

## Assessment of indices of oxidative stress and antioxidant status of buffaloes naturally affected with trypanosomosis

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### Abstract

The study was carried out on thirty six naturally infected buffaloes with *T. evansi*, and six healthy buffaloes to assess the indices of oxidative stress (protein oxidation and lipid peroxidation) and antioxidant status (superoxide dismutase and catalase) parameters alterations. Oxidative stress indices viz. protein carbonyls and lipid peroxidation (LPO) were found to be significantly increased however, significant reduction in the indices of antioxidants enzymes status viz. superoxide dismutase (SOD) and catalase (CAT) were recorded in infected animal, compared to the control ones. It can be concluded that trypanosomosis in buffaloes may lead to severe degree of oxidative stress which might be attributed to various pathological effects of the disease progression.

**Keywords:** Antioxidant status, Buffalo, Trypanosomosis, Oxidative stress, Protein carbonyl

*Trypanosoma evansi* is a protozoan parasite found commonly in the tropical countries causing disease in livestock popularly known as Surra. The disease mostly occurs as an endemic form in northern and eastern India (Laha and Sasmal, 2009). The typical signs are intermittent fever, progressive anaemia, weight loss, oedema, conjunctivitis, marked depression, abortion, petechial haemorrhages, neurologic abnormalities and sudden death (Saleh *et al.*, 2009). It is well known that reactive oxygen species (ROS) produced by several pathological conditions and cause cellular damages such as lipid peroxidation and protein oxidation (Sordillo and Aitken, 2009). The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants (Halliwell, 2006). The same could be responsible for various pathological effects of trypanosomosis in animals. Therefore, the detection of oxidative stress/ antioxidant status parameters is important biomarkers for the host-parasite interactions. Keeping in the view of the above facts present study was planned to determine the indices of oxidative stress and antioxidant enzymes activity in trypanosomosis.

### Material and Methods

The study was performed at teaching veterinary clinical complex (TVCC), DUVASU, Mathura, on 36 positive buffaloes and 6 parasite free healthy buffaloes were used as control. Diagnosis of trypanosomosis

was done on the basis of microscopic examinations of giemsa stained thin blood smear prepared from the ear tip veins. Three thin blood films from every sample were prepared, fixed with absolute methanol (5 min), stained with 10% giemsa solution (45 min) and examined ( $\times 10$ ) to observe various forms of trypanosomes. Blood samples (10 ml) were obtained from all selected buffaloes by the usual technique of collection from jugular vein aseptically with a sterilized disposable syringe and needle. Approximately 5 ml of blood was transferred into vials containing clot activator and serum was harvested. Immediately after harvest, serum samples were transferred into cryovials and kept at  $-20^{\circ}\text{C}$  until the use. For assessment status of protein oxidation; protein carbonyl contents of the diseased and healthy buffaloes, were estimated in serum samples by using specific kits (Sigma-Aldrich). Carbonyls contents was determined using the protein carbonyl content assay kit (Sigma-Aldrich) by the derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of stable dinitrophenyl (DNP) hydrazone adducts, which was detected spectrophotometrically at 375 nm (Chevion *et al.*, 2000).

To evaluate the oxidative stress (lipid peroxidation) and antioxidant status (superoxide dismutase, catalase) parameters, 5ml blood was collected in EDTA vials from healthy and diseased buffaloes for separation of erythrocyte pellets. The

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plasma was harvested by centrifugation of blood samples at 2000 rpm for 10 min and buffy coat were removed. The obtained erythrocyte pellet was washed thrice with 0.15 M NaCl and further RBCs were suspended in PBS which was used for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD) activity, catalase (CAT) activity were estimated using standard methodology (Pandey *et al.*, 2015). The data obtained were analysed using Student's t-test with a P value of <0.05 considered as significant.

