AHC -04

Ameliorative Effects of Kaempferol on Erythrocyte Osmotic Fragility induced by Experimental *Trypanosoma brucei* Infection in Mice

Y. Muhammad[1], M.M. Suleiman[2], I.D. Jatau[3] and A.M. Umar[1]
[1]Department of Animal Health and Production, School of Agriculture, Binyaminu Usman Polytechnic, Hadejia,Nigeria
[2]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, A.B.U Zaria, Kaduna, Nigeria
[3]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, A.B.U Zaria, Nigeria
Corresponding author: Y. Muhammad; E-mail: yamohad@gmail.com, Tel. +2347063249774

Abstract
The interest in the possible health benefits of antioxidants has increased owing to their free radical scavenging activities. The present study was aimed at evaluating the effects of kaempferol, diminazeneaceturate and their combination on erythrocyte osmotic fragility (EOF) induced by experimental *Trypanosomabrucei brucei* infection in mice. Thirty six mice were randomly divided into six groups of six mice each. Mice in group I were neither infected nor treated and served as neutral controls. All mice in groups II to VI were individually infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/ml of blood) intra-peritoneally (I.P) and then treated with kaempferol and/or diminazeneaceturate. Mice in group VI were infected and administered distilled water (DW) only (5 mL/kg). The results showed revealed that EOF reduced significantly ($p< 0.05$) in mice treated with kaempferol only (group V), diminazeneaceturate only (group III) kaempferol and diminazeneaceturate (group IV) when compared to infected untreated mice (group VI).The treatment with kaempferol and or/ diminazeneaceturate ameliorated EOF induced by *Trypanosoma brucei brucei* in mice.

Key words: Mice, erythrocyte osmotic fragility, kaempferol, *Trypanosoma brucei brucei*

Introduction

African trypanosomosis is associated with oxidative stress because oxidative products are generally known to attack the cellular integrity of erythrocytes during trypanosomosis (Anosa & Kaneko, 1983; Igbokwe, 1994; Umar *et al.,* 2007). They particularly attack erythrocyte membrane polyunsaturated fatty acids and proteins (Slater, 1984) or red blood cells directly leading to oxidative haemolysis (Igbokwe, *et al.,* 1994; Umar *et al.,* 2007).Kaempferol has a good free radicals scavenging activity when compared with other phenols and flavonoids (Hoet*et al.,* 2001; Santos and Mira, 2004).

The present study was aimed at evaluating the effect of kaempferol on erythrocyte osmotic fragility and biochemical changes induced by experimental *Trypanosoma brucei brucei* infection in mice.

Materials and methods

Thirty six adult Swiss albino mice of either sex weighing between 18 and 22 grams were used. They were obtained from the Animal House, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.*Trypanosoma brucei brucei* (Federi strain) used for this study was obtained from Nigerian Institute for Trypanosomosis Research, Vom, Plateau State, Nigeria. The parasite was maintained by serial passages in donor mice. The infected blood was collected from the tail of donor mouse at peak parasitaemia and diluted with phosphate buffered saline, pH 7.2. The mice were infected intraperitoneally with 0.2 ml of infected blood from the donor mouse containing 1.0×10^6*T. b. brucei*.

Treatment of experimental animals

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 served as untreated control.
Group II- Mice in this group were pre-treated individually with kaempferol (1 mg/kg) for 14 days. Thereafter, each mouse was infected with *Trypanosoma brucei brucei* (10^6 trypanosomes/ml of blood).
Group III- Each mouse in this group was infected with*Trypanosoma brucei brucei* (10^6 trypanosomes/ml of blood). After infection has been established, each mouse was treated once with diminazeneaceturate (3.5 mg/kg) intra-peritoneally.
Group IV- Mice in this group were infected with *Trypanosoma brucei brucei*(10^6 trypanosomes/ml of blood). After the establishment of infection, each mouse was treated with diminazeneaceturate (3.5 mg/kg) intra-peritoneally, and then treated with kaempferol (1 mg/kg) for 9 consecutive days.
Group V- Mice in this group were infected with *Trypanosoma brucei brucei*(10^6 trypanosomes/ml of blood) and then treated with kaempferol (1 mg/kg) for 9 consecutive days.

Group VI- Mice in this group were infected with *Trypanosoma brucei brucei*(10^6 trypanosomes/ml of blood)and then treated with distilled water at (5 ml/kg) 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 (Herbert and Lumsden, 1976).Erythrocyte osmotic fragilty of blood from the treated mice was determined using method described by Faulker and King (1970) and modified by Oyewale (1987).

