Original Research Article

Vitamin E Ameliorates the Mineralo-Oxidative Stress of Sucking Lice Infestation in Indian Water Buffalo

E. Madhesh1*, Umesh Dimri1, Y. Ajith1, S. Shanmuganathan2, P. Sivasanker3, R. Karthikeyan4, Alok Kumar Choudhary1 and K. Kavitha1

1Division of Medicine, 2Division of Virology, 3Division of Microbiology, 4Division of Biochemistry, ICAR-Indian Veterinary Research Institute, Izatnagar, UP, India-243122

*Corresponding author

A B S T R A C T

Bubaline pediculosis is a common and economically important ectoparasitic condition caused by sucking lice, Haematopinus tuberculatus. This condition in buffalo is characterized by anemia, mineral imbalance, and loss of production performance but its pathobiology is explored less. Aim of the present study was to explore the mineralo-oxidative pathobiology of bubaline pediculosis and its amelioration using the two antioxidants as an adjunct therapeutic modality (N-acetyl cysteine and Vitamin E). Twenty four buffaloes, severely infested with sucking lice (mean total lice count more than 100) were divided into three groups (Group I, II and III) with eight animals per group; another eight animals (Group IV) free from any clinical abnormalities and ectoparasitism were included as healthy control. Lice infested animals (Group I, II and III) animals were given ivermectin therapy (200 µg/kg body weight SC single dose); additionally, group II and group III animals were orally treated with N-acetyl cysteine (12 mg/kg body weight) and vitamin E (Tocopheryl acetate 4000 mg per animal), respectively, once daily for 14 days. The haematological parameters (TEC, Hb, TLC and DLC), oxidant-antioxidant profile (TAC, LPO, GSH, and SOD) and mineral profile (Zinc, Iron, Magnesium, and Copper) were studied on before therapy (Day 0) and post therapy (Day 28). Bubaline pediculosis revealed severe oxidative stress and mineral imbalance along with remarkable anaemia and leukocytosis. Vitamin E given animals demonstrated better recovery from the mineralo-oxidative pathology of bubaline pediculosis and approached post therapeutic normalcy. Vitamin E as an adjunct therapy along with ivermectin alleviated the pathobiological damages in the host system and speed up the clinical recovery, whereas, N-acetyl cysteine was less effective in quenching the mineralo-oxidative response. Future studies on antioxidants therapy may target the influence of mineralo-oxidative response in the host system.

Keywords
Vitamin E, Bubaline pediculosis, Oxidative stress, Mineral balance, N-acetyl cysteine

Accepted: 12 June 2019
Available Online: 10 July 2019

Introduction

Buffaloes considered as “black gold of India”; because buffaloes provide high quality protein, fat rich milk, serve as a source of employment and livelihoods to millions of landless, marginal, and small farmers (Syed Mohmad and Manmohan Singh, 2017).
Ectoparasitism adversely affects the welfare and production performance farm animals. Worldwide buffaloes were commonly infested with sucking lice, *Haematopinus tuberculatus* especially during the winter season can affect cattle also (Bastianetto and Leite, 2005; Egri, 2019; Mamun et al., 2010). Lice infestation in the animal was favored by the risk factors like lack of hygiene, low health status, low temperature-humid weather condition (Taylor et al., 2016). Sucking lice are sebaceous secretion feeders and bloodsuckers, which are less movable and remain tightly attached to the skin of host for a prolonged time. Lice induce hypersensitivity hence, severe *H.tuberculatus* infestations can result in varying levels of scratching, self-excoriation, alopecia, and papulo - crustous dermatitis (Chaudhry, 1978; Egri, 2019; Taylor et al., 2016). Recent findings revealed that inflammation and reactive oxygen species (ROS) induced tissue damage to play an important role in ectoparasitism induced deprivation of host health (Dimri et al., 2010).

Oxidative stress is defined as an imbalance between the production of the oxidants or free radicals and the ability of the body to quenching of their harmful effects through anti-oxidant defense system. The free radicals readily induces oxidative damage to various biomolecules including proteins, lipoprotein, lipids, and DNA and cause a disturbance in normal cellular signaling pathways and homeostasis (Farber, 1994; Kaur et al., 2006; Lykkesfeldt and Svendsen, 2007). Even, ectoparasiticides like cypermethrin, deltamethrin, and flumethrin usage in rats induce a significant oxidative stress (Dubey et al., 2013; Ince et al., 2013; Kanbur et al., 2010). The biomodulators use in the therapeutic management of various diseases is getting universal acceptance because of their vast scope in amelioration of a wide range of diseases conditions by altering the oxidative status. Generally, antioxidants are reducing agents belonging to the class such as thiols, ascorbates and polyphenols that can neutralize ROS and thereby inhibits the oxidation of other biomolecules. The recent finding suggests that antioxidants play a role in immunomodulation (Ajith et al., 2017). N-Acetyl Cysteine (NAC) has been used for the treatment of acute and chronic bronchitis as a mucolytic agent for many years and is an antidote for acetaminophen poisoning. Fat-soluble vitamin E has an antioxidant property through which it can protect the polyunsaturated fatty acids (PUFAs) of the cell membrane from biological oxidation, regulate the production of reactive oxygen species (ROS) and modulate the signal transduction (Lee and Han, 2018).

