Acaricidal potency of polyherbal spray against *Rhipicephalus microplus* and *Hyalomma anatolicum* infestation in cattle

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Abstract Acaricidal potential of polyherbal spray (*Andropogon citrates*, *Cymbopogon citratus*, *Ocimum sanctum*, *Pinus longifoia*, *Calotropis procera*, *Datura stramonium*, *Aegle marmelos*, *Ricinus communis*, *Azadirachta indica*, *Allium sativum*, *Carica papaya*, *Annona squamosa* and *Pongamia glabra*) was assessed against tick infestation in cattle on the basis of measurement of tick count, complete blood count and plasma glucose, total protein, albumin and globulin before treatment and 21 days after treatment. Single application of polyherbal spray over body of 20 randomly selected tick infested cattle revealed significant reduction in mean tick count starting from 3 days post treatment till 21 days post treatment. Highly significant ($P < 0.01$) increase in total erythrocyte count and packed cell volume was observed in treated cows 21 days after application of spray compared to pre-treatment values indicating the reduction in blood loss due to heavy tick infestation before treatment. Plasma biochemical parameters revealed no significant changes in pre-treatment and post treatment values. The results of present study imply the clinical and haematological improvement in tick infested cattle treated with polyherbal spray and it could be potential product for use in livestock as acaricide.

Keywords *Rhipicephalus microplus* · *Hyalomma anatolicum* · Acaricide · Polyherbal spray · Cattle · Treatment

Introduction

Tick infestation produce heavy economic losses to livestock industry due to blood sucking and irritation due to bites. Direct effects like loss of blood and reduction in body weight gain are responsible for decreased productivity of livestock across the world. Some of the tick species are also responsible for transmission of hemoprotezoan and rickettsial diseases like theileriosis, babesiosis, anaplasmosis and ehrlichiosis in animals (Estrada-Pena et al. 2004; Holdsworth et al. 2006; Salih et al. 2015). So tick infestation presents a serious threat to animal farmers across the world (Sumbria et al. 2016). Control and eradication of tick infestation in livestock is a major managerial challenge to reduce the economic losses to dairy farmers from direct and indirect losses (Davey et al. 2010; Rao et al. 2014). Organophosphates, pyrethroids, formamidine and macrocyclic lactones are the major classes of acaricidal chemicals used for tick control since long time (Davey et al. 2010; Rao et al. 2014). Intensive and indiscriminate use of acaricides for control of ectoparasite has culminated in emergence of resistance among different strains of tick populations (Bianchi et al. 2003; Pegram et al. 2000).

To overcome the pressure of resistant strains as well as to reduce the side effects of chemical acaricides, there is need to investigate the efficacy of alternative and eco-friendly products against tick infestation. Few novel preparations of herbal base have been tried against tick

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infestation since last decade. Among the extracts of *Azadirachta indica*, *Prunus persica*, *Mangifera indica* and *Psidium guajava*, the extracts of *A. indica* revealed very high level of efficacy (80%) against *Boophilus microplus* ticks during in vitro studies (Srivastava et al. 2008). Out of eight medicinal plants screened for in vitro acaricidal properties, extracts of seeds of *Annona squamosa* revealed highest efficacy (70.8%) against IVRI isolates of *B. microplus* ticks (Magadum et al. 2009). So taking into consideration the limitations of chemical acaricidal drugs, the present study was undertaken with objectives to evaluate the acaricidal potential of polyherbal spray for the control of tick infestation in cattle and to assess the potential of spray as an alternative to chemical acaricides.

Materials and methods

Twenty cases of tick infestation in cattle irrespective of age, sex and breed of indigenous cattle were randomly selected for the study. The ticks of *B. microplus* (*Rhipicephalus microplus*) and *Hyalomma anatolicum* species were identified in selected cattle.

Single application of polyherbal spray (Clear Ticks Spray provided by Rakesh Pharmaceuticals, Kalol, Gandhinagar, Gujarat, India) containing *Andropogon citrates* (3%), *Cymbopogon citratus* (3%), *Ocimum sanctum* (2%), *Pinus longifoia* (10%) and 1% each of *Calotropis procera*, *Datura stramonium*, *Aegle marmelos*, *Ricinus communis*, *A. indica*, *Allium sativum*, *Carica papaya*, *A. squamosa* and *Pongamia glabra* was applied all over the body of study animals after measurement of tick count. Acaricidal potency of the spray was evaluated on the basis of reduction in tick count compared to pre-treatment counts. Tick counts were measured from seven heavily infested areas of body viz., ear pinna, inguinal region, under tail, back region, wither, dewlap and neck and expressed as mean tick count. Measurements of tick count were performed before application of spray as well as 3, 7 and 21 days after application of spray. Clinical signs like pruritus, scratching, rubbing, alopecia were recorded in tick infested cattle before treatment and 21 days post treatment. Blood samples were collected before treatment and 21 days post polyherbal spray treatment as per standard procedure and analysed for Hb, PCV, TEC, TLC, DLC and platelet count on fully automated haemoanalyzer (Diatron- Abacus Junior 3.11). Plasma samples collected before treatment and 21 days post treatment were analysed for glucose, total proteins, albumin and globulin on semi-automated biochemistry analyser (CA 2005) using diagnostic kits (Austspan Diagnostics Ltd. Surat, India).

