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Enhancing clinical recovery of bovine tropical theileriosis by use of antioxidant supportive therapy

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ABSTRACT

(Graphical abstract available only online)

The present study was conducted to test the hypothesis that oxidative stress plays important role in pathogenesis of bovine tropical theileriosis and the oxidative stress may be mitigated by supportive therapy with anti-oxidants, hastening recovery process from diseased to normal status. Animals (20) clinically infected with bovine tropical theileriosis were divided into 2 groups of 10 animals each and 6 healthy (uninfected) animals were kept as control. Group A animals were treated with buparvaquone alone and group B animals were treated with buparvaquone along with ascorbic acid. These groups were further subdivided into subgroups A_1, A_2 and B_1, B_2 as per schizont and piroplasm stage of disease. All infected animals harboured either schizonts or piroplasms of *T. annulata* and showed high grade fever, anaemia, inappetence and enlarged superficial lymph nodes. Significant decrease in level of haemoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC) and total leucocytes count (TLC) along with lymphocytopenia was observed in infected animals. Treatment of diseased animals with theilericidal drug (buparvaquone) led to disappearance of the clinical symptoms, elimination of parasite population and recovery in hematocrit values. However, recovery was significantly better in animals of group B as compared to group A. Oxidative stress was detected by measuring level of lipid peroxidation [malondialdehyde (MDA) concentration] and antioxidant enzymes viz. glutathione peroxidase (GPx) and superoxide dismutase (SOD) in plasma of infected animals before and after treatment. There was development of oxidative stress in the animals as confirmed by significant increase in plasma MDA, increased activity of GPx and SOD enzymes as compared to corresponding values estimated in plasma of healthy animals. Ascorbic acid was found better in treating clinical cases of bovine tropical theileriosis. However, addition of antioxidant (ascorbic acid) further hastened the recovery process.

Key words: Anaemia, Ascorbic acid, Bovine tropical theileriosis, Glutathione peroxidase, Lipid peroxidation, Malondialdehyde, Oxidative stress, Piroplasm, Schizont, Superoxide dismutase, *Theileria annulata*

Theileria annulata, a tick-borne protozoan parasite, causes bovine tropical theileriosis which is a severe and often fatal disease of *Bos taurus* and crossbred cattle in tropical and subtropical countries (Ahmed *et al*. 2008). The most important vectors for *T. annulata* are the 3-host tick *Hyalomma anatolicum.* The disease in ruminants is characterized by fever and lympho-proliferative disorders, associated with varying degrees of leucopoenia and/or anaemia (Radostits *et al*. 2007).

Haemolytic anaemia is considered a hallmark of *T. annulata* infection in calves (Preston *et al*. 1992). Factors such as haematopoietic precursor cell destruction (Mbassa *et al.* 1994), changes in membrane glycolipid components (Watarai *et al*. 1995), activated complement products (Omer *et al*. 2002), binding of autoantibody (IgG) to red blood cells (RBC) and removal of infected and non-infected RBCs by phagocytosis (Shiono *et al*. 2004) contribute to the theilerial anaemia. Also, there is evidence that oxidative damage to cellular components incorporate in pathogenesis of anaemia in theileriosis (Rezaei and Dalir-Naghadeh 2006, Razavi *et al*. 2011). The activity of antioxidant enzymes such as superoxide dismutase (SOD) (Razavi *et al*. 2011, Hassanpour *et al*. 2013) and glutathione peroxidase (GPx) (Nazifi *et al*. 2009, Razavi *et al.* 2011) may be affected by the parasite. The erythrocyte destruction during oxidative stress is related to lipid peroxidation of RBCs (Grewal *et al*. 2005, El-Deeb and Younis 2009, Saleh *et al.* 2011).

Buparvaquone, the drug of choice for the treatment of bovine tropical theileriosis, kills all the schizonts and piroplasms present in the host body but the survival of animals is not always ensured. Return to normal physiological state and resumption of normal functions in

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sick animals, many a times, is a slow process. It is hypothesized that oxidative stress might be causing the observed delay in return to normal status of such infected animals. Ascorbic acid scavenges reactive oxygen species (ROS) during oxidative stress (Bisla *et al.* 2004, Sunil Kumar *et al.* 2010) and spare other antioxidants in relieving oxidative stress (Frey 1991).Though much work has been done on various aspects of oxidative stress, yet investigations on efficacy of various antioxidants in addition to therapeutic agents in reduction of oxidative stress in bovine tropical theileriosis in cattle had not been fully explored.

