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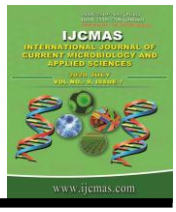

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Vitamin C Moderates the Oxidative Stress of Bubaline Pediculosis in Indian Water Buffaloes

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A B S T R A C T

Bubaline pediculosis caused by the sucking lice, *Haematopinus tuberculatus* is a widespread and economically important ectoparasitic infestation of Indian water buffaloes. This lice infestation in buffalo is characterized by anemia, mineral imbalance and loss of performance but its pathobiology is unexplored. The aim of the present study was to investigate the oxidative pathobiology of bubaline pediculosis and its alleviation through the commonly used two antioxidants as an supportive therapy (N- acetyl cysteine and vitamin C). Twenty four Indian dairy buffaloes with severe bubaline pediculosis were allotted to the three groups (Group 1, 2 and 3).Another eight healthy animals free of any clinical anomalies and ectoparasites have been included as healthy controls (Group 4). Sucking lice infested animals (Group 1, 2 and 3) animals were given with single dose of ivermectin therapy @ 200 microgram/kg body weight Subcutaneously; In addition, group 2 and group 3 animals were treated with N-acetyl cysteine @ 12 mg/kg body weight and vitamin C@2g/ per animal po, respectively, once daily for 15 days. Before therapy (Day 0) and post-therapy (Day 28), haematological parameters like total erythrocyte count (TEC),haemoglobin (Hb), total leukocyte count (TLC) and Differential leukocyte count (DLC), and oxidative stress parameters like total antioxidant capacity(TAC), lipid peroxidation (LPO), reduced glutathione (GSH), and superoxide dismutase (SOD) were evaluated on sucking lice infested buffaloes. Sucking lice infestation in Indian water buffaloes has showed severe anaemia, leukocytosis and along with remarkable oxidative stress response. Vitamin C given animals have demonstrated improved recovery from the bubaline pediculosis induced oxidative stress and reached normalcy post-therapeutically. Adjunct therapy of Vitamin C along with subcutaneous ivermectin has alleviated pathological damage in water buffaloes and hastened clinical recovery, while N-acetyl cysteine has been less effective in alleviating the oxidative stress. In future, therapeutic antioxidant studies should address the influence of the oxidative stress response in the host biological system.

Introduction

Buffaloes considered 'black gold of India;' because buffaloes provide protein, milk, and provide jobs so it provides livelihoods for millions of landless, marginalized and small-scale farmers across India. Ectoparasites adversely affects farm animals in terms of welfare and production performance. Across the world, buffaloes have been commonly infested with sucking lice, *Haematopinus tuberculatus* which can also infest cattle especially during the winter season (Egri, 2019; Mamun *et al.,* 2010). The risk factors such as lack of grooming, poor health status, poor temperature-humid weather conditions have favoured lice infestation in the livestock (Taylor *et al.,* 2016).

Sucking lice feeds sebaceous gland secretion and blood, which are usually less mobile and remain securely attached to the host 's skin for an extended period of time. Lice can induce hypersensitivity, so severe infestations of *H.tuberculatus* can lead to varying levels of scratching, excoriation, alopecia, and papular dermatitis (Taylor *et al.,* 2016). Recent research studies have shown that reactive oxygen species (ROS) and inflammation in ectoparasitism have induced tissue damage, by which it plays an vital role in deprivation of animal health (Dimri *et al.,* 2010). Oxidative stress is defined as an imbalance between oxygen reactive species (ROS) production and the body's ability to avert its harmful effects by means of antioxidant defense mechanism. The ROS easily induce oxidative stress mediated damage to various biomolecules, including proteins, lipoproteins, lipids and DNA and cause disturbance in normal cell signalling and homeostasis pathways (Lykkesfeldt and Svendsen, 2007). In rats, even the normal use of ectoparasiticides such as cypermethrin, and deltamethrin to control ectoparasitism, leads to severe oxidative stress (Dubey *et al.,* 2013; Ince *et al.,* 2013). The biomodulators used in the therapeutic management of various diseases are receiving universal acceptance because of their vast scope in improving a wide range of disease conditions by altering the oxidative stress response in the host system (Ajith *et al.,* 2017a). N-Acetyl Cysteine (NAC) commonly used as a mucolytic agent for several years in the therapeutic management of acute and chronic bronchitis and is an effective remedy for paracetamol toxicity. Vitamin C synthesized in liver of most mammals except by humans and guinea pigs. Vitamin C has antioxidant and immunomodulation properties (Padayatty *et al.,* 2003). However, critical research studies on evaluating these antioxidant compounds in the therapeutic management of oxidative damage inpediculosis of buffaloes, however, are not available. Consequently, the present study centered on exploring the antioxidant capacity of vitamin C and N-acetylcysteine in the management of bubaline pediculosis.

