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Evaluation of Antitrypanosomal Activity of Tetracycline in Animal Model

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***Abstract*-** Trypanosomiasis remains a disease with unsatisfactory medical control. To date, its chemotherapy relies on suramin, pentamidine, melarsoprol and eflornithine. Each of these drugs is expensive, laborious to administer, lacks efficacy against some trypanosomes species and lead to some significant side effects. This study aimed to evaluate the Antitrypanosomal activity of Tetracycline in animal model. A total of 12 adult rats, 7 weeks old of both sexes were randomly divided into six groups (1, 2,3,4,5 and 6) of 2 animals each. All the Groups (1-6) were intraperitoneally infected with 0.1 ml of blood containing the parasites. Groups 1-4 received the tetracycline intraperitoneally at daily doses of 0.01, 0.1, 1.0 and 10mg/ml/kg body weight respectively, while Group 5 received standard Trypanosomal drug of 7.86mg/kg (suramin dose) which served as the Positive control and Group 6 was infected without any treatment (Negative control). Physiological, Physical and Behavioral Changes as well as Hematological parameters were all evaluated. There was decreased in the level of parasitaemia, decrease from higher to normal body temperature, increases in body weight and survival rate, as well as low pallor of the mucus membrane, loss of condition, pyrexia, lacrimation and aggression with high food consumption in all the tetracycline treated groups. More so, a significant decrease in mean PCV, Hgb, RBC, MCV, MCH and MCHC in all the treated groups often comparing with suramin treated group P-value <0.05. It was concluded that, Tetracycline can be considered as potent Antitrypanosomal agent and can be used in the treatment of trypanosomiasis.

***Keywords*-** eflornithine, melarsoprol, parasitaemia, pentamidine, suramin

I. INTRODUCTION

Trypanosomiasis is a neglected tropical disease and a major threat to public health concerns affecting human and animals, which can easily lead to their death [1]. The disease is caused by several species of blood and tissue-dwelling protozoan parasites called trypanosomes which are transmitted by obligatory blood suckers flies called tsetse flies [2]. After infection through the bite of a tsetse fly, the parasite proliferates rapidly by binary fission where it invade lymph and blood vessels of the host and then progressively disseminate to the bone marrow and tissue fluids and finally to the central nervous system causing fever, anemia, loss of condition, reduced productivity and higher mortality [3].

The parasites include *Trypanosoma vivax*, *T. congolense*, and *T. brucei* [1]. The host preferences of each trypanosome species may differ, but *T. vivax*, *T congolense* and *T. brucei* have a wide host range among domesticated animals, Similarly, some species of trypanosomes like *T. simiae*, *T. godfreyi* and *T. suis* occur in pigs, but *T. simiae* is the most important trypanosome in pigs and has also been detected using PCR in camels and horses [4]. Amongst these parasites, the sub-species *T. b. brucei* is reported to be the most virulent trypanosome, this is due to the presence of a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of specific antibodies, thus enabling the parasites to evade the consequences of the host immune reactions by switching the VSG, a phenomenon known as antigenic variation causing severe, and fatal disease in a specific host [5].

Tetracycline is a protein synthesis inhibiter and broad spectrum an antimicrobial drug with iron- chelating property and ability to penetrate the blood brain barrier easily [6]. It exists in different forms, such as chloro tetracyclines, doxycyclines, oxytetracyclines and mono cyclines where they binds with 30S RNA and inhibits proteins synthesis. They also form reversible bond with 30S ribosomal sub units (16S) and inhibit the binding of the newly synthesized amino acids to the growing peptide chains. Therefore, Methylation of 16S ribosomal subs units and alteration of the enzymes binding sites usually lead to the resistance of tetracycline [7]. The objectives of this study were to evaluate the Antitrypanosomal activity of Tetracycline through physiological, physical and behavioral changes in rats infected with *Trypanosoma brucie brucie* and also to evaluate their Hematological indices.

