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RESEARCH ARTICLE

Anthelmintic Effects and Toxicity Analysis of Herbal Dewormer against the Infection of *Haemonchus contortus* and *Fasciola hepatica* in Goat

Rao Zahid Abbas[1], Muhammad Arfan Zaman[2], Zia ud Din Sindhu[1], Muhammad Sharif[3], Azhar Rafique[4], Zohaib Saeed[1*], Tauseef ur Rehman[5], Faisal Siddique[6], Tean Zaheer[1], Muhammad Kasib Khan[1], Muhammad Subbayyal Akram[1], Arslan Javed Chattha[1], Urooj Fatima[7], Tabassum Munir[7] and Muhammad Ahmad[7]

[1]Department of Parasitology, University of Agriculture, Faisalabad-Pakistan; [2]Department of Pathobiology, College of Veterinary and Animal Sciences, Jhang, Pakistan; [3]Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad-Pakistan; [4]Department of Zoology, Government College University, Faisalabad, Pakistan; [5]Department of Parasitology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-Pakistan; [6]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur-Pakistan
[7]Livestock Production and Research Institute, Okara, Pakistan
*Corresponding author: zohaibsaeedahmad@gmail.com

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ABSTRACT

Helminths have been a serious trouble for the farmers due to their adverse effects on small ruminant production. *Hemonchus contortus* (*H. contortus*) and *Fasciola hepatica* (*F. hepatica*) are highly pathogenic and pose serious threat in co-existence. Because of increasing threat of anthelmintic resistance, alternative methods are being investigated and the herbal remedies stand the most prominent due to their efficacy and availability. Anthelmintic activity of the herbal mixture was evaluated in goats. Adult goats were selected at Livestock Production and Research Centre (LPRI) Bahadarnagar, Okara and were examined for helminth eggs in their feces. Twenty-four goats having almost similar egg per gram (EPG) of feces values of *H. contortus* and *F. hepatica* were selected and allocated to four groups of equal size. Herbal mixture was administered at dose rate of 1400, 1200 and 1000 mg/kg to each member of groups G1, G2 and G3, respectively on day 0, 7, 14 and 21 of the trials, while group G4 served as negative control receiving no medicine/herbal mixture. Trials continued for 30 days and data about EPG, fecal egg count reduction, complete blood count and serum biochemistry were collected before administration of herbal mixture at day 15 and day 30 of trial while animals were weighed before initiation of trial (day 0) and at the end of trial (day 30). All treatments showed a significant reduction in fecal egg count as compared to control group. Maximum fecal egg count reduction was observed in animals of group G1, which is 91.35% for *H. contortus* and 82.35% for *F. hepatica.* There was a slight increase in weight gain ratio of the treated animals in respective decreasing order with G1 having highest weight gain. An increase in erythrocyte count, pack cell volume and hemoglobin concentration was recorded while non-significant effect was observed on serum parameters. The results of this trial suggested that herbal dewormer is effective and can be an option for integrated management strategies for nematodes and trematodes control in the goats.

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INTRODUCTION

Goats are capable of providing excellent production efficiencies under low nutritive resources and have major impact on the livelihood of resource poor community of the developing countries. Parasites are among the most common infectious agents in livestock causing a number of problems, which may lead to death (Rehman *et al*., 2016; Naqvi *et al*., 2017; Mehmood *et al*., 2017; Isah *et al*., 2018; Abdel-Saeed and Salem, 2019; Li *et al*., 2019; Hasni *et al*., 2020; Imran *et al*., 2020). Helminths pose great threat to the animals as most of their infections remain latent, causing decrease in production and reproduction losses (El Shanawany *et al*., 2019; Sithole *et al*., 2019; Zafar *et al*., 2019). Helminths may lead the economic losses of 50.67 US dollars per animal per day and 17.94% of total economic costs in animals (Rashid *et al.*, 2018). Clinical signs include emaciation, loss of appetite, dehydration, diarrhea, anemia and edema (Gupta *et al.*, 2017; Satsya *et al.*, 2018). Elimination of the parasites needs a simultaneous control of different classes of helminths.

