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Laxmi Bai
Department of Veterinary Medicine LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India

Parveen Goel
Department of Veterinary Medicine LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India

Ricky Jhambh
Department of Veterinary Medicine LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India

Preeti
Department of Veterinary Public Health LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India

Correspondence
Laxmi Bai
Department of Veterinary Medicine LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India

Ameliorative potential of polyherbal immunomodulator preparation in dogs with canine monocytic ehrlichiosis

Laxmi Bai, Parveen Goel, Ricky Jhambh and Preeti

Abstract
Canine monocytic ehrlichiosis (CME) is an important tick-borne disease of dogs with a worldwide distribution. Doxycycline is considered the treatment of choice for the disease and the most cases show resolution of clinical illness within 24 to 72 hours after institution of treatment; however, the clinical laboratory parameters remain abnormal. Therefore, the present investigation was conducted to evaluate the ameliorative potential of polyherbal preparation containing Guduchi (*Tinospora cordifolia*), Ashvagandha (*Withania somnifera*), Punarnava (*Boerhaavia diffusa*), Shatavari (*Asparagus racemosus*) and Methika (*Trigonella foenum-graecum*) in dogs with canine monocytic ehrlichiosis. A total of 18 dogs diagnosed for CME by nested PCR assay targeting a portion of 16S rRNA gene of *Ehrlichia canis*, were randomly divided into two groups of nine dogs each. Group I was treated with the standard treatment regimen consisting of doxycycline at the rate of 5 mg/kg twice daily for 21 days. Group II was supplemented with polyherbal formulation along with standard therapy. Therapeutic evaluation was done on the basis of haematobiochemical profile on day 0, 7, 14 and 21 of therapy. There was found significant ($P<0.05$) improvement in vital parameters in both the groups. Thus, the present investigation revealed the ameliorative potential of polyhedral immunomodulator preparation in infected dogs.

Keywords: polyherbal formulation, CME, hepatoprotectivity

Introduction
Canine monocytes ehrlichiosis (CME) is an important tick-borne disease with a worldwide distribution (Harrus S and Waner T 2010) [5]. The causative agent is the obligate pleomorphic rickettsia, *Ehrlichia canis* that is transmitted mainly by the bite of the brown dog tick, *Rhipicephalus sanguineus* (Groves MG, Dennis GL, Amyx HL and Huxsoll DL 1975) [3]. It is a multi-systemic disease manifesting in acute, subclinical or chronic form (Harrus S and Waner T 2010) [5].

Molecular techniques like polymerase chain reaction (PCR) using parasite-specific primers provides a better diagnostic tool in terms of both sensitivity as well as specificity and have been widely used in the laboratory diagnosis of canine ehrlichiosis (Iqbal Z, Chaichanasiriwithaya W. and Rikihisa Y 1994; B, Rikihista Y, Mott JM., Grene R., Kim HY, Zhi N *et al* 1997; Lakshmanan B, John L, Gornathinayagam S. and Dhinakarraj G 2007; Milanjeet SH, Singh NK, Singh ND, Singh C and Rath SS 2014) [7, 23, 12, 14].

Doxycycline is considered the treatment of choice, although tetracycline and oxytetracycline were used initially and still work effectively. Doxycycline is a semisynthetic, lipid-soluble tetracycline that is readily absorbed to produce high blood, tissue, and intracellular concentrations. Given that ehrlichiae may persist intra cellularly, drug penetration into the cell is essential in eliminating infection. Doxycycline has the added advantages of alonger half-life and a higher CNS penetration compared with eithertetracycline or oxytetracycline. The standard treatment regimen consists of doxycycline at the rate of 5 mg/kg twice daily or 10 mg/kg once daily for 21-28 days. In addition to antimicrobial therapy, supportive therapy with fluids for dehydration or blood transfusions may be justified if the dog is severely anemic (Harrus S, Waner T and Neer TM 2012) [6]. The most cases show resolution of clinical illness within 24 to 72 hours after institution of treatment; however, the clinical laboratory parameters remain abnormal. To achieve a complete recovery, there is a need to supplement some immunomodulators. A polyherbal formulation containing Guduchi (*Tinospora cordifolia*), Ashvagandha (*Withania somnifera*), Punarnava (*Boerhaavia diffusa*), Shatavari *(Asparagus racemosus*) and Methika (*Trigonella foenum-graecum*), is claimed to be immunomodulator

