Circulating oxidative stress caused by *Psoroptes natalensis* infestation in Indian water buffaloes

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Abstract The present study reports the circulating oxidative stress associated with *Psoroptes natalensis* infestation in Indian water buffaloes. Three non-descriptive water buffaloes, age ranging between 4 and 9 years, presented to Referral Veterinary Polyclinic, IVRI, for treatment served as clinical subject. The infested animals were treated with Ivermectin subcutaneously and Amitraz topically along with antioxidant like ascorbic acid, Vitamin E and selenium. The level of lipid peroxidase was significantly higher (3.94 ± 0.34) in *Psoroptes* infested buffalo and was reduced significantly ($P \leq 0.05$) after treatment (1.56 ± 0.40). The significantly higher levels of MDA before treatment signify the role of lipid peroxide mediated skin lesions in *P. natalensis* infested buffaloes. Similarly the activities of the body antioxidant like GSH and CAT were significantly higher ($P \leq 0.05$) after treatment. The less level of the body antioxidant (GSH) and reduced activities of the antioxidant enzymes like CAT and SOD before treatment imply that *Psoroptes* mite-infested buffaloes were in a state of significant oxidative stress. The study provides information on oxidative stress indices in *P. natalensis* infested buffaloes and gives additional insight regarding the pathogenesis of the disease and its management.

Keywords Buffalo · Oxidative stress · *Psoroptes natalensis*

Introduction

Psoroptes natalensis is one of the emerging ectoparasite causing dermatitis in buffaloes in Indian sub-continent (Sreedevi et al. 2010). Dermatitis caused by *P. natalensis* causes severe economic losses due to weight loss, decreased milk production and increased susceptibility to other diseases in affected animals (Richard and Karin 2006). Free radicals and oxidative stress have been implicated to play an important role in the pathogenesis of various allergic and inflammatory skin diseases in human beings (Okayama 2005). Skin, being the first and the outermost barrier of the body, is exposed to both endogenous and exogenous pro-oxidants (Cross et al. 1998).

It is a well known fact that, whenever there is an over production of reactive oxygen species (ROS) than that of the antioxidant defense mechanism of the body, these free radicals then interact with endogenous macromolecules leading to alteration in cellular functions (Muthukumaran et al. 2008). Free radicals induced adverse effects on the skin includes edema, erythema, wrinkling, inflammation, autoimmune reaction, hypersensitivity and keratinization abnormalities (Bickers and Athar 2006).

Body combat against excess free radicals via antioxidant defense system which comprises of enzymes, like superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and nutritional antioxidants, such as ascorbic acid (vitamin C), zinc, copper, α -tocopherol (vitamin E) and ceruloplasmin. To our knowledge, until date there is no estimation of the oxidative stress status in buffalo suffering from *P. natalensis*. The present study was

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aimed to evaluate the status of oxidative stress indices in blood of Indian water buffalo naturally infested with *P. natalensis* and its management.

Materials and methods

Clinical subjects

The study was conducted on three non-descriptive female water buffaloes age ranging between 4 and 9 years which were naturally infested with *P. natalensis* and were presented to Referral Veterinary Polyclinic, IVRI, for treatment.

Skin scraping

Skin scrapings from 3 to 4 different areas were collected both before and after treatment from the affected animals and were treated with 10% potassium hydroxide (KOH) before examination under microscope and mites were identified on the bases of morphological characteristic as suggested by (Soulsby 1982).

Blood samples

Blood samples were collected both before and after treatment from jugular vein in a glass tubes containing ethylenediamine tetra acetic acid dipotassium salt (EDTA) for routine haematological and biochemical parameters and in heparin for oxidant/antioxidant indices.

Treatment

The treatment in infested animals was instituted with Ivermectin @200 mcg/kg body weight subcutaneously, vitamin E and selenium @10 ml IM, and solution Amitraz for topical application. All medications were given once at weakly interval.

Biochemical assay

Erythrocytic lipid peroxides (LPO) were estimated as described by Placer et al. (1966). The concentration of malonaldehyde (MDA) in nanomoles per millilitre of erythrocytic hemolysate was derived using 1.56×10^5 L mol/cm as extinction coefficient (Utley et al. 1967). The Hb in the hemolysate was estimated by the cyan-methemoglobin method and the LPO concentration in the erythrocytes was expressed in nmol MDA/mg Hb. The glutathione (GSH) level in erythrocytic hemolysate was determined according to the method of Prins and Loos (1969). The superoxide dismutase (SOD) activity was determined by the method of

Menami and Yoshikawa (1979) which is a modification of the method given by Marklund and Marklund (1974). Each unit of SOD activity is defined as the quantity of enzyme that inhibited autooxidation of pyrogallol by 50% under suitable experimental conditions. Activity was expressed as units/mg Hb/ml. The activity of catalase (CAT) was determined from RBC hemolysate as described by Cohen et al. (1970). Decomposition of H_2O_2 was followed directly by the decrease in absorbance per min/mg Hb and it was taken as a measure of the CAT activity. Modified Biuret and Duma's method (Varley 1980) was used for estimating total protein and albumin using commercial kit supplied by M/S Span Diagnostic Limited (Surat) India.

Statistical analysis

The results were reported as mean $\pm$ SE for both pre and post therapy and data were analyzed statistically using independent and paired *t* tests. $P \leq 0.05$ was considered as statistically significant (Snedecor and Cochran 1994).

