Therapeutic Evaluation of Buparvaquone and Peganum harmala against Theileriosis in Cattle

S.A. Lokhande
College of Veterinary and Animal Sciences, MAFSU, Parbhani
M.F. M.F. Siddiqui
drmfmfsiddiqui@gmail.com

College of Veterinary and Animal Sciences Parbhani https://orcid.org/0009-0002-0943-6233
M.P. Sakhare
College of Veterinary and Animal Sciences, MAFSU, Parbhani
G.M. Chigure
College of Veterinary and Animal Sciences, MAFSU, Parbhani
N.D. Jadhav
College of Veterinary and Animal Sciences, MAFSU, Parbhani
S. Sajid Ali
College of Veterinary and Animal Sciences, MAFSU, Parbhani
S.D. Chepte
College of Veterinary and Animal Sciences, MAFSU, Parbhani
S.R. Shaikh
College of Veterinary and Animal Sciences, MAFSU, Parbhani
P.C. Ahire
College of Veterinary and Animal Sciences, MAFSU, Parbhani

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Abstract

Theileriosis is a common tickborne haemoprotozoan disease of dairy cattle in India and the World. Currently, buparvaquone is the only drug used for the treatment and prevention of theileriosis. However, recent reports of buparvaquone resistance in several endemic regions. This study aimed to evaluate clinical, hematological and biochemical alterations in Theileriosis affected cattle and compare the efficacy of *Peganum harmala* and *P. harmala* along with Oxytetracycline with Buparvaquone. Out of 61 cattle screened 21 found positive for Theileriosis. 18 positive cases were randomly divided into three groups (n = 6). Group I treated with Buparvaquone @ 2.5mg/kg body weight, Group II treated with *P. harmala* @ 7 mg/kg body weight and Group III treated with *P. harmala* @ 7mg/kg body weight along with Oxytetracycline @ 20mg/kg body weight. All the affected cattle treated with specific and supportive therapy and B – complex were given for 3 days. Group I, II and III showed significant improvement in Physiological Parameters (heart rate, rectal temperature and respiratory rate), Haemato-biochemical Parameters (total erythrocyte count, packed cell volume, absolute neutrophil, absolute monocyte, total protein globulin, ALT, AST, BUN and creatinine) In group I all the cattle were recovered and there was no parasitemia observed at 14th day of post treatment. Hence, the buparvaquone showed 100% efficacy against *Theileria* parasite. The efficacy of group II was 66.66%. However, group III cattle showed 83.33% as compared to group I. Based on present study it can be concluded that *P. harmala* can be used as alternative medicine against Theileriosis

Introduction

India is the world's leading milk producer, supported by its vast cattle population. However, the average milk yield per animal remains low due to factors such as genetic limitations, poor nutrition, and inadequate management, including ineffective tick control. The country's climate provides a favourable environment for the spread of arthropod-borne parasitic diseases, posing a major challenge to the cattle industry (Zeb et al., 2022). Tick-borne haemoprotozoan diseases, including theileriosis, babesiosis, anaplasmosis, and trypanosomiasis, have significant economic implications, with theileriosis being a major obstacle to livestock productivity.

Theileriosis is a prevalent tick-borne haemoprotozoan disease affecting dairy cattle and buffaloes in India and worldwide. Various species of Theileria are found in domestic and wild ungulates across tropical and subtropical regions, belonging to the Apicomplexa group. Haemoprotozoan infections pose a significant challenge to cattle breeding, causing severe economic losses and reduced productivity. The *Theileria* parasite was first identified by Arnold Theiler and Dschunkowsky, who described the disease in 1904 (Kumar et al., 2018).

Numerous studies have identified several risk factors associated with *T. annulata* infection in bovines. Among these, agro-climatic zone and geographical location are considered significant risk factors as the tropical and subtropical regions provide a hot humid environment for the growth of ticks (Asif et al., 2022)

Currently, buparvaquone is the only drug used for the treatment and prevention of all types of theileriosis. It is a second-generation, hydroxyl-naphthoquinone derivative related to parvaquone, which works by binding to cytochrome-b (cyt-b) and inhibiting the parasite's electron transport chain. Buparvaquone effectively eliminates the parasite, preventing the transmission of signals required for the activation of genes that code for growth factors and receptors involved in signal transduction. However, recent reports of buparvaquone resistance in several endemic regions, including Tunisia, Turkey, and Iran, highlight a growing challenge in controlling theileriosis. Mhathbi et al. (2015) identified two mutations (Pro253Ser and Leu262Ser) responsible for resistance, which affect

68% (13/19) of resistant clones. These mutations replace hydrophobic amino acids (proline and leucine) with hydrophilic serine, potentially reducing buparvaquone's ability to bind to cytochrome-b in the parasite. A Theileria annulata parasite with a single mutation, methionine 128 to isoleucine (M128I), in cytochrome B is resistant to buparvaquone (Tajeri et al., 2024). As a result, it is crucial to explore alternative treatments for theileriosis.

The World Health Organization (WHO) reports that about 11% of medicines are directly derived from plants, with numerous others stemming from natural sources. Around 60% of treatments for various conditions-such as inflammation, neurological disorders, Alzheimer's, pulmonary diseases, diabetes, cardiovascular issues, arthritis, autoimmune disorders, infections, tumors, and cancer-are either derived from nature or are under clinical investigation. Furthermore, research indicates that approximately 15% of plant species have been explored for their photochemical properties, while only 5% have been examined for their biological effects.

Peganum harmala (extract) has demonstrated a high curative rate against tropical theileriosis and is a natural treatment that does not accumulate in the muscles of cattle. The pharmacologically active components of *P. harmala* include a variety of alkaloids, such as β-carbolines like harmaline, harmine, harman, harmatol, and quinazoline derivatives like vasicine and vasicinone. Li et al *(*2024). These alkaloid compounds exhibit diverse antiprotozoal properties like antithilerial properties Mirzaiedehaghi (et al 2006). Saleem et al (2020) evaluate antibabesial activity of *Peganum harmala* in cattle. Motamedifar et al (2016), Iranshahy et al (2019) Wang et al (2022) identified the antibacterial activity of *P. harmala*. It also exhibits anticancer and antioxidant potential Khalid et al (2024). Among them, harmaline (harmidine, C13H14N2O) has been identified as the primary active alkaloid. This compound was not found in the skeletal muscles, ensuring its safety with regard to meat hygiene (Puzii and Seroy, 1983). Beside, certain research has identified the potential capacity of alkaloids and other bioactive components in the seeds of Peganum harmala to protect from inflammatory liver damage. (Abbas et al., 2021; Senhaji et al.2022; Djarmouni et al.2022; Ahmed et al.2021).

