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Efficacy of *Centella asiatica* (Beng Saag) on hemato-biochemical and oxidative stress due to gastro enteritis in pups

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Abstract
Canine parvo virus is an important and potentially contagious disease in India, caused by Canine parvo virus belonging to parvoviridae family. Research was conducted to study the hemato-biochemical and oxidative changes in pups treated with *Centella asiatica*. Twelve pups positive for CPV were selected for the study. Diagnosis of gastroenteritis of parvo virus was made on the basis of history, clinical signs, clinical examination and ELISA test. Twelve selected individuals were randomly divided into 2 groups viz. T1 and T2. The clinical signs were recorded and blood samples were subjected to estimation of hemato-biochemical and oxidative stress parameters on day 0, 3, 5, 7, 10 and 15 from both groups. The haematological evaluation revealed improved values from 7 days onwards in the T1 group. Serum biochemistry revealed decreased value of alkaline phosphatase, alanine aminotransferase as well as increase in albumin and total protein values. Oxidative stress parameters R-GSH and LPO were markedly decreased while SOD increased after oral administation of *Centella asiatica* @ 10mg/kg body weight.

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

Introduction
Canine parvo virus is a highly contagious disease of young pups. The disease is a common cause of morbidity and mortality causing mortality of 16-48% and creating a huge loss among canine population. Parvo virus is a small, non- enveloped, single stranded DNA viruses that spreads rapidly and are known to cause disease in variety of mammalian species. This virus replicates only in certain rapidly dividing cells like intestinal crypt epithelial cells in the bone marrow and myocardiocytes, resulting in cell death and loss due to failure of mitosis (Ettinger and Feldman, 2010) [17]. Gastroenteritis, vomition, bloody diarrhoea and hemoptysis of parvo viral origin are the causes of stress. Oxidative stress is the imbalance between the oxidants and antioxidants in the body either due to excessive formation of ROS or reduced removal (Unuofin and Lebelo, 2020) [47]. Antioxidant molecules such as vitamin E and Se, act as chain-breaking antioxidants, which can scavenge for free radicals, remove them once they are formed, and further halt propagation of peroxidation (Amraoui *et al*., 2018) [4]. Medicinal plants from the ancient are known for their healing and antioxidant properties, amoung those medicinal plants *Centella asiatica* is widely found and continuously consumed through humans and animals throughout Jharkhand. Medicinal properties of *Centella asiatica* were evaluated on rats (Oruganti *et al.*, 2010) [36]. There were no reports on dogs. So, the present study was designed to evaluate the efficacy of *Centella asiatica* (Beng saag) in pups infected with parvo viral infection.

Material and Methods
The leaves of *Centella asiatica* was procured identified and authenticated from Department of Horticulture, BAU, Kanke, Ranchi. These were shade dried and ground in a Willey Grinder at room temperature. For preparation of the aqueous extract, 100 gm each powder of *C. asiatica* was soaked in 1 liter of distilled water for 48 hr at 37 ^{o}C with continuous stirring, the contents were filtered, concentrated at 45-50 °C and reduced pressure using rotatory vacuum evaporator (Idrus *et al*., 2012) [26], lyophilized to get the final extract residue and stored at 40^{o}C till further use.
A total of 12 pups presented at the Department of Veterinary Medicine, Ranchi Veterinary College, Kanke, Ranchi with the history of diarrhoea and vomition were taken for the study.

Selection of the cases was done through detailed history, clinical signs, clinical examination and ELISA test of suspected cases. The selected pups were randomly allotted into two groups namely T1 and T2.

Table 1: Details of allotted treatment in the experiment are given below:

Gr. No.	No. of Pups	Herb used	Dose & Route	Days of Treatment
T1	6	*Centella asiatica*	10mg/kg body weight (orally)	7 days
T2	6	Vit E & Se	25 mg/kg (i/m)	once

*Fluid (RL, DNS), antibiotics (Ceftriaxone + Tazobactam) and supportive therapy (Vit B complex, antiemetic, antihistaminic, haemostatic & iron preparation) were given in all groups along with above treatment.

