Evaluation of phyto-therapeutic efficacy of Artemisia annua and Calotropis procera against bovine tropical theileriosis

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Abstract

Theileriosis is one of the most important ticks borne hemo-protozoan diseases of cattle causing huge economic losses. The current investigation was undertaken to evaluate therapeutic efficacy and impact on hematological indices and biochemical markers of *Artemisia annua* L. and *Calotropis procera* (Aiton) Dryand for the novel treatment of sub-clinical bovine tropical theileriosis. A total 18 sub-clinical theileriosis positive cows were selected and divided randomly into three groups (n = 6). Group I treated with *A. annua* plant extract @ 100 mg/kg BW PO for six days, group II treated with *A. annua* plant crude powder @ 1000 mg/kg PO for six days and group III received *C. procera* flower crude powder @ 100 mg/kg body weight orally 8 doses on alternate days. Clinical study revealed slight increase in body temperature, respiratory and heart rates while hematological analysis revealed decrease in haemoglobin, PCV, platelet count, TEC, TLC and neutrophil and slight increase in percentages of lymphocyte, monocyte, eosinophil and basophil whereas blood biochemical parameters revealed decrease in total serum protein and albumin levels and increase in serum AST, ALT, alkaline phosphatase, creatinine and BUN levels in all sub-clinical theileriosis affected cows. The group I showed 100% efficacy with improvement in clinical parameters and significant improvement in altered PCV, TEC, TLC, monocyte, eosinophil, serum protein and creatinine at 21st day post treatment. However, the drug showed adverse effect on liver and kidney function. The group II and III showed 83.67% recovery rate with improvement in clinical parameters and significant improvement in altered platelet count, TLC, neutrophil, monocyte, serum protein, BUN and creatinine levels at 21st day post treatment. It is concluded that *Artemisia annua* L. plant showed 100% efficacy and *Calotropis procera* (Aiton) Dryand flowers showed promising results for treatment of sub-clinical bovine tropical theileriosis in cattle without any side effects.

Introduction

Theileriosis is a life-threatening tick-borne protozoan disease affecting livestock, especially cattle, prevalent in tropical and subtropical regions, including India due to hot-humid highly favorable climatic conditions for the development and survival of vector ticks (OIE, 2020). It is caused by hemoprotozoan parasites of the genus *Theileria* spp. of Apicomplexa group and *Theileria annulata* is considered to be the most pathogenic species responsible for bovine tropical theileriosis which is commonly transmitted through *Ixodid* tick of genus *Hyalomma* spp.

The *Theileria* spp. life cycle in the mammalian host begins with sporozoites inoculation *via* a tick bite; these sporozoites invade leukocytes during differentiation into schizonts by schizogony which subsequently releases merozoites invading red blood cells leading to clinical manifestations such as fever, lymphadenopathy, anemia, leucopenia, and jaundice resulting from immune-mediated destruction of infected cells and systemic inflammation (Constable et al., 2017). Khawale et al., (2019) have reported a 22.38% overall occurrence of clinical theileriosis while Sachan et al., (2025) found an 11% prevalence of theileriosis. Theileriosis is one of the major diseases of crossbred cattle due to severe economic losses of around 498.7 million dollars per annum due to decreased animal production, and increased susceptibility to secondary bacterial infections and mortalities (Kumar et al., 2018).

Buparvaquone, second-generation hydroxynaphthoquinone, is an anti-protozoal drug commonly use for management of bovine tropical theileriosis around the world. However, recent studies have highlighted concerns regarding the possible emergence of resistance to drug buparvaquone in various regions. The first *in-vivo* evidence of resistance to drug buparvaquone in *T. annulata* in Tunisia was recorded by Mhadhbi et al., (2010) who further demonstrated that when the methionine amino acid at position 128 in the *T. annulata* cyto-b gene is replaced by isoleucine during the M128I mutation, the parasite becomes resistant to the drug buparvaquone (Mhadhbi et al., 2015). Researchers reported non-synonymous point mutation in genes cyto-b (Q at 130–148 and Q at 253–262 regions) and TaPIN1 (A53P and A53T) culpable for anti-protozoal drug resistance from Tunisia, Turkey, Egypt, Sudan, Iran, Pakistan, China, and Germany with concerning the *T. annulata* (Rashid et al., 2024). These findings underscore the importance of monitoring buparvaquone efficacy and exploring alternative therapeutic strategies to manage theileriosis effectively. Medicinal plants are now attracting interest for use of ethno-veterinary therapy (Siddiqui *et al*., 2024), but the literature on herbal treatment of theileriosis is scanty. Considering the expensive cost of chemical therapeutics against theileriosis, there is enormous scope for future research on anti-theilerial medicinal plants.