Previous studies revealed that Vitamin E and N-acetyl cysteine have good antioxidant with immunomodulatory properties (Dekhuijzen, 2004; Lee and Han, 2018). However, studies on the evaluation of these compounds in the management of mineralo-oxidative damage in bubaline pediculosis are not available. Hence, the focus of the current study was to examine the antioxidant potential of Vitamin E and N-acetylcysteine in the management of pathobiology of bubaline pediculosis.

**Materials and Methods**

**Experimental design**

A total of twenty four Indian water buffaloes with “Severe” (cumulative count more than 100) sucking lice infestation were divided into three groups and another eight apparently healthy buffaloes without any parasitic infestation belonging to the 2-6 years age group was kept as healthy control. The severity of sucking lice infestation was carried out by summing of lice counted from different predilection sites; A-cheek (5x10 cm area), ear (5x10 cm area), C-neck and dewlap
(10×20 cm area), D-withers (10×10 cm area), E-foreleg (10×10 cm area), F-back (10×10 cm area), G-hind leg (10×10 cm area), H-tail head and perineum (10×10 cm area) using standard counting technique (Holdsworth et al., 2006; Veneziano et al., 2013, 2003). The *Haematopinus tuberculatus* infested buffaloes (Soulsby, 1982) were allotted three treatment plan; group I (Ivermectin injection @ 200 µg/kg SC single dose), group II (Ivermectin injection @ 200 µg/kg SC single dose and N-acetyl cysteine @ 12 mg/kg SID PO for 14 days) and group III (Ivermectin injection @ 200 µg/kg SC single dose and Vitamin E 4000 mg per animal SID PO for 14 days). The remaining eight healthy animals without any parasitic infestation served as healthy control (group IV). Commercial preparations of Ivermectin (Hitek® injection, 1% w/v; Virbac, India), Vitamin E (Evion® capsule, 400 mg; Merck, India) N-acetyl cysteine (Fluimucil® Tablet, 600 mg; Elder Pharma, India) were used. WAAVP guidelines for evaluation of the ectoparasiticide efficacy was used for clinical evaluation of the different treatment plan (Holdsworth et al., 2006) and was assessed by calculating reduction percentage and assessment of mineral-oxidative status (Veneziano et al., 2013).

**Sample collection and processing**

Animals were evaluated for changes in haematological, oxidant-antioxidant and mineral profile on before the start of therapy (day 0) and post therapy (day 28) of the experiment. Blood samples (12 mL) were collected by external jugular venipuncture in sterile EDTA and heparin coated vials.

Serum samples were collected in sterile vials and stored in a deep freezer for the estimation of mineral profile. About two milliliter of blood collected in sterile EDTA coated vials were utilized for haematological analysis. For estimation of oxidative stress parameters 6 mL of blood collected in heparin coated vial was utilized.

Estimation of catalase (CAT), lipid peroxidation (LPO), superoxide dismutase (SOD) were carried out in the hemolysate obtained from the 6 mL of the heparinized blood sample and whereas, reduced glutathione (GSH) estimation was carried out in RBC suspension obtained from the above blood sample. The cyanohemoglobin method described by Tentori and Salvati, (1981) was used for estimation of hemolysate haemoglobin concentration.

**Evaluation of oxidant and anti-oxidant profile**

The lipid peroxidation (LPO) levels were estimated as per the method described by Placer et al., (1966). DTNB method of Prins and Loos was used for the estimation of reduced glutathione (GSH) concentration in RBCs (Prins and Loos, 1969).

Estimation of superoxide dismutase (SOD) activity was carried out as per the method described by Madesh and Balasubramanian (1998). The method described by Aebi (1974) was used for estimation of Catalase (CAT) activity. Estimation of serum Total antioxidant capacity was carried out as per the manufacturer’s instruction using Total Antioxidant Capacity (TAC) assay kit (Sigma-Aldrich, USA) (Miller and Rice-Evans, 1997).

**Evaluation of hematological profile**

The method described by (Berman, 1919) was used for the estimation of haemoglobin concentration (gm/dl). Haematological parameters like TLC, TEC, and DLC were estimated as per the method described by (Schalm and Jain, 1986).
Statistical analysis

The data were analyzed by two-way ANOVA with Tukey’s post hoc test using IBM SPSS statistics package version 25.0 (Snedecor and Cochran, 1994). The values were expressed as mean ± S.E.

Results and Discussion

The mean lice count in different body regions for different treatment groups and healthy control on day 0 and day 28 is depicted in table 1. The sucking lice of buffalo were mostly concentrated on withers, back and neck and dewlap regions, followed by cheek, foreleg, hind leg, tail head and perineum, which is not available for animal’s self-grooming. The lice infestation was clinically manifested as dermatological lesions related to weakness, hyper-sensitivity reaction, pruritus, alopecia, and seborrhea. However, lice infestation was reduced significantly in all three treated groups on day 28, group III showed better clinical improvement of clinical signs and skin condition.