In the light of above situation, the present study was planned to assess the severity of *T. annulata* infection on some hematological parameters and antioxidant enzymes in naturally infected bovine as well as to study the role of ascorbic acid in diminishing the oxidative stress improving clinical recovery of the animal.

MATERIALS AND METHODS

Clinical cases (20) of bovine tropical theileriosis were used. A total of 26 crossbred cattle were included in the present study, out of which 20 animals were infected with theileria and 6 healthy animals were kept as control (group C). Infected animals were not given any prior treatment and confirmed to be infected by laboratory testing either in schizont stage or piroplasm stage. The animals were divided into group A (10) included *Theileria annulata* infected animals treated with buparvaquone @ 2.5mg/kg body weight i/m once and group B (10) included *Theileria annulata* infected animals treated with buparvaquone @ 2.5mg/kg body weight i/m once and ascorbic acid @ 15 mg/kg body weight I/m for consecutive 3 days. Both group A and group B were further divided in 2 sub-groups of 5 animals each as per schizont stage (A_1 and B_1) and piroplasm stage (A_2 and B_2) of disease. Pre-treatment observations were made once at the time of examination and post treatment observations were made on day 3 and day 14.

Clinical examination: Body temperature, palpation of superficial lymph nodes, determination of the size of lymph node, colour of mucus membrane as indication of anaemic status, presence/identification of ticks attached on animal bodies and other clinical signs including anorexia, dullness, depression, diarrhoea, nasal discharge etc., were recorded.

Hematological parameters: Blood was collected from jugular vein in sterile vials containing ethylene diaminetetra acetic acid (EDTA) for haematological parameters, viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocytes count (TLC) and differential leucocyte count (DLC). Standard procedures were followed for the haematological parameters (Schalm *et al.* 1975).

Parasitological parameters: Lymph node biopsy smears and blood smears were fixed on microslides using methanol and stained by Giemsa stain (1:10 dilution) for 30–40 min. Per cent parasitemia was estimated microscopically by counting the numbers of piroplasm infected erythrocyte in about 10,000 erythrocytes. Presence of schizonts in biopsy smears was semi-quantitated on +1 to +4 scale, wherein +1 stands for rare; +2 means sparse; +3 means high and +4 means very high number. Vector ticks were identified by studying their morphology.

Biochemical assay: Blood with anticoagulant was centrifuged at 3,000 rpm for 15 min in a refrigerated centrifuge to separate plasma. The plasma was stored at –20°C in aliquots for biochemical analysis. The level of total proteins was estimated in plasma using Autopak kits following Biuret method by a model fully automated random access clinical chemistry analyzer. Lipid peroxidation level was assessed in plasma by estimating malondialdehyde (MDA) level by the method of Ohkawa *et al.* (1979). Glutathione peroxidase (GPx) activity in plasma was measured as per Hafeman *et al.* (1974). Superoxide dismutase (SOD) was estimated as per Madesh and Balsubramaniam (1998).

Statistical analysis: The data so obtained was analysed by Duncan test for multiple comparisons using computer software Duncan's Multiple Range Test. $P \leq 0.05$ was taken as the critical criterion for statistically significant differences between the data.

RESULTS AND DISCUSSION

The present study was carried out to check the hypothesis that clinical recovery in theileria infected animals is determined by the oxidative stress induced by the intracellular parasite. The study also envisaged that administration of antioxidants should result into mitigation of damage caused by free radicals during the oxidative stress.

Clinical observations: The clinical signs and symptoms in theileria infected animals were fever, enlargement of superficial lymph nodes, anorexia, nasal discharge, diarrhea, depression and dullness. Similar findings were reported earlier (Brown 1990, Radostits *et al.* 2007, El-Deeb and Younis 2009). The presence of clinical signs in the study population, its intensity and day of its disappearance were recorded and used to monitor the supplementary effect of antioxidant therapy on recovery process. On day 0, fever, enlarged lymph nodes, anorexia, dullness and depression were the consistent finding in all the infected animals while some animals also had nasal discharge and diarrhea. On day 3 post treatment, animals started recovering as elevated normal body temperatures returned to normal, lymph node size started regressing and animals started feeding. However, nasal discharge and diarrhea were found in 1 out of 5 schizont infected animal and 2 out of 5 piroplasm infected animals on day 3 post-treatment but still high in group A animals as compared to group B animals (Table 1). On day 3, dullness and depression were comparatively less in group B animals than the group A animals as compared to day 0, which indicated antioxidant therapy enhancing the recovery in infected animals. On day 14 post treatment, animals did not show any characteristic

sign and symptoms of disease in both groups A and B while recovery in terms of normal body activities, alertness and activeness was faster in group B animals.

