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Ectoparasiticidal Activity of Herbal Product Keetguard Liquid against Riphicephalus Microplus in Ruminants

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Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed and coordinated the study. Author MD assisted in study design. Author AT wrote the draft manuscript. Authors MS and KR designed, advised, evaluated the data and finalized the manuscript for publication. Authors PJ and SA performed and executed the study. All authors read and approved the final manuscript

Research Article

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ABSTRACT

Aims: The present study was aimed to determine the *in vivo* and *in vitro* efficacy of herbal ectoparasiticidal and fly repellant Keetguard liquid (*Supplied by M/s Ayurvet Ltd. Baddi, HP, India*) against *Rhipicephalus microplus*.
Study Design: 24 non descript cows with natural and heavy tick infestation were selected for the trial and divided into four groups of 6 animals Group I, Group II, Group III and Group IV. Each group of animals was kept as control for specific period of time and thereafter they were treated with 1:20 dilution of Keetguard liquid applied over the body coat by spray method once a day and twice weekly.
Place and Duration of Study: Study was conducted under field condition and was undertaken by Department of Veterinary Parasitology, Nagpur Veterinary College,

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Methodology: *In vivo* efficacy of Keetguard liquid on nymphal, larval and adult stages of ticks was determined by treating the cattles with keetguard liquid as per prescription (The dosage was standardized on the basis of preclinical trails). The number of each stage of ticks per square unit was counted. Observations were noted after 6, 12, 18, 24, 30, 48 and 72 hours *in vitro* efficacy of Keetguard liquid towards larval, nymphal and adult stages of ticks from cattle was evaluated by observing mortality of larval, nymphal stages of ticks and adult ticks in filter paper impregnated with keetguard at 24, 48, 72 and 96 hours of exposure to test solution.
Results: Keetguard significantly removed all nymphs in 10 hrs and the adult ticks in 24 hrs in *in vivo* study. A time interval of 24 hrs and 72 hrs was required for complete mortality of nymphal/larval stages of ticks and adult ticks respectively when tested *in vitro.* Besides the immediate effect on adult ticks, the egg-laying property of the survived ticks was also assessed. Keetguard significantly afflicted oviposition percentage in adult female ticks (5%) as compared to untreated females (92.5%). The average egg mass in both groups was 3.2 mg and 12.8 mg respectively. No reifestation was observed till 45 days after treatment signifying the potential residual activity of the Keetguard liquid.
Conclusion: The product was found highly efficacious against the ectoparasite infestation in animals as well safe and eco-friendly. It can effectively reduce the ectoparasites nuisance ultimately turning out to high productive performances in animals. Besides the immediate effect on adult ticks, the egg-laying property of the ticks was also reduced.

Keywords: Ectoparasiticide; ticks; keetguard; herbal.

1. INTRODUCTION

Arthropod parasites (ectoparasites) are a major cause of production losses in livestock throughout the world. Losses caused by these parasites result from exsanguination, toxicosis, arthropod-borne diseases, and reduced animal production and performance [1]. As per the estimate, the control cost of ticks and tick-borne diseases in India has been determined in the tune of about US$498.7 million per annum [2]. Treatment with various drugs to reduce or eliminate ectoparasites is therefore often required to maintain health and to prevent economic losses in animals. Overenthusiastic use of synthetic insecticides led to problems unforeseen at the time of their introduction [3]. Conventional synthetic ectoparasiticides proved to be deterrent for causing environmental contamination, potential harmful residues in food, toxicity to workers and consumers [4]. Meanwhile their intensive use has resulted in the selection of resistant ectoparasite population over a due course of time [4,5]. In order to have a sustainable remedial measure, alternative medicines such as herbal products are increasingly being explored for preventive and therapeutic applications in humans and animals. Plant extracts kill and repel pests, affect insect growth and development, have anti feeding, arresting effects, as well as antifungal, antiviral, and antibacterial properties against pathogens [6,7]. Herbal formulations were found to be effective against ticks resistant to pyrethroids as shown by Ghosh et al. [8]. Juliet et al. [9] observed ethanolic extract of the leaves of *Jatropha curcas* at low concentrations significantly inhibited the hatching of laid eggs and can be considered as a possible alternative for the control of ticks. Literature perusal depicts many reports where individual phytological extracts have been found to be effective against mosquitoes [10,11,12], ticks [13,14,15,16,17,18], mites [19] and as fly repellants [20,21,22]. India possesses 45,000 plant species of which 15,000–20,000 have proven medicinal value [23,24,25,26]. The review of

literature suggests most of the studies remained centered towards testing the acaricidal and ectoparasiticidal activity of individual herbal extracts instead of evaluating the combined potential of herbal formulations. Thus the present study was envisaged in order to regulate the practice of herbalism by assessing Keetguard liquid against tick infestation. The product comprises of oil of herbs viz. *Eucalypta globulus, Cedrus deodara*, *Pinus longifolia* & many others in a fixed concentration. The ectoparasiticidal activity of these plants oils is well established [27,28].