The hematological profile of sucking lice infested buffaloes had significant (p<0.05) anaemia and leukocytosis with neutrophilia, lymphocytopenia, eosinophilia (Table 2). Remarkable improvement of haematological profile (Hb, TEC, TLC, and DLC) by day 28 was observed in all three treatment groups, but, group III values were similar to the healthy control group. Lice infested animals showed significant oxidative stress, with significantly elevated oxidant (LPO) levels and decreased serum TAC, SOD activity, CAT activity and reduced glutathione on day 28, but, the group II and group III showed remarkable improvement closer to the healthy control group.

The mineral profile of lice infested animal revealed a significantly (p<0.05) decreased serum iron, zinc, magnesium (Table 4). However, serum copper levels did not differ significantly between infested and healthy control animals and all the three treatments did not alter serum copper levels much. Group II and group III of the treated animals iron, zinc, magnesium levels were improved on day 28 and values were almost similar to the healthy control.

In the present study, sucking lice infested buffaloes were found to have marked oxidative stress, severe mineral imbalance, iron deficiency anaemia, leukocytosis with neutrophilia, eosinophilia and lymphocytopenia. Similar mineral imbalance, oxidative stress and hematological changes were observed in other ectoparasitism like ovine pediculosis (Dede et al., 2002), caprine pediculosis (Ajith et al., 2017), bubaline pediculosis (El-Moghazy, 2011), demodicosis in dogs (Dimri et al., 2008), psoroptic mange infection in sheep (Aktas et al., 2017), sarcoptic mange in goats (De and Dey, 2010), dogs (Behera et al., 2011; Singh and Dimri, 2013) and buffaloes (Dimri et al., 2007).

Accruing evidence from recent studies is promising the potential use of antioxidants in prophylactic and therapeutic management of the infectious disease. The ameliorative potential of the antioxidants in management of parasitic disease is increasing dramatically, Vitamin C in caprine pediculosis (Ajith et al., 2019), Vitamin E-Selenium for sarcoptic mange in canines (Behera et al., 2011; Singh and Dimri, 2013), Vitamin ADEH for
psoroptic in rabbit (Singh et al., 2012) and herbal essential oils for sarcoptic mange infestation in sheep (Dimri and Sharma, 2004).

N-Acetyl cysteine (NAC), a mucolytic agent, has been used for the treatment of acute and chronic bronchitis in patients for many years and also used as an antidote for acetaminophen toxicity. NAC has been used as a possible chemo-preventive agent is due to its anti-oxidative or detoxifying properties (De Vries and De Flora, 1993). NAC exhibits both direct and indirect antioxidant properties; acts directly by interaction with free electrophilic thiol groups of ROS (Dekhuijzen, 2004). NAC acts indirectly by increasing the intracellular concentrations of cysteine, GSH and oxidant species scavenging. NAC administered orally for seven days in milking goats had quenched the effects of oxidative stress (Jóźwik et al., 2010). Vitamin E, the part of the chain-breaking antioxidant system, scavenges the free radicals and prevents the oxidation of membrane lipids (McDowell, 2000). Vitamin E prevents the peroxidation of phospholipids by acting as the first line of defense against membrane peroxidation. In the present study Vitamin E treated animals had a marked improvement of antioxidant and mineral status compared to N-acetyl cysteine treated animals might be due to NAC mediated activation of Th2 response in lice infestation (Ajith et al., 2019). Vitamin E is an effective antioxidant with anti-inflammatory property might help in improvement of general body condition, mineral balance and oxidative stress in sucking lice infested buffaloes (Tahan et al., 2011).

Table 1: Mean lice count in different body regions of sucking lice (Haematopinus tuberculatus) infested treatment groups on day 0 and day 28

| Body Region       | Group I (n=8) |           | Group II (n=8) |           | Group III (n=8) |           | Group IV (n=8) |           |
|-------------------|--------------|-----------|----------------|-----------|----------------|-----------|----------------|-----------|
|                   | Day 0        | Day 28    | Day 0          | Day 28    | Day 0          | Day 28    | Day 0          | Day 28    |
| Cheek             | 10.86        | 0.25      | 10.78          | 0.33      | 12.58          | 0         | 0              | 0         |
| Ear               | 2.27         | 0         | 2.47           | 0         | 2.17           | 0         | 0              | 0         |
| Neck and dewlap   | 30.52        | 0.78      | 30.82          | 0.54      | 30.42          | 0         | 0              | 0         |
| Withers           | 91.68        | 1.63      | 92.27          | 1.43      | 96.67          | 0         | 0              | 0         |
| Foreleg           | 8.18         | 0         | 8.64           | 0         | 8.08           | 0         | 0              | 0         |
| Back              | 25.22        | 0.53      | 24.78          | 0.45      | 25.58          | 0         | 0              | 0         |
| Hind leg          | 2.28         | 0         | 2.58           | 0         | 2.08           | 0         | 0              | 0         |
| Tail head and perineum | 4.77     | 0         | 6.12           | 0         | 4.17           | 0         | 0              | 0         |
| Cumulative count (Mean) | 175.78   | 3.19      | 178.46         | 2.75      | 181.75         | 0         | 0              | 0         |
| Reduction percentage* | 98.18    |           | 99.01          |           | 100            |           | -              |           |

