

In Vivo Evaluation of Acaricidal Potential of Few Herbs from Hilly Regions of Himachal Pradesh

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Abstract

Synthetic acaricides pose problems of resistance, environmental issues, public health problems and institutional dependence. Herbal medications offer viable alternatives for tick control. We conducted on farm trials on 85 animals and tested crude extracts of plants locally available in the region. The technological know-how was shared by National Innovation Foundation in its earlier limited trials with DGCN college of veterinary & animal sciences, Palampur, Himachal Pradesh. The present study done on a larger scale evaluated effectiveness of herbal technology. The herbal medication had provided relief and showed 40.30 percent efficacy by 24 hours, 81.62 by 48 hours and 89.15 by 72 hours of topical application. This low cost technology based on locally available plants must be propagated for wider dissemination by stakeholders.

Keywords: In-vivo Evaluation, Herbal Extract, Technology Dissemination

Introduction

Tick problem significantly impacts livestock production in tropical and subtropical countries of the world (Abbas, 2014); Dutta *et al.* (2018). Ticks cause immense loss to livestock production directly through blood sucking, milk productivity losses and poor hide value de Castro (1997); Alim *et al.* (2012); Mondal *et al.* (2013); Valente *et al.* (2014); Shyma *et al.* (2019). These organisms open up wounds of cattle, make cattle susceptible to secondary infection and cause toxicity and paralysis under certain situations Rajput *et al.* (2006). Further, indirect effects appear in form of various tick-borne diseases: Theileriosis, Babesiosis and Anaplasmosis de Castro (1997) & Vudriko *et al.* (2018).

Synthetic acaricides are the chemical drugs used to control the problem of cattle ticks. Inconsistent and indiscriminate use of these chemicals has created the problem of acaricide resistance. This has rendered existing acaricides ineffective & limited the efficacy of existing tick control methods (Mendes *et al.*, 2011; Vudriko *et al.* (2016); Pavela *et al.* (2016). Synthetic acaricides being reported ineffective by high percentage of farmers (94%) in the regions of South Africa (Moyo & Masika, 2009). Newer synthetic acaricides require vast amounts of budget for development estimated around 230 million US dollars de Castro (1997).

From farmers viewpoint, they rely on veterinary institutions for the control of tick problem. Veterinary institutions offer limited support due to shortage of inputs and manpower availability (Kataviya *et al.*, 2018). Many times, farmers depend on input dealers who are often ill equipped to educate farmers about proper application & usage. This poses significant environmental & public health danger in the usage of synthetic acaricide (Addah *et al.*, 2018). These problems make it essential to look beyond current methods of tick control. Herbal therapeutic methods offer cost effective and integrated tick control (Ghosh *et al.*, 2007; Babar *et al.*, 2012). These methods are safer for animal use and have less chances of development of tick resistance (Nath *et al.*, 2018). Native and local communities around the world have been using as many as 238 species of plants & many of them have been reported with repellent & acaricidal property (Pavela *et al.*, 2016).

Himachal Pradesh is a hilly state in western Himalayan region of India. The state is rich in faunal & floral biodiversity (Badola & Pal, 2003). The rich biodiversity of the state is under severe threat as awareness level about medicinal plants of the state remains limited. Traditional knowledge about possible use of such plants in animal husbandry practices have eroded and chemical use in hill farming systems has considerably increased. *Vitex negundo* (Banha) is a common plant found in the greater part of India & found mostly at warmer zones and ascending to an altitude of 1500 m in outer Western Himalayas. The plant products of *Vitex negundo* are reported to possess insecticidal properties against stored pests (Chowdhury *et al.*, 2009). *Melia azadirachta* is a tree widely used in the sub Himalayan region up to 2000 m above sea level. This is well known ethno medicinal tree used in Ayurveda & folk medicine across India (Sharma and Paul, 2013). Leaves are used as fodder & are highly nutritious. The leaves have been found to have antihelminthic (AL-Rubae *et al.*, 2009 & Vidyarthi *et al.*, 2013), antifungal (Carpinella *et al.*, 1999) & larvicidal properties (Athanasiadou *et al.*, 2001). The use of *Melia* & *Drek* were tested on limited scale for acaricidal properties through in-situ incubation model demonstration in the region was done before by Thakur *et al.* (2016). The present study involved testing & validation of the herbal technology at much wider scale in the regions of Himachal Pradesh. This is imperative as limited studies have been documented on On farm trials for the control of ticks in the region.