In present study, effort was made to evaluate the therapeutic effect of extract of *P. harmala* against Theileriosis in comparison with buparvaquone and Oxytetracycline

Material and Method

The present research work entitled “Therapeutic evaluation of buparvaquone and *P. harmala* against theileriosis in cattle” was conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, MAFSU, Parbhani from October, 2024 to February, 2025. A total of 61 cattle were screened on the basis of clinical signs such as high fever, pale conjunctival mucous membrane, enlargement of lymph nodes, respiratory distress, presences of ticks over the body and anorexia presented to Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Parbhani. Also, the suspected cattle’s sample was collected from surrounding dispensaries of Parbhani. Peripheral blood smear examination was done to examine the *Theileria annulata* piroplasm’s and lymph node aspiration cytology done to examine Koch’s blue body with Giemsa’s staining method.

Out of 61 animals screened for theileriosis, 21 found positive for theileriosis. Out of 21 positive cases 18 animals selected and were divided randomly and equally into three groups for therapeutic study.

Selection of Cattle and therapeutic protocol

The screening of suspected animals was done by using peripheral blood smear examination The cattle were selected on the basis of clinical signs like fever, lymph node swollen, nasal discharge, pale mucous membrane and ticks present on body.

Eighteen positive cases were randomly divided into three groups (n = 6). Group I treated with Buparvaquone @ 2.5mg/kg body weight, Group II treated with *P. harmala* @ 7 mg/kg body weight and Group III treated with *P. harmala* @ 7mg/kg body weight along with Oxytetracycline @ 20mg/kg body weight. All animals also received supportive therapy for 3 days. Supportive therapy – Fluid therapy consisting of Inj DNS, Inj D5 were given as peer need, Haematinics (Iron dextran), Chlorpheniramine maleate @0.5 mg/kg, Antipyretics (Inj Meloxicam 0.5 mg/kg) and B – complex were given for 3 days intramuscularly. Inj Buparvaquone used for the study was Inj Zokill-Strong (M/s Vet Mankind Pharma) and Inj Oxytetracycline used for the treatment was Inj Steclin (M/s Zenex AH Pharma).

Clinical and Physiological parameters studied

Cattle from all the groups were subjected following clinical and physiological parameters on day '0' (before treatment) and on 7th and 14th day post treatment. Each animal was examined for the, Rectal temperature (°F), Heart Rate(no/min), Respiration rate(no/min), Colour of mucus membrane, General body condition and swollen lymph node.

Collection of blood samples

The blood samples were collected from theileriosis affected eighteen cattle for hematological and biochemical analysis. For hematological analysis, the blood was collected from jugular vein in a clean, sterile tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) at the rate of 2 mg/ml of blood. For diagnosis of theileriosis, blood smear was prepared from the blood drawn from ear tip at the height of temperature. Clear serum samples were carefully drawn and transferred to clean, dry sterilized serum collecting tubes. These tubes were kept at -20 ^{0}C. Blood samples from jugular vein were collected at day 0th, day 7th and 14th day for hemato-biochemical investigation.

Haematological Parameters

Blood samples were collected from all the cows under study from jugular vein with all aseptic precautions in sterile vials containing EDTA as an anticoagulant by using 16gauge hypodermic needle for haematological study.

Biochemical Parameters

For biochemical estimations approximately 5 ml of venous blood was collected in vacutainer. Serum thus separated was centrifuged at 2000 rpm for 10 min. and stored in oven dried glass vials under deep refrigeration at-20˚C until used for biochemical analysis. Biochemical parameters were estimated by using ready to use kits supplied by M/s Mauli Biopath enterprises, Yadav Complex, Station Road, Parbhani, Maharashtra. Estimation of total protein, serum albumin. serum globulin, AST, ALT serum creatinine and BUN were carried out in all the affected cattle on "0" day (Before treatment) and on 7th and 14th day post treatment by using automatic serum biochemistry analyser.

Blood Smear Examination

Preparation of blood smears

A drop of blood was taken on a clean grease free glass slide and thin blood smear was prepared. For identification of smear the animal number was marked on glass slides before fixing with methanol. Blood smears was dried and fixed with methanol for 2–5 min. Each blood smear was made in duplicate.

Staining and microscopic examination of blood smear

Blood smear fixed in methanol was stained with Giemsa's stain and examined for piroplasm in erythrocytes and Koch's blue bodies in lymphocytes as described by Soulsby (1982). The parasitized erythrocytes in stained blood smears were calculated by examining and classifying 400–500 erythrocytes in randomly selected microscopic fields under high power microscope (100x).

Lymph node smear examination (Fine needle aspiration cytology)

Enlarged lymph node was hold and pulled anteriorly. The needle was directly passed into centre of gland and lymph aspired with the help of syringe. If the lymph node was small, then 1–2 ml of normal saline was injected and after slight massage, the fluid was aspired. The aspirated fluid was then placed on slide and a smear was prepared. It was stain with giemsa's staining method and examined under high power microscope for presence of Koch's blue bodies in lymphocytes or organisms of *Theileria.*

Determination of Parasitaemia

Parasitaemia is generally expressed as percentage infected red blood cells. It would seem that all *Theileria* species have defined parasitaemia ranges (Young et al., 1986; Ueti et al., 2012).

The percentage of parasitaemia was determined by counting the infected RBCs in the proportion of total RBCs in Giemsa-stained blood smears under light microscope and at least 15 to 20 microscopic fields was count. Following formula was used for determination of percentage of parasitaemia.

$$\text{Parasitaemia (\%)} = \frac{\text{Total number of Infected Erythrocyte}}{\text{Total number of Erythrocytes}} \times 100$$

Preparation of Extract of Peganum harmala seeds.

Extraction of Peganam harmala

An extract of *P. harmala* was prepared from the seeds of the plant according to the method described by Manske and holmes (1952).

The dried seeds of *P. harmala* (Fig. 2) was crushed in mixer Acetic acid extract was prepared by dissolving 500 gm crushed seeds into the 45 ml of acetic acid (99%) in 1455 ml of distilled water which is three times to the weight of seeds. The extract kept at room temperature with occasional shaking. When the seeds swelled as they could absorb the liquid and form thick dough which was pressed after three days.

The pressed seeds again treated as above with twice, their mass of dilute acetic acid. after nine days the extract was filtered by using sterile Whatman filter paper no–1 the resultant extract was kept in Ultraviolet light for 12 hr. Then the extract was concentrated and evaporated to dryness at 70°C in hot air oven for three days, after total

evaporation the dried powder (Fig. 3) stored in air tight container. The10gm of dried powder of extract dissolved in 90 ml distilled water and filter by 0.2 µm syringe filter.

Preparation of inj Peganum harmala

The 10 gm of dried powder of extract of *Peganum harmala* was dissolved in 90 ml distilled water (Derakhshanfar and Mirzaei 2008) and filtered by 0.2 µm syringe filter which is store in sterile container and then injected to infected animal Inj *Peganum harmala* @7 mg/kg body weight (Fig. 4) intramuscularly for 3 days, this protocol was followed for Group II and Group III animals.