The infected animals had higher body temperature (high grade fever) ranging from 104.3ºF to 104.8ºF as compared to 101.6±0.18ºF in healthy animals (Table 1). The body temperature returned to normal in all the animals after 3 days of buparvaquone treatment. Superficial lymph nodes were highly enlarged at schizont stage of disease in both the groups as compared to piroplasm stage. Size of lymph node started regressing after buparvaquone treatment. The findings are in agreement with those reported earlier (Rakha and Sharma 2003). Color of mucous membrane of all the animals was noticed to ascertain the degree of anaemia. On day 0 and 3 post treatment, anaemia was 'mild' to 'moderate' (i.e. Hb ranging from 7.94±0.56 to 8.56±0.29 g/dl) in animals infected with schizont stage (group A_1 and B_1) and 'severe' to 'very severe', with Hb ranging from 5.80±0.09 to 7.40±0.48 g/dl in animals infected with piroplasm stage (Table 2). On day 14 post treatment, Hb levels raised in animals of both groups (A and B). Vitamin C administration in the theileria infected animals might have reduced the oxidative stress, thereby reducing erythrocyte destruction and improvement in haemoglobin levels of infected animals getting supportive therapy of antioxidants.

Very high tick density was found on all infected animals on the day of observation (day 0). Tick density remained almost similar on day 3 and 14 post treatment in all animals because no acaricidal drug was used in this study. Morphology of ticks was examined and ticks were identified as *Hyalomma anatolicum*. On day 0, the number of schizonts in lymph node smear was 'very high' (+3 to +4) in schizont stage of disease in group A_1 and B_1 animals. Mean piroplasm density measured as percentage was found 0.86 in group A_2 animals and 0.92 in group B_2 animals. After administration of specific drug buparvaquone, animals of both group (A and B) were found free of schizonts and piroplasms by day 3 post treatment. This observation is in agreement with earlier reports of Hudson *et al.* (1985); McHardy *et al.* (1985) and Dhar *et al.* (1986). The study

Table 1. Clinical status of animals infected with *T. annulata* and treated with buparvaquone alone (Group A) or in combination with ascorbic acid (Group B)

Clinical sign	Groups											
	Control (6)	Group A (buparvaquone)						Group B (buparvaquone+ascorbic acid)				
		Subgroup A1schizont (5)			Subgroup A2 piroplasm (5)			Subgroup B1schizont (5)			Subgroup B2 piroplasm (5)	
		Day 0	Day 3	Day 14	Day 0	Day 3	Day 14	Day 0	Day 3	Day 14	Day 0	Day 3	Day 14
Fever	ND	5	2	0	5	2	0	5	1	0	5	2	0
Enlarged lymph node	ND	5	4	0	5	4	0	5	4	0	5	3	0
Anorexia	ND	5	0	0	5	1	0	5	0	0	5	0	0
Nasal discharge	ND	3	1	0	4	2	0	2	0	0	3	1	0
Diarrhoea	ND	2	1	0	3	2	0	3	0	0	3	0	0
Dull	ND	5	5	2	5	5	4	5	2	1	5	2	2
Depressed	ND	5	5	2	5	5	4	5	2	1	5	3	2

• The figures represent the number of animals showing a specific clinical symptom.

