Efficacy Study of Kaempferol, Diminazene Aceturate and their Combination in Stress Induced by Experimental *Trypanosoma brucei brucei* Infection

*[1]Muhammad, Y., [3]Jatau, I. D., [2]Suleiman, M. M., [1]Umar, AM., [4]Chiroma, M.A. and [1]Gumel, M.A.

[1]School of Agriculture, Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia Jigawa State, Nigeria.
[2]Faculty of Veterinary Medicine, Department of Veterinary Pharmacolgy and Toxicology, A.B.U Zaria, Kaduna State Nigeria.
[3]Faculty of Veterinary Medicine, Department of Veterinary Parasitology and Entomology, A.B.U Zaria, Kaduna State Nigeria.
[4]Faculty of Veterinary Medicine, Department of Veterinary Pathology, University of Maiduguri, Borno State, Nigeria.
*Corresponding author: yamohad@gmail.com.

ABSTRACT

The comparative efficacy of kaempferol, diminazene aceturate and their combination against experimental *T.brucei brucei* infection in mice was investigated in this study.Thirty six adult swiss albino mice weighing between 18 and 22 g were randomly divided into six groups of six mice each. Mice in group I were un-treated un-infected. Mice in group II were pre-treated with kaempferol for 14 days prior to infection. Mice in groups II to VI each were inoculated with blood containing *T.brucei brucei* (10^6 trypanosomes/ml of blood/animal) intraperitoneally (I.P). Following detection of parasitaemia, mice in group III were treated once with diminazene aceturate I.P. Mice in group IV were treated with diminazene aceturate once I.P, and then continued with kaempferol for nine days. Mice in group V were treated with kaempferol for nine days. Mice in group VI were given normal saline for nine days. The stress was assessed by determining the serum level of malondialdehyde, antioxidant and liver enzymes on day nine post-infection. The results obtained showed significant ($P < 0.05$) differences between the mean alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in groups II and VI. Superoxide dismutase, catalase and glutathione peroxidase increased significantly ($P < 0.05$) in groups III, IV and V.Serum level of malondialdehyde significantly ($P < 0.05$) increased in groups II and VI. It was observed that kaempferol possessed antitrypanosomal activity, probably due to its ability to scavenge free radicals generated during the course of infection.

Key words: Diminazene aceturate, Kaempferol, *Trypanosoma brucei brucei*

INTRODUCTION

Trypanosomosis is a disease caused by the protozoan parasite from genus Trypanosoma and transmitted by tse- tse fly (Glossina species) and other biting flies [1], making the incidence of the disease to be of great concern in the tropics [2]. The available means of control involves tsetse control, chemoprophylaxis, chemotherapy and use of trypanotolerant livestock [3]. At present, control of trypanosomosis is chiefly done by chemotherapy and chemoprophylaxis using drugs like Diminazine, Homidium, and Isometamidium [4, 5]. Diminazene aceturate is probably the most commonly used therapeutic agent for trypanosomosis in livestock in Sub-Saharan Africa [6]. However, complete dependence on drugs in many situations of trypanosomosis has been hampered in many areas by their toxic effects, high cost and frequent development of resistance to these drugs by the parasites. This is considered a very serious problem in trypanosomosis control in Africa [6]. In Nigeria, the occurrence of drug resistance to available trypanocides has been attributed to the presence of fake drugs, abuse of existing drugs and inadequate dosing of the drugs in trypanosomosis therapy [7]. The current challenge to the majority of African pastoralists is to optimize the use of the relatively old existing drugs [7]. In view of this, the use of drug combinations and new therapeutic regimes of existing trypanocides have been suggested [6].

Secondary metabolites in plants including flavonoids are responsible for a variety of pharmacological activities [8,9]. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds [9]. Antioxidants are important inhibitors of oxidative species generation and scavengers of free radicals. Kaempferol, a flavonoid and antioxidant, has good peroxynitrite scavenging activity when compared with other phenols and flavonoids [10, 11]. Melisa *et al* [12] reported that kaempferol isolated from the leaves of *Schimawallichii* at a concentration of 250 μM inhibited the growth of *Plasmodium falciparum* in a time-dependent manner both *in vivo* and *in vitro.* Kaemperol is also reported to be effective against hepatic fibrosis induced by schistosoma egg [13].

