Biological Forum – An International Journal **(SI-AAEBSSD-2021) 13(3b): 167-172(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Synergistic Efficacy of *Centella asiatica* and *Andrographis paniculata* in the Treatment of Gastroenteritis in Parvo Viral Infection with Special Reference to Oxidative Stress

Lovelin Shweta Xaxa and Praveen Kumar
Department of Veterinary Medicine, Ranchi Veterinary College, BAU, Kanke, Ranchi, India.

(Corresponding author: Lovelin Shweta Xaxa)*
(Received 01 July 2021, Accepted 30 September, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Parvovirus, or canine parvovirus, is a potentially fatal viral disease that affects dogs worldwide. There has been an alarming increase of up to 70% in cases due to the pandemic compared to the previous five years. So, this experimental study was designed to evaluate the antioxidant activity of herbal remedies used for the treatment of gastroenteritis and to document indigenous knowledge about these remedies. Our study involved 18 puppies suffering from gastroenteritis caused by parvovirus. They were divided into three groups by random selection T1, T2 & T3. Group T1 was treated with the combination of *Andrographis paniculata* and *Centella asiatica*, Group T2 was treated with *Andrographis paniculata* alone while the group T3 was treated with known sources of natural antioxidant Vit E & Se. The animals were regularly monitored and blood samples were collected on day 0, 3, 5, 7, 10 & 15 for hemato--biochemical and oxidative stress studies. Group T1 showed a marked improvement by the fifth day with a reduction in oxidative stress markers.

Keywords: Pups, *Andrographis paniculata, Centella asiatica,* gastroenteritis, parvo viral infection, oxidative stress.

INTRODUCTION

Canine parvovirus (CPV) causes canine viral diarrhea, a common health problem affecting dogs across India that is of great concern to veterinarians and dog owners alike. This disease causes a large number of morbidities and deaths in dogs, particularly puppies. Even with vaccines, canine morbidity and mortality remain major problems which need to be addressed (Kubesy *et al*., 2019). It is known that dogs infected with the CPV exhibit symptoms of abdominal pain, vomiting, diarrhea, lethargy, and severe gastroenteritis within three to seven days (Ettinger and Feldman, 2010). In addition to being highly contagious and communicable among puppies, the disease is also very expensive to treat. Lack of immunity, intestinal parasites, and a stressful environment increase its probability. Oxidative stress occurs when there is an imbalance between the production and accumulation of free radical species in an animal body. Medicinal plants like *A. paniculata & C. asiatica* have been evaluated on mice and rats for their wide range of properties (Oruganti *et al*., 2010; Singh *et al*., 2010). Herbs are a great source of antioxidants, and have also been used before to treat gastroenteritis. Since herbs have not previously been used in studies of parvovirus, the current study aimed to examine whether they are effective in treating gastroenteritis caused by this virus.

MATERIAL AND METHODS

Centella asiatica & *Andrographis paniculata* leaves were procured, identified, and authenticated from the Department of Horticulture, BAU, Kanke, Ranchi. These leaves were shade dried individually and ground in a Willey Grinder. For the preparation of the extract, 100 gm powder of *C. asiatica* was soaked in distilled water for 1 liter and 100 gm powder of *A paniculata* in ethanol for 1000 ml for 48 hr at 37oC with continuous stirring. After filtering the contents individually, they were lyophilized to obtain the powdered extract used in the experiment.

The study examined 18 pups brought to Ranchi Veterinary College, Kanke, Ranchi, with a history of diarrhoea and vomition of either gender irrespective of breed. Patients were screened through a detailed history, clinical signs, clinical examination, and an ELISA test of suspected cases. Puppies were randomly assigned to three groups, namely T1, T2, and T3, as shown in table 1.

Table 1: Details of treatment in the experiment.

Gr. No.	No. of Pups	Herb used	Dose & Route	Days of Treatment
T1	6	Combination of *Andrographis paniculata* + *Centella asiatica*	98mg/kg + 10mg/kg body weight (orally)	7 days
T2	6	*Andrographis paniculata*	98mg/kg body weight (orally)	7 days
T3	6	Vit E & Se	25 mg/kg (I/M)	Once

*The following treatments were given in all groups: fluid (RL, DNS), antibiotics (Ceftriaxone + Tazobactam) and supportive therapy (Vit B complex, antiemetic, antihistaminic, haemostatic, and iron preparation).