* Calculated by the formula

\[
Reduction\ percentage = \frac{\text{Lice count before treatment} - \text{Lice count after treatment}}{\text{Lice count before treatment}} \times 100\%
\]
### Table 2: Haematological profile of lice infested goats (Mean ± SE)

| Parameter                                      | Day 0                      | Day 28                     |
|------------------------------------------------|----------------------------|----------------------------|
| **Hemoglobin concentration (gm/dl)**           |                            |                            |
| Group I (n=8)                                  | 9.02±0.28<sup>Bb</sup>     | 12.22±0.23<sup>Ca</sup>    |
| Group II (n=8)                                 | 9.05±0.26<sup>Bb</sup>     | 13.52±0.26<sup>Ba</sup>    |
| Group III (n=8)                                | 9.07±0.25<sup>Bb</sup>     | 15.68±0.24<sup>Ba</sup>    |
| Group IV (n=8)                                 | 15.65±0.35<sup>Aa</sup>    | 15.72±0.32<sup>Aa</sup>    |
| **Total Erythrocyte count-TEC (million cells/µL)** |                            |                            |
| Group I (n=8)                                  | 3.52±0.24<sup>Bb</sup>     | 4.46±0.12<sup>Ca</sup>     |
| Group II (n=8)                                 | 3.63±0.23<sup>Bb</sup>     | 6.52±0.13<sup>Ba</sup>     |
| Group III (n=8)                                | 3.56±0.18<sup>Bb</sup>     | 6.58±0.15<sup>Ba</sup>     |
| Group IV (n=8)                                 | 6.59±0.14<sup>Aa</sup>     | 6.60±0.12<sup>Aa</sup>     |
| **Total Leukocyte count-TLC (Thousand cells/ µL)** |                            |                            |
| Group I (n=8)                                  | 16.08±0.25<sup>Ab</sup>    | 14.63±0.26<sup>Aa</sup>    |
| Group II (n=8)                                 | 16.04±0.22<sup>Ab</sup>    | 12.56±0.24<sup>Ba</sup>    |
| Group III (n=8)                                | 16.07±0.23<sup>Ab</sup>    | 12.25±0.25<sup>Ba</sup>    |
| Group IV (n=8)                                 | 11.58±0.22<sup>Ba</sup>    | 11.56±0.23<sup>Ca</sup>    |
| **Differential Leukocyte Count-DLC (%)**       |                            |                            |
| Neutrophil (%)                                 |                            |                            |
| Group I (n=8)                                  | 45.43±0.52<sup>Ab</sup>    | 42.43±0.53<sup>Aa</sup>    |
| Group II (n=8)                                 | 44.73±0.52<sup>Ab</sup>    | 40.24±0.46<sup>Ba</sup>    |
| Group III (n=8)                                | 45.43±0.52<sup>Ab</sup>    | 38.07±0.55<sup>Ba</sup>    |
| Group IV (n=8)                                 | 37.58±0.38<sup>Ba</sup>    | 37.78±0.30<sup>Ca</sup>    |
| Lymphocyte (%)                                 |                            |                            |
| Group I (n=8)                                  | 42.11±0.68<sup>Bh</sup>    | 52.58±0.45<sup>Ca</sup>    |
| Group II (n=8)                                 | 42.28±0.62<sup>Bh</sup>    | 55.52±0.38<sup>Ba</sup>    |
| Group III (n=8)                                | 42.18±0.63<sup>Bh</sup>    | 58.54±0.42<sup>Ba</sup>    |
| Group IV (n=8)                                 | 59.13±1.03<sup>Ba</sup>    | 59.13±1.03<sup>Aa</sup>    |
| Eosinophil (%)                                 |                            |                            |
| Group I (n=8)                                  | 6.08±0.42<sup>Aa</sup>     | 4.58±0.40<sup>Ba</sup>     |
| Group II (n=8)                                 | 6.08±0.42<sup>Aa</sup>     | 2.35±0.38<sup>Bh</sup>     |
| Group III (n=8)                                | 6.08±0.42<sup>Aa</sup>     | 1.56±0.42<sup>Bh</sup>     |
| Group IV (n=8)                                 | 1.42±0.19<sup>Ba</sup>     | 1.44±0.22<sup>Ca</sup>     |
| Monocyte (%)                                   |                            |                            |
| Group I (n=8)                                  | 2.18±0.22<sup>Aa</sup>     | 1.42±0.18<sup>Ba</sup>     |
| Group II (n=8)                                 | 2.15±0.23<sup>Aa</sup>     | 1.32±0.12<sup>Bh</sup>     |
| Group III (n=8)                                | 2.16±0.20<sup>Ba</sup>     | 1.26±0.16<sup>Ca</sup>     |
| Group IV (n=8)                                 | 1.25±0.13<sup>Ba</sup>     | 1.25±0.15<sup>Ca</sup>     |
| Basophil (%)                                   |                            |                            |
| Group I (n=8)                                  | 1.08±0.15<sup>Aa</sup>     | 0.92±0.18<sup>Ca</sup>     |
| Group II (n=8)                                 | 0.92±0.15<sup>Aa</sup>     | 0.83±0.21<sup>Ca</sup>     |
| Group III (n=8)                                | 0.96±0.13<sup>Aa</sup>     | 0.67±0.14<sup>Ca</sup>     |
| Group IV (n=8)                                 | 0.7±0.15<sup>Aa</sup>      | 0.7±0.19<sup>Aa</sup>      |

