

THERAPEUTIC EVALUATION OF CARICA PAPAYA LEAF EXTRACT AND CROTALUS HORRIDUS AS SUPPORTIVE TREATMENT IN CANINE EHRLICHIOSIS

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DOI 10.29005/IJCP.2024.16.2.125-129}

[Received: 30.05.2024; Accepted: 30.10.2024]

How to cite this article: Game, Y.R., Dhoot, V.M., Bhojne, G.R., and Panchbhai, C.G. (2024). Therapeutic Evaluation of Carica papaya Leaf Extract and Crotalus horridus as Supportive Treatment in Canine Ehrlichiosis. Ind. J. Canine Pract., 16(2): 125-129.

The study titled, “Therapeutic Evaluation of Carica papaya Leaf Extract and Crotalus horridus as Supportive Treatment in Canine Ehrlichiosis” which compared *Crotalus horridus* and *Carica papaya* for treating thrombocytopenia in 20 dogs with Ehrlichiosis at Nagpur Veterinary College. Dogs were diagnosed using blood smear examination and PCR. Haematological and biochemical parameters were analyzed pre- and post-treatment. Initially low levels of Hb, PCV, TEC, TLC, platelets, total protein, albumin, and globulin were normalized post-treatment. Conversely, ALT, AST, BUN, creatinine, and total bilirubin levels were improved post-treatment. *Crotalus horridus* treatment demonstrated better effectiveness, and shorter recovery time compared to *Carica papaya leaf extract* for management of thrombocytopenia with Ehrlichiosis.

Keywords: *Carica papaya* leaf extract, *Crotalus horridus*, Ehrlichiosis, Thrombocytopenia.

Canine monocytic ehrlichiosis (CME) is a globally spread tick-Rhipicephalus sanguineus illness. Initially identified in dogs, Ehrlichia canis is the primary agent responsible for causing CME (Tungnunga *et al.*, 2016). The infection presents in three intracellular forms: primary bodies, small spherical structures (1-2 microns) believed to evolve into larger clusters called morulae, which then break down into elementary bodies. The illness is primarily spread through the bite of the tick, Rhipicephalus sanguineus, and manifests in acute, subclinical, and chronic forms (Roopali *et al.*, 2018). During both the acute and chronic phases, thrombocytopenia consistently presents a significant concern, often occurring concurrently with pancytopenia in the chronic phase. Thrombocytopenia can occur due to various factors such as immune-mediated destruction of platelets, heightened consumption resulting from minor inflammation of blood vessels, trapping of platelets in the spleen, excessive production of a substance inhibiting platelet movement,

bone marrow malfunction in conditions causing decreased blood cell production, or a combination of these factors (Mylonakis *et al.*, 2017).

The objective of the present investigation was to find out hemato-biochemical alterations and to compare the performance between adjunctive homeopathy therapy with conventional therapy.

Materials and Methods

The cases were diagnosed by blood smear examination (Pic - 01) & confirmed by performing PCR. Dogs positive for Ehrlichiosis were subjected to treatment and randomly divided into two groups.

For conducting the PCR assays, genomic DNA was isolated from whole blood using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per protocol.

*Part of M.V.Sc Thesis.