Result and Discussion

The aqueous extract residue was greyish green in colour with characteristic odour. The extract residue showed 5.76 percent yield. The finding was in accordance with Chaitanya *et al.* (2011) [11]; Chauhan and Singh (2011) [12]. Body temperature increased after treatment towards normalcy from subnormal temperature (Table 2). This finding was in accordance with Kumar and Kumar (2017) [32] who reported 75.45% cases and explained that the subnormal body temperature was due to severe fluid and electrolyte losses. The mean value of respiration rate returned towards normalcy after the treatment which was higher before treatment (Table 2). This finding was in accordance with Reddy *et al.* (2015) [39] and Bhat *et al.* (2013) [9]. Increase in respiratory rate during pre-treatment period might be due to progressive development of metabolic acidosis as well as anemic state of the animal. (Saxena *et al*., 2006) [44]. In the present investigation, the variation in mean value of pulse rate decreased and became almost normal after the treatment (Table 2). This finding might be due to effect of catecholamine and other compensatory mechanism of heart to maintain oxygen supply to tissues (Saxena *et al*., 2006 [44] and Kalli *et al*., 2010 [29]).

Table 2: Physical parameters in different groups of pups

Groups	0 day	3 day	5 day	7 day	10 day	15 day
Temperature (^{0}F)						
T1	99.40±0.33	100.13± 0.22	100.87± 0.23	101.10±0.20	101.03±0.14	100.97± 0.15
T2	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	46.83±2.46	45.17± 2.06	36± 1.00	28.17± 1.17	24.33± 0.93	21.5± 1.06
T2	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	97.17±1.01	96.83±1.14	95.17±1.14	93.67± 1.31	92.17± 0.17	92.50± 0.22
T2	98.83±0.40	96.00± 0.37	94.50±0.34	93.17± 0.17	93.5± 0.50	92.83± 0.17

Hemoglobin, packed cell volume and platelets values showed progressive increase after the treatment at different time intervals (Table 3). A marked decrease in hemoglobin and PCV before treatment are indicative of destruction to hematopoietic progenitor cells by the virus causing myeloid and erythroid hypoplasia, severe haemorrhagic enteritis and massive sloughing of intestinal epithelial cells (Kubesy *et al.*, 2019) [31]. Thrombocytopenia can result from decrease of platelet production or as a consequence of direct destruction by viruses or immunological components on platelets or vascular endothelium (Wilson *et al.*, 1982) [49] and increased platelet utilization in the GIT along with destruction of megakaryocyte bone marrow precursor (Rewerts and Cohn 2000) [40]. Oral administration of *C. asiatica* extract increased the hemoglobin, PCV and platelets level that has been reported by Sasikala and Naidu (2019) [43] and Gayathri *et al.* (2011) [19]. It was due to the anti-inflammatory and antioxidative properties of the plant (Adatiya and Jaisawal, 2015 [2]).

There was a decrease in total leucocyte count before treatment (Table 3). The result obtained in the present study were in agreement with the result of Behera *et al.* (2014) [6]. Goddard and Leisweitz (2010) [21] stated that due to destruction of leukocyte progenitor cells in the bone marrow and other lympho proliferative organs such as- thymus, lymph nodes and spleen, the demand of leukocytes in GIT could not be fulfilled. Increase in values of TLC was observed by Doulah and Rafieirad (2017) [15] and Sarumathi and Saravanan (2013) [42] due to oral administration of extract of *C. asiatica* which could be due to antioxidant and anti-inflammatory property of the herb (Hamidpour *et al*.,2015 [24]); (Vaddadi *et al*., 2017 [48]).

Neutrophila observed in the study was due to inflammatory reaction associated with secondary bacterial complication with parvo viral enteritis as earlier reported by Bhargavi *et al.* (2017) [8]. Similar findings with decrease in neutrophil and increase in lymphocytes were also described Berghoff and Steiner (2011) [7]. Same finding was also observed by Sarumathi and Saravanan (2013) [42] with the administration of CA extract in rats. Increase in lymphocyte and decrease in neutrophils may be due to immunomodulatory activity of *C. asiatica* (Jhansi and Kola, 2019 [28] and Prakash *et al*., 2017 [38]).

