





Research Article

Effects of Tobacco, Neem, and Ivermectin against Ectoparasites: A Comparative Study in Black Bengal Goats

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Article History: Received: June 25, 2015 Revised: July 16, 2015 Accepted: July 30, 2015	Article History:	Received: June 25, 2015	Revised: July 16, 2015	Accepted: July 30, 2015
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ABSTRACT

The study was conducted to determine the comparative efficacy of neem oil, tobacco, and ivermectin against ectoparasites in Black Bengal goats. Twenty five goats of both genders infested with ticks were randomly divided into 5 equal groups (T_0 , T_1 , T_2 , T_3 and T_4). Group T_0 kept as control group and group T_1 treated with 4 ml of neem oil topically applied in the affected area of the body once daily for 28 days. In group T_2 , goats were treated once daily for 28 days with 1% tobacco leaves. In group T_3 , goats were treated once daily for 28 days with neem oil and tobacco leaves (spray) combined with the dose rate at 4 ml neem oil and 1% tobacco leaves. In group T_4 , ivermectin was administered at a dose rate of 200 µg/kg body weight subcutaneously on day 0 and 14. The efficacy of neem oil (56.25%), tobacco leaves (60%), neem oil and tobacco leaves combined (68.96%) and ivermectin 100% on the basis of disappearance of ticks. Percentage of total erythrocyte count (TEC), hemoglobin (Hb) and packed cell volume (PCV) increased (p<0.01) at 21 and 28 days of post treatment. Our study suggests that neem, tobacco and combination of both may be used against tick infestation in goats instead of ivermectin.

Key words: Neem, Tobacco, Ivermectin, Ticks, Black Bengal goat

INTRODUCTION

Bangladesh is an agriculture based country in South Asia and its economic backbone is mainly centered on agriculture. Agriculture plays a vital role in national economy, with 16.33% of Gross Domestic Products (GDP) (Bangladesh Economic Review, 2014). Current contribution of livestock sub-sector to overall GDP is about 1.78% (Bangladesh Economic Review, 2014). The export earnings from leather is 3.21% of the total export; 20% of the population is directly and 50% is partly dependent on agriculture sector of Bangladesh (Bangladesh Economic Review, 2014).

Bangladesh leathers have good inherent quality because of warmer climatic condition and food habits. Leather goods made from hides and skins of animals maintained in warmer climates are superior to those of animals raised in colder climates, but such advantages are overshadowed by various defects (FAO, 2004). Surprising leather defects due to skin disease, postmortem defects or curing defects and industrial processing defects cut about 55.2% value of leathers resulting in annual economic loss of approximately Taka 818 core (US\$ 220.95 million). Among such economic losses, US\$ 24.1 million was due to Black Bengal goats skin defects in Bangladesh (FAO, 2004).

In Bangladesh many drugs are being used for long time to combat parasitic infection in livestock. A large number of anthelmintics are now available in the market. The indiscriminate uses of anthelmintics lead to multidrug resistant-parasites (Hannan, *et al.*, 2001).

Various types of herbal extracts showed the larvicidal and acaricidal effects (Chungsamarnyart, et al. 1991). Several indigenous medicinal plants (neem oil, Tobacco) have anthelmintics activity (Nath, 1983, Mostofa, 1983 and Hossain, 1994) and used against both ecto and endo parasites in Bangladesh, (Mostofa, 1983, Safique 1983, Mannan, 1997). Neem has been used historically against several ectoparasites and pests and the action is rapid. Hormone mimicking activities of neem extracts cause interference with the parasitic life cycle, inhibiting their ability to feed as well as inhibit the hatching of eggs (Kumar and Navaratnam, 2013). Neem plant contents have been reported to be an effective bio-insecticide

Cite This Article as: Das K, MR Akanda, MM Rahman, MS Islam and MA Hossain, 2015. Effects of tobacco, neem, and ivermectin against ectoparasites: a comparative study in black bengal goats. Inter J Vet Sci, 4(4): 194-198. www.ijvets.com (©2015 IJVS. All rights reserved)

(Chary, 2011) and found useful in the regulation of many insect species of medical and veterinary significance. However, limited progress was made in this research area in Bangladesh.

