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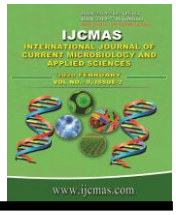

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Evaluation of Haemato-biochemical and Oxidative Stress Parameters in Dogs Affected with Gastroenteritis

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A B S T R A C T

Gastroenteritis in canines is considered as an emergency like disease condition which might be associated with oxidative stress. So this study was planned to correlate oxidative stress with gastroenteritis in dogs. The study included fifty two dogs suffering from vomiting and diarrhoea presented to VCC, COVS, LUVAS, Hisar. Oxidative stress parameters estimated were antioxidant enzymes (SOD, Catalase and GPx) and trace minerals (Fe, Cu and Zn) along with haemato-biochemical parameters and serum electrolytes. The values of the gastroenteritic dogs were compared with healthy control dogs which were brought to clinics for routine vaccination. Majority of the affected dogs showed haemo-concentration with increased packed cell volume, leucocytosis, neutrophilia and thrombocytopenia along with elevated liver and kidney function parameters and decreased serum electrolytes. The gastroenteritic dogs showed significant lower values of antioxidant enzymes i.e. SOD, Catalase, GPx and trace elements Fe, Cu and Zn in comparison with the healthy dogs. The findings of the present study indicated that the dogs with gastroenteritis suffer from acute oxidative stress.

Introduction

Gastroenteritis which is a common illness affecting all species of animals is much more severe in monogastric animals especially in dogs. The patho-physiology of gastroenteritis is quite diverse depending upon the specific etiology. The causes of gastroenteritis may be dietary, viral, bacterial, rickettsial, parasitic and miscellaneous. Gastroenteritis is characterized by varied clinical signs such as diarrhoea, vomition with or without blood, inappetance, lethargy, fever, anaemia and dehydration (Banja *et al.*, 2002). These days oxidative stress is getting a lot of attention for various research studies as it plays very crucial role in the pathogenesis of different diseases. The oxidative stress occurs due to imbalance of production of reactive oxygen/nitrogen species or neutralizing antioxidant enzymes. Excessive production of free radicals and their metabolites, called

reactive oxygen species (ROS) exhibit several harmful effects leading to oxidative damage of lipids, proteins, RNA and DNA. These free radicals can be neutralized by antioxidant system which includes antioxidant enzymes like glutathione peroxidase, catalase and superoxide (Ighodaro and Akinloye, 2018). Zinc and copper are the co-factors of many antioxidant enzymes and are utilized for synthesis of antioxidant enzyme Cu–Zn Superoxide dismutase which catalyses conversion of superoxide radicals to less oxidizing H_2O_2 (Evans and Halliwell, 1994). Iron also serves as co-factor for catalase enzyme. Hence the present study was planned to investigate the oxidative stress indices in dogs suffering from gastroenteritis along with haematological and biochemical alterations

Materials and Methods

The present study was conducted on fifty two dogs presented to Veterinary Clinical Complex (VCC), College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar for a period of one year i.e. from March 2018 to February 2019 with the history and clinical signs of gastroenteritis. The dogs presented for routine health checkup or routine vaccination constituted the healthy control group. Blood samples were collected from cephalic/saphenous veins of the affected and control dogs. Two ml of blood was poured into K_3EDTA coated tube for hematological examination and three ml blood was poured into clot activator coated plain tube for obtaining serum. The separated serum were decanted in 2 ml eppendorf tubes and stored at -20°C till further processing. For the estimation of oxidative stress parameters, blood was poured into a tube coated with sodium heparin. Blood samples collected in heparinised tubes were centrifuged at 3000 rpm for 5 min and the separated plasma were decanted in 2 ml eppendorf tubes and stored at -20°C till further analysis. The blood samples were analyzed in automated hematology cell counter (MS4s, Melet Schlosing Lab). The erythrocytic indices measured were haemoglobin (Hb) g/dl, packed cell volume (PCV) per cent. The leucocytic indices measured were total leucocyte count (TLC) m/mm3 and differential leucocyte count (DLC) (per cent) comprising of neutrophils (N) per cent, lymphocytes (L) per cent, monocytes (M) per cent, eosinophils (E) per cent and basophils (B) per cent were also measured. The thrombocytic indices measured included was total thrombocyte count (THR) m/mm^3. The serum samples were analyzed using automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). The liver function parameters measured were alanine amino transferase (ALT) (U/L), aspartate amino transferase (AST) (U/L) and total protein (g/dl). The kidney function parameters measured in serum were urea (mg/dl) and creatinine (mg/dl). Serum electrolytes were measured in EasyLyte EXPAND analyzer and included sodium (mEq/L), potassium (mEq/L) and chloride (mEq/L). For estimating oxidative stress parameters, blood samples were poured in heparinised tubes and centrifuged at 3000 rpm for 5 min and the separated plasma samples were collected in 2 ml eppendorf tubes and stored at -20°C till further processing. Superoxide dismutase (SOD) enzyme was measured as per the method described by Madesh and Balsubramaniam (1998). Glutathione Peroxidase (GSH-Px) activity was measured by method of Hafeman *et al.,* (1974). The activity of catalase in plasma was determined as per the method of Aebi (1984). For trace elements estimation, one ml of serum sample was taken and to it 10 ml of diacid mixture (HNO_3 and $HClO_4$ in a ratio of 4:1) was added. The samples were kept overnight for digestion and then solutions were heated on

