INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

EFFECTS OF IMMUNACE AND IRON DEXTRAN ON ANEMIA AND IMMUNOSUPPRESSION OF *T. BRUCEI* INFECTED RATS

[1]U.O. ADAMU*, [2]M.K. HARUNA, [1]R.P. OVBAGBEDIA,[1]UZOWURU M, [1]EYITOPE M.G.[3]ANICHEBE F

[1]Trypanosomiasis Research Department, Nigeria Institute for Trypanosomiasis Research, Kaduna Nigeria
[2]Research Extension, M&E Unit, Management Monitoring and Information System sDepartment, NITR Kaduna.
[3]Consultancy and Extension Services Division, Nigeria Institute for Trypanosomiasis Research, Kaduna Nigeria
*Corresponding Author

Abstract

Trypanosomosis has been associated with immunosuppression, anemia and oxidative damage while ImmuneAce and Iron Dextran possess both immunostimulatory, antioxidative and erythrocytes enrichment effects. This study was designed to assess the effect ofImmunace, Iron Dextran, Diminazene Aceturate and a combination of Immunace and Iron Dextran on *T. brucei* experimentally infected rats to check packed cell volume (PCV) and parasitemiam. . Thirty rats, divided into six groups (A-F) of 5 each period and were infected with *Trypanosoma brucei* 3 days post infection.They were treated as follows:3, 6, 9, 12 and 15 days post treatment with 0.02gml-1 immunace, 0.2ml of Iron Dextran, 3.5mg Kg-1 of Diminazene Aceturate and a combination therapy of Iron Dextran and Immuneace. Haematological parametres were significantly ($p<0.05$) higher in all infected and treated groups compared to group E. Hence, overall anti-oxidants capacity mitigated the negative effects observed in the measured parametres in rats better than single administration..

Keywords: African Trypanosomiasis, parasitemia, trace metals, Anaemia, Iron Dextran and Immunosuppression.

Introduction

African trypanosomiasis is a disease of humans and animal caused by several species of trypanosomes and spread by tsetse flies in 37 countries within the sub-Saharan region (Welburn et al., 2001). The disease ismainly transmitted cyclically by the genus *Glossina* spp., but can also be transmitted by several biting flies like tabanids, hippoboscides, stomoxys, etc. (Kneeland et al.,2012; WHO, 2013), and Votypka et al. (2011) have indicted the culex mosquito in the transmission of an avian trypanosome *Trypanosoma culicavium* spp. nov. Two tsetse- transmitted parasites*, Trypanosoma brucei gambiense* and *Trypanosoma bruce irhodensiense,* cause Human African Trypanosomiasis (HAT) which is commonly known as sleeping sickness.

Drug regiments are toxic and cumbersome in addition to being expensive (Kioy and Mattock 2005, Moore 2005). Also with exacerbated drug resistance initiated through a complex mechanism called antigenic variation, the parasites now have a field day in the infected host thereby triggering a host of somatic anomalies and socio-ecomic sufferings. It has been reported that this process is initiated when the parasite penetrate and cleave the erythrocyte cells of the host which in turn cause the release of pyrogen in to the circulatory system (Robinson et al., 1999; Morrison et al., 2009). With increased circulating pyrogen, the host neuro- and immunological mechanism is set in motion to restore homeostasis which leads to unabated pyrexia and consequently oxidative stress if not arrested in time.

Oxidative stress has been implicated in the anaemia observed in trypanosomiasis (Igbokwe et al., 1994; Umar et al., 2000). Oxidative haemolysis is assumed to occur due to high production of free radicals in the body of infected animals (Slater,1984; Baltz et al.,1985) and

depletion of endogenous antioxidant reserves of the body (Zwart et al., 1991).

