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Comparative Anthelmintic Efficacy of *Curcuma longa*, *Citrullus colocynthis* and *Peganum harmala*

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Abstract: The present experiment was designed to evaluate the anti-haemonchosis activities of the aqueous-methanolic extracts rhizome of *Curcuma (C) longa* (P1), fruit of *Citrullus* (Ct) *colocynthis* (P2) and seed of *Peganum* (P)*harmala* (P3). The formulations consisting of a combination of three plants exhibited highest anthelmintic activity in a dose dependent manner and all the worms were found dead 4 hr post-exposure at the dose rate of 100 mg/ml. Mortality of worms was comparable to that levamisole at 1.50 mg/ml after 2 hours post-exposure. There was no mortality of worms in PBS till 12 hours. *In vivo*, efficacy of the study plants were determined using faecal eggs count reduction test in sheep naturally infected with mixed species of nematodes. The maximum reduction (98.7%) in EPG of faeces was recorded on day 18th post-treatment in sheep treated with combination of three plants at the dose rate of 4 gm/kg body weight. The reduction in EPG (99.4%) with levamisole was comparable to that with herbal extract at the dose rate of 4 gm/kg body weight as mentioned. Therefore, HT seems to be promising as an anthelmintic for animals. Large scale trials on efficacy and safety however, are recommended before the HT studies are considered for commercialization.

Key words: *Curcuma longa* · *Citrullus colocynthis* · *Peganum harmala* · Levamisole · Oxfendazole · Sheep · Ethno veterinary medicines · Anthelmintic efficacy · *Haemonchus contortus*

INTRODUCTION

Helminths (Nemathelminthes: Nematoda) are one of the abiding threat to the livestock population of the world [1]. These are responsible for huge economic losses by decreasing milk and meat production [2]. Among these, gastrointestinal(GI)helminths are the most significant members leading to the retarded growth by sharing the nutrients with their host [3]. These are widely prevalent in the world including Pakistan [4, 5]. Control of these parasites largely depends upon the use of synthetic drugs. Few important problems associated with the use of these synthetic drugs are development of resistance in parasites [6]; potential residues of drugs in milk, meat and other animal products [7] and difficult access of herds men to the veterinary personnel. Such kind of constraints forced scientists to explore alternate strategies like phyto therapy over the last decade which could replace the existing chemotherapy against parasites [8]. A handsome data is available on the efficacy of herbal plants from various regions of Pakistan [9, 10, 11]. C. *longa* has been reported as analgesic, antibacterial, antioxidant, expectorant and flavoring agent [12], while *Ct. colocynthis* as anthelmintic, anti-diabetic, antipyretic, anti-rheumatic, carminative and purgative [13]. The seeds of *P. harmala* have been reported as anticestodal, antimalarial [14], insecticidal [15], antibacterial [16], antiparasitic, antifungal, antialgal, antitheilerial [17], analgesic and anti-inflammatory agent [18]. The selected plants are rich in phyto constituents like alkaloids, anthraquionones, flavonoids, glycosides, steroid, tannins and terpenes. In continuation with the previous studies, the present study was designed to determine the anthelmintic activity of the three plants extracts viz; (1) *C. longa*(haldi), *Cit. colocynthis* (tumma) and *P. harmala* (hermal) in various combinations.

MATERIALSAND METHODS

Preparation of Plant Materials: The three Plants were collected from field and identified from an expert in the Department of Botany, University of Agriculture,

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Table 1:Treatments strategy of the study plants for their anti-haemonchosis activity

Sr. No.	Treatments	Formulation	Dosage
1	P1	*Curcuma longa*	50, 25, 12.5, 6.25, 3.12, 1.56 mg/ml
2	P2	*Citrullus colocynthis*	
3	P3	*Peganum harmala*	
4	P1 × P2	*C. longa × Cit. colocynthis*	
5	P1 × P3	*C. longa × P. harmala*	
6	P2 × P3	*Cit. colocynthis × P. harmala*	
7	P1 × P2× P3	*C. longa × Cit. colocynthis × P. harmala*	
8	Levamisole	Nilverm	1.50mg/ml

Table 2: *In vitro*, egg hatch % and LC50 of crude aqueous methanolic extracts against Trichostrongylidae