Values with different superscripts A, B, C, D differ significantly (p < 0.05) in the same column
Values with different superscripts a, b differ significantly (p < 0.05) in the same row
### Table 3
Variation in oxidant-antioxidant profile of treatment groups on day 0 and day 28 (Mean±SE)

| Parameter                               | Day 0              | Day 28             |
|-----------------------------------------|--------------------|--------------------|
| **Lipid per oxidation-LPO (nM MDA/mg Hb)** |                    |                    |
| Group I (n=8)                           | 8.26±0.12<sup>AAa</sup> | 7.58±0.16<sup>Ab</sup> |
| Group II (n=8)                          | 8.34±0.18<sup>AAa</sup> | 6.62±0.15<sup>Bb</sup> |
| Group III (n=8)                         | 8.56±0.16<sup>AAa</sup> | 6.52±0.13<sup>Bb</sup> |
| Group IV (n=8)                          | 6.36±0.18<sup>Ba</sup> | 6.42±0.15<sup>Ba</sup> |
| **Reduced glutathione-GSH (μMol/ml of packed RBC)** |                |                    |
| Group I (n=8)                           | 0.63±0.04<sup>Bb</sup> | 0.68±0.02<sup>Ca</sup> |
| Group II (n=8)                          | 0.65±0.03<sup>Bb</sup> | 0.78±0.04<sup>Ba</sup> |
| Group III (n=8)                         | 0.64±0.02<sup>Bb</sup> | 0.81±0.03<sup>Ba</sup> |
| Group IV (n=8)                          | 0.84±0.03<sup>Aa</sup> | 0.85±0.04<sup>Aa</sup> |
| **Super Oxide Dismutase-SOD (μmol/mg Hb)** |                |                    |
| Group I (n=8)                           | 8.06±0.25<sup>Bb</sup> | 9.28±0.18<sup>Ca</sup> |
| Group II (n=8)                          | 8.07±0.26<sup>Bb</sup> | 9.68±0.24<sup>Ba</sup> |
| Group III (n=8)                         | 8.08±0.27<sup>Bb</sup> | 9.75±0.25<sup>Ba</sup> |
| Group IV (n=8)                          | 10.22±0.16<sup>Aa</sup> | 10.19±0.14<sup>Aa</sup> |
| **Catalase activity-CAT (μmol H₂O₂ decomposed/min/mg Hb)** |                |                    |
| Group I (n=8)                           | 4.69±0.12<sup>Bb</sup> | 6.12±0.12<sup>Ca</sup> |
| Group II (n=8)                          | 4.73±0.15<sup>Bb</sup> | 6.75±0.14<sup>Ba</sup> |
| Group III (n=8)                         | 4.75±0.14<sup>Bb</sup> | 6.79±0.14<sup>Ba</sup> |
| Group IV (n=8)                          | 6.85±0.23<sup>Aa</sup> | 6.82±0.25<sup>Aa</sup> |
| **Total Antioxidant Capacity-TAC (mM/L of serum)** |                |                    |
| Group I (n=8)                           | 0.80±0.23<sup>Bb</sup> | 1.25±0.17<sup>Ba</sup> |
| Group II (n=8)                          | 0.81±0.22<sup>Bb</sup> | 1.73±0.13<sup>Ab</sup> |
| Group III (n=8)                         | 0.82±0.26<sup>Bb</sup> | 1.76±0.15<sup>Ba</sup> |
| Group IV (n=8)                          | 1.78±0.08<sup>Aa</sup> | 1.79±0.10<sup>Aa</sup> |

Values with different superscripts A, B, C, D differ significantly (p < 0.05) in the same Column
Values with different superscripts a, b differ significantly (p < 0.05) in the same row
Table 4: Mineral profile of lice infested buffaloes (Mean ± SE)

| Parameter                  | Day 0               | Day 28              |
|----------------------------|---------------------|---------------------|
| **Serum Iron (µg/dL)**     |                     |                     |
| Group I (n=8)              | 113.30±1.23\textsuperscript{B,a} | 145.23±1.12\textsuperscript{C,a} |
| Group II (n=8)             | 115.25±1.22\textsuperscript{B,a} | 155.44±1.16\textsuperscript{B,a} |
| Group III (n=8)            | 114.28±1.25\textsuperscript{B,a} | 158.58±1.13\textsuperscript{B,a} |
| Group IV (n=8)             | 158.09±2.30\textsuperscript{A,a} | 160.09±2.35\textsuperscript{A,a} |
| **Serum Zinc (µg/dL)**     |                     |                     |
| Group I (n=8)              | 97.28±8.42\textsuperscript{B,b} | 122.32±3.14\textsuperscript{C,a} |
| Group II (n=8)             | 96.30±8.46\textsuperscript{B,b} | 136.22±3.16\textsuperscript{B,a} |
| Group III (n=8)            | 98.32±8.48\textsuperscript{B,b} | 139.36±3.21\textsuperscript{B,a} |
| Group IV (n=8)             | 143.52±1.61\textsuperscript{A,a} | 143.48±1.53\textsuperscript{A,a} |
| **Serum Copper (µg/dL)**   |                     |                     |
| Group I (n=8)              | 68.65±5.43\textsuperscript{A,a} | 73.65±1.23\textsuperscript{A,a} |
| Group II (n=8)             | 68.58±5.42\textsuperscript{A,a} | 72.45±1.28\textsuperscript{A,a} |
| Group III (n=8)            | 68.53±5.42\textsuperscript{A,a} | 74.45±1.23\textsuperscript{A,a} |
| Group IV (n=8)             | 75.33±1.28\textsuperscript{A,a} | 75.38±1.23\textsuperscript{A,a} |
| **Serum Magnesium (mEq/L)**|                     |                     |
| Group I (n=8)              | 0.52±0.03\textsuperscript{B,b} | 1.45±0.06\textsuperscript{B,a} |
| Group II (n=8)             | 0.54±0.02\textsuperscript{B,b} | 1.63±0.05\textsuperscript{A,a} |
| Group III (n=8)            | 0.52±0.05\textsuperscript{B,b} | 1.65±0.03\textsuperscript{A,a} |
| Group IV (n=8)             | 1.66±0.09\textsuperscript{A,a} | 1.67±0.07\textsuperscript{A,a} |

