

Original Research Article

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Oxidative Stress and Imbalance of Mineral Metabolism Contributes to Clinico-pathobiology of Pediculosis in Dairy Buffaloes

E. Madhesh^{1*}, Umesh Dimri¹, Y. Ajith¹, S. Shanmuganathan²,
P. Sivasankar² and R. Karthikeyan³

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

*Corresponding author

ABSTRACT

The present study was aimed to evaluate the mineral metabolism and oxidative pathobiology of lice infestation in buffaloes. Forty-eight buffaloes were divided into four groups; Sucking lice (*Hematopinus tuberculatus*) infested-mild (Group 1, n=12), moderate (Group 2, n=12), severe (Group 3, n=12) and healthy control (Group 4, n=12). Lice infested animals (Group 1, 2 and 3) animals were treated with a single dose ivermectin subcutaneously at 200 µg/kg body weight and healthy control group were treated with 7 mL of distilled water subcutaneously as placebo therapy. To assess the pathological changes, mineral profile (Iron, Zinc, Copper and Manganese), oxidative stress markers (lipid peroxidation-LPO, reduced glutathione-GSH, superoxide dismutase-SOD, Catalase-CAT and total antioxidant capacity-TAC), Mast cell activity (Histamine, Carboxypeptidase A activity, and Chymase activity), endocrine profile (Cortisol, Total thyroxine-TT₄, Total triiodothyronine-TT₃, and free triiodothyronine-FT₃) and haematological status were evaluated. Significant iron deficiency anemia, lymphocytopenia, neutrophilia, eosinophilia was observed in bubaline pediculosis according to the level of severity of infestation. Remarkably increased oxidative stress and mineral imbalance were observed in sucking lice infested buffaloes. Increased mast cell activity was observed in relation to severity of lice infestation. From the present study, it may be concluded that sucking lice infestation produces significant oxidative stress, mineral imbalance and inflammatory responses in dairy buffaloes. Mast cell may modulate host inflammation via mineralo-oxidative mechanism in sucking lice infestation in buffaloes.

Keywords

Mast cell activity,
Bubaline
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Introduction

Buffalo represents an indispensable part of livestock industry in India by providing socio-economical, cultural and religious needs of human being with better adaptability to harsh climate and thriving on low quality roughages

and crop by-products (Syed Mohmad and Manmohan Singh, 2017). Livestock health is the major factor that affects the optimum productivity thus profitability of the livestock industry. Parasitic infestations play a crucial role in direct and indirect losses in domestic animals. Lice infestation in buffaloes or

bubaline pediculosis is a serious problem among the buffalo population worldwide, especially in winter and early spring season (Mamun *et al.*, 2010). Buffaloes are commonly parasitized by hematophagous sucking lice (*Haematopinus tuberculatus*). Sucking lice are blood and sebaceous secretion feeders, which are less moveable and remain tightly attached to the skin of host for long time. Sucking lice infestation in goat causes TGF- β mediated suppression of Th1 and Th2 immune responses whereas chewing lice causes severe oxidative stress and Th2 dominant inflammatory response in goats (Ajith *et al.*, 2017).

The salivary antigens of lice induce irritation and hypersensitivity reaction which attributes to the major clinical manifestations of bubaline pediculosis. Severe sucking lice infestations in animals are manifested as alopecia, self-excoriation, scratching, licking and biting of their skin, erythematous itching areas and papulo-crustous dermatitis (Chaudhry, 1978; Taylor *et al.*, 2016; Egri, 2019). In human pediculosis, the salivary antigens of lice induce severe hypersensitivity reaction thus modifications in host biological system (Fernández *et al.*, 2006). Similar to other ectoparasitic infections bubaline pediculosis also leads to impairments of haemato-biochemical parameters and conferring stress, weakness, anemia, weight loss, and substantial productivity loss (Solouma *et al.*, 2017; Egri, 2019). Lice are considered as the second most potent vector for disease transmission in humans, next to mosquito. Accumulating molecular evidences suggest that sucking lice of small ruminants and buffaloes are involved in the transmission of *Anaplasma ovis*, *Anaplasma marginale*, *Trypanosoma evansi*, *Brucella abortus*, *Bartonella bovis*, *Mycoplasma spp.* and *Rickettsia spp.*, (Hornok *et al.*, 2010; Neglia *et al.*, 2013; Egri, 2019). The salivary antigens present in hematophagous

ectoparasites modulates host immune system response to evade from host immunity, for prolonged attachment and successful blood feeding, and their perpetuation.

