

Assessment of antioxidants as adjunct therapy in management of bovine tropical Theileriosis

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Abstract

The present study evaluated the therapeutic efficacy of antioxidants in bovine tropical theileriosis. Eighteen *Theileria*-positive cattle based on PCR were selected for a therapeutic trial, comprising 6 animals in each three treatment groups (II, III, IV) and a healthy control group (I). Group II received standard treatment, Group III received standard treatment with Vitamin E and selenium, and Group IV received standard treatment with multivitamins lacking selenium. Haematological and biochemical evaluations were conducted on day 0, 7, and 14 post-treatment. Haematological findings revealed anaemia and leucopenia, however the biochemical findings showed hypoproteinaemia, hypoalbuminemia, and increased levels of GGT. Results also indicated significant hematological improvement in treatment groups receiving adjunctive therapy, but animals under Group III showed the highest recovery rates in PCV, hemoglobin, TEC, total protein, albumin, GGT, and antioxidant enzyme levels (SOD, GPx, and catalase). The study confirms that Vitamin E and selenium adjunctive therapy enhances recovery and mitigates oxidative stress in *T. annulata*-infected cattle more effectively than standard treatment alone.

Key words: Anaemia, Buparvaquone, Cattle, Oxidative stress, *Theileria annulata*

Introduction

Bovine tropical theileriosis, caused by *Theileria annulata*, remains a significant health challenge for cattle in tropical and subtropical regions, leading to severe morbidity, productivity losses, and high mortality rates (Narladkar, 2018). The disease, transmitted by *Hyalomma* ticks, manifests as anemia, pyrexia, lymph node enlargement, respiratory distress, and immune suppression (Singh et al., 2014). While standard buparvaquone-based treatment is commonly used, recovery in affected cattle remains slow and unpredictable, often due to underlying oxidative stress (Razavi et al., 2015). The development of anaemia and related complications associated with theileriosis are significantly influenced by oxidative stress (Grewal et al., 2005, Behera et al., 2026). Oxidative stress occurs when reactive oxygen species (ROS) overwhelm the body's natural antioxidant defenses, leading to cellular damage, lipid peroxidation, and RBC destruction, which exacerbates anemia in infected cattle (Medjkane et al., 2014). The primary antioxidants—superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx)—play a crucial role in neutralizing oxidative damage (Ighodaro and Akinloye, 2018). However, research on their alterations during tropical theileriosis and the effect of antioxidants in addition to therapeutic agents in reduction of oxidative stress and accelerating recovery is still limited (Denizhan et al., 2017).

Thus, keeping in view the present study was aimed to assess the effect of *T. annulata* infection on some hematological parameters and antioxidant enzymes in cattle in diminishing the oxidative stress and improving clinical recovery of the animal.

Material and methods

Selection of animal

In the present study, out of total 37 *T. annulata* positive cattle based on PCR results, 18 animals were selected based on their average clinical score of ≥ 2 (Temp 103-105 °F, and Haemoglobin 5-7gm/dl), and subjected to therapeutic trials. For therapeutic trial three treatment groups (II, III and IV) consisting of 6 cattle in each group were taken. Group I comprising of six apparently healthy cattle, negative for blood parasites were taken as healthy control. Group-II comprising of six cattle clinically affected with bovine tropical theileriosis were treated with following therapeutic regimen as standard treatment (inj. Buparvaquone @ 2.5 mg/kg body weight deep intramuscular once; inj. oxytetracycline @ 10mg/kg, IV with NS (500ml) from 2nd day to 6th day; hematinic (Sharkoferrol vet®) @ 50 gram orally once daily for 10 days and antipyretic). Group-III comprising of six clinically infected cattle were given standard treatment as in group II as well as vitamin E and selenium inj. @ 1ml/ 30kg BW, SC on 0th and 7th day as an adjunctive therapy. Group-IV comprising of six infected cattle were given standard treatment as in group II as well as Multivitamins without selenium @ 10 ml, subcutaneously on 0th and 7th days as an adjunctive therapy. Hematological and biochemical examination was performed on blood samples to assess effectiveness of different therapeutic regimen on 0th, 7th and 14th day.

The animals were observed daily for monitoring the clinical symptoms and samples of blood were collected on day 0, day 7 and day 14 for laboratory investigations for monitoring the treatment effect.