II. RELATED WORK

Tetracycline has been found in previous experiments to be effective in the treatment of pneumonia, Respiratory Tract Infections, eye and skin infection, intestinal and Urinary Tract Infections as well as other parasitic infections that are spread by ticks, lice and mites, but there is scarcity literature on its activity in the treatment of Trypanosomiasis [6]. Tetracycline behaves as typical and atypical antibiotics [8]. The typical tetracycline such as tetracycline, doxycycline, minocycline and chlortetracycline are bacteriostatic antibiotics which inhibit the synthesis of protein by binding with ribosomal subunits. Other tetracycline has a typical mechanism which works as bactericidal antibiotics and are very toxic for both prokaryotes and eukaryotes. Atypical tetracycline disrupts cell membrane; inhibit all cellular processes and macromolecules synthesis pathways. Furthermore both typical and atypical tetracycline has pharmacological effects against eukaryotic and prokaryotic cells [9].

Trypanosomiasis has been a major threat to Human and animal health care system and has contributed negatively to public health in Nigeria [1]. It is chronic upon both humans and susceptible mammals leading to fatalities and high economic loses [21]. Accordingly, Billions of dollars are spending each year towards a continuous attempt in effective control and eradication of the menace [1]. However, despite the recent advances in drug research, finding a safe, effective, and easy to use chemotherapy of trypanosomiasis remains a challenging task [10]. The four current anti-trypanosomal drugs (Suramin, Pentamidine, Melarsoprol and Eflornithine) have major disadvantages that limit more widespread use of these drugs in the endemic regions of sub-Saharan Africa [11]. Each of these drugs is expensive, laborious to administer, lacks efficacy against some trypanosomes species and lead to some significant side effects such as toxic effects towards liver and kidney, loss of consciousness during the first administrations, Heavy albuminuria, stomal ulceration, exfoliative dermatitis, severe diarrhea, prolonged high fever and prostration , tiredness, anorexia, malaise and polyuria [10]. Therefore, these problems and other factors increase the need towards a continuous search for safer, potent and affordable drugs to actualize effective control and eradication of the disease.

III. METHODOLOGY

Ethical Approval

All experimental protocols were conducted with strict adherence to guidelines established by the ethical committee for the use of animals, of Nigerian Institute for Trypanosomiasis Research, Kaduna (Ref no: 21.01.13. 1231).

Drugs Collection and Preparation

Tetracycline Hydrochloride (3245 Bach Number) and Suramin Sodiom Salts (3456 Batch Number) both from Shijiazhuang Fengqiang Pharmaceutical, Xinzhaidian Industry Park, Hebei-China, were obtained and used for this study. The drugs were used and prepared according to manufacturer's instruction respectively.

Experimental Animals and Parasite

A total of 12 adult rats of approximately 7 weeks old weighing between 140-200g each, were used in this experiment. They were obtained from the Animal House, Department of Human Anatomy, Bayero University Kano. They were allowed to acclimatize for 14 days in research laboratory, where the experiment was conducted. The rats were housed under standard hygienic conditions in plastic cages, fed commercial feed (Vital feeds LTD, Kano, Nigeria) and given access to clean water ad libitum which was maintained accordingly by the animal curator.

Trypanosoma brucei brucei (Federi strain) was used for this research, it was obtained from a liquid Nitrogen, at Nigerian Institute for Trypanosomiasis and Onchocerciasis Research, Vom, Plateau State, Nigeria. The parasite was first isolated from cattle in 2018 which was identified as *T.b. brucei* and stabilized by four passages in rats before storage in liquid nitrogen. It was confirmed and characterized by the standard trypanosome detection methods of Woo [12], and their modifications [17]. Which include wet blood films, thick and thin blood smears, as well as buffy coat method. The parasite was maintained by serial passages in donor rats, then, the infected blood from the donor rat was collected at peak parasitemia by tail bleeding, and diluted with physiological saline, which was inoculated into the peritoneal cavity of the experimental rats.

Experimental Design

The 12 experimental rats were randomly grouped into six groups (1, 2,3,4,5 and 6) of 2 animals each. All the Groups (1-6) were intraperitoneally infected with 0.1 ml of blood containing approximately 10^6 Trypanosomes/ml. Treatment began the day parasites were first detected by microscopy in the blood stream (day 4), up to (day 14) where the entire negative control died. Groups 1-4 received the tetracycline intraperitoneally at daily doses of 0.01, 0.1, 1.0 and 10mg/ml/kg body weight respectively, while Group 5 received standard Trypanosomal drug of 7.86mg/kg(suramin dose) which served as the Positive control and Group 6 was infected without any treatment (Negative control).

