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Erythrocytic oxidative stress indices, biochemical and trace mineral milieu in yaks with persistent haemorrhagic diarrhoea associated with enterovirulent *Escherichia coli*

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Yak, a unique bovine, is the lifeline for the poor and marginal tribal farmers and brokpas (yak herdsmen) in the Himalayan ranges. Recurrent outbreaks of fatal haemorrhagic diarrhoea often hit the livestock economy of the hilly terrains. Enterovirulent *Escherichia coli* are one of the major causes of diarrhoeal illness in human and animals throughout the globe. Of different pathotypes of diarrhoeagenic *E. coli* recognized, only 3 i.e. shiga-toxin producing (STEC), enteropathogenic (EPEC) and enterotoxigenic *E. coli* (ETEC) were reported in animals (Bandyopadhyay *et al*. 2012). Although, STEC and or EPEC infections are mostly asymptomatic in animals (Bandyopadhyay *et al.* 2011), EPEC was found to have a strong correlation with calf diarrhoea in yaks (Bandyopadhyay *et al.* 2012). Additionally, ETEC was more frequently associated with haemorrhagic diarrhoea in yaks (Bandyopadhyay *et al.* 2012). However, the exact role of these enteropathogens on the health profile of yaks is yet to be uncovered. Accordingly, the present study was carried out to understand the haematobiochemical, antioxidant status and trace mineral profile of yaks with haemorrhagic diarrhoea associated with enterovirulent *E. coli*.

The present study was conducted in yaks from Institutional farm of NRC Yak at Nyukmadung, Arunachal Pradesh. Fifteen yaks of an age between 10–20 years with persistent haemorrhagic diarrhoea and PCR based evidence of enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) infections were selected for the study. All of these animals were tested free from rotavirus infection. For isolation and characterization of pathogenic *E. coli* the previously described method was followed (Bandyopadhyay *et al*. 2011, Bandyopadhyay *et al*. 2012). Another fifteen yaks of the same age group without diarrhoea were randomly taken as healthy control. The Institutional Animal Ethics Committee approved the whole experimental protocol. Blood samples (15 ml) were collected from the jugular vein of all the animals. About 5 ml of blood was kept to separate serum for estimation of biochemical parameters. Rest of the sample was kept in heparinized test tubes (10 IU/ml) for analysis of trace minerals from plasma and for preparation of haemolysate to trace out the antioxidant status. Serum aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, Blood urea nitrogen (BUN) and serum creatinine level were estimated using biochemical kit. The serum samples were analyzed within 24 h of collection for estimation of blood glucose (Frankel *et al*. 1970) and total serum protein (Vatzidis 1977). The lipid peroxide (LPO) (Utley *et al*. 1967), super oxide dismutase (SOD) (Minami and Yoshikawa 1979) and catalase (CAT) activity (Cohen *et al*. 1970) were measured in haemolysate. The presence of nitric oxide (NO) in haemolysis-free plasma was evaluated by estimating the nitrite concentrations, using the Griess reagent (Green *et al*. 1982). Protein carbonyl content was measured in serum according to the method of Levine *et al*. (1994). The concentration of iron, zinc, copper, cobalt and manganese in the digested sample was measured by atomic absorption spectrophotometer (Perkin Elmer Model). Statistical analysis was done with Student's "t" test using SPSS version 17.0 and level of significance was determined at $P<0.05$.

Both the enteropathogenic *E. coli* and enterotoxigenic *E. coli* produces several virulent toxins having severe effects on the cellular structures and associated macromolecules like proteins and nucleic acids. Persistent infection often leads to production of several toxins and metabolites which may further accumulate in the liver. It has been seen in the present study that hemorrhagic diarrhoea affected yaks

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showed a significant (P<0.05) and nonsignificant increase in serum ALT and AST activity respectively compared to non-diarrhoeic ones (Table 1). This was probably due to impending hepatic insults by such toxins or metabolites. Again body's internal water regulatory mechanism prevents loss of water through urine by producing ischemia in the kidney tissue. Persisted ischemia resulted into kidney injury as reflected by the marginally significant (P<0.057) increase in the BUN and serum creatinine level of the diarrhoeic yaks in the present study (Table 1). Many of the Shiga toxin producing *E. coli* and enteropathogenic *E. coli* were detected to cause thrombotic microangiopathies leading to haemolytic uremic syndrome in pediatric patients (Mody *et al*. 2012). A marginally significant (P<0.051) fall in blood glucose level was also recorded in the diarrhoeic yaks compared to the healthy population (Table 1). Total serum protein did not reveal any significant alteration (P<0.05) between the two groups. Prolonged anorexia might be the cause of hypoglycemia in the diarrhoeic population.