	Egg hatch % on different concentrations (µg/ml) of crude aqueous methanolic extracts					
Treatment	1.2	12	120	1200	12000	LC_{50} µg/ml
Plant 1	82	69	56	30	10	69.7543
Plant 2	71	50	34	16	0	62.5321
Plant 3	76	61	52	26	6	76.4326
P1 + P2	73	55	44	22	5	46.1243
P1 + P3	77	63	54	24	7	44.5671
P2 + P3	70	52	45	18	2	41.7831
P1 + P2 + P3	62	48	42	8	0	14.5431
Oxfendazole	60	51	40	6	0	0.0441

Plant 1= *Curcuma longa;* Plant 2= *Citrullus colocynthis;* Plant 3= *Peganum harmala*

Faisalabad Pakistan. Plants were dried in dry and cold place to prepare crude aqueous-methanolic extracts (CAME) following the methods of Tabassum *et al*. [19]. Briefly, dried powdered plant materials were extracted with methanol-aqueous (70:30) for about 72 hours.Solvents of each part of a plant were combined, filtered and evaporated at a reduced pressure in a rotary evaporator (Heidolph). The CAME of three plants were formulated in the treatment plan given in the Table 1.

Adult Motility Assay (AMA): Mature live *Haemonchus (H.) contortus* (females) were collected from the abomasum of freshly slaughtered sheep in the local abattoir. The worms were washed and finally suspended in phosphate buffer saline (PBS). A minimum of ten worms were exposed to each of the following treatments in separate petri dishes at room temperature (25-30°C).

The motility was observed on 0, 2, 4, 6, 8, 10 and 12 hours post treatment. Finally, the treated worms were kept for 30 minutes in the lukewarm PBS to observe the revival of motility. The numbers of dead and survived worms were recorded for each treatment.

Egg Hatch Test (EHT): Sheep faeces (10-15g) were collected from the sheep naturally infected with mixed species of nematodes [20]. The concentration of eggs was estimated in 50µl samples and was adjusted to 100-150 eggs/ml. One milliliter of egg suspension was added in a 24-flat bottomed microtitration plate and mixed with the same volume of the treatment given in the Table 2. The positive control wells received different concentrations of oxfendazole (Systemax®-ICI Pakistan, Ltd., 2.265% w/v). While the negative control wells were filled with the diluents and the egg solution. The eggs were incubated for 48 hours and then two drops of Lugol's iodine solution was added to stop the hatching of eggs. All the eggs (dead and embryonated) and hatched larvae in each well were counted.

Faecal Egg Count Reduction Test (FECRT): Ten sheep (6-12 months old) in each of the nine groups (A through I) having naturally acquired mixed parasitic infestation of gastrointestinal nematodes were selected. Prior to the start of treatment, faecal samples were collected directly from rectum of each animal, at least three times at an interval of three days to determine the number of eggs per gram of feaces. Sheep were grouped considering their live weights and EPG. Group A was considered as negative control sham treated while group B as a positive control drenched with single dose of levamisole at the dose rate of 7.5mg/ml (ICI Pakistan Ltd, Animal Health Division). Groups C to I were treated with different doses of CAME of the three Plants alone or in different combinations at the dose rate of 1, 2 and 4 g/kg body weight.

The body weight of each sheep was recorded weekly. Faecal egg counts were conducted for each animal on day 0, 3, 6, 9, 12, 15, 18 post-treatment and egg count reduction (ECR) was calculated using the following formula:

$$\text{ECR (\%)} = \frac{\text{Pre treatment EPG - post treatment EPG}}{\text{pre treatment EPG}}$$

Data Analysis: The data of AMA, EHT andFECRT were analyzed by analysis of variance (ANOVA) and probit analysis test by using SAS software [21].

