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## Comparative assessment of oxidative stress in dogs regarding CPV infection

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### Abstract

Oxidative stress plays crucial role in the pathogenesis of many disease conditions including CPV infection. This study was planned to compare oxidative stress level in CPV infected and non CPV infected dogs which included a total of thirty six dogs which were diagnosed with molecular technique like polymerase chain reaction for the detection of CPV in faecal samples. Oxidative stress estimation was done by measurement of antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and trace elements like copper, iron and zinc. So, these dogs were further targeted with combined antibiotic and antioxidants therapy to combat oxidative stress. CPV positive dogs showed higher oxidative stress than CPV negative dogs and also showed early clinical recovery because of antioxidant therapy. These findings helped in demonstrating the significance of antioxidants in treatment protocol.

**Keywords:** Oxidative stress, pathogenesis, CPV infection

### 1. Introduction

Canine parvovirus infection is an emerging disease in India and throughout the world, causing high morbidity and frequent mortality in pups (Kumar and Nandi, 2010) <sup>[1]</sup>. The inflammatory changes in the gastrointestinal tract may result in bloody diarrhoea, nausea, inappetence, rectal bleeding, weight loss, fever and anaemia (Juckett and Trivedi, 2011) <sup>[9]</sup>. The oxidative stress occurs whenever there is imbalance of production of reactive oxygen/nitrogen species and neutralizing antioxidant enzymes. Reactive Oxygen species (ROS) can be scavenged by antioxidant enzymes like glutathione peroxidase, catalase and superoxide dismutase and non-enzymatic components involving vitamin E, vitamin C, vitamin A and selenium. The body's ability to counteract oxidative stress depends on the status and activities of antioxidant molecules including the integrity of several enzymes that require adequate supply of trace minerals like zinc, copper, cobalt, selenium and manganese (Evans and Halliwell, 2001) <sup>[6]</sup>. In recent years, emphasis has been given on the antioxidants as the potential drugs of interest for management of viral diseases (Chandrasena *et al.*, 2014 and Crump *et al.*, 2013) <sup>[2, 5]</sup>. A strong association of CPV with oxidative stress suggests incorporation of antioxidants in therapeutic regimen in canine parvoviral diarrhoea that may help in ameliorating the clinical signs. Thus, the health effects caused by the oxidative damages in canine parvovirus diarrhoea can be prevented by ensuring adequate antioxidant defense.

### 2. Materials and Methods

The present study was conducted on thirty six dogs presented to VCC, COVS, LUVAS, Hisar. Six apparently healthy dogs brought for routine health checkup and/or vaccination constituted the healthy control group. Complete history of the affected dogs regarding the duration of illness, appetite, frequency of vomiting and diarrhoea, deworming and vaccination status was recorded. Blood was collected aseptically from each dog from cephalic/saphenous vein with 22/24 gauze scalp vein set. For conducting molecular study, faecal samples were collected from thirty six gastroenteritis dogs by introducing sterile swabs into the rectum. These swabs were then mixed with 1 ml of sterile PBS (pH 7.4), vortexed and stored at -20 °C. Genomic DNA was extracted using commercially available QIAamp DNA Mini Kit (Qiagen) following manufacturer's instructions. The faecal samples were screened for the presence of viral DNA by amplifying the extracted DNA using conventional PCR.

The reaction mixture and cycling conditions were used as per the method described by Kumar *et al.* (2011) [10]. For the estimation of oxidative stress parameters, 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. For the estimation of oxidative stress parameters, 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. Superoxide dismutase (SOD) was estimated as per the method described by Madesh and Balsubramaniam (1998) [12]. Glutathione Peroxidase (GSH-Px) activity in tissue homogenate was measured by method of Hafeman *et al.* (1974) [7], which was a modification of Mill's procedure. The activity of catalase in plasma was determined as per the method of Aebi (1984) [1]. For trace elements estimation, one ml of serum sample was taken and to it 10 ml of diacid mixture (HNO<sub>3</sub> and HClO<sub>4</sub> 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. Therapy was given for five days which included the antibiotics Ceftriaxone-Tazobactam, Ampicillin-Cloxacillin and Metronidazole were administered at the dosage of 25 mg/kg b.wt. i.m. o.d., 10 mg/kg b.wt. i.m. b.i.d. and 15 mg/kg b.wt. i.v. t.i.d. respectively. The antioxidant therapy included Vitamin C @20 mg/kg b.wt. i.v. o.d. and N-acetylcysteine (NAC) @70 mg/Kg b.wt. i.v. o.d. The therapy was supported with supportive and symptomatic treatment in accordance with the clinical conditions of affected dogs. 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. For therapeutic efficacy, within and between the groups, two-way analysis of variance (ANOVA) with repeated measures was applied. The results are presented as Mean ± S.E. at 5 per cent level of significance ( $P < 0.05$ ). Therapeutic evaluation was analyzed on the basis of improvement in clinical signs and restoration of oxidative stress parameters towards normalcy.