Artemisia annua L. is an effective anti-malarial drug used currently in over 50 countries as the first line of treatment against chloroquine-resistant malaria. *A. annua* is traditionally used for the prevention and treatment of many infectious diseases especially malaria (Septembre-Malaterre et al., 2020). The whole *A. annua* plant has antipyretic, anti-helminthic, anti-spasmodic, anti-septic, and anti-malarial properties. Artemisinin, a sesquiterpene lactone, is the active ingredient of this plant containing pharmacological endoperoxide moiety (Wani et al., 2022). Whole plant extract of *A. annua* contains phytochemicals *viz*. tannins, steroids, saponin, phenols, glycosides, flavonoids and alkaloids. The *A. annua* hydro-ethanolic extract administered orally upto 5,000 mg/kg body weight showed no lethal or toxic reactions in Swiss albino mice (Siddiqui et al., 2018). An *in-vitro* study showed *A. annua* extract having promising results against *T. annulata* infection (Girisgin et al., 2023).

Calotropis procera (Aiton) Dryand is a shrub belongs to the Apocynaceae family, having pink violet to white colored flowers. These flowers of *C. procera* contain alkaloids, cardenolides, flavonoids, glycoside, phenols, saponins, steroids, sugars, tannins and terpenoids and which are attributed to hepatoprotective, antioxidant, inflammatory, antimicrobial, and anti-malarial medicinal properties. It is traditionally used for fever, leprosy, eczema, diarrhea, dysentery and jaundice (Batool et al., 2020; Siddiqui et al., 2017). Acute and sub-acute toxicity studies of ethanolic extract of *C. procera* flower in Swiss albino mice showed no-observed-adverse-effect-level @ more than 2000 mg/kg/day (Kumar et al., 2022; Siddiqui et al., 2017). Also, *C. procera* showed therapeutic efficacy against canine babesiosis with complete recovery and restoration of haemto-biochemical parameters to normalcy (Sandeep and Vadari, 2023).

Considering the above facts, the economic losses faced by farming community due to the lack of cost-effective treatment for theileriosis and the efficacy of *A. annua* and *C. procera* against hemoprotozoan diseases, especially theileriosis, the current research work was designed to evaluate the therapeutic efficacy of *A. annua* and *C. procera* in bovine Theileriosis.

Materials and methods

The present research study was meticulously conducted in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Veterinary Clinical Complex, PGIVAS, MAFSU, Akola. Research experiments were performed at Livestock Instructional Farm, Department of Animal Husbandry and Dairy Science, Dr. Punjabrao Deshmukh Krishi Vidhyapeeth, Akola. The plant identification was carried out at the Department of Botany, Shri Shivaji Science College, Akola. The study was undertaken following approval from the Institutional Animal Ethics Committee (IAEC) of PGIVAS, MAFSU, Akola, for practicing standard ethical guidelines during experiments.

Collection and processing of plant material

The mature flowers of *C. procera* were collected between January - February from the campus of the Post Graduate Institute of Veterinary and Animal Science, Akola and the whole plant *A. annua* plant was procured from a local Ayurvedic medicine shop and taxonomically authenticated from expert taxonomist at the Department of Botany, Shri Shivaji Science College, Akola, Maharashtra, under accession number 24402. The collected plant materials were shade-dried considering their phytochemical integrity and finely pulverized using a mechanical pulverizer to obtain a uniform powder. Freshly prepared powder samples were then subjected to cold hydro-ethanolic extraction and the obtained extracts were subsequently utilized for further experimental study.

Selection of animals

The screening of the cows was conducted for sub-clinical theileriosis according to clinical manifestations and blood smear examination, with molecular confirmation performed using PCR technique. A total of 18 cows PCR-positive positive for sub-clinical theileriosis were selected and randomly assigned into three treatment groups, each comprising six cows.