Monitoring of Pharmacological activity in vivo in terms of Parasitaemia, Temperature, Weight and Percentage of Survival

Parasitaemia was observed daily using the rapid matching method of Herbert and Lumsden [13], by preparing a wet mount from the peripheral blood by means of tail-bleeding, in which a drop of blood was placed on a clean glass slide and covered with cover slip. Each slide was prepared separately and observed. The number of parasites was determined microscopically by counting the parasite in each field and match with standard chart [13]. Body Temperature of all the experimental animals was

measured daily, each rat was gently caught and a digital thermometer was inserted 3cm into the anus of each rat, and at the sound of a beep, the thermometer was immediately withdrawn and values obtained was recorded [14]. However, Daily body weight was also determined using a digital weighing balance [7], while percentage of survival was monitored daily and expressed as number of survivor divided by total initial number in the group multiplied by 100% [7].

Monitoring of Physical and Behavioral Changes

Physical and Behavioral changes such as condition of the mucous membranes, loss of condition, Pyrexia, Lacrimation, Food consumptions, Eye redness, Aggression among others, were observed and recorded throughout the experiments [7].

Determination of Hematological Indices

Hematological Indices such as Packed Cell Volume (PCV), Red Blood Cells (RBC), Hemoglobin Concentration (Hbg), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentrations (MCHC) were all determined using automatic hematological blood analyzer [15].

Data Analysis

The results were expressed in mean with standard error. And then analyzed using statistical software: SPSS Version 20, one way ANOVA test at 0.05 degree of freedom.

IV. RESULTS AND DISCUSSION

The Mean Parasitaemia of the experimental animals infected with *Trypanosoma brucie brucie* is presented in Figure 1. There is relatively increased in parasitaemia for group 6 (Negative control), with a very less Parasitaemia in group 1 (0.01mg/kg of Tetracycline), while no and complete disappearance of Parasitaemia was observed in groups 2 (0.1mg/kg Tetracycline), 3 (1mg/kg Tetracycline), 4 (10 mg/kg Tetracycline) and 5 (Positive control) respectively.

The Mean Temperature of the experimental animals is presented in Figure 2. There is stationary increases in The Mean temperature in group 6 (negative control), while groups 1 (0.01mg/kg), 2 (0.1mg/kg), 3 (1mg/kg), 4 (10 mg/kg) and 5 (Positive control) maintained a standard Body Temperature throughout the experiments.

Figure 3 shows the mean Body Weight of the experimental animals. There is slightly increase in mean body weight in Groups 1 (0.01mg/kg), 2 (0.1mg/kg), 3 (1mg/kg), 4 (10 mg/kg) and 5 (Positive control), while a clear trend of decreasing in mean body weight was seen in Group 6 (negative control) respectively.

The percentage survival of the experimental animals infected with *Trypanosoma brucie brucie* is presented in Figure 4. There was 100% Survival throughout the experiments in Groups 1 (0.01mg/kg), 2 (0.1mg/kg), 3 (1mg/kg), 4 (10 mg/kg) and 5 (Positive control) while 0 % Survival was observed and recorded in Group 6 (negative control).

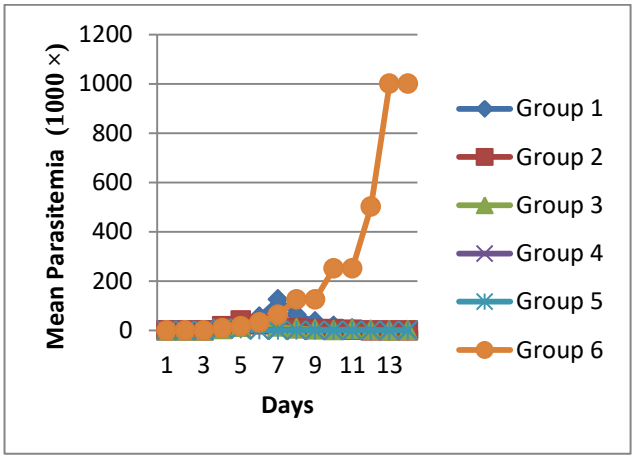

Figure 1: Mean Trypanosome Parasitaemia of the experimental animals in respect to each group within 14 days period