And phagocytosis enhancer. Its efficacy have been evaluated in various clinical conditions in canines such as pyoderma and demodicosis (Bhat and Bhagvat 2010 and Kachhawa JP, Singh AP, Ahuja A, Bihani DK, Kachhawa S, Srivastava M 2014) [1, 9] and found to enhance the clinical recovery, but no such studies have been tried in canine monocytic ehrlichiosis. Individual ingradient importance of the formulation is listed here below:-

Tinospora Cordifolia (Tc)
Immunostimulation, particularly activation of the mononuclear phagocyte system by *Tinospora cordifolia* was observed (Nagarkatti DS, Rege NN, Desai NK and Dahanukar SA 1994; Rege NN, Abraham P, Bapat RD, Ray V, Thatte UM and Dahanukar SA 1999) [19, 20]. Tc given alone exerted an antipyretic effect (Kumar and Shrivastav, 1995).

Withania Somnifera
Hepatoprotective and antioxidant potential of root extract of ashwagandha (*Withania somnifera*) was studied against liver dysfunction in geriatric canine & improved serum ALT, AST, albumin, cholesterol and protein levels were observed (Nabi SU,Wani AR and Dey S 2014) [18].

Boerhhavia Diffusa
It was reported to offer significant protection against kidney disease (Singh SM, Singh N and Shrivastava P 1992). The regenerative effects of *B. diffusa* on kidneys is also reported (Mishra J 1980) [15].

Asparagus racemosus - Its root extract was shown to restore lymphocyte and neutrophils counts in myelosuppressed animals (Thatte and Dahanukar 1988).

Tylophora Indica
Its leaves exhibited significant reduction in serum hepatic enzymes (Mujeeb M, Aeri V, Bagri P and Khan SA 2009) [16].

Terminalia Chebula
It showed hepatoprotective activity against carbon tetrachloride induced toxicity in rat hepatocytes (Tasaduq SA, Singh K, Sethi S, Sharma SC, Bedi KL, Singh J, *et al* 2003) [22]. *Terminalia chebula* extract also showed Hypolipidemic activity.
The aim of the study was to study the therapeutic efficacy of polyherbal formulation on various parameters of blood and serum and to aid the faster recovery of affected dogs along with standard treatment regime.

Materials and Methods
Nested PCR using species-specific primers, 18 dogs were found to be positive for *Ehrlichia canis* infection producing an amplicon of 389 base-pair length. For therapeutic evaluation, these 18 dogs were randomly divided into two groups as follows:

Group	Number of animals	Therapeutic regimen
Group I	9	Doxycycline 5 mg/kg orally BD x 21 days
Group II	9	Doxycycline 5 mg/kg orally BD x 21 days + Polyherbal formulation (Immunol, Himalaya Animal Health) 1-2 tsf orally BD x 21 days

Supportive therapy in the form of fluids and electrolytes, antipyretic (analgin @ 25 mg/Kg IM) acid suppressants (omeprazole 0.7 mg/Kg IV, orally) was given in both groups as per clinical condition of the animal.

Evaluation of therapeutic efficacy
1. Therapeutic evaluation was done on the basis of remission of clinical signs and the following blood parameters on samples collected on day 0, 7, 14 and 21 of therapy:
2. Complete hematological examination using fully automated Heamatology cell counter (MS4s, Melet Schlosing Lab.).
3. Serum biochemical profile using fully automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim G
4. mbH).

Statistical analysis
The data generated was analyzed statistically by suitable statistical methods using statistical software package (SPSS 16.0). The results are presented as Mean±S.E. at the significance level, $P \leq 0.05$.

Results and Discussion
Therapeutic evaluation
Effect of therapy on clinical profile and vital parameters
The clinical signs were improved after treatment in both the groups. All the ill animals showed alertness, improved appetite and regression of the clinical signs like corneal opacity, prepucial and auricular discharge etc. by the end of the therapy. The critical analysis revealed that the polyherbal formulation along with doxycycline had more efficacies with doxycycline to manage the ehrlichiosis infection.
Vital parameters improved after 7 days in both the treatment groups. There was significant reduction of temperature and pulse rate on day 7 post therapy in both the groups. However, more significant decrease was observed in group II. This may be due to antipyretic constituent *Tinospora cordifolia* of polyherbal formulation (Singh SM, Singh N and Shrivastava P 2006) [21].