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

Groups	0 day	3 day	5 day	7 day	10 day	15 day
Hemogobin (g/dl)						
T1	9.13 ± 0.19	9.27 ± 0.23	9.30 ± 0.22	9.80 ± 0.14	11.07 ± 0.23	11.97 ± 0.20
T2	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	27.10 ± 0.60	27.00 ± 0.75	27.50 ±0.69	28.43± 0.61	32.53± 0.75	35.83 ± 0.43
T2	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	95.33 ± 3.53	116.17 ±1.78	139.50 ± 1.64	168.17± 1.97	192.67± 3.72	195.33 ± 1.52
T2	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	5.10 ± 0.20	5.12 ± 0.51	5.47 ± 0.18	5.72±0.17	6.43 ± 0.15	6.68 ± 0.17
T2	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	7.63 ± 0.20	7.98 ± 0.17	9.27 ± 0.37	11.47±0.45	12.15 ± 0.37	13.23 ± 0.18
T2	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	75.83 ± 0.98	73.50 ± 1.18	70.67 ± 0.80	66.67± 0.61	63.33± 0.56	62.17± 0.70
T2	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	14.50 ± 1.20	15.83 ± 1.19	16.17 ± 0.95	21.33± 1.09	25.83± 0.70	27.17 ± 0.75
T2	13.33 ± 1.20	15.50 ± 1.41	17.33 ± 0.88	20.83±1.01	26.00±0.52	27.50 ± 0.76

Decreased serum protein and albumin values in the study were similar to the findings reported by Salem *et al.* (2018) [41] (Table 4). According to Shah *et al.* (2013) [46] the decrease in serum protein through the course of disease were mostly due to the combination of intestinal hemorrhage. Dongre *et al.* (2013) [14] also observed decreased level of serum protein in dogs with parvovirus enteritis. Bhat *et al.* (2013) [9] stated that the significant decrease in albumin values might be due to decline in diet intake, malabsorption and ongoing protein loosing enteropathy. *Centella asiatica* affords restoration of total protein and albumin level towards normal through stimulation of protein synthesis as a contributory mechanism, which accelerates the regeneration and production of liver cells (Alagbe, 2019 [3]; Xavier and Umadevi, 2014 [50]). The levels of sodium and potassium were reduced (Table 4) due to losses in diarrhoeic fluid, vomition and dietary intake resulting in symptoms like depression and weakness (Ettinger and Feldman, 2010 [17]). Above findings were similar to the findings of Kumar and Kumar (2017) [32] and Panda *et al.* (2009) [37]. *Centella asiatica* have fairly adequate concentration of sodium and potassium which resulted to bring the serum sodium and potassium level to normalcy. This herb also reduces enteritis and vomition resulting into absorption of sodium and potassium through GIT tract. The BUN and Creatinine values were marked increased in the experimentation before treatment and reduced to normal by different days of treatment (Table 4). The increased values might be due to prerenal azotemia which might have resulted from reduced glomerular filteration rate due to severe dehydration (Bhargavi *et al.*, 2017) [8]. Goddard and Leisewitz (2010) [21] also observed increased value and suggested to be consequence of dehydration. The elevated values in parvovirus affected dogs were also recorded by Shah *et al.* (2013) [46]. *C. asiatica* leaves extract showed improvement in BUN values through its antioxidant and chelating activities were described by Ghosh and Indra (2015) [20] and Abdulla *et al.* (2010) [1].

ALP and ALT values were higher in affected pups before treatment (Table 4). The similar findings were also recorded by Kumar and Kumar (2017) [32] and Schoeman *et al.* (2013) [45]. In this experiment anemic anoxia may result into the hepatic damage and release of ALT due to the hepatic necrosis. The same finding have been found by Shah *et al.* (2013) [46] and administration of extract of *C. asiatica* increase the level of total protein, albumin value in serum and decrease the ALT and ALP values by regeneration and production of liver cells. These two herbs also help in the reduction of anemia by improving hemoglobin and RBC (Antony *et al.* 2006) [5]. The decrease in ALT values were observed by Xavier and Umadevi (2014) [50] and Antony *et al.* (2006) [5] due to administration of CA which were similar to our study.

