Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.7953

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2020) *8*(1), 120-126

Research Article

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

Evaluating Haemato-biochemical Status in Dogs Suffering from Gastroenteritis after Combined Antioxidant and Antibiotic Therapy

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Received: 2.01.2020 | Revised: 31.01.2020 | Accepted: 6.02.2020

ABSTRACT

Eighteen dogs suffering from gastroenteritis were randomly enrolled into three groups, 6 dogs in each group. These groups were administered with different combinations of antibiotic (ampicillin-cloxacillin with metronidazole) and antioxidants (vitamin C and N-acetylcysteine) along with supportive therapy. Estimation of haemato-biochemical parameters along with serum electrolytes was done to evaluate comparative effect of antioxidants with antibiotic therapy. Haematological and biochemical parameters of affected dogs were compared with six healthy dogs on day 0, 3 and 5 of treatment trial. Increased levels of haemoglobin with increased packed cell volume, leucocytosis/leucopenia, neutrophilia with thrombocytopenia were observed in gastroenteritic dogs. Elevated liver and kidney function parameters along with decreased serum electrolytes were observed in all gastroenteritic dogs. The results of the study concluded that, group which was administered with both the antioxidants along with antibiotic showed better recovery than rest of the two groups although there was not much statistically significant difference observed in the parameters of different groups.

Keywords: *Gastroenteritis, Ampicillin-cloxacillin with metronidazole, Antioxidants, Vitamin C and N-acetylcysteine*

INTRODUCTION

Gastroenteritis is a common disease of multiple etiologies seen in all age groups of dogs. Different etiological factors include infectious and non-infectious causes. Regardless of the pathogenesis, any tissue damage, induces the release of pro inflammatory mediators, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are powerful oxidants and nitrating species that can inactivate enzymes and initiate lipid peroxidation and nitration, which in turn leads to free-radical chain reactions that further damage proteins, membranes and nucleic acids (Muller et al., 2003).

Cite this article: Kataria, D., Agnihotri, D., Kumar, T., & Shunthwal, J. (2020). Evaluating Haemato-biochemical Status in Dogs Suffering from Gastroenteritis after Combined Antioxidant and Antibiotic Therapy, *Ind. J. Pure App. Biosci. 8*(1), 120-126. doi: http://dx.doi.org/10.18782/2582-2845.7953

An antioxidant is defined as "any substance that, when presented at low concentration compared to those of an oxidizable substrate (proteins, lipids, carbohydrates and DNA), significantly delays, or prevents oxidation of that substrate", thus protecting the body by elimination of the superoxide anion and hydroperoxides that may oxidize cellular substrates, and preventing a chain reaction of the destructive effects of free radicals (Rubio et al., 2016). Vitamin C plays an important physiological role in cells as a reducing agent and antioxidant, free radical scavenger and enzyme co-factor (Carr & Frei, 1999). Similarly N-acetylcysteine (NAC), the body's primary cellular antioxidant, is a precursor to glutathione and its role on glutathione maintenance and metabolism is well established (Kelly, 1998). Hence the present study was planned to evaluate the effects of combined antioxidant and antibiotic therapy in dogs suffering from gastroenteritis as compared to alone antibiotic therapy.

MATERIALS AND METHODS

Eighteen dogs which were reported to Veterinary Clinical Complex, LUVAS, Hisar with clinical signs of gastroenteritis such as vomiting, diarrhoea or haemorrhagic diarrhoea, lethargy, dehydration and depression included in the study. Group G1 constituted six apparently healthy dogs which were taken as control dogs. These affected dogs were randomly divided into three different treatment groups G2, G3 and G4. Affected dogs in G2 group were administered with ampicillin-cloxacillin and Metronidazole and NAC while G3 dogs were administered with ampicillin-cloxacillin and Metronidazole alone. Group G4 dogs were administered with two antioxidants vitamin C and N-acetylcysteine along with ampicillin-cloxacillin and Metronidazole. The antibiotics ampicillin-cloxacillin and metronidazole were administered at the dosage of 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 supportive and symptomatic treatment in different groups included the administration of intravenous fluid Ringer's Lactate (RL), Normal Saline Solution (NSS) or Dextrose Normal Saline (DNS) on the basis of dehydration status and the clinical condition, antacids (pantaprazole @1 mg/kg i.v.), antiemetic (prochlorperazine @0.2 mg/kg b.wt. i.m.), antihistaminic (pheniramine maleate @0.5 mg/kg b.wt. i.m.), antipyretic (analgin @25 mg/kg b.wt. i.m.), vitamin B complex, amino acid preparation, antifibrinolytic agents (Tranexamic acid @10 mg/kg b.wt.) were administered as per the clinical condition of the animal. The blood samples collected in tubes coated with K_3EDTA 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/mm^3 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). Therapeutic evaluation was done on the basis of remission of clinical signs and normalization of haemato-biochemical values. The blood and serum parameters were analyzed on day 0 (pre-treatment), day 3 and day 5 of therapy (post-treatment). The data obtained was analyzed by applying suitable statistical methods using