Treatment groups

Cows in group I were administered *A. annua* plant extract @ 100 mg/kg BW PO daily for 6 days. In group II, cows were treated with *A. annua* plant crude powder @ 1,000 mg/kg BW PO daily for 6 days. While cows, in group III, received C. *procera* flower crude powder @ 100 mg/kg body weight orally in 8 doses on alternate days. In group IV, six normal healthy cows negative for theileriosis were kept as normal healthy control.

Blood and serum sample collection

For hematological analysis, blood samples were aseptically collected from the jugular vein of selected cows using a 16-gauge hypodermic needle and transferred into sterile vials containing EDTA as an anticoagulant. For biochemical assessments, approximately 10 mL of venous blood was drawn into vacutainers. The separated serum was centrifuged at 2,000 RPM for 10 minutes and stored in oven-dried glass vials at -20°C until further use for biochemical evaluation.

Parameters studied

Cows from all the groups were subjected to the clinical examination and haemato-biochemical analysis on the '0' day (before treatment) and on the 7th and 21st day (after treatment). Clinical examination included body temperature, heart rate and respiratory rate. A comprehensive hematological evaluation was performed by quantifying Hb, PCV, TLC, TEC, thrombocyte count and differential leukogram. Biochemical parameters included total protein, albumin, globulin, AST, ALT, ALP, creatinine, BUN and total bilirubin. Blood analysis was performed using an automated hematoanalyzer, while biochemical parameters were estimated using ready-to-use diagnostic kits from M/s Span Diagnostics Pvt. Ltd., Surat, India, on a Semi-Auto Analyzer (Autochem-2011).

Statistical analysis

The data obtained in this study for various parameters were statistically analyzed using a Two-Way Factorial Design with the Web-Based Agricultural Statistics Software Package (WASP-2.0), developed by the ICAR Research Complex Goa, India (Version 2.0). Results were presented as mean ± standard error (SE).

Results

The results of clinical examination and hemato-biochemical analysis of sub-clinical Theileriosis-positive cows treated with *A. annua* and *C. procera* at different intervals are presented in Table 1-5.

As per results, before treatment on day 0, theileriosis-affected cows in all groups (I, II, III) exhibited slightly elevated body temperature, respiratory rate, and heart rate compared to the normal healthy control group (IV). These findings of increased values of clinical parameters in theileriosis positive cows are in agreement with Aktas *et al.*, (2023) and Sakhare *et al.*, (2018). The statistical analysis revealed a significant ($P<0.05$) decrease in mean body temperature but non-significant variations in respiratory rate and heart rate of theileriosis affected cows in all groups at 21st day post-treatment towards normalcy at par with normal healthy control group (table 1). In normal healthy control group (IV), the mean body temperature, respiratory rate and heart rate were within the normal range and remained constant with minor fluctuations during the entire experimental period.

The values of Hb, PCV, TEC, TLC and platelet count were slightly lower in sub-clinical theileriosis-infected cows in all groups before initiation of treatment and as compared to the normal healthy control group (IV). These findings are in agreement with earlier reports of Yadav *et al.*, (2024) and Khawale *et al.*, (2019) who stated anemia in theileriosis.

Cows in group I showed non-significant but apparent improvement in Hb, PCV, TEC and TLC values while significant ($P<0.05$) variations in platelet count were recorded after treatment. In group II, theileriosis-infected cows showed non-significant but apparent improvement in PCV, TEC and platelet count whereas a

significant (P<0.05) increase observed in Hb and TLC values at 21st day post treatment. While, cows in group III showed non-significant but apparent improvement in Hb, PCV, TEC and a significant (P<0.05) increase was observed in platelet count and TLC values at 21st day post-treatment. In normal healthy control group (IV), Hb, PCV, TEC and TLC values did not differ significantly and remained almost constant with marginal fluctuation (Table 2).