Chemical pharmaceutical substances have been widely used for the control of helminths (Saddiqi *et al*., 2006). These products are proven effective agents for the control of helminthiasis in last decades, but they are costly for the farmers (Gupta *et al.*, 2016). Anthelmintics are being used up to 53% of total veterinary drugs (Lira *et al.*, 2008) and this excessive use of anthelmintics poses a great economic stress to the farmer. Besides this, alarming threat of anthelmintic resistance is major hindrance in development of small ruminant industry (Qamar *et al.*, 2011 Falzon *et al.,* 2014; Gasbarre, 2014). The most dangerous aspect is that these drugs are hazardous for public health due to their metabolic residues in meat and milk of animals. Hence the demand for search of suitable, potent and safe alternative is overwhelming (Zaman *et al*., 2017; Abbas *et al*., 2018; Fayyaz *et al*., 2019). Although lot of efforts is being made to find suitable alternatives including vaccines, use of organic acids and immune modulators but use of herbsis most promising of them.

Herbal products have been used as therapeutic agents since centuries. Modern studies proved antibacterial, antiviral, antiparasitic and anti-inflammatory effects of various plants (Zaman *et al*., 2017, 2020; Abbas *et al*., 2018, 2019; Khater *et al*., 2018; Mahmood *et al*., 2018; Fayyaz *et al*., 2019; Luce, 2019; Salman *et al*., 2020). Plants are economical, safe and easily available. Many plant families have been proved for anthelmintic activity because of presence of a number of compounds having anthelmintic properties (Newman and Cragg, 2016; Romero-Benavides *et al.*, 2017).

The present work was designed to evaluate a mixture of plants from a number of families used in combination. Various parts of plants belonging to families *Lamiaceae, Combretaceae, Rosaceae, Apiaceae, Fabaceae, Gentianaceae, Linaceae, Nitrariaceae, Asclepiadaceae, Cucurbitaceae*, *Theaceae*and *Brassicaceae* with already proven anthelmintic activities were selected (Santos *et al.*, 2019). A mixture was formed to evaluate their effect on nematode (*H. contortus*) and trematode (*F. hepatica*) infection on goats.

MATERIALS AND METHODS

P*lant material:* Parts of selected plants were purchased from local market of Faisalabad. Identification of plants was performed by Department of Botany, University of Agriculture Faisalabad. Plant material was partially ground and mixed to make herbal dewormer. The detail about the composition of herbal dewormer has been given in Table 1.

Table 1: Composition of herbal dewormer including families, scientific names and common names of plants

Family name	Scientific name	English name	Weight (%)
Lamiaceae	*Mentha spicata*	Mint	2
Combretaceae	*Combretum indicum*	Chinese honeysuckle	2
	Terminalia chebula	Chebulic myrobalan	2
Rosaceae	*Rosa sericea*	Rose	2
Apiaceae	*Foeniculumvulgare*	Fennel	18
	Trachyspermumammi	Bishop's weed	18
	Cuminumcyminum	Cumin	3
Fabaceae	*Glycine soja*		10
	Sansevieriatrifasciata	Viper's bowstring hemp	10
	Casia fistula	Golden rain tree	2
Gentianaceae	*Swertia L.*	Felworts	1
Linaceae	*Linumusitatissimum*	Linseed	2
Nitrariaceae	*Peganumharmala*	Harmel	2
Asclepiadaceae	*LeptadeniaReticulata*	Beaumont root/, black root	3
Theaceae	*Camellia sinensis*	Camilla	4
Brassicaceae	*Lepidium sativum*	Garden cress	1
Cucurbitaceae	*Citrulluscolocynthis*	Korrtumma	2

Animals: Non-pregnant, non-lactating adult goats of non-descript type having an age of 2-3 years were selected randomly at Livestock Production and Research Institute, Bahadarnagar, Okara. Fecal samples of hundred goats were collected and examined for EPG at day 0 of experiment. Among them twenty-four goats having similar EPG values were selected as per recommendations of World Association for Advancements of Parasitology (Sunandhadevi *et al*., 2017; Joachim *et al*., 2018).