Table 1: Alteration in vital parameters of dogs with CME in response to therapy (Mean ± S.E.)

Vital parameter	Day post treatment	Group I (n=9)	Group II (n=9)
Temperature (°F)	d 0	103.63 ± 0.58^{a}	103.99 ± 0.72^{a}
	d 7	103.08 ± 0.41^{ab}	101.61 ± 0.25^{a}
	d 14	102.49 ± 0.31^{abx}	102.18 ± 0.16^{ay}
	d 21	102.36 ± 0.17^{b}	101.90 ± 0.26^{b}
Pulse rate (per minute)	d 0	92.77 ± 3.75^{a}	99.22 ± 0.72^{a}
	d 7	78.88 ± 3.67^{a}	83.33 ± 2.41^{ab}
	d 14	76.22 ± 2.97^{a}	82.44 ± 1.97^{b}
	d 21	74.11 ± 1.51^{b}	73.11 ± 1.85^{c}

Values with superscript a, b, c differ significantly (0.05) in a column and superscript x, y differ significantly ($P<0.05$) in a row for a parameter

Effect of therapy on haematological profile

The Hb, packed cell volume, total erythrocyte count, platelet count and platelet crit was significantly improved on day 21 in both group I and group II after therapy. Increase in Hb, TEC and packed cell volume was more significant in group II as compared to group I. MCH, MCHC and MCV values increased in both the treated groups by end of follow-up. This may be due to homeostasis effect of *Withania somnifera* root extract (Kumari *et al*., 2014) [10].

Table 2: Alteration in erythrocytic indices of dogs with CME in response to therapy (Mean±S.E.)

Parameter	Day post treatment	Group I (n=9)	Group II (n=9)
Hb (g/dl)	d 0	9.28 ± 0.88^{a}	7.52 ± 1.12^{a}
	d 7	9.94 ± 0.59^{ax}	9.01 ± 1.15^{ay}
	d 14	11.78 ± 0.47^{bx}	9.56 ± 1.23^{aby}
	d 21	12.91 ± 0.38^{bx}	12.40 ± 0.61^{by}
TEC (m/mm^3)	d 0	5.77 ± 0.79^{ab}	4.45 ± 0.59^{a}
	d 7	5.32 ± 0.44^{a}	5.73 ± 0.82^{ab}
	d 14	7.35 ± 0.54^{bc}	5.81 ± 0.76^{ab}
	d 21	7.61 ± 0.61^{cx}	7.19 ± 0.67^{by}
Hct (%)	d 0	33.31 ± 3.10^{a}	28.08 ± 4.14^{a}
	d 7	39.32 ± 5.28^{ab}	39.10 ± 6.02^{ab}
	d 14	48.56 ± 3.63^{bc}	40.48 ± 5.23^{b}
	d 21	53.32 ± 4.34^{c}	47.80 ± 4.88^{b}
MCV (fl)	d 0	65.20±8.61	63.08±2.97
	d 7	72.65±4.12	68.07±2.43
	d 14	66.96±3.76	70.86±3.36
	d 21	70.07±3.71	67.91±4.34
MCHC (g/dl)	d 0	27.89 ± 0.38^{x}	26.67 ± 1.08^{y}
	d 7	26.95±1.67	24.20±1.86
	d 14	25.01±1.42	23.99±1.78
	d 21	25.32±1.80	27.40±2.20
MCH (pg)	d 0	16.58±1.10	16.16±0.89
	d 7	17.40±1.13	17.80±0.81
	d 14	18.15±2.45	19.56±0.54
	d 21	19.09±0.81	20.19±1.47

Values with superscript a, b, c differ significantly (P<0.05) in a column and superscript x, y differ significantly (P<0.05) in a row for a parameter

Increase in platelet count was more significant in group II after treatment follow-up. Significant decrease in mean platelet volume was observed in both the treated groups. The results clearly indicated that both doxycycline and polyherbal (formulation) therapy improved the hematological values in ehrlichiosis-infected dogs. This effect may be due to *Tinospora cordifolia* (Nagarkatti DS, Rege NN, Desai NK and Dahanukar SA 1994; Rege NN, Abraham P, Bapat RD, Ray V, Thatte UM and Dahanukar SA 1999) [19, 20].