Materials and Methods

Study Locale

The study was conducted in villages of Gunehar, Chowgan, Keori, Deol, Paprola, Lalahar, Saliana and Paprola & Sonpur Gaushala with the support of Veterinary Hospitals Paprola, Deol, Bir in Kangra District of Himachal Pradesh. The on-farm trials were conducted over 87 animals from July 2019- November 2019. These areas lie at an altitude of 1472 meters to 1525 meters.

Preparation of Plant Extract

The medications was prepared by collecting 2.5 kg fresh leaves of *Drek* (*Melia azedarach*) which was soaked in 4 litres of lukewarm water and 2.0 kg fresh leaves of Banha or Mala (*Vitex negundo L.*) in 1 litre of lukewarm water

and kept overnight. The crude extract of both medicinal ingredients were prepared in this manner. The supernatant fluid of these individual extracts were collected and stored at room temperature. About 300 ml supernatant fluid from Drek (*Melia azedarach*) and 100 ml of Banha or mala (*Vitex negundo L.*) were mixed in 3.6 litres of normal water. The prepared medication was topically applied over affected site of animal two times daily for three days and observed the impact. Thakur *et al.* (2016) had earlier used this methodology earlier on limited basis in villages around Palampur and assessed acaricidal properties in 8 animals.

Selection of Animals and Clinical Examination

The selection of villages was done based on consultation of local veterinary doctors in the region. Thus, purposive sampling method was used in selection of villages. In the villages, the livestock owners who reported infestation of ticks in their animals to veterinarians were individually contacted. Farm and home visits were conducted to initiate trials among the animals. Animals infested with ticks were identified and upon examination these were found to be of *Rhipicephalus* sp. Results confirmed that affected animal were infested with hard tick.

Application of Medication and Method of Examination

- Number of ticks attached in a unit area of 30 cm² [10 cm X 3 cm] on each animal were counted with the help of specially designed plastic hand tool prior to spraying the animals with herbal acaricide.
- Identification of tick to determine the efficacy of medication against different species, preferably single host tick. In case of three host tick, only adult ticks were tried.
- Ticks were counted on next day morning to see the effect of the medications of the previous day. After completion of the counting, the next applications were undertaken.
- All animals were observed daily (twice daily) for presence of ticks, tick movements, new attachments, any signs of tick borne or other diseases.
- The per cent efficacy of the application was calculated.
- Animals were observed with the support of investigator, local clinician and later with para veterinary professional upto 72 hours.

Evaluation of Poly-herbal Acaricidal Medication

A total of 87 naturally infested animals of different age group and sex were part of experimental study during the period between July 2019 to November 2019. About 9 demonstration were held during the period by preparing this medicine in-situ at farmers field. The medication was applied topically twice daily. These animals were observed for ectoparasite counts, efficacy at interval of 24, 48 and 72 hours in field situation. The ticks were counted in an area of 30 cm² on the respective sites of infestation. Before after method of experimental study design was used to study the effect of plant extract on animals.

Results

The study was conducted in villages of Gunehar, Chowgan, Keori, Deol, Paprola, Lalahar, Saliana and Paprola & Sonpur Gaushala with the support of Veterinary Hospitals of Paprola, Deol, Bir in Kangra District of Himachal Pradesh (Table 1).

Table 1: Details of villages in which on farm trials were conducted

S. No.	Village	Veterinary Hospital	Farmers	Total Animals (N=87)	Duration of Observation
1	Paprola	Paprola	Nil	2	Upto 4 days
2	Lalahar	Bir	6	12	5
3	Paprola Gaushala	Paprola	Nil	4	3
4	Sonpur Gaushala	Paprola	0	1	2
5	Chowgan	Bir	6	11	3
6	Keori	Bir	16	21*	2
7	Deol	Bir	9	12	4
8	Gunehar	Bir	8	13	4
9	Saliana	Saliana	5	11**	4
		Total	50	87	

*30 infested sites, **12 infested sites

Table 2: Nature of animals under experimentation

S. No.	Description of Animals		Frequency (N=87)	Percent
1.	Sex	Female	85	97.7
		Male	2	2.29
2.	Breed	Jersey	78	89.65
		Holstein Friesian	5	5.74
		Non-descript	4	4.59
3.	Adult		60	68.96
	Heifer		15	17.24
	Calf		12	13.79
4.	Body part N=97	Head & Neck	28	28.87
		Dewlap	23	23.17
		Wither	1	1.03
		Foreleg	20	20.62
		Inguinal region	1	1.03
		Udder	15	15.46
		Whole body	2	2.06
5.	Average age in years			2.96