RESULT AND DISCUSSION

Freshly prepared *C. asiatica* extract yielded 5.76 percent and was greyish green in color and had a characteristic odor. This is in agreement with Ghosh and Indra (2015). Crystalline dark green colored extract of *Andrographis paniculata* was collected around 11.8%, which was similar to the findings of Joselin & Jeeva (2014).

As shown in Table 2, 15 (83.33%) cases showed subnormal temperature, while 3 (16.66%) cases had elevated temperatures that returned to normal after treatment. According to Kumar and Kumar (2017), 75.45% of patients had subnormal body temperatures due to severe fluid and electrolyte losses. Infected pups had a higher respiratory rate before treatment (Table 2). The results are in accordance with Reddy *et al.* (2015). The increase in respiratory rate may be due to anemic hypoxia as well as metabolic acidosis (Datta *et al.*, 1991). The reduced pulse rate (Table 2) observed during the study may be due to catecholamines and other compensatory mechanisms of the body to maintain oxygen supply to cells and tissues Shah *et al.*, (2013). After the experiment, the drop in pulse rate and increase in respiratory rate returned to normal.

Table 2: Physical parameters in different groups of pups.

Groups	0 day	3 day	5 day	7 day	10 day	15 day
Temperature (ºF)						
T1	99.23±0.34	100.07± 0.31	101.03± 0.30	100.93±0.24	101.13±0.30	101.20± 0.48
T2	100.33± 0.55	100.67±0.32	101.27± 0.41	101.20± 0.31	101.80± 0.22	101.07± 0.30
T3	100.33± 0.39	100.17± 0.19	101.33± 0.22	101.07±0.16	101.43±0.29	101.03± 0.29
Respiration (Breathe/ min)						
T1	44.67± 1.84	42.50± 0.99	34.33± 1.51	26.83±1.22	23.33±0.34	20.83± 0.48
T2	43.67± 1.52	43.33± 1.38	35.17± 1.08	27.33±1.63	23.83±1.32	21.17± 1.42
T3	44.17±1.83	42.67± 1.69	34.83±1.72	27.83± 0.95	23.67± 0.46	21.67± 0.76
Pulse (Beats/min)						
T1	96.83±1.47	96.17± 0.79	95.67±0.80	92.33± 0.21	92.17± 0.40	92.33± 0.21
T2	96.17±1.19	97.33± 0.61	95.50±0.67	94.83± 0.40	93.83± 0.98	92.67± 0.42
T3	98.83±0.40	96.00± 0.37	94.50±0.34	93.17± 0.17	93.5± 0.50	92.83± 0.17

A marked significant decrease (Table 3) in hematological parameters (Hb, PCV, Platelets) is indicative of destruction caused by the virus to hematopoietic progenitor cells causing myeloid and erythroid hypoplasia with severe haemorrhagic enteritis and massive sloughing of intestinal epithelial cells (Agnihotri *et al.*, 2017). From Table 3, it is clear that significant hematological changes in T1 took place from the 5th day onwards, while T2 and T3 showed the increase from the 7th day onward. Hb, platelets, TEC and hematocrit values in T2 showed marked increase in 7 days followed by T3 in 10 days. The oral administration of *A. paniculata* stimulates the bone marrow to produce blood cells (Kusmardi *et al.*, 2017). Ghosh and Indra (2015) reported that *C. asiatica* extract increased hemoglobin, PCV, and platelet levels due to its anti-inflammatory and antioxidative properties.

The leukopenia observed in this experiment (Table 3) was consistent with that observed by *Agnihotri et al.* (2017), which was caused by the destruction of leukocyte progenitor cells in the thymus, lymph nodes, and spleen. TLC values significantly increased in T1 after five days, followed by T2 and T3 after seven days.