The value of neutrophil was slightly lower while the values of lymphocyte, monocyte and eosinophil were slightly higher in all sub clinical theileriosis affected cows in all groups before initiation of treatment. Basophil count was found zero in groups I, II and III. Similar observations were also documented by Yadav *et al.*, (2024).However, few researchers has found neutrophilia and lymphocytopenia in Theileria infected cattle (Aktas *et al.*, 2023). The theileriosis affected cows in groups I and III showed non-significant improvements in neutrophil, lymphocyte and monocyte but significant (P<0.05) decrease in eosinophil recorded at 21st day post treatment. In group II, theileriosis affected cows showed significant improvements in neutrophil and eosinophil but non-significant improvements in lymphocyte and monocyte recorded after treatment. In normal healthy control group (IV), DLC values did not differ significantly and remained almost constant with marginal fluctuation (Table 3).

The levels of serum total protein, albumin and globulin were slightly lower while AST, ALT and ALP levels were higher in sub-clinical theileriosis affected cows in all groups before initiation of treatment (Yadav *et al.*, 2024). The results showed significant (P<0.05) improvement in total protein level and non-significant but apparent improvement in albumin, globulin and AST levels in all treatment groups (Aktas *et al.*, 2023). The ALT and ALP values improved significantly (P<0.05) in group I and non-significantly improved in groups I and III on 21st day after treatment as compared to pre treatment level. In normal healthy control group (IV), AST, ALT and ALP values did not differ significantly and remained almost constant with marginal fluctuation (Table 4).

The mean serum creatinine, BUN and bilirubin levels were higher in all sub clinical theileriosis affected cows in all groups before initiation of treatment. These findings are in accordance with the findings of Yadav *et al.*, (2024).The serum creatinine level was significantly (P<0.05) decreased while BUN and bilirubin levels showed non-significant variations in all treatment groups at on 7th and 21st day post treatment compared to the pre treatment level of corresponding groups (Singh *et al.*, 2024). In normal healthy control group (IV), creatinine, BUN and bilirubin values did not differ significantly and remained almost constant with marginal fluctuation (Table 5).

Table 6 presents the comparative efficacy of *A. annua* and *C. procera* treatments in sub-clinical theileriosis-positive cows. The results indicate a 100% recovery rate in Group I signifying its superior therapeutic potential. While, Groups II and III were demonstrated recovery rates of 83.67% at 21st day post-treatment.

Discussion

In the present research, a total of 18 cows diagnosed with sub-clinical theileriosis, confirmed through blood smear examination and PCR, were selected. These cows were then randomly assigned into three treatment groups, with six animals (n = 6) in each group.

A slight increase in body temperature, respiratory rate, and heart rate was observed in cows affected by sub-clinical theileriosis. The rise in body temperature may be attributed to inflammation triggered by *Theileria* parasites, which induce TNF-α production and disrupt physiological functions (Aktas et al., 2023). Additionally, the increased heart and respiratory rates in affected animals could be linked to cytokines such as TNF-α, IL-1, and IL-6, which are produced by infected mononuclear cells (Kacchawa *et al.*, 2016).

Also, there were slightly decrease in the hematological parameters viz. Hb, PCV, TEC, TLC and platelet count in sub-clinical theileriosis infected cows. The decline in Hb concentration and PCV % might be due to the destruction of infected erythrocytes by macrophages in the spleen during parasite multiplication, ultimately leading to anemia (Ganguly et al., 2015). Constable et al. (2017) documented that damage is caused by both scizonts in lymphocyte and piroplasm in erythrocyte. Furthermore, oxidative stress weakens the erythrocyte membrane, increasing its susceptibility to hemolysis or macrophage-mediated clearance. Activated complement proteins may also play a role in RBC destruction by marking them for immune clearance (Saravanan et al., 2017). Differential leukocyte count (DLC) analysis indicated neutropenia, lymphocytosis, monocytosis, and eosinophilia in *Theileria*-infected cows. Similar findings were reported by Ganguly et al. (2015), who observed a significant increase in lymphocyte, eosinophil, and monocyte counts, accompanied by a decline in neutrophil levels in infected animals. The rise in lymphocytes is likely a defense response, driven by their proliferation in lymphoid organs to counter the protozoan infection (Abdel-Hamied et al., 2020).

