Original Research Article

Evaluation of the Antioxidants as Adjunct Therapy in Cattle Naturally Infected with Bovine Tropical Theileriosis

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A B S T R A C T

The present study was planned to evaluate the effect of supplementing non-enzymatic antioxidants as adjunct therapy in enhancing clinical recovery of cattle naturally infected with bovine tropical theileriosis. A total of 18 crossbred dairy cattle with the clinical signs consistent of bovine tropical theileriosis and confirmation by detection of piroplasm infected erythrocytes in blood smears, were randomly divided into 3 groups (A, B, C) of 6 animals each. Animals in group A were treated with specific therapy, buparvaquone @ 2.5 mg/kg intramuscular once; group B treated with vitamin C @ 15 mg/kg intramuscular for nine days along with specific therapy and group C treated with vitamin E @ 1.5 mg/kg plus selenium @ 0.05 mg/kg intramuscular in three doses at three days interval along with specific therapy. The therapeutic evaluation was done on day 0, 3, 6 and 9 of therapy based upon alteration in haematologicochemical profile and oxidative stress indices. Administration of buparvaquone alone led to disappearance of clinical signs; however, antioxidant supplementation adjunct to specific therapy hastened the clinical recovery. Quicker revival in haematologicochemical profile was observed in animals of group C which were supplemented with vitamin E-selenium as compared to animals of group B. The oxidative stress indices in blood showed significant improvement in animals which were supplemented with antioxidants; however, more improvement was observed in animals of group C as compare to animals of group B. Thus, the present findings suggest vitamin E-selenium as better adjunct antioxidant therapy than vitamin C in bovine tropical theileriosis.

Keywords

Theileria annulata; bovine tropical theileriosis; vitamin C; vitamin E and selenium; malondialdehyde; glutathione peroxidase; superoxide dismutase

Accepted: 30 October 2017
Available Online: 10 November 2017

Introduction

Bovine tropical theileriosis, a disease of global economic importance, caused by haemoproteozan parasite *Theileria annulata* and transmitted by ticks of genus *Hyalomma* (Preston, 2001) is characterized by lymphadenopathy, spleenomegaly, fever, anaemia, weakness and loss of body weight (Omer *et al.*, 2002; El-Deeb and Younis, 2009). A significant feature of the disease is anaemia due to overproduction of cytokines and reactive oxygen species (Nazifi *et al.*, 2009; Saleh *et al.*, 2011), haematopoietic precursor cell destruction (Mbassa *et al.*, 1994), activated complement products (Omer *et al.*, 2002; El-Deeb and Younis, 2009).
et al., 2002), binding of autoantibody (IgG) to red blood cells (RBC) and removal of infected and non-infected erythrocytes by phagocytosis (Shiono et al., 2004). Hepatic tissue damage in this disease includes coagulative necrosis, destruction of hepatic cords and heavy infiltration of lymphocytes in peri-portal area, which indicates severe damage to hepatobiliary system due to hypoxia resulting from anaemia and jaundice (Stockham et al., 2000).

Oxidative stress due to increase in reactive oxygen species in cells of hosts infected with parasite *T. annulata* is well established (Grewal et al., 2005; Nazifi et al., 2008; El-Deeb and Younis, 2009). Oxidative stress evident by the reduction in activity of antioxidant enzymes and decreased level of antioxidant vitamins in blood of parasitized animals has been reported in theileria infected cattle (Issi and Gul, 2001; Shiono et al., 2001; Nazifi et al., 2008). Reactive oxygen species can be scavenged by antioxidant system which includes antioxidant enzymes glutathione peroxidase and superoxide dismutase and non-enzymatic components involving vitamin E, vitamin C, selenium and glutathione. α-tocopherol (vitamin E) and ascorbic acid (vitamin C) acts as cellular antioxidant vitamins which are present in the cell membrane and plasma lipoproteins (Bast et al., 1991).

The antioxidant mechanisms of ascorbic acid are based on the donation of hydrogen atom to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen (Rumsey et al., 1999). Vitamin E effectively minimizes oxidative stress, lipid peroxidation and toxic effects of reactive oxygen species in biological systems (Ogutcu et al., 2006). Selenium (Se) as co-antioxidant) seem to be primarily associated with its presence in the selenoenzymes, which are known to protect DNA and other cellular components from oxidative damage (Valko et al., 2006).