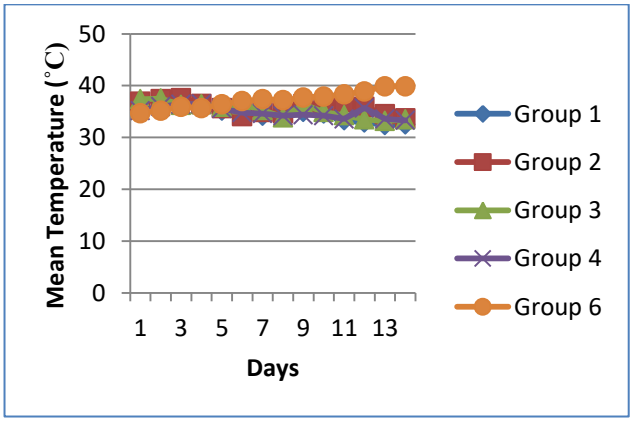

Figure 2: Mean Temperature of the experimental animals in respect to each group within 14 days period

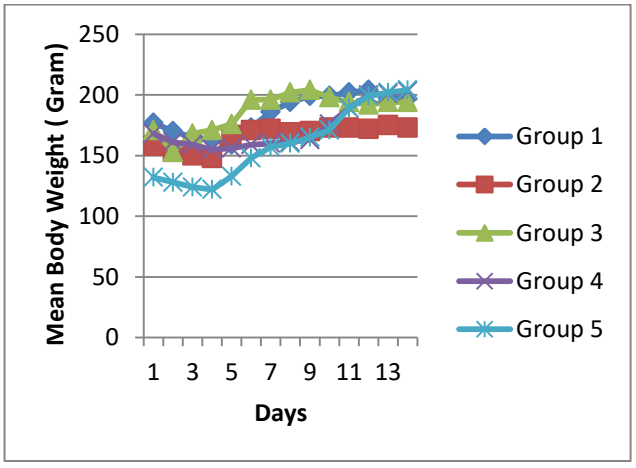

Figure 3: Mean Body Weight of the experimental animals in respect to each group within 14 days period

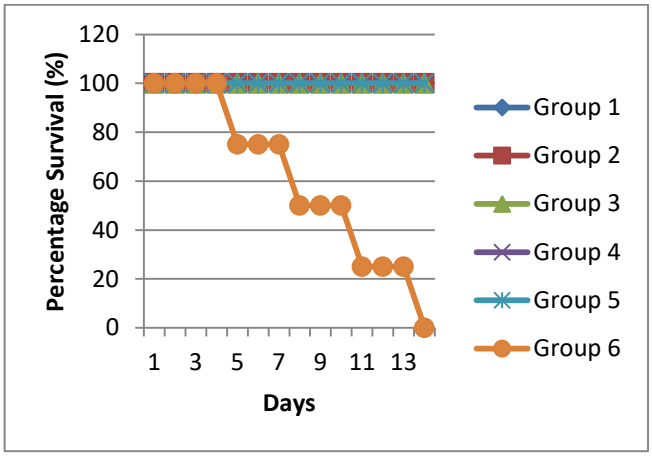

Figure 4: Percentage Survival of the Experimental Animals in respect to each group within 14 days period

Physical and Behavioral Changes of the experimental animals is presented in Table 1. There was low pallor of the mucus membrane, loss of condition, pyrexia, lacrimation and aggression with high food consumption in Groups 1 (0.01mg/kg), 2 (0.1mg/kg), 3 (1mg/kg), 4 (10 mg/kg) and 5 (Positive control), while opposite of these parameters were observed in Group 6 (negative control). However, Eye redness, Alopecia, Ringtail and Gangrene were found to be absent in all the experimental groups.