Values with different superscripts A, B, C, D differ significantly (p < 0.05) in the same column
Values with different superscripts a, b differ significantly (p < 0.05) in the same row

The proper functioning of the antioxidant defense needs several trace minerals, thus mineral imbalance contributes to the development of oxidative stress. Magnesium act as an antioxidant by participating as a cofactor for several enzymes, maintaining the stability of cell membranes and mitigating the effects of oxidative stress (Morais et al., 2017). Magnesium deficiency enhances the generation of ROS, free radicals and increases the substrates available for radical oxidation (Zheltova et al., 2016). The normal functioning of the enzymes involved in antioxidant defense system requires zinc as a cofactor and zinc also protects cells by stabilization of cell membranes, inhibition of nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase) enzyme and synthesis of metallothionein proteins that are effective in sequestration of ROS and reduction of hydroxyl radicals (Chasapis et al., 2012; Ruz et al., 2013).

Increased oxidative stress has been associated with magnesium and zinc deficiencies; magnesium deficiency increases ROS level and simultaneously decreasing the expression of antioxidant enzyme whereas zinc plays an indirect antioxidant role by serving as a structural cofactor and essential catalytic for superoxide dismutase and other enzymes (Eide, 2011). For Cu–Zn superoxide dismutase, the antioxidant enzyme, both zinc and copper are act as essential components.
Therefore, utilization or sequestration of Zn and Cu to neutralize the excessively produced ROS and the general poor condition of affected animals might be responsible for their lower level in the blood of pediculosis affected animals (Dede et al., 2002; Dimri et al., 2010). Anaemia in sucking lice infested buffaloes might have resulted from prolonged blood loss and iron deficiency. The loss of essential nutrients, iron deficiency and oxidative stress induced erythrocyte damage might have attributed to anaemia of the lice infested buffaloes. For iron deficiency anaemia development, oxidative stress plays a crucial role (Yoo et al., 2009).

In conclusion, ubaline pediculosis is associated with severe anaemia, oxidative stress, mineral imbalance and leukocytosis with neutrophilia, lymphocytopenia, and eosinophilia. N-Acetyl cysteine as an adjunct antioxidant is less effective than Vitamin E in reducing mineralo-oxidative pathology of bubaline pediculosis. Future studies on antioxidants therapy may target the influence of mineralo-oxidative response in the host system.

Acknowledgements

The authors sincerely thank SEED division, Department of Science and Technology, Ministry of Science and Technology, Government of India for funding this work under DST-SARTHI (Grant no. SEED/SARTHI/HP/19/2012) scheme. The authors are also thankful to Dr. Anil P. Joshi, Dr. Rakesh Kumar and Dr. Kiran Negi from Himalayan Environmental Studies and Conservation Organisation (HESCO), Dehradun.