Buparvaquone is the most effective and safest drug for treatment of *Theileria* in cattle, and this drug has been thoroughly investigated both in vitro and in vivo (Dhar et al., 1988; McHardy, 1990; Keles et al., 2001). Kumar et al., (2016) measured the oxidative stress in *Theileria* infected cattle and reported that there were significantly altered levels of enzymes indicating a high degree of oxidative stress in theileria infected animals.

Further, the administration of buparvaquone (drug of choice) alone led to further increase in levels of oxidative stress. Study for the role of antioxidant therapy along with anti theilerial drug for three days in bovine tropical theileriosis revealed that there was significant reduction in oxidative stress levels and there was faster clinical recovery in infected-animals. However, when the vitamin C administration was stopped after 3 days, oxidative stress levels again rose beyond normal values.

So keeping in view these observations, the present study was planned to evaluate the effect of supplementing non enzymatic antioxidants and that too given for a longer duration to further enhance clinical recovery of theileriosis in bovines.

**Materials and Methods**

The study was conducted in 18 crossbred (9 adults and 9 young) dairy cattle naturally infected with *T. annulata*. Animals found infected with theileriosis in laboratory diagnosis, not given any prior treatment and haemoglobin level >6 g/dl were selected for therapeutic evaluation.
**Therapeutics regimen**

*T. annulata* infected animals were randomly divided into three groups. Group A (n = 6) animals treated with buparvaquone only @ 2.5 mg/kg body weight intramuscular once, group B (n = 6) animals treated with buparvaquone @ 2.5 mg/kg body weight intramuscular once and ascorbic acid @ 15mg/kg body weight intramuscular daily for 9 days and group C (n = 6) animals treated with buparvaquone @ 2.5 mg/kg body weight intramuscular once with vitamin E @ 1.5mg/kg body weight and selenium @ 0.05mg/kg body weight intramuscular on day 0, 3 and 6.

The samples were collected on day 0, 3, 6 and 9 to monitor - clinical recovery, presence/absence of a clinical sign and effect of supplementing antioxidant therapy on recovery process.

**Clinical observations**

Clinical vital parameters rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute) were recorded. The superficial lymph node(s) enlargement graded as 1 to 4. The lymph node enlargement was graded as 1, 2, 3 or 4 corresponding to ‘very small’, ‘small’, ‘large’, or ‘very large’ lymph nodes respectively (Rakha and Sharma, 2003).

**Sampling**

Blood samples were collected in triplet, in ethylenediamine-tetraacetic acid (EDTA) vial for haematological examination, in vial without anticoagulant for harvesting serum and in centrifuge tube containing heparin for separation of plasma and haemolysate. The plasma was separated in aliquots and 10% stock haemolysate was prepared form blood containing heparin. The plasma, haemolysate and serum were stored at -20°C in aliquots till analysis.

**Parasitological observations**

Thin blood smears and lymph node aspirate smears from swollen prescapular lymph nodes were fixed in methanol at the site of sample collection and were stained by Giemsa stain using 1:10 dilution for 30 min. Percent parasitaemia was estimated microscopically by counting the numbers of piroplasm infected erythrocyte in total of about 1,000 erythrocytes.

Presence of schizonts in biopsy smears was semi-quantitatively measured on the scale of 1 to 4. Wherein ‘1’ stands for rare; ‘2’ means sparse; ‘3’ means high and ‘4’ means very high in numbers in the smear.

**Haemato-biochemical examinations**

The blood samples collected in EDTA vials were used for complete haematological examination using fully automated Haematology Cell Counter (MS4s, Melet Schlosing Lab.). The serum samples were analyzed for estimation of biochemical profile using fully automated random access Clinical Chemistry Analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). Blood samples collected in heparin were used for measurement of oxidative stress indices both in plasma and haemolysate. Lipid peroxidation in terms of malondialdehyde (MDA) levels was assessed by method of Ohkawa et al., (1979). Glutathione peroxidase (GPx) activity was measured by method of Hafeman et al., (1974). Superoxide dismutase (SOD) activity was measured by method of Madesh and Balsubramanian (1998). The haemoglobin in the haemolysate was estimated spectrophotometrically by the cyanomethemoglobin method (Vankampen and Ziglstra, 1961).
Statistical analysis

The data generated was analyzed statistically by suitable statistical methods using statistical software package (SPSS 16.0). For analysis of various parameters observed for therapeutic efficacy, within and between groups, two-way analysis of variance (ANOVA) was applied. The results are presented as Mean±S.E. at the significance level, p≤ 0.05.