### 3. Results and Discussion

Out of thirty six gastroenteritis dogs, twenty one dogs were found positive for canine parvo virus infection on diagnosis

with PCR for CPV-2 variant.

#### 3.1 Oxidative stress parameters in dogs suffering from canine parvo viral gastroenteritis

Comparative evaluation of antioxidant parameters in CPV positive and CPV negative dogs is depicted in Table 1. Mean values of superoxide dismutase (SOD) activity in the dogs having canine parvo virus infection were found to be significantly decreased ( $P < 0.05$ ) as compared to the healthy control group of dogs at day 0 before the start of treatment. Similarly, the mean values of SOD activity in CPV negative dogs was found to be significantly decreased ( $P < 0.05$ ) on day 0 as compared to the control group. A significant increase ( $P < 0.05$ ) in the mean levels of SOD was observed on day 5 within both the groups i.e. CPV positive and CPV negative in response to the treatment. On day 3 and day 5 of therapy the mean SOD activity remained significantly lowered ( $P < 0.05$ ) in the CPV positive and CPV negative groups as compared to the control group. Significantly decreased ( $P < 0.05$ ) mean levels of catalase activity was observed in CPV positive and CPV negative dogs on day 0 as compared to the control group. Significant increase ( $P < 0.05$ ) in the mean levels of catalase was observed on day 5 of therapy within both the groups i.e. CPV positive and CPV negative dogs. The mean catalase activity on day 3 and day 5 of treatment remained significantly lowered ( $P < 0.05$ ) in CPV positive and CPV negative dogs as compared to the control group. A significant decrease in the activity ( $P < 0.05$ ) of glutathione peroxidase in CPV positive and CPV negative dogs was observed on day 0 as compared to the healthy control group of dogs. Significant increase ( $P < 0.05$ ) in the mean values of glutathione peroxidase was observed within both the groups i.e. canine parvo virus affected and CPV negative dogs on day 3 and day 5 in response to the therapy. On day 3 and day 5 of therapy the mean glutathione peroxidase activity remained significantly low ( $P < 0.05$ ) as compared to the control group. Similar findings were reported by Crnogaj *et al.* (2017) [4] in their study on *B. canis* where they have found that the activities of SOD, GPx and CAT were reduced in the dogs affected with *Babesia canis canis* as compared to the healthy control dogs. On the contrary, Panda *et al.* (2009) [13] reported higher activities of SOD and CAT in the dogs affected with canine parvoviral gastroenteritis. Significant reduction in antioxidant biomarkers found in diseased dogs could be attributed to the consumption of antioxidants that act as "scavengers" of free radicals during the oxidative processes (Crnogaj *et al.*, 2017) [4]. SOD, CAT and glutathione peroxidase are the major enzymes present in RBC to counteract the toxic effects of reactive oxygen species such as superoxide radicals and hydrogen peroxides.

**Table 1:** Oxidative stress parameters (Mean ± S.E.) in dogs suffering from canine parvo virus gastroenteritis

Parameters	Day	Healthy control	CPV Positive (n=21)	CPV Negative (n=15)
SOD (Units/g protein)	0	12.67±0.95 <sup>B</sup>	7.78±0.45 <sup>Aa</sup>	9.17±0.93 <sup>Aa</sup>
	3	12.67±0.95 <sup>B</sup>	8.41±0.29 <sup>Aa</sup>	8.54±0.52 <sup>Aa</sup>
	5	12.67±0.95 <sup>B</sup>	10.53±0.71 <sup>Ab</sup>	9.87±0.27 <sup>Ab</sup>
Catalase (mmol H <sub>2</sub> O <sub>2</sub> utilised/min/g protein)	0	403.67±15.69 <sup>B</sup>	272.71±11.53 <sup>Aa</sup>	260.38±9.08 <sup>Aa</sup>
	3	403.67±15.69 <sup>B</sup>	295.03±7.80 <sup>Aa</sup>	283.24±6.56 <sup>Aa</sup>
	5	403.67±15.69 <sup>B</sup>	327.33±7.76 <sup>Ab</sup>	321.2±69.09 <sup>Ab</sup>
GPx (Units/g protein)	0	3889.43±103.23 <sup>B</sup>	1779.53±101.52 <sup>Aa</sup>	2080.01±160.17 <sup>Aa</sup>
	3	3889.43±103.23 <sup>B</sup>	2218.70±295.64 <sup>Ab</sup>	2523.02±121.15 <sup>Ab</sup>
	5	3889.43±103.23 <sup>B</sup>	3063.32±112.52 <sup>Ac</sup>	2921.86±103.21 <sup>Ac</sup>
GSH (µmol/ml plasma)	0	77.17±2.94 <sup>B</sup>	44.81±2.24 <sup>Aa</sup>	45.48±2.48 <sup>Aa</sup>
	3	77.17±2.94 <sup>B</sup>	52.45±1.98 <sup>Ab</sup>	53.29±2.08 <sup>Ab</sup>
	5	77.17±2.94	67.29±1.96 <sup>c</sup>	65.33±2.23 <sup>c</sup>

The means bearing different superscripts (a, b and c) differ significantly ( $P < 0.05$ ) within the groups. The means bearing different superscripts (A, B) differ significantly ( $P < 0.05$ ) between the groups.

