

## Evaluation of Anthelmintic Efficacy of Ethanolic Extract of *Punica granatum* in Goats

Avinash M. Gadhav<sup>1</sup>, Vikas V. Karande<sup>2\*</sup>, B. C. Ghumare<sup>3</sup>, K. Kundu<sup>4</sup>,  
S. N. Jadhav<sup>5</sup> and P. P. Mhase<sup>6</sup>

<sup>1</sup>M.V.Sc scholar, Department of Pharmacology and Toxicology,

<sup>2\*,3</sup>Assistant Professor, Department of Pharmacology and Toxicology,

<sup>4</sup>Department of Parasitology,

<sup>5</sup>Assistant Professor, Department of Biochemistry,

<sup>6</sup>Assistant Professor, Department of Microbiology,

Krantisinh Nana Patil College of Veterinary Science, Shirwal Dist- Satara,

Bombay Veterinary College, Parel, Mumbai -400012, India

\*Corresponding Author E-mail: [drvikas111081@gmail.com](mailto:drvikas111081@gmail.com)

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### ABSTRACT

As per the 19<sup>th</sup> animal census of India, total population of goat is around 135.17 million contributing 26.40% of total animal population. Maharashtra shares about 6.24% of the total goat population. Helminthic infections are one of the constraints reducing the productivity of these small ruminants and manifested by symptoms like anaemia, emaciation, enteritis, dehydration and death in kids. Chemotherapeutic agents mainly from benzimidazole group, imidathiazole and avermectins are used for the treatment of helminth infections. However, the cost effectiveness and increasing resistance to these chemotherapeutics is the largest field problem. Hence the present study was undertaken in order to evaluate the anthelmintic efficacy of *Punica granatum* in goats. Total 30 infected goats were divided into three groups comprising 10 animals in each. Pre-treatment EPG counts (Mean  $\pm$ SE) of faecal samples from the groups A, B, and C were recorded as 665 $\pm$ 177.81, 790 $\pm$ 210.79 and 720 $\pm$ 136.87 respectively. Group A consisted of ten helminthic infested goats (Mean EPG of 665). Group B animals (Mean EPG of 790) received treatment with fenbendazole @ 5 mg/kg b.wt orally. Group C animals (Mean EPG of 720) received treatment with ethanolic extract of *P. granatum* peel @ 200 mg/kg b.wt orally. Treatment group (B and C) showed significant ( $p \leq 0.01$ ) increase in Hb, TEC, PCV and while the EPG of faeces and TLC were found to be decreased significantly ( $p \leq 0.01$ ) at 15<sup>th</sup> day and 28<sup>th</sup> day in gastrointestinal nematodes infected goats

**Keywords:** *P. granatum*, Benzimidazole, Anthelmintic, Goats

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## INTRODUCTION

Livestock is an integral part of the farming system. In the traditional agricultural economy, livestock plays a crucial role in providing the most needed manure, valuable protein and food like meat and milk. India occupies second position in terms of goat population, fifth position in meat production, growth rate of meat production is 5.21% and situated at first position in milk production. Chevon (goat meat) is most preferred and widely consumed meat in the country contributing 14.25% to total meat production of India. Goats have earned a special place in the lives of poor and landless farmer and therefore it is also known as "poor man's cow". Population of goats is about 135.17 millions, which is 26.40 % of total livestock population in India out of these Maharashtra share 6.24% involving about 79.71 lakh as per 19th census of India (FAOSTAT, 2014).

Helminthic infection of Gastrointestinal (GI) tract is one of the major causes of decreased productivity in goat worldwide, particularly under grazing conditions. GI parasitic infection in goat is characterized clinically by anaemia, emaciation, enteritis, dehydration and death in kids. Parasitic infection in goats also has an adverse effect on blood enzyme levels, which decreases goat production in different biological ways.

*Punica granatum* (*P. granatum*) a member of family Punicaceae, is a favorite table fruit of the tropical and subtropical regions of the world. It is native from Iran to the Himalayas in India and has been cultivated over the whole Mediterranean region since ancient times (Holland *et al.*, 2009). Pomegranate can be grown throughout India but due to its better adaptability towards arid climate, the commercial cultivation is being done in Maharashtra, Karnataka and Rajasthan. Maharashtra is the leading producer of pomegranate in the country and accounts for 66.2% of the total production of pomegranate in the country. Maharashtra state is producing 0.49 million ton of pomegranate

from an area of 0.08 million hector, which is the highest in the country.

Control of gastrointestinal nematode in goat is a big challenge due to the emergence of drug resistance, environmental pollution and food residues. Therefore, there is urgent need for alternate methods of control and to reduce worm burden, which should be less toxic, cheaper with wide margin of safety, easily available, and eco-friendly (Raje & Jangde, 2003). Hence the anthelmintic activity of *P. granatum* was investigated against GI roundworms in goats.

