International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 3 (6), pp. 001-007, June, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Depletion of parasitaemia by halofantrine Hydrochloride and artemether in rats infected with African trypanosomes

Adeyemi Oluyomi S.[1]*, Ekanem Justine T.[2] and Sulyman Faoziyat[3]

[1]Department of Chemical Sciences, Bells University of Technology, Ota, Nigeria.
[2]Department of Biochemistry, University of Uyo, Nigeria.
[3]Department of Biochemistry, University of Ilorin, Nigeria.

Accepted 12 March, 2012

Two antimalarial drugs; Halofantrine HCl and Artemether whose mechanism of action has been shown to depend on the disruption of the red blood cells during malarial infections were investigated for possible trypanocidal activity *in vivo*. Pre-treatment with Artemether before infection with the parasite had no effect on infected rat when compared to the control. Treatment at the early stage of infection extended the lifespan from 11 days for control to 13th and 14th days post infection for dose treatments at 4.6 and 2.3 mg/kg rat weight respectively. Similar results were obtained for late stage treatment. Pre-treatment with Halofantrine HCl extended the life span to 16 days at 7.1 mg/kg rat weight. Early stage treatment with 7.1 and 14.2 mg/kg rat weight extended life span to 14 and 13 days respectively even though parasitaemia kept decreasing until death of the animals. The late stage treatment however extended the life span to 18 days. A combination of both drugs maintained low parasitaemia and extended life span to 16 days for prophylactic treatment, 15 and 16 days for early stage treatment at 2.3 mg Artemether and 7.1 mg/Kg Halofantrine HCl; 4.6 and 14.2 mg/Kg rat weight respectively, and 19 days for late stage treatment. Results suggest that Halofantrine HCl and possibly Artemether could be useful in the management of trypanosomosis since both drugs were able to maintain low parasitaemia. Parasitaemia has been shown to correlates with the severity of infection.

Key words: Halofantrine, artemether, parasitaemia, trypanosomosis, red blood cells.

INTRODUCTION

African trypanosomes is the causative agent for trypanosomiasis, for which about 300,000 new cases are reported annually in some 36 developing African countries (Chretien and Smoak, 2005; Ekanem and Yusuf, 2008). Trypanosomiasis is fatal if left untreated and chemotherapy which forms the most important and major aspect of control and eradication of the disease in African countries is beset with problems of toxicity and increasing incidence of resistance among the trypanosomes to the existing drugs (Kioy and Mattock, 2005; Moore, 2005). The search for new drugs and formulations that are safe, affordable and effective against both early and late stages of the disease is recommended (Jannin and Cattand, 2004; Chibale, 2005; Pink et al, 2005). This scourge remains a pressing challenge especially to African medical scientists for possible action plan that would be basic on the poor resources of these communities. The articulation of such plan would include both preventive measures and treatment modalities (Okochi et al., 2003). Current drugs used in the management of African trypanosomiasis are toxic and can encounter parasite resistance, hence the need for urgent, less toxic and readily available alternative source of trypanocide (Tijani et al., 2009) . Despite the understanding of this disease, there is little or no interest in developing new drugs for its treatment due to lack of financial reward. It therefore seems rational to look at alternative source of remedy for this disease. Earlier studies suggest that the antimalarial activity of artemether and halofantrine HCl is dependent on the formation of complex with heme iron which is a bye product of red blood cells degradation (Blauer, 1988; Meshnick, 2001).

*Corresponding author. E-mail: 05190592@uni.massey.ac.nz.

Erythrocyte lysis is a common feature in African trypanosomosis (Orhue et al., 2005). This complex formation is believed to be toxic to the parasite (Pandey et al., 1999; Villers et al., 2008) . These antimalarials are generally believed to interfere with haemoglobin digestion in blood stages of malaria parasite life cycle.

In this study we have investigated the effect of two antimalarial drugs, artemether and halofantrine HCl on the parasitaemia of rats infected with *Trypanosoma brucei brucei*.

