 31±0.12 37±0.17	13±0.01 11±0.03 12±0.10 11±0.07 13±0.16 11±0.11 12±0.03 13±0.13	5.30±0.10 5.14±0.40 4.29±0.05 5.31±0.01 6.31±0.01 4.31±0.31 3.20±0.31 3.61±0.02	8.88±0.10 1.30±0.40 18.75±0.30 15.85±0.09 17.15±0.24 14.43±0.01 10.13±0.13 12.85±0.03	3.37±0.30 0.30±0.80 9.38±0.10 9.83±0.10 11.41±0.11 10.41±0.17 8.53±0.13 9.72±0.13	4.88±0.30 0.78±0.30 6.75±0.20 4.12±0.70 52.12±0.70 41.20±0.20 40.32±0.17 32.14±0.40	0.27±0.60 0.22±0.40 0.56±0.60 0.48±0.80 0.21±0.20 0.51±0.13 0.42±0.10 0.34±0.24	0.00±0.00 0.00±0.00 0.19±0.50 0.48±0.60 0.61±0.40 0.60±0.26 0.53±0.27 0.46±0.23	0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00
	Ref. Values		**30-47**	**10-16**	**3.8-6.7**	**3.6-14.0**	**13-61**	**55-86**	**0.0-1.0**	**0.0-8.0**	**0.0-2.0**

KEY: PCV- Packed cell volume, **Hb**- Haemoglobin concentration, **RBC**- Red blood cells, **WBC**- White blood cells, **N**- Neutrophils, **L**- Lymphocytes, **M**- Monocytes, **E**- Eosinophils, **B**- Basophils, **Ba**- Band cells, * Statistically significant. Reference (Delwata *et al.,* 2018

Table 7: Hepatobiochemical Indices in Models after intraperitonel infection With Triad of *T. brucei brucei, T. congolense, T. vivax* and Treatment with Binary of Pyrimethamine-Sulphadoxine and Artemether Separately

			Scr (mg/dL)	**BUN (mg/dL)**	**BL ((mg/dL)**	**AST (UL)**	**ALT (UL)**	**TCH (mg/dL)**	**HDL (mg/dL)**	**ALP (UL)**	**TP (g/dL)**	**GL (mg/dL)**
PYRIMETHAMINE *T. brucei brucei*	**PyrX+SulX**	**Group1**	2.9±0.10	22.1±0.10	1.3±0.12	*450.0±0.10	108.0±0.02	43.03±0.08	0.32±0.06	0.32±0.30	4.70±0.30	40.1±0.2*
PYRIMETHAMINE *T. Congolense*	**PyrX+SulX**	**Group3**	3.2±0.13	19.5±0.17	1.2±0.10	510.1±0.07	72.3±0.12	74.24±0.16	1.41±0.30	0.60±0.60	3.90±0.12	22.01±0.18
PYRIMETHAMINE	**PyrX+SulX**	**Group5**	3.6±0.23*	21.0±0.13	1.7±0.11	413±0.12	90.23±0.13	66.16±0.10	0.22±0.70	0.00±0.00	2.31±0.11	20.66±0.13

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T. vivax												
ARTEMETHER *T. brucei bruce*	**ARTX**	**Group2**	1.2±0.01	11±0.03	0.82±0.02	94.90±0.17	47.01±0.30	18±0.10	33±0.10	33±0.10	3.30±0.02	71.00±0.12
ARTEMETHER *T. congolense*	**ARTX** **ARTX**	**Group4**	1.5±0.31 0.9±0.15	09±0.11 19±0.05	1.12±0.10 0.90±0.14	69.13±0.80 79±0.14	32.11±0.20 29±0.21	19.2±0.12 18.4±0.01	35±0.15 31±0.07	42±0.23 31±0.15	3.9±0.13 4.30±0.17	96.03±0.04 90.12±0.15
ARTEMETHER *T. Vivax*	**Ref. Values**	**Group6**	**0-2**	**17.5-45.5**	**0-0.7**	**30-130**	**40-80**	**20-87**	**10.0-42.5**	**12.0-96.0**	**5.4-7.5**	**75.0-160**

KEY: Scr-serum creatinine, **BUN-** Blood Urea Nitrogen, **BL-** Billirubin, **AST-** Aspartate aminotransferase, **ALT-** Alanine aminotransferase, **TCH-** Total cholesterol, **HDL-** High density cholesterol, **ALP-** Alkaline phosphatase, **TP-** Total protein, **Ba-** Glucose, * Statistically significant. Reference normal ranges: (MSD; Delwata *et al.,* 2018)