Table 2. Haematological values of animals infected with *T. annulata* and treated with buparvaquone alone (Group A) or in combination with ascorbic acid (Group B)

Parameter	Groups											
	Control (6)	Group A (buparvaquone)						Group B (buparvaquone+ascorbic acid)				
		Subgroup A1 schizont (5)			Subgroup A2 piroplasm (5)			Subgroup B1 schizont (5)			Subgroup B2 piroplasm (5)	
		Day 0	Day 3	Day 14	Day 0	Day 3	Day 14	Day 0	Day 3	Day 14	Day 0	Day 3	Day 14
Haemoglobing(g/dl)	10.80^{a}	7.94^{b}	8.00^{b}	8.80^{ab}	6.06^{d}	5.80^{d}	6.60^{c}	8.56^{b}	8.20^{b}	9.28^{ab}	7.12^{b}	7.40^{b}	8.60^{ab}
	±0.32	± 0.56	± 0.86	± 0.87	± 0.10	± 0.09	± 0.26	± 0.29	± 0.40	± 0.46	± 0.55	± 0.48	± 0.56
PCV (%)	32.20^{a}	25.00^{b}	25.60^{b}	27.80^{b}	19.60^{cd}	18.4^{d}	20.80^{cd}	25.80^{bc}	25.40^{bc}	28.80^{b}	22.00^{c}	23.80^{c}	26.80^{bc}
	± 0.80	± 1.60	± 2.50	± 2.30	± 0.70	± 0.50	± 0.50	±0.90	± 1.10	± 1.50	± 1.60	±2.10	±1.80
TEC (10^9/ml)	5.44^{a}	4.15^{c}	4.18^{c}	4.54^{bc}	3.31^{d}	3.18^{d}	3.44^{d}	4.02^{c}	4.16^{c}	4.80^{b}	3.67^{cd}	3.80^{cd}	4.39^{bc}
	± 0.14	± 0.29	± 0.38	± 0.41	± 0.09	± 0.07	± 0.07	± 0.28	± 0.20	± 0.24	± 0.25	± 0.37	± 0.29
TLC (10^6/ml)	5.67^{a}	4.64^{ab}	3.88^{b}	4.93^{ab}	3.44^{b}	3.45^{b}	4.42^{a}	4.50^{ab}	4.41^{ab}	4.56^{ab}	3.53^{b}	3.56^{b}	4.42^{ab}
	± 0.23	± 0.78	± 0.27	± 0.95	± 0.32	± 0.30	± 0.99	± 0.54	± 0.54	± 0.86	± 0.27	± 0.24	± 0.52

Means with different lower case superscripts indicate significant difference ($P \leq 0.05$) within a row for a parameter.

suggested that single dose of buparvaquone is sufficient to kill all the schizonts and piroplasms of *T. annulata*. To ensure better and faster clinical recovery, antioxidant therapy should be undertaken rather than repeating administration of buparvaquone.

Haematological parameters: The values of haematological parameters in healthy as well as infected animals treated with either buparvaquone alone (group A) or in combination with ascorbic acid (group B) are presented in Table 2. TEC, Hb concentration and PCV markedly declined in the infected animals. Estimated Hb in infected animals on day 0 was low (6.06 g/dl to 8.56 g/dl) that showed significant anaemic condition in infected animals. Similar observations were also reported by Rezaei and Dalir-Naghadeh (2006) and Razavi *et al.* (2011). Anaemia was more pronounced in animals at piroplasm stage of theileriosis as compared to animals at schizont stage. Till day 3 post-treatment, the Hb levels increased non-significantly but significant rise was observed on day 14. Similar pattern was observed in PCV, TEC and TLC. Lymphocytopenia in infected animals was a significant finding. Lymphocyte number further decreased on day 3 post treatment and returned to normal by day 14. These findings are in accordance with the earlier reports (Preston *et al*. 1992, Nichani *et al.* 1994, Omer *et al.* 2002). This might be due to destruction of lymphocytes in lymphoid organs and an infiltration of these cells into various organs. Decrease in neutrophils, basophils and monocytes was also observed in infected animals on day 0. Several other researchers (Osman and Al-Gaabary 2007, El-Deeb and Younis 2009) have also reported a pronounced lymphocytopenia, neutropenia and monocytopenia in the theileria infected animals, which is in agreement with our findings.

