



Evaluation of oxidative stress and efficacy of antioxidant therapy in dogs with haemorrhagic gastroenteritis



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Abstract

The present study was conducted to evaluate the alterations in oxidative stress parameters in dogs suffering from haemorrhagic gastroenteritis (HGE). Dogs presented with vomiting and diarrhoea were screened and fifteen animals with signs suggestive of HGE were included in the study. The oxidative stress parameters, serum malondialdehyde (MDA) level, total antioxidant status (TAS) and plasma glutathione peroxidase (GSH-Px) activity were studied. The values were compared with the values from six apparently healthy dogs. A significant increase was noticed in the mean values of serum MDA and TAS of diseased animals at the time of presentation when compared to healthy animals whereas the activity of plasma GSH-Px was found to be lower than in healthy dogs. Supplementation with N-acetyl cysteine @ 70 mg/kg or five days was found effective in managing the oxidative injury in the affected animals.

Keywords: Haemorrhagic gastroenteritis, oxidative stress, N-acetyl cysteine

Haemorrhagic gastroenteritis (HGE) is defined as sudden onset of blood-tinged diarrhoea frequently associated with vomiting (Unterunteer and Busch, 2021). The multifactorial aetiology of the condition often makes the treatment difficult. The major infectious agents causing HGE in dogs enlisted by Abd El-Baky *et al.* (2017) included viral agents like parvovirus, coronavirus and rotavirus, bacterial agents such as *Salmonella* spp., *Escherichia coli* and *Clostridium* spp. or endoparasites

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like *Dipylidium caninum* and *Ancylostoma caninum*. Haemorrhagic gastroenteritis can also occur during heat stroke, stress, food allergy, intoxication or use of certain drugs like non-steroidal anti-inflammatory drugs. The inflammation of the gastrointestinal (GI) mucosa often leads to generation of free radicals and their metabolites known as reactive oxygen species (ROS).

Kataria *et al.* (2020b) reported that the excess production of ROS causes oxidative damage to lipids, proteins, ribonucleic acids and deoxy-ribonucleic acids. Oxidative stress occurs when there is an imbalance in the production of ROS or antioxidant enzymes that neutralises them. Oxidative stress plays a crucial role in the pathogenesis and progression of majority of diseases. Thus, the present study was carried out to evaluate the oxidative stress indices in dogs with HE and to assess the efficacy of N-acetyl cysteine in the management of oxidative stress.

Materials and methods

Selection of animals

Fifteen animals with clinical signs suggestive of haemorrhagic gastroenteritis were included in the study. Six apparently healthy animals were taken as control group. The oxidative stress parameters of animals in study group were compared with healthy animals. Aetiology was identified from history, clinical examination of the animal and laboratory evaluation of the samples which included analysis of whole blood and serum samples, identification of parasitic ova and detection of viral antigens (canine parvovirus and canine coronavirus) by standard lateral flow kits supplied by VetCetera Animal Health.

Oxidative stress parameters

Whole blood was collected in clot accelerator tubes for assessing malondialdehyde level and total antioxidant status. Serum was separated by centrifuging at 2400 RPM for 15 min. Blood samples collected in heparin vials (75 USP) were used for glutathione peroxidase estimation. Oxidative

stress indices were analysed on day 0 and 7 of treatment.

Level of lipid peroxides in serum was determined by the method of Yagi (1984) by estimating malondialdehyde (MDA) level. In the present study, MDA levels were detected by the measurement of thiobarbituric acid reactive substance, which were produced during lipoperoxidation (Manisha *et al.*, 2017).

Total antioxidant status (TAS) was measured by using Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996).

The Glutathione peroxidase activity in plasma was measured by a method elaborated by Pleban *et al.* (1982).

The absorbance was measured using Perkin Elmer Lambda 25 UV-VIS Spectrometer.

Treatment

Eight animals (53.33 per cent) included in the study group were diagnosed with parvoviral enteritis based on the results of CPV lateral flow antigen test kit (Fig. 1). Concurrent infection with *Ancylostoma caninum* (Fig. 2) was identified in three dogs (20 per cent). Haemorrhagic gastroenteritis was of dietary origin in the other four dogs (26.66 per cent).

Based on the aetiology and clinical signs, animals were treated with antibacterial agent amoxicillin- clavulanate @ 12.5 mg/kg BID, antiemetic ondansetron @ 0.5 mg/kg BID, proton pump inhibitor pantoprazole @ 1mg/kg OD and antiparasitic fenbendazole @ 50 mg/kg PO OD for three consecutive days. The medications were initiated parenterally followed by oral medications when vomiting reduced. Supportive therapy included N-acetyl cysteine @ 70 mg/kg bodyweight intravenously q24 hours for five days, fresh plasma/fresh frozen plasma @ 6 mL/kg bodyweight intravenously once and racecadotril @ 1mg/kg bodyweight orally q8 hours till diarrhoea subsided for all animals.



Fig.1. CPV Antigen test kit- Positive

Results and discussion

The mean values of oxidative stress parameters, viz. lipid peroxidation level, total antioxidant status and glutathione peroxidase level in the healthy (Group II) and diseased animals (Group I) are depicted in table 1. As done in the present study, Revathiet *et al.* (2020) also estimated methods Total antioxidant status (TAS) by Ferric Reducing Antioxidant Power (FRAP) assay and Level of lipid peroxides in serum by the method of Yagi (1984) by estimating the malondialdehyde (MDA) level.