Table 3: Alteration in thrombocytic indices of dogs with CME in response to therapy (Mean ± S.E.)

Parameters	Day post treatment	Group I (n=9)	Group II (n=9)
Thrombocytes (m/mm^3)	d 0	109.56 ± 11.72^{a}	83.33 ± 10.03^{a}
	d 7	117.56 ± 9.35^{a}	161.17 ± 13.35^{a}
	d 14	143.00 ± 6.60^{ax}	320.89 ± 33.74^{by}
	d 21	265.00 ± 15.61^{bx}	452.22 ± 31.27^{cy}
MPV (fl)	d 0	8.38 ± 0.20^{a}	8.32 ± 0.19^{a}
	d 7	7.57 ± 0.19^{ab}	8.08 ± 0.23^{ab}
	d 14	7.35 ± 0.29^{a}	7.48 ± 0.16^{a}
	d 21	7.10 ± 0.35^{bx}	7.53 ± 0.20^{by}
Pct (%)	d 0	0.16±0.02	0.13 ± 0.02^{a}
	d 7	0.25 ± 0.09^{x}	0.17 ± 0.02^{ay}
	d 14	0.23±0.02	0.13 ± 0.01^{ab}
	d 21	0.32±0.30	0.22 ± 0.02^{b}

Values with superscript a, b, c differ significantly (P<0.05) in a column and superscript x, y differ significantly (P<0.05) in a row for a parameter

Monocyte count reduced in group II of dogs receiving doxycycline and polyherbal formulation as experimental therapy. A standard therapy. However, changes were also found in group I of dogs receiving doxycycline as a standard therapy but in a lesser intensity.

Neutrophils increased significantly in both the treated groups. This change was more obvious in group II as compared to group I. Lymphocytes count decreased in both the treated groups and was significant in group I by the end of treatment. Basophil and eosinophil counts were also found improved after 21 days therapy in both the groups of dogs.

PCV increased significantly from respective pre-treatment values nearing towards normal. Among various RBC indices, the post treatment mean values of MCHC and MCH decreased from respective pre-treatment values nearing towards normal. These findings suggested improved status of blood and blood forming system post-treatment. Among various serological parameters, post-treatment mean values of total protein increased significantly from pre-treatment values. These findings support the statement on use of

Doxycycline given by Van Heerden and Immelman (1979).

Table 4: Alteration in leucocytic indices of dogs with CME in response to therapy (Mean±S.E.)

Parameter	Day post treatment	Group I (n=9)	Group II (n=9)
TLC (m/mm^3)	d 0	15.09±2.73	18.07±3.03^{a}
	d 7	12.43±2.15	14.85±2.55^{ab}
	d 14	10.65±2.41	14.78±2.42^{ab}
	d 21	9.19±1.45	9.29±2.34^{b}
L (%)	d 0	38.38±3.95^{a}	43.04±6.86
	d 7	26.90±3.80^{ab}	31.74±9.18
	d 14	28.06±4.29^{ab}	29.16±5.12
	d 21	21.73±3.52^{b}	23.06±5.64
N (%)	d 0	55.04±4.19^{a}	51.04±7.20^{a}
	d 7	65.27±3.56^{ab}	61.88±8.85^{ab}
	d 14	65.47±3.81^{ab}	64.22±4.89^{ab}
	d 21	69.57±3.10^{b}	70.36±5.64^{b}
M (%)	d 0	5.51±0.54	5.17±0.52
	d 7	5.47±0.44^{x}	5.00±0.53^{y}
	d 14	4.51±0.49^{x}	4.88±0.20^{y}
	d 21	4.35±0.34	3.97±0.41
E (%)	d 0	1.44±0.47	1.34±0.47
	d 7	2.00±1.54	0.93±0.30
	d 14	1.73±0.85^{x}	1.41±0.47^{y}
	d 21	2.81±1.45	1.60±0.33
B (%)	d 0	0.36±0.13^{x}	0.22±0.07^{ay}
	d 7	0.34±0.06	0.25±0.05^{ab}
	d 14	0.36±0.10	0.31±0.08^{b}
	d 21	0.36±0.05	0.33±0.09^{b}

Values with superscript a, b differ significantly (P<0.05) in a column and superscript x, y differ significantly (P<0.05) in a row for a parameter

Effect of therapy on serum biochemical profile

There was decrease in the value of ALT, AST and ALP on day 21 in both the treated groups. There was significant decrease in the values of ALP in group II. Dogs of both groups were depicting steady improvement in the ALT, AST and ALP values as compared to healthy one.