The sub-clinical theileriosis infected cows exhibited marginal reductions in serum albumin, globulin and total protein levels accompanied by a slight elevation in AST, ALT and ALP enzymatic activities. These biochemical perturbations may be attributed to impaired hepatic synthetic capacity, likely compounded by reduced feed intake and compromised liver function. The causative agent, *T. annulata*, exhibits distinct cytopathic effects on hepatocytes. This direct cellular damage contributes to hepatic dysfunction, disrupting normal protein metabolism and enzymatic homeostasis. The observed reductions in serum protein fractions reflect a diminished capacity for protein synthesis, while the elevated liver enzymes indicate hepatocellular damage (Goud *et al.*, 2021). Hepatic injury in bovine tropical theileriosis arises from a complex, multifactorial pathogenesis encompassing both direct and indirect mechanisms. Firstly, direct hepatocellular injury occurs due to the release of toxic metabolites by the *Theileria* protozoan, resulting in metabolic dysregulation and oxidative stress. Secondly, indirect damage is mediated by profound inflammatory responses. These responses are triggered by the sequestration of parasitized and structurally compromised erythrocytes and lymphocytes within hepatic sinusoids, leading to immune-mediated hepatocellular injury. Finally, severe anemia, a hallmark of theileriosis, induces ischemic-hypoxic insult, culminating in degenerative and apoptotic changes in hepatocytes. This cascade of events progressively impairs hepatic function, exacerbating the overall disease pathology (Abubakar et al., 2019). The observed increase of AST level in serum reflects hepatocellular injury, characterized by extensive coagulative necrosis, disruption of hepatic trabeculae, and peripherally accentuated lymphocytic infiltration. These histopathological features suggest severe hepatobiliary dysfunction driven by a complex interplay of sustained inflammatory insult, leading to hepatocyte apoptosis and subsequent compromise of sinusoidal integrity and function (Modi et al., 2015).

The sub-clinical theileriosis infected cows were having higher levels of creatinine, BUN and bilirubin values. The elevation in BUN values were likely a functional impairment of both hepatic and glomerular tissues as a secondary to inflammatory changes affecting cellular integrity and physiological processes of hepatic and renal systems (Sarma et al., 2016). This observed changes in BUN levels likely attributed to a cascade initiated by *Theileria* protozoa derived toxic metabolism, which induce hepatocellular injury, subsequently impairing hepatic function, and ultimately disrupting renal enzyme activity (Hamed et al., 2016).

Group I treated with *A. annua* plant extract showed 100% efficacy as all the animals recovered and none of the animal was found positive for theileriosis by PCR as well as blood smear examination on 21st day post treatment. The cows in group II and III, treated with crude extracts of *A. annua* plant and *C. procera* respectively, 5 cows showed complete recovery, indicating the recovery rate of 83.67%. All the animals showed improvement in clinical parameters and significant improvement in altered PCV, TEC, TLC, monocyte %, eosinophil %, total serum protein and serum creatinine. The improvement brought by the treatment could be due to the elimination of both piroplasmic and lymphocytic stages of the protozoan parasite from both blood and lymphnodes (Al-Hosaryet al., 2015).

The study demonstrates that treatment with *A. annua* had no adverse effects on renal and hepatic functions at the administered dose, confirming its safety profile. Furthermore, the treatment effectively eliminated the *Theileria* parasite while significantly improving clinical and hemato-biochemical parameters within the experimental period. Notably, there is a lack of prior literature on the therapeutic efficacy of *A. annua* and its derivatives against theileriosis, making this investigation a pioneering effort in the field. This research represents the first documented evaluation of the hydro-ethanolic extract and crude powder of *A. annua* against sub-clinical theileriosis in cattle, offering promising insights into its potential as an alternative therapeutic agent. Given the increasing concerns over drug resistance in conventional anti-protozoal treatments, further studies exploring the pharmacokinetics, optimal dosing, and long-term efficacy of *A. annua* are warranted to establish its role in the treatment of bovine theileriosis.

The administration of *A. annua* extract and crude powder demonstrated promising efficacy in promoting recovery in *Theileria*-infected cattle without any adverse effects. This therapeutic potential may be attributed to the plant's diverse biological activities, including its well-documented anti-malarial and antioxidant properties. The active compounds in *A. annua* likely play a crucial role in combating the parasite and mitigating oxidative stress. However, the precise mechanism of action against *Theileria* remains unclear, warranting further investigation to elucidate its pharmacological pathways and therapeutic potential. However, it could be attributed to the anti-malarial action due to active principle, artemisinines, which involves the formation of heme-mediated decomposition of the endoperoxidase bond to produce carbon-centered free radicals which mediates eradication of the parasites species (Ho et al., 2013). To explore more about the various active principles in *A. annua* and their mode of action against *Theileria* parasite is further promising field of study in future. To validate the present findings in large group of cattle and other species and to study the efficacy of *A. annua* against other protozoal and parasitic disease further research experiments are suggested.

