Anti-Trypanosomal Activities of in Goats with Experimental *Trypanosoma brucei brucei* Infection

***Muhammad, Y[1]., Dahiru, M[1]., Muhammad, A.S[1] and Musa, B.S[1]**

[1]School of Agriculture, Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia, Jigawa State, Nigeria.
*Corresponding author: yamohad@gmail.com, Tel. +2347063249774

Abstract
The effect of kaempferol on level of parasitaemia in goats with experimental *Trypanosoma brucei brucei* infection was investigated. Thirty-six one year old goats both sexes weighing between 18 and 22 kilograms (kg) were randomly divided into six groups (I, II, III, IV, V and VI) of six goats each. Goats in group I were neither treated nor infected (un-infected, normal control). Goats in group II were pre-treated prophylactically with kaempferol (1 mg/kg per os) for 14 days prior to infection. Goats in groups II to VI each were inoculated with blood containing *Trypanosoma brucei brucei* (10^6 trypanosomes/ml of blood/animal) intravenous (I.V). Following detection of parasitaemia the infected goats (groups II to VI), goats in group III were treated once with diminazene aceturate (3.5 mg/kg) I.P only. Goats in group IV were treated with diminazene aceturate (3.5 mg/kg) once I.V, and then continued with kaempferol (1 mg/kg per os) for nine days. Goats in group V were treated with kaempferol (1 mg/kg per os) only for nine days. Goats in group VI were given normal saline (5 ml/kg per os) only for nine days. The level of parasitaemia increased progressively in mice pre-treated with kaempferol (group II) and those that were treated with kaempferol only (group V) and normal saline (group VI) up to day nine post-infection. The parasites were cleared completely from the blood stream of goats in group III group IV on day six post-infection. Although the onset of parasitaemia was not different from that in other groups, there was a significant ($P < 0.05$) reduction in the level of parasitaemia in goats treated with kaempferol only (group IV) when compared to goats pre-treated with kaempferol (group II) and those that were administered normal saline (group VI). In conclusion, kaempferol possesses antitrypanosomal activity and probably due to its ability to scavenge free radicals generated during the course of infection.

Key words: Goats, Kaempferol, Parasitaemia, *Trypanosoma brucei brucei*, Free-radicals

Introduction

Trypanosomosis is a disease caused by the protozoan parasite from genus Trypanosoma and trans mitted by the tse- tse fly (Glossina species) and other biting flies (Masocha *et al.,* 2012), making the incidence of the disease to be of great concern in the tropics (Anosa *et al*., 1983). The available means of control involves tsetse control, chemoprophylaxis, chemotherapy and use of trypanotolerant livestock (Muhammad *et al.,* 2017). In Nigeria, the occurrence of drug resistance to available trypanocides has been attributed to the presence of fake drugs, abuse of the existing drugs and inadequate dosing of the drugs in trypanosomosis therapy (Ezeokonkwo *et al.,* 2010).

Secondary metabolites in plants including flavonoids are responsible for a variety of pharmacological activities (Mahomoodally *et al.,* 2005; Pandey, 2007), recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds (Pandey, 2007). In present study, effect of kaempferol on level of parasitaemia, haematological parameters and biomarkers of oxidative Stress in goats with experimental *Trypanosoma brucei brucei* infection was evaluated.

Material and Methods

Location of the Research

This work was conducted in the School of Agriculture, Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia, Jigawa State, Nigeria.

Experimental Animals

Thirty-six one year old goats of either sex weighing between 18 and 22 kilograms were used in this study. They were reared in the animal complex of the Department of Animal Health and Production, Binyaminu

Usman Polytechnic Hadejia. They were managed under intensive system at room temperature, 25 ^{o}C. Wood shavings were used as beddings and changed once every week so as to prevent secondary infection. The experimental animals were allowed free access to feed and water *ad-libitum*. All animal experiments were carried out according to international guidelines as approved by the ethical committee of the Polytechnic.

The Parasite

Trypanosoma brucei brucei was 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 rats. Parasitaemia was monitored by use of wet mount viewed under × 400 magnifications (Herbert and Lumsden, 1976).

Drugs, Sources and Preparation

Kaempferol was sourced from whitehead scientific (pty) limited, South Africa, it came along 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 pharmacy unit of the Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia.

The drugs (kaempferol and diminazene aceturate) were dissolved in distilled water and administered to each goat 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 Goats

Trypanosomes infected blood was obtained from the tail of the infected donor rats at peak of parasitaemia (10^9) and used to maintain parasite suspension in phosphate buffer saline glucose solution, which was inoculated into jugular vein of uninfected goats. 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 (2008). The Thirty-six one-year old goats were randomly divided into six groups of six goats each and were treated as follows:

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

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

Group III- Each of the goat in this group was infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/ml of blood i.v). After infection was established, each goat was treated once with diminazene aceturate (3.5 mg/kg i.m) intramuscularly. Animal in this group served as treated controls.