Statistical analysis

The analysis of data of the present research work was statistically analysed with various statistical tools such as percentages and Completely Randomised Design (CRD) using Web Agri Stats Package (WASP 2.0) and values were expressed as Mean ± SE.

Results

Theileriosis affected cattle showed the various clinical symptoms such as presence of tick over the body (100%), prescapular lymph node enlargement (90.47%), high temperature > 103˚F (85.71%), pale conjunctival mucous membrane (80.95%), anorexia (66.66%), nasal discharge (57.14), dull and depressed behaviour (52.38%), weakness (49.85%), decreased milk production (28.57%), emaciation (9.52%) and sternal recumbency (9.52%). Clinical signs and symptoms noticed in theileriosis affected cattle is depicted in Fig. 13.

By blood smear examination intraerythrocytic piroplasm (Fig. 6) and by Fine needle aspiration cytology Koch's blue bodies (Fig. 7) seen in smear which was examined under microscope.

In all the theileriosis affected groups increase in rectal temperature, heart rate, respiration rate was observed on day '0' (before treatment) Theileriosis affected cattle showed significant decrease in haematological parameters such as in Hb, PCV, TEC, TLC and neutrophil count and non-significant increase in absolute lymphocyte, absolute monocyte, absolute eosinophil count. Theileriosis affected cattle showed significant decrease in biochemical parameters such as decrease in total protein and albumin levels whereas significant increase in AST, ALT, creatinine and BUN levels. Similar observations were reported by Siddiqui et al., (2017), Khawale et al., (2019).

Group I treated with buparvaquone showed significant improvement in clinical parameters such as rectal temperature and heart rate but non-significant improvement was observed in respiratory rate. Group II treated with *P. harmala* showed significant improvement in rectal temperature, heart rate and respiratory rate. This suggests a broader therapeutic effect, improving both cardiovascular and respiratory functions. Group III treated with *P. harmala* and oxytetracycline showed significant improvement in rectal temperature, heart rate and highly significant improvement in respiratory rate. This suggests that the treatment was particularly effective in improving respiratory function, along with rectal temperature and heart rate which proves the synergistic effect of *P. harmala* and oxytetracycline. The results of clinical parameters seen in theileriosis affected cattle in Group I, Group, II and Group III at 0 (before treatment),7th and 14th day (after treatment) are depicted in Table 1

Table 1
Results of clinical parameters of theileriosis positive cattle treated with Buparvaquone, *Peganum harmala, Peganum harmala* with oxytetracycline at different interval

Parameters	Group I			Group II			Group III		
Day	**0**	**7th**	**14th**	**0**	**7th**	**14th**	**0**	**7th**	**14th**
Rectal temperature	104.08^{c} ± 0.35	101.38^{b} ± 0.34	100.43^{a} ± 0.21	103.98^{b} ± 0.35	100.41^{a} ± 0.33	99.9^{a} ± 0.21	103.56^{b} ± 0.80	100.76^{a} ± 0.52	100.5^{a} ± 0.34
Heart rate	73.33^{c} ± 1.820	64.17^{b} ± 1.30	57.5^{a} ± 1.23	72.83^{b} ± 1.973	62.17^{a} ± 0.946	60.83^{a} ± 0.477	77.50^{b} ± 2.717	68.00^{ab} ± 3.606	64.17^{a} ± 3.710
Respiration rate	34.67 ± 1.60	30.50 ± 2.34	28.67 ± 2.15	38.67^{b} ± 3.00	33.00^{ab} ± 1.86	28.00^{a} ± 1.91	42.83^{c} ± 2.136	36.33^{b} ± 1.783	29.17^{a} ± 1.40

Similar superscript indicates a non-significant difference

Group I affected cattle were treated with buparvaquone @ 2.5 mg/kg body weight. Following treatment, improvements were observed in hematological parameters, including a significant increase in the total erythrocyte count and absolute neutrophil count. However, non-significant improvements, were seen in haemoglobin levels, packed cell volume, total leukocyte count, absolute counts of lymphocytes, monocytes and eosinophils.

In Group II treated cattle the most notable and statistically significant changes were observed in the hematological parameters such as absolute neutrophil and monocyte counts. While there was a slight, non-significant improvement in haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, absolute lymphocyte count, and absolute eosinophil count. These results indicate a positive therapeutic effect of the treatment regimen on the cattle's blood profile. In Group III treated cattle with *Peganum harmala* @ 7 mg/kg body weight, intramuscularly along with Oxytetracycline @ 20 mg/kg body weight daily for three days, along with supportive therapy, showed significant improvements in hematological parameters following treatment. Notable enhancements were observed in packed cell volume, total erythrocyte count, and absolute neutrophil count. Additionally, there was a non-significant improvement in haemoglobin levels, total leukocyte count, and absolute counts of lymphocytes, monocytes and eosinophils. The results seen in theileriosis affected cattle for haematological parameters at different interval at 0,7th and 14th day are depicted in Table 2

Table 2
Results of Haematological parameters of theileriosis positive cattle treated with Buparvaquone, *Peganum harmala, Peganum harmala* with oxytetracycline at different interval

Parameters	Group I			Group II			Group III		
Day	**0**	**7th**	**14th**	**0**	**7th**	**14th**	**0**	**7th**	**14th**
Hb (g/dl)	7.13 ± 0.87	7.60 ± 0.76	7.83 ± 0.76	6.88 ± 0.51	7.67 ± 0.61	8.11 ± 0.56	6.03 ± 0.44	6.28 ± 0.45	6.56 ± 0.498
PCV %	20.28 ± 1.95	22.90 ± 1.84	26.76 ± 2.69	22.82 ± 1.29	24.77 ± 1.17	26.87 ± 1.28	19.65^{a} ± 0.41	20.62^{a} ± 0.98	23.85^{b} ± 1.18
TEC	4.42^{a} ± 0.50	5.28^{ab} ± 0.47	6.29^{b} ± 0.48	4.70 ± 0.28	5.43 ± 0.39	5.91 ± 0.38	3.93^{a} ± 0.15	4.60^{a} ± 0.25	5.41^{b} ± 0.25
TLC	6.08 ± 0.54	6.33 ± 0.54	6.51 ± 0.52	7.03 ± 0.69	7.28 ± 0.69	7.35 ± 0.59	7.05 ± 1.09	7.41 ± 1.07	7.90 ± 1.06
Absolute lymphocyte	4.42 ± 0.45	4.28 ± 0.37	4.10 ± 0.36	5.05 ± 0.52	4.75 ± 0.46	4.54 ± 0.36	4.95 ± 0.69	4.78 ± 0.69	4.89 ± 0.66
Absolute neutrophil	1.27^{a} ± 0.12	1.66^{ab} ± 0.14	2.07^{b} ± 0.17	1.44^{a} ± 0.14	2.15^{b} ± 0.22	2.49^{b} ± 0.22	1.43^{a} ± 0.28	2.15^{ab} ± 0.28	2.64^{b} ± 0.33
Absolute monocyte	0.36 ± 0.04	0.26 ± 0.03	0.23 ± 0.04	0.36^{a} ± 0.05	0.27^{b} ± 0.03	0.16^{b} ± 0.019	0.46 ± 0.12	0.34 ± 0.08	0.23 ± 0.05
Absolute eiosinophil	0.13 ± 0.02	0.12 ± 0.01	0.07 ± 0.02	0.16 ± 0.02	0.10 ± 0.02	0.12 ± 0.02	0.16 ± 0.01	0.13 ± 0.03	0.12 ± 0.03
Absolute basophil	0.017 ± 0.07	0.00 ± 0.00	0.017 ± 0.07	0.017 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.017 ± 0.007
Similar superscript indicates a non-significant difference									

