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Therapeutic management of oxidative stress in cattle, naturally affected with bovine tropical theileriosis by vitamin e and selenium

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Abstract
Topical Theileriosis is a blood protozoan disease of cattle which not only causes high mortality among the animals but also impacts huge economic losses to the farmers due to reduced quality and quantity of milk yield and impaired reproductive performances. Oxidative stress and lipid peroxidation in erythrocytes of cattle infected with *Theileria annulata* causes increased erythrocyte fragility and membrane lysis. For the present study, 20 Theileria affected cattle were selected and divided into two groups 10 cattle each i.e. Group II (treated with Buparvaquone with Marbofloxacin) and Group III (treated with vitamin E and selenium along with Buparvaquone with Marbofloxacin). Another 10 healthy cattle were taken as the healthy control group (Group I). It was found that there was a significant increase ($p<0.05$) of LPO and a significant decrease in SOD, Catalase, Hb, TEC, PCV, TLC in the group II and Group III as compared to Group I at day 0 of study. It is also shown that the Group III animals revealed a rapid recovery from typical clinical signs, oxidative stress and from haemato-biochemical alterations, as compared to the Group II. This indicated that the administration of vitamin E and selenium along with Buparvaquone and Marbofloxacin in tropical Theileriosis potentiated the efficacy of the anti-theilerial drug by elevating the antioxidant level of the affected animal.

Keywords: Theileriosis, oxidative stress, vitamin e, selenium, bupaquone, marbofloxacine

1. Introduction
The protozoan parasite *Theileria annulata* bestowing the tick-borne disease Bovine Tropical Theileriosis (BTT), intricate enormous economic losses about US$ 384.3 million annually in Indian livestock sector [1] It is transmitted by *Hyalomma anatolicum* ticks which survive better and multiply faster in hot and humid climatic conditions like Odisha. The previous study shows that normally 27- 36% of cattle in costal and non-coastal areas of Odisha are suffered from sub-clinical Tropical Theileriosis as evidenced by examination of peripheral blood smears. Furthermore, the incidence of the Tropical Theileriosis was 23%, 35% and 22% in rainy, summer and winter season respectively in Odisha [2].The major clinical manifestations of natural acute Theileriosis are pyrexia (104º-106º F rectal temperature), generalized lymphadenopathy, pallid mucus membrane, anaemia, anorexia, cachexia, respiratory distress, petechiae in the conjunctiva, oral and nasal mucosa and unilateral or bilateral exophthalmia [3, 4].
Oxidative stress is one important indicator of the health when there is an excess production of reactive oxygen species (ROS) that can be countered by natural antioxidant mechanism exist in the body. This includes antioxidant enzymes like SOD, catalase, GSH-PX and antioxidant vitamins like vit-A, E, C. [5]. Lipid peroxidation is a general mechanism whereby ROS induce tissue damage and are implicated under several diverse pathological conditions [6]. The increased level of oxidative stress and lipid peroxidation in erythrocytes of cattle infected with T. Annulata causes increased erythrocyte fragility and membrane lysis which leads to severe anaemia and death [7].
Antioxidants like vitamin E and selenium bring faster clinical recovery with normalization of productive performances within a shorter duration of time in the affected cattle. A higher level of erythrocytic antioxidant enzymes (Catalase and Glutathione peroxidase) is recorded in bovines by supplementation of selenium [8] and intramuscular injection of vitamin E brings an increased level of erythrocyte GSH-Px enzyme [9]. So vitamin E and selenium supplementation can be included as principal therapeutic agents for limiting the oxidative stress.

So in this study, estimation of the oxidative stress at the erythrocytic level, assessment of haemato-biochemical alterations and evaluation of the efficacy of parenteral antioxidant for the prevention of Tropical Theileriosis was thoroughly investigated to diminish the economic loss of the farmer as well as the country.

2. Materials and Methods

2.1. Ethical approval

The experimental procedures have been con-ducted in accordance with the guidelines laid down by the Institutional Ethics Committee.

2.2. Area of study

This study was carried out in the animals affected with tropical Theileriosis of Bhubaneswar, Khurda, Puri, Konark and the presented cases of Teaching Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry, OUAT, Bhubaneswar, Odisha.