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. The various antioxidants exert their effect by scavenging superoxide, or by activating a battery of detoxifying/defensive proteins [14]. The prevention of oxidation is an essential process in all the aerobic organisms, as decreased antioxidant protection may lead to cytotoxicity [15]. Although, the precise molecular mechanism of action of flavonoid have not yet been demonstrated [16], there are various ways in which reactive oxygen species (ROS) can be produced. Flavonoids (e.g quercetin) can induce the production of superoxide anion, hydrogen peroxide, and other ROS [17]. ROS are generated in cells infected by pathogens to combat infection [16]. ROS can also be generated in response to some drugs, and the same principle works for certain antiprotozoan drugs in killing parasites in an infected cell [18].

In the present study, the efficacy of kaempferol, diminazene aceturate and their combination in stress induced by experimental *Trypanosoma brucei brucei* infection were evaluated.

MATERIALS AND METHODS

Experimental Animals

Thirty six adult swiss albino mice of either sexes weighing between 18 and 22 g were used in this study. These mice were reared in the animal house, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. These animals were housed in locally fabricated mice cages at room temperature, 25 ^{o}C. Wood shavings were used as beddings and changed once every week. The experimental mice were allowed free access to rat chow and water *ad-libitum*.

All animal experiments were carried out according to international guidelines as approved by the Postgraduate ethical committee of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

The Parasite

*Trypanosoma brucei brucei*were obtained from the National Veterinary Research Institute (N.V.R.I) Vom, Jos, Plateau State, Nigeria. The parasites were maintained by continuous

passage in a donor mouse. Parasitaemia was monitored by the use of wet mount viewed under × 400 magnifications [19].

Drugs, Sources and Preparation

Kaempferol was sourced from Whitehead Scientific (Pty) Limited, South Africa. It came with the following details: CAS number- 520-18-3, Catalog number -3603, EC number -208-287-6 and Batch number - 3. Diminazene aceturate was purchased from the Pharmacy unit of the Veterinary Teaching Hospital (VTH).

The drugs, kaempferol and diminazene aceturate, were dissolved in distilled water and administered to each mouse according to the body weight. The concentrations of kaempferol and diminazene aceturate used were 0.5 mg/mL and 3 mg/12.5 mL, respectively.

Experimental Infection of the Mice

Trypanosomes infected blood was obtained from the tail of the infected donor mice at the peak of parasitaemia (10^9) and used to maintain parasite suspension in phosphate buffer saline glucose solution, which was inoculated into peritoneal cavity of uninfected mice. The suspension contained 3 or 4 trypanosomes per microscopic field at × 100 magnification (approximately 10^6 trypanosomes per mL) as described by Ekanem and Yusuf [20]. The thirty six adult mice were randomly divided into six groups of six mice each and were treated as follows:

Group I- The mice in this group were neither infected with the parasites nor treated with any substance and therefore served as neutral control group.

Group II- Each mouse in this group was pre-treated individually with kaempferol (1 mg/kg *per os*) for 14 days. Thereafter, each mouse was infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/mL of blood i.p).

Group III- Each mouse in this group was infected with *Trypanosoma bruceibrucei* (10^6 trypanosomes/mL of blood i.p). After infection was established, each mouse was treated once with diminazene aceturate (3.5 mg/kg i.p) intraperitoneally. Animals in this group served as treated controls.

Group IV- Each mouse in this group was infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/mL of blood). After the establishment of infection, each mouse was treated with diminazene aceturate (3.5 mg/kg i.p) intraperitoneally, and then treated with kaempferol (1 mg/kg *per os*) for 9 consecutive days.

Group V- Each mouse in this group was infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/mL of blood i.p) and then treated with kaempferol (1 mg/kg *per os*) for 9 consecutive days.

Group VI- Each mouse in this group was infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/mL of blood i.p)and then administered normal saline at (5 ml/kg *per os*) for 9 consecutive days. Animals in this group served as untreated controls.

The parasitaemia in the infected and treated groups was monitored daily using the rapid matching counting method [19].