Group I showed improvements in biochemical parameters following a single dose of Inj. Buparvaquone Significant improvement were observed in globulin, ALT, and creatinine levels, while albumin, AST, and BUN demonstrated non-significant improvements, highlighting the effectiveness of the treatment. these findings are in accordance with findings of Siddiqui et al.,2017, Khawale et al.,2019.

Cattle infected with Theileriosis in Group II demonstrated notable improvements in Clinical signs such as nasal discharge before and after treatment (Fig. 7 and Fig. 8), Shedding of ticks after treatment (Fig. 9 and Fig. 10), biochemical parameters following treatment with Inj. *P. harmala* at a dosage of 7 mg/kg body weight, administered intramuscularly for three days there were significant improvement in total protein, AST, BUN, and creatinine level. While non-significant improvements were observed in albumin, globulin, and ALT levels, indicating a positive therapeutic response.

Cattle infected with Theileriosis in Group III showed significant improvements in biochemical parameters following treatment with Inj. *P. harmala* along with Inj. Oxytetracycline, Significant improvements were observed in rectal temperature (Fig. 11and Fig. 12) ALT, BUN, and creatinine levels. Whereas, non-significant improvements were noted in total protein, albumin, globulin, and AST indicating a favorable therapeutic response. The results seen in theileriosis affected cattle for biochemical parameters at different interval at 0,7th and 14th day are depicted in Table 3

Table 3
Results of Biochemical parameters of theileriosis positive cattle treated with Buparvaquone, *Peganum harmala, Peganum harmala* with oxytetracycline at different interval.

Parameters	**Group I**			**Group II**			**Group III**		
Day	**0**	**7th**	**14th**	**0**	**7th**	**14th**	**0**	**7th**	**14th**
Total protein	5.20^{a} ± 0.28	6.10^{b} ± 0.08	6.73^{c} ± 0.18	5.59^{a} ± 0.32	5.99^{a} ± 0.21	6.85^{b} ± 0.19	5.90 ± 0.44	6.88 ± 0.45	7.49 ± 0.52
Albumin (g/dl)	2.29 ± 0.345	2.61 ± 0.12	3.10 ± 0.23	2.97 ± 0.39	3.26 ± 0.37	3.96 ± 0.31	2.56 ± 0.33	2.93 ± 0.48	3.30 ± 0.44
Globulin(g/dl)	3.01^{a} ± 0.19	3.50^{b} ± 0.12	3.83^{Bb} ± 0.10	2.61 ± 0.26	2.73 ± 0.25	2.89^{A} ± 0.24	3.05 ± 0.26	3.61 ± 0.21	3.85 ± 0.21
AST	123.83 ± 13.84	118.73 ± 15.58	102.03 ± 7.26	119.43^{b} ± 4.46	94.65^{a} ± 5.32	84.16^{a} ± 2.39	122.55 ± 2.95	104.29 ± 11.93	93.55 ± 7.84
ALT	38.20^{b} ± 4.25	28.18^{ab} ± 4.44	23.25^{a} ± 2.90	35.68 ± 2.07	31.11 ± 2.27	30.33 ± 1.18	49.08^{b} ± 5.14	39.56^{ab} ± 3.48	28.21^{a} ± 3.36
Creatinine (mg/dl)	2.70^{b} ± 0.29	2.04^{ab} ± 0.21	1.50^{a} ± 0.14	2.96^{c} ± 0.22	2.06^{b} ± 0.16	1.25^{a} ± 0.10	3.05^{b} ± 0.24	1.72^{a} ± 0.24	1.25^{a} ± 0.14
BUN (mg/dl)	31.67 ± 2.83	44.94 ± 5.15	34.06 ± 3.10	35.85^{b} ± 3.84	27.21^{ab} ± 2.44	23.30^{a} ± 2.18	39.45^{b} ± 4.86	32.93^{ab} ± 4.09	24.51^{a} ± 1.76
Similar superscript indicates a non-significant difference									

In group I highly significant (P < 0.01) reduction in parasitemia was seen at 7th day (post treatment) it indicates buparvaquone is 100% effective against theileriosis. While in group II cattle treated with *P. harmala* the highly significant reduction (P < 0.01) observed at 7th and 14th day as compared to day '0' (before treatment) but mild level of parasitemia was seen at 14th day in two cattle. which indicated the low efficacy of *P. harmala* than buparvaquone. In group III the level of parasitemia showed highly significant reduction (P < 0.01) at 7th and 14th day and mild level of parasitemia was seen at 14th day in one cattle. (Fig. 11) it indicates *P. harmala* and Oxytetracycline have synergistic effect on theileriosis but the efficacy is low than buparvaquone. The results seen in theileriosis affected cattle for Parasitemia at different interval at 0,7th and 14th day are depicted in Fig. 14

In group I all the cattle were recovered and there was no parasitemia observed at 14th day of post treatment. Hence, the buparvaquone showed 100% efficacy against *Theileria* parasite. In group II out of six cattle four cattle showed complete recovery whereas two cattle showed mild parasitemia upto 14th day after treatment. the efficacy of group II was 66.66%. However, in group III out of six cattle five cattle showed complete recovery whereas one 1 cattle showed mild parasitemia at 7th and 14th day, the efficacy of group III cattle was 83.33% as compared to group I. The efficacy of therapeutic treatment is depicted in Table 4.