The results indicate that *C. procera* exhibits therapeutic efficacy against sub-clinical theileriosis in cattle, potentially due to the active compounds present in its flowers. Flavonoids and phenolic compounds, known for their antioxidant properties, may contribute to the plant's effectiveness in mitigating disease effects. However, the exact mechanism by which *C. procera* promotes recovery remains unclear, necessitating further research to elucidate its mode of action and potential role as an alternative treatment for bovine theileriosis. However, it could help in eliminating the *Theileria* parasite by their anti-oxidant, anti-protozoal and immunomodulatory properties through their active principles as reported by several workers (Durrani et al., 2009).

The comparative therapeutic efficacy results demonstrated that *A. annua* plant extract was 100% effective in eliminating *Theileria* parasites. The crude extracts of *A. annua* and *C. procera* exhibited remarkable efficacy in promoting recovery from sub-clinical theileriosis. Additionally, the herb was found to be safe and non-toxic throughout the experiment. Notably, the hydro-ethanolic extract of *A. annua* proved to be superior in enhancing recovery, making it a promising alternative to other treatments for sub-clinical theileriosis in cattle.

Conclusions

It is concluded that *Artemisia annua* plant showed 100% efficacy and *Calotropis procera* (Aiton) Dryand showed promising results for treatment of sub-clinical theileriosis in cattle without any side effects.

Declarations

Acknowledgement

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Authors' contributions

Siddiqui M.F.M.F.: Conceptualization, Methodology, Investigation, Resources, Data Curation, Review, Writing. **S.P. Waghmare**: Conceptualization, Supervision, Resources, Writing - review and Editing. **S. Sajid Ali**: Software, Proof reading, Statistical analysis. **S.D. Chepte**: Resources, Editing. **S.R. Shaikh**: Writing – original draft, Visualization, Proof reading.

Ethics Approval

The research study protocol have been approved by the Institutional Animal Ethics Committee (IAEC), Post Graduate Institute of Veterinary and Animal Sciences, Akola and Board of Studies of University and the experiment was performed in accordance with internationally accepted standard ethical

guidelines for animal use and care.

Conflict of Interest

All authors declare that they have no conflicts of financial or personal interests for this manuscript

Availability of data and material

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

Code availability

Not applicable

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Tables

Table 1: The results of clinical parameters of sub-clinical Theileriosis positive cows treated with *A. annua* and *C. procera* at different intervals

Treatment	Parameter	Body temperature (⁰F)			Respiratory Rate (per minute)			Heart rate (per minute)		
	Interval	BT	AT		BT	AT		BT	AT	
	Group	'0' day	7th day	21st day	'0' day	7th day	21st day	'0' day	7th day	21st day
A. annua plant extract @ 100 mg/kg BW PO	I	102.50[b]± 0.60	101.63[a] ± 0.28	101.57[a] ± 0.34	19.67 ± 0.84	20.00 ± 0.73	18.83 ± 0.87	62.67± 3.71	61.67± 3.05	61.33± 3.11
A. annua plant crude powder @ 1000 mg/kg BW PO	II	103.25b± 0.55	101.68[a]± 0.39	101.62[a]± 0.49	19.83 ± 1.90	19.83 ± 0.95	20.67 ± 1.85	58.67± 2.38	58.17± 1.47	58.17± 1.62
C. procera plant crude powder @ 100 mg/kg BW PO	III	102.57 [b] ± 0.51	100.85 [a] ± 0.17	100.87 [a] ± 0.41	20.67 ± 1.31	21.17 ± 1.11	20.17 ± 1.01	61.33± 2.23	62.17± 2.60	60.50± 2.57
Healthy Control	IV	101.12± 0.55	102.00± 0.56	101.5± 0.38	19.17 ± 1.17	19.50 ± 0.76	19.00 ± 1.15	56.33± 1.94	58.17± 2.76	59.17± 3.42

- Similar superscript (a, b, c) indicates non-significant differences between groups