Serum Analyses

About 2.0 mL of blood was collected from each mouse and allowed to clot. The clotted blood was centrifuged at 1000 × *g* for 10 min. The supernant (serum) from each tube was decanted into clear test tube and was used to determine serum level of liverenzymes and markers of oxidative stress.

Determination of Liver Enzymes

Serum level of alanine amino transferase (ALT), aspartate amino trasferase (AST), and alkaline phosphatase (AP) were determined using chemical auto-analyzer (Bayer Clinical Chemistry Analyzer, Germany).

Determination of Serum Malondialdehyde Concentration

About 50 µL of serum from each animal were used to determine the level of serum malondialdehyde by double heating method as described by Draper and Hadley [21].

Determination of Serum Superoxide Dismutase (SOD) Specific Activity

The specific activity of SOD was measured using the method described by Misra and Fridovich [22].

Determination of Serum Catalase (CAT) Specific Activity

The specific activity of catalase in the serum collected from each mouse was determined using method described by Beers and Sizer [23].

Determination of Serum Glutathine Peroxidase (GPx) Specific Activity

A modified method of Paglia and Valentine [24] was used to determine glutathione peroxidase (GPx) activity spectrophotometrically.

This work was conducted in the Departments of Veterinary Pharmacology and Toxicology and Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University (A.B.U), Zaria, Kaduna State, Nigeria

Statistical Analysis

Data obtained were expressed as means ± standard errors of mean (S.E.M), subjected to one-way analysis of variance (ANOVA) and compared with Tukey post-hoc test. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Effects of the Treatments with Kaempferol and/or Diminazene Aceturateon the Activities of Liver Enzymes

Figure 1 shows the effects of treatments with kaempferol and/or diminazene aceturate on the activities of liver enzymes of mice infected with *T.brucei brucei.* There were significant ($P < 0.05$) increase in the mean of ALT, AST and ALP in mice administered with normal saline (group VI) when compared to those that were treated with diminazene aceturate only (groups III), kaempferol and diminazene aceturate (group IV) and kaempferol only (group V). Similarly, no significant ($P < 0.05$) difference was recorded in the mean ALT, AST and ALP in mice administered with normal saline (group VI) when compared to mice pre-treated with kaempferol (group II).