According to Ghosh and Indra (2015), TLC values increased after oral administration of extract of *C. asiatica*, which may be a result of its antioxidant and anti-inflammatory properties (Vaddadi *et al.*, 2017). In their study, Sonwane *et al.* (2017) reported that *A. paniculata* increases lymphocytes (%) and therefore TLC. These findings are consistent with Samy *et al.* (2007), who reported antiviral activity in *A. paniculata*. However, T2 exhibited a higher recovery rate compared with T3.

Table 3: Hematological Parameters in different treatment groups of pups.

Groups	0 day	3 day	5 day	7 day	10 day	15 day
Hemoglobin (g/dl)						
T1	8.43±0.24	9.10±0.18	10.53±0.22	10.90±0.21	11.73±0.11	12.97± 0.22
T2	8.90±0.33	9.17±0.31	9.73±0.19	10.63±0.15	11.43±0.32	12.47± 0.19
T3	8.67±0.23	8.90±0.22	9.37±0.18	9.97±0.22	11.37±0.17	12.33± 0.30
Packed Cell Volume (%)						
T1	24.83±0.83	27.03±0.63	30.80±0.64	31.83±0.57	34.87±0.36	38.57± 0.70
T2	26.20±1.18	26.83± 0.86	28.70±0.42	31.20± 0.42	33.80± 0.95	36.63± 0.55
T3	25.50±0.87	26.10±0.84	27.87±0.47	29.63±0.64	33.60±0.65	36.83± 0.80
Platelets (10^3/µL)						
T1	93.33±2.80	119.00±1.44	149.83± 1.05	194.00±2.24	195.50±1.98	201.33± 1.99
T2	94.67±2.87	118.50± 0.87	142.33± 1.30	190.50±0.62	193.17±0.90	196.67± 1.65
T3	94.00±2.07	117.17±1.79	141.00± 0.99	170.00± 0.60	185.67± 2.70	197.17± 1.10
TEC (10^6/µL)						
T1	4.60±0.19	4.53±041	6.42±0.19	6.75±0.12	6.88±0.12	7.12±0.15
T2	4.93± 0.13	4.68± 0.37	5.63± 0.29	6.63± 0.22	6.65± 0.18	6.72± 0.19
T3	4.75±0.19	4.72±0.16	5.78±0.14	5.87±0.13	6.57±0.18	6.93±0.08
TLC (10^3/µL)						
T1	7.85±0.31	8.08±0.31	10.6±0.40	13.15±0.32	13.35±0.24	14.12± 0.30
T2	7.22±0.21	8.18±0.22	9.65± 0.13	12.88±0.18	13.17±0.24	13.8±0.24
T3	7.72±0.14	8.12±0.27	9.52±0.22	11.73±0.23	12.93±0.20	13.43± 0.22
Neutrophils (%)						
T1	77.50±0.67	74.33±1.43	64.50±1.02	62.83±0.87	61.17±0.70	60.83± 0.60
T2	74.17±0.83	73.33± 0.76	68.17±1.08	63.17± 0.79	61.50± 0.76	61.50± 0.56
T3	76.50±1.06	74.17± 0.95	70.50±0.89	67.17± 0.95	63.17± 0.48	61.67± 0.56
Lymphocytes (%)						
T1	12.33±0.61	14.67±1.50	20.83±0.91	24.50±1.09	28.17±0.75	27.83± 0.79
T2	15.00±0.52	15.67± 1.12	17.67±1.09	24.17± 0.54	27.00±1.10	27.50 ± 0.62
T3	13.33±1.20	15.50± 1.41	17.33±0.88	20.83±1.01	26.00±0.52	27.50 ± 0.76

Protein and albumin levels decreased in all the groups before treatment (Table 5). This may be due to viral replication causing damage to villi and intestinal hemorrhages, resulting in protein-wasting enteropathies (Prittie, 2004). Serum protein levels and albumin values significantly differed among groups on the fifth day of treatment, with significant increases observed in group I as compared to Gr. 2 & Gr. 3 from 5th day of treatment. A progressive significant rise in mean values of serum total protein and albumin was observed in Gr 2 in 7 days in comparison to Gr 3. In dogs with parvovirus enteritis, Kalli *et al.* (2010) also observed a decrease in serum protein levels. *Centella asiatica* contributes to the restoration of total protein level and albumin level to a normal range through stimulation of protein synthesis, which promotes the regeneration of liver cells (Alagbe, 2019). As Sivakumar and Rajeshkumar (2015) reported, decreased protein indicates polyphagia and loss of weight, but treatment with an ethanolic extract of *A. paniculata* stimulates protein synthesis to restore total protein levels to normal.