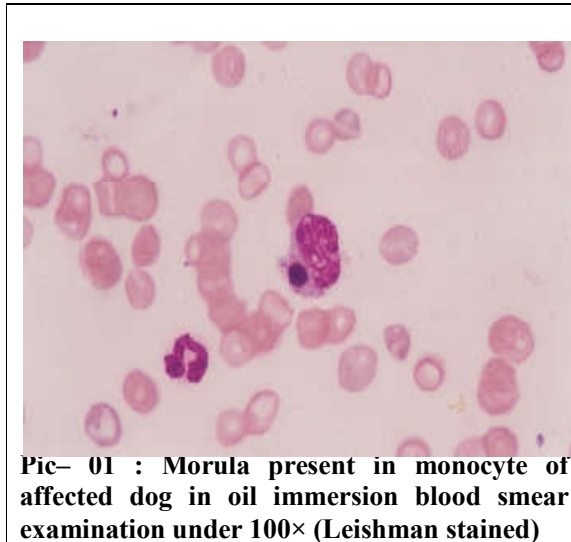


Table:1 Treatment Groups

<p>Group T1 (n=10)</p>	<p>Ten Ehrlichia positive dogs were treated with <i>Carica papaya</i> leaf extract @1100mg orally twice a day with Inj. Doxycycline @ 10 mg/kg BW I/V once a day for 5 days followed by Tab Doxycycline @10 mg/kg orally once a day for 16 days along with fluid therapy, Inj.Vetalgin, vitamin B-complex, Sharkoferrol 1tsf BD .</p>
<p>Group T2(n=10)</p>	<p>Ten Ehrlichia positive dogs were treated with homeopathy <i>Crotalous horridus</i> 0/1, five ml twice a day orally, Inj. Doxycycline @ 10 mg/kg BW I/V once a day for 5 days followed by Tab Doxycycline @10 mg/kg orally once a day for 16 days along with fluid therapy, Inj. Vetalgin, vitamin B-complex, Sharkoferrol 1 tsf BD.</p>

Clinico-physiological parameters were recorded for 5 consecutive days from 1st day (before treatment) 3rd, 7th, 14th and 22nd days of post-treatment. Haematological estimates Hb, PCV, TEC, TLC, DLC and Platelets were carried out using a Haematology Analyser (Horiba Make ABX Micros ESV-60) on the principle of photometry numeric integration and electronic impedance variation. Manual Platelet count estimates from a blood smear examination stained with Leishman

stain. Platelet count was done manually with a commercial diluting system, a hemocytometer, and a microscope. The serum was separated for biochemical study on 0 days (before treatment), 3, 7, 14 and 22nd day of post-treatment which included Total protein (gm/dl), Albumin (gm/dl), Globulin (gm/dl), ALT (Alanine transaminase) (IU/L), AST (Aspartate transaminase) (IU/L), BUN (mg/dl), Creatinine (mg/dl) and Total bilirubin (mg/dl).

Results and Discussion

HAEMATOLOGY

In the present study, haematological parameters such as Hb, PCV, TEC, TLC, DLC, Platelets and mean platelets were studied before and after treatment. The results

obtained from all these parameters were analysed and are discussed below. Haematological parameters studied 20 positive cases were randomly selected and divided into two respective groups. Estimates were made for haemoglobin, PCV, TEC, TLC, DLC and platelet counts. The results of the current investigation showed that dogs with Ehrlichia infection had decreased levels of haemoglobin, PCV, and TEC before therapy, indicating anaemia. Dogs with ehrlichiosis had a mean TLC that was within acceptable limits, however, in DLC initially the monocyte and eosinophils were slightly higher before the treatment. Substantial improvement was observed on the 22nd day post-treatment, In dogs with ehrlichiosis, the mean platelet count was low (110.4±11.21 and 75.8±6.11) before treatment, suggesting thrombocytopenia. The statistical analysis revealed highly significant variation (P< 0.01) in platelet count between groups & intervals. The average platelet count exhibited a remarkably substantial enhancement in group T2.

Similar to us Roopali, 2018, reported significant decrease in platelet count observed

in their study which could be due to a reduction in the lifespan of circulating platelets, platelet dysfunction, the generation of anti-platelet antibodies, or an increase in platelet destruction. Clinical investigations utilizing *Carica papaya* leaf extract indicated a rise in platelet counts from day two onwards, with a notable increasing trend observed until day seven, highlighting the thrombopoietic effects of *Carica papaya* Leaf Extract juice in dogs as also reported by Baranidharan and Nambi (2023). Treatment with doxycycline alongside *Crotalus horridus* resulted in clinical improvement, reduced parasitaemia, improved hemogram values, increased thrombocyte count, decreased monocyte count, and a faster return of liver function to normal levels. Similar to us in their investigation, Tunnunga *et al.* (2016) explored the therapeutic potential of the homeopathic remedy *Crotalus horridus* 200C in managing ehrlichiosis in dogs. He also demonstrated that both doxycycline and *Crotalus horridus* 200C treatment effectively enhanced the hematological parameters in dogs infected with ehrlichiosis.