Results and Discussion

The present study was planned and conducted to check the hypothesis that oxidative stress and liver damage caused by *T. annulata* need to be reversed to ensure faster and complete clinical recovery and administration of different antioxidants should result into mitigation of damage caused by free radicals during the oxidative stress.

Clinical profile

Monitoring of clinical profile is depicted in table 1. All animals which were anorectic on day zero started feeding except one animal in group A which started feeding on day 6. Diarrhoea was present in three animals in group A, four animals in group B and C each. In animals of group B and group C there was no diarrhoea on day 3, while one animal in group A was diarrhoeic on day 3 and recovered from diarrhoea on day 6. All animals were dull on day 0. There was slow improvement and on day 9 three animals in group A, one animal in group B and one animal in group C were dull. Recovery in terms of anorexia, diarrhoea and dullness was faster in animals of group B and group C as compare to animals of group A. Clinical recovery observed in the present study was similar to that reported by Kumar *et al.*, (2016). There was almost no difference in recovery and clinical profile parameters in animals of group B and group C, but the recovery was faster in these animals as compare to animals of group A.

Clinical Vital parameters

Clinical vital parameters are depicted in table 2. After 3 days of buparvaquone treatment there was significant (p≤ 0.05) reduction in rectal temperature in all the animals and remained normal up to 9 days. As far as body temperature of infected animals is concerned, administration of antioxidants, vitamin C and vitamin E along with selenium did not make any significant impact. In our findings reduction in rectal temperature was due to the specific drug called buparvaquone alone, since no antipyretic/analgesic was administered to infected animals. Kumar *et al.*, (2016) also reported reduction in temperature due to buparvaquone alone in cattle infected with *T. annulata*.

Pulse rate on day 3 increased non significantly (p≤ 0.05) in animals of group A and decreased significantly in animals of group B and C. From day 3 to day 9, there was continuous significant decrease pulse rate of animals in all the three groups. Respiration rate increased significantly in animals of group A and decreased significantly in animals of group C and non-significantly in animals of group B. Maximum significant decrease among these three groups was observed in group C, followed by group B and least in group A. It may be due to decrease in harmful pathogenic effect of *T. annulata* and reduction in degree of anaemia.

Gradual reduction in size of lymph node in all the theileria infected animals was observed from day 0 to day 9, however lymph nodes remained palpable throughout the course of study. It is to be mentioned here that in all the animals which have suffered from bovine tropical theileriosis previously, lymph nodes always remained palpable (Rakha and
Sharma, 2003; Kumar et al., 2016). As administration of theilericidal drug buparvaquone, kills all Koch’s blue bodies and consequently regression of lymph node is faster. Additional supplementation of antioxidant therapy did not make additional measurable impact on size of lymph node(s).

Parasitological observations

Percent parasitemia in blood smear of 18 diseased animals was 0.42 ± 0.04 and schizont density was 0.61 ± 0.20. The results were in agreement with observations of Al-Temeimy (1982), Al-Robayi (1999) and Stockham et al., (2000). After administration of specific drug buparvaquone, animals of all groups were found to be free from schizonts and piroplasm on day 3 and remained so up to day 9. It revealed that buparvaquone administration killed all schizonts and piroplasm of *T. annulata*. This observation is in agreement with earlier reports of Sharma et al., (1987) and Singh (1990).