Table 5: Alteration in liver function profile of dogs with CME in response to therapy (Mean ± S.E.)

Serum biochemical parameter	Day post treatment	Group I (n=9)	Group II (n=9)
ALT (U/L)	d 0	36.29±10.72	27.76±6.86
	d 7	28.04±8.60	27.14±6.16
	d 14	23.70±4.87	22.91±4.41
	d 21	15.38±2.80	19.47±3.26
AST (U/L)	d 0	28.52±5.04	32.33±8.08
	d 7	24.15±4.17	27.35±5.29
	d 14	18.68±2.72	24.37±7.96
	d 21	18.52±2.05^{x}	20.48±5.79^{y}
GGT (U/L)	d 0	7.34±1.37	17.63±3.08
	d 7	6.91±1.21	15.92±3.41
	d 14	6.48±1.57	12.67±2.20
	d 21	6.15±1.33	9.96±1.56
Alkaline Phosphatase (U/L)	d 0	113.78±5.16	183.56±17.71^{a}
	d 7	122.00±6.70	138.78±14.65^{a}
	d 14	126.78±8.26	104.11±16.87^{a}
	d 21	113.33±11.69	99.33±10.04^{b}
Bilirubin total (mg/dl)	d 0	0.36±0.09^{a}	0.34±0.10^{a}
	d 7	0.26±0.07^{abx}	0.16±0.02^{by}
	d 14	0.22±0.02^{abx}	0.14±0.03^{by}
	d 21	0.14±0.02^{bx}	0.13±0.02^{by}
Bilirubin Indirect mg/dl	d 0	0.26±0.08^{ax}	0.24±0.10^{ay}
	d 7	0.11±0.02^{b}	0.06±0.02^{ab}
	d 14	0.13±0.02^{ab}	0.08±0.02^{ab}
	d 21	0.08±0.02^{b}	0.08±0.01^{b}
Bilirubin direct (mg/dl)	d 0	0.10±0.01	0.09±0.01
	d 7	0.15±0.06	0.10±0.01
	d 14	0.09±0.01	0.06±0.01
	d 21	0.06±0.01	0.05±0.00

Values with superscript a, b differ significantly (P<0.05) in a column and superscript x, y differ significantly(P<0.05) in a row for a parameter

Total protein values were increased in both the treated groups after therapy. However, more significant increase in total protein was observed in group II. Albumin level was increased in group I and group II on day 21of treatment follow-up. Decrease in globulin level was analysed in both the groups. A G ratio decreased in both the treated groups but more significantly in group II.

Table 6: Alteration in serum protein profile of dogs with CME in response to therapy (Mean±S.E.)

Parameter	Day post treatment	Group I (n=9)	Group II (n=9)
Total Protein (g/dl)	d 0	4.84±0.40	4.59±0.41^{a}
	d 7	4.71±0.32	6.09±0.41^{b}
	d 14	5.40±0.35	6.93±0.29^{b}
	d 21	5.60±0.42	7.03±0.29^{b}
Albumin (g/dl)	d 0	2.44±0.25	2.02±0.14
	d 7	2.28±0.16	2.15±0.18
	d 14	2.37±0.16	2.35±0.20
	d 21	2.40±0.20	2.50±0.20
Globulin (g/dl)	d 0	3.23±0.31	4.58±0.30^{a}
	d 7	2.94±0.28^{x}	4.52±0.32^{by}
	d 14	2.43±0.27	3.94±0.43^{b}
	d 21	2.40±0.28	2.57±0.42^{b}
A:G	d 0	0.76±0.06	0.43±0.03^{a}
	d 7	0.85±0.10^{x}	0.56±0.04^{by}
	d 14	1.02±0.12	0.63±0.04^{b}
	d 21	1.09±0.15	0.95±0.15^{b}

Values with superscript a, b differ significantly (P<0.05) in a column and superscript x, y differ significantly (P<0.05) in a row for a parameter