statistical software package (SPSS 16). For analysis of various haemato-biochemical parameters observed in affected dogs as compared with the healthy control dogs, the independent t-test was applied. For analysis of various haemato-biochemical parameters observed 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).

RESULTS AND DISCUSSION

Changes in hematological parameters of the dogs (n=18) in therapeutic groups are represented in Table 1. Non-significant (P<0.05) higher mean values of hemoglobin were observed in the treatment groups G3 and G4 than the healthy control group at day 0 before the start of the treatment except for group G2 where the mean values of hemoglobin were lower than the control group (G1) on day 0 before the start of the treatment. A non-significant decrease (P<0.05) in mean values of hemoglobin were observed on day 3 and day 5 of therapy within all the treatment groups (G2, G3 and G4). Similar findings of haemoglobinemia in gastroenteritic dogs were also reported in earlier studies (Goddard & Leisweitz, 2010; Agnihotri et al., 2017 and Bhargavi et al., 2017). On the other hand increased levels of haemoglobin were reported in various studies on CPV gastroenteritis (Gaykwad et al., 2016) which might be due to excessive fluid loss resulting in dehydration. The mean values of packed cell volume were found to be non- significantly higher (P<0.05) in all the treatment groups than the control group on day 0 before the start of the treatment. The mean values of packed cell volume non-significantly (P<0.05) decreased within all the treatment groups from day 0 to day 5 of therapy. Increased PCV levels observed might be due to severe dehydration and fluid losses through vomition and diarrhoea as also reported by Biswas et al. (2005) and Bhargavi et al. (2017). Non-significant (P<0.05) higher mean levels of leucocytes were observed in group G4 before the start of treatment on day 0 as compared to the healthy control group while groups G2 and G3 showed non-significant lower mean levels of leucocytes on day 0. Non-significant (P<0.05) decrease in mean values of total leucocyte count within groups G4 was observed on day 3 and day 5 of therapy. On the other hand a non-significant increase (P<0.05) in the mean total leucocyte count was observed within groups G2 and G3 on day 3 and day 5 of therapy. Leucopenia in the gastroenteritic dogs was reported frequently in gastroenteritis by Castro et al. (2013) and Agnihotri et al. (2017). Leucopenia in enteritis is attributable to the destruction of haematopoetic progenitor cells of the various leucocyte types in the bone marrow and other lymphoproliferative organs such as thymus, lymph nodes and spleen which resulted in inadequate supply for the massive demand for leucocytes in the inflamed gastrointestinal tract (Goddard & Leisweitz, 2010). Leucocytosis during gastroenteritis could be due to secondary bacterial invasion in the damaged intestinal epithelium. Non-significant higher (P<0.05) mean values of neutrophil count were observed in all the treatment groups on day 0 before the start of treatment as compared to the healthy control group. Neutrophil count within the treatment groups G2, G3 and G4 decreased non-significantly (P<0.05) on day 3 and day 5 post-treatment towards normalization. Neutrophilia in this study might be associated with secondary bacterial complications as also observed by Decaro and Buonavoglia (2012). A non-significant low (P<0.05) mean levels of lymphocyte count was observed in all the treatment groups on day 0 before the start of treatment as compared to the healthy control group. Mean values of lymphocyte count increased non-significantly (P <0.05) in groups G2 and G3 in response to therapy on day 5 of therapy. A significant increase (P<0.05) was observed in group G4 in the mean lymphocyte levels on day 5 from day 0 in response to therapy. The treatment groups G2 and G4 showed non-significantly low mean levels of monocyte count than the control group at day

0 before the start of treatment. Non-significant differences in the mean levels of monocyte and eosinophil count were observed within all the treatment groups on day 3 and day 5 of therapy. Changes in lymphocytic indices are relative to the neutrophil count observed. All the treatment groups showed non-significantly low mean values of thrombocyte count than the control group at the start of therapy on day 0. Mean values of total thrombocyte count increased non-significantly (P< 0.05) within all the treatment groups from day 0 to day 5 in response to therapy. Thrombocytopenia in the dogs suffering from gastroenteritis might be due to the loss of blood through vomitus and faeces, increased destruction and/ or aggregation, decreased production and disseminating intravascular coagulation. It might also result from increased platelet utilization in the gastrointestinal tract combined with destruction of megakaryocyte bone marrow precursors (Rewerts & Cohn, 2000).

