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In vivo Evaluation of the Acaricidal Efficacy of Polyherbal Aqueous Formulation against *Rhipicephalus microplus* Infested Cattle Calves

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Abstract

The study evaluated the acaricidal potency of a Multi-herbal aqueous preparation in controlling *Rhipicephalus microplus* ticks in 12 naturally infested calves. The formulation, made from a blend of garlic, tobacco, tulsi, lantana, sweet flag, neem, and turmeric, was applied in two groups of calves. Group I received a single treatment, while Group II received two treatments (on days 0 and 7). Results showed that the double application was more effective, with significant improvements in hemato-biochemical parameters, bringing values like Hb, PCV, TEC, and TLC closer to normal levels. The findings suggest that the Multi-herbal aqueous preparation is a promising acaricide. The study of a polyherbal aqueous formulation against *Rhipicephalus microplus*-infested calves offers a natural, eco-friendly, and cost-effective alternative to chemical acaricides. It helps reduce tick burden, improves calf health and productivity, minimizes the risk of resistance, and supports sustainable livestock management, especially in rural and resource-limited settings.

Key Words: *Rhipicephalus microplus*, Polyherbal spray, Tick count, Calves

Introduction:

Ticks, especially *Rhipicephalus microplus*, are major ectoparasites that harm both livestock and humans, leading to significant economic and health issues. Synthetic acaricides are widely used; their environmental risks, health concerns, and tick resistance are major challenges. Natural alternatives, such as plant extracts and essential oils exhibit promising acaricidal effects. Products like neem oil, garlic, and polyherbal formulations have demonstrated effective tick control and improved cattle health. This study seeks to assess the in vivo mite-killing effectiveness of a water-based polyherbal formulation on tick-infested calves, offering a potentially safer alternative to conventional synthetic acaricides.

De Almeida et al. (2021) tested various concentrations of dehydrated garlic (0.1%, 0.5%, and 1%) and found it effective against *Rhipicephalus microplus*. Suraj et al. (2020) compared the efficacy of amitraz, fipronil, and neem (*Azadirachta indica*), showing significant differences in tick survival post-treatment, with Oil and extract from neem leaf (*Azadirachta indica*) achieving mortalities ranging from 72.7% to 95.3%. Nasreen et al. (2020) found that extracts of *Allium sativum* and *Cannabis sativa* significantly reduced tick populations after 96 hours of treatment. Bhikane et al. (2018) observed a notable reduction in tick count and an increase in erythrocyte count and packed cell volume in cattle treated with a polyherbal spray, demonstrating its potential for tick control.

Nasreen et al. (2020) reported that extracts of *Allium sativum* and *Cannabis sativa* significantly decreased tick populations within 96 hours of application. Similarly, Bhikane et al. (2018) observed a marked decline in tick numbers, along with increased erythrocyte counts and packed cell volume in cattle treated with a polyherbal spray, highlighting its potential as an effective tick control strategy.

Materials and Methods:

The study, conducted at the Department of Medicine, College of Veterinary Science and A. H. in Mhow, aimed to assess the acaricidal effectiveness of a polyherbal aqueous formulation. The study was conducted for a period of six months, from May to October, *ie.* mid-summer, rainy and winter season.

Polyherbal aqueous formulation

The following ingredients were mixed together in the grinder: 10 gm each of Garlic pearls (*Allium sativum*), Tobacco leaves (*Nicotiana tabacum*), Tulsi leaves (*Ocimum tenuiflorum*), Lantana camara leaves (*Shrub verbena*), Sweet flag rhizome (*Acorus calamus*) and 20 gm of Neem leaves (*Azadirachta indica*) and Turmeric powder (*Curcuma longa)*. These ingredients were grind and dissolved in one liter of water.

In vivo evaluation

Twelve calves of both sexes, aged between 1 and 6 months and naturally infested with *Rhipicephalus microplus* ticks, were randomly assigned to two groups: Group I received a single application on day 0, while Group II received two applications on days 0 and 7, with six calves in each group. The polyherbal formulation was applied to the entire body of the calves after measuring the tick count.