Haematological examinations

Erythrocytic indices are depicted in table 3. Hematological parameters viz. Hb, TEC, PCV and TLC revealed non-significant (p≤ 0.05) changes after administration of therapy. It might be due to our selection of mild cases of theileriosis in this study. However the significant changes in relative leucocytes count in the group B and group C were found up on administration of antioxidant therapy. Decrease in relative lymphocyte count may be due to removal of infected lymphocytes by phagocytic system. Increase in neutrophil count could be related to the protection of cell membrane and intracellular organelles by the antioxidant effect (Smith et al., 1997).

| Clinical sign | Day post treatment | No. of Diseased animals depicting clinical sign during 9 days of study period |
|---------------|-------------------|----------------------------------------------------------------------------|
|               |                   | Group A (n=6) | Group B (n =6) | Group C (n = 6) |
| Anorexia      | d 0               | 6/6           | 6/6            | 6/6             |
|               | d 3               | 1/6           | 0/6            | 0/6             |
|               | d 6               | 0/6           | 0/6            | 0/6             |
|               | d 9               | 0/6           | 0/6            | 0/6             |
| Diarrhoea     | d 0               | 3/6           | 4/6            | 4/6             |
|               | d 3               | 1/6           | 0/6            | 0/6             |
|               | d 6               | 0/6           | 0/6            | 0/6             |
|               | d 9               | 0/6           | 0/6            | 0/6             |
| Dullness      | d 0               | 6/6           | 6/6            | 6/6             |
|               | d 3               | 6/6           | 4/6            | 4/6             |
|               | d 6               | 4/6           | 2/6            | 2/6             |
|               | d 9               | 3/6           | 1/6            | 1/6             |
Table 2 Changes in clinical vital parameters in cattle infected with *T. annulata* during 9 days of treatment period (Mean ± S.E.)

| Parameter                  | Day post treatment | Group A (n=6)  | Group B (n=6)  | Group C (n=6)  |
|----------------------------|--------------------|----------------|----------------|----------------|
| Temperature (°F)           | d 0                | 104.12±0.20a   | 104.57±0.16b   | 104.63±0.23b   |
|                           | d 3                | 102.23±0.20a   | 101.93±0.08b   | 102.77±0.12b   |
|                           | d 6                | 102.00±0.10a   | 101.70±0.12b   | 101.43±0.12b   |
|                           | d 9                | 101.77±0.08b   | 101.67±0.20b   | 101.47±0.10b   |
| Pulse rate (per min)       | d 0                | 68.00±2.59a    | 74.33±2.54a    | 77.33±2.67a    |
|                           | d 3                | 74.67±1.96a    | 60.00±2.39b    | 63.50±2.74b    |
|                           | d 6                | 54.17±3.11a    | 58.83±1.64a    | 54.00±3.13a    |
|                           | d 9                | 48.00±3.17a    | 45.00±2.38a    | 42.67±2.47a    |
| Respiration rate (per min) | d 0                | 38.17±1.99a    | 47.33±1.45b    | 47.67±2.70b    |
|                           | d 3                | 45.33±1.52a    | 40.83±1.58c    | 39.17±1.64d    |
|                           | d 6                | 25.16±1.30b    | 25.17±2.65b    | 20.33±1.23c    |
| Lymph node size (scale 1 to 4) | d 0              | 2.50±0.22a    | 2.83±0.31a    | 3.17±0.31a    |
|                           | d 3                | 1.83±0.17a    | 2.17±0.17a    | 2.33±0.21b    |
|                           | d 6                | 1.83±0.17a    | 1.83±0.17a    | 1.83±0.17a    |
|                           | d 9                | 1.00±0.00a    | 1.00±0.00a    | 1.00±0.00a    |

Values with superscript a, b, c differ significantly (p<0.05) in a column and superscript x, y differ significantly (p<0.05) in a row for a parameter

Table 3 Changes in haematological parameters in cattle infected with *T. annulata* during 9 days of treatment period (Mean ± S.E.)