Results of oxidative indices shows a significantly higher (P<0.05) level of MDA in the yaks with persistent diarrhoea. However, SOD and catalase levels were significantly reduced (P<0.05) in the diarrhoeic yaks than that of healthy ones. Significantly (P<0.05) increased nitrite and protein carbonyl concentration were also evident in the diseased population (Table 1). Moreover, iron, copper and zinc concentration in plasma was significantly (P<0.05) reduced in the diarrhoeic yaks, but cobalt and manganese concentrations remained unaltered (Table 1).

Interaction of bacterial toxins with unsaturated fatty acid leads to production of excessive free radicals. Erythrocytes are rich in polyunsaturated fatty acids in their membrane. Therefore, free radicals generated during stress, act on the fatty acid of erythrocyte membrane and leads to production of MDA, a biomarker of oxidative stress. It is observed from the present study that there was significant (P<0.05) increase in MDA production in the diarrhoeic yaks compared to the healthy one. Endotoxins and other metabolites released from cellular interactions of potentially virulent organisms like, STEC, EPEC and ETEC might have been responsible for the stress associated with diarrhoea. De *et al*. (2014) also reported a higher MDA production in diarrhoeic pigs. Protein carbonyl assay is a sensitive and early marker of oxidative stress as compared with lipid peroxidation. The results suggested that there was significant drop in plasma iron, copper and zinc in yaks with persistent haemorrhagic diarrhoea. Panda *et al*. (2009) also reported a reduction in plasma iron and copper level in severely affected diarrhoeal calves.

Higher level of plasma nitric oxide as observed in infected yaks reflected the production of reactive nitrogen species following interaction of the bacterial toxins and metabolites with cellular macromolecules. Catalase and SOD are the two major enzymes to counteract the toxic effect of reactive oxygen species (ROS) such as, superoxide radicals and hydrogen peroxides. Free radicals generated during stress initially resulted into higher activity of these two enzymes to counteract the cellular damage but after a certain time their activity started to decline due to their down regulated synthesis (Dimri *et al*. 2014). Thus in the present study, significant (P<0.05) reduction in SOD and catalase activity in diseased group was probably due to persistent haemorrhagic diarrhoea.

Table 1. Biochemical, trace mineral and erythrocytic oxidative milieu in yaks with persistent haemorrhagic diarrhoea associated with STEC/EPEC and ETEC

Parameters (Unit)	Healthy	Diarrhoea
ALT (IU/l)	27.07±1.40	34.60±3.20*
AST (IU/l)	33.07±1.1	44.33±5.8
BUN (mg/dl)	16.7±0.9	25.47±4.2
Creatinine (mg/dl)	0.65±0.01	0.94±0.14
Glucose (mg/dl)	59.3±1.5	55.47±1.13
Protein (g/dl)	6.9±0.12	6.5±0.2
MDA (nM /mg Hb)	3.64±0.1	5.44±0.12*
SOD (unit/mg Hb)	5.2±0.18	4.28±0.09*
Catalase (unit/mg Hb)	5.56±0.21	4.6±0.09*
Protein carbonyls (mM/ mg Hb)	3.62±0.028	7.41±0.52*
Nitrite concentration (mM/ml)	35.00±0.03	54.00±0.07*
Fe (ppm)	4.5±0.24	2.8±0.11*
Cu (ppm)	1.92±0.07	1.52±0.02*
Co (ppm)	5.34±0.36	5.27±0.3
Zn (ppm)	1.61±0.04	0.99±0.05*
Mn (ppm)	0.44±0.017	0.45±0.01*

*The statistical comparison was determined by unpaired t test and significance was determined at P<0.05.

Thus it may be concluded from the present study that persistent haemorrhagic diarrhoea in yak could deteriorate the body's self-regulating mechanism that leads to changes in most of the biochemical parameters, certain trace mineral profile (e.g. Cu, Zn and Fe level) and oxidative indices. This might be helpful for the veterinarian in supplementation therapy during treatment of persistent haemorrhagic diarrhoea in yaks associated with enterovirulent *Escherichia coli*.

SUMMARY

The present study was designed to enumerate the antioxidant status and trace mineral profile of yaks with haemorrhagic diarrhoea associated with enterovirulent *E. coli*. The study was conducted in 15 yaks with haemorrhagic diarrhoea and with PCR based evidence for enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) infection. A significant rise of ALT activity, marginally significant increase of BUN and serum creatinine level, marginally significant fall of blood glucose level, and no significant changes in AST activity and serum protein were recorded in affected yaks. Persistent haemorrhagic diarrhoea resulted into significant increase in MDA, protein carbonylation and reactive nitrogen species level, but decreased SOD and catalase activity. Iron, copper

and zinc concentrations in plasma were reduced, whereas cobalt and manganese concentrations remain unaltered in the affected population.

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