Materials and Methods

Experimental study design:

Total of twenty-four Indian water buffaloes with "severe bubaline pediculosis" (cumulative count more than 100) caused sucking lice infestation were divided into three groups and further, eight apparently healthy buffaloes without any internal and ectoparasitic infestation, which arein the 2-8 years age group had served as healthy controls. The severity of sucking lice infestation was assessed by following the standard lice counting technique (Holdsworth *et al.,* 2006; Veneziano *et al.,* 2013, 2003). Three treatment plans were followed for the *Haematopinus tuberculatus* infested buffaloes (Soulsby, 1982); group I was treated with single dose of Ivermectin injection @ 200 microgram / kg subcutaneously), group II was

administered with single dose of Ivermectin injection @ 200 microgram / kg subcutaneously along with oral N-acetyl cysteine @ 12 mg / kg SID for 15 days) and group III was treated with single dose of subcutaneous Ivermectin injection @ 200 microgram/ kg along with oral vitamin C2g per animal SID for 15 days).Commercially available preparations of Ivermectin injection (Neomec®, 1% w/v; Intas, India), Vitamin C (Limcee® tablet, 500 mg; Abbott, India) N-acetyl cysteine (Fluimucil® Tablet, 600 mg; Elder Pharma, India) were utilized for this study. The WAAVP recommendations for determining the efficacy of ectoparasiticides were used for the clinical assessment of the various treatment groups (Holdsworth *et al.*, 2006) and were assessed by calculating the percentage of lice reduction and evaluating the oxidative stress parameters (Veneziano *et al.*, 2013).

Collection of samples for processing

Experimental animals were subjected for haematological and oxidative evaluation on before the start of therapy (day 0) and post therapy (day 28) period. Blood samples (12 mL) were obtained from external jugular in sterile EDTA and heparin-coated vials. For haematological analysis, approximately two millilitres of blood collected in sterile EDTA-coated vials were used. About 6 mL of blood collected in heparin-coated vials are used for the estimation of oxidative stress parameters. In the hemolysate obtained from the 6 mL heparinized blood sample, catalase (CAT), lipid peroxidation (LPO), superoxide dismutase (SOD) were estimated. Whereas, in the case of reduced glutathione (GSH) estimation, the RBC suspension obtained from the above blood sample was utilized. The cyanohemoglobin method described in Tentori and Salvati, (1981) was used to estimate the concentration of hemolysate haemoglobin.

Evaluation of oxidative stress profile

The levels of lipid peroxidation (LPO) were estimated according to the method Placer *et al.*, (1966) described. Superoxide dismutase (SOD) activity estimation was conducted according to the method described by Madesh and Balasubramanian (1998). DTNB method was used for estimating the reduced concentration of glutathione (GSH) in RBCs (Prins and Loos, 1969). The method described by Aebi (1974) was used for estimating the Catalase activity (CAT).The estimation of serum total antioxidant potential was conducted utilizing the Total Antioxidant Ability (TAC) assay kit (Sigma-Aldrich, USA) as per the manufacturer 's guidance (Miller and Rice-Evans, 1997).

Hematological profile evaluation

The method described by (Berman, 1919) was followed for the estimation of haemoglobin concentration (gm / dl). Haematological parameters such as TLC, TEC, and DLC have been estimated according to the method described by (Schalm and Jain, 1986).

Statistical analysis

The experimental data were subjected to statistical analysis using IBM SPSS statistics software version 25.0 (Snecdecor and Cochran, 1994), following two-way ANOVA with Tukey's post-hoc test. The values were stated as mean ± S.E.