## MATERIALS AND METHODS

The present investigation was carried out at Punyashlok Ahilyadevi Sheep & Goat Development Farm, Mahud, Tal- Sangola, Dist- Solapur to access in vivo evaluation of anthelmintic activity of *A. indica* in caprines by the egg per gram (EPG) count method before and after treatment.

### Collection and Extraction of plant material:

*P. granatum* plant was identified botanically and authenticated from the Department of Botany Shripatarao Kadam Mahavidyalaya, Shirwal. *P. granatum* peels were selected for the ethanolic extraction and extraction was done at Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science Shirwal Dist Satara.

### Experimental animals:

Osmanabadi goats (n=30) of 8 to 12 months age were randomly selected and divided in three groups comprising 10 animals in each. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The helminthic infection was confirmed before beginning of the study by fecal examination of each animal, as per the standard procedure described by Soulsby (1982). The animals having more than 600 eggs per gram (EPG) count were included in this experiment. All groups of experimental goats were housed in separate pens at Punyashlok Ahilyadevi Sheep

and Goat Development Farm Mahud, Tal-Sangola, Dist- Solapur. The goats were allowed free grazing daily early in the morning. Then fed with fresh grass and concentrate diet and ad-libitum potable drinking.

### Experimental groups and treatments:

Total 30 goats were selected on the basis of EPG count and divided in three groups as A, B and C. The experimental groups and schedule of treatment are detailed in Table 1.

**Table: 1 Details of groups and treatment schedules**

Sr. No.	Group	No of Animals	Treatment
1	A	10	Control –untreated
2	B	10	Fenbendazole @ 5 mg/kg b.wt dosed orally once at day '0'.
3	C	10	<i>P. granatum</i> peels extract @ 200 mg/kg b.wt for first five days and on 16 <sup>th</sup> day

### Sample collection and Processing:

The egg per gram (EPG) count of faeces of each fecal sample was determined by modified McMaster Technique (Holland et al., 2009) and calculated as follows:

Egg per gram (EPG) = Number of egg in the chamber x 50

$$\text{FECR \%} = \frac{\text{Pre-treatment EPG} - \text{Post-treatment EPG}}{\text{Pre-treatment EPG}} \times 100$$

Anthelmintic efficacy was calculated by the faecal egg count reduction (FECR) test (Raje, & Jangde, 2003) according to the following formula:

### Statistical analysis

All the values in the test groups are presented as Means  $\pm$  SE and the data was analysed by using Completely Randomized Design (CRD) by using WASP software 'P' value  $p \leq 0.01$  or  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

**General observations:** The body weights were taken on day '0' (before start of experiment) and day 28<sup>th</sup> i.e. termination day of the experiment. All the treatment groups found to be apparently healthy without any side effects or behavioural signs throughout the experimental duration

**Phytochemical analysis:** Phytochemical analysis of ethanolic extract of *P. granatum* peels had showed the presence of alkaloids, reducing sugars, tannins and glycoside.

**Anthelmintic efficacy:** Anthelmintic efficacy of *P. granatum* compared with control and standard group is depicted in Table 2. Pre-treatment EPG counts (Mean $\pm$ SE) of fecal samples from the groups A, B and C were

recorded as 665 $\pm$ 177.81, 790 $\pm$ 210.79 and 720 $\pm$ 136.87 respectively. Pre-treatment EPG count (Mean $\pm$ SE) among the groups did not vary significantly at  $p \leq 0.05$ . Control group A did not show any significant variation in EPG count on 15<sup>th</sup> and 21<sup>st</sup> day of the study.

Pre-treatment EPG count (Mean $\pm$ SE) in group B (standard drug) was 790 $\pm$ 210.79. EPG counts on days 15 and 28 post treatment were 125 $\pm$ 39.612 and 100 $\pm$ 34.155 respectively. Statistically significant decrease ( $p \leq 0.01$ ) in fecal egg counts were recorded on day 15 and day 28 post treatment when compared with pre-treatment EPG count. Post treatment FEC reduction was 84.2% on day 15 and 87.4% on day 28.

The present findings are further supported by the previous studies of Singh<sup>[9]</sup> who reported that fenbendazole @ 10 mg/kg b.wt orally achieved 99.52% reduction in fecal egg count of infected goats on 13<sup>th</sup> day post-treatment. The animals treated with fenbendazole revealed 83% efficacy by fecal egg count reduction test in goats<sup>[8]</sup>. Goats

treated with fenbendazole at a dosage rate of 5 mg/kg body weight orally achieved 71.08% reduction in FEC on 14th day post treatment Lalhmingchhuanmawii et al. (2014) reported that fenbendazole exhibited 85 and 100 % efficacy on day 7th and 14th of post treatment.