Anaemia is a major clinical and laboratory finding in trypanosomosis and is characterized by a pronounced decrease in white blood cell, red blood cell, packed cell volume, and haemoglobin levels (LososandIkede, 1972). *Trypanosoma brucei* infection like other trypanosome infections precipitate increased red blood cell destruction which results in anaemia as well as tissue damage (Ekanem and Yusuf, 2008; Akanji et al., 2009). It has been hypothesized that the large amounts of peroxides and free radicals generated by trypanosome and activated mononuclear phagocytes predispose erythrocytes to early ageing and fragmentation. However, several reports claim that drug supplements containing vitamin E (α- tocopherol) is an indispensable lipid soluble non-enzymatic antioxidant that protects cell membranes from oxidation, thus stabilizing and maintaining their selective permeability (Herrera and Barbas, 2001; Trabes and Atkinson, 2007). . Hence the Onus is to continue to seek inexpensive and less cumbersome approaches to the management and treatment of the disease (Moore, 2005).

Immunace is a specialist food supplement providing advance nutritional support for all round health and vitality as well as specific nutrients for the normal function of the immune system. Immunace provides comprehensive formula including Vitamins E&D, Zinc and Selenium which contribute to the normal functioning and strengthening of the Immune system, plusfolate which contribute to the normal blood formation. Immunace was the first immune system supplement to pioneer and recognize that Vitamin D does not only contribute to the maintenance of normal bones and muscle function, but also contribute to the normal function of the immune system.

Also, Iron Dextran is commercially available as a sterile solution of Iron Dextran complex containing 5% Iron and 20% Dextran. It contains 50mg/Ml of elemental Iron, most of which is present in the ferric state. Iron Dextran is a stable, clear brown solution available in 2 and 5 –ml samples and a 10Ml multiple close – vial. Because of the phenol content, the multiple close vial should only be used for intramuscular administration (http://www.revital.co.uk/vitabiotics-immunace-selenium-30-tablets-29083)

This study was therefore designed to determine the effect of Immunace, Iron Dextran and a combination therapy of both ImmuneAce and Iron Dextran treatment on the parasitemia and PCV of *T. brucei* infected rats.

Materials and Methods

Test Organisms

T.bbrucei was obtained from the stables maintained at the Nigeria institute for Trypanosomiasis Research (NITR) Kaduna, Nigeria. The parasites were maintained in the laboratory by continuous passaging in rats until when required. Passaging was considered necessary when parasitemia was in the range of 16 – 32 parasites per field usually 3 days post infection. In passaging, 1 x 10^{-3}parasites were inoculated intramuscularly into rats in 0.1 – 0.2 blood / PBS solution.

Experimental Animals

A total of thirty (30) rats of both sexes used for the study were purchased form NITR Kaduna Nigeria. They were maintained in clean rat cages in a 12hrs light/dark cycle with litter changed every day. They were fed with pelleted commercial rats feed (ECWA Nigeria Plc Jos, Plateau State Nigeria). Standard protocol was observed in accordance with Good Laboratory Practices (GLP). Regulation of the WHO (1997) the Animal Laboratory Care was strictly followed.

Preparation of Drug Sample

The Immunace tablet was pulverized with a grinder. Afterwards, 0.02g of the powdered Immunace was weighed using a sensitive digital weighing balance. 0.02g of the powdered Immunace was dissolve in 0.5ml distilled water to form a solution of the drug.

In vivo Antitrypanosomal Activities of Immunace, Diminazene Aceturate, Iron Dextran and Immunace / Iron Dextran Combination

Thirty (30) rats of both sexes were randomly selected and separated into six groups (A – F) of five rats each and were kept in clean rat cages. Five groups of rats (A – E) were each inoculated intraperitonially with 1 x10^{3}*T.b brucei* parasites. Day 3 post infection. 0.2ml of Iron Dextran was intramuscularly used to treat rats in group A while the rats in group B were given oral dose of 0.02g/ml of Immunace. Group C received a combination therapy of 0.02ml Iron Dextran (Intramuscularly) and 0.2mg/mtImmunace (Oraly), rats in group D received a single dose of 3.5mg/kg b.w of Diminazene Aceturateintraperitonialy as the infected treated control. While rats in groups E and F were used as negative and positive controls respectively.