Haemato-biochemical estimation

Blood samples were collected from the affected cattle as well as the healthy ones from the jugular vein. About 2.5 ml of blood was collected aseptically in non-anticoagulant plain vials for separation of serum for biochemical analysis; another 2.5 ml of blood was collected in EDTA vial for haematology and estimation of plasma oxidative indices. Hematological (Hemoglobin, PCV, Total erythrocyte count and Total leucocyte) estimation was performed using an automatic blood analyzer (Nihon Kohden, Europe) on 0th, 7th and 14th day of treatment (Table 1).

The biochemical parameters total protein, albumin, and gamma-glutamyl transferase (GGT) were analyzed using automatic veterinary biochemical analyzer (Dri-chem Nx600v) using the kits provided by the same. Antioxidant enzymes (Catalase, SOD and GPx) levels were estimated in blood plasma with commercially (Cayman Chemical Company, USA) available kits as per manufacturer protocols on 0th, 7th and 14th day of treatment.

Statistical analysis

One-way analysis of variance (ANOVA) and post hoc test using Tukey-HSD was performed using IBM SPSS version 27.0 software for multiple comparisons between groups, periods, time intervals and their interactions for different parameters; these findings were displayed as mean \pm standard error (SE). Any differences were considered statistically significant considering significance threshold of $p < 0.05$ (Snedecor and Cochran, 1994).

Results and discussion

The goal of the current study was to evaluate the oxidative stress induced by the intracellular parasite (*T.annulata*), and also to assess the effect of parental administration of antioxidants that could mitigate the damage caused by free radicals during oxidative stress.

It is important to note that anaemia is one of the main symptoms of tropical theileriosis. The study revealed significant ($p < 0.05$) reduction in packed cell volume (PCV), Hb concentration, and TEC levels in all treatment groups (Gr. II, III and IV) as compared to the healthy control group (Table 1). Similar findings of low Hb, TEC and PCV values in cases of infected cattle with bovine tropical theileriosis were documented by Singh et al. (2014), and Baghel et al. (2023). The proposed mechanism of anemia in theileriosis could be owing to removal of erythrocytes by phagocytosis rather than parasite-induced lysis or involvement of an auto-immune reaction or by suppressing haematopoietic progenitors. Anemia in theileriosis is caused by extravascular hemolysis due to enzymatic, immune-mediated, mechanical, toxic, erythroid hyperplasia, and enhanced hemagglutinin processes. The toxic metabolites of *T. annulata* have a detrimental effect on bone marrow by interfering with the process of erythropoiesis as well as ongoing blood loss brought on by permanent blood sucking ticks, which may be the cause of the normocytic hypochromic anemia that leads to low blood Hb, TEC and PCV values seen in cattle with bovine tropical theileriosis (Al-Emarah et al., 2012). There was significant increase ($p < 0.05$) in Hb and TEC concentration on day 7 in group III and group IV. Similar reflection of therapeutic efficacy on Hb, TEC and PCV were observed by Kumar et al. (2016) and Saravanan et al. (2017) using vitamins as adjunctive therapy. Group III revealed improvement in blood parameters compared to other treatment groups on day 7 and day 14. Therefore, adjunctive therapy of vitamin E and Selenium found more efficacious over multivitamins as adjunctive therapy (Table 1). Similar finding was observed by Neelam et al. (2017). Comparing the various treatment groups of *T. annulata*-positive cattle to healthy control, it was revealed that the TLC values on day 0 was significantly low ($p < 0.05$), and the Findings was in close accordance with Agina et al. (2020) and Sally et al. (2024). Leucopenia in the case of theileriosis could be due to spread of *T. annulata* quickly through the lymphoid system and other organs, causes excessive lymphocyte destruction by schizogony in lymphoid organs and cause these cells to infiltrate into different organs, which lowers the number of lymphocytes in the peripheral circulation (Farhang, 2017). On the other hand, a number of investigations have revealed that, in comparison to the controls, the total WBC count in all animals with bovine tropical theileriosis increased significantly ($p < 0.05$) (Yousef et al., 2020). Changes in leucograms may result from the long-term harm that *Theileria* spp. toxic metabolites cause to hemopoietic organs, especially the bone marrow and their interference with the leucogenesis process (Sarma et al., 2016).