hot plate until converted into transparent solution. The transparent solutions were poured into fresh plastic tubes and the final volume was made to 10 ml by adding distilled water. These samples were analyzed in Atomic Absorption Spectrophotometer (AAS) machine (PerkinElmer Atomic Absorption Spectrophotometer PinAAcle 900T) after performing calibration with standard solutions. The data obtained was analyzed by suitable statistical methods using statistical software package (SPSS 16). To compare various parameters obtained in diseased dogs with the healthy control dogs, the independent t-test was applied. The results are presented as Mean ± S.E. at 5 per cent level of significance ($P<0.05$).

Results and Discussion

Changes in hematological parameters of the affected dogs (n=52) are presented in Table 1. Non-significant lowered mean values of haemoglobin and packed cell volume were observed in the dogs suffering from gastroenteritis as compared to healthy control dogs. Decreased haemoglobin levels before the start of treatment might be due to loss of blood through intestinal and gastric haemorrhages. Similarly, decreased haemoglobin levels in gastroenteritic dogs were also reported in earlier studies (Agnihotri *et al.,* 2017 and Bhargavi *et al.,* 2017). On the contrary increased levels of haemoglobin were reported by Gaykwad *et al.,* (2016) which might be due to excessive fluid loss resulting in dehydration. The decreased PCV levels are also observed by Bhat *et al.,* (2013) who suggested that decreased mean levels of PCV might be correlated with the intestinal bleeding and haemorrhages during gastroenteritis. On the other hand, increased PCV levels were observed by Biswas *et al.,* (2005) which might be due to severe dehydration and fluid losses through vomition and diarrhoea. Mean values of total leucocyte count was found to be non-significantly higher in affected dogs than the healthy control dogs while neutrophil count was found to be significantly increased ($P<0.05$) in the gastroenteritic dogs. Mean lymphocyte count was found significantly lowered ($P<0.05$) in the gastroenteritic dogs than the healthy control dogs. Leucocytosis and neutrophilia in this study could be due to secondary bacterial invasion in the damaged intestinal epithelium (Decaro and Buonavoglia, 2012). Changes in lymphocytic indices are relative to the neutrophil count observed. Non- significant difference in the mean values of monocyte and eosinophil count in gastroenteritic dogs compared to the healthy dogs was observed while mean values of thrombocyte count were found to be significantly decreased ($P<0.05$) in affected dogs than healthy control dogs. Thrombocytopenia in the affected dogs suffering could be due to blood loss through vomitus and faeces, increased destruction and/ or aggregation, decreased production and disseminating intravascular coagulation.

Alteration in biochemical parameters and serum electrolytes of dogs suffering from gastroenteritis as compared to the dogs of healthy control group is presented in Table 2. Non-significant ($P<0.05$) higher mean values of ALT and AST were observed in the dogs suffering from gastroenteritis as compared to the control group. Mean values of total protein were also found to be non-significantly lowered ($P<0.05$) in the affected dogs than the healthy control group. The increased levels of liver function parameters could be due to reactive hepatopathy as also suggested by Berghoff and Steiner (2011) and due to hepatic damage caused by infectious agents which can lead to increased levels of enzyme activity in serum. Elevation in ALT level of affected dogs might be due to the result of hepatic hypoxia secondary to severe hypovolumia or the absorption of toxic

substances due to damage of the gut barrier (Shah *et al.,* 2013). Mean levels of blood urea nitrogen were found to be significantly higher (P<0.05) in the dogs suffering from gastroenteritis than the healthy control group while mean creatinine levels showed a non-significant increase in the affected dogs than the control group.

The increased values of BUN are suggestive of pre renal azotemia which might be because of reduced glomerular filtration rate (Biswas *et al.,* 2005 and Bhat *et al.,* 2015). Elevated values of BUN due to decrease tissue perfusion and dehydration are also observed by Salem (2014). Non-significant (P < 0.05) lower mean values of serum sodium, potassium and chloride ions were observed in dogs suffering from gastroenteritis as compared to the control group. Agnihotri *et al.,* (2017) also observed that hypokalaemia might be due to the loss of potassium in the diarrhoeic fluid along with sodium and bicarbonate. Haligur *et al.,* (2009) and Joshi *et al.,* (2012) also suggested that hyponatremia might be due to severe vomition, diarrhoea and dehydration in the dogs affected with gastroenteritis of varied etiologies. Hypochloremia might be due to the loss of chloride ions through vomition and diarrhoea and resulting intestinal villous atrophy (Burchell *et al.,* 2014).