DISCUSSION

Lack of available reference values and deviations secondary to diseases in research settings that is resource limited can be a significant drawback for scientists and clinicians (Delwata *et al.,* 2018). This has impacted on quality of care provided in the various point of care (POC) for both veterinary and human patients in pertinent areas, it has also adversely contributed to poor overall patient outcome. Trypanosomosis has a wide spectrum of transmission with lack of readily available chemotherapy options within research area (personal experience). Trypanosomosis is endemic within the area of study with fulminant cases presenting in both humans and animals. The vegetation in the Sahel belt few kilometers from the research area, the scarcity of mainstream trypanosides, and preponderance of cases due to multiplication of the vectors provide fertile ecosystem for potential epidemic. It is imperative researchers in life sciences think of innovative alternatives of chemotherapy to mainstream drugs continuously been restricted for importation. The hope of vaccination against trypanosomosis remains distant as no known large-scale production policy and study is known in national and international partner platforms, Tesfalem (2017) reports that the hope of getting a vaccination for trypanosomosis in animals in the near future remains bleak. Chemotherapy remains the effective means of treatment and control of the disease in both humans and animals (Giordani *et al.,* 2016).

Antimalarials are numerous with several options with recent advances in combination therapies. Two commonly used and very affordable as well as available options is the Artemisinin combination therapy (ACT) with artemether being the most commonly used and sulpha-based agents with pyrimethamine and sulphadoxine being the commonest example. These drugs have documented promise of having efficacy on *Trypanosoma* species (Nok, 2005). Their mechanism of action includes free radical production in the parasites food vacuole (Eckstein-Ludwig *et al.,* 2003) and inhibition of parasitic calcium ATPase (Omotosho *et al.,* 2014). Pyrimethamine acts by interfering with folic acid synthesis pathway in protozoan parasites. It specifically targets the enzyme dihydrofolate reductase (DHFR), which is essential in folic acid metabolism pathway. By inhibiting DHFR, pyrimethamine effectively stops the conversion of dihydrofolate to tetrahydrofolate, which leads to depletion of folate derivatives in the parasite (Gatton *et al.,* 2004). This combination, known as sulfadoxine-pyrimethamine (SP), provides a synergistic effect that enhances the overall efficacy of the treatment and delays the development of resistance. (Gatton *et al.,* 2004)

A number of modeled studies provided proof of concept on the use of antimalarials for therapeutics on animal trypanosomosis and, clinicians in several point of care have leveraged this finding in prescription and prophylaxis in different veterinary settings. Studies have shown that despite the indispensable value of

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

antimalarials as viable alternative to mainstream trypanosides, the drugs possess hemato-renal as well as hepatic toxicities worthy of studies (Omotosho *et al.,* 2014). At the moment, there's paucity of research-borne data on side effects of these alternative trypanosides in models or from clinical documented observations, reports are anecdotal within the area of study and in general literature. The design of the experiment featuring three replicates (A, B, C corresponding to Infected and later treated; treated prophylactically then later infected and infected but never treated respectively) covers the hemato-biochemical changes both in prophylactic as well as curative instances of patient management.

First, the antimalarials have proven efficacy with quantified signified parasite suppression (data on this aspect considered in another publishing platform). The various species of Trypanosome caused various alterations in hematology at standard inoculum in models as shown in Table 6. This is consistent with the reports of Omotosho *et al.* (2014) and Jolayemi *et al.* (2021) who reported consistent decrease in the Red Blood Cell count (RBC), Packed Cell Volume (PCV) and hemoglobin concentration with antimalarial use. This decrease is borne from intravascular haemolysis, impaired hematopoiesis due to bone marrow depression and impaired folic acid synthesis (Mbajiorgu *et al.,* 2007). The clinical implication of continuously decreasing RBC and Hb is anemia. Jolayemi *et al.* (2021) documented the binary relationship between parasitaemia and anemia with an external complicating factor being the antimalarial in use, clinicians and diagnosticians in life sciences should expect this dynamic to play out in patient management, modeled study and laboratory diagnosis. The hemato-suppression is dose dependent and tolerable from general literature consensus. However, this study did not investigate progressive hemo-biochemical changes proportional to varying doses of the different classes of antimalarials used nor considered varying inoculum of the pathogen. The study focused on fixed previously reported and generally used dose of the drugs. There was non-random consistent depression in the PCV, RBC and Hb in most groups especially the group treated with Pyrimethamine and sulphadoxine with statistically significant ($P<0.05$) changes as reported by Nok, (2005). There was also marked but non-statistically significant ($P>0.05$) leukopenia associated with neutropenia (Table 4, 5 and 6) especially in the pyrimethamine-sulphadoxine treated groups as reported by Omotosho *et al.* (2014), suggesting immunosuppression with continuous use. Clinicians should therefore be wary of indiscriminate use and abuse especially in subjects with underlining medical conditions. While this decrease was unsurprising, the tolerance by models was informative pointing to the possibility of safe drug use in patients with anticipated debilities from these deviations. This finding will be valuable to clinicians and covers doubts and fears of use as well as leveraged to provide improved overall patient outcome. It is important to note, as shown in Table 5, the non-random exclusion of artemether compared to sulphadoxine-pyrimethamine combination in causing consistent depression of RBC, PCV and Hb values. The forecast from this result is the non-likely eventual development of anemia when artemether is used compared to sulphadoxine combination. Clinicians therefore, have the leeway, based on empirical data to choose from range of antimalarial for the management and prophylaxis of cases of trypanosomiasis presenting.