| Parameter     | Day post treatment | Group A (n=6)  | Group B (n=6)  | Group C (n=6)  |
|---------------|--------------------|----------------|----------------|----------------|
| Hb (g%)       | d 0                | 8.63±0.57      | 8.28±0.91      | 7.48±0.20      |
|               | d 3                | 8.38±0.53      | 8.06±0.21      | 7.18±0.33      |
|               | d 6                | 8.30±0.49      | 7.72±0.27      | 7.28±0.26      |
|               | d 9                | 8.21±0.66      | 8.00±0.35      | 7.68±0.24      |
| TEC (M/mm³)   | d 0                | 7.52±0.25      | 6.58±0.31      | 7.04±0.52      |
|               | d 3                | 7.43±0.33      | 6.48±0.25      | 7.08±0.63      |
|               | d 6                | 7.37±0.36      | 6.39±0.22      | 7.76±0.48      |
|               | d 9                | 7.39±0.56      | 6.62±0.27      | 8.09±0.52      |
| PCV (%)       | d 0                | 31.23±3.60     | 32.52±2.52     | 38.84±1.05     |
|               | d 3                | 33.51±1.87     | 30.53±0.63     | 38.93±0.75     |
|               | d 6                | 30.83±2.30     | 29.97±0.64     | 27.76±0.69     |
|               | d 9                | 32.78±2.81     | 31.08±1.14     | 30.05±0.66     |
| TLC (mm³/mm³) | d 0                | 6.76±0.96      | 7.27±0.62      | 7.14±1.08      |
|               | d 3                | 6.86±0.93      | 7.29±0.84      | 7.33±1.21      |
|               | d 6                | 7.31±0.42      | 7.67±0.87      | 7.88±1.34      |
|               | d 9                | 7.45±0.83      | 7.90±0.47      | 7.95±1.35      |
| Lymphocytes   | d 0                | 81.95±8.78     | 93.05±1.16     | 79.87±0.58     |
|               | d 3                | 73.53±9.19     | 88.47±1.30     | 91.78±0.56     |
|               | d 6                | 82.30±7.51     | 66.93±10.31c   | 80.35±10.63    |
|               | d 9                | 80.41±7.20     | 59.40±10.85    | 69.36±10.03    |
| Monocytes (%) | d 0                | 5.98±2.99      | 1.60±0.21      | 7.05±3.65      |
|               | d 3                | 8.98±3.43      | 2.58±0.29      | 7.30±3.57      |
|               | d 6                | 6.95±3.40      | 6.07±1.89      | 7.13±3.30      |
|               | d 9                | 8.67±2.99      | 10.87±2.77     | 7.88±3.37      |
| Neutrophils (%)| d 0                | 10.42±5.40     | 2.68±0.35      | 11.92±5.82     |
|               | d 3                | 13.03±5.77     | 7.15±1.64b     | 12.85±5.62     |
|               | d 6                | 9.20±3.64      | 18.93±7.25a    | 6.60±3.15      |
|               | d 9                | 11.82±3.87     | 20.37±6.49a    | 11.10±3.83     |
| Eosinophils (%)| d 0                | 1.45±0.59      | 2.52±1.16      | 1.07±0.62      |
|               | d 3                | 2.27±1.02      | 1.68±1.06      | 0.67±0.67      |
|               | d 6                | 1.55±0.79      | 7.58±3.66      | 5.62±2.27      |
|               | d 9                | 5.55±2.87b     | 8.92±2.24a     | 1.90±1.05b     |
| Basophils (%)  | d 0                | 0.17±0.08      | 0.15±0.08      | 0.10±0.05b     |
|               | d 3                | 0.18±0.07      | 0.72±0.66      | 0.02±0.02b     |
|               | d 6                | 0.10±0.04      | 0.38±0.11      | 0.28±0.19b     |
|               | d 9                | 0.25±0.09      | 0.35±0.14      | 0.15±0.07b     |

Values with superscript a, b, c differ significantly (p<0.05) in a column and superscript x, y differ significantly (p<0.05) in a row for a parameter

Hb=Haemoglobin content; TEC=Total erythrocyte count; PCV=Packed cell volume; TLC=Total leucocyte count.
**Table 4** Changes in liver function tests in cattle infected with *T. annulata* during 9 days of treatment period (Mean ± S.E.)