This may be due to hepatoprotective activity of *Withania somnifera* root extract of polyherbal formulation in improving serum ALT, AST, albumin, cholesterol and protein levels (Nabi SU, Wani AR and Dey S 2014) [18]. Alcoholic extract of root of *Asparagus racemosus* has been shown to significantly reduce the enhanced levels of ALT, AST and ALP in CC14-induced hepatic damage in rats, indicating antihepatotoxic potential of *Asparagus racemosus* (Muruganadan S, Garg H, Lal J, Chandra S and Kumar D 2000; Chawla A, Chawla P, Mangalesh R and Roy C 2011) [17, 2]. The methanolic extract of *Tylophora indica* leaves also has shown hepatoprotective activity (Gujrati V, Patel N, RaoVanket N, Nandakumar K, Gauda TS, Mohamad S *et.al* 2007) [4].

The level of both creatinine and BUN decreased by the end of therapy and significant decrease in BUN between the groups was also observed. This decrease was more pronounced in group II as compared to group I. This may be due to *Boerhaavia diffusa* regenerative effect on kidneys (Mishra J 1980) [15].

Table 7: Alteration in kidney function parameters of dogs with CME in response to therapy (Mean±S.E.)

Serum biochemical parameter	Day post treatment	Group I (n=9)	Group II (n=9)
Urea (mg/dl)	d 0	36.11±7.33	44.28±9.38
	d 7	32.48±1.89^{x}	38.67±8.59^{y}
	d 14	32.02±2.12	33.85±7.04
	d 21	31.84±1.48^{x}	30.24±3.00^{y}
Creatinine (mg/dl)	d 0	2.23±1.27	2.27±1.32
	d 7	1.51±0.56	1.17±0.13
	d 14	0.97±0.15	1.04±0.12
	d 21	0.83±0.08	0.78±0.07

Superscript x, y differ significantly (P<0.05) in a row for a parameter

The post treatment mean values of ALT, AST and serum creatinine decreased significantly from respective pre-treatment values nearing towards normal suggestive of improved hepatic and renal functions of dogs treated under the group. These findings support the statement on use of Doxycycline given by Van Heerden and Immelman (1979).

Hypolipidemic activity of *Terminalia chebula* extract against experimentally induced atherosclerosis have been documented (Murugundan S, Garg H, Lal J, Chandra S and Kumar D 2000) [17].

Table 8: Alteration in serum lipid parameters of dogs with CME in response to therapy (Mean±S.E.)

Parameter	Day post treatment	Group I (n=9)	Group II (n=9)
Triglycerides (mg/dl)	d 0	104.46±17.38	101.47±17.27
	d 7	132.82±25.46	92.67±15.97
	d 14	118.23±17.80	88.34±15.08
	d 21	108.41±14.06	79.33±7.82
Cholesterol (mg/dl)	d 0	237.78±41.41	201.44±22.79
	d 7	233.89±20.44	190.08±15.79
	d 14	241.11±31.23	143.56±17.35
	d 21	200.67±17.02	184.22±25.35

Conclusion

Dogs receiving Doxycycline along with polyherbal formulation showed faster recovery in vital, blood and serum parameters as compared to the ones who were only receiving the Doxycycline as standard treatment regime.

References

1. Bhat RU, Bhagvat VG. Study to assess the beneficial effects of immunol liquid in the management of canine pyoderma. Veterinary world. 2010; 3:78-81.
2. Chawla A, Chawla P, Mangalesh R, Roy C. *Asparagus racemosus*. Biological Activities & its Active Principles. Indo-Global Journal of Pharmaceutical Sciences. 2011; 1(2):113-120.
3. Groves MG, Dennis GL, Amyx HL, Huxsoll DL. Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). American Journal of Veterinary Research. 1975; 36:937-940.
4. Gujrati V, Patel N, RaoVanket N, Nandakumar K, Gauda TS, Mohamad S *et.al*. Indian Journal of Pharmacology, 2007, 39-43.
5. Harrus S, Waner T. Diagnosis of canine monocytotropic ehrlichiosis: An overview. The Veterinary Journal. 2010; 187:292-296.
6. Harrus S, Waner T, Neer TM. *Ehrlichia canis* infection. In: Greene CE: Infectious dieseases of the dog and cat, 4th edition, St Louis, MO: Elsevier, 2012, 227-238.
7. Iqbal Z, Chaichanasiriwithaya W, Rikihisa Y. Comparison of PCR with other tests for early diagnosis of canine ehrlichiosis. Journal of Clinical Microbiology. 1994; 32:1658-1662.
8. Israni DA, Patel KV, Gandhi TR. Anti-hyperlipidemic activity of aqueous extract of *Terminalia chebula* and Gaumutra in high cholesterol diet fed rats. Int J Pharm Sci. 2010; 1(1):48-59.
9. Kachhawa JP, Singh AP, Ahuja A, Bihani DK, Kachhawa S, Srivastava M *et al*. Evaluation of efficacy of herbal immunomodulators in therapy for canine Demodicosis. Journal of Immunology and Immunopathology. 2014; 15:83-116.
10. Kumari P, Singh SK, Dimri U, Kataria M, Ahlawat S.

