

Effects of Kaempferol on oxidative Stress Biomarkers in Goats Experimentally Infected with *Trypanosoma brucei brucei*

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Abstract

The effects of kaempferol on biomarkers of oxidative Stress in goats with experimental *Trypanosoma brucei brucei* infection was evaluated. Thirty-six 1 year old goats both sexes weighing between 18 and 22 kilograms (kg) were randomly divided into 6 groups (I, II, III, IV, V and VI). 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* (10⁶ trypanosomes/ml of blood/animal) intravenous (I.V). Following detection of parasitaemia, 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 9 days. Goats in group V were treated with kaempferol (1 mg/kg per os) only for 9 days. Goats in group VI were given normal saline (5 ml/kg per os) only for 9 days. The stress induced by *Trypanosoma brucei brucei* was assessed by determining the serum level of malondialdehyde and antioxidant enzymes on day 9 post-infection. The results obtained showed significant ($P < 0.05$) increase in the serum level of malondialdehyde in groups II and VI when compared to groups III, IV and V. Antioxidant enzymes; superoxide dismutase, catalase and glutathione peroxidase increased significantly ($P < 0.05$) in groups III, IV and V when compared to groups II and VI. In conclusion, kaempferol possesses antitrypanosomal activity and probably due to its ability to scavenge free radicals generated during the course of infection and boost goats immunity.

Key words: Catalase, Goats, Glutathione peroxidase, Kaempferol, Malondialdehyde, *Trypanosoma brucei brucei*, Superoxide dismutase,

Introduction

Pathogenic trypanosome infections of domestic animals are still a major scourge in sub-Saharan Africa and they largely account for the low livestock productivity of the continent (Umar *et al.*, 2001), thus making it an important priority for biomedical and public agencies, agricultural sector and the scientific community (Kuriakose *et al.*, 2012). The disease caused by the *Trypanosoma brucei* subgroup is associated with anaemia, hepatocellular degeneration and glomerulonephritis (Umar *et al.*, 2019) which is largely attributed to the large amount of free radicals and superoxides generated by the trypanosomes that attack membrane polyunsaturated fatty acids and proteins, resulting in cellular injuries and consequently affecting vital tissues and organs of the infected animals (Umar *et al.*, 2010).

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 of a battery of detoxifying/defensive proteins (Cao *et al.*, 2020). The prevention of oxidation is an essential process in all the aerobic organisms, as decreased antioxidant protection may lead to cytotoxicity (Miura, *et al.*, 2021). Reactive Oxygen Species (ROS) are generated in cells infected by pathogens to combat infection (Fonseca-Silva *et al.*, 2021). 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 (Mamani-Matsuda *et al.*, 2022).

for production of antitrypanosomal vaccine in the near future because of antigenic variation exhibited by the parasites.

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 °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 $\times 400$ 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 $\times 100$ 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 Serum Malondialdehyde Concentration

Fifty microlitres of serum from each animal were used to determine the level of serum malondialdehyde by double heating method as described by Draper and Hadley (1990).

Determination of Serum Superoxide Dismutase (SOD) Specific Activity

The specific activity of SOD was measured using the method described by Misra and Fridovich (1972).

Determination of Serum Catalase (CAT) Specific Activity

The specific activity of catalase in the serum collected from each goat was determined using method described by Beers and Sizer (1952).

Determination of Serum Glutathione Peroxidase (GPx) Specific Activity

Modified method of Paglia and Valentine (1967) was used to determine glutathione peroxidase (GPx) activity spectrophotometrically.

Statistical Analyses

Data obtained were expressed as means $\pm$ 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

Table 1: Mean $\pm$ SEM of serum biomarkers of oxidative stress parameters in goats infected with *Trypanosoma brucei brucei* and treated with Kaempferol and/ or diminazene aceturate

Test	Treatments					
	NC	PKF	DZ	KF+DZ	KF	NS
SOD	69.67 $\pm$ 4.302 ^a	48.33 $\pm$ 3.442 ^b	71.17 $\pm$ 4.615 ^a 73.50.5 $\pm$ 6.29	75.83 $\pm$ 4.936 ^a	75.31 $\pm$ 3.992 ^a	46.67 $\pm$ 3.211 ^b
CAT	72.67 $\pm$ 3.474 ^a	46.5 $\pm$ 3.085 ^b	^a	75.33 $\pm$ 2.362 ^a	77.17 $\pm$ 3.790 ^a	48.33 $\pm$ 3.981 ^b
GPx	32.33 $\pm$ 1.358 ^a 19.17 $\pm$ 0.7923	24.15 $\pm$ 1.478 ^b	30 $\pm$ 2.989 ^a	30.33 $\pm$ 1.145 ^a 21.13 $\pm$ 0.9458	33.5 $\pm$ 1.803 ^a 21.67 $\pm$ 0.6667	24.67 $\pm$ 1.544 ^b 30.67 $\pm$ 1.476 ^c
MDA	^a	29.87 $\pm$ 1.579 ^b	22.11 $\pm$ 0.577 ^b	^b	^b	

Discussions

A significant ($P < 0.05$) increase in the serum level of specific antioxidants enzymes were recorded in goats treated with diminazene aceturate and kaempferol and kaempferol only when compared to those given normal saline. This finding agrees with the report of Koo *et al* (2014) 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. One of the end products of lipid peroxidation is MDA, which is measured by concentration of MDA in body tissues or serum (Umar *et al.*, 2010). 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 (Kagira *et al.*, 2022). The end result of these changes on the surface of erythrocytes is increased erythrocyte osmotic fragility (Samhan-Arias *et al.*, 200220). 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 (Eze *et al.*, 2008). The significant reduction in the serum level of MDA in goats 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* (2016) who reported significant reduction in goats 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, the results in oxidative stress.

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