Results and Discussion

Table 1 shows the mean lice count on different body regions for three different treatment groups and healthy control on before (day 0) and after therapy (day 28). The buffalo sucking lice, *Haematopinus tuberculatus* were highly concentrated on withers, back and neck and dewlap regions,

followed by cheek, foreleg, hind leg, tail head and perineum which are usually unavailable for self-grooming by animals. The lice infestation has been clinically expressed as dermatological related problem with lethargy, hyper-sensitivity reactions, pruritus, alopecia, and seborrheic lesions. However, on Day 28, lice infestation in all three treatment groups was significantly reduced, among the treatment groups, group 3 showed better clinical improvement of clinical signs and skin lesions. The hematological evaluation of sucking lice infested buffaloes revealed significant ($p<0.05$) leukocytosis with neutrophilia, eosinophilia. lymphocytopenia and anaemia (Table 2).Marked improvement of haematological parameters (Hb, TEC, TLC, and DLC) by day 28 were observed in all three treatment groups, but group 3had haematological values similar to the healthy control group. Buffaloes infested with sucking lice displayed significant oxidative stress, with elevated amounts of oxidant (LPO) and decreased serum TAC, SOD, diminished GSH, and CAT activities (Table 3). Furthermore, in the three treatment groups on day 28, lipid peroxidation biomarker malondialdehyde level was significantly reduced ($p<0.05$), and group 2 and group 3 of the treatment animal value were comparable to the healthy control group. All the three treatment groups revealed significant improvement in their antioxidant defense status such as serum TAC, SOD activity, CAT, and reduced glutathione activities on day 28, but group 2 and group 3 showed significant improvement, which were closer to the healthy control group.

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 1(n=8)		Group 2 (n=8)		Group 3 (n=8)		Group 4 (n=8)	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Cheek	10.76	0.20	10.98	0.28	13.48	0	0	0
Ear	2.25	0	3.75	0	2.15	0	0	0
Neck and dewlap	31.52	0.74	32.42	0.45	29.62	0	0	0
Withers	91.48	1.54	94.22	1.27	98.27	0	0	0
Foreleg	7.58	0	8.56	0	7.78	0	0	0
Back	25.28	0.48	24.58	0.44	27.68	0	0	0
Hind leg	2.20	0	2.65	0	2.58	0	0	0
Tail head and perineum	4.56	0	6.25	0	4.57	0	0	0
Cumulative count (Mean)	175.63	2.96	183.41	2.44	186.13	0	0	0
Reduction percentage*	**98.31**		**98.66**		**100**		**-**	

* Calculated by the formula

$$\textit{Reduction percentage} = \frac{\textit{lice count before treatment} - \textit{Lice count after treatment}}{\textit{Lice count before treatment}} \times 100$$

T**able.2** Haematological profile of lice infested goats (Mean ± SE)