Table 1: Alterations in hematological parameters (Mean ± S.E.) of gastroenteritic dogs in different therapeutic groups

Parameters	Day	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	Group 4 (n=6)
Hemoglobin (g/dl)	0	10.67±0.69	10.50±0.20	12.50±1.20	11.12±0.41
	3	10.67±0.69	10.36±0.65	11.75±1.17	10.23±0.69
	5	10.67±0.69	10.07±0.65	10.96±1.28	10.10±0.52
PCV (%)	0	34.83±2.71	34.90±1.08	36.25±2.69	36.92±0.76
	3	34.83±2.71	33.20±1.46	32.80±3.50	34.80±2.75
	5	34.83±2.71	31.23±1.75	31.02±3.44	32.50±2.77
TLC (m/mm^3)	0	13.02±0.54	7.73±2.15	9.37±1.16	16.74±6.71
	3	13.02±0.54	7.98±1.86	10.00±1.14	16.69±5.67
	5	13.02±0.54	8.55±1.41	10.67±1.47	14.53±2.94
Neutrophil (%)	0	72.17±2.95	79.67±3.69	80.83±2.44	84.17±3.05
	3	72.17±2.95	76.40±3.12	80.33±1.80	80.60±2.68
	5	72.17±2.95	76.50±1.69	78.00±0.71	76.00±1.48
Lymphocyte (%)	0	23.17±3.71	18.17±2.88	15.17±2.33	14.33±2.64^a
	3	23.17±3.71	19.40±2.32	14.00±2.27	14.80±1.98^a
	5	23.17±3.71	20.00±1.45	17.25±0.85	21.83±1.82^b
Monocyte (%)	0	3.50±0.62	2.00±1.06	3.50±0.87	1.50±1.31
	3	3.50±0.62	4.20±1.56	4.50±1.15	4.60±1.44
	5	3.50±0.62	3.00±1.10	4.00±0.82	3.50±1.31
Eosinophil (%)	0	1.17±0.54	0.00±0.00	1.67±0.84	0.00±0.00
	3	1.17±0.54	0.00±0.00	1.17±0.54	0.00±0.00
	5	1.17±0.54	0.60±0.60	0.75±0.75	0.00±0.00
Thrombocyte (m/mm^3)	0	418.00±59.03	146.83±25.31	325.33±76.09	195.60±39.54
	3	418.00±59.03	196.60±26.20	329.75±80.78	212.67±25.22
	5	418.00±59.03	210.20±12.24	399.00±130.72	217.33±53.63

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

Alteration in biochemical parameters of dogs suffering from gastroenteritis in different therapeutic groups (n=18) on days 0, 3 and 5 of treatment is presented in Table 2. Non-significant ($P<0.05$) higher mean values of ALT were observed on day 0 in groups G2 and G4 as compared to the control group G1 while group G3 showed non-significant low mean levels of ALT than control group on day 0. Non-significant higher values of mean AST were observed in group G3 than the control group on day 0 while groups G2 and G4 showed non-significant ($P<0.05$) lowered values than the control group. Changes were non-significant in the mean values of ALT and AST within all the treatment groups was observed on day 5 in response to therapy towards normalcy. These elevated levels of liver function parameters could be due to reactive hepatopathy as also observed by Berghoff and Steiner (2011) and due to hepatic damage caused by viral infections which can lead to increased levels of enzyme activity in serum. Treatment groups G2, G3 and G4 showed non-significant lower mean total protein levels than the healthy control group on day 0. Following therapy a non-significant ($P<0.05$) decrease in the mean levels of total protein was observed on day 3 and day 5 of therapy in all the treatment groups. Similar findings of decreased levels of total protein in the gastroenteritic dogs were also reported by Biswas et al. (2005) and Baruah et al. (2007). While, non-significant higher values of total protein was observed by Surendhar et al. (2018) which could be because of dehydration caused by diarrhoeic losses in CPV infection.