TLC concentrations in groups II, III, and IV increased significantly ($p < 0.05$) on days 7 and 14 following treatment. Recovery pattern using vitamins in group IV was similar to Kumar et al. (2016) and Saravanan et al. (2017) using vitamins as adjunctive therapy. By the fourteenth day, group III significantly ($p < 0.05$) recovered, followed by group IV, and group II had recovered the least. Similar finding was observed by Neelam et al. (2017).

When comparing the different treatment groups of *T. annulata*-positive cattle to healthy control, the mean values of total protein and albumin on day 0 was found to be significantly low ($p < 0.05$). Similar finding of decrease in total protein as well as albumin was recorded by Naik et al. (2016), which might be due to harmful influence of toxic metabolites on hepatocytes leading to disturbed hepatic function or may be due to decreased dietary intake, intestinal malfunction, and kidney damage. The results also revealed a significant ($p < 0.05$) increase in total serum protein as well as serum albumin levels after 7th and 14th day post treatment in Group III, and the findings were in accordance with the findings of Nayak et al. (2018), however, a significant ($p < 0.05$) increase in total serum protein as well as serum albumin level was observed on 14th day post treatment in Group IV (Table 2). Vitamin E and selenium have antioxidant properties that minimize the harmful effects of protozoal metabolites, and thus indicating hepatoprotective effect. These findings suggest that improved hepatic function could lead to better protein biosynthesis (Neelam et al., 2017). Using vitamins as an adjuvant therapy, the recovery pattern was also comparable to that of Kumar et al. (2016) and Saravanan et al. (2017).

GGT levels on day 0 were significantly higher ($p < 0.05$) in *T. annulata*-positive cattle treatment groups as compared to healthy controls (Table 2). These findings of significant increase in GGT in infected cattle in present investigations are in accordance with findings earlier reported by Alam et al., (2011) and Prajapati et al. (2022). The elevation in the activities of these enzymes possibly due to the hepatic damage and leakage of these enzymes in the blood due to hypoxia resulting from anemia and jaundice in cattle infected with bovine tropical theileriosis (Col and Uslu, 2006). Significant improvement in GGT levels was observed in all therapeutic groups on 14th day post treatment this might be due to reason that therapeutic measures applied might have overcome pathological alterations produced by the parasite, however a significant decrease in mean GGT concentration on the 7th and 14th days post-treatment in Group III, which received vitamin E and selenium as adjunctive therapy hastened the recovery process than the other treatment groups. These findings suggest that improved hepatic function could lead to better protein biosynthesis (Neelam et al., 2017).

Antioxidant enzymes

The Mean values of GPx, SOD and Catalase on day 0 were found to be significantly low ($p < 0.05$) in the various treatment groups of *T. annulata* positive cattle in comparison to healthy control. This was comparable to the results of Razavi et al. (2015), who found that *T. annulata* infected cows had lower levels of the erythrocyte antioxidant enzymes SOD, GPX, and catalase than non-infected controls. This suggested that endogenous GSH was being used extensively and that its production had decreased. In a study on tropical theileriosis in cattle, Nazifi

et al. (2008) found a similar outcome. According to Grewal *et al.* (2005), cows with *T. annulata* showed a marked increase in GPx activity and this increase could be due to body producing more GSH in response to increased oxidative stress.

However, there was a significant increase ($p < 0.05$) in the GPx and catalase concentration at day 7 after the treatment, in group III. After 14 days of treatment only group III showed significant ($p < 0.05$) improvement from 0 to 7th day and then after 7 to 14th day. At day 7 following treatment, the SOD concentrations in groups III and IV increased significantly ($p < 0.05$) in comparison to group II. As compared to standard treatment group, other groups given adjunctive therapy were shown to recover from oxidative stress and levels of Catalase, SOD and GPx were comparable to healthy control. Group IV using multivitamins recovered better in comparison to standard treatment and results were in accordance with Ashoori and Saedisomeolia, 2014. This could be due to, B group vitamins having pro-oxidant and antioxidant effects on lipid peroxidation. Riboflavin has antioxidant qualities both on its own and as part of the glutathione redox cycle as riboflavin shields the body from oxidative stress, particularly lipid peroxidation and reperfusion oxidative injury. It is probable that vit C has maintained the