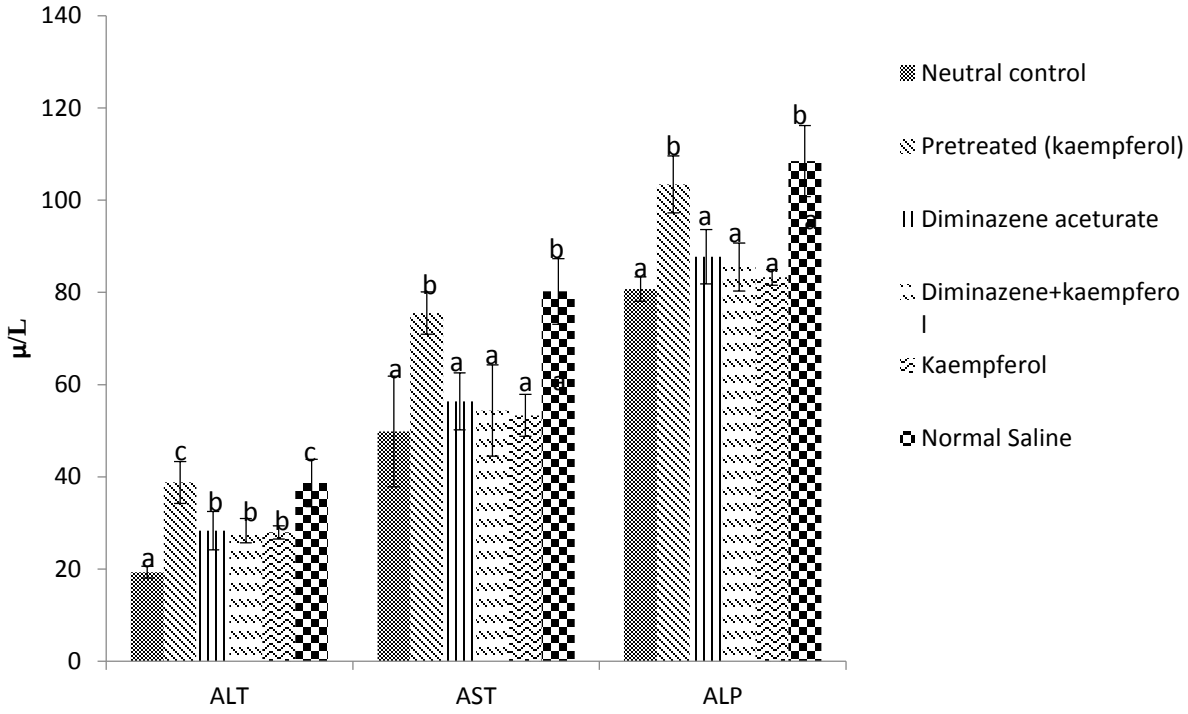

Figure 1: Effects of treatments with kaempferol and/ or diminazene aceturate on the activities of liver enzymes (ALT, AST and ALP) in *Trypanosoma brucei brucei* experimentally infected mice

Means with different letters differ significantly ($P < 0.05$). Values are expressed as mean ± SEM (6 animals per group).

Effects of Treatments with Kaempferol and/ or Diminazene Aceturate on the Serum Level of Malondialdehyde (MDA)

Figure 2 shows the effects of the treatments with kaempferol and/ or diminazene aceturate on the serum level of malondialdehyde of mice infected with *T. brucei brucei.* A significant ($P < 0.05$) increase in mean serum MDA concentration were recorded in mice pre-treated with kaempferol (group II) and those that were administered normal saline (group VI) when compared to mice diminazene aceturate only (group III), kaempferol and diminazene aceturate (group IV) and kaempferol only (group V).

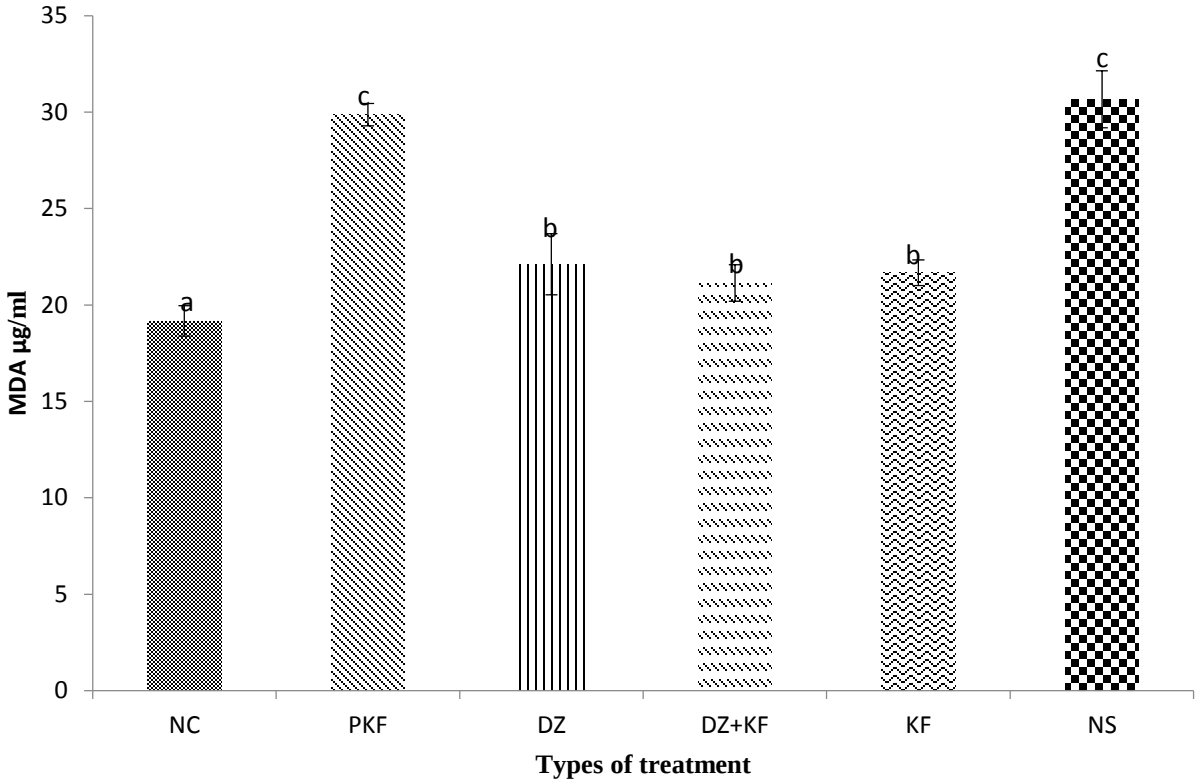

Figure 2: Effects of treatments with kaempferol and/ or diminazene aceturate on the serum level MDA concentration in *Trypanosoma brucei brucei*experimentally infected mice