| Serum biochemical parameter | Day post treatment | Group A (n=6) | Group B (n=6) | Group C (n=6) |
|-----------------------------|-------------------|--------------|--------------|--------------|
| ALT (U/L)                   | d 0               | 35.90±2.16a  | 38.05±3.32a  | 36.20±3.12a  |
|                             | d 3               | 30.30±2.51an | 30.13±1.69an | 19.72±3.21y |
|                             | d 6               | 29.45±2.11an | 28.33±1.29an | 13.58±3.95y |
|                             | d 9               | 28.48±2.41an | 27.83±0.94an | 12.23±0.80y |
| AST (U/L)                   | d 0               | 65.63±0.70   | 80.35±17.48  | 70.15±5.15   |
|                             | d 3               | 51.67±7.66   | 73.54±14.62  | 65.58±5.01   |
|                             | d 6               | 50.49±6.13   | 66.82±9.19   | 57.58±11.30  |
|                             | d 9               | 48.17±5.91   | 59.80±6.98   | 48.52±7.08   |
| GGT (U/L)                   | d 0               | 18.32±0.80a  | 24.65±2.61a  | 33.38±3.14a  |
|                             | d 3               | 18.46±3.29   | 20.06±2.61   | 30.87±5.76   |
|                             | d 6               | 17.70±3.31   | 17.28±3.21   | 29.82±5.98   |
|                             | d 9               | 17.60±2.12y  | 16.46±1.64y  | 28.43±5.62y  |
| Bilirubin total (mg/dl)     | d 0               | 0.15±0.02    | 0.13±0.02    | 0.21±0.04a   |
|                             | d 3               | 0.17±0.03x   | 0.09±0.01x   | 0.12±0.02xy  |
|                             | d 6               | 0.13±0.01    | 0.13±0.01    | 0.12±0.01h   |
|                             | d 9               | 0.12±0.01    | 0.10±0.01    | 0.09±0.01h   |
| Bilirubin direct (mg/dl)    | d 0               | 0.12±0.02    | 0.09±0.01x   | 0.11±0.02    |
|                             | d 3               | 0.11±0.02x   | 0.05±0.01xy  | 0.08±0.01xy  |
|                             | d 6               | 0.09±0.01    | 0.08±0.01x   | 0.07±0.02    |
|                             | d 9               | 0.09±0.01    | 0.07±0.01xy  | 0.06±0.01    |
| Bilirubin indirect (mg/dl)  | d 0               | 0.03±0.00an  | 0.03±0.01x   | 0.10±0.03y   |
|                             | d 3               | 0.06±0.02a   | 0.04±0.01    | 0.04±0.01b   |
|                             | d 6               | 0.03±0.01an  | 0.05±0.01    | 0.05±0.01b   |
|                             | d 9               | 0.03±0.01a   | 0.05±0.01    | 0.03±0.01b   |

Values with superscript a, b differ significantly (p<0.05) in a column and superscript x, y differ significantly (p<0.05) in a row for a parameter

ALT=Alanine aminotransaminase; AST=aspartate aminotransaminase; GGT=Gamma glutamyl transferase.

**Table 5** Changes in protein profile in cattle infected with *T. annulata* during 9 days of treatment period (Mean ± S.E.)

| Serum biochemical parameter | Day post treatment | Group A (n=6) | Group B (n=6) | Group C (n=6) |
|-----------------------------|-------------------|--------------|--------------|--------------|
| Total protein (g/dl)        | d 0               | 6.02±0.94a   | 7.40±0.33a   | 5.83±0.56a   |
|                             | d 3               | 8.00±0.38b   | 8.05±0.35ab  | 8.10±0.49b   |
|                             | d 6               | 8.15±0.35b   | 8.34±0.36ab  | 8.35±0.51b   |
|                             | d 9               | 8.35±0.28b   | 8.58±0.31b   | 8.55±0.39b   |
| Albumin (g/dl)              | d 0               | 3.13±0.12    | 2.82±0.20    | 2.90±0.19    |
|                             | d 3               | 3.20±0.08    | 3.19±0.18    | 2.92±0.18    |
|                             | d 6               | 3.31±0.12    | 3.27±0.18    | 2.98±0.13    |
|                             | d 9               | 3.33±0.12    | 3.35±0.14    | 3.00±0.15    |
| Globulin (g/dl)             | d 0               | 2.88±0.89a   | 4.58±0.41    | 2.93±0.66a   |
|                             | d 3               | 4.80±0.37a   | 4.86±0.40    | 5.18±0.63a   |
|                             | d 6               | 4.84±0.35b   | 5.07±0.40    | 5.37±0.60b   |
|                             | d 9               | 5.02±0.34b   | 5.23±0.39    | 5.55±0.41b   |