The direct effects of pediculosis include the reduced quality of buffalo products like leather, milk yield, body weight, and decreased production performance. While, the indirect consequences of bubaline pediculosis on buffalo health in context of oxidative stress and mineral imbalance and immuno regulations are equally important and remain less explored. Accumulating evidence indicates a close bidirectional communication and regulation between the neuroendocrine and immune systems. Thyroid hormones exert its responses in various immune cells, thus affects the several inflammatory processes of host animals (such as, chemotaxis, cytokines production, phagocytosis and reactive oxygen species generation). Mast cells can modulate thyroid function. Therefore, evaluation of oxidative stress and mineral imbalance along with mast cell activity and neuroendocrine system in buffalo lice infestations could throws lights on host-parasite interactions and immuno-pathology of bubaline pediculosis. Therefore, the present study was aimed to evaluate the oxidative stress, mineral balance, and mast cell activity containments of sucking lice infestations in buffaloes.

Materials and Methods

Experimental design

Thirty-six lice infested buffaloes were divided into three groups (Group 1-3) having twelve infested buffaloes each. Sucking lice (*Haematopinus tuberculatus*) infested buffaloes were divided into three groups considering its severity of lice infestation; mild (Group 1, n = 12), moderate (Group 2, n = 12) and severe (Group 3, n = 12). Twelve buffaloes free of any other ectoparasite

infestations were kept as healthy control (Group 4). The lice from buffaloes were collected in 70% ethanol and identification of lice were carried out microscopically based on the morphological characters (Soulsby, 1982). The severity of sucking lice infestation was carried out by summing the of lice counted from different predilection sites (Fig.1) using standard counting technique (Veneziano *et al.*, 2003, 2013; Holdsworth *et al.*, 2006). Based on the total count, the severity of infestation was graded as mild (less than 10), moderate (10-100) and severe (more than 100).

Sample collection and processing

Blood samples (12 mL) were collected by jugular venipuncture in sterile vials containing clot activator, EDTA and heparin. Serum samples were harvested from the blood samples (5 mL) and stored in deep freezer until the estimation of mineral, mast cell activity and hormone profile. Blood samples (4 mL) collected in sterile EDTA vials were used for haematology. The hemolysate obtained from the heparinized blood sample (3 mL) was used for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT). Whereas, the RBC suspension obtained from the same sample was used to estimate the reduced glutathione (GSH). Haemoglobin concentration of hemolysate was estimated by cyanohemoglobin method (Tentori and Salvati 1981).

Evaluation of oxidant-antioxidant profile

The LPO level in the RBC hemolysate was determined by the method described by Placer *et al.*, (1966). The concentration of malondialdehyde (MDA) per mg haemoglobin was calculated using the extinction coefficient of 1.56×10^5 M/cm (Utley *et al.*, 1967). The GSH content of

RBCs was estimated using the suggested Prins and Loos (1969) method. SOD activity was estimated as per the method described by Madesh and Balasubramanian (1998). CAT activity was estimated as per the method of Aebi (1974). The TAC of serum samples was estimated using TAC assay kit (Sigma-aldrich, USA) following the instruction protocols suggested by the manufacturer.

