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EVALUATION OF ANTHELMINTIC, HEMATOLOGICAL AND SERUM BIOCHEMICAL EFFECTS OF HERBAL DEWORMER ON THE CATTLE

Zohaib Saeed[1], Abdullah Alsayeqh[2]*

[1]Department of Parasitology, University of Agriculture, Faisalabad, [2]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah 51452, Qassim, Saudi Arabia

*Corresponding author, E-mail: a.alsayeqh@qu.edu.sa

Abstract: Cattle play an important role in the food chain as a major milk and meat producer. Helminths affect the health and production of livestock which need to be controlled efficiently and economically. The high usage of anthelmintic drugs has increased anthelmintic resistance to all available classes resulting in failed treatment options and economic losses. Herbs, which may be better options for animal and food safety, are among the best candidates to control these parasites. For this purpose, a research experiment was conducted. Twelve Sahiwal heifers were used for the evaluation of the anthelmintic activity of herbal dewormers composed of various parts of local herbs. Animals were divided into 4 groups A, B, C and D, all the groups, except group D were given herbal dewormer powder. In an experiment of 30 days, doses were given on a weekly basis. Fecal samples for egg counts, blood and serum samples were obtained fortnightly and subjected to statistical analysis. Results revealed that the animals receiving herbal dewormer @ 1400 mg/Kg had the best results and egg counts and hematological values were significantly ($P<0.05$) better than the control animals. The results of the study suggested that the herbal dewormer was efficient in reducing the worm counts as well as beneficial for the hematological profile of the cattle.

Key words: cattle; helminths; EPG; ECR; anthelmintic; herbal

Introduction

Infectious agents i.e., viruses, bacteria, fungi, protozoa, and helminths, etc. are the major causes of death in life on the earth (1, 2). Among the infectious agents, parasites are the major causes of mortalities and morbidities in animals. Helminths are among the dangerous parasites which affect a wide range of animals and pose a threat to their productive and reproductive efficiencies (3). They are among the major threats to food security because of their great impact on the milk and meat production of animals (4). Among the food animals, cattle have vital importance because of their huge share in milk and meat production globally (5). Cattle are greatly affected by helminths, which reduce their milk and meat production and may even cause mortalities (6). The surety of food security through efficient cattle milk and meat production needs proper control of these parasites (2, 7). Among helminths, nemathelminths and platyhelminths are two phyla that contain the parasites of the helminths. *Trichostrongylus* from nemathelminths and *Paramphistomum cervi* from phylum platyhelminths are among the major species found in cattle (8). Multiple researchers have reported about these parasites are found frequently in cattle (9).

Routine control of these parasites is dependent on the chemical anthelmintics which are being used practically (10, 11). Progressively, helminths

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are being resistant to these drugs which makes it impossible to use these drugs in near future (12). Multiple drug resistance has been reported in various infectious agents including bacteria, protozoa, and helminths (13, 14). Additionally, these drugs are harmful to milk and meat consumers due to their toxic residual metabolites in these products. These issues are issues of great concern for scientists, so they are trying to find alternatives (15). Vaccines, organic acids, and many other alternatives have been suggested by scientists (14, 15). Researchers are focusing to develop commercial products for proper control of these drugs (16, 17).

Plants have been among the most prominent options for their use because of their safe mechanism and ease of availability (18, 19). Multiple plants have been tested to control helminths, but the use of a single plant may not be sufficient to control these varieties of parasites (20, 21). This phenomenon needs a proper herbal product made up of various plants belonging to different families having diverse active compounds for their safe and effective use (19). For this purpose, a plant-based herbal dewormer was formulated to find out the cumulative effect of various plants simultaneously. In this research effect of herbal dewormer was evaluated against total helminths, *Trichostrongylus* spp. and *Paramphistomum* spp. along with evaluation of the effects of these herbs on blood and chemical procedures.

Material and methods

Plant material:

Various parts of the plants were obtained from commercial resources and verified at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. They were ground to make a powder for use in the cattle. Detailed composition of the plants used for the formulation of herbal dewormer is given in the Table (1).