In all treatment groups, sodium and potassium levels were reduced (Table 4). The decrease in values is a direct consequence of gastroenteritis induced vomiting and diarrhea in pups (Ettinger and Feldman, 2010). An increasing trend from base line values of Na & K+ was observed in all groups with a non-significant (P>0.05) difference on subsequent days of treatment. Similar findings have been reported previously by Kumar and Kumar (2017). *Centella asiatica & Andrographis paniculata* are fairly adequate sources of sodium and potassium, resulting in normalization of serum sodium and potassium levels. Similar findings were also observed by Alagbe, 2019.

BUN, Creatinine, ALP and ALT values were significantly elevated before treatment and returned to normal after treatment (Table 4). T1 showed a significant decrease compared to T2 and T3 after 5 days of treatment. On the 7th day of treatment, a significant decline was observed in T2 compared with other groups. After 10 days of treatment, all groups returned to normal, with T2 showing a faster recovery than T3. According to Basanti *et al.* (2004), dehydration associated with fluid loss may contribute to the elevation in creatine and BUN levels. Similarly, Goddard & Leisewitz (2010) reported similar findings. Using *C. asiatica* leaves extract showed antioxidant and chelating properties, as demonstrated in Ghosh and Indra's (2015) study.

In T1 and T2 from the 5th day on, the ALT and ALP values decreased significantly, whereas in T3 from the 7th day on, a significant decrease in ALT and ALP values was observed. Shah *et al.* (2013) report that anemic anoxia leads to an increase in ALT & ALP levels due to hepatic damage. Kumar and Kumar (2017) reported similar findings. Ukpanukpong *et al.* (2018) reported that diarrhoea is the main cause of elevated ALP & ALT values and that *A. paniculata* can restore normal values. Those findings were in accordance with Nasir *et al.* (2013). The administration of *C. asiatica* extract leads to an increase in total protein, albumin value in serum and a decrease in ALT and ALP levels by regeneration and production of liver cells in the liver. The decrease in ALT levels was also observed by Xavier and Umadevi (2014) after administration of *C. asiatica*, which was similar to our findings. According to the findings, all of the values within all treated groups were within range by the 15th day of treatment.

Table 4: Biochemical Parameters in different treatment groups of pups.