Lipid peroxidation: Oxidative stress results when the production of reactive metabolites of oxygen exceeds their safe disposal by antioxidant mechanisms. The erythrocytic membrane is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals and is very susceptible to lipid peroxidation (May *et al*. 1998, Devasena *et al*. 2001). The values of lipid peroxidation (MDA concentration) and antioxidant enzyme activities in healthy and infected animals were compared and it was observed that mean MDA concentration (nmol/ml) on day 0 was 14.15 in animals suffering from schizont stage (group A_1) and 18.74 in animals suffering from piroplasm stage (group A_2) of disease. Mean MDA values in group B were very high i.e. 14.81nmol/ml and 19.35 nmol/ml, respectively, in animals infected with schizont stage and piroplasm stage of disease. This indicated increase in oxidative stress in the infected animals. MDA values further elevated significantly ($P<0.05$) in group A animals on day 3, while declined significantly in group B animals. The reduction in MDA values was clearly attributable to administration of ascorbic acid for 3 days continuously. On day 14 post treatment, mean MDA values were again elevated in infected animals of both groups. Also, the MDA values were higher in piroplasm stage as compared to those in schizont stage of the disease. The significantly higher levels of lipid peroxidation (MDA) seen in the present study were similar to those reported in the plasma and erythrocytic samples of cattle naturally infected with theileriosis (Rezaei and Dalir-Naghadeh 2006, El-Deeb and Younis 2009, Razavi *et al.* 2011). Naziroglu *et al.* (1999) also reported significantly higher levels of lipid peroxidation (MDA) in plasma and erythrocyte samples after treatment with buparvaquone. Buparvaquone persists longer in plasma (half-life 7 days) due to the presence of tertiary-butyl moiety which results in far slower metabolism to the hydroxy derivative at the 4-position on the cyclohexyl ring (McHardy 1989) and functions in the treatment of *Theileria annulata* by forming free radicals. Thus elevated level of MDA at day 14 post treatment in the present study animals might be due to residual effect of buparvaquone in animals.

Glutathione peroxidase enzyme (GPx) level was significantly ($P<0.05$) higher on day 0 in infected animals as compared to healthy animals. Mean GPx values on day 3 were significantly ($P<0.05$) lower in group B animals as compared to the GPx values on day 0 while in animals of group A, GPx level elevated further by day 3. On day 14 post treatment, mean GPx values in animals were again elevated in both the groups. GPx reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water, thus protecting the animals from oxidative damage. Mean plasma GPx enzyme level, on day 0, were significantly higher in all the theileria infected animals than the base values indicating oxidative stress in the infected animals. However, GPx values were higher in piroplasm stage compared to schizont stage. The significant rise in GPx activity was not in line with the findings of Naziroglu *et al.* (1999), who reported that GPx activities in plasma and erythrocytes did not differ significantly between a group of cattle naturally infected with *T. annulata* and treated with buparvaquone as well as uninfected, untreated controls. Similarly, Rezaei and Dalir-Naghadeh (2006), Baghshani *et al.* (2011) and Razavi *et al.* (2011) also reported activities of erythrocyte glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase were significantly lower in the theileria affected cattle than in healthy ones. However, the significant rise in GPx activity in our study could be due to the fact that GPx appeared to be the major mechanism for intracellular decomposition of lipid peroxides (Christophersen 1966, Flohe 1971). Hafeman *et al.* (1974) also proposed that GPx played a crucial role in preventing membranes from peroxide damage induced by lipid peroxides. Grewal *et al.* (2005) also reported that infection with theileria led to increased oxidative stress in the animals and even a significant rise in the activities of antioxidant enzymes could not lower the oxidative stress. The mean GPx values were observed to be elevated after 3 days of start of buparvaquone treatment in both stages of disease in group A animals. However, on day 14, mean GPx values were still higher than that at day 3, although the increase at

Table 3. Differential leucocyte count (10^6/ml) of animals infected with *T. annulata* and treated with buparvaquone alone (Group A) or in combination with ascorbic acid (Group B)

Group	Control (6)	Group A (buparvaquone) (10)			Group B (buparvaquone+ ascorbic acid) (10)		
		Day 0	Day 3	Day 14	Day 0	Day 3	Day 14
Neutrophils	1.77± 0.25	1.39± 0.16	1.32± 0.08	1.63± 0.21	1.48 ± 0.10	1.509± 0.11	1.698± 0.15
Lymphocytes	3.56^{a}± 0.13	2.26^{b}± 0.26	2.011^{b}± 0.11	2.41^{b}± 0.33	2.28^{b}± 0.18	2.236^{b}± 0.17	2.532^{b}± 0.27
Eosinophils	0.17± 0.04	0.19± 0.05	0.133± 0.02	0.12± 0.02	0.19± 0.03	0.142± 0.03	0.154± 0.03
Basophils	0.06^{a}± 0.01	0.054^{ab}± 0.01	0.038^{b}± 0.003	0.04^{ab}± 0.01	0.04^{ab}± 0.009	0.048^{ab}± 0.01	0.053^{ab}± 0.007
Monocytes	0.08^{a}± 0.01	0.051^{b}± 0.01	0.047^{b}± 0.006	0.04^{b}± 0.006	0.04^{b}± 0.007	0.046^{b}± 0.005	0.052^{b}± 0.012