Table 4
Mean of Parasitaemia of Theileriosis affected cattle in Buparvaquone, P. harmala and P. harmala with oxytetracycline treated group before treatment and at different intervals after treatment

Parameters	Group I			Group II			Group III		
Day	0	7th	14th	0	7th	14th	0	7th	14th
Parasitemia (%)	27.16^{b} ± 4.99	0.00^{a} ± 0.00	0.00^{a} ± 0.00	28.13^{b} ± 5.08	3.42^{a} ± 2.30	1.80^{a} ± 1.40	21.80^{b} ± 5.89	2.00^{a} ± 2.00	1.10^{a} ± 1.10
Similar superscript indicates a non-significant difference									

Discussion

The findings suggest that, *P. harmala* @ 7 mg/kg body weight, does not induce harmful or toxic effects in cattle, and by extension, may be considered safe for use in cattle when administered intramuscularly at the recommended dosage. Rezzagui et al. (2020) reported that *Peganum harmala* extract is fairly toxic with LD50 500 mg/kg body weight, and the use of this extract is safe at doses ≤ 100 mg/kg. *P. harmala* could be a potential source of bioactive compounds with antioxidant and antiproliferative potential.

In Group I, II and III treated cattle the mean values for rectal temperature were showed highly significant (P < 0.01) reduction at 7th day and 14th day of post treatment as compared to day '0' before initiation of treatment. El-Rifaei et al., (1980) and Arif et al., (2022) stated that the compounds extracted from *P. harmala* plant have a variety of medicinal properties, including anti-inflammatory effects, antipyretic and antiparasitic effects. *P. harmala* seeds exhibits notable anti-inflammatory activity, accompanied by a mild peripheral analgesic effect. This may be primarily attributed to its high content of linoleic acid, γ-tocopherol, and polyphenols, as well as its significant antioxidant properties. (Khadhar et al., 2017).

In Buparvaquone treated (Group I) Cattle infected with theileriosis in group I showed improvement in various clinical and physiological, haematological and biochemical parameters, including significant improvement in rectal temperature heart rate, respiratory rate, total erythrocyte count, absolute neutrophil, globulin, ALT and creatinine

and non-significant improvement in haemoglobin, packed cell volume, total leukocyte count, absolute lymphocyte, absolute monocyte, absolute eosinophil, albumin, AST and BUN following treatment with single dose of Inj Buparvaquone @ 2.5mg/kg body weight intramuscularly with supportive therapy for three days. these findings are in accordance with findings of Siddiqui et al., 2017, Sakhare et al., 2018 Khawale et al., 2019.

In this study, the improvement observed with treatment may be attributed to recovery from the disease state, supported by both specific and adjunctive therapies. In the *P. harmala* treated group, a significant improvement in heart rate was noted.

P. harmala having antiinflammory, antiplasmodial, antibacterial and monoamine oxidases (MAO) inhibitor effect. Kumar et al., (2015), Ahmed et al., (2021) and Zhu et al (2022) Various pharmacological studies have also demonstrated that the extract of *P. harmala* and its primary active alkaloids—harmine, harmaline, harman, and harmalol-exert diverse cardiovascular effects. These include bradycardia, a reduction in systemic arterial blood pressure and total peripheral vascular resistance, as well as an increase in pulse pressure, peak aortic flow, and cardiac contractile force. The improvement in respiratory rate in group II and group III affected cattle was due to response to the specific therapy and supportive therapy. Moloudizargari., (2013) pointed out the bronchodilator and expectorant effect of *P. harmala.*

In group II and III the mean values of parasitaemia reduces significantly. Results for *P. harmala* were showed similar with Derakhshanfar and Mirzaei (2008), Moghaddan *et al*., (2011) Farah et al., (2014) and Saleem et al., (2014). reported that harmaline was found in the highest concentration in the extract, followed by harmine. Numerous studies have demonstrated that various protozoan infections exhibit different levels of susceptibility to *P. harmala* extract. Alkaloid compounds from the plant effectively highlight the diversity of antiprotozoal agents present in *P. harmala*.

Therefore, the combined use of *Peganum harmala* along with Oxytetracycline in Group III is likely the more effective therapy, as it resulted in both statistically significant improvements in more hematological parameters and a broader overall response as compared to buparvaquone.

Combination of *Peganum harmala* and Oxytetracycline can be considered a viable alternative treatment for Theileriosis, offering strong efficacy with significant clinical improvements and fewer concerns about kidney function compared to Buparvaquone. Additionally, it is more cost-effective than Buparvaquone, making it a practical option for widespread use, especially in resource-limited settings. While Buparvaquone remains the most effective treatment in terms of parasitemia reduction, the combination therapy in Group III provides a safer and more economical alternative, with good therapeutic outcomes at a lower cost. However, further research, including clinical trials, is needed to confirm its safety and efficacy for these uses.

Conclusions

Based on present study it can be concluded that, *P. harmala* along with oxytetracycline shows better efficacy and it can be used as an alternative medicine against theileriosis in cattle.

Declarations

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Authors' contributions

Conceptualization – Siddiqui M.F.M.F., Data curation & analysis –, S. Sajid Ali, Lokhande SA, Shaikh SR,; Methodology- Siddiqui MFMF, Lokhande SA, Sakhare MP, Chigure GM, Jadhav N.D; Validation & Investigation- Lokhande SA, Siddiqui M.F.M.F.; Writing, review & editing – Lokhande SA Siddiqui MFMF, Sakhare M.P. ; SD Chepte, Shaikh S.R. Ahire P.C.

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Data availability

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Code availability

Not applicable

Ethics approval

All the experimental procedures and the study protocol have been approved by the Institutional Committee and Board of Studies of College of Veterinary & Animal Sciences, Parbhani, Approved as on clinical cases (Resolution No: IAEC 146/25) and the experiments were performed in accordance with the internationally accepted standard ethical guidelines for animal use and care.

Conflict of interest

No any conflict of interest relevant to this article was reported from all authors or any other source.