Table.1 Haematological parameters (Mean ± S.E.) of gastroenteritic dogs

Parameters	Healthy control (n=6)	Dogs suffering from Gastroenteritis (n=52)
Hemoglobin (gm/dl)	10.67±0.69	10.47±0.39
PCV (%)	34.83±2.71	33.09±1.19
TLC (m/mm^3)	13.02±0.54	16.61±3.94
Neutrophil (%)	72.17±2.95^A	83.73±1.16^B
Lymphocyte (%)	23.17±3.71^B	13.79±1.04^A
Monocyte (%)	3.50±0.62	2.80±0.36
Eosinophil (%)	1.17±0.54	2.19±0.26
Thrombocytes (m/mm^3)	418.00±59.03^B	224.62±21.17^A

The means bearing different superscripts (A, B) differ significantly (P<0.05) between the groups.

Table.2 Biochemical and electrolyte alterations (Mean ± S.E.) in gastroenteritic dogs

Parameters	Healthy control (n=6)	Dogs suffering from gastroenteritis (n=52)
ALT (IU/L)	29.95±2.59	31.48±3.99
AST (IU/L)	39.27±3.74	53.99±12.04
Total Protein (g/dl)	6.38±0.29	5.62±0.32
BUN (mg/dl)	20.72±2.17^A	51.77±6.21^B
Creatinine (mg/dl)	0.82±0.09	1.15±0.10
Sodium (mEq/L)	143.15±2.10	136.72±1.34
Potassium (mEq/L)	5.20±0.22	4.25±0.11
Chloride (mEq/L)	107.80±1.05	102.50±81.43

The means bearing different superscripts (A, B) differ significantly (P<0.05) between the groups.

Table.3 Comparative evaluation of oxidative stress parameters (Mean ± S.E.) between healthy control and gastroenteritic dogs

Parameters	Healthy control (n=6)	Dogs suffering from Gastroenteritis (n=52)
SOD (Units/g protein)	12.67 ± 0.95^{B}	8.34 ± 0.46^{A}
Catalase (mmol H_2O_2 utilised/min/g protein)	403.67 ± 15.69^{B}	286.00 ± 7.74^{A}
GPx (Units/g protein)	3889.43 ± 103.23^{B}	1900.88 ± 90.02^{A}
Copper (ppm)	4.55 ± 0.29^{B}	2.80 ± 0.06^{A}
Iron (ppm)	57.82±0.42	55.75±0.27
Zinc (ppm)	3.92 ± 0.28^{B}	3.10 ± 0.06^{A}

The means bearing different superscripts (A, B) differ significantly (P<0.05) between the groups.

Comparative evaluation of oxidative stress parameters between the dogs suffering from gastroenteritis (n=52) and control group is depicted in Table 3. Mean levels of all the antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were found to be significantly (P<0.05) lowered in the gastroenteritic dogs as compared to the control dogs. In a study of Rautray *et al.*, (2016), decreased enzymatic activities of SOD and CAT in erythrocyte lysate of dogs suffering from gastroenteritis was reported. Similarly, Singh and Dimri (2013) also observed decreased activities SOD, GPx, CAT, glutathione-S-transferase (GST) in dogs affected with sarcoptic mange. On the contrary, higher activities of SOD and CAT in the dogs affected with canine parvoviral gastroenteritis were reported by Panda *et al.*, (2009).

In the present study, lower activities of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in dogs affected with gastroenteritis were observed as compared to control dogs which is indicative of a state of oxidative stress which might be because of continual assault beyond the auto-regulatory mechanism which causes a decline in enzyme as suggested by Rautray *et al.*, (2016). Significantly lowered (P < 0.05) mean levels of trace minerals i.e. copper and zinc were observed in the gastroenteritic dogs as compared to the control group of dogs while non-significant lower mean values of iron were noticed in the affected dogs as compared to the control group. Chaudhuri *et al.*, (2008) in their study also found significant lower levels of iron, copper and zinc in dogs affected with babesiosis. Mahadappa and Dey (2018) found decreased levels of GSH, SOD and CAT in *Toxocara canis* infected dogs as compared to the healthy control dogs. Levels of zinc and iron were also found to be decreased in infected dogs than healthy control dogs in their study. Destruction of intestinal absorptive surface by free radicals impairs absorption of macro and micronutrients (Rahman *et al.,* 2002) which might be the reason for low values of iron, copper and zinc in infected dogs.

In conclusion the gastroenteritic dogs showed haemo-concentration with increased packed cell volume, leucocytosis, neutrophilia with thrombocytopenia. Dogs suffering from gastroenteritis showed significantly low activities of antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and reduced levels of glutathione and trace elements copper, iron and zinc as compared to the healthy dogs indicating oxidative stress in the affected dogs. Significant low levels of

antioxidant enzymes and trace minerals are significant oxidative stress markers in gastro-enteric infections of dogs.

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