Table 1: Haematological values on 0th, 7th and 14th day post treatment

Parameters	Group	0 th day	7 th day	14 th day
HB (g/dl)	I	10.2 ± 0.49 ^{aA}	10.06 ± 0.40 ^{aA}	11.0 ± 0.33 ^{aA}
	II	6.4 ± 0.13 ^{bB}	5.8 ± 0.29 ^{cB}	7.46 ± 0.09 ^{dA}
	III	5.8 ± 0.33 ^{bC}	7.5 ± 0.11 ^{bB}	9.96 ± 0.24 ^{bA}
	IV	6.3 ± 0.16 ^{bC}	7.2 ± 0.08 ^{bB}	8.48 ± 0.18 ^{cA}
TEC (x10 ⁶ /μL)	I	6.56 ± 0.24 ^{aA}	6.43 ± 0.18 ^{aA}	6.80 ± 0.10 ^{aA}
	II	4.26 ± 0.08 ^{bB}	4.23 ± 0.06 ^{cB}	5.13 ± 0.04 ^{dA}
	III	4.30 ± 0.10 ^{bC}	5.01 ± 0.07 ^{bB}	6.31 ± 0.11 ^{bA}
	IV	4.23 ± 0.10 ^{bC}	4.81 ± 0.06 ^{bB}	5.68 ± 0.14 ^{cA}
PCV (%)	I	28.43 ± 1.06 ^{aA}	30.56 ± 1.13 ^{aA}	31.88 ± 0.64 ^{aA}
	II	18.45 ± 0.37 ^{bB}	17.81 ± 0.40 ^{cB}	21.73 ± 0.43 ^{dA}
	III	18.65 ± 0.44 ^{bC}	21.68 ± 0.31 ^{bB}	28.18 ± 0.50 ^{bA}
	IV	18.35 ± 0.48 ^{bC}	20.71 ± 0.26 ^{bB}	25.13 ± 0.89 ^{cA}
TLC (x 10 ³ cells/μL)	I	9.63 ± 0.47 ^{aA}	10.21 ± 0.53 ^{aA}	10.46 ± 0.28 ^{aA}
	II	4.35 ± 0.08 ^{bC}	4.66 ± 0.07 ^{cB}	5.40 ± 0.06 ^{dA}
	III	4.40 ± 0.09 ^{bC}	6.65 ± 0.48 ^{bB}	9.11 ± 0.38 ^{bA}
	IV	4.30 ± 0.10 ^{bC}	5.72 ± 0.40 ^{bcB}	7.96 ± 0.13 ^{cA}

Table 2: Mean serum biochemical values of all groups on 0th, 7th and 14th day post treatment