Monitoring of Infected and Control Animals

Determination of parasitemia

Parasitemia was monitored in blood obtained from the tail, pre-sterilized with methylated spirit. The number of parasites were determines microscopically at X 40 magnification using the "Rapid matching" method of Herbert and Lumsden (1976).

Determination of Packed Cell Volume

PCV values were determined for each group of rats using the Haematocrit Centrifugation Techniques (HCT)

Results and Discussion

Following infection of all the groups with *T.bbrucei* the survival rate in the infected – untreated rats was found shorter ($p > 0.05$) than that in the infected but treated groups.

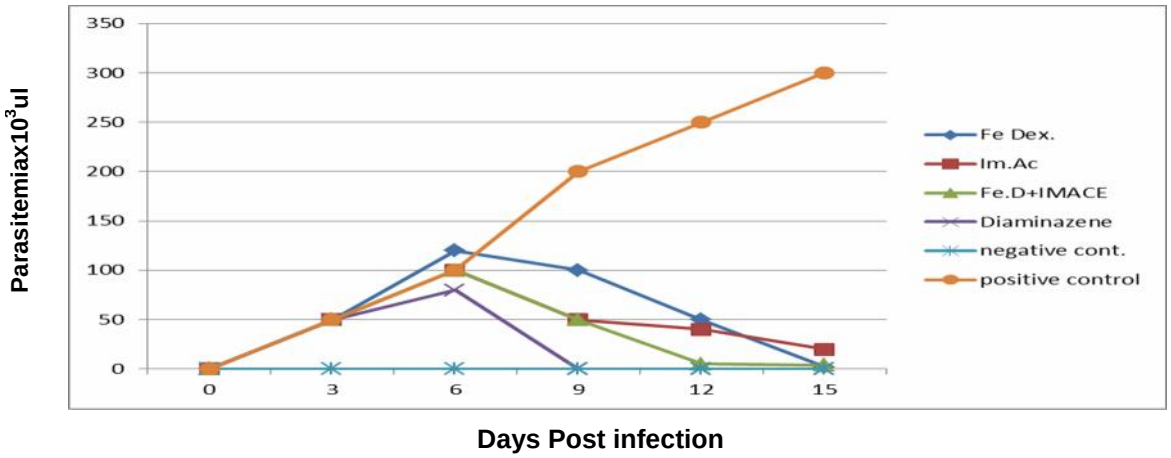
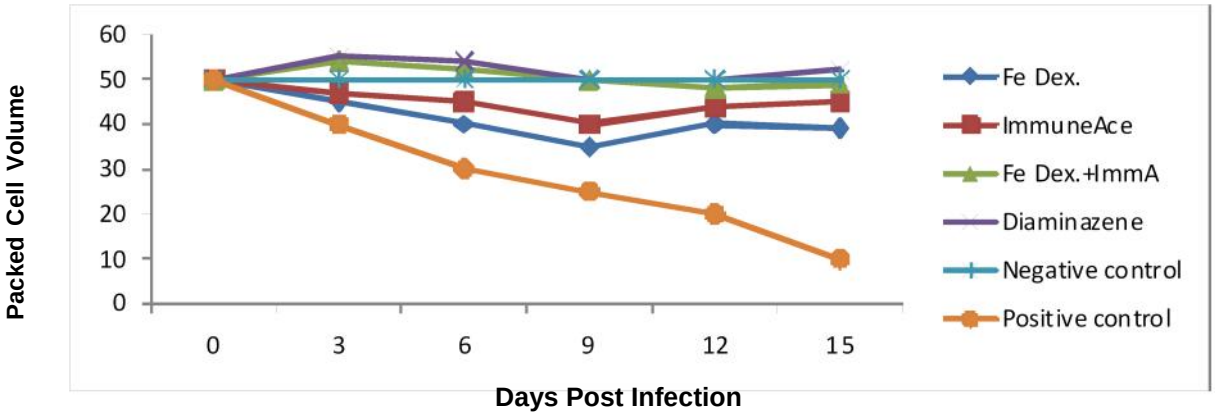