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Figures

Figure 1

Plant *P. harmala*

Figure 2

Seeds of *P. harmala*

Figure 3

Dried extract

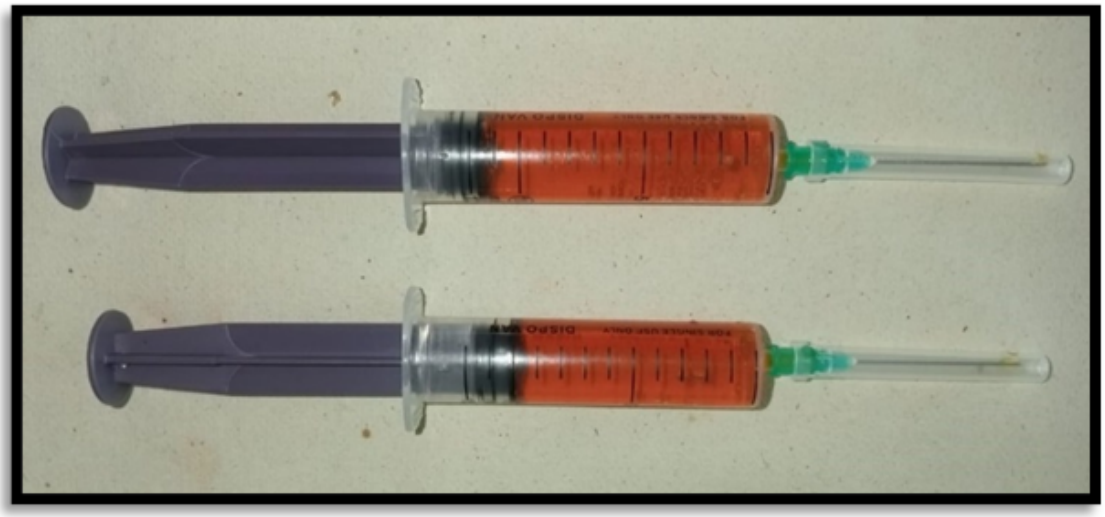

Figure 4

Inj *P. harmala*

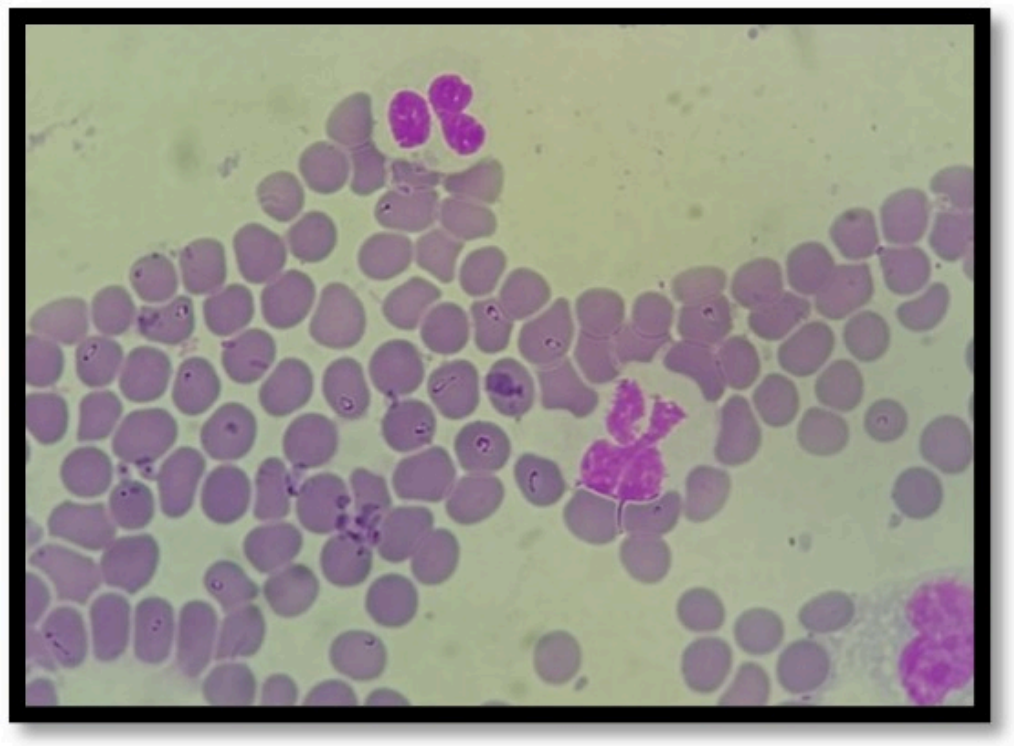

Figure 5

Intraerythrocytic piroplasm of *T. annulata*

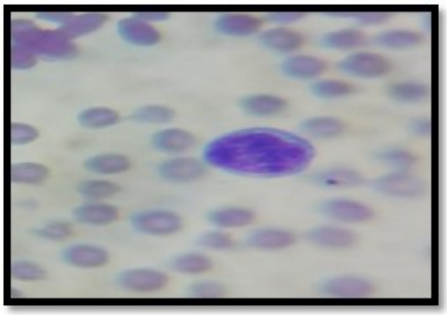

Figure 6

Kochs blue bodies in lymphocyte

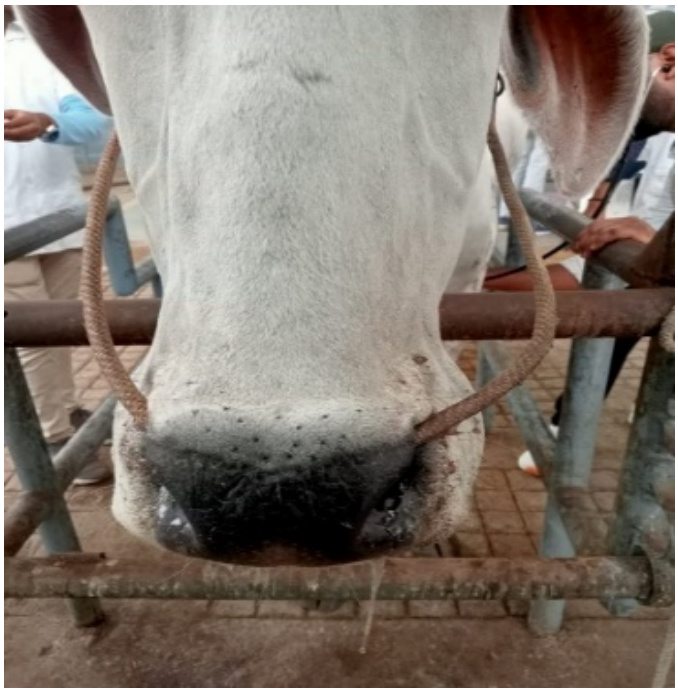

Figure 7

Nasal discharge in theileriosis affected bull. (Before treatment)

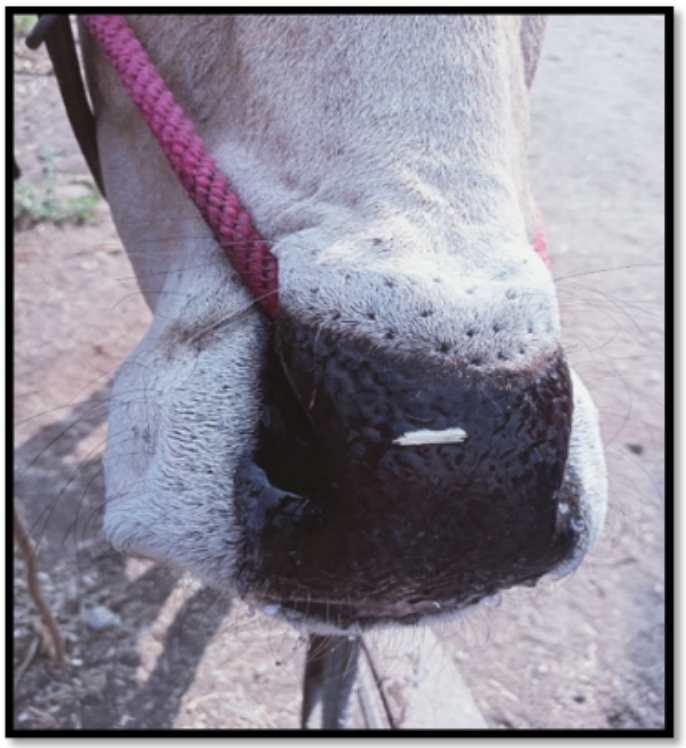

Figure 8

Improvement seen after treatment With Inj *P. harmala*

Figure 9

Presences of ticks in theileriosis affected cattle

Figure 10

After treatment with *P. harmala*

Figure 11

High rectal temperature (Before treatment)

Figure 12

Reduction in body temperature after treating with Inj *P. harmala* and Oxytetracycline.