Table 4. Biochemical parameters of animals infected with *T. annulata* and treated with buparvaquone alone (Group A) or in combination with ascorbic acid (Group B)

Parameter	Control (6)	Groups											
		Group A (buparvaquone)						Group B (buparvaquone+ascorbic acid)					
		Subgroup A1schizont (5)			Subgroup A2 piroplasm (5)			Subgroup B1schizont (5)			Subgroup B2 piroplasm (5)		
		Day 0	Day 3	Day 14	Day 0	Day 3	Day 14	Day 0	Day 3	Day 14	Day 0	Day 3	Day 14
Protein (g/dl)	6.93^{a}	5.95^{bc}	5.62^{c}	6.11^{bc}	5.74^{bc}	5.63^{c}	6.03^{bc}	5.96^{bc}	5.92^{bc}	6.34^{ab}	6.93^{a}	5.95^{bc}	5.62^{c}
	±0.243	±0.344	±0.195	±0.236	±0.110	±0.104	±0.144	±0.163	±0.187	±0.339	±0.243	±0.344	±0.195
MDA (nmol/ml)	4.13^{a}	14.15^{c}	22.57^{d}	28.09^{ef}	18.74^{d}	26.90^{e}	33.46^{f}	14.81^{c}	8.92^{b}	20.72^{cd}	4.13^{a}	14.15^{c}	22.57^{d}
	±0.135	±1.05	±0.93	±2.23	±0.68	±1.34	±2.35	±0.79	±0.86	±3.44	±0.135	±1.05	±0.93
GPx (U/mg protein)	1.37^{a}	2.30^{b}	3.49^{c}	3.79^{c}	3.82^{c}	4.70^{d}	5.05^{d}	2.36^{b}	1.38^{a}	1.59^{ab}	1.37^{a}	2.30^{b}	3.49^{c}
	±0.33	±0.34	±0.34	±0.35	±0.36	±0.39	±0.40	±0.40	±0.37	±0.38	±0.33	±0.34	±0.34
SOD (U/mg protein)	0.305^{a}	0.331^{ab}	0.367^{b}	0.379^{b}	0.491^{bc}	0.522^{c}	0.521^{c}	0.347^{ab}	0.297^{a}	0.342^{ab}	0.305^{a}	0.331^{ab}	0.367^{b}
	±0.007	±0.019	±0.031	±0.031	±0.053	±0.054	±0.069	±0.032	±0.021	±0.030	±0.007	±0.019	±0.031

Means with different lower case superscripts indicate significant difference ($P \leq 0.05$) within a row for a parameter.

day 14 was non-significant.

In group B animals, mean GPx values were significantly lower on day 3 as compared to day 0 values which might be attributed to the administration of ascorbic acid continuously for 3 days. On day 14 post treatment, mean GPx values in animals suffering from either schizont or piroplasm stages were again elevated. Mean GPx values on day 14 post treatment with antioxidant i.e., in group B, were non-significantly higher in schizont stage but significantly higher in piroplasm stage as compared to corresponding values on day 3 post treatment but less than the values on day 1 of observation. Though the values in animals of group B were higher but still lower than the animals of group A indicated that ascorbic acid lowered the oxidative stress when administered for three consecutive days but oxidative stress somehow increased after arresting the ascorbic acid supplementation.

SOD values were higher in all the animals suffering from bovine tropical theileriosis as compared to those of control group, though the differences were statistically non-significant at schizont stage and significant ($P<0.05$) at piroplasm stage of disease (Table 4). On day 3 post treatment, mean SOD values in animals of group A showed an increasing trend while SOD values decreased in animals of group B. On day 14 post treatment, mean SOD values in group A animals were similar to those observed at day 3 post treatment while mean SOD values in group B animals were comparable to those on day 0 although slightly higher than the value recorded on day 3 post treatment.The SOD catalyzes the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide. Thus, it is an important antioxidant defense in nearly all cells exposed to oxygen. Significant increase was observed at piroplasm stage of disease. Grewal *et al.* (2005) also reported non-significant changes in SOD levels in theileria infected animals. However, significant rise in SOD activity in the present study was not in line with the findings of Rezaei and Dalir-Naghadeh (2006) and Razavi *et al.* (2011). They reported significantly lower activities of erythrocyte glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase in theileria affected cattle than in healthy ones. This might be due to the fact that the rise in GPx activity in the present study was probably a consequence of the activation of the RBCs safeguard mechanisms in response to the oxidative damages in the earlier stages of the disease, but the activity of the antioxidant enzymes gradually decreased as a squeal of insufficient capability of such mechanisms to neutralize the oxidant agents in the later stages of the acute disease.