At present, there are no available effective drugs without toxicities. Although ivermectin is safe and effective against both ecto and endo parasites of animals but it is expensive. In contrast, the alternative cheapest and available source of drug is herbal therapy. Experimental investigations, therefore, is imperative to assess the therapeutic value of indigenous herbal plants and oil. In order to bridge this knowledge gap, this research work was designed to investigate the comparative efficacy of indigenous medicinal plants (tobacco and neem oil) and ivermectin against ectoparasites (ticks) of goats; and study any adverse effects.

MATERIALS AND METHODS

Study area and animals

The experiment was conducted from January 1, 2014 to January 28, 2014, at Sylhet Government Goat Farm, Sylhet and The Department of Pharmacology and Toxicology of Sylhet Agricultural University, Sylhet. Twenty five Goats of both genders aged between 12 to 24 months were primarily selected in this study. All goats were examined for the presence of ectoparasites and the efficacy of Ivermectin and indigenous medicinal plant (Neem oil and Tobacco) were studied against these naturally infested ectoparasites.

Procurement of drugs

Injectable Ivermectin (Vermic[®], S/C formulation, Techno Drugs Limited, Bangladesh) was purchased from local market at Sylhet. The tobacco leaves were collected from Sylhet town and brought to the laboratory of the Department of Pharmacology and Toxicology. After collection, all leaves were washed in running tap water and cut into small pieces. Tobacco leaves were dried in shade and then dried in the oven at 55-60^oC for 72 hours to gain constant weight and also apparently free from moisture. Neem oil was purchased from Modern Herbal Company, Sylhet.

Preparation of tobacco dust and solutions

Dusts were prepared by pulverizing the dried leaves with the help of a manual grinder. A 25-mesh diameter sieve was used to obtain fine dust and preserved them in airtight plastic container till their use in extract preparation. From the dust, the aqueous extract was prepared. 20g of tobacco leaves was added with 100ml distilled water for homogenous mixture. The mixture was then evaporated while boiling at water bath at 50° C. Final volume of the mixture was brought down to 10ml and stored at this concentration in refrigerator as a stock solution until its use for preparation of solution. The solution of 1% was prepared by adding required amount of water in the stock solution. All solutions were prepared freshly.

Reagents

Saturated salt solution, normal saline (0.9%), anticoagulant (sodium citrate 3.8%), Hayem's solution,

0.14% Hydrochloric acid (HCL) solution and these were prepared in the Pharmacology and Toxicology Laboratory, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet.

Detection of ectoparasite

The ectoparasites (ticks, *Boophilus microplus*) were detected by physical examination of the animals and the presences of the ectoparasites were recorded. Tick infestation was examined physically on individual animal. The procedure of examination was to count the adult ticks of paralumbar region with an area of 5×4 i.e. 20 square cm. The selected areas were marked with a permanent colour and ticks within this area were counted at pretreatment and post-treatment days.

Severity of infestation

The severity of infestation of ectoparasites (ticks) was observed by counting the number of ectoparasites in a selected area of the individual goat. The ectoparasites were detected by visual examination with the help of magnifying glass and the number of parasites was recorded. The ticks within the selected areas were counted at pre-treatment (0 day) and (7th, 14th, 21st and 28th) post treatment period.

Body weight

To observe the effects of three drugs on body weight goats of treated and non-treated control group, body weights were recorded before treatment (day '0') and 14 - and 28 - post treatment.

Total erythrocyte count (TEC), hemoglobin (gm %) and packed cell volume (PCV)

The counting and calculation of red blood cells were performed as per methods indicated by Coffin (1955). The hemoglobin estimation was performed by the acid Haematin method with the Hellige Hemometer (Coffin, 1955) and the PCV was also determined as per method described by Coffin (1955).

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) procedures (Steel and Torrie, 1980) appropriate for a completely randomized design by the GLM procedure of SAS (1995).

The comparisons of data were made between different days within a group.