Parameter	Day 0	Day 28
Hemoglobin concentration (gm/dl)		
Group 1 (n=8)	8.02 ± 0.27^{Bb}	11.22 ± 0.23^{Ca}
Group 2 (n=8)	8.05 ± 0.25^{Bb}	12.56 ± 0.26^{Ba}
Group 3 (n=8)	8.07 ± 0.23^{Bb}	16.58 ± 0.26^{Aa}
Group 4 (n=8)	16.65 ± 0.35^{Aa}	16.72 ± 0.28^{Aa}
Total Erythrocyte count-TEC (million cells/µL)		
Group 1 (n=8)	4.52 ± 0.23^{Bb}	5.46 ± 0.14^{Ca}
Group 2 (n=8)	4.63 ± 0.24^{Bb}	7.52 ± 0.14^{Ba}
Group 3 (n=8)	4.56 ± 0.18^{Bb}	7.58 ± 0.16^{Ba}
Group 4 (n=8)	7.59 ± 0.14^{Aa}	7.72 ± 0.13^{Aa}
Total Leukocyte count-TLC (Thousand cells/ µL)		
Group 1 (n=8)	17.08 ± 0.25^{Ab}	13.63±0.24Aa
Group 2 (n=8)	17.04 ± 0.22^{Ab}	12.86 ± 0.23^{Ba}
Group 3 (n=8)	17.07 ± 0.23^{Ab}	12.55 ± 0.20^{Ba}
Group 4 (n=8)	12.58 ± 0.22^{Ba}	12.56 ± 0.23^{Ca}
Differential Leukocyte Count-DLC (%)		
Neutrophil (%)		
Group 1 (n=8)	45.43 ± 0.52^{Ab}	42.43 ± 0.53^{Aa}
Group 2 (n=8)	44.73 ± 0.52^{Ab}	40.24 ± 0.46^{Ba}
Group 3 (n=8)	45.43 ± 0.52^{Ab}	38.07 ± 0.55^{Ca}
Group 4 (n=8)	37.58 ± 0.38^{Ba}	37.78 ± 0.36^{Ca}
Lymphocyte (%)		
Group 1 (n=8)	42.11 ± 0.68^{Bb}	52.58 ± 0.45^{Ca}
Group 2 (n=8)	42.28 ± 0.62^{Bb}	55.52 ± 0.38^{Ba}
Group 3 (n=8)	42.18 ± 0.63^{Bb}	58.54 ± 0.42^{Aa}
Group 4 (n=8)	59.13 ± 1.03^{Aa}	59.13 ± 1.03^{Aa}
Eosinophil (%)		
Group 1 (n=8)	7.08 ± 0.42^{Aa}	5.56 ± 0.40^{Ab}
Group 2 (n=8)	7.05 ± 0.42^{Aa}	2.36 ± 0.36^{Bb}
Group 3 (n=8)	6.28 ± 0.42^{Aa}	1.55 ± 0.44^{Cb}
Group 4 (n=8)	1.35 ± 0.19^{Ba}	1.35 ± 0.22^{Ca}
Monocyte (%)		
Group 1 (n=8)	2.12 ± 0.22^{Aa}	1.43 ± 0.18^{Ab}
Group 2 (n=8)	2.13 ± 0.23^{Aa}	1.35 ± 0.14^{Bb}
Group 3 (n=8)	2.17 ± 0.20^{Aa}	1.27 ± 0.16^{Ca}
Group 4 (n=8)	1.28 ± 0.13^{Ba}	1.28 ± 0.15^{Ca}
Basophil (%)		
Group 1 (n=8)	2.08 ± 0.15^{Aa}	2.02 ± 0.18^{Aa}
Group 2 (n=8)	1.92 ± 0.15^{Aa}	1.90 ± 0.21^{Aa}
Group 3 (n=8)	1.96 ± 0.13^{Aa}	1.78 ± 0.14^{Aa}
Group 4 (n=8)	1.23 ± 0.15^{Aa}	$1.22.\pm0.18^{Aa}$

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 1 (n=8)	7.26 ± 0.12^{Aa}	7.13 ± 0.16^{Ab}
Group 2 (n=8)	7.34 ± 0.18^{Aa}	5.62 ± 0.15^{Bb}
Group 3 (n=8)	7.56 ± 0.16^{Aa}	5.52 ± 0.13^{Bb}
Group 4 (n=8)	5.36 ± 0.18^{Ba}	5.42 ± 0.15^{Ba}
Reduced glutathione-GSH (µMol/ml of packed RBC)		
Group 1 (n=8)	0.73 ± 0.04^{Bb}	0.76 ± 0.02^{Ca}
Group 2 (n=8)	0.75 ± 0.04^{Bb}	0.80 ± 0.04^{Ba}
Group 3 (n=8)	0.65 ± 0.02^{Bb}	0.81 ± 0.03^{Ba}
Group 4 (n=8)	0.86 ± 0.03^{Aa}	0.86 ± 0.04^{Aa}
Super Oxide Dismutase-SOD (µmol/mg Hb)		
Group 1 (n=8)	9.06 ± 0.24^{Bb}	10.28 ± 0.18^{Ca}
Group 2 (n=8)	9.08 ± 0.26^{Bb}	11.10 ± 0.24^{Ba}
Group 3 (n=8)	9.07 ± 0.27^{Bb}	11.15 ± 0.25^{Ba}
Group 4 (n=8)	11.23 ± 0.16^{Aa}	11.22 ± 0.14^{Aa}
Catalase activity-CAT (µmol H_2O_2 decomposed/min/mg Hb)		
Group 1 (n=8)	4.89 ± 0.12^{Bb}	7.12 ± 0.12^{Ca}
Group 2 (n=8)	4.83 ± 0.15^{Bb}	7.75 ± 0.14^{Ba}
Group 3 (n=8)	4.75 ± 0.14^{Bb}	7.79 ± 0.14^{Ba}
Group 4 (n=8)	7.85 ± 0.23^{Aa}	7.84 ± 0.25^{Aa}
Total Antioxidant Capacity-TAC (mM/L of serum)		
Group 1 (n=8)	0.83 ± 0.23^{Bb}	1.45 ± 0.12^{Ba}
Group 2 (n=8)	0.82 ± 0.22^{Bb}	1.83 ± 0.14^{Aa}
Group 3 (n=8)	0.82 ± 0.26^{Bb}	1.86 ± 0.13^{Aa}
Group 4 (n=8)	1.93 ± 0.08^{Aa}	1.95 ± 0.12^{Aa}