Quantification of the number of ticks

The acaricidal efficacy of the polyherbal aqueous formulation against *Rhipicephalus microplus* in infested calves was evaluated by counting ticks on each animal. Tick counts were conducted through direct palpation at seven heavily infested body regions: the ear pinna, inguinal region, under the tail, back, withers, dewlap, and neck. The effectiveness of the spray was determined by comparing post-treatment tick counts with those recorded before treatment. Tick counts were recorded before the spray application, as well as on the 3rd, 7th, and 21st days after treatment. The results were expressed as mean tick count.

Haemato-biochemical examination

Haematological parameters were carried out by Automatic Blood Cell Counter Make Diatron, Model Abacus 380. Differential leukocyte count was done by staining blood smears with Wright's stain as per the method described by Jain (1986). Alterations in the values were observed by using commercial semi auto analyzer. Samples were centrifuged at 2500 rpm for 10 minutes to separate the serum for biochemical analysis, following the method described by Brar et al. (2002). Hematological parameters were assessed on day 0 and again 21 days after treatment.

Results and Discussion:

In vivo acaricidal effectiveness of Water-based polyherbal mixture against *Rhipicephalus microplus* infested calves

The tick count significantly decreased on the 3rd and 7th days compared to day 0 (group I), but In Group II, the mean tick count showed a significant increase on day 21 compared to day 0. However, the mean tick count showed a significant reduction on day 21 compared to day 0 (Table 01). Similar observations were reported by Bhikane et al. (2018) and Nasreen et al. (2020). Costa and Furlong (2011) investigated the effectiveness of garlic extract and stated overall efficacy of the garlic extract was 64% in controlling the cattle tick *Rhipicephalus microplus*, Ghosh et al. (2011) evaluated the acaricidal efficacy of *Acorus calamus* extracts against *Rhipicephalus microplus* through both *in vitro* and *in vivo* studies. Similarly, Rodríguez et al. (2017) found that a 5% neem extract exhibited repellent and acaricidal effects in cattle, lasting for at least 42 days.

Table 01: Mean (± SE) Total Tick Count in Calves

Days	Groups	
	Ⅰ	Ⅱ
0	$118.5^{b} \pm 22.38$	$116.5^{c} \pm 23.19$
3	$63.5^{a} \pm 19.32$	$57.33^{b} \pm 17.17$
7	$39.6^{a} \pm 13.46$	$30.33^{ab} \pm 10.40$
21	$98.6^{Bb} \pm 21.24$	$15^{Aa} \pm 6.07$

Different superscript ($p<0.05$) shows significant difference, same superscript shows non-significant difference (a-c = within group), (A-B = between groups)

Haematological observations

By the 21st day post-treatment, hemoglobin levels had significantly increased in both groups. The packed cell volume (PCV) also significantly increased in both groups, along with a significant rise in total erythrocyte count (TEC) in both groups (Table 02). The changes observed in hemoglobin, PCV, TEC, and TLC in tick-infested animals were consistent with the findings of previous studies by Rajendran and Hafeez (2003), Kaur et al. (2017) and Jain et al. (2021). Bhikane et al. (2018) investigated the acaricidal potential of a polyherbal spray and found that a single application on tick-infested cattle led to a significant reduction in the mean tick count beginning on day 3 post-treatment and continuing through day 21. A highly significant increase ($P<0.01$) in total erythrocyte count and packed cell volume was observed in treated animals 21 days after application, indicating a decrease in blood loss previously caused by heavy tick infestation. No significant changes were noted in plasma biochemical parameters.

Table 02: Mean values of Hb (gm /dl), PCV (%), and TEC (millions/ cumm) before and after treatment

Parameters	Groups		
	Days	Ⅰ	Ⅱ
Hb	0	$8.74^{a} \pm 0.16$	$8.83^{a} \pm 0.24$
	21	$10.34^{b} \pm 0.33$	$11.24^{b} \pm 0.30$
PCV	0	$26.22^{a} \pm 0.48$	$26.49^{a} \pm 0.73$
	21	$31.02^{b} \pm 1.00$	$33.72^{b} \pm 0.91$
TEC	0	$5.24^{a} \pm 0.096$	$5.29^{a} \pm 0.146$
	21	$6.19^{b} \pm 0.201$	$6.74^{b} \pm 0.183$