Means with different letters differ significantly ($P < 0.05$). Values are expressed as mean ± SEM (6 animals per group).

Key**:** NC= Neutral control, DZ= Diminazene aceturate, PKf= pre-treated with kaempferol, DZ+Kf= Diminazene aceturate and kaempferol, Kf= kaempferol and NS= Normal saline

Effects of the Treatments with Kaempferol and/or Diminazene Aceturate on the Activities of Antioxidant Enzymes

Figure 3 shows the effects of the treatments with kaempferol and/or diminazene aceturate on antioxidant enzymes of mice infected with *T. brucei brucei.* There was significant ($P > 0.05$) decrease in the mean SOD, CAT and GPx in mice pre-treated with kaempferol (groups II) to untreated uninfected mice (group I). There were significant ($P < 0.05$) increase in the mean of

SOD, CAT and GPx in mice treated with diminazene aceturate only (groups III), kaempferol and were administered normal saline (group VI).

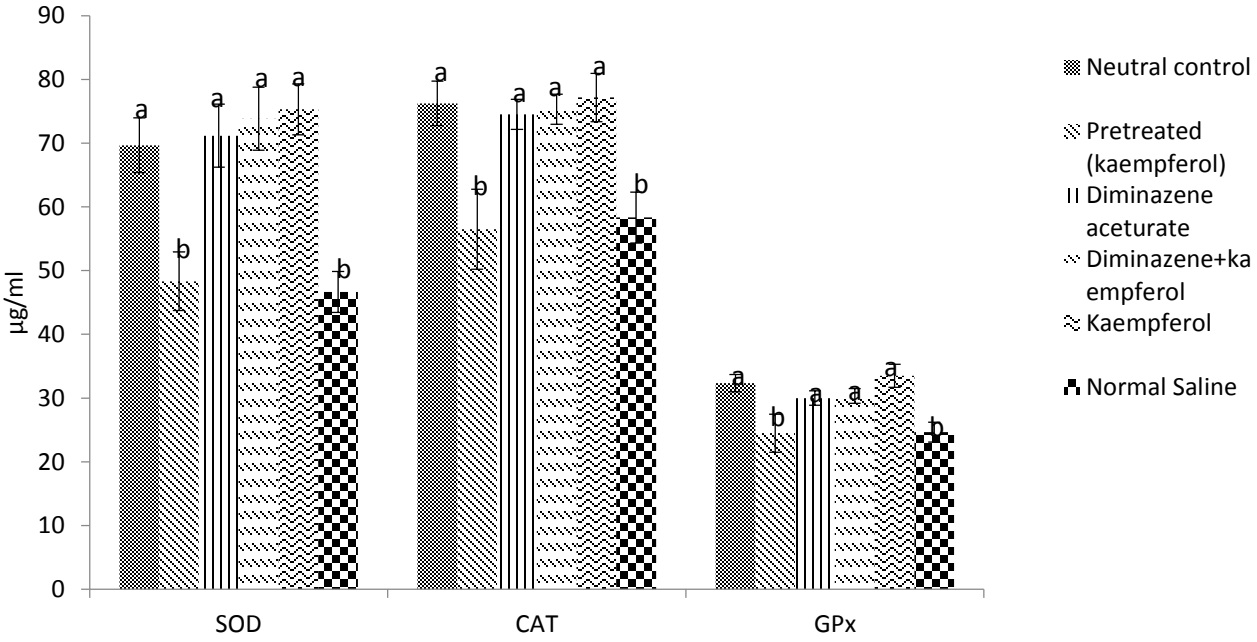

Figure 3: Effects of treatments with kaempferol and/ or diminazene aceturate on antioxidant enzymes (SOD, CAT and GPx) in *Trypanosoma brucei bruceibruceibrucei*experimentally infected mice

Means with different letters differ significantly ($P < 0.05$). Values are expressed as mean ± SEM (6 animals per group).