Data analysis

Tick counts were analyzed for mean, standard error and analysis of variance (ANOVA) at $P < 0.05$ significance level while haematology and biochemical parameters were analysed for mean, standard error and level of significance ($P < 0.05$ and $P < 0.01$) with paired 't' test by using SPSS software (Version: 16).

Results

A total of 20 cases of tick infestation in cattle were selected randomly for study. Various clinical observations in tick infested cattle were itching, alopecia, redness, rubbing and pruritus in areas with higher density of ticks. Skin coat was coarse with varying degree of hair fall. Treatment of cattle with polyherbal spray revealed significant reduction in clinical signs and hair fall from skin coat of affected cattle. Treated animals revealed improved lustre or shine on their hair coat on 21st days post treatment.

Acaricidal potency of polyherbal spray against tick infestation in cattle

Total tick count in cattle before treatment and over a period after treatment is given in Table 1. Total tick count in area of ear pinna, inguinal region, under tail, back region, wither, dewlap and neck was 180.9 ± 32.6 , 105.7 ± 12.8 , 47.0 ± 7.86 , 61.10 ± 12.26 , 119.45 ± 22.18 and 70.60 ± 11.86 respectively with mean total tick count of 94.97 ± 17.15 on day 0. Significant ($P < 0.05$) reduction in average mean total tick count from 94.97 ± 17.15 to 26.42 ± 5.97 , i.e. by 72.18% was observed on 3rd day of treatment which was further reduced up to 4.62 ± 1.60 (95.13%) on 7th day of treatment. However, average total tick count was increased from 4.62 ± 1.60 to 25.34 ± 3.12 on day 21 after treatment.

Application of polyherbal spray revealed no adverse reactions like irritation, itching, salivation in study animals. Application of polyherbal spray revealed reduction in the degree of fly infestation on the body of cattle and so reported to have additional fly repellent activity.

Effect of polyherbal spray on haematological parameters

Complete blood count was performed on blood samples collected before start of treatment and 21 days post treatment in study cattle. Highly significant ($P < 0.01$) increase in total erythrocyte count and hematocrit was observed on

Table 1 Mean $\pm$ SE values of total tick count in cattle infested with *Rhipicephalus microplus* and *Hyalomma anatolicum* over a period of treatment (n = 20)

S. No.	Area of tick infestation	Total tick count			
		Before treatment	After treatment		
		Day 0	Day 3	Day 7	Day 21
1.	Ear pinna	180.9 $\pm$ 32.6 ^a	55.6 $\pm$ 13.2 ^b (69.11)	12.6 $\pm$ 4.27 ^b (93.03)	34.0 $\pm$ 7.04 ^b (81.20)
2.	Inguinal region	105.7 $\pm$ 12.8 ^a	25.1 $\pm$ 5.09 ^b (76.25)	3.70 $\pm$ 5.17 ^c (96.49)	21.75 $\pm$ 10.4 ^{ab} (79.42)
3.	Under tail	47.0 $\pm$ 7.86 ^a	10.25 $\pm$ 3.15 ^{ab} (78.19)	0.7 $\pm$ 0.45 ^c (98.51)	14.4 $\pm$ 2.69 ^b (69.36)
4.	Back region	80 $\pm$ 16.31 ^a	26.75 $\pm$ 10.08 ^b (66.56)	5.35 $\pm$ 2.1 ^b (93.3)	31.55 $\pm$ 8.79 ^b (60.56)
5.	Wither	61.10 $\pm$ 12.26 ^a	16.25 $\pm$ 6.6 ^b (73.40)	2.55 $\pm$ 1.31 ^b (95.82)	21.0 $\pm$ 4.52 ^b (65.63)
6.	Dewlap	119.45 $\pm$ 22.18 ^a	37.35 $\pm$ 9.88 ^b (68.73)	7.0 $\pm$ 3.04 ^b (94.13)	35.65 $\pm$ 7.71 ^b (70.15)
7.	Neck	70.60 $\pm$ 11.86 ^a	13.60 $\pm$ 3.83 ^b (80.73)	0.45 $\pm$ 2.01 ^b (99.36)	19.05 $\pm$ 19.86 ^b (73.01)
Mean total tick count		94.97 $\pm$ 17.15 ^a	26.42 $\pm$ 5.97 ^b (72.18)	4.62 $\pm$ 1.60 ^b (95.13)	25.34 $\pm$ 3.12 ^b (73.31)

Values with different superscripts between columns differ significantly $P < 0.05$ (a, b, c)

Figures in parenthesis indicates percentage (%) reduction in tick count compared to '0' day values

Table 2 Mean $\pm$ SE values of haematological values before and after treatment in cattle with *Rhipicephalus microplus* and *Hyalomma anatolicum* infestation (n = 20)