Data obtained were expressed as mean ± SEM and subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post-hoc test, using Graph Pad Prism version 5.0 for windows (Graph Pad Software, San Diego, California, USA). Values of $P < 0.05$ were considered significant.

Results and Discussion

Figure I shows the effect of the treatment on erythrocyte osmotic fragility. At 0.3, 0.5, 0.7 and 0.9 % NaCl concentrations, the mean percentage of red blood cell haemolysis increased significantly ($P < 0.05$) in infected untreated mice(group vi) when compared to mice treated with diminazeneaceturate (group ii), diminazeneaceturate and kaempferol (group iv) and kaempferol only (Group v).

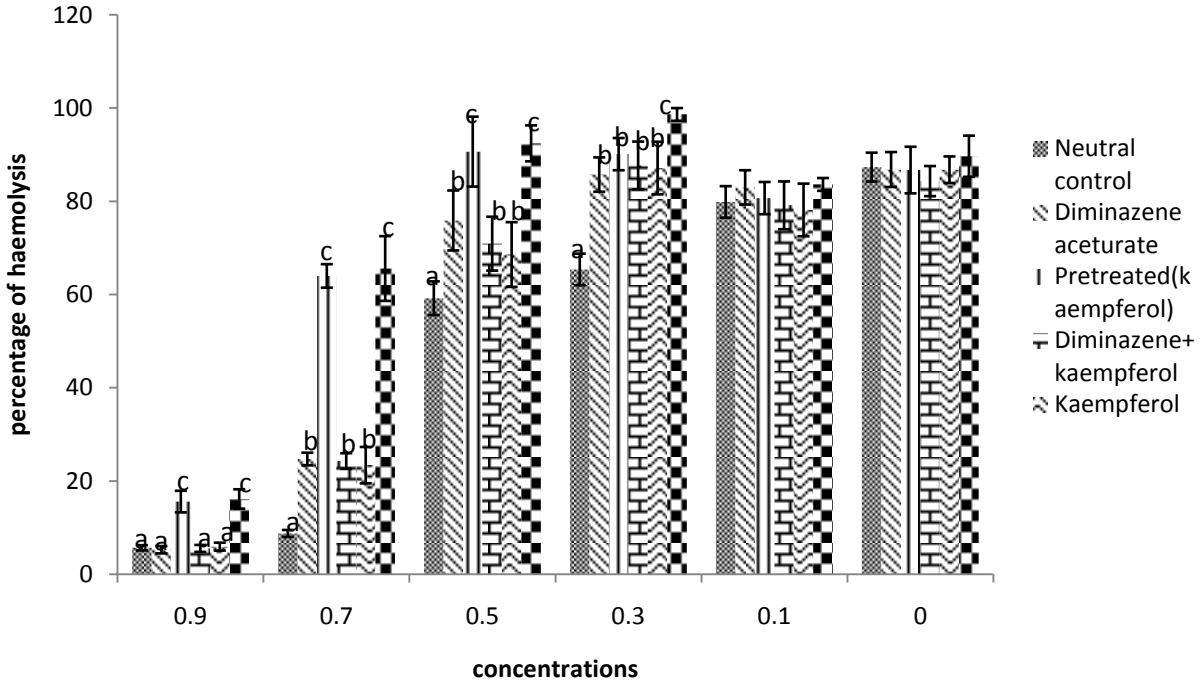

Fig.I: Effects of treatments with kaempferol and/or diminazeneaceturate on erythrocyte osmotic fragility in *Trypanosoma brucei brucei* experimentally infected mice. Means with different superscript letters are statistically significant ($p< 0.05$).

The significant ($p< 0.05$) reduction in the mean percentage of haemolysis in mice treated with diminazeneaceturate (group iii), diminazeneaceturate and kaempferol(group iv) and kaempferol only(group v) when compared to infected untreated mice (group vi) and those that were pre-treated with kaempferol group ii)agrees with the work of Kobo *et al* (2014), which showed the ameliorative effects of flavonoid mixture (hesperidin and daflon®) on erythrocyte osmotic fragility in rats infected with *T. b. brucei*, and the effect was attributed to the scavenging effect of antioxidant on free radicals that are detrimental to erythrocyte membrane. Lipid peroxidation in the major finding in animals with trypanosomosis, and by products of lipid peroxidation are known to decrease membrane fluidity, increase membrane permeability, inactivate membrane bound enzymes and loss of essential fatty acid which eventually increase erythrocyte osmotic fragility (Zhu *et al.,* 2000; Mbaya *et al.,* 2012)

Conclusion

Kaempferol has significantly reduced erythrocyte osmotic fragility and lipoperoxidative changes induced by *T. brucei brucei* in this study.

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