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 current study showed that bubaline pediculosis caused by *H. tuberculatus* infestation had severe oxidative stress, anaemia, leukocytosis with neutrophilia, eosinophilia and lymphocytopenia. Other ectoparasitic infestations in various domestic animals; ovine pediculosis (Dede *et al.*, 2002), pediculosis in goats (Ajith *et al.*, 2017b), pediculosis in buffaloes (El-Moghazy, 2011), generalized canine demodicosis (Dimri *et al.*, 2008), psoroptic mange infection in sheep (Aktas *et al.*, 2017), sarcoptic mange in domestic goats (De and Dey, 2010), dogs (Behera *et al.*, 2011; Singh and Dimri, 2013) and dairy buffaloes (Dimri *et al.*, 2007) had similar oxidative stress and haematological changes observed in bubaline pediculosis. Growing body of evidence from recent studies promises the potential use of antioxidants for prophylactic and therapeutic management of various infectious diseases. The potential use of the antioxidants in the various ectoparasitic conditions in domestic animals are increasing significantly, Vitamin E in bubaline pediculosis (Madhesh *et al.*, 2019),Vitamin C in pediculosis of goats (Ajith *et al.*, 2019).Combination of Vitamin E-Selenium for sarcoptic mange in dogs (Behera *et al.*, 2011; Singh and Dimri, 2013), Combined Vitamin ADEH was used for control of psoroptic mange in domestic rabbits (Singh *et al.*, 2012) and essential oils also used to control sarcoptic mange in sheep (Dimri and Sharma, 2004).

Ascorbic acid or Vitamin C act as a powerful antioxidant with reducing properties, prevents the oxidation of lipids, proteins, DNA molecules in biological host system (Padayatty *et al.*, 2003). Dimri and Sharma (2004) had reported that, Vitamin C supplementation along with miticidal therapy recovered the sarcoptic mange infestation in sheep. Vitamin C administration had the ability to suppress Th2 immune response by increasing the interferon gamma with simultaneous reduction of IL-4, reduced serum histamine and IgE levels (Sun *et al.*, 2009) and Ajith *et al.*, (2019) also had reported the similar result with Vitamin C supplementation in caprine pediculosis.

N-Acetyl cysteine (NAC), a mucolytic agent with antioxidant properties, has been successfully used in human patients for several years to treat acute and chronic bronchitis, and had been used as effective antidote for paracetamol toxicity. It also used as possible chemo-preventive agent owing to its antioxidant or detoxifying properties (De Vries and De Flora 1993).The NAC had both direct and indirect antioxidant properties and direct antioxidant properties are mainly due tointeraction with free electrophilic thiol groups of reactive oxygen species (Dekhuijzen, 2004).NAC acts as indirect antioxidant by improving the intracellular cysteine concentration, increasing the GSH activity along with enhanced oxidant species scavenging.Oral NAC administration in dairy goats had ameliorated the oxidative stress (Jóźwik*et al.*, 2010).

Anemia in bubaline pediculosis might be a consequence of prolonged blood loss, iron deficiency mediated by oxidative stress, loss of vital nutrients, oxidative stress induced RBC damage (Madhesh *et al.*, 2019; Yoo *et al.*, 2009).

In conclusion, bubaline pediculosis caused by sucking lice is associated with severe oxidative stress, and haematological changes such as anemia, leukocytosis with neutrophilia, lymphocytopenia, and eosinophilia. Oral N-Acetyl cysteine as an adjunct antioxidant is less effective than Vitamin C in reducing oxidative stress mediated pathology of sucking lice infestation in Indian dairy buffaloes. In future, therapeutic antioxidant studies on ectoparasitism should address the influence of the oxidative stress response in the host biological system.

Compliance with ethical standards

This work was undertaken in compliance with the universal ethical standards

Conflict of interest statement

The authors declare that they have no conflict of interest.

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