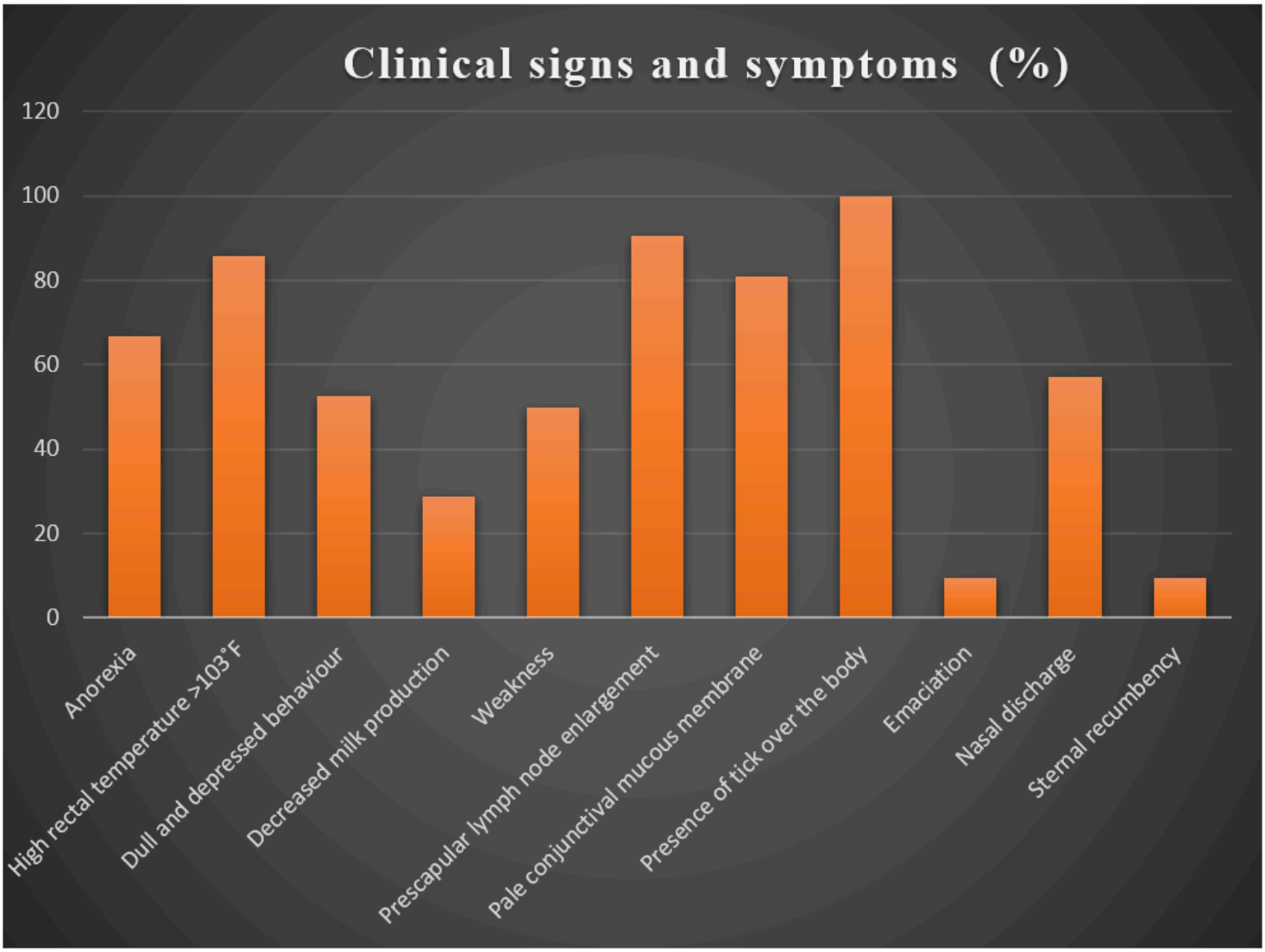

Figure 13

Clinical signs and symptoms noticed in theileriosis affected cattle

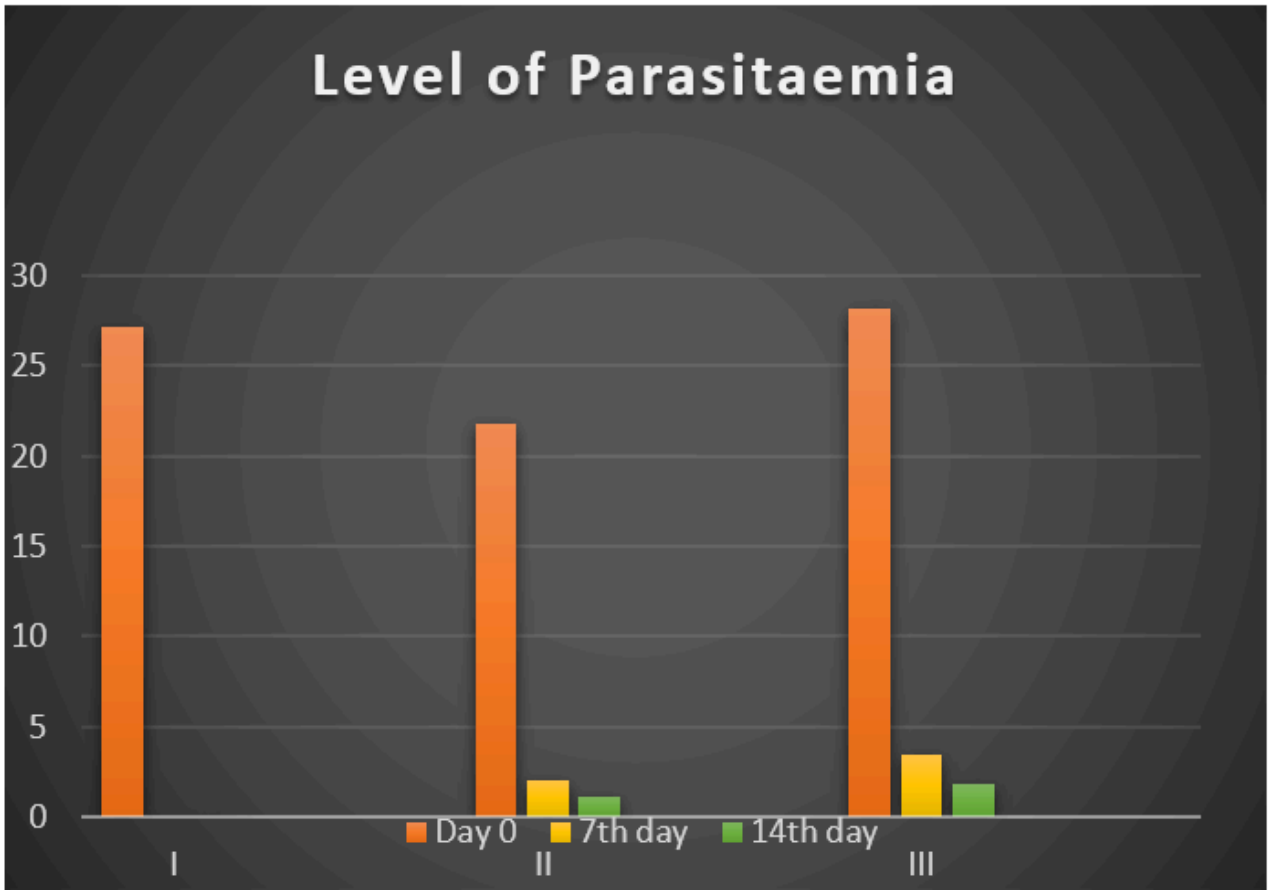

Figure 14

Mean of Parasitaemia of Theileriosis affected cattle in Buparvaquone, *P. harmala and P. harmala* with oxytetracycline treated group before treatment and at different intervals after treatment.