Fig.1 Mean Parasitemia level of T.bbrucei experimentally infected rats

The positive control (Group F) developed clinical trypanosomal manifestations by pyrexia, weakness, rough hair coats and parlour of the mucous membrane. The clinical signs were progressive with mortalities in the group, 5 -7 days post infection. Treatment with the drug and supplements (Immunace, Iron Dextran, DiaminazeneAceturate and Combination therapy of immunace/Iron Dextran), suppressed parasite establishment and the manifestation of mild clinical diseases. There was a partial clearance of the parasite from circulating on day 15 post treatment with those in groups A and B. Treatment with a combination of Iron Dextran / Immunace produce better clearance of the parasites 15 days post infection. Treatment of group D with DiaminazeneAceturate at 3.5mg/kg (Samore Animal Care) cleared the parasite from circulating within 48hrs and suppressed the manifestation of clinical signs of the disease with no relapse or death within the experimental period.



Mean Packed Cell Volume of all the groups with days of Post Infection

Pyrogenic stimuli released during infection leads to pyrexia and oxidative stress observed in the positive control (Group E). This is because pyrexia is a direct response to successive waves of parasitaemia (Stephen, 1986; Baracos et al., 1987). However, the significant decrease observed in all infected and treated groups compared to group E suggest that the drugs and supplementpossess anti-pyretic capacity, since this activity is achieved through their ability to boost immune response to disease. (Passmoore and Eastwood, 1986).

Haematological parameters such as RBC and PCV have been shown to be important indices of trypanosomiasis this is further supported by the report of Ekanem et al. (2005; 2006) which documented that the measurement of anaemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals. PCV is a measure of anaemia and the reduction in PCV indicator observed in this study particularly in group E suggests that the anaemia in this infection is haemolytic and that it involves a significant increase in the rate of erythrocyte destruction.

Our findings in this study is in agreement with the reports of Preston et al. (1979) and Igbokwe and Nwosu (1997) who concluded in their studies that trypanosomiasis may cause anaemia as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host. Inaddition, several authors have reported that severity of anaemia usually reflects the intensity and duration of parasitaemia in trypanosomal infection (Ogusanmi and Taiwo, 2001; Umar et al., 2007; Ekanem et al., 2008; Saleh et al., 2009), as it has been reported by Mbaya et al. (2009) in their study on red fronted gazelles experimentally infected with Trypanosomabrucei and/or Haemonchuscontortus, that there is an inverse relationship between parasitaemia and anaemia. The anaemia observed in this experiment decreased progressively without any period of drop, another indication of an acute phase of the disease. Similar observation has been previously reported with a different strain (Basa) of the parasite (Umar et al., 1999).

Several factors contribute to the development of anaemia among which is the oxidative damage of RBC membranes by free radicals and peroxides generated during the course of the infection (Igbokwe et al., 1994) on one hand, but on the other hand, infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the red blood cell (Igbokwe, 1994; Taiwo et al., 2003; Akanji et al., 2009). The administration of the drugs and supplement as shown in fig.2, ameliorated the disease induced anaemia compared to group E, and may be attributable tothe antioxidant activity of the supplements and Iron Dextran; by scavengingthe trypanosome-generated free radicals; thus reducing the free radical load (Kaikabo and Salako, 2006; Eze and Ochike, 2007).

However, the inability of the theimmuneAce and Iron Dextran to completely prevent the disease-induced anaemia compared to diminazene suggests the involvement of other aetiological factors in the development of anaemia in the infected rats.

Conclusion

In conclusion Immunace supplementation and Iron dextran treatment had a significant effect on the PCV, parasitemia and reduction in the severity of trypanosome infections in rats as evidenced by the higher survival times, lower level of parasitaemia and less severe anaemia in the trypanosome infected supplemented rats compared to the infected-untreated control. The result obtained in this study has demonstrated that combination of both Immuneace (immune booster) and Iron Dextran suppress parasitaemia than ImmuneAce and Iron Dextran in their individual application. It is our recommendation therefore, that further work should be carried out to evaluate the antitrypanosoma lace metals of compexed drugs with trace metals to evaluate the impact of drug combination on the effective control the menace of this disease.