2. MATERIALS AND METHODS

In vivo and *in vitro* efficacy of herbal ectoparasiticidal and fly repellant Keetguard liquid (*Supplied by M/s Ayurvet Ltd. Baddi, HP, India)* against commonly occurring bovine ticks *Rhipicephalus microplus* (formerly *Boophilus microplus*) was evaluated. A field study was conducted in natural and heavily ectoparasite infested cattles from tropical conditions of Nagpur region. Trial was conducted as per standard methods of insecticide/Pesticide evaluation recommendation of World Health Organization (WHO) after approval from Committee for the purpose of control and supervision of experimentation on animals (CPCSEA). Resultant efficacy of the product was assessed on the basis of tick counts/intensity before and after treatment, protection period against reinfestation in treated group. The percentage efficacy was determined by comparing the treated group and control group using the following formula:

$$\%\, Efficacy = \frac{C - T}{C} \times 100$$

Where C = mean of the controlled group, T = mean of the treated group.

The reproductive potential such as preoviposition and oviposition periods, output of eggs, hatchability rate and number of larvae of ectoparasites recovered from the animals before and after treatment was compared to draw relevant conclusions. Lethal concentration of the Keetguard liquid for 50% and 90% tick mortality (LC50 and LC90) alongwith the residual activity, against different breeding sites and livestock dwellings (huts, sheds etc) was also determined.

2.1 *In vivo* Studies

Before *in vivo* trial on farm animal the herbal test product was subjected to toxicity trial as per the research guidelines given by the World Health Organization, WHO [29] in order to assess its safety of administration. The product was found safe with no harmful effects on animal health and environment.

Non descript cattles (n=24) with heavy tick infestation were selected for the trial and *in vivo* efficacy of Keetguard liquid on nymphal, larval and adult stages of ticks was determined. Animals were divided into four equal groups Group I, Group II, Group III and Group IV. Each group was kept as control for specific period of time and thereafter treated with Keetguard liquid as prescribed. The number of each stage of ticks per square unit area was counted. Observations were noted after 6, 12, 18, 24, 30, 48 and 72 hours.

2.2 *In vitro* Studies

In vitro efficacy of Keetguard liquid towards larval, nymphal and adult stages of ticks from non-descript cattle was evaluated. Filter papers impregnated with Keetguard liquid of recommended concentration were kept in a petridishes. Ten adult ticks of both sexes (5 each) were released on filter paper and petridishes was covered with muslin cloth and kept at room temperature in four replicates. Filter paper moistened in distilled water kept in another petridishes was treated as control. Mortality of ticks was recorded at 24, 48, 72 and 96 hours of exposure to test solution. Death of ticks was assumed when they failed to react to the mechanical stimulation by entomological pin.

The female ticks which survived after exposure from treatment as well as control groups were collected from the body of the animal and were kept in test tubes tied with muslin cloth for oviposition at a temperature of 28ºC and 85% relative humidity [30]. The oviposition period of both the groups was noted. The no. of eggs laid in treated and untreated ticks were compared to find out the efficacy of Keetguard liquid as oviposition deterrent alongwith the percentage hatchability.

2.3 Statistical Analysis

The data from both the studies were pooled and analysed by two way analysis of variance and the significance of difference between means was determined as per the method described by Snedecor and Cochran. [31].

3. RESULTS

3.1 *In vitro* Studies

Results of *in vitro* studies are summarized in Table 1, 2, 3, 4 and 5. The average numbers of ticks in all the four replicates of Keetguard liquid treated group were 10 which showed 100% mortality at 72 hours (3 days) post treatment. From the observation the test product is 100% effective on adult stages of ticks at 72 hours post treatment. All the larvae in the Keetguard liquid treated group were found dead after 12 hours post treatment. Each larva in untreated control group was alive at a time interval 96 hours. A drastic mortality pattern was observed in the Keetguard treated group and no nymph was found alive at 24 hour interval. All replicates with average 10 numbers of nymphs in untreated control group were found alive at all time intervals of observation. The efficacy of Keetguard liquid on nymphal and larval stages of ticks was recorded as 100% at 12 hours post treatment.