Lipid peroxidation level

A significant increase in the mean lipid peroxidation level value was observed in the animals before treatment ($3.94 \pm 0.26\text{nM/mL}$) compared to healthy animals ($2.2 \pm 0.09\text{nM/mL}$) ($p < 0.01$). At the end of treatment, the value declined to $3.21 \pm 0.25\text{nM/mL}$. The oxidation of polyunsaturated fatty acids of erythrocyte cell membrane by the free radicals resulted in lipid peroxidation. In agreement with the present findings, elevation in MDA levels associated with HGE was also reported by Elsayed *et al.* (2020). After treatment, all the animals showed a significant decrease in the mean MDA value compared to pre-treatment value. The decrease in serum MDA values after treatment denotes the reduction in oxidative stress. It might be due to the inclusion of N-acetyl cysteine (NAC) to the therapeutic regimen. Candellone *et al.* (2022) also recorded similar outcome after the administration of NAC in dogs with gastroenteritis.

Total antioxidant status

The mean total antioxidant status level of animals prior to treatment was $540.21 \pm 51.32\mu\text{M/L}$ and the value was significantly higher compared to the healthy animals ($295.69 \pm 21.56\mu\text{M/L}$). This was in accordance with the

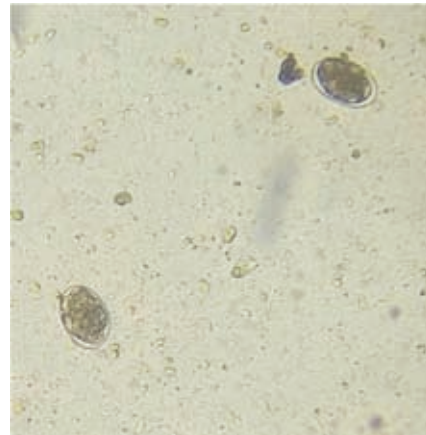


Fig.2. Ova of *Ancylostoma caninum* by direct faecal sample examination (Magnification-10x)

observations of Elsayed *et al.* (2020). After treatment, the mean value significantly reduced to $402.59 \pm 33.05\mu\text{M/L}$. Rao *et al.* (2015) defined total antioxidant status (TAS) or total antioxidant capacity (TAC) as the measured total antioxidant response of a sample. It is one of the most popular analytical techniques for assessing the antioxidant balance in biological materials (Rubio *et al.*, 2016). Elevated TAC levels in the beginning of study indicative of marked oxidative stress might be the compensatory response to the predominant oxidative status of body (Camkerten *et al.*, 2009). Sies (1991) pointed out that in order to maintain homeostasis between the pro-oxidants and the antioxidants, continuous regeneration of antioxidant capacity was required. So, the elevated TAC in the diseased animals in the study might be associated with the mechanism to maintain homeostasis. After treatment, the mean TAC level was found to be significantly reduced. It might be due to the interaction of NAC with the electrophile groups of ROS (Zhang *et al.*, 2018).

Glutathione peroxidase level

The values of GSH-Px in animals before treatment were significantly less ($2732 \pm 182.98\text{U/L}$ of plasma) compared to healthy animals. By the end of treatment, the mean value increased to $3537.07 \pm 177.13\text{U/L}$ of plasma, which was significantly higher compared to the enzyme activity before

Table 1. Oxidative stress parameters of dogs with HGE and apparently healthy dogs

Variable	Period	Group I (Mean±SE)	Group II (Mean±SE)	t value	p value
Lipidperoxidation level (nM/mL)	Day 0	3.94 ± 0.26	2.2 ± 0.09	6.418**	< 0.001
	Day 7	3.21 ± 0.25			
	t value	4.163**			
	p value	0.001			
Total antioxidant status (µM/L)	Day 0	540.21 ± 51.32	295.69 ± 21.56	4.392**	< 0.001
	Day 7	402.59 ± 33.05			
	t value	3.413**			
	p value	0.004			
Glutathione peroxidase level (U/L of plasma)	Day 0	2732 ± 182.98	5280 ± 173.82	8.161**	< 0.001
	Day 7	3537.07 ± 177.13			
	t value	7.107**			
	p value	< 0.001			

**Significant at 0.01 level ($p < 0.01$)

treatment. Kataria *et al.* (2020a) also reported reduced GSH-Px activity in diarrhoeic animals. Ighodaro and Akinloye (2018) described that the free radicals produced as a result of oxidative damage of lipids, proteins, RNA and DNA was neutralised by antioxidant enzyme glutathione peroxidase. The decreased levels could be due to the utilisation of the antioxidant enzymes to counter the ROS. After administration of NAC, a significant increase in the mean GSH-Px level was noticed in the present study. Supplementation of NAC caused an increase in plasma cysteine concentration. Cysteine might have contributed to the replenishment of intracellular glutathione, which is the substrate for enzyme glutathione peroxidase (Viviano and Vander Wielen, 2013).

Conclusion

Following the administration of NAC, significant increase in GSH-Px activity and decrease in the levels of serum MDA and TAS were observed. Hence, administration of N-acetylcysteine @ 70 mg/kg bodyweight intravenously once daily for five days was found to be effective in alleviating oxidative stress during HGE in dogs which in turn reduces the gastrointestinal damage and thereby aid in rapid recovery of patient.

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Conflict of interest

The authors declare that they have no conflict of interest.

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