Experiment design: Selected animals were then placed into four groups namely G1, G2, G3 and G4. Group G4 was kept as control while other three were administered with herbal mixture (Bio-dewormer) at dose rate of 1400 mg/Kg, 1200 mg/Kg and 1000 mg/Kg to group G1, G2 and G3 respectively. Treatments continued for four weeks at an interval of 7 days. Fecal samples and blood samples were collected fortnightly while animals were weighed at initiation and termination of trial.

Evaluation of fecal egg count reduction and egg per gram of feces: Fecal samples were collected in polythene bags and carried to the Department of Parasitology, University of Agriculture Faisalabad where samples were subjected to floatation (*H. contortus*) and sedimentation technique (*F. hepatica*) to determine EPG by modified McMaster technique (MAFF, 1986; Gupta *et al.*, 2017) and eggs were identified using standard keys (Soulsby, 1982; Zaman *et al.*, 2012). Fecal Egg count reduction was determined by following the equation (Dash *et al.* 1988).

$$\text{FECR} = \frac{(Pre - TreatmentEPG - PostTraetmentEPG)X100}{Pre - TreatmentEPG}$$

Weight gain: Animals were weighed at day 0 and day 30 of trial. Average weight gain of animals was recorded by calculating difference in weight at day 30 and day 0.

Hematology and serum biochemistry: Blood samples were collected at days 0, 15 and 30 of trial, sera were separated and subjected to hematological and serum biochemistry analyses to estimate the toxic effects of herbal mixture on blood parameters including hemoglobin value, leukocyte count, erythrocyte count, packed cell volume and serum parameters (Gupta *et al.*, 2017). Serum chemistry was done by using standard kits and results were obtained to evaluate the toxic effects of dewormer (Gupta *et al*., 2017).

Statistical analysis: Hierarchial design was used for the analysis of the data of this experiment and Duncan Multiple Range Test was used for the comparison of means (Gupta *et al*., 2017). Data were statistically analyzed using SAS statistical software (SAS, 1998).

RESULTS

Effect on fecal egg count: Reduction in egg counts was observed over the period of thirty days. Significant reduction in fecal egg counts was recorded in all three groups treated with serially decreasing dose of herbal mixture in comparison to control group, which was negative control. Egg counts were found to be reduced up to 90.91, 80.60 and 53.62% in groups G1, G2 and G3, respectively. Highest reduction in egg counts was calculated in group G1 treated with highest dose of herbal mixture that is 91.35 and 82.33% in the egg count of H. contotus and F. hepatica, respectively (Table 2 & 3).

Effect on hematological parameters: Significant effects on blood parameters were observed at day 30 of experiment. Significant increase in PCV & Hb was observed in members of group G1 at day 30 when compared with those of control group (Table 4). RBCs counts were found to be significantly higher in members of group G1 and G2 in comparison to control group. However, WBCs were found to be significantly lower in groups G1 and G2 than control group.

Effect on serum biochemistry: Serum values obtained at the initiation and termination of the trial indicated the positive effects on the serum. Values of AST (SGOT/ serum glutamic-oxaloacetic transaminase) (Fig. 1, ALT (SGPT/serum glutamic-pyruvic transaminase) (Fig. 2), T. Bilirubin (Total Bilirubin) (Fig. 3), ALK Phos. (alkaline phosphate) (Fig. 4), Total Serum Protiens (Fig. 6) and Total albumins in the serum (Fig. 7) were increased significantly. While values of GGT (Gema Glutamyl Transferase), (Fig. 5) were decreased, LDH (lactate dehydrogenases) (Fig. 8), Creatinine (Fig. 9) and BUN (Blood Urea Nitrogen) (Fig. 10) significantly decreased in group G1 and G2 while there was no significant effect on their values in G3 and G4. No toxic effect of herbal mixture on serum biochemistry of goats was observed in all doses.

Effect on weight gain: Significant difference in weight gain of all treated groups from control was observed with maximum weight gain in group G1 along with gain in weights of group G2, G3 and G4 in decreasing order (Table 5).