Figure 15

Dull, depressed, recumbent body condition (Before treatment)

Figure 16

Improvement seen after treating with Inj *P. harmala* and oxytetracycline

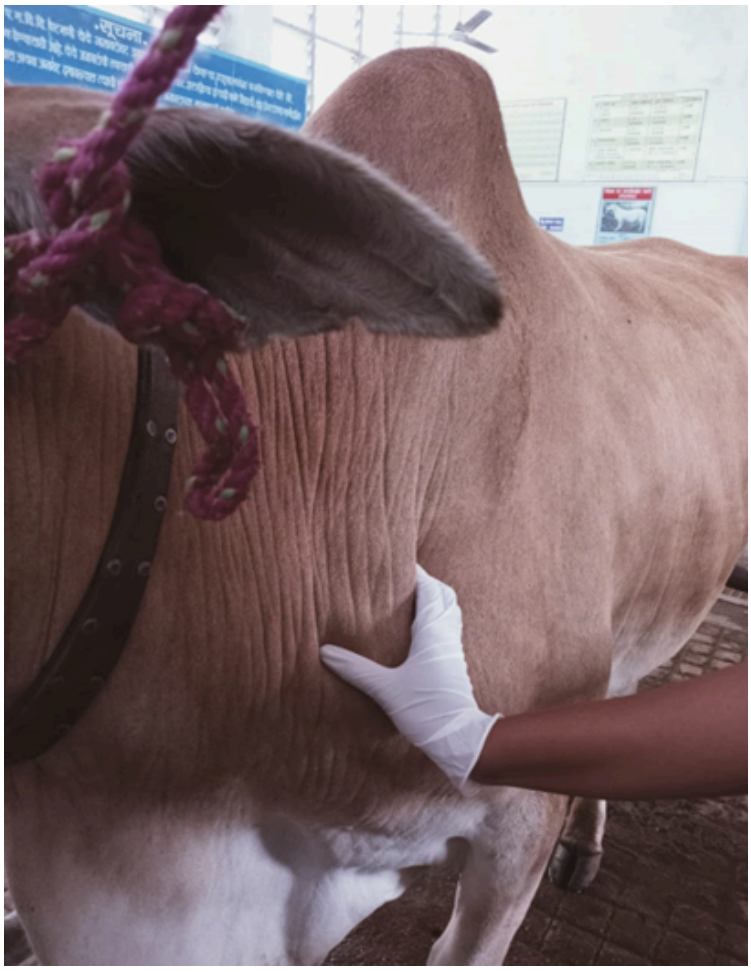

Figure 17

Swollen lymph node (Before treatment)

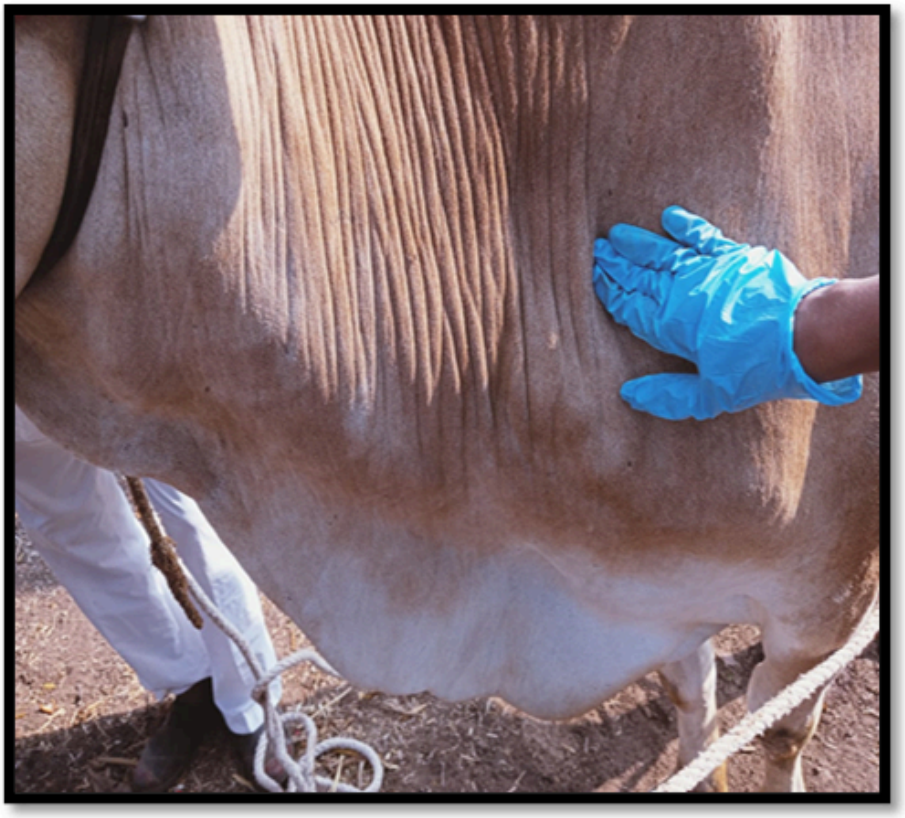

Figure 18

Reduced swelling after treating with Inj *P. harmala*