The Mean Standard Error Haematological indices of the experimental animals are presented in Table 2. There was significant decrease in mean PCV, Hgb, RBC, MCV, MCH and MCHC in groups 1 (0.01mg/kg), 2 (0.1mg/kg), 3 (1mg/kg), 4 (10 mg/kg) and 6 (negative control), when comparing with group 5 (Positive control) P-value <0.05.

Table 1: Physical and Behavioral Changes of Experimental Animals

Parameters	G1	G2	G3	G4	G5	G6
Pallor of Mucous membrane	+	-	-	-	-	+++
Loss of conditions	-	-	-	-	-	+++
Pyrexia	-	-	+	-	-	+++
Lacrimation	-	+	-	-	-	+++
Eye redness	+	-	-	+	+	+
Aggression	-	-	-	++	++	-
Alopecia	-	-	-	-	-	-
Ringtail	-	+	-	-	-	-
Gangrene	-	-	-	-	-	-
Food consumption	++	++	+++	+++	+++	+

Key: +++ =High, ++ = Moderate, + = Low, - = Absent

Table 2: Mean Standard Error Hematological indices of Experimental Animals

Parameters	G1	G2	G3	G4	G5	G6	P-value
PCV (%)	28.5±1.56	30.0±0.0	32.75±0.95	33.75±0.25	36.0±0.58	14.5±0.29	< 0.001
Hgb (g/dL)	9.65±0.59	12.9±2.44	11.05±0.29	11.15±0.05	12.15±0.14	4.7±0.06	< 0.001
RBC (10^6/µL)	4.73±0.11	4.85±0.05	5.98±0.1	5.18±0.03	5.4±0.14	2.85±0.03	< 0.001
MCV (fl)	60.2±1.89	62.89±0.38	64.5±0.69	63.72±0.38	65.45±0.38	50.8±0.46	< 0.001
MCH (pg)	20.38±0.75	21.48±0.18	21.75±0.15	21.25±0.05	22.05±0.03	16.5±0.06	< 0.001
MCHC (g/dL)	33.83±0.24	34.1±0.1	33.73±0.13	33.75±0.15	33.73±0.14	32.4±0.23	< 0.001

Means are significantly different when P-value <0.05.

Key: PCV=Parked Cell Volume, Hgb=Hemoglobin Concentration, RBC=Red blood cells counts, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration.

Discussion

The pathogenesis and clinical manifestation of Trypanosomiasis is always associated with; increase in parasitaemia, increases in body temperature, decrease in body weight and even death if there is no drug intervention [5]. Thus, any Antitrypanosomal agent is expected to show the reverse pattern of the case [16]. However, many researchers have been reporting similar pattern (Higher parasitaemia, increase in body temperature and decrease in body weight) when evaluating the Antitrypanosomal activity of a particular agent [3]. In this study, no and very less parasitaemia, decrease from higher to normal body temperature and increase in body weight were all observed and recorded in Group 1 (0.01mg/kg Tetracycline), group 2 (0.1mg/kg Tetracycline), group 3 (1mg/kg Tetracycline), group 4 (10 mg/kg Tetracycline) and group 5 (Positive control [Treated with Suramin]), while there reverse pattern were observed and recorded in Group 6 (negative control). Therefore, these results indicate the Antitrypanosomal activity of Tetracycline in rats infected with *Trypanosoma brucie brucie.* However, The Result of the Physical and Behavioral Changes corresponds with the report from [17], who reported that, Trypanosomiasis is characterized by loss of condition, pyrexia, lacrimation and pallor of the mucus membrane. Furthermore, it was reported that, anemia is a useful and common indication of Trypanosomiasis and its degree determines the severity of trypanosomal infections [4]. Accordingly, groups 1 (0.01mg/kg), 2 (0.1mg/kg), 3 (1mg/kg), 4 (10 mg/kg) 5 (Positive control [Treated with Suramin]) have no signs of anemia because all their red parameters (PCV, Hgb, RBC, MCV, MCH and MCHC) are within the normal range, while Group 6 (negative control) have red parameters bellow the normal range, as such, morbidity is higher in this group since no treatment was given to the animals in the group, therefore the continuous peroxidative damage to red blood cells and the movement of the parasite in the blood stream can be found in this group, resulting to obstruction to the blood cells and the removal of red blood cells by the expanding mononuclear phagocytic system leading to anemia which can be used as a useful indicator for Trypanosomiasis [4, 20]. Accordingly, The Antitrypanosomal activity of Tetracycline in this experiment might be attributed to its ability in penetrating the blood brain barrier and its probable role in inhibiting ribonucleotide reductase through iron chelation [18]. More so, the broad spectrum antimicrobial activity of tetracycline and its iron–chelating activity have been reported to contribute to its antimicrobial activity [7, 19], as such, the antitrypanosomal activity of tetracycline in this experiment might be related to this property. However, the excellent distribution of the drug in body fluids and tissues could also be a key reason towards its Antitrypanosomal activity [18], this is because tetracycline can penetrate to cerebrospinal and tissue fluid resulting to the gradual appearance of the drug in the spinal fluid, making it easy to react with the trypanosomes and act upon them [18, 19, 20].