2.3. Experimental design

10ml blood was collected from jugular vein with EDTA from 35 suspected cows and blood smear examination was carried out by standard staining method [10] to differentiate the positive cases from the healthy animal. This study was carried out by dividing the 20 number positive animals into two groups 10 number each (Group II and III). In Group I, 10 apparently healthy animal showing blood smear negative for protozoa were taken as control group. The therapeutic regimen i.e. Group II animals treated with Buparvaquone with Marbofloxacin and Group III animal treated with antioxidant like vitamin E and selenium along with Buparvaquone and Marbofloxacin was followed for 28 days. There was no treatment followed for Group I animals.

2.4. Parameters studied

During these 28 days treatment period, different investigations like detection of blood protozoa, estimation of oxidative stress (MDA, SOD, Catalase), haematological parameters (Hb, PCV, Neutrophil, Lymphocyte, TLC, TEC) and biochemical parameters (AST, ALT, Total protein, A-G ratio) were carried out from serum sample on 0th, 14th, 28th day of the study. Estimation of oxidative stress parameters MDA, SOD, Catalase was carried out by the manual method using double beam UV-VIS spectrophotometer [11]. Haematobiochemical parameters were estimated by fully automatic analyzer using the standard kit method. Alleviation of clinical signs of treated animals was assessed to know the efficacy of the therapeutics and the progress of recovery of the animals.

2.5. Statistical analysis

All the data generated in the above experiments were statistically analyzed using SPSS (1996) computer package. For comparison of groups, Generalized Linear Model, ANOVA procedure and Duncan's multiple range tests were used [12].

3. Results

The results of haematobiochemical alternation in all three group of animal during the period of study are shown in Table 1. The mean value of Hb, PCV (%), TEC, TLC, neutrophills showed significantly higher value ($P \leq 0.05$) i.e 10.2gm/dl, 30.60%, 5.10, 5.67, and 46.17% respectively in Group III animals on 28th day as compared to Group II. Similarly the mean Lymphocyte and AST value decreased significantly in Group III than Groups II animals during course of treatment. Total protein and A/G ratio showed increasing trend in values in both groups during this study, but ALT showed no significant difference in both the groups during this study.

Table 1: Effect of therapeutics on haemato-biochemical changes of animals in different groups on 0th, 14th, 28th day

Parameters	Groups n=10	Mean± SE		
		0 DAY	14TH DAY	28TH DAY
Hb (g/dl)	G I	11.28^{b} ±0.18	11.11^{b}±0.22	11.18^{c}±0.21
	G II	06.72^{aA} ±0.37	07.78^{aB}±0.32	08.73^{aC}±0.25
	GIII	06.74^{aA}±0.43	08.63^{aB}±0.37	10.20^{bC}±0.29
PCV (%)	G I	33.84^{b}±0.54	33.33^{b}±0.65	33.54^{c}±0.63
	G II	20.16^{aA}±1.10	23.34^{aB}±0.96	26.19^{aC}±0.76
	GIII	20.22^{aA}±1.29	25.89^{aB}±1.11	30.60^{bC}±0.88
TEC (*10^6))	G I	05.64^{b}±0.09	05.56^{b}±0.11	05.59^{c}±0.11
	G II	03.36^{aA}±0.18	03.89^{aB}±0.16	04.37^{aC}±0.13
	GIII	03.37^{aA}±0.22	04.32^{aB}±0.18	05.10^{bC}±0.15
TLC (*10^3)	G I	06.05^{b}±0.16	06.11^{c}±0.08	06.19^{c}±0.07
	G II	03.76^{aA}±0.26	04.18^{aA}±0.20	04.89^{aB}±0.20
	GIII	03.71^{aA}±0.22	04.61^{bB}±0.13	05.67^{bC}±0.10
NEUTROPHIL (%)	G I	41.17^{b}±0.95	38.83^{b}±0.98	41.83^{a}±0.98
	G II	20.17^{aA}±1.19	33.67^{aB}±0.61	38.50^{aC}±1.23
	GIII	20.83^{aA}±1.58	39.83^{bB}±1.35	46.17^{bC}±1.74
LYMPHCYTE (%)	G I	53.83^{a}±0.95	56.17^{a}±0.98	53.17^{b}±0.98
	G II	74.83^{bC}±1.19	61.33^{bB}±0.61	56.50^{bA}±1.23
	GIII	74.17^{bC}±1.58	55.17^{aB}±1.35	48.83^{aA}±1.74
TOTAL POTEIN (g/dl)	G I	07.03^{b}±0.05	6.97^{b}±0.03	06.87±0.07
	G II	05.22^{aA}±0.11	5.78^{aB}±0.10	06.70^{C}±0.12
	GIII	05.23^{aA}±0.10	5.88^{aB}±0.07	06.75^{C}±0.13
A/G Ratio	G I	01.25^{b}±0.03	1.27^{b}±0.05	01.27±0.06
	G II	00.37^{aA}±0.03	0.77^{aB}±0.08	01.13^{C}±0.02
	GIII	00.41^{aA}±0.01	0.78^{aB}±0.02	01.16^{C}±0.06
AST (IU/l)	G I	93.30^{a}±4.57	96.70^{a}±3.44	96.00^{a}±3.73
	G II	149.80^{bC}±5.80	132.70^{bB}±4.18	119.00^{Ac}±3.69