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

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

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

Determination of Parasitaemia in goats

Parasitaemia was monitored in the goats from blood obtained from the jugular vein. The number of parasites were determined microscopically at × 400 magnification using rapid matching method (Herbert and Lumsden, 1976).

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

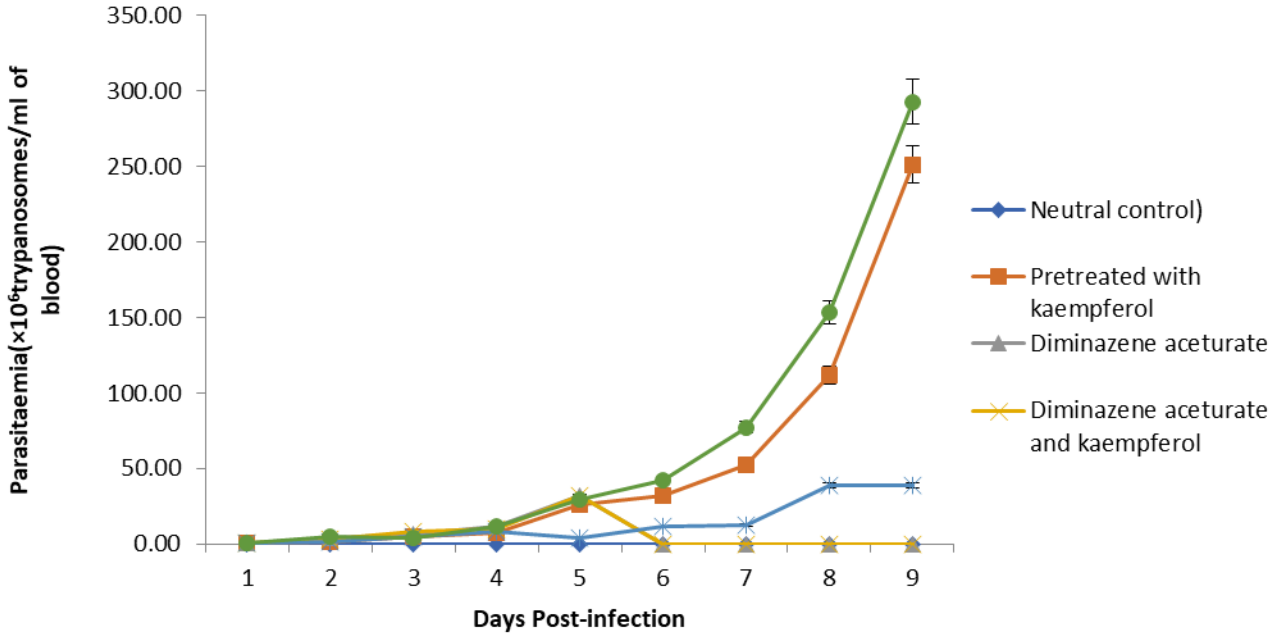

Figure 1: Effect of treatments with kaempferol and/or diminazene aceturate on the level of parasitaemia in *Trypanosoma brucei brucei* experimentally infected goats.

Discussions

There was a significant ($P < 0.05$) decrease in the parasite count in goats treated with kaempferol only when compared to goats pretreated with kaempferol and those that were treated with normal saline. Similarly, there was a progressive increase in parasitaemia in goats pre-treated with kaempferol and those that were treated with kaempferol and normal saline up to nine days post-infection. This result is consistent with that reported by Muhammad *et al* (2017) where mixture of two different flavonoids; reduced parasitaemia in goats infected with *Trypanosoma brucei brucei* by preventing the rate of the parasite proliferation. In addition, some flavonoids like kaempferol, vitamin E and carotenoids have ability to regenerate other antioxidants with known immune-enhancing activity (Pandey, 2007; Zhu *et al.*, 2013). This may explain why kaempferol was effective at reducing the level of parasitaemia in this study.

Acknowledgements

We sincerely appreciate TETFund for giving me the resources to carry out this research. Also our gratitude goes to the laboratory technologists of the Departments; Animal Health and Production, Binyaminu Usman Polytechnic, Hadejia, is highly appreciated, who have contributed immensely for the success of this work.

References

Anosa, V.O. (1983). Mammalian blood: cells in health and in trypanosomiasis. *Tropical Veterinary Journal*, 1: 177-199.

Ezeokonkwo, R.C., Ezeh, I.O., Onunkwo, J.I., Obi, P.O., Onyenwe, I.W. and Agu, W.E. (2010). Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. *Journal of Veterinary Parasitology*, 173: 48-54.

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

Mahomoodally, M. F., Gurib-Fakim, A., and SubrattY, A.H. (2005). "Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius," *Pharmaceutical Biology*, 43(3): 237–242.

Muhammad, Y., Umar, A.M., Mohammed, H., Ahmed, G., Gumel, M.A. and Alhassan, M. (2022). 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. E-ISSN: 2278-4136 P-ISSN: 2349-8234.

Pandey, A. K. (2007). "Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Pariheniumhisterophorus*: an *in vitro* study," *National Academy Science Letters*, 30 (11-12): 383–386.

Zhu, 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.