RESULTS

In control group T_0 , ticks were gradually increased on study period. On the other hand, groups of T_1 , T_2 , T_3 and T_4 showed variable efficacy against ectoparasitic infestation by killing ticks i.e. Neem oil-56.25%, tobacco leaves-60% and combined with neem oil-tobacco leaves-68.96% whereas ivermectin showed 100% efficacy in group T_4 within 7th day of treatment (Table 1).

Body weight

In control group (T₀), body weight of goats was decreased up to 12.48% on 28^{th} day of treatment. On the other hand, the body weight was increased in all groups to

 Table 1: Effects of neem oil, tobacco leaves, neem oil-tobacco leaves combined, and ivermectin against tick infestation in goats.

Group	NO. OI LICKS	No. of ticks (Post-treatment)					
_	(Pre-treatment)						
_	0 day	7 th day	14 th day	21 st day	28 th day		
T ₀	6.2±0.37	6.8±0.37	7.4±0.24	7.8±0.37	8.4±0.24		
T_1	6.4±0.50	5±0.54	4 ± 0.54	3.2±0.58**	2.8±0.37** (56.25)		
T_2	6±0.44	5±0.44	3.8±0.37	3.2±0.20**	2.4±0.24** (60.00)		
T ₃	5.8±0.48	4.6±0.50	3.6±0.24	2.8±0.20**	1.8±0.20** (68.96)		
T ₄	5.4±0.67	0.00	0.00	0.00	0.00 (100%)		

** Significantly decreased (P<0.01)

Table 2: Effects of neem oil, tobacco leaves, combination of neem oil and tobacco leaves and ivermectin on body weight (Kg).

Groups	Pre-treatment	Post-treatment			
	0 day	7th day	14th day	21st day	28th day
T ₀	15.86±0.51	14.98±0.14	15.27±0.55	14.18 ± 0.08	13.88±0.11 (12.48% ^b)
T_1	15.60 ± 0.50	15.85±0.10	16.20±0.05	15.03 ± 0.02	15.85±0.10 (1.60% ^a)
T_2	14.57±0.51	16.08±0.13	16.38±0.55	15.28 ± 0.07	14.99±0.12 (2.88% ^a)
T ₃	14.45 ± 0.45	14.82 ± 0.07	15.03±0.03	15.17±0.01	15.65±0.04 (5.53% ^a)
T_4	14.79±0.20	15.10 ± 0.40	15.50±0.20	15.35 ± 0.55	15.95±0.15 (7.88% ^a)
Level of sig.	NS	**	**	**	**

** = Significant at 1 percent level (P<0.01); ($\%^{a}$) = Percent of increased; ($\%^{b}$) = Percent of decreased

Table 3: Effects of neem oil, tobacco leaves, combination of neem oil and tobacco leaves and ivermectin on TEC (million/cu. mm) in goat

Groups Pr	Pre-treatment	Post-treatment			
	0 day	7 th day	14 th day	21 st day	28 th day
T ₀	7.55±0.07	7.23±0.02	7.06±0.01	6.91±0.02	6.85±0.01
T_1	6.81±0.04	7.17±0.02	7.63 ± 0.07	8±0.05	8.63±0.06 (26.72%)
T_2	7.88±0.07	8.59±0.06	9.17±0.06	9.46±0.07	9.75±0.03 (23.73%)
T ₃	7.13±0.06	7.85±0.07	8.43±0.07	8.83±0.03	9.32±0.03 (30.71%)
T_4	7.28±0.03	8.83±0.01	9.76±0.06	10.18±0.06	10.5±0.06 (44.23%)
Level of sig.	NS	**	**	**	**

** Significantly increased (P<0.01)

 Table 4: Effects of neem oil, tobacco leaves, combination of neem oil and tobacco leaves and ivermectin on hemoglobin content (gm %) in goat