Acknowledgments

The Authors of this research work wish to thank the Director General of the Nigeria Institute of Trypanosomiasis Research (NITR) Professor Muhammed Mamman for his technical assistance. Also we wish to appreciate the encouragement given to us by the Head of Department, Trypanosomiasis Research Department, NITR Kaduna Nigeria. Finally, our gradiyude goes to the staff of NITR laboratory for their immense technical support to making this work a reality.

References

Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F (2009).Psidiumguajava extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI J. 8: 148-154.

.Baltz T, Baltz D, Giround C, Crockett J (1985). Cultivation in semi defined medium of animal infected forms of Trypanosomabrucei, T. equiperdium, T.evansi, T. rhodosiense, and T. gambiense. EMBO. J. 4: 1273-1277.

Baracos VE, Whitmore WT, Gale R (1987). The metabolic cost of fever.Can. J. Physiol. Pharmacol. 65: 1248-1254. Dacie JV, Lewis SM (1991). Practical haematology. 7th (eds). Churchill Livingston, Edinburg.

Ekanem JT, Majolagbe OR, Sulaiman FA, Muhammad NO (2006).Effects of honey supplemented diet on the parasitaemia and some enzymes of Trypanosomabrucei-infected rats. Afr. J. Biotechnol. 5: 1557-61.

Ekanem JT, Sulyman FA, Adeyemi OS (2005). Therapeutic properties and serum iron in T. brucei infected rats treated with amodiaquineand mefloquine. Biokemistri, 17: 115-121.

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

Eze JI Ochike BO (2007).I Effect of dietary vitamin E supplementation on Trypanosomabrucei infected mice. Trop. Vet. 25: 18-25.

Herbert WJ, Lumsden WHR (1976). Trypanosomabrucei: A rapid matching method for estimating the host's parasitaemia. Exp. Parasitol. 40: 427-431.

Herrera E, Barbas C (2001). Vitamin E, action, metabolism and perspectives. J. Physiol. Biochem. 57: 43-56.

Igbokwe IO, Esievo KA, Saror DI, Obagaiye OK (1994).Increased susceptibility of erythrocytes to in vitro peroxidation in acute Trypanosomabrucei infection in mice. Vet. Parasitol. 55: 279-86.

Igbokwe IO, Nwosu CO (1997). Lack of correlation of anaemia with splenomegaly and hepatomegaly in Trypanosomabrucei and Trypanosomacongolense infections of rats. J. Comp. Pathol. 117: 261-265.

Kaikabo AA, Salako MA (2006). Effects of vitamin E supplementation on anaemia and tissue pathology in rats infected with Trypanosomabrucei (Federe strain). Trop. Vet. 24: 46-51.

Kneeland KM, Skoda SR, Hogsette JA, Li AY, Molina-Ochoa J,Lohmeyer KH, Foster JE (2012). A Century and a Half of Research on the Stable Fly, Stomoxyscalcitrans (L.) (Diptera: Muscidae), 1862-2011: An Annotated Bibliography. United States Department of Agriculture, Agricultural Research Service ARS- 173.

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

Losos GJ, Ikede BO (1972). Review of pathology of disease in domestic and laboratory animals caused by Trypanosomacongolense, Trypanosomavivax, Trypanosomabruceibrucei, Trypanosomarhodesiense and Trypanosomagambiense. J. Vet. Pathol. 9: 1-17.

Mbaya AW, Aliyu MM, Nwosu CO, Egbe-Nwiyi T (2009).The relationship between parasitaemia and anaemia in concurrent Trypanosomabrucei and Haemonchuscontortus infections in red fronted gazelles (Gazellarufifrons). Vet. Arhiv. 79(5): 451-460.

Morrison LJ, Marcello L, McCulloch R. (2009). Antigenic variation in the African trypanosome: Molecular mechanisms and phenotypic complexity. Cellular Microbiol. 11(12): 1724-1734.