	GIII	163.30^{bC}±4.74	143.60^{bB}±4.24	109.9^{bA}±5.73
ALT (IU/l)	G I	31.40±2.38	32.10±2.05	31.20±2.34
	G II	33.70±3.01	33.40±2.02	33.50±1.64
	GIII	31.60±2.81	33.00±1.72	29.50±2.45

Values are expressed as Mean± S.E. Parenthesis denotes range. Mean values with different superscripts (A, B, C) differs in a row significantly within a group at P≤0.05 and superscripts (a, b, c) differs in a column between the groups significantly at (P≤0.05)

The results of oxidative stress parameters MDA, SOD and Catalase was shown in Table No 2. The mean MDA values of Group III animals showed significantly lower **(**P≤0.05**)** value i.e 1.01nmol/mg Hb on 28th day as compared to Group II. Mean SOD and catalase value showed significantly higher (P≤0.05) values in Group III than Group II on 28 days i.e 4.23 U/mgHb and 2.70 U/mgHb respectively.

Table 2: Effect of therapeutics on oxidative stress parameters of animals in different groups on 0th, 14th, 28th day

Parameters	**Groups n=10**	**Mean± SE**		
		0 DAY	**14TH DAY**	**28TH DAY**
MDA (nmol/mg Hb)	G I	1.12^{a}±0.04	1.14^{a}±0.03	1.14^{a}±0.03
	G II	4.77^{bC}±0.14	3.04^{cB}±0.07	1.92^{bA}±0.08
	GIII	4.72^{bC}±0.17	1.91^{bB}±0.07	1.01^{aA}±0.03
SOD (units /mgHb)	G I	4.05^{b}±0.15	3.99^{b}±0.16	4.08^{b}±0.16
	G II	1.87^{aA}±0.12	2.66^{aB}±0.17	3.06^{aB}±0.18
	GIII	1.89^{aA}±0.11	3.21^{aB} ±0.21	4.23^{bC}±0.32
CATALASE (units /mgHb)	G I	2.12^{b}±0.05	2.09±0.04	2.01^{a}±0.05
	G II	1.47^{aA}±0.12	1.82^{AB}±0.13	2.02^{aB}±0.16
	GIII	1.50^{aA}±0.11	2.15^{B}±0.15	2.70^{bC}±0.17

Values are expressed as Mean± S.E. Parenthesis denotes range. Mean values with different superscripts ($^{A, B, C}$) differs in a row significantly within a group at P≤0.05 and superscripts ($^{a, b, c}$) differs in a column between the groups significantly at (P≤0.05)

The assessment of clinical signs in Group I, Group II and Group III upon treatment on 0th, 14th and 28th day animals was done and showed in Table No 3. It was seen that almost all the clinical symptoms were diminished in Group III animals on 28th day of this study.

Table 3: Showing the alleviation of clinical signs of three groups on 0th, 14th and 28th day of study

	Day 0			**Day 14**			**Day 28**		
Clinical signs	**Group I**	**Group II**	**Group III**	**Group I**	**Group II**	**Group III**	**Group I**	**Group II**	**Group III**
Fever	-	+++	+++	-	-	-	-	-	-
Enlargement of lymph nodes	-	+++	+++	-	++	+	-	+	-
Lacrimation and nasal discharge	-	++	++	-	+	-	-	-	-
Respiratory manifestations	-	++	++	-	-	-	-	-	-
Anorexia	-	+++	+++	-	++	+	-	+	-
Corneal opacity	-	+	+	-	-	-	-	-	-
Pale mucus membrane	-	+++	+++	-	++	+	-	+	-
Diarrhoea	-	++	++	-	-	-	-	-	-
Dullness and depression	-	+++	+++	-	++	+	-	+	-