References

Aebi, H., 1974. Catalase, in: Methods of Enzymatic Analysis. Elsevier, pp. 673–684.
Ajith, Y., Dimri, U., Singh, S.K., Gopalakrishnan, A., Devi, G., Verma, M.R., Joshi, V., Alam, S., 2017. Lice induced immuno-oxidative wreckage of goats. Vet. Parasitol. 242, 24–30.
Ajith, Y., Dimri, U., Dixit, S.K., Singh, S.K., Gopalakrishnan, A., Madhesh, E., Rajesh, J.B., Sangeetha, S.G., 2017. Immunomodulatory basis of antioxidant therapy and its future prospects: an appraisal. Inflammopharmacology 25, 487–498.
Ajith, Y., Dimri, U., Gopalakrishnan, A., Devi, G., Junaid, N., Madhesh, E., 2019. Host immunomodulation by ascorbic acid ameliorates oxidative stress in caprine pediculosis—a pilot study. Small Rumin. Res. 176, 65–69.
Aktas, M.S., Kandemir, F.M., Kirbas, A., Hanedan, B., Aydin, M.A., 2017. Evaluation of oxidative stress in sheep infected with Psoroptes ovis using total antioxidant capacity, total oxidant status, and malondialdehyde level. J. Vet. Res. 61, 197–201.
Bastianetto, E., Leite, R.C., 2005. Control of the louse (Haematopinus tuberculatus) in herds of water buffalos (Bubalus bubalis) raised for milk and meat. Rev Bras Reprod Anim, Belo Horiz. 29, 118–121.
Behera, S.K., Dimri, U., Singh, S.K., Mohanta, R.K., 2011. The curative and antioxidative efficiency of ivermectin and ivermectin+ vitamin E-selenium treatment on canine Sarcoptes scabiei infestation. Vet. Res. Commun. 35, 237–244.
Berman, L., 1919. The determination of hemoglobin by the acid hematin method. Arch. Intern. Med. 24, 553–556.
Chasapis, C.T., Louisdou, A.C., Spiliopoulou, C.A., Stefanidou, M.E., 2012. Zinc and human health: an update. Arch. Toxicol. 86, 521–534.
Chaudhry, N.I., 1978. Common disease problems in buffalo calves. Pak. J. Sci. 30, 120–126.
De Vries, N., De Flora, S., 1993. N- acetyl- l- cysteine. J. Cell. Biochem. 53, 270–277.
De, U.K., Dey, S., 2010. Evaluation of organ function and oxidant/antioxidant status in goats with sarcoptic mange. Trop. Anim. Health Prod. 42, 1663–1668.
Dede, S., Deger, Y., Deger, S., 2002. Serum
Copper, Zinc, and Calcium Concentrations in Lice-Infested Sheep. 274 Biol. Trace Elem. Res. 88, 87–90.

Dekhuijzen, P.N.R., 2004. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. Eur. Respir. J. 23, 629–636.

Dimri, U., Ranjan, R., Kumar, N., Sharma, M.C., Swarup, D., Sharma, B., Kataria, M., 2008. Changes in oxidative stress indices, zinc and copper concentrations in blood in Canine demodicosis. Vet. Parasitol. 154, 98–102.

Dimri, U., Ranjan, R., Singh, S.K., Sharma, M.C., Swarup, D., Dwivedi, P., Sharma, A.K., Kataria, M., 2007. Clinico-pathological and haematobiological changes in buffaloes with sarcoptic mange. Indian J. Vet. Pathol. 31, 160–162.

Dimri, U., Sharma, M.C., 2004. Effects of Sarcoptic Mange and its Control with Oil of Cedrus deodara, Pongamia glabra, Jatropha curcas and Benzyl Benzoate, both with and without Ascorbic Acid on Growing Sheep: Epidemiology; Assessment of Clinical, Haematological, Cell-Mediated Humoral Immune Responses and Pathology. J. Vet. Med. Ser. A 51, 71–78.

Dimri, U., Sharma, M.C., Yamdagni, A., Ranjan, R., Zama, M.M.S., 2010. Psoroptic mange infestation increases oxidative stress and decreases antioxidant status in sheep. Vet. Parasitol. 168, 318–322.

Dubey, N., Khan, A.M., Raina, R., 2013. Subacute deltamethrin and fluoride toxicity induced hepatic oxidative stress and biochemical alterations in rats. Bull. Environ. Contam. Toxicol. 91, 334–338.

Egri, B., 2019. Louse Infestation of Ruminants, in: Bovine Science - A Key to Sustainable Development. InTechOpen, Rijeka, p. Ch. 6.

Eide, D.J., 2011. The oxidative stress of zinc deficiency. Metallomics 3, 1124–1129.

El-Moghazy, F.M., 2011. Effect of parasitic infestation on oxidant/antioxidant status in buffaloes. Middle East J. Sci. Res. 7, 585–593.

Farber, J.L., 1994. Mechanisms of cell injury by activated oxygen species. Environ. Health Perspect. 102, 17–24.

Holdsworth, P.A., Vercruysse, J., Rehbein, S., Peter, R.J., Letonja, T., Green, P., 2006. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of ectoparasiticides against biting lice, sucking lice and sheep ked on ruminants. Vet. Parasitol. 136, 45–54.

Ince, S., Kucukkurt, I., Demirel, H.H., Turkmen, R., Zemheri, F., Akbel, E., 2013. The role of thymoquinone as antioxidant protection on oxidative stress induced by imidacloprid in male and female Swiss albino mice. Toxicol. Environ. Chem. 95, 318–329.

Jóźwik, A., Bagnicka, E., Strzałkowska, N., Śliwa-Jóźwik, A., Horbańczuk, K., Cooper, R.G., Pyzel, B., Krzyżewski, J., ŚwiergIEL, A.H., Horbańczuk, J.O., 2010. The oxidative status of milking goats after per os administration of N-acetylcysteine. Anim. Sci. Pap. Reports 28, 143–152.

Kanbur, M., Eraslan, G., Sarica, Z.S., Altinordulu, Ş., 2010. The effects of Saw palmetto on flumethrin-induced lipid peroxidation in rats. Pestic. Biochem. Physiol. 97, 43–46.

Kaur, G., Alam, M.S., Jabbar, Z., Javed, K., Athar, M., 2006. Evaluation of antioxidant activity of Cassia siamea flowers. J. Ethnopharmacol. 108, 340–348.

Lee, G., Han, S., 2018. The Role of Vitamin E in Immunity. Nutrients 10, 1614.