One of the end products of lipid peroxidation is MDA, which is measured by concentration of MDA in body tissues or serum [25]. These products of lipid peroxidation produce changes in the structures and functions of the cell membrane, leading to decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids [26]. The result of these changes on the surface of erythrocytes is increased erythrocyte osmotic fragility [27]. The increase in the concentration of MDA in normal saline group suggests an increase in the production of free radicals by *T. brucei brucei* resulting from imbalance

between radical generating and radical scavenging activities. This observation agrees with the findings of earlier workers [28]. The significant reduction in the serum level of MDA in mice treated with diminazene aceturate only, diminazene aceturate and kaempferol and kaempferol only when compared to those that were administered normal saline also agrees with the work reported by Kobo *et al* [29] who reported significant reduction in wistar rats infected with *Trypanosoma brucei brucei* and then treated with vitamin C. The effect was attributed to the action of *T. brucei brucei* on the erythrocyte membranes. Infection by *T. brucei brucei* may alter the host's antioxidant defence system against free radicals, which results in an imbalance between radical-generating and radical-scavenging activities and, hence, results in oxidative stress.

There was a significant increase in the levels of ALT, AST and ALP in mice pretreated with kaempferol and those that were administered normal saline. The elevated serum levels of ALT and AST in mice in this study is in agreement with earlier reports [30-32], in various trypanosome-infected animals. The elevation may be due to tissue death (necrosis) and inflammation in the host, particularly of the liver, heart, muscle and kidney [32, 33]. Another possibility of increased levels of ALT and AST is the lyses of RBCs by trypanosomes [32, 33] at different stages of the infection. Serum levels of ALT and AST are two of the most useful indications of liver cell injury, although the AST is less liver specific than is ALT [34]. Elevations of the AST level may also be seen in acute injury to cardiac or skeletal muscle [34]. Therefore, AST, ALT, and AP tests are useful in making a distinction between hepatocellular and damage in other organs [34]. *Trypanosoma brucei brucei* may induce stress, thereby weakening the immune system and eventually alter the serum biochemical parameters. Awobode [35] reported that many enzymes were found in the serum and the level of their activity can be determined, and that depression or elevation of particular enzyme indicates the presence of disease or damage to specific tissue or organ.

A significant ($P < 0.05$) increase in the serum level of specific antioxidants enzymes were recorded in mice treated with diminazene aceturate + kaempferol and kaempferol only when compared to those given normal saline. This finding agrees with the report of Kobo*et al* [36] in

rats with experimental *Trypanosoma brucei brucei* infection. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are enzymatic antioxidants that mop-up free radicals. SOD are the first line of defense against oxidative stress. It dismutates superoxide anion to H_2O_2 and water. The H_2O_2 generated from the activity of SOD will be detoxified to water and oxygen by CAT and GPx [36]. Hence, CAT and GPx are positioned in the second line of defence to scavenge free radical and ROS [37]. Trypanosomes were reported to induce oxidative stress and deplete the body antioxidants. Exogenous antioxidants such as Vitamin C and dimethyl sulphoxide gave a better clinical improvement and survival in *T.brucei brucei* infection in rats [37]. ROS are produced during normal cellular function. ROS include hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide. They are transient species due to their high chemical reactivity that leads to lipid peroxidation [16]. Under normal conditions, antioxidant systems of the cell minimize the perturbations caused by ROS. When ROS generation is increased to an extent that overcomes the cellular antioxidants, the result is oxidative stress [16].

Acknowledgement

We sincerely appreciate laboratory technologists of the Departments of Veterinary Pharmacology and Toxicology, Veterinary Parasitology and Entomology and Veterinary Pathology); especially the assistance of S. Musa, A. H. Ya'u Abdulwahab, A. Sani, D. Otie, Y. Idris, Mal. Yunusa and U. Lawal, is highly appreciated, who have contributed immensely to the success of this work.