DISCUSSION

The present work reports the *in vitro* evaluation of anthelmintic activity of this herbal mixture for concurrent infection of haemonchosis and fasciolosis. The mixture used contained a number of plants, all are locally available and economical to the village population which rears the goats. Helminth species identified in goats of Okara are similar to previous reports (Khan *et al*., 2010; Rashid *et al*., 2016) in which *H. contortus* was the most prevalent species.

Table 2: Eggs per gram values of total helminths, *H. contortus* and *F. hepatica* in four groups of goats at Day 0, 15 and 30

Group	Total Helminths			*H. contortus*			*F. hepeatica*		
	D0	D15	D30	D0	D15	D30	D0	D15	D30
G1	2383.3± 116.7a	616.7± 72.6cd	216.7± 44.1d	566.7± 44.10a	216.7± 44.10def	49.0± 22.8f	283.3± 88.19bcd	133.3± 33.33cde	50.0± 28.87e
G2	2233.3± 101.4a	950.0± 50.0bc	433.3± 16.7d	516.7± 16.67ab	250.0± 28.87de	80.3± 11.27ef	383.3± 60.09ab	216.7± 16.67b-e	83.3± 16.67de
G3	2283.3± 116.7a	1266.7± 159.0b	1066.7± 164.1bc	500.0± 28.87ab	333.3± 60.09bcd	300.0± 28.87cd	366.7± 33.33ab	275.0± 14.43bcd	258.3± 36.32b-e
G4	2183.3± 92.8a	2216.7± 83.3a	2200.0± 86.6a	483.3± 44.10abc	510.7± 35.3ab	531.8± 9.10a	300.0± 28.87bc	516.7± 33.33a	533.3± 44.10a

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 3: Percent fecal egg count reduction (FECR) of total helminths, *H. contortus* and *F. hepatica* in four groups of goats at day 15 and 30

Group	Total Helminths FECR (%)		*H. contortus* FECR (%)		*F. Hepatica* FECR (%)	
	D0-15	D0-30	D0-15	D0-30	D0-15	D0-30
G1	74.13	90.91	61.76471	91.17647	52.94118	82.35294
G2	57.46	80.60	51.6129	83.87097	43.47826	78.26087
G3	44.53	53.28	33.33333	40	25	29.54545
G4	-1.53	-0.76	-6.89655	-10.3448	-72.2222	-77.7778

Table 4: Blood parameter values of four groups of goats

Group	PCV			Hb			RBC			WBC		
	D0	D15	D30	D0	D15	D30	D0	D15	D30	D0	D15	D30
G1	25.93± 0.19^{bc}	26.54± 0.27ab	27.23± 0.15a	8.87± 0.20bc	9.37± 0.26ab	10.06± 0.10a	9.85± 0.17bc	10.45± 0.25ab	10.95± 0.11a	11.25± 0.13ab	10.74± 0.14bc	9.82± 0.06c
G2	25.90± 0.06^{bc}	26.12± 0.34bc	26.38± 0.20abc	8.61± 0.26bc	8.98± 0.11bc	9.09± 0.11bc	9.86± 0.13bc	10.08± 0.19bc	10.31± 0.25ab	11.63± 0.28ab	11.22± 0.22ab	10.71± 0.28bc
G3	25.54± 0.20*	25.74± 0.14bc	25.84± 0.06bc	8.83± 0.23bc	8.93± 0.21bc	9.03± 0.18bc	9.80± 0.09bc	9.87± 0.09bc	9.97± 0.08bc	11.63± 0.22ab	11.46± 0.23ab	11.26± 0.18ab
G4	25.64± 0.20bc	25.59± 0.20bc	25.40± 0.18c	8.77± 0.12bc	8.53± 0.11bc	8.27± 0.22c	9.93± 0.05bc	9.79± 0.08bc	9.45± 0.14c	11.69± 0.34ab	11.82± 0.32ab	12.04± 0.15a

Means sharing similar letter in a row or in a column within a box are statistically non-significant (P>0.05). Where PCV stands for Packed Cell Volume, Hb stands for Hemoglobin concentration, RBC stands for Red Blood Cells (Erythrocyte) and WBC stands for White Blood Cells (Leukocytes).