The mean values of BUN and serum creatinine were found higher in all the treatment groups on day 0 as compared to the control group before the start of therapy. A decreasing trend in the mean values of blood urea nitrogen and creatinine was observed within all the treatment groups following therapy from day 0 to day 3 and day 5. 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). Castro et al. (2013) in their study on gastroenteritis caused by CPV and CCoV found increased values of BUN and creatinine due to dehydration as a result of vomition and diarrhoea caused by these viruses. Elevated BUN might also be due to metabolic breakdown of tissues due to viral and bacterial infections (Surendhar et al., 2018). Mean values of the electrolyte parameters i.e. sodium, potassium and chloride were found non-significantly lower in all the treatment groups i.e. G2, G3 and G4 in comparison to the healthy control group before the start of therapy on day 0. Non-significant increase ($P<0.05$) in the mean values of sodium was observed within group G3 on day 5 of therapy while non-significant decrease was observed within groups G2 and G4 on day 5 of therapy. The mean value of potassium and chloride increased as a result of therapy on day 3 and day 5 within all the therapeutic groups but the increase was non-significant. Decrease in electrolytes was also reported by Bhargavi et al. (2017); Haligur et al. (2009) and Joshi et al. (2012) in diarrhoeic dogs which might be due to the loss of potassium, sodium and chloride in severe vomition and diarrhoea resulting intestinal villous atrophy.

Antibiotic ampicillin has a special significance in treatment of haemorrhagic gastroenteritis in dogs because it retains the typical gram positive activity of benzyl penicillin, and simultaneously possesses greatly enhanced activity against gram negative bacteria (Brander et al., 1991). Antibiotic treatment along with adequate fluid therapy and supportive therapy proved to be effective in managing canine gastroenteritis in various studies (Bhat et al., 2015; Agnihotri et al., 2017 and Bhargavi et al., 2017).

Table 2: Biochemical alterations (Mean ± S.E.) in gastroenteritic dogs in different therapeutic groups

Parameters	Day	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	Group 4 (n=6)
ALT (IU/L)	0	29.95±2.59	30.62±3.60	25.88±4.30	30.80±2.30
	3	29.95±2.59	28.86±6.81	19.77±3.75	29.25±2.48
	5	29.95±2.59	27.22±5.05	17.40±2.34	27.92±4.23
AST (IU/L)	0	39.27±3.74	29.86±6.70	42.04±2.94	35.15±7.94
	3	39.27±3.74	26.95±6.35	33.88±5.45	27.94±6.82
	5	39.27±3.74	29.26±5.36	28.96±6.33	30.55±1.28
Total Protein (g/dl)	0	6.38±0.29	4.82±0.58	5.17±0.77	6.32±1.15
	3	6.38±0.29	4.62±0.58	4.74±0.60	5.90±1.06
	5	6.38±0.29	4.56±0.56	4.56±0.72	5.07±0.87
BUN (mg/dl)	0	20.72±2.17	53.22±11.97	51.05±12.13	63.75±33.96
	3	20.72±2.17	42.36±11.19	38.24±7.10	46.74±19.88
	5	20.72±2.17	37.38±6.42	35.42±9.09	34.20±8.14
Creatinine (mg/dl)	0	0.82±0.09	1.22±0.41	0.95±0.08	1.15±0.29
	3	0.82±0.09	1.23±0.32	0.79±0.02	1.14±0.36
	5	0.82±0.09	0.95±0.21	0.85±0.06	1.05±0.17
Sodium (mEq/L)	0	143.15±2.10	141.50±2.29	134.08±3.60	139.78±2.34
	3	143.15±2.10	141.96±4.10	137.48±3.65	134.64±3.57
	5	143.15±2.10	137.88±3.52	136.96±2.49	137.92±2.31
Potassium (mEq/L)	0	5.20±0.22	4.33±0.39	4.24±0.15	4.05±0.17
	3	5.20±0.22	4.67±0.41	4.37±0.24	4.14±0.18
	5	5.20±0.22	4.69±0.38	4.70±0.34	4.16±0.27
Chloride (mEq/L)	0	107.80±1.05	107.38±3.09	97.90±4.63	106.05±2.77
	3	107.80±1.05	112.88±2.24	107.40±5.65	109.32±3.27
	5	107.80±1.05	113.68±2.57	111.12±1.43	112.60±2.54

CONCLUSION

The group which was administered with the antioxidants along with antibiotic showed better recovery in dogs suffering from gastroenteritis. In agreement with the findings of the present study it can be inferred that adjunctive therapy with antioxidants vitamin C and NAC helped in early clinical recovery of dogs affected with gastroenteritis as all the haematological parameters were restored towards normalcy after five days of treatment along with the remission of clinical signs.

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