Table 3.1 - Mean ± SE of haematological parameters in group T1 & T2 on pre-treatment '(0)' day and 3rd, 7th, 14th and 22nd day of post-treatment.

PARAMETER	GROUP N=10	MEAN ±SE					Pooled mean
		0 TH DAY	3 RD DAY	7 TH DAY	14 TH DAY	22 ND DAY	
Hb	T1	8.37±0.83	9.2±0.90	10.53±0.99	11.52±0.94	12.57±0.82	10.43±0.44 ^A
	T2	9.95±1.04	10.11±0.80	11.15±0.90	11.79±0.86	12.56±0.62	11.11±0.39 ^A
PCV	T1	26.45±3.24	28.85±3.39	32.78±3.46	35.28±3.09	38.77±2.65	32.42±1.50 ^A
	T2	28.66±2.77	30.04±2.56	34.17±2.80	35.54±2.45	37.4±1.58	33.16±1.16 ^A
TLC	T1	6.8±1.38	7.55±0.77	9.54±0.86	10.34±0.77	10.47±0.41	8.93±0.43 ^A
	T2	6.7±1.55	8.48±1.42	9.02±1.47	9.41±1.19	10.04±0.82	8.73±0.58 ^A
TEC	T1	3.72±0.42	4.38±0.46	4.87±0.41	5.01±0.42	5.55±0.30	4.70±0.19 ^A
	T2	3.66±0.47	4.14±0.35	4.87±0.35	5.05±0.31	5.68±0.21	4.6±0.18 ^A
PLT	T1	110.4±11.21	134±10.55	151.5±7.31	173.9±5.99	205.9±4.02	155.14±5.88 ^A
	T2	75.8±6.11	134.6±9.26	215.7±10.33	275.6±10.71	336.7±8.76	207.68±13.97 ^B
M. PLT	T1	107.7±11.48	129.4±10.22	146.15±7.24	167.2±5.99	197.6±4.17	149.61±5.66 ^A
	T2	72.5±6.15	103.15±8.91	215.4±12.4	260.15±15.04	331.7±9.35	201.98±13.93 ^B
Neutrophils (%)	T1	70.86±1.5	73.75±0.99	74.77±1.13	75.72±1.13	73.97±0.87	73.81±0.55 ^A
	T2	68.91±1.85	72.45±1.71	75.18±1.45	74.67±1.71	72.57±1.72	72.75±0.79 ^A
Lymphocytes (%)	T1	16.82±1.49	15.44±0.90	16.11±0.95	15.5±1.39	18.18±1.01	16.41±0.42 ^A
	T2	19.79±1.77	18.67±1.93	16.28±1.45	17.63±1.87	21.59±1.68	18.79±0.79 ^B
Monocyte	T1	9.09±0.62	7.78±0.52	6.34±0.53	6.02±0.46	5.54±0.28	6.95±0.28 ^A

s (%)							
	T2	8.86±0.57	6.96±0.48	5.67±0.51	4.73±0.51	3.74±0.48	5.95±0.33 ^B
Eosinophil (%)	T1	4.09±0.71	3.52±0.47	3.22±0.30	3.28±0.49	2.25±0.18	3.27±0.21 ^A
	T2	3.04±0.11	2.77±0.10	2.52±0.12	2.37±0.14	2.07±0.10	2.55±0.06 ^B
Basophils (%)	T1	0.22±0.03	0.28±0.03	0.25±0.04	0.38±0.14	0.25±0.06	0.27±0.03 ^A
	T2	0.23±0.04	0.23±0.04	0.24±0.03	0.20±0.05	0.24±0.03	0.23±0.01 ^A

Capital alphabets denote the difference for the group. Different Capital alphabets indicate significant differences.

BIOCHEMISTRY

Before starting therapy, the mean levels of serum albumin, serum globulin, serum ALT, serum AST, serum BUN, serum creatinine, and total bilirubin were measured in groups T1 and T2 of the current study on '0' and day 22 post-treatment. These results showed that in dogs with ehrlichiosis, serum albumin levels decreased while those of total serum protein, serum globulin, ALT, AST,

BUN, creatinine, and total bilirubin increased. The mean values of creatinine, AST, ALT, and total bilirubin, however, were all within the normal physiological range. The results of the statistical analysis showed that there was a significant improvement in all groups' total serum protein, serum globulin, BUN, serum creatinine, and total bilirubin levels on the 22nd day post-treatment compared to the pre-treatment level (P<0.05).