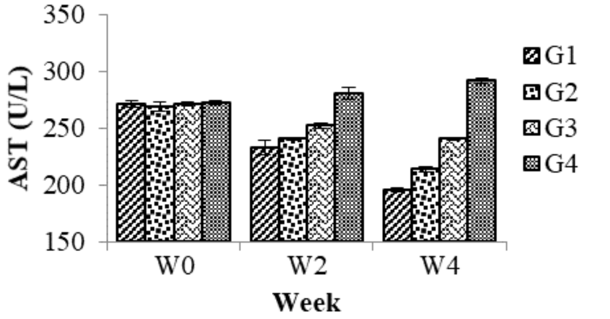

Fig. 1: Figure indicating the effect of herbal dewormer on AST (SGOT) in the serum of the goats. Values sharing means are statistically non-significant (P>0.05).

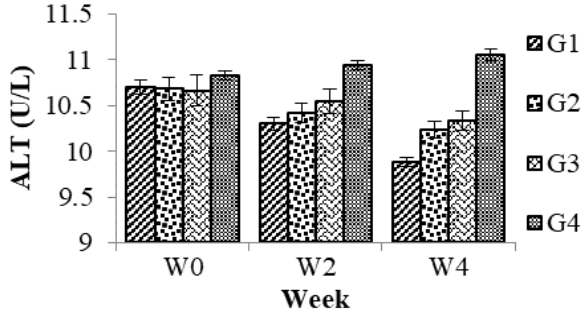

Fig. 2: Figure indicating the effect of herbal dewormer on serum values of ALT (SGPT/ serum glutamic-pyruvic transaminase) of goat. Values sharing means are statistically non-significant (P>0.05).

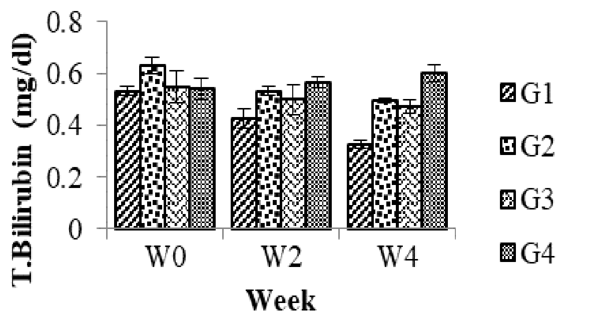

Fig. 3: Figure indicating the effect of herbal dewormer on T. Bilirubin (Total Bilirubin) values in serum of goat. Values sharing means are statistically non-significant (P>0.05).

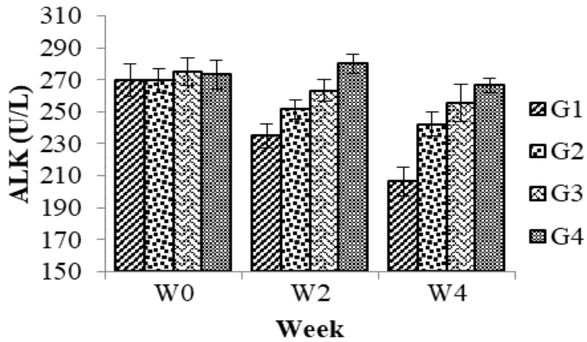

Fig. 4: Figure indicating the effect of herbal dewormer on ALK Phos. (Alkaline Phosphatase) in the serum of goat. Values sharing means are statistically non-significant (P>0.05).

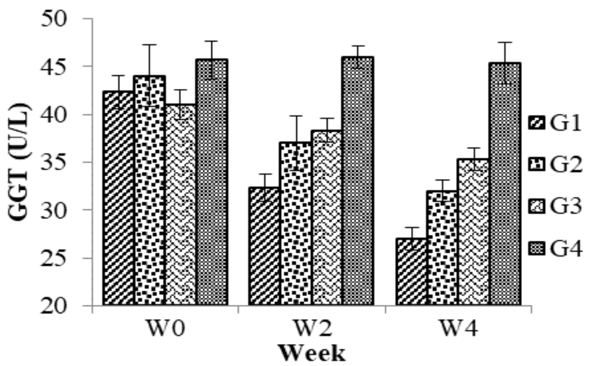

Fig. 5: Figure indicating the effect of herbal dewormer on GGT (Gamma Glutamyl transferase) in the serum of goat. Values sharing means are statistically non-significant (P>0.05).