S. No.	Parameter	Group I		't' value
		0 day	21st day	
1.	Total erythrocyte count ($\times 10^{12}/l$)	6.34 $\pm$ 0.23	7.71 $\pm$ 0.24	– 4.632**
2.	Haemoglobin (gm/dl)	10.43 $\pm$ 0.26	10.33 $\pm$ 0.30	0.322 ^{NS}
3.	Haematocrit (%)	27.10 $\pm$ 0.64	31.05 $\pm$ 0.75	– 4.284**
4.	Total leukocyte count ($\times 10^9/l$)	9.97 $\pm$ 0.68	9.90 $\pm$ 0.53	0.156 ^{NS}
5.	Granulocyte %	31.70 $\pm$ 2.00	36.52 $\pm$ 2.84	– 1.575 ^{NS}
6.	Monocytes %	1.40 $\pm$ 0.16	2.62 $\pm$ 0.53	– 2.451*
7.	Lymphocytes %	66.89 $\pm$ 2.03	60.85 $\pm$ 2.74	1.934 ^{NS}
8.	Platelets ($\times 10^9/l$)	203.1 $\pm$ 16.16	194.4 $\pm$ 12.15	0.573 ^{NS}

NS non-significant

* Significant ($P < 0.05$); ** highly significant ($P < 0.01$)

21 day post treatment in cattle treated with polyherbal spray compared to pre-treatment values (Table 2). Monocyte count also revealed significant ($P < 0.05$) increase 21 days post treatment compared to day 0 values. No significant difference was observed in pre and post-treatment values of total leukocyte count, haemoglobin, granulocyte, lymphocyte and platelet count in treated cattle.

Effect of polyherbal spray on biochemical parameters

No significant difference ($P < 0.05$) was observed in values of plasma total proteins, albumin, globulin and glucose in cattle infested with tick on day 0 and 21 days post treatment (Table 3).

Discussion

Ticks are blood sucking parasites of livestock which are also responsible for transmission of several dreadful haemoprotozoan diseases causing heavy financial losses to livestock industry. Female *R. microplus* tick ingests around 1 ml of blood from host during parasitic phase causing weight loss of around 1 g and loss of milk production by 8.9 ml in dairy cattle thereby reducing the milk yield and economic losses to the tune of 7.30 USD/head/year (Jonsson 2006; Jonsson et al. 1998). Taking into consideration the drawbacks of conventional chemical acaricides, herbal acaricides/phytopesticides are now emerging as a potential option for control of various ectoparasitic infestations (Habeeb 2010). Very few studies are published signifying

Table 3 Mean $\pm$ SE values of plasma biochemical parameters in cattle with *Rhipicephalus microplus* and *Hyalomma anatolicum* infestation (n = 20)

S. No.	Parameters	Group I		't' value
		0 day	21st day	
1.	Total proteins (gm/dl)	8.6 $\pm$ 0.35	8.2 $\pm$ 0.46	0.622 ^{NS}
2.	Albumin (gm/dl)	3.82 $\pm$ 0.09	3.91 $\pm$ 0.11	– 0.595 ^{NS}
3.	Globulin (gm/dl)	4.78 $\pm$ 0.37	4.30 $\pm$ 0.47	0.748 ^{NS}
4.	Glucose (mg/dl)	50.37 $\pm$ 1.37	52.25 $\pm$ 0.86	– 0.158 ^{NS}

NS non-significant

the in vivo efficacy of herbal acaricides for control of tick infestation in cattle. Promising acaricidal activity of *A. squamosa* seed oil comparable to pyrethrins and moderate efficacy with *A. indica* seed oil was observed against *B. microplus*, *H. anatolicum* and *R. haemaphysalis* both in vitro and in vivo (Kalakumar et al. 2000). Rapid knockdown effect of mixture of aqueous extracts of five herbs (*A. indica*, *M. indica*, *Polyalthina longifolia*, *A. squamosa*, *Ficus benghalensis*) in 32 s was observed against *R. microplus* ticks in vitro compared to extracts of same plants alone indicating additive/synergistic effect in mixture (Parate et al. 2014). Efficacy of Keetguard Liquid, a polyherbal ectoparasiticide against *R. microplus* tick infestation was evaluated in buffaloes at 1:20 dilution. Thrice application of preparation per week revealed 95% efficacy on 7th day post treatment which persisted up to 90% till 30 days post treatment (Tripathi et al. 2016).

In the present study polyherbal spray prepared from 13 different herbs might be having additive effect in significantly reducing tick burden as well as improving red blood cell parameters. Mean tick count was significantly reduced up to 95.14% on 7 days post treatment in study cattle while it showed increasing trend up to 73.31% on 21 days. Increase in tick count on day 21 might be due to short residual effect or entry of new ticks on body from breeding places in cattle sheds.

Use of polyherbal spray against *R. microplus* and *H. anatolicum* infestation in cattle proved effective in reducing the tick count and thereby significant improvement in haematological parameters demonstrating the acaricidal potential of these herbs as well as potential alternative ectoparasiticide for controlling tick infestations in cattle.

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Compliance with ethical standards

Conflict of interest Authors declare that there is no conflict of interest.

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