Parameters	Group	0 th day	7 th day	14 th day
Total protein (g/dl)	I	7.05 ± 0.07 ^{aA}	6.97 ± 0.22 ^{aA}	7.06 ± 0.20 ^{aA}
	II	5.10 ± 0.07 ^{bA}	5.00 ± 0.07 ^{cA}	5.20 ± 0.11 ^{cA}
	III	5.11 ± 0.09 ^{bC}	5.83 ± 0.11 ^{bB}	6.25 ± 0.09 ^{bA}
	IV	5.06 ± 0.08 ^{bB}	5.43 ± 0.10 ^{bcB}	5.91 ± 0.11 ^{bA}
Albumin (g/dl)	I	3.46 ± 0.11 ^{aA}	3.57 ± 0.19 ^{aA}	3.43 ± 0.20 ^{aA}
	II	1.56 ± 0.01 ^{bB}	1.86 ± 0.11 ^{cA}	2.02 ± 0.07 ^{cA}
	III	1.53 ± 0.06 ^{bB}	2.55 ± 0.11 ^{bA}	2.86 ± 0.09 ^{bA}
	IV	1.56 ± 0.08 ^{bB}	2.47 ± 0.06 ^{bA}	2.65 ± 0.09 ^{bA}
GGT (U/L)	I	13.65 ± 1.43 ^{bA}	13.33 ± 1.45 ^{cA}	13.78 ± 0.49 ^{cA}
	II	29.66 ± 3.61 ^{aAB}	32.00 ± 1.65 ^{aA}	22.00 ± 0.81 ^{aB}
	III	35.00 ± 1.39 ^{aA}	24.00 ± 1.65 ^{bB}	18.33 ± 1.05 ^{bC}
	IV	34.16 ± 3.41 ^{aA}	27.16 ± 1.57 ^{abA}	18.66 ± 0.42 ^{bB}
Glutathione peroxidase (nmol/ml)	I	181.00 ± 7.41 ^{aA}	176.00 ± 5.32 ^{aA}	171.50 ± 4.08 ^{aA}
	II	121.83 ± 4.43 ^{bA}	122.33 ± 1.74 ^{cA}	123.66 ± 3.36 ^{cA}
	III	126.00 ± 4.78 ^{bC}	150.66 ± 0.88 ^{bB}	164.33 ± 1.30 ^{aA}
	IV	119.50 ± 9.35 ^{bB}	137.00 ± 4.56 ^{bAB}	146.16 ± 3.58 ^{bA}
Catalase activity (ng/ml)	I	3.90 ± 0.13 ^{aA}	4.00 ± 0.16 ^{aA}	4.25 ± 0.14 ^{aA}
	II	2.71 ± 0.36 ^{bA}	2.85 ± 0.17 ^{cA}	2.76 ± 0.24 ^{bA}
	III	2.50 ± 0.08 ^{bC}	3.48 ± 0.04 ^{abB}	3.78 ± 0.07 ^{aA}
	IV	2.66 ± 0.19 ^{bB}	3.15 ± 0.12 ^{bcAB}	3.74 ± 0.20 ^{aA}
Superoxide dismutase (U/ml)	I	35.00 ± 3.65 ^{aA}	37.03 ± 1.46 ^{aA}	38.87 ± 1.38 ^{aA}
	II	24.78 ± 2.13 ^{bA}	24.06 ± 1.09 ^{cA}	27.50 ± 0.40 ^{cA}
	III	25.00 ± 0.81 ^{bC}	29.33 ± 1.22 ^{bB}	34.00 ± 1.63 ^{abA}
	IV	21.40 ± 1.70 ^{bC}	26.81 ± 1.24 ^{bcB}	32.25 ± 1.57 ^{bcA}

Means bearing different superscripts (a, b, c and d) in a column differ significantly ($p < 0.05$). Means bearing different superscripts (A, B and C) in a row differ significantly ($p < 0.05$).

activities of SOD and GPx and played a significant role as an antioxidant in the group that received ascorbic acid supplements Kumar et al. (2016). Treatment groups with highest recovery was in group III followed by group IV and minimum in group II.

In terms of improvement in GPx in treated groups of cattle, best recovery was assessed in group III. Animals in Group III were recovered better than all treatment groups. This finding is similar to other researchers who also studied effect of selenium and vit E and its effect on levels of GPx and SOD (Mattioli et al., 2020 and Vedovatto et al., 2020). It may be due to the fact that body uses selenium to help protect itself from hydrogen peroxide accumulation caused by cellular metabolism, antioxidant vitamin E and selenium greatly aid in the rapid clinical recovery of infected animals from bovine tropical theileriosis. Vitamin E is a lipid soluble antioxidant that can inhibit lipid peroxidation and stabilize membranes within cells. It is the only antioxidant that is present in the lipid membrane layer and the only compound that is able to break lipid peroxide chain reactions by neutralizing lipid peroxides in addition to oxidants. Vitamin E can alter oxidants level and is the first line of defense against lipid peroxidation, and other hydrophobic antioxidants function mainly in and around the membrane lipid bilayers, which halt lipid peroxidation through peroxide radical trapping chain breaking antioxidation (Ighodaro and Akinloye, 2018).

Conclusion

In treating clinical cases of bovine tropical theileriosis, the groups received adjunctive therapy along with standard therapy were shown to recover from oxidative stress, with catalase, SOD and GPx levels comparable to group that received only standard therapy. However, parenteral injection of Vitamin E and selenium along with standard therapy was found to be more effective in reducing oxidative stress than multivitamins without selenium. Further, it also hastened the recovery process.

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Conflict of Interest

There is no conflict of interest.

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