MATERIALS AND METHOD

Parasite

T. b. brucei, lafia strain was obtained from Veterinary and Livestock studies Department, Nigerian Institute for Trypanosome Research (VLS-NITR), Vom, Jos, Plateau State, Nigeria. The parasite was injected intraperitoneally into rats and maintained by repeated passaging into other rats.

Animals

A total number of 39 albino rats of an average weight of 250 g were used for the experiment. The rats were of the species of R*attus novergicus* obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were acclimatized for 14 days before the commencement of the experiment. The animals were fed with rat pellets (obtained from Bendel Feed and Flour Mills Ltd. Ewu, Edo State, Nigeria) and clean water was provided *ad libitum*.

Experimental drugs

Artemether was a product of Novartis Pharma Limited, Beijing, China and halofantrine HCl was a product of SmithKline Beecham, France. Both were purchased from a local pharmacy store in Ilorin, Nigeria.

Inoculation of parasite

The tail of an infected rat was cleaned with a damp cloth and the tip of the tail was cut with a clean pair of scissors. The blood was extruded into about 0.5 ml normal saline. The extruded blood and saline was swirled to mix and drawn into 1 ml syringe. A drop of the solution was placed on a microscopic slide and observed under the light microscope to ascertain the presence of the parasite in the solution. Inoculation into the peritoneal cavity of an uninfected rat was carried out when parasite suspension contained 3 or 4 trypanosomes per view at x100 magnification.

Preparation of experimental drug solutions

Artemether: 1600 mg of artemether was dissolved in distilled water and made up to 400 mls. Volumes corresponding to 2.3 mg/Kg rat weight were administered to the rats. Halofantrine HCl: 2000 mg of halofantrine hydrochloride was dissolved in distilled water and made up to 250 mls. Volumes corresponding to 7.1 mg/Kg rat weight were administered to the rats.

In both cases the weight of the rats were taken into consideration before arrival at the dosages used.

Animal grouping/drug administration

The animals were randomly distributed into thirteen (13) groups of three rats each. Drugs were introduced daily into the peritoneal cavity of the infected rats using a 1 ml syringe. Parasitaemia was recorded daily until death of the animals. Administration of drugs was proportional to body weight of rats used.

Treatment with halofantrine HCl

The first group of rats were treated with halofantrine HCl (7.1 mg/Kg rat weight) three days before infection and as the infection progressed until death. The second and third groups were treated with 7.1 and 14.2 mg/Kg rat weight respectively from the day parasite was first sighted in the blood until the animals died.

Treatment with artemether

The fourth group of rats were treated with artemether (2.3 mg/Kg rat weight) three days before infection and as the infection progressed until death. The fifth and sixth groups were treated with 2.3 and 4.6 mg/Kg rat weight respectively from the day parasite was first sighted in the blood until death of animals.

Treatment with combination of both drugs

The seventh groups of animals were treated with artemether 2.3 mg/kg and halofantrine HCl 7.1 mg/Kg rat weight three days before infection and as the infection progressed until death. The eight and ninth groups were treated with artemether and halofantrine HCl 2.3 mg and 4.6, 7.1 and 14.2mg/kg rat weight respectively from the day parasite was first sighted in the blood until the animals died.

Late stage treatments

These groups of rats were treated from the 10^{th} day post infection until death. The tenth, eleventh and twelfth groups were treated with artemether (2.3 mg/Kg), halofantrine HCl (7.1 mg/Kg) and combination of both drugs respectively.

Control

This group was infected but not treated. The parasitaemia was recorded daily until death of animals.

Parasitaemia

Parasitaemia was obtained by counting the number of parasites per view under a light microscope at x100 magnification from a thin blood smear obtained from the tip of the tail of an infected rat.

RESULTS

The control

The parasitaemia of the untreated rats increased until the rats died on day 11-post infection.