Table 1. *In vitro* efficacy of keetguard liquid against ticks

Time of observation post treatment	Number of adult ticks died (n=40)		No. of larvae died (n=40)		No. of nymphs died (n=40)		No. of female ticks laid eggs (n=40)	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
6 hr	00	00	00	03	00	11	00	00
12 hr	00	10	00	37	00	29	08	00
24 hr	00	04	00	00	00	00	08	00
30 hr	00	07	00	00	00	00	07	00
48 hr	00	08	00	00	00	00	05	02
72 hr	00	21	00	00	00	00	09	00
96 hr	00	00	00	00	00	00	00	00
Total	**00**	**40****	**00**	**40****	**00**	**40****	**37**	**02****

*** Values differ significantly (P<0.01) as compared to control values.*

Table 2. *In vitro* efficacy of Keetguard Liquid on adult stages of ticks of non descript cattle

Group of animals	No. of adult ticks per sq. unit area exposed	Number of adult ticks died post treatment at time interval														Percent efficacy/ mortality
		Untreated (Control) group							Treated group							
		6 *hr.	12 hr.	18 hr.	24 hr.	30 hr.	48 hr.	72 hr.	6 hr.	12 hr.	18 hr.	24 hr.	30 hr.	48 hr.	72 hr.	Total
I	10	0	0	0	0	0	0	0	00	0	0	2	0	1	7	10	100%
II	10	0	0	0	0	0	0	0	00	0	0	0	3	2	5	10	100%
III	10	0	0	0	0	0	0	0	00	0	0	1	3	2	4	10	100%
IV	10	0	0	0	0	0	0	0	00	0	0	1	1	3	5	10	100%
Mean	10	0	0	0	0	0	0	0	**00**	0	0	1	1.75	2	5.25	10	**100%**

**Time of treatment*

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.990 CD(0.05) = 0.729

Table 3. *In vitro* efficacy of keetguard liquid on larval stages of ticks of Non descript cows

No. of observations	No. of larvae of ticks exposed	No. of larvae died post treatment at time interval						No. of larvae died post treatment at time interval							Percent mortality/ Efficacy
		Untreated (Control) group						Treated (Control) group							
		6 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.	96 Hr.	6 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.	96 Hr.	Total	
I	10	0	0	0	0	0	0	1	9	0	0	0	0	10	100%
II	10	0	0	0	0	0	0	0	10	0	0	0	0	10	100%
III	10	0	0	0	0	0	0	0	10	0	0	0	0	10	100%
IV	10	0	0	0	0	0	0	2	8	0	0	0	0	10	100%
Mean	10	0	0	0	0	0	0	0.75	9.25	0	0	0	0	10	100%

Table 4. *In vitro* efficacy of keetguard liquid on nymphal stages of ticks of Non descript cows

No. of observations	No. of larvae of ticks exposed	No. of nymphs died post treatment at time interval						No. of nymphs died post treatment at time interval						Percent mortality/ Efficacy
		Untreated (Control) group						Treated (Control) group						
		6 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.	96 Hr.	6 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.	96 Hr.	
I	10	0	0	0	0	0	0	2	8	0	0	0	0	100%
II	10	0	0	0	0	0	0	3	7	0	0	0	0	100%
III	10	0	0	0	0	0	0	2	8	0	0	0	0	100%
IV	10	0	0	0	0	0	0	4	6	0	0	0	0	100%
Mean	10	0	0	0	0	0	0	2.75	7.25	0	0	0	0	100%

**Time of treatment.*

Coefficient of Variation = 57.499.

Treatments found Significant at 5% level of Significance CD (0.05)= 2.196.

Table 5. *In vitro* efficacy of keetguard liquid on egg laying of ticks of Non descript cows

No. of observations	No. of female ticks exposed	No. of female ticks lay at time interval								No. of female ticks lay eggs post treatment at time interval							Percent Oviposion Efficacy
		Untreated (Control) group								Treated (Control) group							
		6 Hr	12	24	48 Hr	72	96	Total	Percent oviposition	6 Hr	12 Hr	24 Hr	48 Hr	72 Hr	96 Hr	Total	
I	10	0	3	2	2	2	0	9	90	0	0	0	1	0	0	1	10%
II	10	0	0	0	4	5	0	9	90	0	0	0	0	0	0	0	0%
III	10	0	1	2	5	2	0	10	100	0	0	0	0	0	0	0	10%
IV	10	0	4	4	1	0	0	9	90	0	0	0	1	0	0	1	0%
Mean	10	0	2	2	3	2.25	0	9.25	92.5	0	0	0	0.5	0	0	0.5	5%

**Time of treatment coefficient of variation = 182.571.*

Treatments found significant at 5% level of significance CD (0.05) = 1.405.