Clinical examination of 87 infested animals of average age of 2.9 years had shown hard tick infestation. These animals were observed for maximum of 5 days. High prevalence were seen in adult (68.96%) followed by heifer (17.24%) and calf (13.79%). Yakhchali and Hasanzadehzarza (2004) reported high incidence of tick infestation in adult cows than in calves which coincide with the present study. Kabir *et al.* (2014) reported tick infestation 2.23 times higher in young cattle than adult cattle in Pakistan. The most affected sites were head and neck (28.87%), dewlap (23.71%), withers (1.03%), forelegs (20.62%), inguinal region (1.03%), udder (15.46%) and whole body (2.06%) (Table 2). External genital was most affected sites followed by dewlap, inner thigh, neck and back (Atif *et al.*, 2012). Nady *et al.* (2014), reported that the preferred sites of ticks' attachment to infested animals were udders and external genitalia (70.7% of each) then Neck & chest (63.0% of each), inner thighs (61.1%), perineum (41.7%), ears (14.6%) and around eyes (11.7%). Average tick count was 24.20 ± 2.18 before medication for total sample size of 85 infested sites. Subsequently after topical application of medication, it was reduced to 14.45 ± 1.66 by 24 hours and 2.62 ± 1.20 by 72 hours (Table 3). The herbal medication had provided relief and showed 40.30 percent efficacy by 24 hours, 81.62 by 48 hours and 89.15 by 72 hours of topical application (Table 3). Vitex negundo leaves contain Aucubinaginaside, Alkaloids: Nishindine, Hydrocotylene, Glyoflavonoids, Orientin, Isoorientin, 5-Hydroxy, 3,6,7,31,41 pentamethoxy flavone (Maurya, and Rao, 2019). Similarly leaves of melia azedarach contain nimbinene, meliacin, quercetrin, quercetin-3-O- β -rutinoside, kaempferol-3-O- β -rutinoside, rutin and kaempferol-3-L-rhamno-Dglucoside (Rana,2008). Thus, poly-herbal nature of formulation from these two medicinal plants explain their acaricidal property .

Table 3: Control of tick infestation (before & after medication) n = 85

Duration of treatment	Total tick count (Before medication)	After 24 hrs	After 48 hrs	After 72 hrs
Mean \pm S.E	24.20 \pm 2.18	14.45 \pm 1.66	4.45 \pm 1.31	2.62 \pm 1.20
Percent efficacy	-	40.3	81.62	89.15

It was found that on 32 locations or infestation sites there was complete cure against tick worry through application of this medicine by 2nd day of observation. Similar on farm trials conducted by Ravikumar *et al.* (2016) in Gujarat demonstrated the efficacy of indigenous veterinary medications among 24 out of 38 animals.

Table 4: Control of tick infestation (before & after medication) n = 53

Duration of treatment	Total tick count [Before medication]	After 24 hrs	After 48 hrs	After 72 hrs
Mean± S.E	23.83±2.38	13.91±1.85	7.13±1.66	4.21±1.51
Percent efficacy	-	41.64	70.07	82.34

These sites (n=53) were assessed separately to appreciate the nature of curation and found that the rate of infestation (23.83±2.38) was reduced to 4.21±1.51 by 72 hours. The medicine was found to possess 82.34 per cent efficacy (Table 4). Kataviya *et al.* (2018) in parts of Gujarat & Himachal Pradesh among 18 animals found 92.95% acaricidal property within 24 hours of application of medication. Jadhav *et al.* (2018) reported acaricidal properties of polyherbal spray containing extracts of *Andropogon citrates*, *Cymbopogon citratus*, *Ocimum sanctum*, *Pinus longifolia*, *Calotropis procera*, *Datura stramonium*, *Aegle marmelos*, *Ricinus communis*, *Azadirachta indica*, *Allium sativum*, *Carica papaya*, *Annona squamosa* and *Pongamia glabra* against tick infestation in buffaloes. Phytoacaricides or herbal acaricides are beneficial in fighting resistance due to their rapid degradation and lack of persistence as well as bioaccumulation in the environment compared to synthetic chemical acaricides (Ghosh and Nagar, 2014).

Conclusion

On farm trials successfully demonstrated the efficacy of herbal medications against the control of ticks. The standardized herbal technology is based on readily available plants in the prevailing natural ecosystem. The state animal husbandry departments, dairy producer organization & farmer organization need to take up this technology as alternative to synthetic acaricides to reduce problem of resistance, environmental problems and institutional dependence of farmers.

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Conflict of Interests

There is no conflict of interest.

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