RESULTS

The formulations consisting of a combination of three plants (*C. longa* x *Cit. colocynthis* x *P. harmala*) exhibited highest anthelmintic activity in a dose dependent manner and all the worms were found dead 4 hr post-exposure at the dose rate of 100 mg/ml (Figure 9). Mortality of worms was comparable to that levamisole (reference drug) at 1.50 mg/ml after 2 hours post-exposure. There was no mortality of worms in PBS till 12 hours. All the selected herbal extracts inhibited egg hatching and thus indicated their ovicidal effects. A comparison of the LC_{50} of different plants revealed that the combined effect of these three plants (*C. longa, Cit. colocynthis, P. harmala*) was the best (LC_{50}= 14.54 µg/ml) in order by P2xP3 (41.78 µg/ml), P1xP3 (44.56 µg/ml), P1xP2 (46.52), P2 (62.53µg/ml) and P1 (69.75µg/ml); whereas, individual impact of *P. harmala* was recorded having lowest efficacy (LC_{50}=76.43) as has been depicted in Table 2 and it is well supported by the linear Probit analysis in Figure 1, 2, 3, 4, 5, 6 and 7 for plants and 8 for oxfendazole. Herbal extracts exhibited anthelmintic activity in a time and dose-dependent manner as compared to negative control. The maximum reduction (98.7%) in EPG of faeces was recorded on day 18th post-treatment in sheep treated with combination of three plants at the dose rate of 4 gm/kg body weight. The reduction in EPG (99.4%) with levamisole was comparable to that with herbal extract at the dose rate of 4 gm/kg body weight as mentioned in Figure 10.

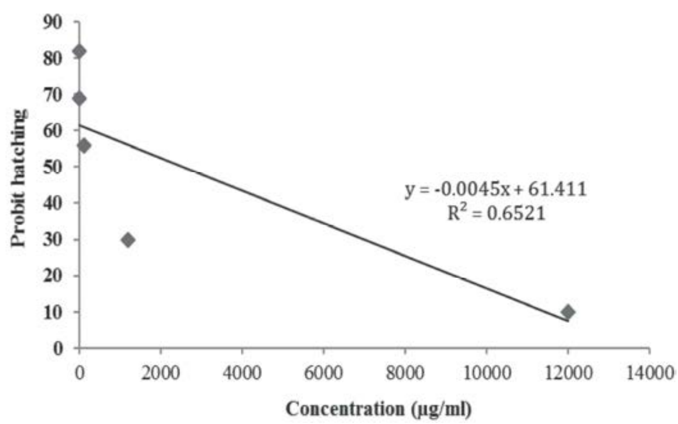


Fig. 1: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and *Curcuma longa* aqueous methanolic extract concentration (µg/ml)

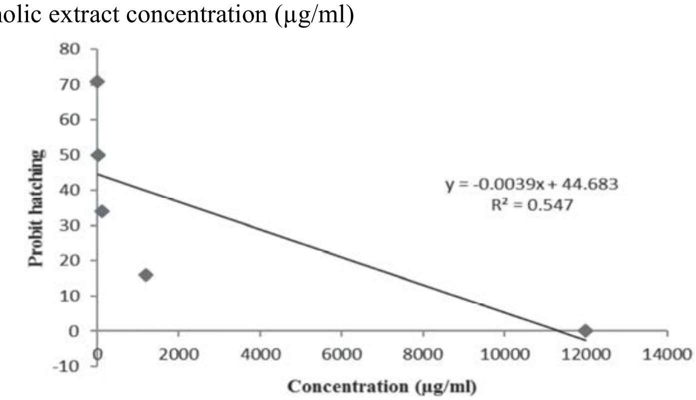


Fig. 2: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and *Citrullus colocynthis* aqueous methanolic extract concentration (µg/ml)

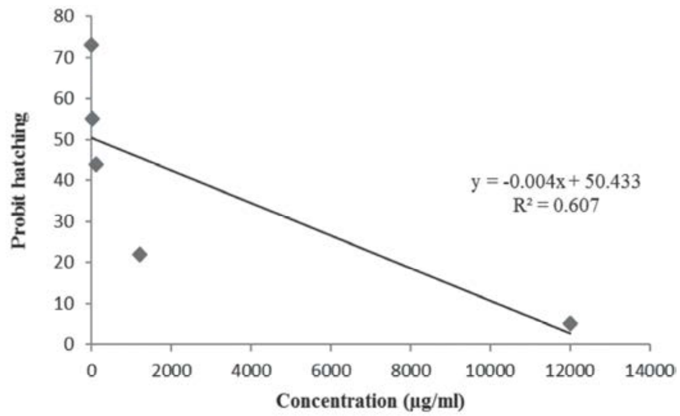


Fig. 3: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and *Peganum harmala* aqueous methanolic extract concentration (µg/ml)