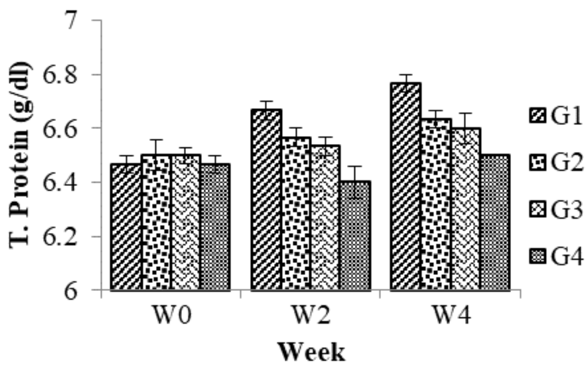

Fig. 6: Figure indicating the effect of herbal dewormer total serum proteins values in serum of goat. Values sharing means are statistically non-significant (P>0.05).

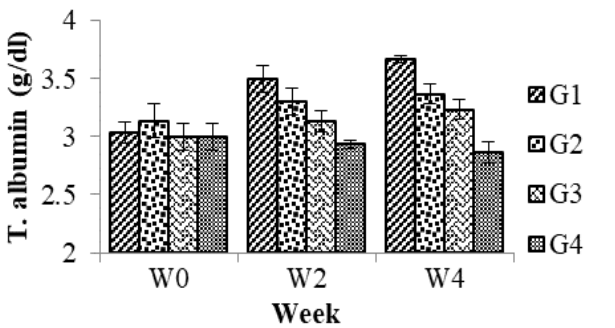

Fig. 7: Figure indicating the effect of herbal dewormer on total albumin values in serum of goat. Values sharing means are statistically non-significant (P>0.05).

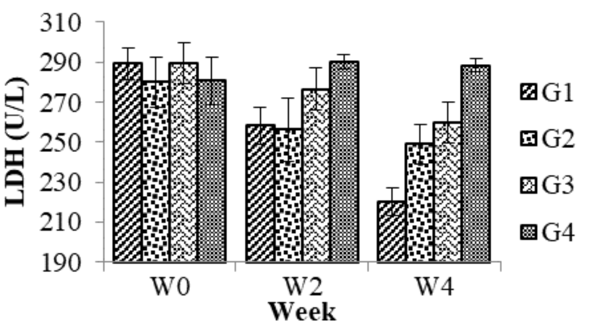

Fig. 8: Figure indicating the effect of herbal dewormer on LDH (lactate dehydrogenases) values in serum of goat. Values sharing means are statistically non-significant (P>0.05).

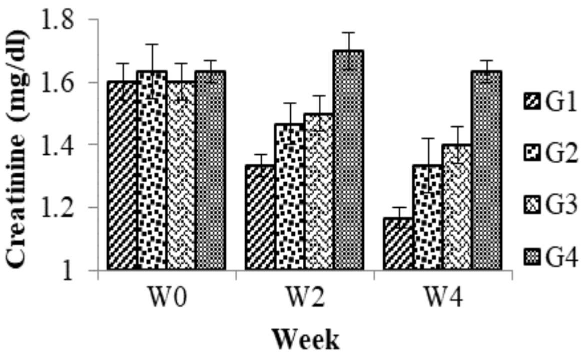

Fig. 9: Figure indicating the effect of herbal dewormer on creatinine values in serum of goat. Values sharing means are statistically non-significant (P>0.05).

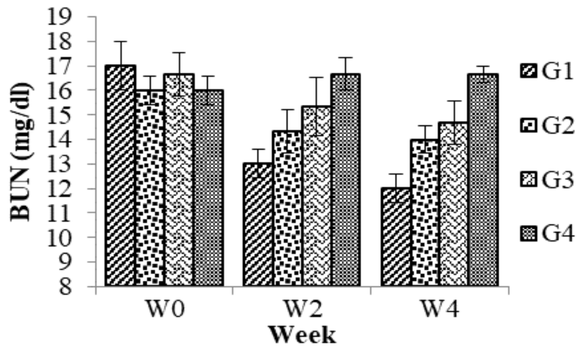

Fig. 10: Figure indicating the effect of herbal dewormer on BUN (Blood Urea Nitrogen) of serum of goat. Values sharing means are statistically non-significant (P>0.05).