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

Groups	0 day	3 day	5 day	7 day	10 day	15 day
Serum Total Protein (g/dl)						
T1	5.09± 0.19	5.17± 0.19	5.44± 0.10	5.83± 0.13	6.70± 0.12	7.03± 0.12
T2	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	2.20±0.14	2.42±0.14	2.60± 0.26	3.23±0.10	3.67±0.11	4.00±0.13
T2	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	138.83± 2.18	140.33± 2.20	141.67± 2.08	142.67± 2.10	145.83± 1.41	146.83± 1.20
T2	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	3.67± 0.30	3.70± 0.32	3.88± 0.32	3.98± 0.32	4.08± 0.31	4.25± 0.30
T2	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	55.50±1.45	48.83±1.72	47.17±1.33	43.83±0.83	35.33±1.58	32.83± 1.47
T2	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	2.10±0.14	1.97±0.12	1.90±0.12	1.58±0.09	1.20±0.11	0.77±0.08
T2	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	154.00± 4.94	144.17± 5.58	131.00± 2.71	113.17±2.41	95.50± 2.34	96.00± 5.61
T2	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	96.50±4.56	92.17± 3.79	75.17±2.00	72.33± 1.21	54.67± 1.23	50.17± 1.17
T2	97.67±2.50	91.00± 1.43	73.50±2.27	71.00± 0.48	53.67± 1.16	49.83± 1.08

A significant decrease in the SOD values were observed before treatment which came to normal after treatment (Table 5). Increased SOD in the present investigation might be due to enhanced synthesis of antioxidant enzymes in moderately severe cases of gastroenteritis as in-built compensatory mechanism. Increased oxidative stress indices in dog have also been reported in other infections by Kiral *et al*. (2005) [30] and Bildik *et al*. (2004) [10]. Super oxide dismutase represents the front line of defence against oxidative damage. Kumari *et al*. (2016) [34] and Jayashree *et al*. (2003) [27] observed the increase in SOD due to the oral administration of *Centella asiatica* extract which is due to antioxidant properties of the herb. There was an increase in the LPO values before treatment (Table 5). Oxidative stress could be mediated by an early phase of liberation of pro-inflammatory cytokinine and production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 and results in increased LPO (Halliwell, 1994) [23]. Hydroxyl radicals are the major active oxygen species causing LPO and enormous biological damage (Chidambarum *et al*. 2013) [13]. This might be one of the reasons for significant alteration in LPO in dogs (Elsayed *et al*., 2019 [16] and Panda *et al*., 2009 [37]). Increased LPO was observed by Schoeman *et al*. (2013) [45] due to gastroenteritis caused by parvovirus. Changes in lipid peroxidation are thought to be indicative of stress or damage resulting from toxic effects in cells. Significant reduction in LPO was observed after treatment in the present study which is in coordination with Elsayed *et al*. (2019) [16] and Panda *et al*. (2009) [37]. Flora and Gupta (2007) [18] and Kumar and Gupta (2002) [33] observed the reduction in LPO activity due to the administration of *Centella asiatica*. Oxidative stress refers to the cytotoxic consequences of oxygen radicals like SOD, hydroxyl radical and hydrogen peroxide which are generated as byproducts of normal aberrant metabolic processes during aging and other neuro degenerative diseases and act on polyunsaturated fatty acids (PUFA). Gupta *et al*. (2003) [22] observed the increase of free radical generation and also observed that the administration of CA reduces the LPO level in pentylenetetrazole- induced toxicity in rats. Hussin *et al*. (2007) [25] recorded the decreased LPO and increased catalase activity in erythrocytes of CA treated rats during H_2O_2 induced oxidative stress in rats. Lipid peroxidation involves the generation of free radicals and hydroxyl radicals. Oyenihi *et al*. (2017) [36] indicated that the CA possesses potent antioxidant properties due to its inhibition of the formation of the LPO end product MDA in the liver of Type-II diabetic rats.

The observation taken in the study signifies that the GSH content was greatly increased before treatment and came towards normalcy on treatment (Table 5). These finding were common in parvo viral infection (Elsayed *et al*., 2019 [16] and Panda *et al*., 2009 [37]). Glutathione plays a protective role in tissue by detoxification of xenobiotics. GSH values were decreased through oral administration of CA indicating immunomodulatory and antioxidant property of the plant. The finding of the present study on antioxidant effect of plant extracts as evidenced by GSH was in accordance with the reports of earlier workers viz. Chauhan and Singh (2012) [12] and Flora and Gupta (2007) [18].