Pre-treatment EPG count (Mean±SE) in group C was 720±136.87. EPG counts of faeces on days 15 and 28 post treatment were 210±76.666 and 180± 64.637, respectively. Significant reduction in fecal egg counts were recorded on day 15 and day 28 post treatment when compared with pre-treatment count ( $p \leq 0.01$ ). This fecal egg count reduction was 69.4% on day 15 and 73.7% on day 28.

The present findings are further supported by the previous studies of Boonmasawai (2013) who reported that

alcoholic extract from pomegranate peels reduces worm egg production significantly at day 1, 3 and 7 ( $45 \pm 11\%$ ,  $57 \pm 14\%$  and  $64 \pm 7\%$ , respectively) against gastrointestinal nematodes of infected goats.

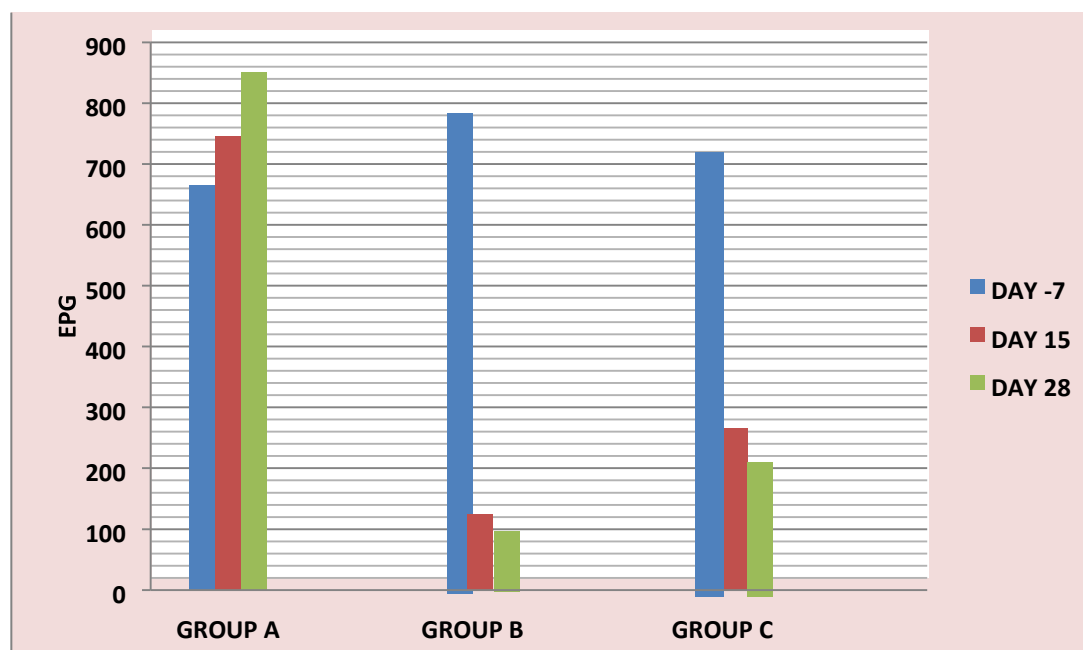
Similarly Lalhmingchhuanmawii et al. (2014) also reported that ethanol extract of *P. granatum* rind achieved maximum significant reduction (97.95 %) in EPG count on day 21 post-treatment with 50 mg/ml concentration of extract against paramphistomes infected sheep.

## CONCLUSION

*P. granatum* (pomegranate) is proved to be a good anthelmintic activity and further studies should be conducted in order to make a feasible pharmaceutical formulation.

**Table 2: EPG count of different groups on Pre-treatment Day (-7<sup>th</sup>) and post treatment 15<sup>th</sup> and 28<sup>th</sup> day**

Group	Treatment	Mean EPG count ± (Mean SE)				
		Pre-treatment Day (-7 <sup>th</sup> )	Day 15 <sup>th</sup>		Day 28 <sup>th</sup>	
		EPG count	EPG count	FECR %	EPG count	FECR %
A	Control	665± 177.81 <sup>ap</sup>	745±186.26 <sup>ap</sup>	-	850± 184.4 <sup>ap</sup>	-
B	Fenbendazole	790± 210.79 <sup>ap</sup>	125± 39.61 <sup>bq</sup>	84.2	100± 34.15 <sup>bq</sup>	87.4
C	Ethanol extract of <i>Punica granatum</i>	720± 136.87 <sup>ap</sup>	275± 95.23 <sup>bq</sup>	61.9	210± 88.75 <sup>bq</sup>	70.9



**Fig. 1: Groupwise EPG count on Day 7, 15 and 28<sup>th</sup> of study**

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