3.2 *In vivo* Studies

Results of *in vivo* studies are summarized in Table 6, 7, 8 and 9. Total adult tick count in all treatment groups of non-descript cattle was found to have decreasing trend after 6, 12, 18, 24, 30, 48 and 72 hours. Treatment with the Keetguard liquid led to mortality of all ticks in treated groups after 24 hrs of application exhibiting 100% efficacy. All the nymphal stages of ticks showed 100% mortality at 10 hours while nymphs in control group remained unaffected. No side effects of the product on the animals under experimentation could be observed. The reinfestation time noted on the same animals was 45 days. This may be due to mixed grazing, common pasture land, sharing of huts, sheds etc, with ectoparasitic load.

Table 6. *In vivo* efficacy of Keetguard liquid against ticks

Time of observation post treatment	Number of adult ticks died (n=60)		No. of nymphs died (n=60)		No. of female ticks laid eggs (n=20)	
	Control	Treatment	Control	Treatment	Control	Treatment
6 hr	00	00	00	16	00	00
8 hr	00	23	00	23	00	00
10 hr	00	25	00	21	00	00
12 hr	00	10	00	00	00	00
18 hr	00	02	00	00	00	00
24 hr	00	00	00	00	04	00
48 hr	00	00	00	00	06	01
72 hr	00	00	00	00	06	00
96 hr	00	00	00	00	03	01
Total	**00**	**60****	**00**	**60****	**19**	**02****

*** Values differ significantly (P<0.01) as compared to control values.*

In vitro studies

Table 7. *In vivo* efficacy of keetguard liquid on adult stages of ticks of non descript cattle

Group of animals	No. of adult ticks per sq. unit area exposed	Number of adult ticks died post treatment at time interval																	Percent efficacy/ mortality
		Untreated (control) group									Treated group								
		6 *hr.	8 hr	10 hr	12 hr.	18 hr.	24 hr.	30 hr.	48 hr.	72 hr.	6 hr.	8 hr	10 hr	12 hr.	18 hr.	24 hr.	30 hr.	48 hr.	72 hr.	
I	16	0	0	0	0	0	0	0	0	0	0	7	6	2	1	--	--	--	--	100%
II	14	0	0	0	0	0	0	0	0	0	0	6	7	1	0	--	--	--	--	100%
III	16	0	0	0	0	0	0	0	0	0	0	4	6	6	0	--	--	--	--	100%
IV	14	0	0	0	0	0	0	0	0	0	0	6	6	1	1	--	--	--	--	100%
Mean	**15**	0	0	0	0	0	0	0	0	0	**0**	**5.75**	**6.25**	**2.5**	**0.5**	**--**	**--**	**--**	**--**	**100%**

**Time of treatment*

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.990 CD (0.05) = 0.729

Table 8. *In vivo* efficacy of keetguard liquid on nymphal stages of ticks of non-descript cattle

Group of animals	No. of nymphs per 64 sq. cm area of skin of neck	Number of nymphs died post treatment at time interval										Percent efficacy / mortality
		Untreated (Control) group					Treated group					
		6 *hr.	8 hr.	10 hr.	12 hr.	24 hr.	6 hr.	8 hr.	10 hr.	12 hr.	24 hr.	
I	14	0	0	0	0	0	4	6	4	-	-	100%
II	15	0	0	0	0	0	3	7	5	-	-	100%
III	16	0	0	0	0	0	3	7	6	-	-	100%
IV	15	0	0	0	0	0	6	3	6	-	-	100%
Mean	**15**	0	0	0	0	0	**4.0**	**5.75**	**5.25**	**-**	**-**	**100%**

**Time of treatment Treatments found Significant at 1% and 5% level of significance*

CD(0.01) = 2.005 CD(0.05) = 1.453

Table 9. *In vivo* efficacy of keetguard Liquid on preoviposition and oviposition of ticks of non descript cattle

Group of animals	No. of engorged females	Number of females depositing eggs after treatment at time interval													Percent oviposition
		Preoviposition (Control)							Oviposition (Treated)						
		24 *hr.	48 hr.	72 hr.	96 hr.	120 hr.	Total	Percent oviposition	24 *hr.	48 hr.	72 hr.	96 hr.	120 hr.	Total	
I	5	0	0	3	2	0	5	83.33	0	0	0	0	0	0	16.66
II	5	2	2	0	0	0	4	80.00	0	0	0	1	0	1	0
III	5	0	3	1	1	0	5	100.00	0	0	0	0	0	0	16.66
IV	6	2	1	2	0	0	5	83.33	0	1	0	0	0	1	0
Mean	**5.25**	**1**	**1.5**	**1.5**	**0.75**	**0**	**4.75**	**86.66**	**0**	**0.25**	**0**	**0.25**	**0**	**0.50**	**8.33**

**Time of treatment*

Treatments found Significant at 1% and 5% level of significance CD(0.01) = 1.362 CD(0.05) = 0.974

Keetguard liquid efficiently hindered the egg laying capacity alongwith the egg hatchability of tick females. Only 5% females from treated group could successfully lay eggs but zero recorded hatchability. Rest of ticks showed no oviposition, became comatose and died. In untreated control group egg laying capacity of the tick females remained unaffected with recorded 92.5% average oviposition and significant hatchability.