4. Discussion

Theileriosis is the most important blood protozoan disease in cattle as there is every chance of death along with reduced quantity and quality of milk yield for a prolonged period, immuno-suppression leading to high susceptibility to various diseases, reduced reproductive performances leading to heavy economic losses to dairy farmers [13]. In this study, the preliminary screening through blood smear examination revealed all forms of *Theileria annulata* piroplasms (cocci, rod, comma, signet-ring, and pear-shaped) with abnormalities in erythrocyte structure which was similar to the findings of Al-Emarah *et al.* [14]. The observed clinical findings like fever, anorexia, corneal opacity, enlarged superficial lymph nodes in cattle affected with Theileriosis were in good agreement of Radostits *et al.* [15]. The high rise in body temperature is due to the liberation of endogenous pyrogens because of cellular lysis and high level of parasitemia leading to the stimulation of thermoregulatory center in the hypothalamus [16]. Anorexia attributed to persistent fever; moreover the enlargement of superficial lymph node could be explained by lymphoid hyperplasia in early stage of disease that occurs due to increases of proliferation of micro-schizonts inside the lymphocyte causing inflammatory reaction in the infected lymph node [17] and the corneal opacity as a result of white blood cells infiltration [18].

Administration of Buparvaquone and Marbofloxacin was followed in this study due to positive effect on the haematological parameters like Hb, TEC and PCV and high accuracy and parasite specificity of the mode of action against the protozoa *Theileria annulata* [19]. The natural antioxidant Vitamin E and selenium was supplemented along with the above therapeutic regimen to the Group III animals by exploiting the chain breaking properties of Vit E which prevents free radical damage in biological membranes [20] and being integral part of enzyme glutathione peroxidase (GSH-Px) selenium was administered to reduce the production and lower the serum accumulation of hydrogen peroxide and also LPO value [8].

The haematobiochemical and oxidative stress parameters of all animals in Group I, Group II, and Group III are thoroughly investigated at 0th, 14th and 28th day of this study. It was shown that the values of mean Hb, PCV and TEC were increased significantly ($p<0.05$) on day 28 in Group III as compared to Group II which was in accordance with Kumar *et al.* [21] indicating treatment with buparvaquone and marbofloxacin in tropical theileriosis may enhance the Hb, PCV and TEC concentration but additional therapy of vitamin-E and selenium as antioxidants may reduce the erythrocyte destruction and enhance the Hb concentration more quickly. There was significant ($p<0.05$) increase in the TLC of Group III as compared to Group II on day 14 and 28, gradually reaching the normal value as in group I, it may be due to the effect of vitamin E and selenium, stimulating the immune system as well as potentiating the phagocytic effect of leukocytes to fight against the infection [21]. Similarly, the lymphocyte count was significantly ($p<0.05$) lower in group III as compared to Group II on day 14 and 28, approaching the normal value which may reveal the antioxidant property and immunomodulatory effect of vitamin E and selenium[22].

The LPO values of Group III animals was significantly lower ($p<0.05$) than Group II on 28th day. It is due to selenium being an integral part of the enzyme glutathione peroxidase (GSH-Px), which reduces of peroxides level in the RBC cells [21].The mean catalase level showed a significant ($p<0.05$) increase in Group III on day 14 as compared to its day 0, but no significance difference was found in Group II with respect to its day 0 reflecting the potent antioxidant properties of vitamin E and selenium substantiating the earlier findings of Rajeesh *et al.* [22]. The mean SOD value showed a significant increase on 14th and 28th day in Group III as compared to Group II indicating the intrinsic antioxidant property of vitamin E and selenium and its role in ameliorating oxidative stress in this study. The mean AST value showed a significant ($p<0.05$) decrease in Group III on 28th day as compared to Grouo II indicating hepatoprotective effect of Vit E and selenium but mean ALT values showed no significant difference between group I, group II and group III on 14th and 28th indicating AST in cattle is more liver specific than ALT in accordance with the findings of [23].

The mean total protein and albumin showed significant ($p<0.05$) increase in Group III as compared to Group II on day 14 and 28 indicating the antioxidative property of vitamin E and selenium which reduces the effect of toxic metabolites of the protozoa in agreement with results of Ganguly *et al.* [24]. The mean serum albumin-globulin ratio in group III was found non-significantly higher than group II on day 14 and 28 indicating the immunomodulatory as well as antioxidative property of vitamin E and selenium [25].

The results of the present study suggested that the administration of vitamin E and selenium along with Buparvaquone and Marbofloxacin in tropical Theileriosis potentiated the efficacy of the anti-theilerial drug by elevating the antioxidant level of the affected animal.

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