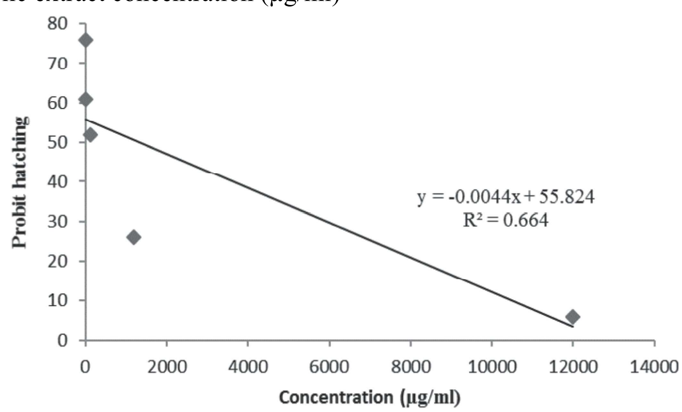


Fig. 4: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and *Curcuma longa* and *Citrullus colocynthis* aqueous methanolic extract concentration (µg/ml)

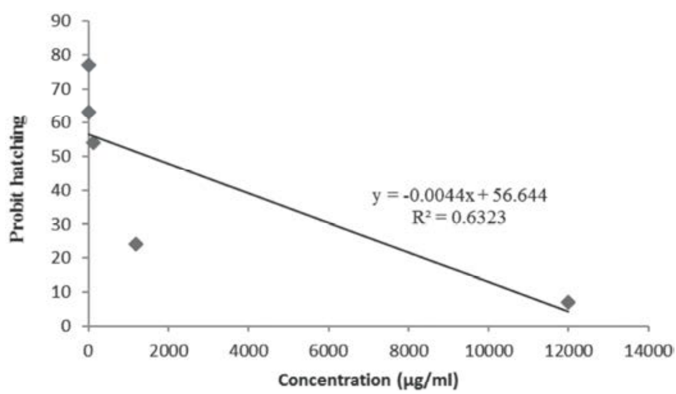


Fig. 5: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and *Curcuma longa* and *Peganum Harmala* aqueous methanolic extract concentration (µg/ml)

DISCUSSION

The current study revealed comparative individual and combined anthelmintic efficacy of three plants viz; *C. longa, Ct. colocynthis, P. harmala.* Highest efficacy of the three plants in combination could be due to synergistic effect of active phyto-constituents i.e alkaloids, saponins, flavonoids, teroenes, steroids, etc. present in the plant extract [22]. Individual effects of *C. longa* as an anthelmintic has revealed that some parts of cucurbits process anthelmintic properties due to secondary metabolite cucurbitacin contents.

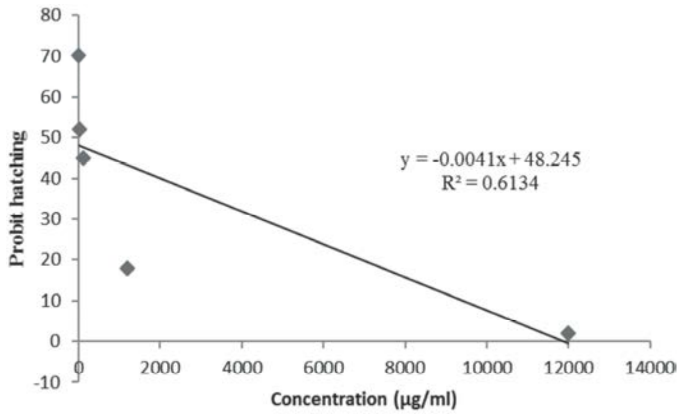


Fig. 6: Linear relationship between egg hatching % on the Probit scale of *H. contortus* and *Citrullus colocynthis* and *Peganum Harmala* aqueous methanolic extract concentration (µg/ml)