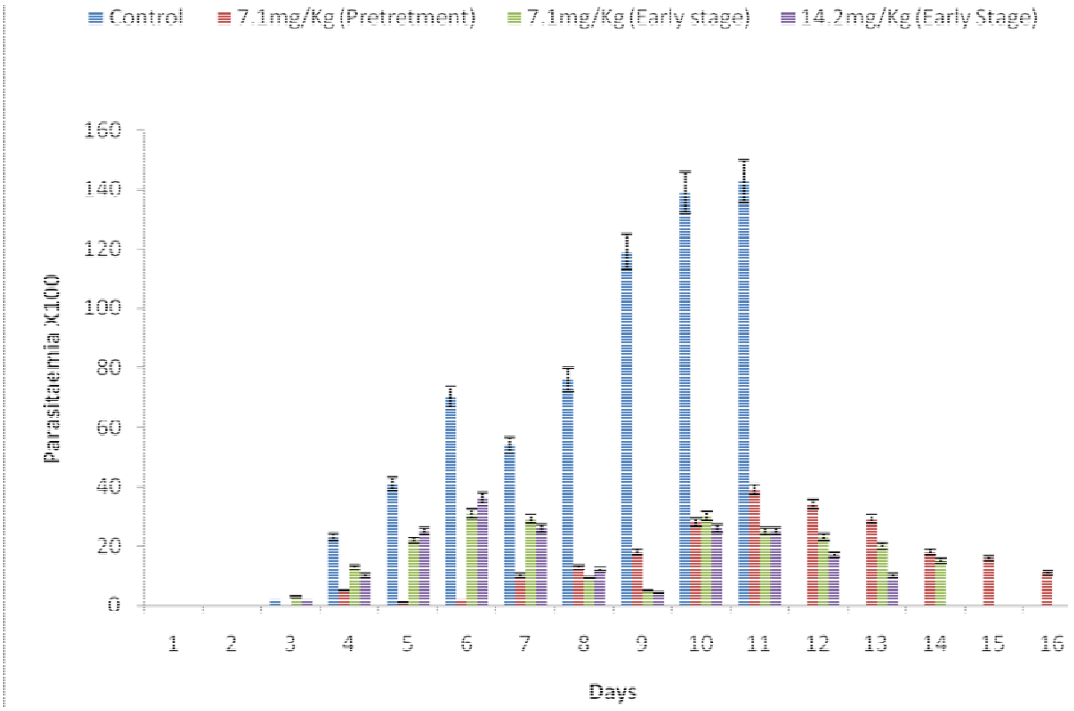

Figure 1. Effects of halofantrine HCl on the parasitaemia of rats experimentally infected with *T. brucei* (Pre-treatment and Early Stage). Each point is an average count from 3 rats.