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

Ogunsanmi AO, Taiwo VO (2001). Patho-biochemical mechanisms involved in the control of the disease caused by Trypanosomacongolense in African grey duiker (Sylvicapragrimmia). Vet. Parasitol. 96: 51–63.

Passmoore R, Eastwood MA (1986). Human Nutrition and dietetics 8th(eds). Churchill Livingstone, Edinburgh. pp. 371-376. Preston JM, Wellde BT, Kovatch RM (1979).Trypanosomacongolense: calf erythrocyte survival. Exp. Parasitol. 48: 118-125.

Preston JM, Wellde BT, Kovatch RM (1979).Trypanosomacongolense: calf erythrocyte survival. Exp. Parasitol. 48: 118-125.

Robinson NP, Burman N, Melville SE, Barry JD (1999). Predominance of duplicative VSG gene conversion in antigenic variation in African trypanosomes Mol. Cell. Biol. 19(9): 5839-5846.

Saleh MA, Bassam MA, Sanousi SA (2009). Oxidative stress in blood ofcamels (Camelus dromedaries) naturally infected with Trypanosomaevansi. Vet. Parasitol. 2162: 192–199.

Slater TF (1984). Free radical mechanism in tissue injury. Biochem. J. 222: 1-15.

Stephen LE (1986). Trypanosomiasis- A Veterinary Perspective.1st(eds). Pergamon Press, New York.

Taiwo VO, Olaniyi MO, Ogunsanmi AO (2003). Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental Trypanosomacongolense and Trypanosomabrucei infections in sheep.Israel J. Vet.Med.58(4):1-5.

Traber MG, Atkinson J (2007) Vitamin E, antioxidant and nothing more. Free Radic. Biol. Med. 43: 4-15.

Umar IA, Ogenyi E, Okodaso D, Kimeng E, Stancheva GI, Omage JJ, Isah S, Ibrahim MA (2007b). Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamins A and C to trypanosomabruceibrucei-infected rats. Afr. J. Biotech. 6 (18): 2083-2086.

Umar IA, Toh ZA, Igbalajobi FI, Igbokwe IO, Gidado A (2000). The effects of orally administered vitamins C and E on severity of anaemia in Trypanosomabrucei-infected rats. Trop. Vet. 18: 71-77.

Umar IA, Toma I, Akombum CA, Nnadi CJ, Mahdi MA, Gidado A, Igbokwe IO, Buratai LB (2007a). The role of intraperitoneallyadministered vitamin C during Trypanosomacongolense infection of rabbits.Global. J. Pur. Appl. Sci. In press.

Umar IA, Wuro-Chekke AU, Gidado A, Igbokwe IO (1999). Effects of combined parenteral vitamin C and E administration on the severity of anaemia, hepatic and renal damage in T. brucei infected rabbits. Vet. Parasitol. 85: 43-47.

Votypka J, Szabova J, Radova J, Zidkova L, Svobodova M (2011). Trypanosomaculicavium sp. nov., an avian trypanosome transmitted by Culex mosquitoes. IJSEM.doi: 10.1099/ijs.0.032110-0 IJSEM .

Welburn SC, Coleman PG, Fevre E, Mandlin I (2001). Sleeping sickness- a tale of two diseases.Trends Parasitol. 17: 19-24.

WHO (2013).Human African trypanosomiasis (sleeping sickness). URL: Fact sheet N°259: African trypanosomiasis or sleeping sickness. Accessed 27th August, 2013.

WHO, (1992) GOOD LABORATORY PRACTICE AND COMPLIANCE MONITORING Number 1 OECD Principles on Good Laboratory Practice (as revised in 1997) 61011

Zwart D, Brouwer BC, VanderHel W, Van den Akker HN, VersegenMWA (1991). Effect of Trypanosomavivax infection on body temperature, feed intake and metabolic rate of West African Dwarf goat. J. Anim. Sci. 69: 3780-3788

Internet:http://www.revital.co.uk/vitabiotics-immunace-selenium-30-tablets-29083