Evaluation of hormone profile and mast cell activity

The serum levels of hormones Cortisol, Total Thyroxine-TT₄, Total Triiodothyronine-TT₃, Free triiodothyronine-FT₃ were estimated using Cortisol (IM1841, Immunotech, Czech Republic), TT₄ (IM1447, Immunotech, Czech Republic), TT₃ (IM1699, Immunotech, Czech Republic) and FT₃ (IM1579, Immunotech, Czech Republic) RIA kits respectively, working on radio immunoassay technique. The hormone levels were obtained in Gamma counter. To assess mast cell activity, Carboxypeptidase A activity, Chymase activity and histamine levels were estimated. Chymase activity in the serum samples was estimated using Chymase activity Assay Kit (CS1140, Sigma-aldrich, USA) - Substrate A (N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide) and Substrate B (N-benzoyl-L-tyrosine ethyl ester-BTEE) method as per Ferry *et al.*, (2001). Serum histamine concentration was estimated by using modified method of Stoner (1985).

Evaluation of haematological and mineral profile

Haematological panels including haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated by routine methods.

Table.1 Mean lice count in different body regions of sucking lice (*Haematopinus tuberculatus*) infested buffaloes

Body region	Before treatment			After treatment		
	Mild (n=12)	Moderate (n=12)	Severe (n=12)	Mild (n=12)	Moderate (n=12)	Severe (n=12)
Cheek	0.50	2.75	10.58	0	0	0
Ear	0.25	2.42	2.17	0	0	0
Neck and dewlap	1.08	8.00	30.42	0	0	0
Withers	3.67	26.67	91.67	0	0	0
Foreleg	0.42	2.33	8.08	0	0	0
Back	1.67	7.83	24.58	0	0	0
Hind leg	0.25	2.33	2.08	0	0	0
Tail head and perineum	0.08	1.83	4.17	0	0	0
Cumulative count (Mean)	7.92	54.17	173.75	0	0	0

Bold value indicates cumulative count and is mean value of each group.

Table.2 Oxidant-antioxidant profile of lice infested buffaloes (Mean ± SE)

Parameter	Before treatment			After treatment			Control
	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 4 (n=12)
Lipid per oxidation-LPO (nM MDA/mg Hb)	6.89±0.16 ^c	7.76±0.22 ^b	8.46±0.17 ^a	6.32±0.24 ^d	6.49±0.18 ^d	6.66±0.18 ^d	6.08±0.16 ^d
Reduced glutathione-GSH (µMol/ml of packed RBC)	1.22±0.04 ^b	0.86±0.03 ^c	0.64±0.04 ^d	1.34±0.05 ^a	1.27±0.05 ^a	1.23±0.04 ^a	1.43±0.07 ^a
Super Oxide Dismutase-SOD (µmol/mg Hb)	9.53±0.18 ^b	8.77±0.19 ^c	8.04±0.22 ^d	10.2±0.11 ^a	10.14±0.12 ^a	9.96±0.12 ^a	10.32±0.13 ^a
Catalase activity-CAT (µmol H ₂ O ₂ decomposed/min/mg Hb)	6.07±0.21 ^b	5.48±0.12 ^c	4.79±0.15 ^d	6.56±0.10 ^a	6.49±0.19 ^a	6.28±0.13 ^a	6.85±0.23 ^a
Total Antioxidant Capacity-TAC (mM/L of serum)	1.43±0.05 ^b	1.16±0.06 ^c	0.80±0.09 ^d	1.61±0.1 ^a	1.55±0.09 ^a	1.38±0.11 ^a	1.71±0.08 ^a

Values with different superscripts a, b, c, d differ significantly (p < 0.05) in the same row

Table.3 Mineral profile of lice infested buffaloes (Mean ± SE)