Among the 3 biochemical parameters to determine the oxidative stress, MDA and GPx levels are sensitive as well as more responsive to the presence of *T. annulata* parasites and buparvaquone treatment. Enhanced antioxidant administration had led to significant reduction in oxidative stress. Thus, it is likely that in ascorbic acid supplemented group, ascorbate has played a major role as an antioxidant and maintained the activities of GPx and SOD.

REFERENCES

Ahmed J S, Glass E J, Salih D A and Seitzer U. 2008. Review: innate immunity to tropical theileriosis. *Innate Immunology* **14**: 5–12.

Baghshani H, Razmi GR, Saeed Yaghfouri and Dezaki AA. 2011. Status of some oxidative stress biomarkers in sheep naturally infected with theileriosis. *Research Opinions in Animal* and *Veterinary Sciences* **1** (8): 499–504.

Bisla R S, Singh J, Chawla S K and Krishnamurthy D. 2004. Assessment of ascorbic acid as an antioxidant in buffaloes during diaphragmatic herniorrhaphy. *Indian Journal of Veterinary Surgery* **25**: 94–97.

Brown CGD. 1990. Control of tropical theileriosis (*Theileria annulata* infection) of cattle. *Parasitologi*a **32**: 23–31.

Christophersen BO. 1966. Oxidation of reduced glutathione by sub cellular fractions of rat liver. *Biochemistry Journal* **100**: 95–101.

Devasena T, Lalitha S and Padma K. 2001. Lipid peroxidation, osmotic fragility and antioxidant status in children with acute post-streptococcal glomerulonephritis. *Clinica Chimica Acta* **308**: 155–61

Dhar S, Malhotra D V, Bhushan C and Gautam O P. 1986. Chemotherapy of *Theileria annulata* infection with buparvaquone. *Veterinary Record* **119**: 635–36.

El-Deeb W M and Youni E E. 2009. Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with particular orientation to oxidative stress and ketosis relationship. *Veterinary Parasitology* **164**: 301–05.

Flohe L. 1971. Glutathione peroxidase: enzymology and biological aspects. *Klinische Wochenschrift* **49**: 669–83.

Frey B. 1991. Vitamin C protects lipids in human plasma and low density lipoprotein against oxidative damage. *American Journal of Clinical Nutrition* **54**: 113.

Grewal A, Ahuja C S, Singha S P S and Chaudhary K C. 2005. Status of lipid peroxidation, some antioxidant enzymes and erythrocyte fragility of crossbred cattle naturally infected with *Theileria annulata*. *Veterinary Research Commun*ication **29**: 387–94.

Hafeman D G, Sunde R A and Hoekstra W G. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in rat. *Journal of Nutrition* **104**: 580–87.

Hassanpour A, Sabegh Y G and Sadeghi-nasab. 2013. Assessment of serum antioxidant enzymes activity in cattle suffering from Theileriosis. *European Journal of Experimental Biology* **3** (1): 493–96.

Hudson A T, Randall A W, Fry M, Ginger C D, Hill B, Latter V S, McHardy N and Williams R B. 1985. Novel anti-malarial hydroxynaphthoquinones with potent broad spectrum protozoal activity. *Parasitology* **90**: 45–55.

Kumar-Sunil B V, Singh G and Mear S K. 2010. Effects of addition of electrolyte and ascorbic acid in feed during heat stress in buffaloes. *Asian Australian Journal of Animal Science* **23** (7): 880–88.

Madesh M and Balsubramanian K A. 1998. Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry and Biophysics* **35**: 184–88.

May J M, Qu Z C and Mendiratta S. 1998. Protection and recycling of alfa tocopherol in human erythrocytes by intracellular ascorbic acid. *Archives of Biochemistry and Biophysics* **349**: 281–89.