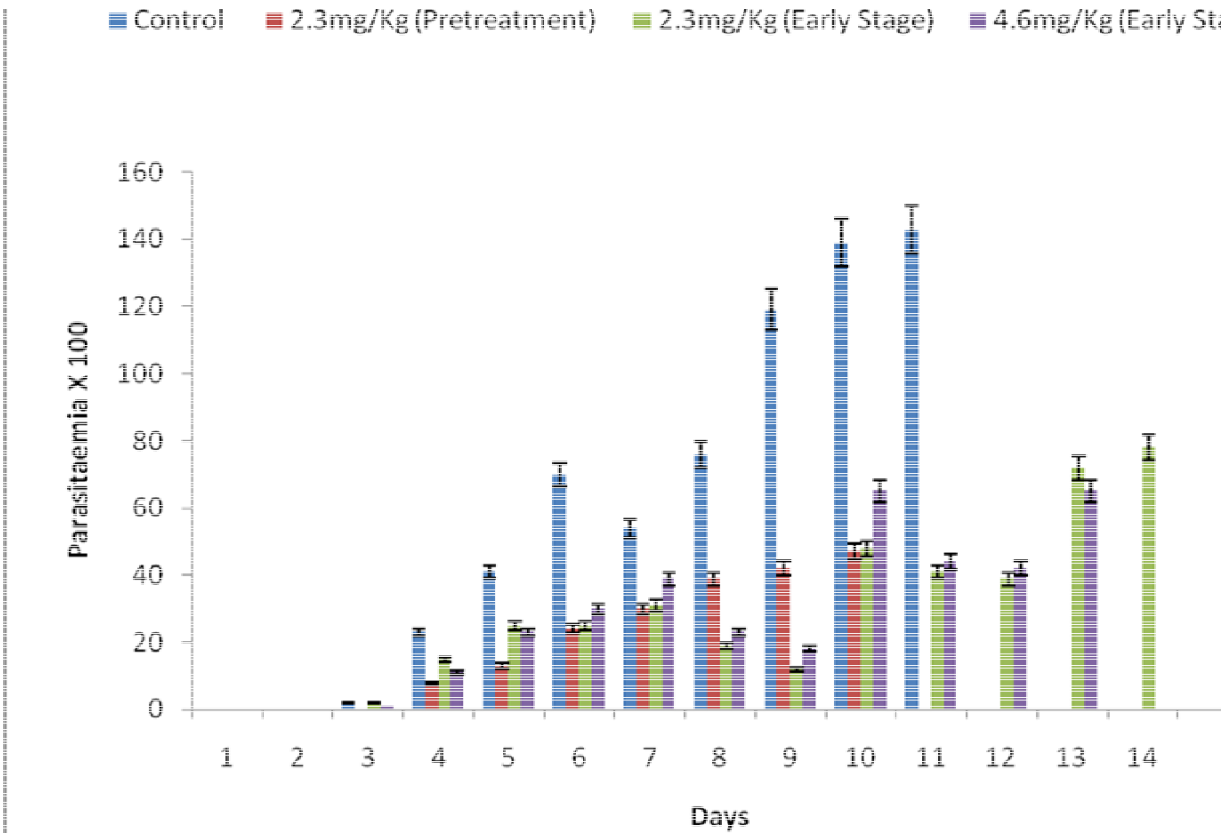

Figure 2. Effects of Artemether on the parasitaemia of rats experimentally infected with *T. brucei* (Pre-treatment and Early Stage). Each point is an average of count from 3 rats.

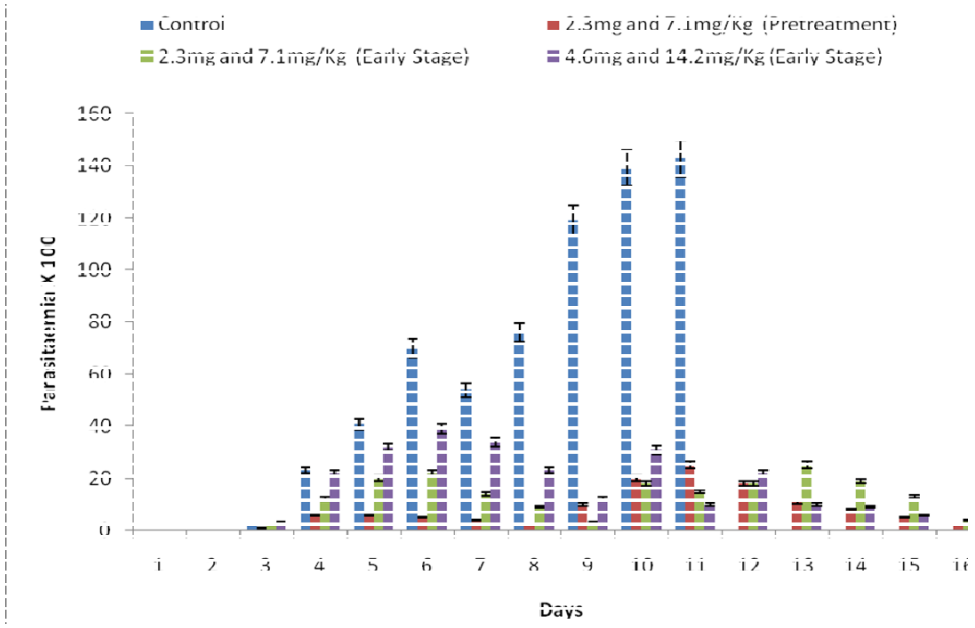

Figure 3. Effects of the combination of Artemether and Halofantrine HCl on the parasitaemia of rats experimentally infected with *T. brucei* (Pre-treatment and Early Stage). Each point is an average of count from 3 rats.