V. CONCLUSION AND FUTURE SCOPE

This work evaluates the Antitrypanosomal activity of Tetracycline in rats infected with *Trypanoma brucei brucei,* findings from this research obtained through Physiological, Physical and Behavioral changes and other Hematological Parameters, indicated that Tetracycline is bioactive on *Trypanoma brucei brucei* in vivo with potency level up to 10mg/kg, hence, can be considered as a potent Antitrypanosomal agent. Moreover, Future research and more trials should be carried out using different species and sub-species of trypanosome and other Higher animals that are natural host of Trypanosome (such as Goats, Pigs, Horses and Cattle) before adopting the result of this finding for laboratory use. Additionally, further research is needed to elucidate the mechanisms by which genes mediate resistance to Trypanosomal drugs, so as to overcome the spread of drug resistance.

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REFERENCES

[1]. F. A. Rufa'i, A.I. Zakari, A. Umar, M. Shuaibu, A.A. Sani, " Clinical signs and pathogenesis of trypanosomal infections in human and animals, *''Asian Journal of Pharmaceutical Research and Development*, Vol. **9,** Issue. **3, pp**. **57 -61, 2021.**

[2]. N.A. Dyer, K.S. RavelSchoi, A.C. Darbym, "cryptic diversity within the major trypanosomiasis vector glossina,'' *Journal of Tropical Diseases,* Vol. **5,** Issue. **8,** pp. **126-138, 2011.**

[3]. A. Mbaya, H. Kumshe, C.O. Nwosu, " The Mechanims of Anaemia in Trypanosomosis : A Review,'' *Donald Silverberg Publication, **Nigeria** ,* pp. **26-34, 2000.**

[4]. A. K. Maigari, N.T. Dabo, "Prevalence and source distribution of *Trypanosoma evansi* in trade camels in Kano state,'' *Fudma Journal of Microbiology*. Vol. **1,** Issue. **1,** pp. **234-239, 2018.**

[5]. J. B. Balogun, Z. Abubakar, T.B. Ibrahim, S. U. Balogn, I.S. Sadiq, M. M. Dogara *et al.,* "In Vitro anti-Trypanosomal Potential of Methanol Root Extract of *Terminalia macroptera* in *Trypanosoma brucie brucie* infected Wister Rats,'' *IOSR Journal of pharmacy and Biological Sciences*, Vol. **12,** Issue. **1,** pp. **34- 39, 2017.**

[6]. D. Fucco, "Classification frame work and chemical biology of tetracycline,'' *Structure based drug antibiotics*, Vol. **3,** Issue. **1,** pp. **42- 49, 2012.**

[7]. D. Grenier, M. Hout, D. Mayrand," Iron-chelating activity of tetracyclines and its impact on the susceptibility of *Actinobacillus actinomycetemcomitants* to these antibiotics,'' *Antimicrobial agents and chemotheraphy,* Vol. **106,** Issue. **1,** pp. **763-766**, **2000.**

[8]. J. T. Ekanem, T.O. Johnson, "Effect of Tetracycline on late stage African Trypanosomiasis in Rats,'' *International jornal of Expirementel Bilogy,* Vol. **14,** Issue. **2,** pp. **51-56**, **2003.**