Mbassa G K, Balembo O, Maselle R M and Mwaga N V. 1994. Severe anemia due to haematopoietic precursor cell destruction in field cases of East coast fever in Tanzania. *Veterinary Parasitol*ogy **52**: 243–56.

McHardy N. 1989. Buparvaquone, the new antitheilerial: a review of its efficacy and safety. (Ed.) Dolan T T. Theileriosis in Eastern, Central and Southern Africa. *Proceedings of Workshop on East Coast Fever Immunisation*. pp. 158–65.Lilongwe, Malawi, 20– 22 September 1988. ILRAD, Nairobi,

McHardy N, Wekesa L S, Hudson A T and Randall A W. 1985. Antitheilerial activity of BW 720C (buparvaquone): a comparison with parvaquone. *Research in Veterinary Science* **39**: 29–33.

Nazifi S, Razavi SM and Esmailnejad Z. 2009. Evaluation of serum proteins in cows naturally infected with *Theileria annulata*. *Journal of Applied Animal Research* **36** (1): 81–83.

Naziroglu M, Saki C E and Sevgili M. 1999. The effect of buparvaquone treatment on the levels of some antioxidant vitamins, lipid peroxidation and glutathione peroxidase in cattle with theileriosis. *Journal of Veterinary Medicine* **46**: 233–39.

Nichani A K, Craigmile S C, Thorp B H, Ritchie M, Brown C G D and Spooner RL. 1994. Response of *Theileria annulata* infection in efferent lymph leaving the node draining the site of infection. *Proceedings of European Union Third Coordination Meeting on Tropical Theileriosis. Antalya, Turkey*, October 4–9, 1994. pp 117–22.

Ohkawa H, Ohishi N and Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annals of Biochemistry* **95**: 351–58.

Omer O H, El-Malik K H, Mahmouda O M, Haroun E M, Hawas A, Sweeney D and Magzoubd M. 2002. Haematological profiles in pure bred cattle naturally infected with *Theileria annulata* in Saudi Arabia. *Veterinary Parasitology* **107**: 161–68.

Osman S A and Al-Gaabary M H. 2007. Clinical, haematological and therapeutic studies on tropical theileriosis in water buffaloes in Egypt. *Veterinary Parasitology* **146**: 337–40.

Preston P M, Brown C G D, Bell-Sakyi L, Richardson W and Sanderson A. 1992. Tropical theileriosis in Bos taurus and Bos taurus cross Bos indicus calves : response to infection with graded doses of sporozoites of *Theileria annulata*. *Research in Veterinary Sciences* **53**: 230–43.

Radostits O M, Gay C C, Hinchcliff K W and Constable P D. 2007. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*.10th edn. Elsevier, Philadelphia.

Rakha N K and Sharma R D. 2003. Current epidemiological status of bovine tropical theileriosis in Haryana (India). *Intas Polivet* **4** (1): 15–22.

Razavi S M, Nazifi S, Batenia M and Rakhshandehrooa E. 2011. Alterations of erythrocyte anti-oxidant mechanisms: Antioxidant enzymes, lipid peroxidation and serum trace elements associated with anaemia in bovine tropical

Theileriosis. *Veterinary Parasitology* **180**: 209–14.

Rezaei A S and Dalir-Naghadeh B. 2006. Evaluation of antioxidant status and oxidative stress in cattle naturally infected with *Theileria annulata*. *Veterinary Parasitology* **142**: 179–86.

Saleh M A and Al-salahy M B. 2011. Circulating oxidative stress status in dromedary camels infested with sarcoptic mange. *Veterinary Research Communications* **35**: 35–45.

Schalm O W, Jain N C and Carrol E J. 1975. *Veterinary Haematology*. 3rd edn. pp 197–99. Lea and Febiger, Philadelphia.

Shiono H, Yagi Y, Kumar A, Yamanaka M and Chikayama Y. 2004. Accelerated binding of autoantibody to red blood cells with increasing anaemia in cattle experimentally infected with *Theileria sergenti*. *Journal of Veterinary Medicine and Surgery* **51**: 39–42.

Watarai S, Kushi Y, Shigeto R, Misawa N, Eishi Y, Handa S and Tasuda T. 1995. Production of monoclonal antibodies directed to hanganutziu-deicher active gangliosides, N-glycolylneuraminic acid containing gangliosides. *Journal of Biochemistry* **117**: 1062–69.