Different superscript ($p<0.05$) shows significant difference (a-b = within group)

On the 21st day post-treatment, a significant decrease in total leukocyte count (TLC) was observed in both groups. The decrease in total leukocyte count after treatment with the polyherbal solution may be due to its anti-inflammatory and immunomodulatory properties, which help reduce the stress and immune response triggered by

tick infestation, leading to normalization of elevated leukocyte levels. A significant increase in neutrophil count and a significant decrease in lymphocyte count were observed in both groups. In group II, the monocyte count significantly increased. Additionally, the eosinophil count significantly decreased in both groups, and the basophil count significantly decreased in group II (Table 03). Findings were in support with Rajendran and Hafeez (2003), evaluated Haemato-Biochemical changes in naturally tick infested crossbred cattle based on the treatment and concluded a significant reduction in Hb, PCV, TEC in tick infested groups as compared to control group. The eosinophil count was significantly high ($P<0.01$) in tick infested groups than lymphocytes, neutrophils, and monocytes.

Table 03: Mean (± SE) values of total leucocyte count (thousands / cumm) and differential leucocyte count before and after treatment

Parameters	Groups		
	Days	Ⅰ	Ⅱ
Total leucocyte count	0	$12.94^{b} \pm 0.423$	$13.03^{b} \pm 0.333$
	21	$11.79^{Ba} \pm 0.27$	$10.97^{Aa} \pm .150$
Neutophils	0	$32.18^{a} \pm 0.68$	$30.79^{a} \pm 1.31$
	21	$36.97^{Ab} \pm 0.6$	$39.29^{Bb} \pm 0.68$
Lymhocytes	0	$61.79^{b} \pm 0.73$	$62.14^{b} \pm 0.37$
	21	$58.41^{Ba} \pm 0.69$	$56.23^{Aa} \pm 0.68$
Monocytes	0	$0.92^{a} \pm 0.07$	$0.81^{a} \pm 0.03$
	21	$1.27^{Aa} \pm 0.18$	$1.93^{Bb} \pm 0.14$
Eosinophils	0	$4.83^{b} \pm 0.20$	$5.01^{b} \pm 0.18$
	21	$3.14^{Ba} \pm 0.09$	$2.36^{Aa} \pm 0.14$
Basophils	0	$0.28^{a} \pm 0.04$	$0.25^{b} \pm 0.01$
	21	$0.21^{Aa} \pm 0.03$	$0.19^{Aa} \pm 0.01$

Different superscript shows significant difference, same superscript shows non significant difference (a-b= within group), (A-B = between groups)

Biochemical observations

At the 21st day post-treatment, the levels of SGPT, SGOT, and creatinine showed a non-significant decrease in both groups (Table 04). These findings were also observed by Malviya (2016).

Table 04: Mean (± SE) Values of SGPT (IU/L), SGOT (IU/L), and Creatinine (mg/dL) Pre- and Post-Treatment

Parameters	Groups		
	Days	Ⅰ	Ⅱ
SGPT	0	26.64 ± 3.72	25.10 ± 2.75
	21	26.32 ±3.46	23.92 ± 2.40
SGOT	0	98.08 ± 9.09	107.66 ± 7.32
	21	95.86 ± 8.95	106.65 ± 6.95
Creatinine	0	1.61 ± 0.10	1.65 ± 0.12
	21	1.55 ± 0.10	1.54 ± 0.12

Conclusions:

The efficacy of polyherbal formulation with double application was found to be higher than single application. Haemato - biochemical parameters like Hb, PCV, TEC, TLC, SGPT, SGOT and creatinine of affected calves were towards normal limit after treatment. The values of Hb, PCV, TEC, TLC significantly changed and in values of SGPT, SGOT and creatinine non significantly changed towards normal limit.

Conflict of Interest:

The authors declare no competing interests.

Data Availability:

Data will be made available on reasonable request.

Author's Contribution:

All authors contributed in Methodology, Data curation, Investigation; Conceptualization, Formal analysis, Project administration, Writing – original draft. All authors approved the final version of this manuscript.

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