Animals

The research was conducted on the cattle from cattle section, Livestock Production and Research Institute, Bahadurnagar, Okara, Pakistan. Adult female heifers of the Sahiwal breed (18-24 months old) which were not pregnant, were selected and screened for egg counts. 12 cattle were selected for the research.

Table 1: Chemical composition of herbal dewormer

Sr. No	Family name	Scientific name	composition (%)
1	*Apiaceae*	*Foeniculumvulgare*	18
2			
3		*Trachyspermumammi*	18
4		*Cuminumcyminum*	3
5	*Fabaceae*	*Glycine soja*	10
6		*Sansevieriatrifasciata*	10
7			
8		*Casia fistula*	4
9	*Combretaceae*	*Combretum indicum*	4
10		*Terminalia chebula*	4
11	*Linaceae*	*Linumusitatissimum*	4
12	*Nitrariaceae*	*Peganumharmala*	4
13	*Asclepiadaceae*	*Leptadenia Reticulata*	3
14	*Theaceae*	*Camellia sinensis*	4
15	*Brassicaceae*	*Lepidium sativum*	3
16	*Cucurbitaceae*	*Citrulluscolocynthis*	3
17	*Rosaceae*	*Rosa sericea*	4
18	*Lamiaceae*	*Mentha spicata*	2
19	*Gentianaceae*	*Swertia* L.	2

Experimental design

Selected animals were grouped equally distributed into four groups A, B, C and D. Groups A, B and C were administered the herbal formulation at a rate of 1000 mg/Kg, 1200mg/Kg and 1400 mg/Kg orally in the powder form. Group D was maintained as negative control. Trial continued for 30 days. The animals were given the dose at a week interval and the fecal samples were collected fortnightly. Weight gains, blood and serum samples were also collected fortnightly.

Eggs per gram of feces and percent reduction in egg count

Standard, sterile methods of collection of fecal samples were observed and collected samples were transported to the university of Agriculture, Faisalabad. MAFF (22) method of egg counting was used, and the following equation was used for the determination of egg counts.

Weight Gains

The weights of the animals under study were taken initially and at the end of the trial. Data obtained was subjected for estimation of weight gain, average daily weight gains and percent weight gains (23).

Hematology and serum biochemistry

Blood and serum samples were collected on day 0, 15 and day 30. Standard procedures were adopted to evaluate effects on blood parameters i.e., Red blood cells (RBCs) white blood cells (WBCs), Packed Cell Volume (PCV) and hemoglobin concentration. Spectrophotometric kits were used to analyze the serum parameters of liver and renal efficiency (3).

Statistical analysis

Egg per gram, serum and blood values were subjected to the analysis of variance and means were compared through the Tukey test using Minitab® 26.0. Percent egg count reduction and graphs were formed using Excel® 365 (24).

Results

Eggs per gram of feces and percent reduction in egg count

Significant (P<0.05) reduction in EPG was observed in the treated groups when compared to the control, over the period of time. A dose-dependent response was observed in the treated groups (Table 2). Group C had the lowest EPG among all the groups and the highest percent reduction was also observed in all the groups. Group C had the highest percent Reduction of eggs i.e., 80.97% for total helminths 83.8% for *Trichostrogylus* and 81.43% for *Paramphistomum* (Table 3).

Hematology and serum biochemistry

Group C animals showed significantly (P<0.05) higher RBC, PCV and Hb values at the end of the trial. Lower WBC counts were observed in the treated groups and group C had significantly

Table 2: Helminths, *Trichosrtongylus*, and *Paramphistomum* egg counts per gram of feces in Sahiwal cattle on days 0, 15, and 30 of trial