Immunostimulatory activities of Withania somnifera root extract in dexamethasone induced immunocompromised mice and *in vitro* model. Asian Journal of Complementary and Alternative Medicine. 2014; 2(2):06-10.
11. Komnenou AA, Mylonakis ME, Kouti V, Tendoma L, Leontides L, Skountzou, E *et al*. Ocular manifestations of natural canine monocytic ehrlichiosis (*Ehrlichia canis*): a retrospective study of 90 cases. Veterinary Ophthalmology. 2007; 10:137-142.
12. Lakshmanan B, John L, Gornathinayagam S, Dhinakarraj G. Molecular detection of *Ehrlichia canis* from blood of naturally infected dogs in India. Veterinarski Archive. 2007; 83:353-354.
13. Maruthappan V, Shree KS. Hypolipidemic activity of Haritaki (*Terminalia chebula*) in atherogenic diet induced hyperlipidemic rats. J Adv Pharm Tech Res. 2010; 1:229-235.
14. Milanjeet SH, Singh NK, Singh ND, Singh C, Rath SS. Molecular prevalence and risk factors for the occurrence of canine monocytic ehrlichiosis. Veterinarni Medicina. 2014; 59:129-136.
15. Mishra J. Studies on the effect of indigenous drug *Boerhavia diffusa* Rom. on kidney regeneration. Indian Journal of Pharmacology. 1980; 12:59.
16. Mujeeb M, Aeri V, Bagri P, Khan SA. International Journal of green Pharmacy, 2009, 125.
17. Muruganadan S, Garg H, Lal J, Chandra S, Kumar D. Studies on the immunostimulant and antihepatotoxic activities of *Asparagus racemosus* root extract. Journal of Medicinal and Aromatic Plant Science. 2000; 22:49-52.
18. Nabi SU, Wani AR, Dey S. Therapeutic Effect of Ashwagandha in liver dysfunction of old dogs. Applied Biological Research. 2014; 16(2):232-236.
19. Nagarkatti DS, Rege NN, Desai NK, Dahanukar SA. Modulation of Kupffer cell activity by *Tinospora cordifolia* in liver damage. Journal of Postgraduate Medicine. 1994; 40:65-67.
20. Rege NN, Abraham P, Bapat RD, Ray V, Thatte UM, Dahanukar SA. Clinical prospects of *Tinospora cordifolia*: An immunomodulator plant. In *Immunopharmacology*: Strategies for Immunotherapy, Upadhyay SN (ed.). Narosa Publishing House. India, 1999, 105-116.
21. Singh SM, Singh N, Shrivastava P. Effect of alcoholic extract of Ayurvedic herb *Tinospora cordifolia* on the proliferation and myeloid differentiation of bone marrow precursor cells in tumor bearing host. Fitoterapia. 2006; 77:1-11.
22. Tasaduq SA, Singh K, Sethi S, Sharma SC, Bedi KL, Singh J *et al*. Hepatocurative and antioxidant profile of HP-1, a polyherbal phytomedicine. Human and Experimental Toxicology. 2003; 22(12):639-645.
23. Wen B, Rikihista Y, Mott JM, Grene R, Kim HY, Zhi N *et al*. Comparison of nested PCR with immunofluorescent-antibody assay for detection of *Ehrlichia canis* infection in dogs treated with doxycycline. Journal Clinical Microbiology. 1997; 35:1852-1855.