Group	Pre-treatment	Post-treatment				
	0 day	7 th day	14 th day	21 st day	28 th day	
T ₀	7.70±0.07	7.54±0.08	7.32±0.06	7.21±0.04	7.14±0.06	
T_1	7.51±0.09	7.70±0.11	7.9±0.10	8.02±0.10	8.21±0.09 (9.32%)	
T_2	7.76±0.13	8.00±0.12	8.21±0.12	8.50±0.10	8.71±0.09 (12.24%)	
T_3	7.26±0.15	7.53±0.14	7.76±0.11	8.15±0.10	8.42±0.06 (15.97%)	
T_4	7.28±0.09	7.57±0.07	7.91±0.05	8.48±0.09	8.72±0.10 (19.78%)	
Level of sig.	NS	**	**	**	**	

** Significantly increased (P<0.01); NS = Non-significant

Table 5: Effects of neem oil, tobacco leaves, combination of neem oil and tobacco leaves and ivermectin on PCV (%) in goat

Pre-treatment	Post-treatment			
0 day	7 th day	14 th day	21 st day	28 th day
30.74±0.05	30.59±0.05	30.41±0.04	30.15±0.02	29.8±0.06
30.57±0.09	30.78±0.10	30.87±0.10	31.00±0.09	31.27±0.09 (2.29%)
30.71±0.08	30.9±0.08	31.11±0.07	31.46±0.08	31.59±0.07 (2.87%)
30.53±0.08	30.83±0.09	31.08±0.09	31.46±0.10	31.66±0.09 (3.70%)
30.34±0.07	30.73±0.05	31.23±0.08	31.67±0.08	32.4±0.09 (6.79%)
NS	NS	**	**	**
	$\begin{array}{c} \hline 0 \text{ day} \\ \hline 30.74 {\pm} 0.05 \\ \hline 30.57 {\pm} 0.09 \\ \hline 30.71 {\pm} 0.08 \\ \hline 30.53 {\pm} 0.08 \\ \hline 30.34 {\pm} 0.07 \\ \hline \end{array}$	$\begin{array}{c ccccc} 0 \ day & 7^{th} \ day \\ \hline 0 \ day & 7^{th} \ day \\ \hline 30.74 \pm 0.05 & 30.59 \pm 0.05 \\ 30.57 \pm 0.09 & 30.78 \pm 0.10 \\ 30.71 \pm 0.08 & 30.9 \pm 0.08 \\ \hline 30.53 \pm 0.08 & 30.83 \pm 0.09 \\ \hline 30.34 \pm 0.07 & 30.73 \pm 0.05 \\ \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

** Significantly increased (P<0.01); NS = Non-significant

the extent of 1.60%-7.88%. So, body weight of goats increased significantly in all treated groups on 28^{th} day of treatment (Table 2).

TEC

The mean values of TEC on '0' day in control group T_0 was 7.55 ± 0.07 which decreased gradually and it was 6.85 ± 0.01 on 28^{th} day of treatment. On the other hand, neem oil, tobacco leaves, neem oil-tobacco leaves combined and ivermectin increased the TEC values in

treated goats. On the 28^{th} day of the post-treatment, the mean values of TEC were significantly (p<0.01) (Table 3).

Hb

The mean values of Hb (gm) on '0' day in control group T_0 was 7.70 ± 0.07 and decreased to 7.14 ± 0.06 on the 28^{th} day of treatment. On 28^{th} day of the post-treatment, the mean values of Hb (gm %) were significantly (P<0.01) increased up to 8.21 ± 0.09 ,

8.71 \pm 0.09, 8.42 \pm 0.06 and 8.72 \pm 0.10 in T₁, T₂, T₃ and T₄ groups, respectively (Table 4).

PCV

The mean values of packed cell volume of infected control group T_0 on '0' day was 30.74 ± 0.05 which decreased to 29.80 ± 0.06 on the 28^{th} day of treatment. PCV values were significantly increased in T_1 , T_2 , T_3 and T_4 groups (Table 5).

DISCUSSION

After the treatment with neem oil, tobacco leaves, neem oil-tobacco leaves combined and ivermectin, all the four treated and control group of goats were kept 28 days for observation.