	Eggs per gram								
	Total helminths			Trichostrongylus			Parsmphistomum		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
A	2300 ± 217.94^{a}	1716.66 ± 125.83^{b}	1116.66 ± 125.83^{b}	783.33 ± 104.08^{a}	650 ± 50^{a}	400 ± 50^{b}	533.33 ± 76.37^{a}	400 ± 50^{ab}	300 ± 50^{ab}
B	2316.66 ± 125.83^{a}	1500 ± 50^{b}	916.66 ± 152.75^{b}	800 ± 132.28^{a}	550 ± 50^{a}	333.33 ± 76.37^{bc}	516.66 ± 125.83^{a}	316.66 ± 28.86^{ab}	166.66 ± 28.86^{b}
C	2266.66 ± 175.59^{a}	1100 ± 50^{c}	433.33 ± 76.37^{c}	816.66 ± 104.08^{a}	333.33 ± 28.86^{b}	133.33 ± 28.86^{c}	516.66 ± 104.08^{a}	233.33 ± 28.86^{b}	100 ± 50^{b}
D	2283.33 ± 301.38^{a}	2316.66 ± 225.46^{a}	2333.33 ± 251.66^{a}	716.66 ± 125.83^{a}	716.66 ± 104.08^{a}	733.33 ± 125.83^{a}	500 ± 180.27^{a}	483.33 ± 175.59^{a}	516.66 ± 175.59^{a}

Values with similar letters within a column are statistically nonsignificant (p>0.05)

lower white blood cells over the passage of time (Table 4). There was a non-specific trend in the serum biochemical values except serum protein and albumins among the groups with the passage of time (Figure 1-9). Serum albumins and proteins were significantly (P<0.05) higher in group C (Figure 5, 6).

Weight Gains

Weight gain, average daily weight gain and percent weight gain of all the groups was evaluated and it was estimated that the group C had the best weight gains among all the groups. All the treated groups had the dose dependent response across the concentrations (Table 5).

Table 3: Percent reduction in egg counts on day 0, 15 and 30 of the trial

	Fecal Egg count reduction (%)								
	Total helminths			Trichostrongylus			Parsmphistomum		
	Day 0-15	Day 15-30	Day 0-30	Day 0-15	Day 15-30	Day 0-30	Day 0-15	Day 15-30	Day 0-30
A	25.21 ± 3.71	34.95 ± 5.42	51.48 ± 1.79	16.61 ± 4.88	38.61 ± 2.97	48.88 ± 1.92	24.83 ± 2.52	23.74 ± 17.94	42.67 ± 13.64
B	35.09 ± 4.88	38.62 ± 12.13	60.49 ± 5.81	29.25 ± 18.7	38.53 ± 17.19	58.63 ± 2.99	37.05 ± 10.88	47.61 ± 4.12	67.24 ± 4.12
C	51.33 ± 3.22	60.61 ± 6.56	80.97 ± 2.05	59.02 ± 1.99	60.31 ± 5.49	83.8 ± 1.72	54.29 ± 4.17	58.33 ± 17.55	81.43 ± 6.25
D	-1.76 ± 3.48	-0.65 ± 1.13	-2.4 ± 2.5	-0.42 ± 6.52	-2.08 ± 3.6	-2.38 ± 4.12	3.43 ± 13.09	-8.11 ± 8.34	-3.7 ± 6.41

Table 4: Hematological parameters evaluated in the cattle on day 0, 15 and 30 of the experiment

	Red Blood Cells (10^6/uL)			Hemoglobin(g/deciliter)			PCV (%)			White Blood cells ($x10^3$/uL)		
	DAY 0	DAY 15	DAY 30	DAY 0	DAY 15	DAY 30	DAY 0	DAY 15	DAY 30	DAY 0	DAY 15	DAY 30
A	3.2 ± 0.09a	3.36 ± 0.05b	3.56 ± 0.11b	8.54 ± 0.09a	9.4 ± 0.16b	9.82 ± 0.12c	23.8 ± 0.6a	25.63 ± 0.86bc	27.6 ± 1.95c	5.23 ± 0.08a	5.03 ± 0.16ab	4.77 ± 0.04ab
B	3.26 ± 0.2a	3.43 ± 0.15ab	4.2 ± 0.1a	8.44 ± 0.22a	9.63 ± 0.13b	10.33 ± 0.15b	25.7 ± 0.87a	27.28 ± 1.47b	33.5 ± 2.83b	5.41 ± 0.4a	4.98 ± 0.06b	4.82 ± 0.05b
C	3.46 ± 0.15a	3.73 ± 0.15a	4.46 ± 0.05a	560.75 ± 478.48a	10.33 ± 0.15a	10.96 ± 0.2a	24.2 ± 2.33a	32.5 ± 0.95a	39.53 ± 0.95a	5.55 ± 0.12a	4.41 ± 0.05c	4.22 ± 0.08c
D	3.2 ± 0.09a	3.33 ± 0.05b	3.33 ± 0.15b	8.47 ± 0.21a	8.39 ± 0.18c	8.36 ± 0d	24.46 ± 1.95a	24.23 ± 1.02c	24.4 ± 0.81c	5.39 ± 0.19a	5.46 ± 0.2a	5.35 ± 0.2a