Parameter	Before treatment			After treatment			Control
	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 4 (n=12)
Serum Iron (µg/dL)	138.49±0.81 ^B	123.67±2.18 ^C	113.30±1.01 ^D	152.15±2.87 ^A	150.53±2.46 ^A	147.32±30 ^A	157.09±2.90 ^A
Serum Zinc (µg/dL)	125.45±1.39 ^B	112.46±0.85 ^C	97.38±8.82 ^D	138.57±2.43 ^A	135.32±3.13 ^A	133.07±2.65 ^A	141.92±1.61 ^A
Serum Copper (µg/dL)	72.35±1.05 ^a	70.48±0.91 ^a	64.65±5.43 ^a	73.01±0.89 ^a	72.85±1.06 ^a	72.53±0.59 ^a	74.33±1.28 ^a
Serum Magnesium (mEq/L)	1.29±0.10 ^B	0.90±0.05 ^C	0.52±0.03 ^D	1.55±0.08 ^A	1.46±0.08 ^A	1.39±0.07 ^A	1.63±0.09 ^A

Values with different superscripts a, b, c, d differ significantly (p < 0.05) in the same row
 Values with different superscripts A, B, C, D differ significantly (p < 0.01) in the same row

Table.4 Mast cell activity of lice infested buffaloes (Mean ± SE)

Parameter	Before treatment			After treatment			Control
	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 4 (n=12)
Chymase activity assay- Substrate-A method (Units/mgP)	0.73±0.04 ^c	0.97±0.04 ^b	1.23±0.09 ^a	0.54±0.02 ^d	0.56±0.03 ^d	0.6±0.03 ^d	0.50±0.05 ^d
Chymase activity assay- Substrate-B method (Units/mgP)	0.26±0.01 ^c	0.32±0.02 ^b	0.38±0.01 ^a	0.18±0.02 ^d	0.2±0.02 ^d	0.21±0.02 ^d	0.15±0.02 ^d
Carboxypeptidase-A assay (milliunits/mL)	0.84±0.04 ^c	0.97±0.02 ^b	1.12±0.04 ^a	0.74±0.05 ^d	0.79±0.03 ^d	0.81±0.03 ^d	0.70±0.03 ^d
Serum Histamine (µmol/L)	0.30±0.02 ^c	0.36±0.01 ^b	0.41±0.02 ^a	0.27±0.02 ^d	0.28±0.02 ^d	0.29±0.02 ^d	0.25±0.01 ^d

Values with different superscripts a, b, c, d differ significantly (p < 0.05) in the same row.

Table.5 Haematological profile of lice infested goats (Mean ± SE)

Parameter	Before treatment			After treatment			Control
	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 4 (n=12)
Hemoglobin concentration (gm/dl)	11.68±0.40 ^B	10.38±0.33 ^C	9.02±0.28 ^D	13.29±0.24 ^A	13±0.26 ^A	12.85±0.21 ^A	14.61±0.39 ^A
Total Erythrocyte count-TEC (million cells/ µL)	5.45±0.14 ^C	4.18±0.22 ^C	3.51±0.16 ^D	5.23±0.15 ^A	4.98±0.14 ^A	4.97±0.14 ^A	6.49±0.14 ^A
Total Leukocyte count-TLC (Thousand cells/ µL)	13.06±0.59 ^c	14.63±0.25 ^b	16.02±0.25 ^a	12.19±0.35 ^d	12.55±0.3 ^d	12.56±0.3 ^d	11.61±0.26 ^d
Differential Leukocyte Count-DLC (%)							
Neutrophil (%)	40.58±0.44 ^c	42.85±0.55 ^b	45.43±0.52 ^a	38.03±0.73 ^d	38.72±0.55 ^d	39.24±0.46 ^d	37.71±0.38 ^d
Lymphocyte (%)	50.25±1.10 ^B	46.06±0.89 ^C	42.11±0.72 ^D	57.89±0.68 ^A	56.65±0.83 ^A	56.49±0.78 ^A	59.13±1.03 ^A
Eosinophil (%)	3.17±0.41 ^C	4.58±0.40 ^B	6.08±0.42 ^A	1.58±0.23 ^D	2.08±0.23 ^D	2.17±0.24 ^D	1.42±0.19 ^D
Monocyte (%)	1.33±0.14 ^a	1.42±0.19 ^a	2.08±0.23 ^a	1.33±0.14 ^b	1.58±0.15 ^b	1.75±0.18 ^b	1.25±0.13 ^b
Basophil (%)	0.75±0.18 ^a	0.92±0.15 ^a	1.08±0.15 ^a	0.67±0.14 ^a	0.83±0.21 ^a	0.92±0.19 ^a	0.5±0.19 ^a