Values with superscript a, b differ significantly (p<0.05) in a column
### Table 6

Changes in oxidative stress indices (in plasma and hemolysate) in cattle infected with *T. annulata* during 9 days of treatment period (Mean ± S.E.)

| Parameter                       | Day post treatment | Group A (n=6) | Group B (n =6) | Group C (n = 6) |
|--------------------------------|--------------------|---------------|----------------|-----------------|
| **MDA plasma (nmol/ml)**       |                    |               |                |                 |
| d 0                            | 17.06±3.01         | 13.87±1.30a   | 19.13±5.17     |
| d 3                            | 20.48±4.79         | 9.79±1.34b    | 15.00±5.29     |
| d 6                            | 23.88±5.40a        | 6.84±1.40bcy  | 9.74±4.48b     |
| d 9                            | 26.42±5.06a        | 5.46±0.61c     | 5.45±1.06c     |
| **MDA haemolysate (nmol/ml)**  |                    |               |                |                 |
| d 0                            | 38.16±7.76         | 40.35±1.63a   | 42.25±4.04a    |
| d 3                            | 48.24±8.73         | 36.63±2.04a   | 36.13±3.11as   |
| d 6                            | 56.16±10.08a       | 32.27±4.89bcy | 24.64±4.97bcy  |
| d 9                            | 63.29±7.14a        | 24.61±4.05bcy | 17.13±4.45bcy  |
| **GPx plasma (U/mg protein)**  |                    |               |                |                 |
| d 0                            | 3.18±0.53a         | 3.45±0.20a    | 3.79±0.21a     |
| d 3                            | 3.87±0.29ab       | 2.44±0.46by   | 1.90±0.29by    |
| d 6                            | 4.03±0.56ab       | 2.29±0.27by   | 1.85±0.35by    |
| d 9                            | 4.79±0.51b        | 2.15±0.12by   | 0.94±0.11bc    |
| **GPx haemolysate (U/mg Hb)**  |                    |               |                |                 |
| d 0                            | 6.06±1.20a        | 6.81±1.47     | 7.68±1.03s     |
| d 3                            | 7.92±0.57a        | 6.47±1.29s    | 4.62±0.93s     |
| d 6                            | 9.48±1.33a        | 5.16±0.62y    | 3.56±1.24y     |
| d 9                            | 10.20±1.12a       | 3.85±0.30y    | 1.92±0.83z     |
| **SOD plasma (U/mg protein)**  |                    |               |                |                 |
| d 0                            | 0.38±0.01         | 0.44±0.02a    | 0.50±0.08a     |
| d 3                            | 0.38±0.02         | 0.32±0.04ab   | 0.36±0.02b     |
| d 6                            | 0.40±0.02         | 0.28±0.06b    | 0.34±0.02b     |
| d 9                            | 0.41±0.02a        | 0.23±0.05by   | 0.30±0.07by    |
| **SOD haemolysate (U/mg Hb)**  |                    |               |                |                 |
| d 0                            | 0.12±0.01         | 0.26±0.05ys   | 0.16±0.02abyz  |
| d 3                            | 0.12±0.02         | 0.16±0.04ab   | 0.11±0.00b     |
| d 6                            | 0.13±0.01         | 0.14±0.02b    | 0.09±0.02b     |
| d 9                            | 0.15±0.02a        | 0.12±0.02abyz | 0.07±0.01yz    |

Values with superscript a, b, c differ significantly (p<0.05) in a column and superscript x, y, z differ significantly (p<0.05) in a row for a parameter.

MDA=Malondialdehyde level; GPx=Glutathione peroxidase activity; SOD=Superoxide dismutase activity.

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**Serological examinations**

Changes in liver function tests are depicted in table 4. Significant (p ≤ 0.05) decrease in levels of ALT in all groups was observed and among groups maximum decrease in animals of group C was found. Along with it, total bilirubin and indirect bilirubin decreased significantly in group C. Rest of the parameters altered non-significantly. However slight decrease in levels of other hepatic enzymes was observed in all groups with maximum decrease in group C. In group A, it may be due to the removal of parasitic burden on hepatocytes, which were deteriorating the functions of hepatocytes. In group B and C improved functions of liver may be due to ability of vitamin C and vitamin E-selenium to protect the hepatic cells from oxidative damage and lipid peroxidation, which is mediated by oxygen-free radicals. The protective effect may be due to stabilization of plasma membrane thereby preserving the structural integrity of hepatocytes.