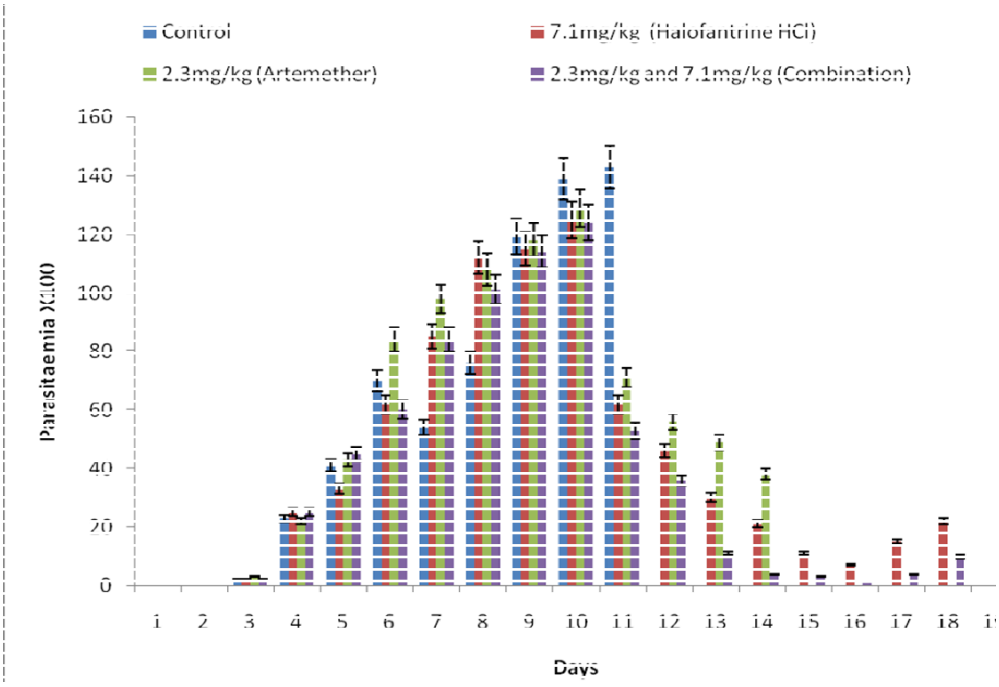

Figure 4. Effects of Artemether and Halofantrine HCl on the parasitaemia of rats experimentally infected with *T. brucei* and the combination of both drugs (Late Stage treatment). Each point is an average of count from 3 rats.

Treatment with halofantrine HCl

In the pre-treatment, with halofantrine HCl (Figure 1) the life span of infected rats was extended from 11 days for control to 16 days. The parasitaemia remained low compared with control throughout the experiment. The results for early stage treatment (Figure 1) both at 7.1mg and 14.2mg per kg rat weight showed that the animals

died on day 14 and 13 post infection respectively.

Treatment with artemether

In the pre-treatment, the parasitaemia increased until the death of rats on day 10 post infection (Figure 2). The results for early stage treatment (Figure 2) both at 2.3 mg and 4.6 mg/Kg rat weight, showed that the parasitaemia fluctuated until the death of rats on day 14 and 13 post infection respectively.

Treatment with combination

In the pre-treatment, with combination of both drugs artemether and halofantrine HCl (Figure 3) the life span of infected rats was extended from 11 days for control to 16 days. The parasitaemia remained low compared with control throughout the experiment. The results for early stage treatment (Figure 3) both at 2.3 and 7.1 mg; 4.6 and 14.2 mg per kg rat weight showed that the animals died on days 16 and 15-post infection respectively.