## Results and Discussion

The values of, protein carbonyls, lipid peroxidation and antioxidant status of buffaloes with trypanosomosis and healthy control revealed that protein carbonyls content of diseased buffaloes was significantly higher ( $P \leq 0.05$ ) as compared to healthy buffaloes. Protein carbonyl content is a most general indicator and by far most commonly used marker for protein oxidation. Accumulation of protein carbonyls has been observed in several human diseases including Alzheimer's disease (AD), diabetes, inflammatory bowel disease (IBD), and arthritis (Chevion *et al.*, 2000). Protein CO groups can be induced by almost all types of ROS and may be reflective of more severe cases of oxidative stress. Indeed, detection of elevated levels of protein carbonyls is generally a sign not only of oxidative stress but also of a protein dysfunction (Donne *et al.*, 2003). The results of the current study revealed remarkable elevation in serum protein carbonyls content of buffaloes infected with *T. evansi* which strongly suggests the involvement of overproduced ROS in pathology and progression of trypanosomosis in buffaloes. Till date, there was no scientific report on estimating protein carbonyls contents and demonstrating oxidative damage marker of protein during the course of trypanosomosis in bovines. The results of the present study demonstrated an oxidative damage of protein in

buffaloes for the first time.

Serum lipid peroxidase (LPO) contents of diseased buffaloes were remarkably higher ( $P \leq 0.05$ ) in comparison to healthy buffaloes. Antioxidant enzymes *viz* superoxide dismutase (SOD) and catalase (CAT) activity were found to be significantly reduced in diseased buffaloes in comparison to the healthy ones. Bovine trypanosomosis induced oxidative stress was measured as mean lipid peroxides (LPO) in form of malonyl dialdehyde (MDA) (n mol MDA/ mgHb). Reactive oxygen species (ROS) lead to both lipid and protein oxidation and liberates MDA and protein carbonyls respectively. It was documented that mean LPO values in infected group is significantly higher than control group which is in agreement with the findings of Ranjithkumar *et al.* (2011) and Yusuf *et al.* (2012). *T. evansi* infection releases sialidase and phospholipase that damages the erythrocyte membrane leading to formation of insulted RBCs which produces Reactive Oxygen Species (ROS) causing oxidative stress and enhanced lipid peroxidation in RBC (Pandey *et al.*, 2015).

The measurement of antioxidant enzyme activities like SOD and CAT are appropriate indirect ways to assess the status of antioxidant defence (Adeyemi and Sulaiman, 2012). Increase in serum LPO and decrease in antioxidant enzymes as in present study have been reported by many workers in trypanosomosis in various species in recent past (Ranjithkumar *et al.*, 2011, Saleh *et al.*, 2010). In this regard Trypanosomosis affected buffaloes showed significantly ( $P < 0.05$ ) lower erythrocytic catalase concentration and SOD activities in infected group compared to that of the healthy control which is agreement with the findings of Ranjithkumar *et al.* (2011) and Eze *et al.* (2016). These findings suggest that trypanosomosis in buffaloes leads to severe protein oxidation, lipid peroxidation and reduction in the antioxidant enzyme activity.

**Table 1:** Markers of protein oxidation, oxidative stress and antioxidant enzymes alterations in *T. evansi* infected buffaloes

Variables	Healthy control (n=6)	Infected buffaloes (n=36)
Protein Carbonyls (nM carbonyl/mg protein)	8.96 ± 1.28	15.24 ± 2.8*
Lipid peroxidation (nM MDA/ml packed RBCs)	2.28±0.52	5.48±0.82*
Superoxide dismutase (U/mg protein)	8.30±0.88	4.34±0.64*
Catalase (mM H <sub>2</sub> O <sub>2</sub> utilized/min/mg protein)	5.58±0.76	2.30±0.92*

\*Means bearing superscript differ significantly ( $p \leq 0.05$ )

In conclusion, the findings of present study suggest that containment of systemic antioxidant defence and remarkable oxidative injuries might be associated with the pathology and progression of trypanosomiasis in buffaloes. Veterinarians may therefore recommend adjunct antioxidants along with anti-trypanosomal medications for therapeutic management of the disease in buffaloes.

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