Values with different superscripts a, b, c, d differ significantly (p < 0.05) in the same row

Values with different superscripts A, B, C, D differ significantly (p < 0.01) in the same row

Table.6 Hormone profile of lice infested buffaloes (Mean ± SE)

Parameter	Before treatment			After treatment			Control
	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 1 (n=12)	Group 2 (n=12)	Group 3 (n=12)	Group 4 (n=12)
Total Thyroxine-TT ₄ (ng/mL)	19.58±0.27 ^a	19.67±0.30 ^a	19.13±0.26 ^a	20.4±0.46 ^a	20.34±0.48 ^a	20.26±0.47 ^a	20.51±0.45 ^a
Total triiodothyronine-TT ₃ (ng/mL)	1.17±0.05 ^a	1.12±0.04 ^a	1.07±0.06 ^a	1.21±0.04 ^a	1.19±0.05 ^a	1.18±0.05 ^a	1.22±0.05 ^a
Free triiodothyronine-FT ₃ (pM/L)	17.73±0.26 ^a	17.31±0.32 ^a	17.17±0.36 ^a	18.08±0.25 ^a	17.98±0.27 ^a	17.91±0.29 ^a	18.19±0.18 ^a
Cortisol (ng/mL)	66.98±0.32 ^c	68.29±0.31 ^b	69.48±0.34 ^a	66.21±0.44 ^a	66.42±0.40 ^a	66.76±0.39 ^a	65.79±0.24 ^d

Values with different superscripts a, b, c, d differ significantly (p < 0.05) in the same row