Table 5: Effect of herbal dewormer on weight gain of various groups of goat

Groups	Average Initial weights (grams)	Average Final weights (grams)	weight Gain (%)
G1	2213.33±280.42	2306.67±266.89	4.05
G2	2170±153.95	2226.67±168.03	2.54
G3	2176.67±257.75	2240±310	1.36
G4	2183.33±125.0333	2200±130	0.78

The herbal mixture proved a fecal egg count reduction up to 90.91% at maximum concentration in group G1 receiving 1400mg/kg dose of herbal mixture. At maximum concentration, decrease was more than 90%, which indicates that it is an effective dewormer as per World Association for Advancements in Veterinary Parasitology definition (Sunandhadevi *et al.,* 2017; Joachim *et al.,* 2018). Dose dependent response of herbal mixture in this study is in agreement to the results of other studies verifying anthelmintic activity of other plants (Lateef *et al.,* 2006; Zaman *et al.,* 2012; Kimani *et al.,* 2014; Gupta *et al.,* 2017). Zaman *et al.* (2012) reported more than 96% reduction in egg counts and Sunandhadevi *et al.* (2017) reported 97% reduction in egg counts, which are comparable to results of present combination whereas lower percent reduction by some other plants was recorded by Lateef *et al.* (2006), Kimani *et al.* (2014) and Gupta *et al.,* (2017). Synergistic activity of constituents of herbal mixture used in this study may reinforce anthelmintic activity of this dewormer.

This anthelmintic activity may be suggested due to the presence of active proved anti parasitic compounds in the plants (Wamburu *et al.*, 2013). Our herbal extract showed a marked activity against the intestinal parasites of the goat when given to the animals along with their feed. This activity may be connected to presence of a large number of active compounds in the plants which are hydrophilic and act in the water containing medium. Presence of saponins, alkaloids, taninins, flavonoids and triterpenes or sterols in the various parts of plants has been confirmed (Wamburu *et al.*, 2013). These compounds may act in a number of ways against the parasites such as the condensed tannins, which lead to impaired feeding and reproduction activities of the parasite and also cause parasite cuticle disruption. A number of studies suggest the cytolitic action of saponins against parasite by effecting membrane associated sterols and increasing the permeability of cell (Geidam *et al.*, 2007). Alkaloids are lethal for nematodes as they disturb their nervous activities and affect their gastric motility also (Lateef *et al.*, 2006).

Significantly different weight gain at highest dose than control might be result of lowered burden of parasitic fauna of goats. These results were totally different from Agaie and O`nyeyili, (2007) who determined no gain in weight after administering plant dewormer.

Positive correlation in mixture dose and RBC count, Hemoglobin concentration and PCV is suggestive of no toxic effects at all doses. All the hematological parameters were comparable to the results of Gupta *et al.* (2017). Increase in RBCs, PCV and hemoglobin may be attributed to the decrease in blood loss caused by *Haemonchus* and *Fasciola* (Agaie and O `nyeyili, 2007).

Results of serum biochemistry indicated that there was individual to individual variation in theses parameters but there was no specific pattern of variation among the groups in contrast to Gupta *et al.* (2017) where herbal mixture had significantly positive results on various parameters. This was obvious that all the plants had no adverse effects on liver and kidney, and these may be declared as safe for use at these doses and for given period.

Conclusions: Results of this study are suggestive of promising anthelmintic activity of herbal dewormer for both nematodes and trematodes of goats. Large scale evaluation of anthelmintic efficacy of this herbal dewormer is suggestive to explore its potential against other parasites too.

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Authors contribution: RZA, MAZ, ZDS, ZS, MKK conceived and designed the trial. ZS, MSA, AJC, UF, TM and MA executed study. MS, TZ, TUR, AR analyzed the data. FS critically improved the manuscript. All authors critically read the manuscript and have no conflict of interest.

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