Lykkesfeldt, J., Svendsen, O., 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet. J. 173, 502–511.

Madesh, M., Balasubramanian, K.A., 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J. Biochem. Biophys. 35, 184–188.

Mamun, M.A.A., Begum, N., Shahadat, H.M., Mondal, M.M.H., 2010. Ectoparasites of buffaloes (Bubalus bubalis) in Kurigram district of Bangladesh. J. Bangladesh Agric. Univ. 8, 61–66.

McDowell, L.R., 2000. Vitamins in animal and human nutrition. 2nd edn., Iowa State University Press, Ames, p.793.

Miller, N.J., Rice-Evans, C.A., 1997. Factors influencing the antioxidant activity determined by the ABTS + radical cation assay. Free Radic. Res. 26, 195–199.

Morais, J.B.S., Severo, J.S., Santos, L.R. Dos, de Sousa Melo, S.R., de Oliveira Santos, R., de
Oliveira, A.R.S., Cruz, K.J.C., do Nascimento Marreiro, D., 2017. Role of Magnesium in Oxidative Stress in Individuals with Obesity. Biol. Trace Elem. Res. 176, 20–26.

Placer, Z.A., Cushman, L.L., Johnson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16, 359–364.

Prins, H.K., Loos, J.A., 1969. Glutathione, in: Biochemical Methods in Red Cell Genetics. Academic Press New York, pp. 115–137.

Ruz, M., Carrasco, F., Rojas, P., Codyceo, J., Inostroza, J., Basfi-Fer, K., Valencia, A., Vásquez, K., Galgani, J., Pérez, A., 2013. Zinc as a potential coadjuvant in therapy for type 2 diabetes. Food Nutr. Bull. 34, 215–221.

Schalm, O.W., Jain, N.C., 1986. Schalm’s veterinary haematology, 4th edn., Lea and Febiger, Philadelphia, PA, p. 1221.

Singh, S.K., Dimri, U., 2013. Amelioration of sarcoptic mange-induced oxidative stress and apoptosis in dogs by using Calendula officinalis flower extracts. ISRN Oxidative Med. 2013.

Veneziano, V., Rinaldi, L., Giannetto, S., Cringoli, G., 2003. The first record of Haematopinus tuberculatus on Bubalus bubalis (water buffalo) in Italy. Bubalus bubalis 9, 69–75.

Yoo, J.-H., Maeng, H.-Y., Sun, Y.-K., Kim, Y.-A., Park, D.-W., Park, T.S., Lee, S.T., Choi, J.-R., 2009. Oxidative status in iron-deficiency anaemia. J. Clin. Lab. Anal. 23, 319–323.

Zheltova, A.A., Kharitonova, M. V, Iezhitsa, I.N., Spasov, A.A., 2016. Magnesium deficiency and oxidative stress: an update. BioMedicine 6.

Oliveira, A.R.S., Cruz, K.J.C., do Nascimento Marreiro, D., 2017. Role of Magnesium in Oxidative Stress in Individuals with Obesity. Biol. Trace Elem. Res. 176, 20–26.

Placer, Z.A., Cushman, L.L., Johnson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16, 359–364.

Prins, H.K., Loos, J.A., 1969. Glutathione, in: Biochemical Methods in Red Cell Genetics. Academic Press New York, pp. 115–137.

Ruz, M., Carrasco, F., Rojas, P., Codyceo, J., Inostroza, J., Basfi-Fer, K., Valencia, A., Vásquez, K., Galgani, J., Pérez, A., 2013. Zinc as a potential coadjuvant in therapy for type 2 diabetes. Food Nutr. Bull. 34, 215–221.

Schalm, O.W., Jain, N.C., 1986. Schalm’s veterinary haematology, 4th edn., Lea and Febiger, Philadelphia, PA, p. 1221.

Singh, S.K., Dimri, U., 2013. Amelioration of sarcoptic mange-induced oxidative stress and apoptosis in dogs by using Calendula officinalis flower extracts. ISRN Oxidative Med. 2013.

Veneziano, V., Rinaldi, L., Giannetto, S., Cringoli, G., 2003. The first record of Haematopinus tuberculatus on Bubalus bubalis (water buffalo) in Italy. Bubalus bubalis 9, 69–75.

Yoo, J.-H., Maeng, H.-Y., Sun, Y.-K., Kim, Y.-A., Park, D.-W., Park, T.S., Lee, S.T., Choi, J.-R., 2009. Oxidative status in iron-deficiency anaemia. J. Clin. Lab. Anal. 23, 319–323.

Zheltova, A.A., Kharitonova, M. V, Iezhitsa, I.N., Spasov, A.A., 2016. Magnesium deficiency and oxidative stress: an update. BioMedicine 6.

How to cite this article:

Madhesh, E., Umesh Dimri, Y. Ajith, S. Shanmuganathan, P. Sivasankar, R. Karthikeyan, Alok Kumar Choudhary and Kavitha, K. 2019. Vitamin E Ameliorates the Mineralo-Oxidative Stress of Sucking Lice Infestation in Indian Water Buffalo. Int.J.Curr.Microbiol.App.Sci. 8(07): 1538-1548. doi: https://doi.org/10.20546/ijcmas.2019.807.183