References

1. Masocha, W., &Kristensson, K. (2012). Passage of parasites across the blood brainbarrier.*Virulence*,3(2): 202–12.
2. Anosa, V.O. (1983). Mammalian blood: cells in health and in trypanosomiasis. *Tropical Veterinary Journal*, 1, 177-199.
3. Muhammad, Y., Umar, A.M., Mohammed, H., Ahmed, G., Gumel, M.A. & Alhassan, M. (2017). Effect of Crude Methanol Extract of Senna occidentalis on Biomarkers

of Oxidative Stress of wistar Rats with Experimental Trypanosoma congolense Infection. *Journal of Pharmacognosy and Phytochemistry.* 6(5), 2699-2705.

4. Leach, T.M.& Roberts, C.J. (1981). Present status of Iranian Journal of Veterinary Research, Shiraz University chemotherapy and chemoprophylaxis of animaltrypano-somiasis in eastern hemisphere. *Journal Pharmacology and Therapeutics*, 13, 91-147.
5. ILRAD, Reports. (1990). Chemotherapy for trypanosomiasis.The International Laboratory for Research on Animal Trypanosomiasis in the Eastern Hemisphere. Nairobi, Kenya. *Pharmacology and Therapeutics*, 13, 91-147.
6. Geerts, S. and Holmes, P.H. (1998). Drug management and parasite resistance in animal trypanosomiasis in Africa.Programme Against African Trypanosomiasis (PAAT) Technicaland Scientific Series 1, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
7. Ezeokonkwo, R.C., Ezeh, I.O., Onunkwo, J.I., Obi, P.O., Onyenwe, I.W. & Agu, W.E. (2010).Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei bruceibrucei*. *Journal of Veterinary Parasitology*, 173, 48-54.
8. Mahomoodally, M. F., Gurib-Fakim, A. & SubrattY, A.H. (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius, *Pharmaceutical Biology*, 43(3), 237–242.
9. Pandey, A. K. (2007). Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Pariheniumhisterophorus*: an *invitro*study, *National Academy Science Letters*, 30 (11-12), 383–386.
10. Heijnen, C.G. M., Haenen,G.R.M.M., van Acker,F.A.A., van der Vijgh, W .J. · A. & Bast, A (2001). Flavonoids as peroxynitrite scavengers: The role of the hydroxyl groups. *Environmental Toxicology and Pharmacology*, 6, 55-8.
11. Santos, M. R. & Mira, L. (2004). Protection by flavonoids against the peroxynitrite-mediated oxidation of dihydrorhodamine. *Free Radical Research*, 38, 1011–1018

12.Melisa, I.B.,Eka, W.S., Ajeng, D., Toshimitsu, H., Junko, N., Anas, S. and Hiroshi, K. (2014).Antiplasmodial properties of kaempferol-3-*O*-rhamnoside isolated from the leaves of *Schimawallichii* against chloroquine-resistant *Plasmodium falciparum.Biomedical Reports*, 2(4), 579–583.

13. Zhou,Y-P., Zhang, S-L.& Cheng, D. (2013).Preliminary Exploration on Anti-Fibrosis Effect of Kaempferol in Mice with *Schistosoma Japonicum* Infection. *European Journal of Inflammation*, 11, 2213-17.

14. Cao, G., Sofic, E. & Prior, R.L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships, *FreeRadical Biology and Medicine*, 22, 749–760

15. Miura, Y.H., Tomita, I., Watanabe, T., Hirayama, T. & Fukui S. (1998). Active oxygens generation by flavonoids.*Biology and Pharmaceutical Bulletin*, 21,93–96.

16. Fonseca-Silva, F.,Job D. F.., Inacio, M.M., Canto-Cavalheiro. & Elmo Eduardo, A. (2011). Reactive Oxygen Species Production and Mitochondrial Dysfunction Contribute to Quercetin Induced Death in *Leishmaniaamazonensis*.*PLoS One*, 6(2), e14666.

17. Bischoff, S.C. (2008). Quercetin: potentials in the prevention and therapy of disease. *Current Opinion in Clinical Nutrition and Metabolism*, 11,733–740.

18. Mamani-Matsuda, M., Rambert, J., Malvy, D., Lejoly-Boisseau, H. & Daulouede, S. (2004). Quercetin induces apoptosis of *Trypanosoma brucei bruceigambiense* and decreases the proinflammatory response of human macrophages. *Antimicrobial Agents Chemotherapy*, 48:924–929.