Table 2: The results of hematological parameters of sub-clinical Theileriosis positive cows treated with *A. annua* and *C. procera* at different intervals

	Hb (g/dl)			PCV (%)			TEC (x10^6/ µL)			TLC (x10^3/µL)			Platelet Count (x10^3/µL)		
Interval	BT	**AT**		**BT**	**AT**		**BT**	**AT**		**BT**	**AT**		**BT**	**AT**	
Group	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th da
I	7.42± 0.63	7.83± 0.36	8.35± 0.38	27.89 ± 2.23	30.87 ± 2.41	30.95 ± 3.69	6.31 ± 0.36	6.69 ± 0.37	7.47 ± 0.18	8.67± 0.84	9.85± 1.07	10.41± 1.15	211.17^{a} ± 23.81	323.50^{ab} ± 49.37	514.33 ± 106.63
II	6.93^{a} ± 0.43	7.87^{ab} ± 0.82	8.27^{b} ± 0.86	26.96 ± 1.36	27.96 ± 1.73	30.07 ± 4.02	5.50 ± 0.50	6.01± 0.18	7.06± 0.20	8.13^{a} ± 0.68	9.89^{ab} ± 0.62	10.88^{b} ± 0.60	134.33 ± 21.92	206.17 ± 55.54	340.83 ± 130.56
III	6.80± 0.29	7.35± 0.53	8.03± 0.43	27.37 ± 1.52	31.45 ± 2.65	31.90 ± 4.21	6.18± 0.45	6.68± 0.25	7.83± 0.27	8.16^{a} ± 0.65	8.92^{a}± 0.52	10.44^{b}± 0.86	139.67^{a} ± 19.34	225.67^{ab} ± 61.51	445.83 ± 139.33
IV	9.35± 0.47	9.37± 0.34	9.52± 0.39	32.79 ± 1.68	33.61 ± 3.00	32.99 ± 4.98	8.19± 0.73	8.28± 0.82	8.65 ± 0.66	10.31± 0.79	10.39± 0.64	10.51 ± 1.08	368.17 ± 72.20	371.17 ± 82.38	382.33 ± 98.8

- Similar superscript (a, b, c) indicates non-significant differences between groups

Table 3: The results of DLC parameters of sub-clinical Theileriosis positive cows treated with *A. annua* and *C. procera* at different intervals

DLC	Neutrophil (%)			Lymphocyte (%)			Monocyte (%)			Eosinophil (%)			Basophil (%)		
Interval	**BT**	**AT**		**BT**	**AT**		**BT**	**AT**		**BT**	**AT**		**BT**	**AT**	
Group	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day
I	32.66± 4.77	37.83± 5.45	40.17± 3.65	62.50± 5.19	58.17± 3.41	56.83± 3.21	1.67 ± 0.33	1.67 ± 0.33	2.00 ± 0.52	3.17^{b} ± 0.40	2.33^{b} ± 0.33	1.00^{a} ± 0.36	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
II	30.00^{a} ± 3.26	39.33^{ab} ± 5.06	45.00^{b} ± 2.41	63.83± 3.79	55.67± 5.47	52.83± 2.70	2.00 ± 0.36	1.33 ± 0.21	1.50 ± 0.34	4.17^{b} ± 0.79	3.33^{b} ± 0.49	0.67^{a} ± 0.49	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
III	31.17 ± 5.94	36.17± 3.41	37.84± 3.22	63.67± 5.86	59.67± 3.71	56.50± 3.40	2.00 ± 0.36	1.33 ± 0.21	1.33 ± 0.33	3.17 ± 0.48	2.83 ± 0.48	1.67 ± 0.33	0.00^{a} ± 0.00	0.00^{a} ± 0.00	0.00^{a} ± 0.00
IV	31.67 ± 3.11	32.34± 4.91	35.00± 6.27	64.67± 2.91	64.00± 4.97	62.67± 6.15	2.00 ± 0.45	1.50 ± 0.22	1.67 ± 0.33	1.66 ± 0.67	1.50 ± 0.62	0.67 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

- Similar superscript (a, b, c) indicates non-significant differences between groups

Table 4: The results of LFT parameters of sub-clinical Theileriosis positive cows treated with *A. annua* and *C. procera* at different intervals