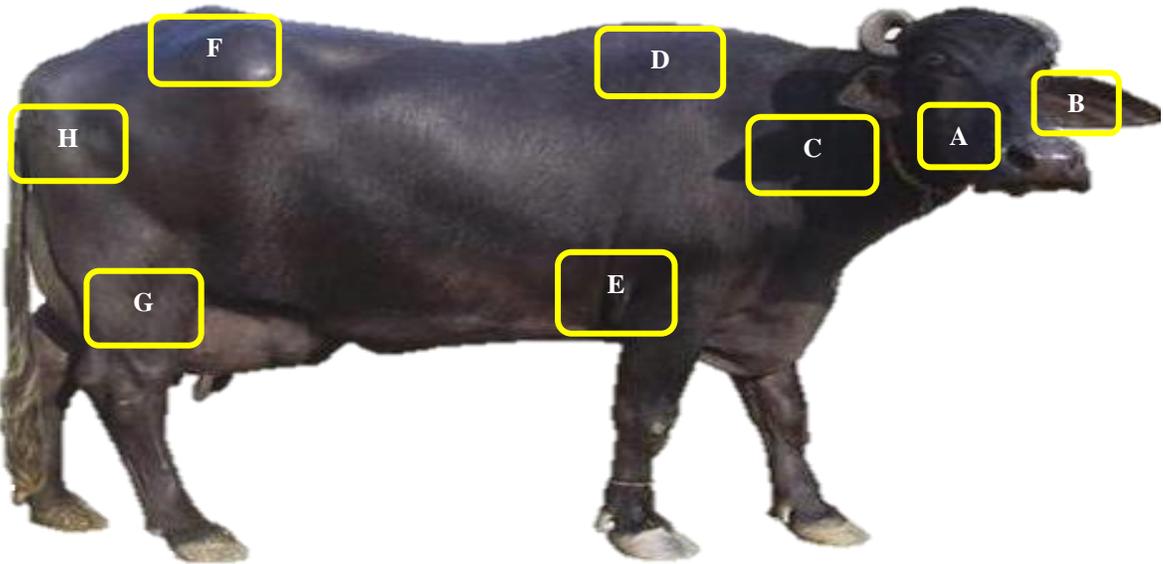


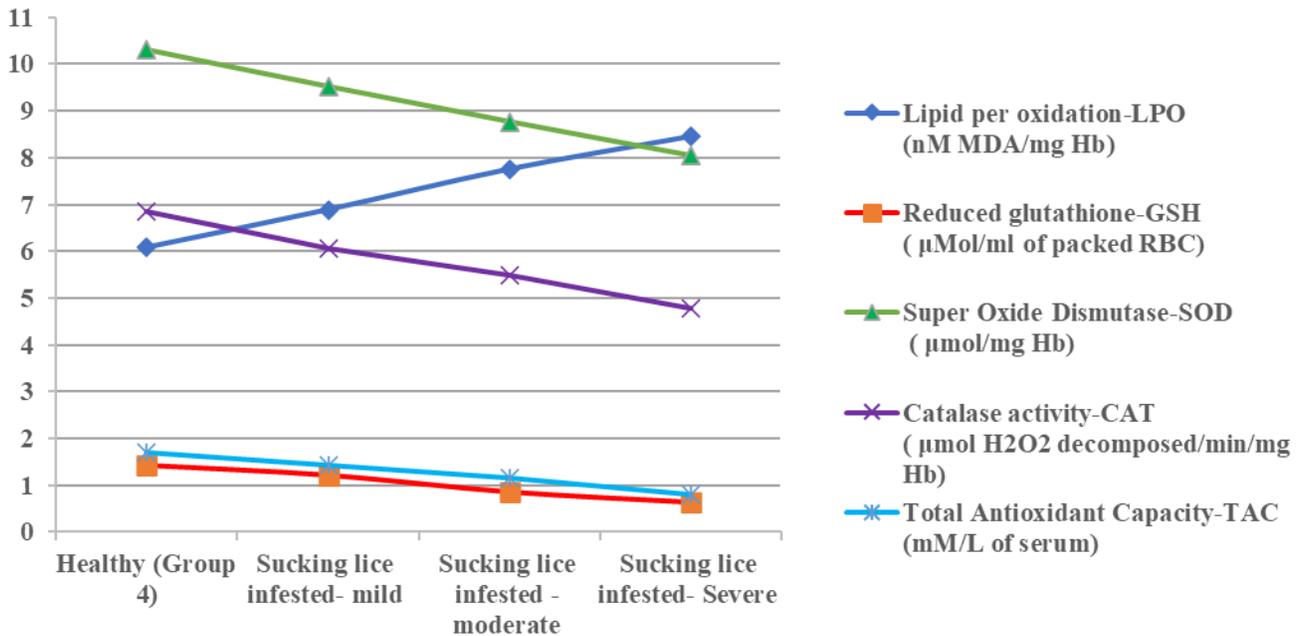
Fig. 1. Standard lice count from different predilection sites in buffalo.

A Cheek (5×10 cm); B Ear (5×10 cm); C Neck and dewlap (10X20 cm); D Withers(10X10 cm); E Foreleg (10X10 cm); F back(10X10 cm); G Hind leg (10X10 cm); H Tail head and perineum (10X10 cm)



Fig.2 Magnified view (10x) of Adult sucking louse (*Haematopinus tuberculatus*)

Fig.3 Levels of oxidative stress markers in relation to severity of sucking lice infestation in buffaloes



Moreover, serum mineral profile including iron, magnesium, zinc and copper were estimated using commercial specific biochemistry analysis kits following the protocols suggested by the kit manufacturers.

Statistical analysis

The results were analyzed using SPSS 25.0. The values were expressed as Mean ± SE. One way Multiple Analysis of Variance (MANOVA) along with Tukey post hoc test was used to compare the significance of variance between the groups. A value of $p < 0.05$ was considered as statistically significant.