There was marked elevation of various hepato-biochemical markers expressing varying degrees of cyto-toxicities with continuous use of the drugs although generally tolerant in models. There was a statistically significant ($P<0.05$) increase in Serum creatinine (Scr) and total glucose (tGL) with the use of the pyrimethamine-sulphadoxine combination unlike the artemether group (Table 7). This is in tandem with report of Omotosho *et al.* (2014), Jolayemi *et al.* (2021) and Nok (2005) which attributed this to renal damage from generally tolerable and reversible epithelial damages upon discontinuation of therapy. Medications with pyrimethamine-sulphadoxine for prophylactic or curative clinical use will depress appetite in the patient. Anorexia of 48 to 72 hours course is likely to lower blood glucose beyond which gluconeogenesis pathways become activated to synthesize glucose with attendant emaciation as seen in clinical cases of the disease. Kennedy (2013) and Beverley (2016) all reported significant weight loss caused by activity of the *Trypanosoma* and depression caused by the chemotherapy in the initial stages, their studies showed models gaining weight after suppression of parasitaemia and discontinuation of therapy generally within one week to 10days. The low glucose generally restores with discontinuation of therapy and patient feeding naturally. There was a non-random non-statistically significant ($P>0.05$) increase of blood urea nitrogen (BUN), bilirubin (BL), and alanine aminotransaminase (ALT) (Table 7). These deviations is related to hepatic as well as skeletal muscle damages seen more in the pyrimethamine-sulphadoxine group unlike the artemether group emphasizing choice of drug amongst chemotherapy options. Similarly, there was a statistically significant ($P<0.05$) elevation of aspartate aminotransferase (AST) in the sulphadoxine group which was not seen in the artemether group. This is attributed

to damages in hepatocytes well documented in the modeled study of Ekam *et al.* (2019). Vasudevan and Skrecumari (2007) also emphasized that increases in serum AST, ALT and ALP is associated with necrotic damages to hepatic musculature and can be seen in other diseases and toxicities like liver cirrhosis, exposure to toxic substances such as prolonged antimalarial therapy, heavy metals toxicities or even chronic alcoholism. Use of sulphadoxine based antimalarial causes epithelial and hepatocytes damages, concurrent use of this medication in patients with hepatic conditions or prolonged use for prophylaxis or curative purpose should be done cautiously in tandem with the earlier studies of Ben-Harari *et al.* (2017) who reported that high doses or prolonged use of pyrimethamine can inhibit DHFR leading to potential side effects such as bone marrow suppression and folate deficiency (Ben-Harari *et al.*, 2017). Liver function test (LFT) should precede prolonged use of this class of antimalarials or should guide clinicians and diagnosticians in post therapy toxicities and complications

CONCLUSION

There was marked but tolerable statistically significant ($P<0.05$) depression of the PCV, total RBC, Hb, total WBC and neutrophils mostly in the groups in which trypanosomosis was treated with pyrimethamine and sulphadoxine combination but sparing the artemether group, this is independent of what species of trypanosome used for the experimental infection. Combination of Pyrimethamine-sulphadoxine used as trypanoside produced various hemodynamic as well as hepato-biochemical changes, which was generally tolerated by models used.

There was a non-random statistically significant ($P<0.05$) increase in serum creatinine (Scr) in the group treated with pyrimethamine-sulphadoxine which was not seen in the group treated with artemether. Similarly, There was a non-random non-statistically significant ($P>0.05$) increase of blood urea nitrogen (BUN), bilirubin (BL), and alanine amino-transaminase (ALT) in the pyrimethamine-sulphadoxine group but less the artemether group.

This study provides data that can be leveraged upon to improve patient care and treatment values. The data will guide clinicians on anticipated hemodynamic as well as hepatic activities on predictive platform in the management of animal and human trypanosomosis using affordable, available and efficacious group of antimalarials as viable alternatives to mainstream trypanosides.

Limitation: inability to use graduated doses to see variability of efficacy in a broad rage of doses, absence of in-depth toxicity studies using histopathology to comprehensively ascertain drug safety and residues. This limitations is partly imposed by funding complications

ACKNOWLEDGEMENT

The authors acknowledge the funding of this research by the Institutional Based Research Grant (IBR) Batch 8, of the Usmanu Danfodyo University Sokoto, Nigeria with ID: TETFUND/UDUS/COMB/2021 BATCH D

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