LFT	Total Protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)			AST (IU/L)			ALT (IU/L)			ALP (IU/L)	
Interval	BT	AT		BT	AT		BT	AT		BT	AT		BT	AT		BT	AT
Group	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th
I	8.50 a ± 0.51	9.55 ab ± 0.43	9.85 b ± 0.53	3.92 ± 0.23	4.48 ± 0.20	4.68 ± 0.42	4.58 ± 0.60	5.07 ± 0.43	5.10 ± 0.67	108.88 ± 14.73	92.35 ± 13.68	81.30 ± 9.35	51.38a ± 6.84	41.60 ab ± 5.38	28.30 b ± 6.20	115.30a ± 11.39	84. ± 1
II	8.12 a ± 0.16	9.20 ab ± 0.33	8.87 b ± 0.22	3.58 ± 0.21	4.62 ± 0.23	4.50 ± 0.20	4.53 ± 0.17	4.58 ± 0.46	4.37 ± 0.24	121.68 ± 13.76	98.97 ± 12.75	92.72 ± 12.86	21.23 ± 5.63	13.45 ± 2.21	16.72 ± 3.07	100.22 ± 17.62	88. ± 9
III	7.42 ± 0.49	8.9 ± 1.28	9.53 ± 1.17	3.15 ± 0.21	4.28 ± 0.45	4.28 ± 0.23	4.27 ± 0.39	4.62 ± 1.10	5.25 ± 1.03	105.45 ± 17.14	80.97 ± 10.82	92.32 ± 9.49	38.05 ± 6.80	28.12 ± 9.42	23.75 ± 7.71	92.25 ± 16.53	82. ± 9
IV	8.72 ± 0.48	8.9 ± 0.52	9.07 ± 0.73	4.25 ± 0.50	4.48 ± 0.51	4.25 ± 0.34	4.47 ± 0.41	4.42 ± 0.51	4.82 ± 0.59	77.27 ± 8.78	77.73 ± 8.53	78.78 ± 6.15	16.03 ± 3.54	17.67 ± 2.31	17.92 ± 4.26	76.30 ± 10.77	77. ± 1

- Similar superscript (a, b, c) indicates non-significant differences between groups

Table 5: The results of KFT parameters of sub-clinical Theileriosis positive cows treated with *A. annua* and *C. procera* at different intervals

KFT	Serum Creatinine (mg/dl)			BUN (mg/dl)			Total Bilirubin (mg/dl)		
Interval	BT	AT		BT	AT		BT	AT	
Group	'0' day	7th day	21th day	'0' day	7th day	21th day	'0' day	7th day	21th day
I	3.68 b ± 0.53	2.18 a ± 0.19	1.50 a ± 0.22	30.57 ± 6.48	23.13 ± 4.42	22.42 ± 5.45	0.38 ± 0.13	0.37 ± 0.10	0.28 ± 0.06
II	3.40 b ± 0.28	1.92 a ± 0.20	1.32 a ± 0.25	23.45 ± 3.25	20.08 ± 5.51	19.13 ± 1.93	0.37 ± 0.12	0.35 ± 0.08	0.20 ± 0.05
III	3.42 b ± 0.27	1.63 a ± 0.07	1.33 a ± 0.22	33.70 ± 7.16	24.98 ± 4.07	22.40 ± 6.27	0.42 ± 0.12	0.28 ± 0.06	0.18 ± 0.03
IV	1.55 ± 0.46	1.88 ± 0.34	1.65 ± 0.46	21.82 ± 3.18	22.73 ± 3.28	23.38 ± 2.41	0.28 ± 0.09	0.23 ± 0.04	0.20 ± 0.04

- Similar superscript (a, b, c) indicates non-significant differences between groups

Table 6: The results of comparative efficacy of *A. annua* and *C. procera* treatments against sub-clinical theileriosis positive cows at different intervals

Group	Treatment	No. of cases treated (sub clinical theileriosis)	No. of cases cured	Recovery (%)
I	*A. annua* plant extract @ 100 mg/kg BW PO	6	6	100.00
II	*A. annua* plant crude powder @ 1000 mg/kg BW PO	6	5	83.67
III	*C. procera* plant crude powder @ 100 mg/kg BW PO	6	5	83.67