Late Stage Treatment: In the late stage treatment with halofantrine HCl (Figure 4), the life span of the treated animals was extended from 11 days for control to 18 days. The parasitaemia declined drastically compared with the control. The late stage treatment with artemether (Figure 4) did induce reduction in parasitaemia as well though the rats died on day 14 post infection, which was 3 days after the control died. And in the late stage treatment with combination of both drugs halofantrine HCl and artemether (Figure 4), the life span of the treated animals was extended from 11 days for control to 19 days. The parasitaemia declined drastically compared with the control.

DISCUSSION

Atawodi et al. (2003) reported that the complete elimination of motility or reduction in motility of parasites when compared to the control could be taken as indices of activity. Artemether is an effective antimalarial (Vennerstrom et al., 2000) . Results from this study (Figures 1, 3 and 4) showed that artemether was able to suppress the parasitaemia level in the treated rats relative to the untreated control though it could not extend the life span of the treated animals appreciably. The ability of artemether to induce decline in parasitaemia could probably be due to its ability to form complex with heme which is believed to be toxic to the parasites (Pandey et al., 1999; Meshnick, 2001).

Although, studies have shown that there are death related treatment with halofantrine HCl as a result of sudden cardiac toxicity (Nosten et al., 1993; Simooya et al., 1998). The death of treated animals on days 14 and 15 (Figure 1) for the double and single concentration respectively might have been the result of infection. No death was recorded when halofantrine HCl was administered to uninfected rats for 14 days at the therapeutic dosages.

Parasitaemia has been shown to correlate with severity of infection (Ekanem and Yusuf, 2008). The effects of halofantrine HCl and its ability to maintain low parasitaemia in treated animals (Figures 1, 3 and 4) could be due to the fact that halofantrine HCl can form complex with heme iron (Blauer, 1988; Villers et al., 2008) which is believed to be lytic to parasites amongst others. Several reports have shown that infection with trypanosomes induce erythrocytes membrane destruction (Taiwo et al., 2003) thus releasing the constituents of the red blood cell.

Another possibility is the prolonged half-life of halofantrine HCl ranging from 1 to 4 days (Milton et al., 1989), thereby giving halofantrine HCl a high bioavailability.

In the late stage treatment (Figure 4), halofantrine HCl was able to induce a sharp reduction in parasitaemia compared with the control to a near zero level and also extended the life span appreciably. The combination can be said to owe its trypanocidal effects to the activity of halofantrine HCl. Since similar results were obtained for both halofantrine HCl and combination therapy. Earlier studies showed that trypanosomes release extra-cellular factors (Ekanem, 1989), which are believed to be toxic to the host. The death of treated animals even at low parasitaemia could have been the result of the toxic activities of these extra-cellular factors. More so when the extra-cellular factors have been reported to have pathological effects on host rats (Ekanem et al., 1996).

Conclusion

The results of this study suggest that halofantrine HCl and artemether could be useful in the management of African sleeping sickness since both drugs were able to reduce the parasitaemia level in the infected and treated rats. This is of significant importance especially when one considers the fact that reduction may also lead to low inducement of biochemical lesions usually associated with proliferating parasites. Several studies have shown that parasitaemia correlates with infection. We also speculate that halofantrine HCl and artemether may have caused the depletion of parasitaemia in the infected and treated rats owing to their ability to form complex with heme iron which is a bye product of red blood cell lysis due to trypanosoma infection induced erythrocyte membrane damage.

REFERENCES

Aldhous P (1994). Fighting parasites on a show string. Science 264: 1857-1859.

Anosa VO (1988). Haematological and biochemical changes in human

and animal trypanosomiasis. Review Elev. Med. Vet. Pays Trop. 41(42): 151 –164.

Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M (2003). *In vitro* trypanocidal effect of methanolic extract of some Nigerian Savannah plants. Afr. J. Biotech. 2(9): 312-321.

Biryomumaisho S, Katunguka-Rwakishaya E (2007). The pathogenesis of anaemia in goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*: Use of the myeloid:erythroid ratio. Vet. Parasitol. 143: 354-357.