Table 5: Oxidative stress parameters in different groups of pups

Groups	0 day	3 day	5 day	7 day	10 day	15 day
SOD (µmol/ml)						
T1	1.56± 0.04	1.74± 0.04	1.82± 0.05	1.84± 0.05	2.14± 0.05	2.40±0.03
T2	1.52± 0.06	1.73± 0.04	1.80± 0.03	1.84± 0.04	2.14± 0.02	2.42± 0.05
LPO (µmol/ml)						
T1	6.09± 0.24	5.21±0.20	3.02± 0.21	1.29± 0.19	0.86± 0.06	0.75± 0.05
T2	5.99± 0.20	4.92±0.18	2.92± 0.22	1.26± 0.13	0.85± 0.09	0.74± 0.04
R-GSH (µmol/ml)						
T1	1.02± 0.06	0.83± 0.08	0.66±0.05	0.55± 0.05	0.47± 0.05	0.33± 0.03
T2	1.05± 0.05	0.80± 0.02	0.65±0.04	0.52±0.03	0.45± 0.03	0.33± 0.02

Conclusion

CPV is a highly contagious in nature and spreads from dog to dog by direct or indirect contact with their feces. In untreated cases the mortality can reach upto 91%. The disease induces stress among canines which may further aggravate the condition. There is a need for antioxidants for quick recovery. *C. asiatica* (Beng saag) having marvelous properties and a good antioxidant could be used in the treatment of CPV.

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References

1. Abdulla MA, AL-Bayaty FH, Younis LT, Abu Hassan MI. Anti-ulcer activity of *Centella asiatica* leaf extract against ethanol-induced gastric mucosal injury in rats.