The average egg mass in Keetguard treated group reduced to 3.2 mg while that for untreated control group was 12.8 mg. From the observations, the test product Keetguard liquid showed 95% efficacy against the oviposition/egg laying capacity of ticks whereas 100% efficacious against the hatchability of egg of treated females.

4. DISCUSSION

The results of the present study are in accordance with results found in experiments conducted on various other herbal plants for their efficacy against ectoparasitic infestation. In an experiment conducted to evaluate the efficacy of different herbal plants against ectoparasites. Eight plant extracts were screened for efficacy against *Boophilus microplus,* the extracts prepared from the *A. indica* seed showed very high level of efficacy (80%) after 5 h of treatment. Besides the immediate effect on adult ticks, the egg-laying properties of the survived ticks was also assessed, and a significant reduction ($P < 0.01$) in the reproductive index of ticks fed on animals treated with *A. indica* seed extracts was noted in comparison to control [15]. Similarly, in another study on efficacy of herbal plants against ectoparasites, after 24 h of treatment, the highest acaricidal activity of 70.8% was recorded in the ticks treated with 8% extract of *A. squamosa* followed by *Nicotiana tobacum* (45.8%) and *Tamarindus indica* (41.7%) extracts, whilst 29.8% and 20.8% mortality, respectively, was recorded in ticks treated with *Eucalyptus globulus* and *Citrus limonum* extracts against *R. microplus* [14]. It was observed that at 10% concentration of extracts, 35% of the ticks treated with *A. calamus* died within 24 hrs of treatment (8). Earlier tick infested buffaloes and cattle treated five times at 6-day intervals (0, 6, 12, 18 and 24) with (AV/EPP/14) (containing the active ingredients: *Cedrus deodara, Pongamia glabra, P. pinnata, Azadirachta indica, Eucalyptus globulus* and *Acorus calamus*) resulted in elimination of 65.3, 87.6, 96.5, 99.6 and 100% of the ticks, respectively. The treated animals were free of ticks for a period of 30 days after the last treatment in the experiment conducted by Kumar et al. [32].

The high rate of mortality with Keetguard may have resulted from cumulative effect of herbal extracts in contrast to individual efficacy of the different phytological ingredients. It was observed earlier that extract of C. serrata proved to be toxic killing 100% of the larvae of R. microplus at the concentrations of 50, 25, 12.5 and 6.25 mg/mL after 48 h [33]. The essential oil of Cymbopogon winterianus Jowitt was tested against larvae and engorged females. Total inhibition of eclosion was observed at a concentration of 7.14% and of egg conversion at 10%. All the larvae died at concentrations between 5.5 and 7.14% [34). Duarte et al. [35] have reported that effect of six hyacinthacine analogues derived from pyrrolizidine alkaloids were toxic to the larvae of the ticks and inhibited the eggs' hatchability at 5µg/ml. The plant *Sapindus saponaria* also demonstrated larvicidal activity for the *Rhipicephalus sanguineus* (Acari: Ixodidae), with LC50 and LC99 values of 1,994 and 3,922 ppm, respectively [36]. In a different study conducted by Srivastava et al. [15] mean egg mass produced from the ticks fed on animals treated with neem extract alone, Cypermethrin and untreated control groups were 26.7 mg. 19.5 mg and 60.2 respectively. The ethanolic extract of *Jatropha curcas* leaves at all concentrations tested (50-100 mg/176 ml) considerably blocked the hatchability of eggs when compared to control. Percent hatchability in treated groups reduced significantly ($P<0.05$) to 10% as compared to control, 100% [9].

5. CONCLUSION

Finally it can be concluded that the test product comprising of oil of herbs viz. *Eucalypta globulus, Cedrus deodara, Pinus longifolia* etc. in a fixed concentration is highly efficacious against the ectoparasite infestation in animals. It's useful ectoparasiticidal properties can effectively reduce the nuisance of ectoparasites. Besides the immediate effect on adult ticks, the product also reduced the egg-laying property of the ticks.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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