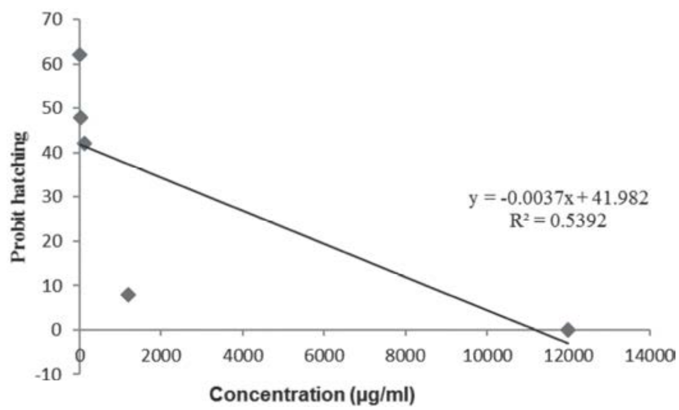


Fig. 7: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and *Curcuma longa,Citrullus colocynthis* and *Peganum Harmala* aqueous methanolic extract concentration (µg/ml)

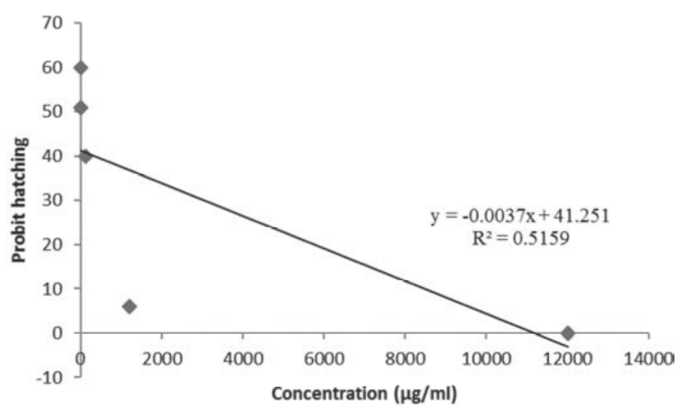
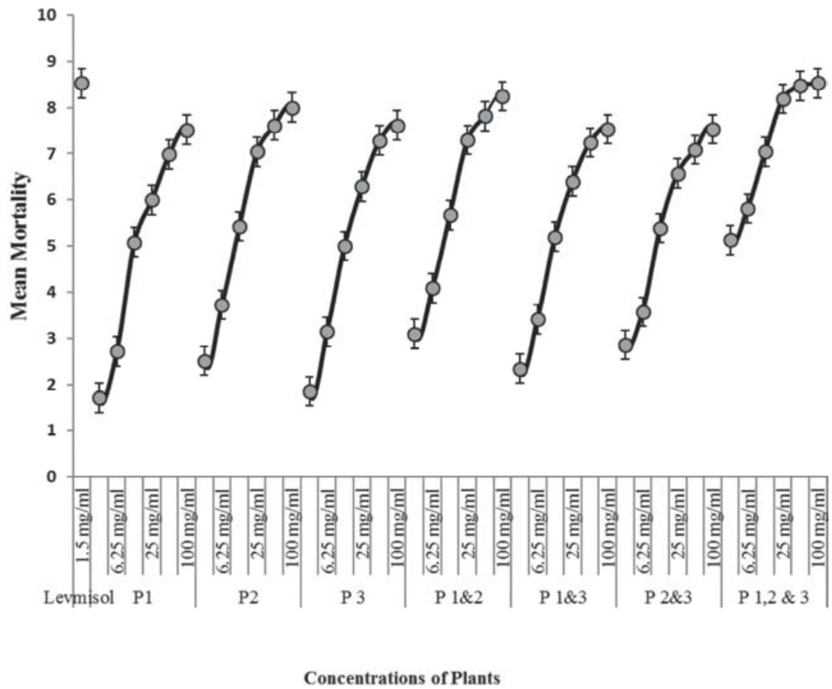


Fig. 8: Linear relationship between egg hatching % on the Probit scale of Trichostrongylidae and Oxfendazole

Moreover, constituents of *C. longa* exert several protective effects on the gastrointestinal tract e.g. sodium curcuminate inhibited intestinal spasm and p-tolymethylcarbinol, a turmeric component, increased gastrin, secretin, bicarbonate and pancreatic enzyme secretion [23].Turmeric has also been shown to inhibit ulcer formation caused by helminthes in the form of stress, release of alcohol, indomethacin, pyloric ligation which significantly increases gastric wall mucus in animals subjected to these gastrointestinal insults [24].