Results and discussion

In the present study animals had very distinct and characteristic lesions of superficial dermatitis comprising of alopecia, pruritis, and small crusts formation in neck, shoulder and rump region. On microscopic examination the skin scarping were found positive for mite and identified morphologically as *P. natalensis* (Soulsby 1982). Similarly Sreedevi et al. (2010) reported *P. natalensis* infestation in buffalo manifested as severe skin lesions distributed all over the body in form of alopecia, thickened skin, dry exudative crusts on the upper neck, face, upper eyelids, poll and ears.

Oxidative stress is believe to be a major contributor in genesis of skin diseases and is considered as integral part in patho-physiology of skin diseases expressed as erythema, edema, wrinkling, hypersensitivity, keratinization and cancer (Portugal et al. 2007). Oxidative stress is a situation where the production of endogenous free radicals exceeds capacity of antioxidant defense of the body resulting in molecular disruption and tissue damage (Niki 2009). The measurement of activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) are appropriate indirect ways to assess the status of antioxidant defense and estimation of malondialdehyde (MDA), a byproduct of lipid-peroxidation, continues to be a reliable method to assess the degree of oxidative damage to cell membrane (Gurer et al. 1998).

In the present study on day 0 that is before treatment (3.94 ± 0.34), the levels of lipid peroxides (LPO) were significantly higher ($P \leq 0.05$) when compared post

Table 1 Comparison of the markers of oxidative stress in buffaloes infested with *P. natalensis* before and after treatment (mean $\pm$ SE)

Parameters	LPO (nmol MDA/mg Hb)	GSH (μ mol/mg Hb)	CAT (U/mg Hb)	SOD (U/mg Hb)
Before treatment	3.94 $\pm$ 0.34	0.18 $\pm$ 0.02	1.11 $\pm$ 0.19	1.10 $\pm$ 0.45
After treatment	1.56 $\pm$ 0.40*	0.34 $\pm$ 0.01*	4.92 $\pm$ 0.59*	2.00 $\pm$ 0.20

* Differ significantly ($P \leq 0.05$)

treatment (1.56 $\pm$ 0.40). Lipid per oxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxidation can be harmful for skin due to alterations in the structure and permeability (Portugal et al. 2007). The significantly higher levels of MDA before treatment signify the role of lipid peroxide-mediated skin lesions in *P. natalensis* infested buffaloes.

Similarly in the present study the activities of the body antioxidant like GSH and enzymes CAT were significantly higher ($P \leq 0.05$) after treatment whereas there is non-significant increase in activity of SOD post treatment as detailed in Table 1. The less level of the body antioxidant GSH and reduced activities of the antioxidant enzymes like CAT and SOD before treatment imply that *P. natalensis* infested buffaloes were in a state of significant oxidative stress. Similarly oxidative stress have been demonstrated in various species associated with sarcoptic mange (Camkerten et al. 2009; De and Dey 2010; Saleh et al. 2011) and psoroptic mange (Kanbur et al. 2008).

The hematological values like neutrophil, TLC and lymphocytes count varies significantly before and after treatment of the infested buffaloes where as there was no significant change in Hb, PCV and TEC before and after treatment as detailed in Table 2.

Table 2 Hematological and biochemical values of buffaloes infested with *P. natalensis* before and after treatment (mean $\pm$ SE)

Parameters	Before	After
Hemoglobin (gm/dl)	11.6 $\pm$ 0.52	12.13 $\pm$ 0.29
Total erythrocytes count ($\times 10^6$ μ l)	6.93 $\pm$ 1.88	7.62 $\pm$ 0.58
Packed cell volume (%)	40.4 $\pm$ 1.14	40 $\pm$ 1.15
Total leukocyte count ($\times 10^3$ μ l)	7.05 $\pm$ 0.67	7.5 $\pm$ 0.66*
Neutrophil (%)	27.67 $\pm$ 2.40	37 $\pm$ 0.58*
Lymphocyte (%)	65.67 $\pm$ 2.19	57.67 $\pm$ 0.67*
Monocyte (%)	2 $\pm$ 0.58	1.67 $\pm$ 0.33
Eosinophil (%)	4.67 $\pm$ 0.88	3.67 $\pm$ 0.33
Total protein (gm/dl)	5.07 $\pm$ 0.44	5.4 $\pm$ 0.2
Albumin (gm/dl)	2.67 $\pm$ 0.27	3.2 $\pm$ 0.12
Globulin (gm/dl)	2.4 $\pm$ 0.42	2.07 $\pm$ 0.27
A:G ratio	1.18 $\pm$ 0.22	1.49 $\pm$ 0.20

* Differ significantly ($P \leq 0.05$)

The marked neutropenia in present study before treatment reflect suppression of bone marrow by serum factors related to *P. natalensis* infestation. Similar finding was observed by Ramprabhu et al. (2001) in a case of concurrent sarcoptic and psoroptic mange infestation in buffaloes. Whereas eosinophilia may due to antigen antibody interaction in tissues with abundant mast cells, such as skin, as well as to prolonged intimate host-parasite control (Duncan and Prasse 1979).

Antioxidant vitamins, such as vitamins E and C, act by quenching singlet oxygen and prevent free radical formation. Their supplementation in diseases associated with oxidative stress hastens clinical recovery (Morita et al. 2006). Vitamin E is considered the first line of defense against lipid peroxidation, protecting polyunsaturated fatty acids in cell membranes from the free radical attack.

The present paper provides information on oxidative stress indices in *P. natalensis* infested buffaloes and gives additional insight regarding the pathogenesis of the disease and its management.

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