Journal of Medicinal Plants Research. 2010; 4:1253-1259.
2. Adatiya P, Jaisawal P. Protective effects of aqueous extract of *Centella asiatica* against cyclophosphamide induced toxicity in experimental rats. International Journal of Pharmacy and Pharmaceutical Research. 2015; 4:396-413.
3. Alagbe JO. Effect of dried *Centella asiatica* leaf meal as a herbal feed additive on the growth performance, haematology and serum biochemistry of broiler chicken. International Journal of Animal Research. 2019; 3:23.
4. Amraoui W, Adjabi N, Bououza F, Boumendjel M, Taibi F, Boumendjel A *et al*. Modulatory role of Selenium and Vitamin E, natural antioxidants, against Bisphenol A-induced Oxidative stress in wistar albinos rats. Toxicological Research. 2018; 34:231-239.
5. Antony B, Santhakumari G, Merina B, Sheeba V, Mukkadan J. Hepatoprotective effect of Centella asiatica (L) in carbon tetrachloride-induced liver injury in rats. Indian Journal of Pharmaceutical Sciences. 2006; 68:772-776.
6. Behera M, Panda SK, Sahoo PK, Acharya AP, Patra RC, Das D et al. Clinico-pathological findings in naturally infected cases of canine parvovirus infection. Indian Journal of Veterinary Pathology. 2014; 38:226-230.
7. Berghoff N, Steiner JM. Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. Veterinary Clinics of Small Animal. 2011; 41:311-328.
8. Bhargavi M, Shobhamani B, Kumari N, Srilatha C. Diagnostic aspects and hematobiochemical changes associated with canine parvo viral enteritis in dogs. International Journal of Current Microbiology and Applied Sciences. 2017; 6:3357-3364.
9. Bhat AA, Wadhwa DR, Singh SP, Singh I. Haematological and biochemical analysis in canine enteritis. Veterinary world. 2013; 6:380-383.
10. Bildik A, Kargin F, Seyrek K, Pasa S, Ozensoy S. Oxidative stress and non- enzymatic antioxidative status in dogs with visceral Leishmaniasis. Research in Veterinary Science. 2004; 77:63-66.
11. Chaitanya KV, Lakhtakia R, Ramji MT, lavanya K, Rajesh K, Jayakumar K, Sneha C et al. The role of antioxidants in human health maintainance small molecules with infinite functions. International Journal of Pharmaceutical Science and Research. 2011; 2:1395-1402.
12. Chauhan PK, Singh V. Acute and subacute toxicity study of the acetone leaf extract of Centella asiatica in experimental animal models. Asian Pacific Journal of Tropical Biomedicine, 2012, S511-S513.
13. Chidambarum U, Pachamuthu V, Natarajan S, Elango B, Narayanan S, Ramkumar KM. In vitro evaluation of free radical scavenging activity of Codariocalyxmotorius root extract. Asian Pacific Journal of Tropical Medicine. 2013; 6:188-194.
14. Dongre J, Mehta HK, Maheswari P. Rapid diagnosis and clinical management of canine parvovirus infection. Intas Polivet. 2013; 14:155-156.
15. Doulah A, Rafieirad M. The effect of *Centella asiatica* aqueous extract on some blood parameters in rat model of Alzheimer's disease. Report of Health Care. 2017; 3:10-16.
16. Elsayed NM, Kubesy AA, Salem NY. Altered blood oxidative stress biomarkers in association with canine parvovirus enteritis. Comparative Clinical Pathology, 2019, 1-5.
17. Ettinger JS, Feldman EC. Textbook of veterinary internal medicine: diseases of dog and cat. W.B. Saunders Company, Philadelphia, 2010, 1310-1408.
18. Flora SJS, Gupta R. Beneficial effects of Centella asiatica aqueous extract against arsenic-induced oxidative stress and essential metal status in rats. Phytotherapy research. 2007; 21:980-988.
19. Gayathri V, Lekshmi P, Padmanabhan RN. Anti-diabetes activity of ethanol extract of *Centella asiatica* (L.) Urban (whole plant) in Streptozotocin-induced diabetic rats, isolation of an active fraction and toxicity evaluation of the extract. International Journal of Medicinal and Aromatic Plants. 2011; 1:278-286.
20. Ghosh K, Indra N. Efficacy of *Centella asiatica* ethanolic extract on cadmium induced changes in blood haematology, hepatic and nephritic function markers in albino rats. International Journal of Current Research. 2015; 7:18283-18288.
21. Goddard A, Leisewitz AL. Canine parvovirus. Veterinary Clinics: Small Animal Practice. 2010; 40:1041-1053.
22. Gupta YK, Kumar MHV, Srivastava AK. Effect of *Centella asiatica* on pentylenetetrazole- induced killing, congenital and oxidative stress in rats. Pharmacology, Biochemistry and Behaviour. 2003; 74:579-585.
23. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause and consequence. Lancet. 1994; 344:721-724.
24. Hamidpour R, Hamidpour S, Hamidpour M, Zarabi M, Sohraby M, Hamidpour R. Medicinal property of *Gotu kola* (*Centella asiatica*) from the selection of traditional applications to the novel phytotherapy. Archives in Cancer Research. 2015; 3:42.
25. Hussin M, Abdul-Hamid A, Mohamed S, Saari N, Ismail M, Bejo MH. Protective activity of *Centella asiatica* and powder on oxidative stress in rats. Food Chemistry. 2007; 100:535-541.
26. Idrus RBH, Chowdhury SR, Manan NABA, Fong OS, Adenan MI, Saim AB. Aqueous extract of *Centella asiatica* promotes corneal epithelium wound healing in vitro. Journal of Ethno pharmacology. 2012; 140:333-338.
27. Jayashree G, Kurup MG, Sudarslal S, Jacob VB. Anti-oxidant activity of *Centella asiatica* on lymphoma-bearing mice. Fitoterapia. 2003; 74:431-434.
28. Jhansi D, Kola M. The antioxidant potential of *Centella asiatica*: a review. Journal of Medicinal Plants Studies. 2019; 7:18-20.
29. Kalli I, Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T, Koutinas AF. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. Research in Veterinary Science. 2010; 89:174-178.
30. Kiral F, Karagenc T, Pasa S, Yenisey C, Seyrek K. Dogs with hepatozoon canis respond to the oxidative stress by increased production of glutathione and nitric oxide. Veterinary Parasitology. 2005; 131:15-21.
31. Kubesy AA, Saleh IA, Torad FA, Salem SI, Ali ME. The prognostic value of electrocardiography for canine parvo virus infection in german shepherd puppies. Bioscience Research. 2019; 16:234-241.
32. Kumar A, Kumar R. Clinico-hematobiochemical changes