Groups	0 day	3 day	5 day	7 day	10 day	15 day
Serum Total Protein (g/dl)						
T1	4.93±0.17	5.44±0.15	5.92±0.08	6.47±0.08	6.97±0.10	7.18±0.11
T2	5.12±0.16	5.27± 0.15	5.56± 0.09	6.29± 0.11	6.84± 0.15	7.12±0.18
T3	4.89± 0.16	5.15± 0.15	5.50± 0.08	5.90± 0.15	6.78±0.22	7.05± 0.22
Serum Albumin (g/dl)						
T1	2.18±0.13	2.50±0.17	3.21±0.19	3.75±0.15	3.95±0.17	4.24±0.17
T2	2.22±0.13	2.47±0.11	2.73±0.08	3.63±0.13	3.79± 0.12	4.13±0.15
T3	2.19±0.13	2.45±0.13	2.76±0.13	3.30±0.13	3.70±0.11	4.05±0.15
Serum Sodium (mEq/l)						
T1	137.67± 2.66	142.07±1.58	143.83± 0.84	145.17±0.75	148.17±0.91	148.67± 0.41
T2	139.50± 2.27	141.33± 2.50	141.83± 2.33	143.50±1.99	146.00±1.23	147.50± 0.88
T3	139.50± 2.39	141.83±2.95	142.00± 1.48	143.50± 1.48	145.83± 1.05	147.67± 1.29
Serum Potassium (mEq/l)						
T1	3.18±0.32	3.40±0.33	4.10±0.22	4.28±0.20	4.45±0.21	4.58±0.20
T2	4.20±0.29	4.27±0.25	4.28±0.22	4.67±0.18	4.73±0.17	4.78±0.19
T3	3.27±0.39	3.32±0.39	3.63±0.35	3.75±0.32	3.98±0.28	4.37±0.25
BUN (g/dl)						
T1	53.17±1.51	47.67±1.54	42.83±0.83	39.67±0.99	34.67±1.28	31.83± 0.87
T2	53.83±1.80	47.17±1.38	46.67±1.26	40.33±0.88	34.17±0.65	32.17± 0.65
T3	52.33±1.63	48.17±1.58	46.83±1.58	43.17±2.14	34.83±1.62	32.5±1.15
Creatinine (mg/dl)						
T1	2.05±0.10	1.72±0.11	1.47±0.10	1.22±0.12	0.82±0.11	0.53±0.09
T2	1.98±0.10	1.88±0.11	1.85±0.12	1.32±0.07	0.92±0.13	0.60±0.10
T3	2.02±0.15	1.95±0.16	1.88±0.16	1.52±0.13	1.12±0.15	0.65±0.11
ALP (IU/L)						
T1	162.33± 4.83	142.00± 4.16	97.33±1.08	89.50±1.67	86.00±1.65	82.17± 2.57
T2	156.00± 3.53	139.67±3.46	121.83± 5.24	94.17±1.25	89.50±4.53	88.33± 4.47
T3	143.17± 5.59	132.50± 3.01	129.33± 2.44	108.83±4.11	94.33± 1.78	94.00± 1.73
ALT (IU/L)						
T1	103.17± 4.32	89.67± 1.98	59.67±0.51	53.50±0.98	49.83±0.27	48.83± 0.48
T2	100.83± 4.59	91.00± 1.31	70.00±1.89	55.50±1.76	52.33±1.46	49.17± 0.29
T3	97.67±2.50	91.00±1.43	73.50±2.27	71.00±0.48	53.67±1.16	49.83±1.08

SOD values were significantly decreased before treatment and returned to normal after treatment at different intervals and on different days of treatment (Fig. 1). Prasad *et al.* (2018) explain that SOD is an enzyme family that catalyzes the reduction of oxygen radicals (O2-) to molecular oxygen and hydrogen peroxide, which in turn provides cellular defenses against ROS. In the

present study, there was a decline in values after the 5th day in Gr 1 and after the 7th day in Gr 2. Gr 1 recovered much faster than Gr 2, then Gr 3 and then Gr 4.

The increased level of SOD in this study may be attributed to the decreased immune status of the pups that were affected by a severe viral infection. Kiral *et al.* (2005) have also observed increased oxidative stress indices in dogs with other infections. Jhansi and Kola (2019) observed an increase in SOD due to the oral administration of *Centella asiatica* extract, which is attributed to its antioxidant properties. A similar finding was also reported by Kumari *et al.* (2016). Sivakumar and Rajeshkumar (2015) have observed an increase in SOD values in the extract of *A. paniculata*. Yin and Guo (1990) reported that the active constituent andrographolide found in the leaves of the plant acts directly on cortisol, a neutral antistress agent.

The LPO values of all three groups were elevated before treatment but returned to normal on day 5 in Gr 1 and 7 in Gr 2 and on day 10 in Gr 3 (Fig. 2). According to Chidambarum *et al*. (2013), hydroxyl radicals are the most significant active oxygen species that cause LPO and enormous biological damage. This might be one reason for significant changes in LPO in dogs (Khinchi *et al.*, 2019). In dogs infected with parvovirus-induced gastroenteritis, Schoeman *et al.* (2013) observed increased SOD levels. The antioxidant properties of *Centella asiatica* are responsible for the reduction of LPO activity observed by Adatiya and Jaiswal (2015). The same result was also recorded by Oyenihi *et al.* (2017). Trivedi and Rawal (2007) observed a significant reduction in MDA values in mice treated with *Andrographis paniculata*. The findings of Thakur *et al.* (2016) also revealed reduced values.