**Protein profile**

Changes in protein profile is depicted in table 5. Total protein increased significantly (p≤ 0.05) in animals of all groups. Globulin increased significantly in group A and group C. Albumin level increased non-significantly in all groups. These findings might be due to
the improvement in hepatic function which leads to improvement in biosynthesis of proteins.

**Evaluation of oxidative stress and antioxidants in response to therapy**

Oxidative stress indices in terms of MDA, GPx and SOD measured in plasma and haemolysate, are depicted in table 6.

**MDA levels**

MDA was found to be gradually increased, both in plasma and haemolysate from day 0 to day 9 in group A. This finding is in agreement with Naziroglu et al., (1999) and Kumar et al., (2016). This finding might be due to buparvaquone administration which might function by forming free radicals to kill *T. annulata* infection. McHardy (1989) also reported that buparvaquone persists for a long time in plasma (half-life 7 days). Hence, elevated level of MDA after treatment with buparvaquone in the present study might be due to residual effect of buparvaquone in body of animals. Vitamin C and vitamin E-selenium supplementation caused significant decrease in level of MDA (lipid peroxidation) both, in plasma and haemolysate. Vitamin C acts as an inhibitor/chain blocker of lipid peroxidation (Tanaka et al., 2007). Vitamin E-selenium provides protection against damaging effect of free radicals on cellular components (Papas, 1999). Therefore, the decrease in the level of MDA might be due to the reduction in cellular damage caused by free radicals due to supplementation with antioxidants.

**GPx levels**

In group A, level of GPx increased significantly both in plasma and haemolysate from day 0 to day 9. It might be due to the fact that, animal body was still under oxidative stress and levels of GPx increased to combat the effects of free radicals. While antioxidant therapy significantly decreased the level of GPx in group B and group C (both, in plasma and haemolysate) indicating that the oxidative stress was reduced in both groups. As compared to group B significant decrease in GPx levels in plasma was found in animals of group C. As Vitamin E-selenium is the important part of antioxidant defense system of living tissues (Gerloff, 1992). Vitamin E has the capacity to quench free radicals which causes the initiation and propagation of lipid oxidation, and the Se-containing antioxidant enzyme (GPx) catalyses the decomposition of lipid hydroperoxides into less-reactive products (DeVore et al., 1983, Faustman et al., 1989). Glutathione peroxidase a selenoenzymes, and the Se status of the body influences its activity (Brown and Arthur, 2001).

**SOD levels**

SOD level non-significantly increased from day 0 to day 9 in group A. In animals of group B and group C which were administered antioxidant therapy, level of SOD were significantly lower after treatment in both plasma and haemolysate. SOD level in haemolysate was significantly lower in group C as compared to group B. The activity of enzyme SOD could also be assigned as a major factor in preventing the RBC membrane from peroxide damage, induced by lipid peroxidation.

Antioxidants (vitamin C and vitamin E-selenium) greatly help in recovery of infected animals from theileriosis and leads to fast clinical recovery when supplemented with specific drug therapy. Antioxidants greatly help in reduction of damage caused by free radicals and plays important role in improvement in liver functions. Vitamin E-selenium given 3 times at 3 days interval
proved to be better antioxidant therapy as compared to vitamin C given every day for 9 days. So antioxidants should be given as adjunct therapy for treatment of bovine tropical theileriosis for fast clinical recovery of animals.

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**How to cite this article:**

Neelam, Naresh Kumar Rakha, Ricky Jhambh, Meenakshi Virmani, Parveen Goel and Ramkaran. 2017. Evaluation of the Antioxidants as Adjunct Therapy in Cattle Naturally Infected with Bovine Tropical Theileriosis. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 5373-5384. doi: [https://doi.org/10.20546/ijcmas.2017.611.514](https://doi.org/10.20546/ijcmas.2017.611.514)