Blauer G (1988). Interaction of ferriprotoporphyrin IX with the antimalarials amodiaquine and halofantrine. Biochem. Int. 17: 729-734.

Bodley AL, Shapiro TA (1995). Molecular and cytotoxic effects of camptothecin, a topoisomerase I inhibition on trypanosomes and leshmania. Proc. Natl. Acad. Sci. (USA) 92: 3272-3730.

Bodley AL, Wani MC, Wall ME, Shapiro TA (1995). Antitrypanosomal activity of camptothecin analogs. Structure-activity correlations. Biochem. Pharmacol. 50: 937-942.

Brun R, Schumacher R, Schid C, Kunz C, Burri C (2001). The phenomenon of treatment failures in human African Trypanosomiasis. Trop. Med. Int. Health 6: 906-914.

Chibale K (2005). Economic drug discovery and rational medicinal chemistry for tropical diseases. Pure Appl. Chem. 77: 1957-1964.

Chretien JPL, Smoak BL (2005). African Trypanosomiasis: Changing epidemiology and consequences. Curr. Infec. Dis. Reports 7: 54-60.

Ekanem JT (1989). Extra cellular fractions derived from *trypanosoma brucei* activate erythrocyte Ca^{2+} ATPase. Med. Sci. Res. 17: 739-40.

Ekanem JT, Akanji MA, Odutuga AA (1996). Extracellular proteins of Trypanosoma brucei origin lyse erythrocytes of rats *in vitro*. Biochem. 6(1): 21-29.

Ekanem JT, Yusuf OK (2008). Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. Afr. J. Biomed. Res. 11: 79 – 85.

Gibson WC, Marshall TF de C, Godfrey DG (1980). Numerical analysis of enzyme polymorphism: a new approach to the epidemiology and taxonomy of trypanosomes of the subgenus trypanozoon. Adv. parasit. 18: 175-246.

Jannin J, Cattand P (2004). Treatment and control of human African trypanosomiasis. Curr. Op. Infec. Disease 17: 565-571.

Khattab MM, Nagi MN (2007). Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. Phytother. Res. Published online: 2007. 10: 1002-2083.

Kioy D, Mattock N (2005). Control of sleeping sickness – time to integrate approaches. Lancet 366(9487): 695-696.

Kökdil G, Tamer L, Ercan B, Çelik M, Atik U (2006). Effects of Nigella orientalis and N. Segetalis fixed oils on blood biochemistry in rats. Phytother. Res. 20(1): 71 – 75.

Kuzoe FAS (1993). Current situation of African Trypanosomiasis. Acta Trop. 54: 153-162.

Legros D (2002). Treatment of human African Trypanosomiasis-present situation and needs for research and development. Lancet Infect. Dis. 2: 437-440.

Meshnick SR (2001). Artemisinin and its derivatives. In Antimalarial chemotherapy: Mechanisms of Action, resistance and New Directions in Drug Discovery (ed. P.J. Rosenthal): 191-201. Ottawa. N.J. Human Press.

Milton KA, Edwards G, Ward SA, Orme MLE, Breckendridge AM (1989). Pharmacokinetics of halofantrine in man: effects of food and dose size. British J. Clin. Pharmacol. 28: 71-77.

Moore AC (2005). Prospects for improving African trypanosomiasis chemotherapy. J. Infec. Dis. 191: 1793-1795.

Murray M, Morrison WY, White-Kiv DV (1988). Host susceptibility to African Trypanosomiasis, trypanotolerance: Advance in parasitol. 21: 52-57.

Nosten F, Kuile FO, Luxemburger C, Woodrow C, Kyle DE, Siddhi C, White NJ (1993). Cardiac effects of Antimalarial treatment with halofantrine. The Lancet. 341: 1054-1056.

Okochi VI, Okpuzor J, Okubena MO, Awoyemi AK (2003). The influence of African Herbal Formula on the haematological parameters of trypanosome infected rats. Afr. J. Biotechnol. 2(9): 312-316.