### 3.2 Trace minerals (Mean $\pm$ S.E.) in dogs suffering from canine Parvo virus gastroenteritis

Comparative evaluation of trace minerals in CPV positive and CPV negative dogs in response to therapy at days 0, 3 and 5 are depicted in Table 2. Comparative evaluation of trace minerals in CPV positive and CPV negative dogs in response to therapy at days 0, 3 and 5 are depicted in Table 2. Significantly lowered mean values of copper were observed in both CPV affected and CPV negative dogs on day 0 at the start of therapy as compared to the control group. Significant increase ( $P < 0.05$ ) in the mean values of copper was observed within CPV affected positive group on day 3 and day 5 of therapy whereas CPV negative dogs showed a non-significant increase in the mean levels of copper on day 3 and day 5 of therapy. Non-significant low mean levels of iron was observed in both CPV affected and CPV negative dogs as compared to the control group at the start of therapy on day 0. Significant increase in the mean levels of iron ( $P < 0.05$ ) was observed within both the groups of canine parvo virus affected and CPV negative dogs on day 5 of therapy. Significantly lower mean levels ( $P < 0.05$ ) of zinc were observed in both CPV positive and CPV negative dogs as compared to the healthy control group of dogs at the start of therapy on day 0. Significant increase ( $P < 0.05$ ) in the levels of zinc was observed on day 5 in response to therapy, irrespective of their status of canine parvo virus infection. The mean levels of zinc remained significantly lower ( $P < 0.05$ ) in the CPV positive and CPV negative dogs on day 3 and day 5 of therapy as compared to the control group. The findings of the present study are in accordance with the study of Chaudhuri *et al.* (2008) [13] who have found significant lower levels of iron, copper and zinc in dogs affected with babesiosis. Heidarpour *et al.* (2013) [8] found lowered levels of copper, iron and zinc in sheep affected with liver cystic echinococcosis. Similarly Souza *et al.* (2014) [15] also reported significantly reduced iron and zinc levels in dogs affected with canine visceral leishmaniasis. On the contrary they found higher levels of copper in the affected dogs as compared to the healthy control dogs. Destruction of intestinal absorptive surface by free radicals impairs absorption of macro and micronutrients (Rahman *et al.*, 2002) [14] which explain for low values of iron, copper and zinc in infected dogs.

**Table 2:** Trace minerals (Mean  $\pm$  S.E.) in dogs suffering from canine parvo virus gastroenteritis

Parameters	Day	Healthy control	CPV Positive (n=21)	CPV Negative (n=15)
Copper (ppm)	0	4.55 $\pm$ 0.29 <sup>B</sup>	2.71 $\pm$ 0.07 <sup>Aa</sup>	2.94 $\pm$ 0.11 <sup>A</sup>
	3	4.55 $\pm$ 0.29 <sup>B</sup>	2.84 $\pm$ 0.07 <sup>Aab</sup>	2.78 $\pm$ 0.08 <sup>A</sup>
	5	4.55 $\pm$ 0.29 <sup>B</sup>	3.02 $\pm$ 0.06 <sup>Ab</sup>	2.90 $\pm$ 0.09 <sup>A</sup>
Iron (ppm)	0	56.83 $\pm$ 0.43	55.48 $\pm$ 0.35 <sup>a</sup>	56.14 $\pm$ 0.41 <sup>ab</sup>
	3	56.83 $\pm$ 0.43	55.58 $\pm$ 0.33 <sup>a</sup>	55.37 $\pm$ 0.33 <sup>a</sup>
	5	56.83 $\pm$ 0.43	56.87 $\pm$ 0.29 <sup>b</sup>	56.86 $\pm$ 0.30 <sup>b</sup>
Zinc (ppm)	0	3.92 $\pm$ 0.28 <sup>B</sup>	3.17 $\pm$ 0.06 <sup>Aa</sup>	3.05 $\pm$ 0.11 <sup>Aa</sup>
	3	3.92 $\pm$ 0.28 <sup>B</sup>	3.17 $\pm$ 0.04 <sup>Aa</sup>	3.07 $\pm$ 0.05 <sup>Aa</sup>
	5	3.92 $\pm$ 0.28 <sup>B</sup>	3.45 $\pm$ 0.08 <sup>Ab</sup>	3.42 $\pm$ 0.06 <sup>Ab</sup>

The means bearing different superscripts (a, b) differ significantly ( $P < 0.05$ ) within the groups.

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

### 4. Conclusion

Both the CPV positive and negative dogs also showed decreased activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and trace

elements (copper, iron and zinc). However, with the administration of combination therapy of antioxidants and antibiotics all the oxidative stress parameters were found to be significantly increased towards comparable normal values in CPV positive and negative dogs when compared with healthy unaffected dogs.

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