Results and Discussion

On microscopic examination, the sucking lice from buffaloes were identified as *Haematopinus tuberculatus* (Fig. 2). The mean lice count among the different lice predilection sites of sucking lice infested

buffaloes was presented in Table 1. The count was more in the withers, back and neck and dewlap regions, followed by cheek, foreleg, hind leg, tail head and perineum. The “nits” or lice eggs were commonly found attached with long hairs of wither and neck region. Sucking lice infestation was clinically manifested as dermatological lesions related to weakness, hyper-sensitivity reaction, pruritis, alopecia, and seborrhea.

Sucking lice infestation affects the both oxidant and antioxidant defence system in buffaloes (Table 2). The MDA level of sucking infested buffaloes (Group 1,2 and3) were significantly higher compared healthy control group and the levels were directly proportional to the level of severity of lice infestation ($p < 0.05$). In addition, antioxidant parameters like R-GSH content, SOD and CAT activities in RBC and serum TAC level of sucking lice buffaloes (Group 1, 2 and 3) were significantly lowered as compared to control and the levels were directly

proportional to the levels of sucking lice infestation ($p<0.05$) (Fig.2). Additionally serum iron and zinc levels of sucking lice infested buffaloes (Group 1, 2 and 3) were remarkably reduced as compared to control group and the levels were directly proportional to the level of severity of lice infestation ($p<0.01$) (Table 3). Serum copper level in sucking lice infested animals were not differ from control group however, magnesium level in sucking lice infested animals (Group 1, 2 and 3) was significantly lower in comparison to control and level was directly proportional to the levels of sucking lice infestation in buffaloes ($p<0.05$). Mast cell activity profile of sucking lice infested animals and healthy control was presented in Table 4. Mast cell activity like Chymase activity (Substrate A and B), Carboxypeptidase A and serum histamine levels in lice infested buffaloes (Group 1, 2 and 3) were significantly higher in comparison to control and levels were elevated in relation to the levels of sucking lice infestation in buffaloes ($p<0.05$).

Sucking lice infested buffaloes were more anaemic compared to control. Sucking lice infested buffaloes also revealed significantly lowered Hb content and total erythrocyte count (TEC) as compared to control and levels were directly proportional to the levels of lice infestation ($p<0.05$) (Table 5). The total leukocyte count (TLC) was significantly elevated in sucking lice infested buffaloes ($p<0.05$). In addition, lice infested animals (Group 1, 2 and 3) reveals a significant neutrophilia ($p<0.05$) along with significantly lymphocytopenia and eosinophilia ($p<0.01$) as compared to control and the levels were directly proportional to the level of severity of sucking lice infestation. Cortisol level in sucking lice infested buffaloes (Group 1, 2 and 3) significantly higher in comparison to control and the levels were directly proportional to the severity of lice sucking

lice infestation ($p<0.05$) (Table 6). Moreover, Total thyroxine, total triiodothyronine and free triiodothyronine levels in sucking lice infested were did not differ significantly as compared to control but levels were reduced to lower normal range of healthy control according to the level of sucking lice infestation.

Accruing evidence suggests that, there is a close bidirectional communication and regulation between the neuroendocrine and immune systems. Thyroid hormones can exert its responses in various immune cells (monocytes, macrophages, natural killer cells, and lymphocytes) thus affecting several inflammation-related processes, such as, chemotaxis, phagocytosis, reactive oxygen species generation, and cytokines production. The endocrine and immune system interactions have been contributed to the pathophysiological conditions like sepsis, inflammation, autoimmune diseases and viral infections (Jara *et al.*, 2017). The relationship between thyroid hormones and immune cells is complex and thyroxine (T_4) and triiodothyronine (T_3) can modulate immune responses through both genomic and nongenomic mechanisms. Recent evidence indicates that cells of the immune system, including mast cells can synthesize and store hormones among which are thyroid stimulating hormone (TSH) and the thyroid hormone T_3 (Csaba and Pállinger 2009; Thangam *et al.*, 2018). Further, evidence indicates that mast cells express T_3 receptors and that tissue mast cells population increased in hypothyroidism (Siebler *et al.*, 2002); mast cells can also modulate the thyroid function. Hypothyroidism causes enhancement of phagocytosis and increased levels of reactive oxygen species generation by decreasing the levels of antioxidant enzymes in host (Chakrabarti *et al.*, 2016; Mancini *et al.*, 2016).