Methanolic extract of *Ct. colocynthis* showed a good sensitivity against *S. parasitica* (helminth) [13, 25] revealed the effect of various extracts of leaves of *Ct. colocynthis* on Pheretim Posthuma. The ethanol and



P1= *Curcuma longa;* P 2= *Citrullus colocynthis;* P3= *Peganum harmala*

Fig. 9: *In vitro* effect of crude-aqueous methanolic extracts on survival of Trichostrongylidae

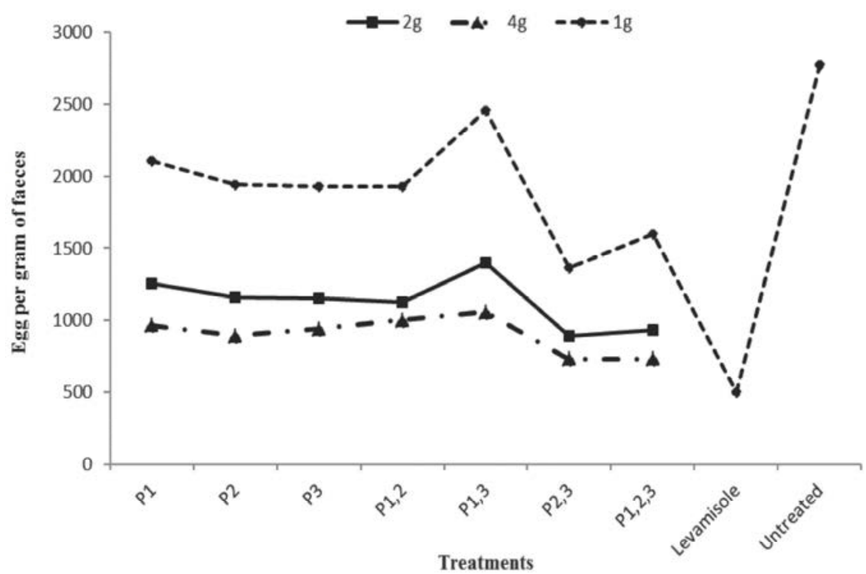


Fig. 10: Anthelmintic activity of various concentrations of plants (P1= *Curcuma longa;*P2= *Citrullus colocynthis;* P3= *Peganum harmala*)

aqueous extracts of leaves of *Ct. colocynthis* showed better helmintholytic activity. These extracts required the least time for causing paralysis and death of the earthworms followed by other extracts. Leaf of *Ct. colocynthis* displayed intrinsic helmintholytic properties with at the dose rate of 40 mg/ml giving a shortest time of paralysis and death.

Fruit of *Ct. colocynthis* had been used as a strong laxativeto treat *Trypanosomalewisi*, refractory oedema, amenorrhoea, nerves pain, fever and snake-bite, parasites and muscle pain in hand and feet. In many countries, seeds were used by nomads in traditional medications applied to the parasite [26]. The function of the anthelmintic drugs like albendazole is to cause paralysis of worms so that they are expelled in the faeces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at the concentration of 40 mg/ml as compared to that with albendazole [13].

[27] reported anthelmintic efficacy of *P.harmala* against GI cestodes of goats. Powder of *P. harmala* seed at the dose rate of 3 g/kg, its equivalent water and methanol extract and Nilzan®at the dose rate of 5 ml/15 kg resulted in 100±0, 89±32, 92±41 and 98±62% reduction in EPG, respectively. Results of all the three tests indicated anthelmintic efficacy of the herbal extract. In adult motility assay, herbal extract(HT) exhibited anthelmintic effects in a dose dependent manner and worms were found dead at 2hours post-exposure at 3.125 mg/ml and above. Similarly, ovicidal effects of HT were recorded in a dose dependent manner with LC_{50} value of 76.43µg/ml, which was far greater than that of standard drug oxfendazole, i.e.0.0441µg/ml. *In vivo*, maximum reduction (98.6%) in eggs per gram of faeces (EPG) was recorded on day 18 post-treatment in sheep treated at the dose rate of 4 g/kg body weight compared to 99.2% with levamisole, which was comparable to that with combined herbal extract. These findings have suggested that, (a) plant extracts areappropriate for the resource-poor farmers as broad spectrum anti-parasitic, (b) herbal crude products are cheaper and easily available, (c) assimilation of these formulations in integrated parasitic management practices will add to the sustainability and thus generate income of the farmers, (d) all the plants used in this study are commonly used and available in the rural areas and farmers can easily use the combination of these plants. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane [28], is attributed to their ability to interclate with DNA [29].

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