19. Herbert, W.J.& Lumsden, W.H. (1976). *Trypanasoma brucei*: A rapid "matching" method for estimating the host's parasitaemia. *Experimental Parasitology*, 40,427–31.

20. Ekanem, J.T. and Yusuf, O.K. (2008). Some biochemical and haematological effects of black seed (Nigella sativa) oil on T. brucei-infected rats. *African Journal of Biomedical Research*. 11,79–85.

21. Draper, H.H. & Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation.*Methods in enzymology*, 186, 421-431.

22. Misra, H.P. & Fridivich, I. (1972).The role of superoxide union in auto-oxidation of epinephrine and sample assay for superoxide dismutase.*Journal of Biological Chemistry*, 247, 3170-3175.

23. Beers, R.F. & Sizer, I.W. (1952). A Spsectrophotometric method for measuring the breakdown of H_2O_2 by catalase.*Journal of Biological Chemistry*, 195, 133-140.

24. Paglia, D.E. & Valentine, W.N. (1967).Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Laboratory Clinical Medicine*, 70, 158-169.

25. Umar, I.A., Toma, I., Akombum, C.A., Nnaji, C.J., Mahdi, M.A. & Gidado A. (2010).The role of intraperitoneally administered vitamin C during Trypanosoma congolense infection of rabbits. African *Journal of Biotechnology*, 9,5224–8.

26. Kolanjiappan, K., Manoharan, S. &Kayalvizhi, M. (002). Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients.*ClinicalChimActa*, 326,143–9.

27. Saluja, P.S., Gupta S.L., Malhotra, D.V. &Ambawat, H.K. (1999).Status of plasma malondialdehyde in experimental T. annulata infection in crossbred bovine calves. *Indian Veterinarian*, 76,379–781.

28. Eze, J.I., Anene, B.M., Chukwu, C.C. (2008). Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index in Trypanosoma brucei - infected rats.*Comparative Clinical Pathology*, 17, 67–72.

29. Kobo,P.I., Ayo.J.O., Aluwong, T., Zezi, A.U., Maikai, V.& Ambali, S.F. (2014). Flavonoid mixture ameliorates increase in erythrocyte osmotic fragility and malondialdehyde concentration induced by *Trypanosoma brucei brucei*-infection in Wistar rats. *Research in Veterinary Science*, 96, 139–142.

30. Eghianruwa, K.I. (2012). The effect of supplemental antioxidants vitamin C and dimethyl sulfoxide on weight gain and survival in *Trypanosoma brucei bruceibrucei*infected and diminazene treated rats. *Veterinarski Archive*, 82, 519–529.

31. Srivastava, P., Yadav, N., Lella, R., Schneider, A., Jones, A. & Marlowe, T. (2012). Neem oil limonoids induces p53-independent apoptosis and autophagy. *Carcinogenesis*, 33, 2199-2207.

32. Taiwo, V.O., Olaniyi, M.O. &Ogunsanmi, A. O. (2003).Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosoma congolenseand Trypanosoma brucei brucei* infection in sheep. *Israel Journal of Veterinary Medicine.* 58(4), 435-443.

33. Takeet, M. I. & Fagbemi, B. O. (2009). Haematological, Pathological and Plasma biochemical Changes in rabbits experimentally infected with *trypanosoma congolense*, *Science World Journal*, 4, 2.

34. Murali, R.A. (2014). Liver Test Interpretation - Approach to the Patient with Liver Disease: A Guide to Commonly Used Liver Tests. *Journal Centre for Continuing Eeducation.*

35. Awobode, H. O. (2006). The biochemical changes induced by natural human African trypanosomes infections. *African Journal of Biotechnology*, 5(9), 738-42.

37. Mandal, A.K., Lee, P., Chen, J.A., Nillegoda, N., Heller, A., DiStasio, S., Oen, H., Victor, J., Nair, D.M., Brodsky, J.L. & Caplan, A.J.(2007). Cdc37 has distinct roles in protein kinase quality control that protect nascent chains from degradation and promote posttranslational maturation. *Journal of Cell Biology*, 176(3), 319-28.