In the present study, sucking lice infested buffaloes were found to be in increased mast cell activity which was proportional to the levels of severity of lice infestation which was similar to the mouse infested with sucking lice showed sustained increase of mast cell numbers and degranulation (Nelson *et al.*, 1972). Increased mast cell activity revealed an elevated levels of serum histamine, chymase activity and carboxypeptidase A activity in sucking lice infestations which could lowered thyroid hormone levels to lower end of normal range. Thyroid hormone synthesis requires trace elements like zinc, copper and selenium, and deficiency of these trace elements can result in hypothyroidism (Betsy *et al.*, 2013). Oxidative stress defined as imbalance between pro-oxidants and antioxidant. It is associated with increased oxidizing species production or decreased effectiveness of antioxidant defence system and results in tissue damage. Lowered thyroid hormones like thyroxine and triiodothyronine in sucking lice infestation may enhanced the generation of reactive oxygen species (ROS) generation by decreasing antioxidant enzyme activities (R-GSH, SOD, CAT, and TAC) in sucking lice infested buffaloes; hypothyroidism enhances the generation of ROS by decreasing the antioxidant enzyme activities (Chakrabarti *et al.*, 2016; Mancini *et al.*, 2016). Thus increased mast cell activity modulates thyroid function and produces severe oxidative stress and inflammatory response in bubaline pediculosis. Magnesium deficiency contributes to the development of oxidative stress as it plays a role as an antioxidant, participates as a cofactor of several enzymes, maintains cell membrane stability and mitigates the effects of oxidative stress (Morais *et al.*, 2017). Zinc acts as a cofactor for important enzymes involved in the proper functioning of the antioxidant defence system. In addition, zinc protects cells against oxidative damage, acts in the stabilization of membranes and inhibits the enzyme

nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase). Zinc also induces the synthesis of metallothionein, which are proteins effective in reducing hydroxyl radicals and sequestering reactive oxygen species (ROS) produced in stressful situations (Chasapis *et al.*, 2012; Ruz *et al.*, 2013). The deficiencies of magnesium and Zinc have been associated with increased oxidative stress, by increasing ROS and decreasing antioxidant enzyme expression (Mg) or by an indirect antioxidant role as an essential catalytic and structural cofactor for superoxide dismutase and many enzymes (Zn) (Hans *et al.*, 2002; Eide, 2011).

Sucking lice infestation caused the elevation of serum cortisol levels which indicates the affected animals were in severe stress which could lower the productivity of buffaloes by reducing body weight, milk yield and leather quality. Magnesium deficiency contributes to the overproduction of cortisol and epinephrine (Simental-Mendía *et al.*, 2009; Günther 2010). Anemia in sucking lice infested buffaloes might be resulted from prolonged blood losses and iron deficiency. The loss of essential nutrients, iron deficiency and oxidative stress induced erythrocyte damage might have attributed to anemia of the lice infested buffaloes. Oxidative stress plays an important role in development of iron deficiency anemia. Leukocytes are soldiers of animal body and involved in function like identification, opsonisation, and phagocytic destruction of foreign invaders. The sucking lice infestation in buffaloes cause lymphocytopenia, neutrophilia, eosinophilia and monocytosis which was similar to the findings of (Ahmed *et al.*, 2009).

It could be concluded that sucking lice infestation in buffaloes elicited severe oxidative stress and inflammatory response which are mediated through mast cell modulation via mineralo-oxidative

mechanism. Sucking lice affected buffaloes are in severe mineral imbalance with remarkable oxidative stress and anemia. Bubaline Pediculosis causes severe oxidative stress which could lower the productivity of animal.

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