A very significant increase in GSH was observed in the study before treatment and gradually decreased after treatment (Fig. 3). Panda *et al.* (2009) reported that these findings were common in parvovirus infection. According to Checconi *et al.* (2019), viruses alter the intercellular redox state of animals to prooxidant conditions which aggravate the disease. The excessive production of GSH may be due to protective mechanisms of the body for controlling viral replication. As found in Khinchi *et al.* (2019), oral administration of *C. asiatica* was observed to normalize GSH values, indicating immunomodulatory and antioxidant properties of the plant. Our results are consistent with these findings. Jhansi and Kola (2019) reported similar findings. An excellent response was observed in Gr 1 followed by Gr 2 and Gr 3

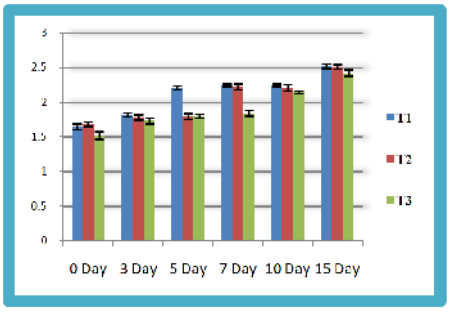

Fig. 1. Graph showing SOD values groups.

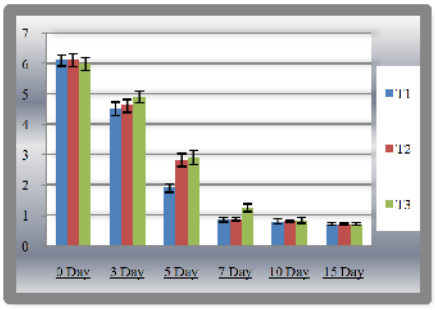

Fig. 2. Graph showing LPO values in different in different groups.

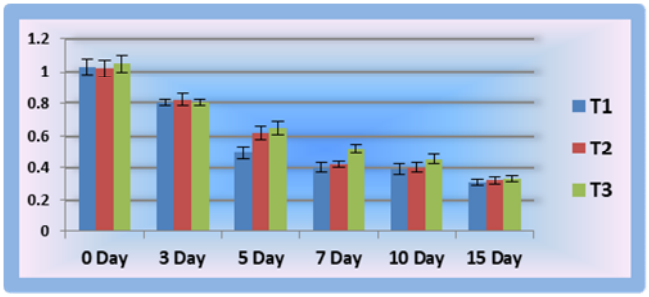

Fig. 3. Graph showing reduced glutathione values in different groups.

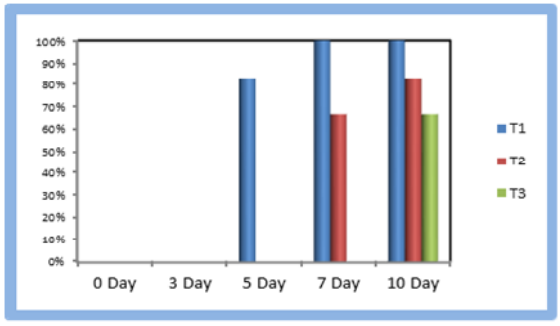

Fig. 4. Graph showing post treatment recovery in different groups.

CONCLUSION

In the experiment, *Centella asiatica* and Andrographis *paniculata*, two herbal medicines that act synergistically, had superior therapeutic efficacy and antioxidant property than all the other treatments. The antioxidant properties of *Andrographis paniculata*

alone were also better than those of Selenium and Vit E, which is a widely known antioxidant. The efficacy of herbs could be studied further in order to formulate a herbal drug for animals.

Acknowledgment. We the authors are thankful to the Vice Chancellor, Director Research, BAU and the Dean Ranchi Veterinary College, for providing facilities and encouragement.

Conflict of Interest. The authors declare that they have no conflict of interest in the present work.

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