Efficacy of neem

The efficacy of neem oil was 56.25% against tick infestation in goats. Sanjib (2007) stated that efficacy of neem oil was 70% against tick infestation in goats. Muraleedharan *et al.* (2009) reported that undiluted neem oil provided 94.42% reduction of ticks on day 4 in goats. Kalakumar *et al.* (2000) reported that neem oil was found 60.75% effective in cattle infested with ticks which are 7% lower than the result of the present study in goats. However, Nahar (2004) reported that aqueous extract of neem showed 86.67% efficacy in vitro on spray method using 2% concentration.

Efficacy of tobacco

The efficacy of tobacco at 7 days interval was evaluated against tick infestation in goats. Tobacco at 1% concentration showed 60% efficacy against tick infestation. However, Hossen and Mostofa (1999) reported that the efficacy of tobacco-leaf extract (2% solution) and tobacco-copper sulfate (1% tobacco and 1% copper sulfate) formulation against ticks and lice were found to be 100% effective at 21 day post treatment in cattle.

Combined efficacy of neem oil-tobacco leaves

The efficacy of neem oil-tobacco leaves combined at 7 days interval was evaluated against tick infestation in goats. Combined spray with 1% tobacco solution and 4 ml of neem oil showed 68.96% efficacy against tick infestation. However, Hossen *et al.* (2002) examined that tobacco vaseline, tobacco-termeric and Neem-tobacco ointment were found to be 54.41%, 66.38% and 74.80% effective against hump sore in cattle, respectively.

Effectiveness of ivermectin

The effectiveness of ivermectin (200 µg/kg body weight) against ticks was found to be 100% effective at 7th post treatment day and remained effective up to 28th day of post treatment. This result was in agreement with the earlier record of Aziz *et al.* (2012), Hassan *et al.* (2012), Fahima (2003), Yazwinski *et al.* (1997), Sangwan *et al.*, (1995), Vizzio and Caro (1995), Pedroso *et al.*, (1994), Thomson *et al.*, (1994). However, some studies have shown 86.6% (Unur and Irmark, 1993) and 90% (Imrul, 1997) efficacy of ivermectin in sheep and goat, respectively. On comparing the efficacy of neem oil,

tobacco leaves, neem oil-tobacco leaves combined and ivermectin on the basis of disappearance of ticks, ivermectin was found to be the best drug and was effective within 7 days of post treatment. On the other hand, other drugs showed relatively lower efficacy against tick infestation within 7 days of post treatment.

Body weight

Body weight changes were determined pre and post treatment with all groups. The body weight of treated group (T_1 , T_2 , T_3 and T_4) significantly (P<0.01) increased to the extent of 1.60-7.88% respectively. Ryan *et al.* (1997) found that the mean total weight gain was 33.9 kg greater for the ivermectin treated Black Bengal goats than those of untreated control animals (P<0.02); a 34% increase. However, ivermectin influenced the weight gain by limiting the impact of subclinical parasitism.

Hematological parameters

Hematological changes were determined pre and post treatment with neem oil, tobacco leaves, neem oil-tobacco leaves combined and ivermectin. The Hb content and TEC of treated group (T_1 , T_2 , T_3 and T_4) significantly (p<0.01) increased to the extent of 9.32-19.78% and 26.72-44.23% respectively. Increase in PCV values might be due to removal of parasites from the surface of the body. Changes in hematological parameters were also observed in previous studies (Aziz *et al.* (2012), Hassan *et al.* (2012), (Hanif *et al.*, 2005), (Fahima, 2003), (Imrul, 1997) and (Kumar and Jashi, 1992).

Conclusion

Neem, tobacco and their combination are cost effective and easily applicable and available in Bangladesh. Although ivermectin is most effective against ectoparasite (tick), the cost of ivermectin is expensive; administration of the drug requires skilled personnel and may not be available in areas of Bangladesh. This study shows the potential of neem oil, tobacco leaves, combination of both neem oil and tobacco leaves as an effective treatment options against ectoparasite and as a replacement of ivermectin against tick infestation in Black Bengal goat.

Acknowledgement

Authors are highly grateful to the personnel of Sylhet Govt. goat development farm and Dept. of pharmacology and Toxicology to give the opportunity for conducting this study.

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