Table 3.2 - Mean ± SE of biochemical parameters in group T1 & T2 on pre-treatment '0' day and 3rd, 7th, 14th and 22nd day of post-treatment.

PARAMETER	GROUP N=10	MEAN ±SE					Pooled mean
		0 TH DAY	3 RD DAY	7 TH DAY	14 TH DAY	22 ND DAY	
TP	T1	4.83±0.25	5.14±0.24	5.32±0.23	5.66±0.12	6.1±0.08	5.41±0.10 ^A
	T2	5.19±0.28	5.27±0.20	5.6±0.25	6.12±0.22	6.6±0.23	5.79±0.13 ^B
Ser. Alb.	T1	1.72±0.2	1.91±0.17	2.05±0.14	2.17±0.13	2.57±0.11	2.08±0.07 ^A
	T2	1.87±0.17	1.86±0.16	2.07±0.17	2.31±0.17	2.80±0.16	2.18±0.08 ^B
Ser. Glob.	T1	3.11±0.15	3.23±0.16	3.27±0.16	3.49±0.16	3.48±0.13	3.31±0.07 ^A
	T2	3.32±0.14	3.48±0.15	3.62±0.19	3.83±0.19	3.84±0.29	3.62±0.09 ^B
ALT	T1	56.45±18.5	52.87±17.10	45.09±16.00	39.36±12.25	31.93±9.64	45.14±6.5 ^A
	T2	54.95±7.75	54.28±7.38	47.66±7.19	37.96±5.94	33.75±4.69	45.72±3.11 ^A
AST	T1	76.51±11.94	74.58±10.90	59.7±9.11	49.26±7.22	38.06±6.43	59.61±4.5 ^A
	T2	57.44±16.49	51.79±13.88	41.71±11.85	37.57±11.27	28.53±7.02	43.41±5.55 ^B
BUN	T1	28.57±9.1	24.46±7.16	21.73±6.53	18.33±5.77	17.44±4.11	22.10±2.94 ^A
	T2	25.55±3.97	23.46±2.81	18.61±2.06	17.94±2.65	15.95±3.11	20.30±1.38 ^A
Ser. Creat.	T1	1.18±0.18	1.14±0.12	1.01±0.13	0.91±0.10	0.84±0.13	1.02±0.06 ^A
	T2	1.33±0.44	1.30±0.38	1.15±0.33	1.07±0.31	1.02±0.24	1.18±0.15 ^A
TB	T1	0.87±0.06	0.73±0.05	0.63±0.05	0.51±0.04	0.38±0.05	0.62±0.03 ^A
	T2	0.73±0.07	0.63±0.05	0.43±0.03	0.29±0.02	0.18±0.01	0.45±0.03 ^B

Capital alphabets denote the difference for the group. Different Capital alphabets indicate significant differences.

The findings of this study suggest that *Crotalus horridus*, when used alongside allopathic drugs, proves to be an effective treatment for managing thrombocytopenia in comparison to conventional therapy for canine ehrlichiosis. As the cost of treatment is

Indian Journal of Canine Practice
ISSN: 2277-6729 e-ISSN: 2349-4174

less with *Crotalus horridus* it can be used for treatment of thrombocytopenia in dogs suffering with ehrlichiosis. Anaemia, thrombocytopenia, neutropenia and lymphocytosis, hyperproteinaemia, hypoalbuminemia, hyperglobulinemia, and a

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slight elevation in BUN and creatinine level were the major hemato-biochemical alterations observed in Ehrlichia infected dogs. The treatment showed improvement in both the groups. However, the platelets counts were come to normal on post 7th day treatment in Group- T2 as compared to groups T1, which showed normal platelet counts on 22nd post treatment. *Crotalus horridus* has been shown to be an effective adjunctive homeopathic treatment for managing thrombocytopenia due to dengue, as reported by Nayak *et al.* (2019).

The treatment with *Crotalus horridus* lowers the recovery period as compared to available papaya extract.

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