in parvo viral infection in dog. International Journal of Science, Environment and Technology. 2017; 6:2837-2841.
33. Kumar MHV, Gupta YK. Effect of different extracts of Centella asiatica on cognition and markers of oxidative stress in rats. Journal of Ethnopharmacology. 2002; 79:253-260.
34. Kumari S, Deori M, Elancheran R, Kotoky J, Devi R. In vitro and In vivo Antioxidant, Anti-hyperlipidemic Properties and Chemical Characterization of *Centella asiatica* (L.) Extract. Frontiers in Pharmacology. 2016; 7:400.
35. Oruganti M, Roy BK, Singh KK, Prasad R, Kumar S. Safety assemment of *Centella asiatica* in albino rats. Pharmacognosy Journal. 2010; 2:5-13.
36. Oyenihi AB, Chegou NN, Oguntibeju OO, Bubuya. *Centella asiatica* enhances hepatic antioxidant status and regulates hepatic inflammatory cytokines in type 2 diabetic rats. Pharmaceutical Biology. 2017; 55:1671-1678.
37. Panda D, Patra RC, Nandi S, Swarup D. Oxidative stress indices in gastroenteritis in dogs with canine parvoviral infection. Research in Veterinary Science. 2009; 86:36-42.
38. Prakash V, Jaiswal N, Srivastava M. A review on medicinal properties of *Centella asiatica.* Asian Journal of Pharmaceutical and Clinical Research. 2017; 10:69-74.
39. Reddy KB, Shobhamani B, Sreedevi B, Prameela DR, Reddy BS. Canine parvo viral infection in dogs and their treatment. International Journal of Veterinay Science. 2015; 4:142-144.
40. Rewerts JM, Cohn LA. CVT update: diagnosis and treatment of canine parvovirus. In: Kirk RW current veterinary therapy XIII, small animal practice, W.B. Saunders, Philadelphia, London, 2000, 629-632.
41. Salem NY, Yehia SG, Farag HS, Soliman SM. Evaluation of Hepcidin level and clinic-pathological modifications in canine parvovirus enteritis. International Journal of Veterinary Science. 2018; 7:93-96.
42. Sarumathi A, Saravanan N. A study on the hematological parameters and brain acetylcholine esterase activity in immobilization induced stress and co- treatment with *Centella asiatica* leaves extract in winstar rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2013; 3:102-107.
43. Sasikala S, Naidu MD. Evaluation of protective effect of *Centella asiatica* leaves on pancreas function in diabetic rats. International Journal of Herbal Medicine. 2019; 7:55-60.
44. Saxena R, Dua K, Uppal SK, Saini N, Kumar A. Effect of fluid therapy and lignocaine in management of gastro-enteritis in dogs. Indian Journal of Veterinary Medicine. 2006; 26:81-85.
45. Schoeman JP, Goddard A, Leisewitz AL. Biomarkers in canine parvovirus enteritis. New Zealand Veterinary Journal. 2013; 61:217-222.
46. Shah SA, Sood NK, Wani N, Gupta K, Singh A. Haemato-biochemical changes in canine parvoviral infection. Indian Journal of Veterinary Pathology. 2013; 37:131-133.
47. Unuofin JO, Lebelo L. Antioxidant effects of mechanisms of medicinal plants and their bioactive compounds for the treatment of Type 2 Diabetes: An update review. Oxidative Medicine and Cellular Longevity, 2020, 1-36.
48. Vaddadi S, Agrawal P, Das A, Kotagiri D, Kolluru VC. Antimicrobial and Antioxidant Activities in the Root, Stem and Leaf Extracts of *Centella asiatica*. Advances in Biotechnology & Microbiology. 2017; 3:555618.
49. Wilson JJ, Neame PB, Kelton JG. Infection- induced thrombocytopenia. Seminors in Thrombosis Hemostasis. 1982; 8:217-233.
50. Xavier SM, Umadevi D. Hepatoprotective effect of gotu koa (*Centella asiatica* linn.) on carbon tetra chloride induced liver injury in rats. International Research Journal of Pharmacy. 2014; 5:929-931.