Values with similar letters within a column are statistically nonsignificant (p>0.05)

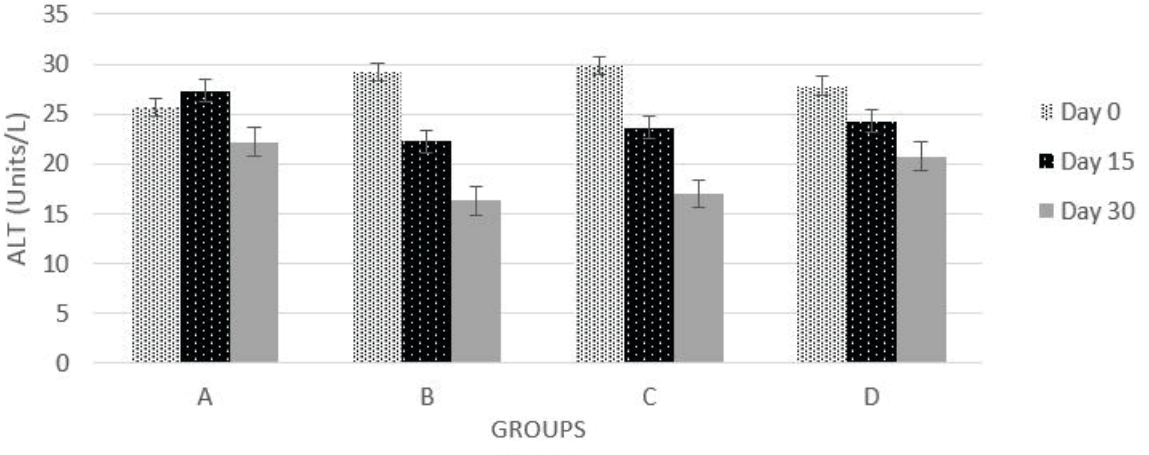

Figure 1: Alanine aminotransferase (ALT) values of Sahiwal cattle on days 0, 15 and 30 of trial

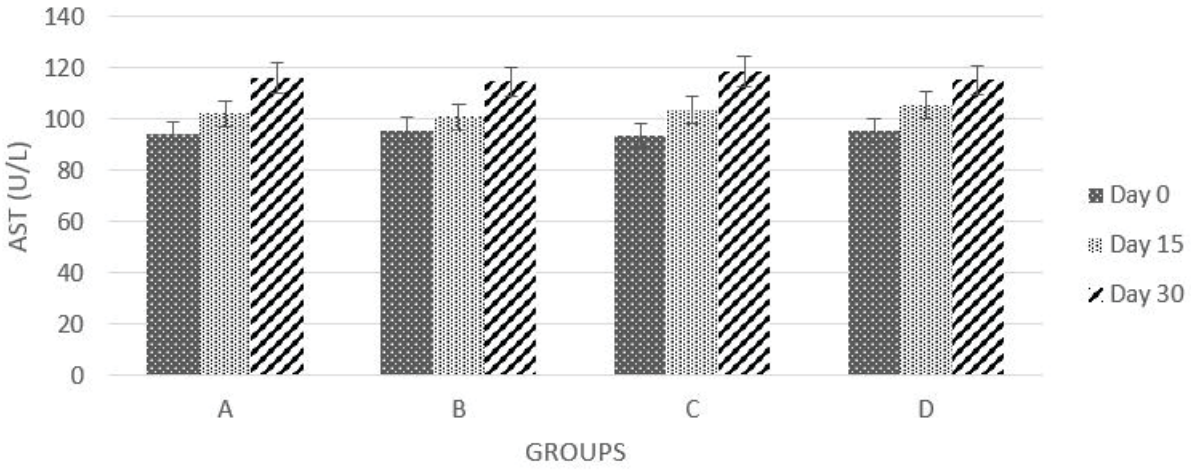

Figure 2: Aspartate Transferase (AST) values of Sahiwal cattle on days 0, 15 and 30 of trial