[9]. D. Grenier, M. Hout, D. Mayrand, "Iron-chelating activity of tetracyclines,'' *Antimicrobial agents and chemotheraphy,* Vol.**106,** Issue. **1,** pp. **763 -766**, **2000.**

[10]. B. Peter, O. Adekunle, A. Sanyaolu, F. Oyibo, C. Fagbenro-Beyioku, "A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis,''

Pathogens and Global Health, Vol. **107,** Issue. **5,** pp. **124 -139**, **2013.**

[11]. P.P. Simarro, J. Franco, A. Diarra, J. A. Postigo, J. Jannin, "Update on field use of the available drugs for the chemotherapy of human African trypanosomiasis,'' *Jornal of Parasitology*. Vol. **139,** Issue. **1,** pp. **842-846**, **2012.**

[12]. P. T. Woo, "The Haematocrit Centrifuge for the Detection of Trypanosomes in Blood,'' *Canadian Journal of Zoology*, Vol. **47,** Issue. **2,** pp. **921-923**, **1969.**

[13]. W. J. Herbert, W. A. Lumsden, " *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitaemia,'' *Journal of Experimental Parasitology*. Vol. **40,** Issue. **1,** pp. **427-431**, **1976.**

[14]. J.J. Ajakaiye, A. A. Muhammad, M.R. Mazadu, Y. Shuaibu, B. A. Kugu, B. Mohammad *et al.*, " Trypadim, Trrpamidium and Novidium can eliminate the negative effects on the body temperature and serum chemistry in Wister rats infected with *Trypanosoma brucie brucie'' International journal of Biochemistry and Bioinformatics*, Vol. **4,** Issue. **4,** pp. **371-341**, **2014.**

[15]. A. K. Maigari, S. B. Liman, A. M. Musa, A. Sani, S. Abubakar, Z. H. Jega, " Mixed Occurrence of Trypanosomes in Trade Cattle Slaughtered at Kano Abattoir, Northwestern Nigeria,'' *International Journal of Current Research*, Vol. **7,** Issue. **9,** pp. **9I6-9I9**, **2015.**

[16]. J. N. Abenga, F. N. Enwezor, F. A. Lawani, C. Ezebuiro, J. Sule, K.M. David, "Prevalence of Trypanosomiasis in Trade Cattle at Slaughter in Kaduna, Nigeria,'' *Nigerian Journal of Parasitol*ogy, Vol. **23,** Issue. **1,** pp. **107-110**, **2017.**

[17]. N.T. Dabo, A.K. Maigari, "Soft Options for Effective Diagnosis of African Animal Trypanosomiasis: A Review,'' *International Journal of Medical Evaluation and Physical Report*, Vol. **2 ,** Issue. **2,** pp. **257-263**, **2017.**

[18]. T. Saleha, F. Syed, R. Askari, A. Ummar, "Tetracycline: Classification, Structure, Activity Relationship and Mechanism of Action ; A Mini Review, '' *Biomedical Journal of Scientific & Technical Research.* Vol. **7,** Issue. **2,** pp. **123-129, 2018**.

[19]. A. Sani, U. Abdullahi, A. Mahe, D. Sinhg, " In Vitro study of Antytripanosomal activity of Ethanolic Leaf extract of Garcini a kola against *Trypanosoma brucei brucie*,'' *Asian journal of pharmaceutical and clinical research*, Vol. **6,** Issue. **2,** pp. **234-239**, **2018.**

[2O]. L. Roland, M. Drillich, and M. Iwersen, " Hematology as a Diagnostic Tool in Bovine Medicine," *Journal of Veterinary Diagnostic Investigation,* Vol. **26,** Issue. **5,** pp. **592-598**, **2014.**

[21].A. Abubakar, M.A.Yaro, G. Abdu and F.A. Rufa'i. "In Vivo and In Vitro Antitrypanosomal activities of Nigerian Medicinal Plants," I*nternational Journal of Scientific Research in Chemical Sciences,* Vol. **6,** Issue **4,** pp. **4-9, 2019.**

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