Onyeyili, RA, Egwu GO (1995). Chemotherapy of African Trypanosomiasis: A historical review. Protozol Abstr. 5: 229-243.

Orhue N, Nwanze E, Okafor A (2005). Serum total protein, albumin and globulin levels in *Trypanosoma brucei*-infected rabbits: Effect of orally administered *Scoparia dulcis.* Afr. J. Biotechnol. 4(10): 1152-1155.

Pandey AV, Tekwani BL, Singh RL, Chauhan VS (1999). Artemisinin, an Endoperoxide Antimalarial, Disrupts the Hemoglobin Catabolism and Heme Detoxification Systems in Malarial Parasite. J. Biol. Chem. 274(27): 19383-19388.

Pink R, Hudson A, Mouries MA, Bendig M (2005). Opportunities and challenges in antiparasitic drug discovery. Nature Rev. Drug Discovery 49: 727-740.

Simooya OO, Sijumbil G, Lennard MS, Turker G (1998). Halofantrine and chloroquine inhibit CY2D6 activity in healthy Zambians Br. J. Clin. Pharmacol. 45: 315-317.

Suliman HB, Feldman BF (1989). Pathogenesis and aetiology of anaemia in trypanosomiasis with special reference to *T. brucei* and *T. evansi*. Protozool. Abtr. 13: 37- 45.

Taiwo VO, Olaniyi MO, Ogunsanmi AO (2003). Comparative plasma biochemical changes and susceptility of erythrocytes to in vitro peroxidation during experimental trypanosoma Congolese and T. brucei infections in sheep. Israel J. Vet. Med.

Tijani AY, Uguru MO, Salawu OA, Abubakar A, Onyekwelu NO, Akingbasote JA (2009). Effect of *Faidherbia albida* on some biochemical parameters of rats infected with *Trypanosoma brucei brucei.* Afr. J. Pharm. Pharmacol. 3(1): 026-030.

Vennerstrom JL, Dong V, Anderson SL, Ager AL, Jr Fu H, Miller REE, Wesche DL, Kyle DE, Gerena L, Walters S (2000). Synthesis and antimalarial activity of sixteen dispiro-1,2,4,5-tetraoxanes: alkyl-substituted 7(8): 15-16. tetraoxadispiro [5.2.5.2) hexadecanes. J. Med. Chem. 43: 2753-2758.

Villiers KA, Marques HM, Egan TJ (2008). The crystal structure of halofantrine– ferriprotoporphyrin IX and the mechanism of action of arylmethanol antimalarials. J. Inorg. Biochem. 102(8): 1660-1667.

Warren KS (1998). The global impact of parasitic diseases. In: The Biology of Parasitism (Eds. England P.T., Sher A.) Alan R. Liss, New York.

WHO (1986). Epidemiology and control of African Trypanosomiasis. Report of a WHO expert committee. WHO Geneva Switzerland. Technical Report Series 739: 122.

Appendix

Calculation of different concentrations of drugs

For Halofantrine HCl:
Average weight of rats -250 g
Concentration of drugs used 7.1 and 14.2 mg/Kg
250 g =250/1000 =0.25 Kg
If 1kg takes 7.1 mg

$$\therefore \text{ 0.25 Kg will need } = \frac{0.25 \times 7.1}{1} = 1.8 \text{ mg}$$

But 2000 mg of halofantrine HCl was dissolved in 250 ml.

$$\therefore \text{ 1.8 mg will be dissolved in } = \frac{1.8 \times 250}{2000} = 0.2 \text{ ml}$$

For Artemether:

Average weight of rats -2.50 g
Concentration of drugs used 2.3 and 4.6 mg/Kg
250 g = 250/1000 = 0.25 Kg

If 1 Kg needs 2.3 mg

$\therefore$ 0.25 kg needs = $\frac{0.25 \times 2.3}{1}$ = 0.6 mg

But 1600 mg was dissolved in 400 mls

$\therefore$ 0.6 mg will be dissolved in = $\frac{0.6 \times 400}{1600}$ = 0.15 ml