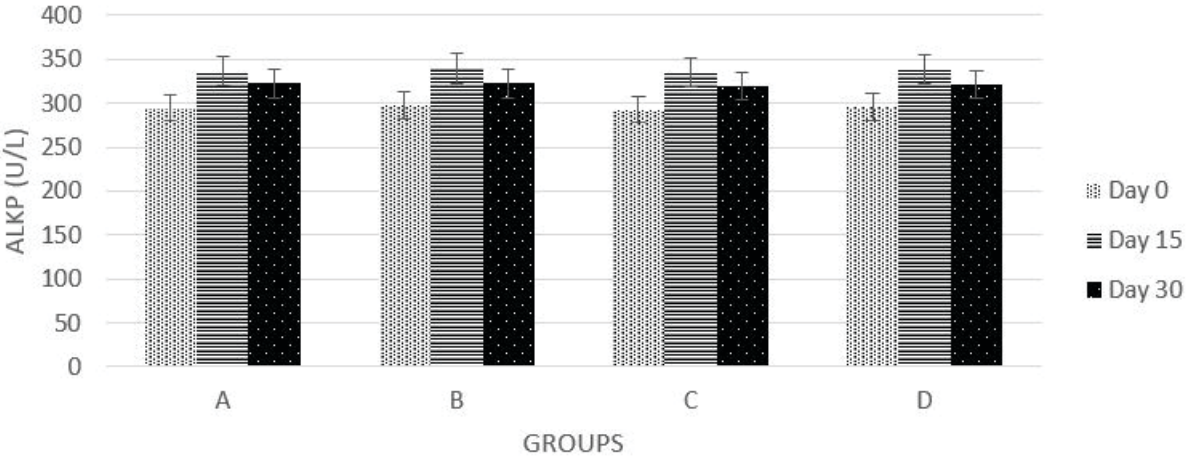

Figure 3: Alkaline Phosphatase (ALKP) values of Sahiwal cattle on days 0, 15 and 30 of trial

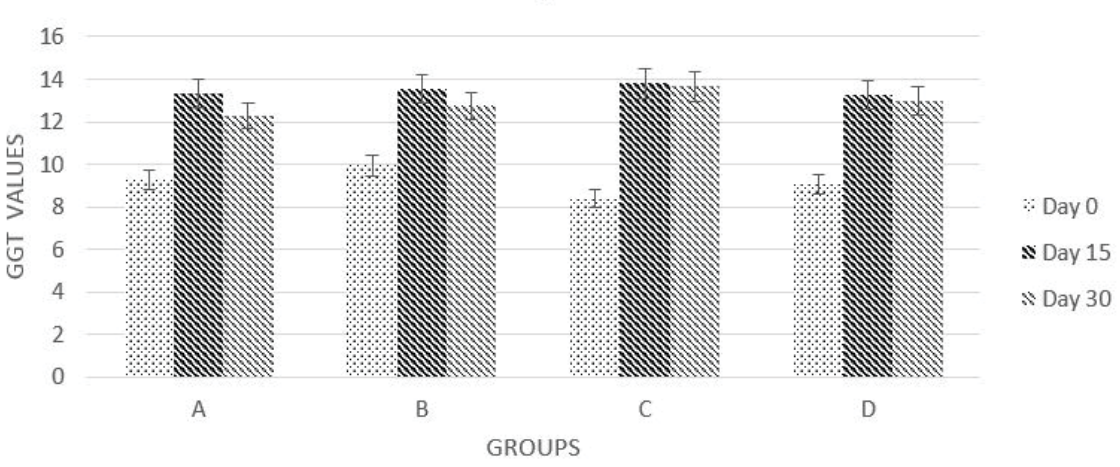

Figure 4: Gamma Glutamyl Transferase (GGT) values of Sahiwal cattle on days 0, 15 and 30 of trial

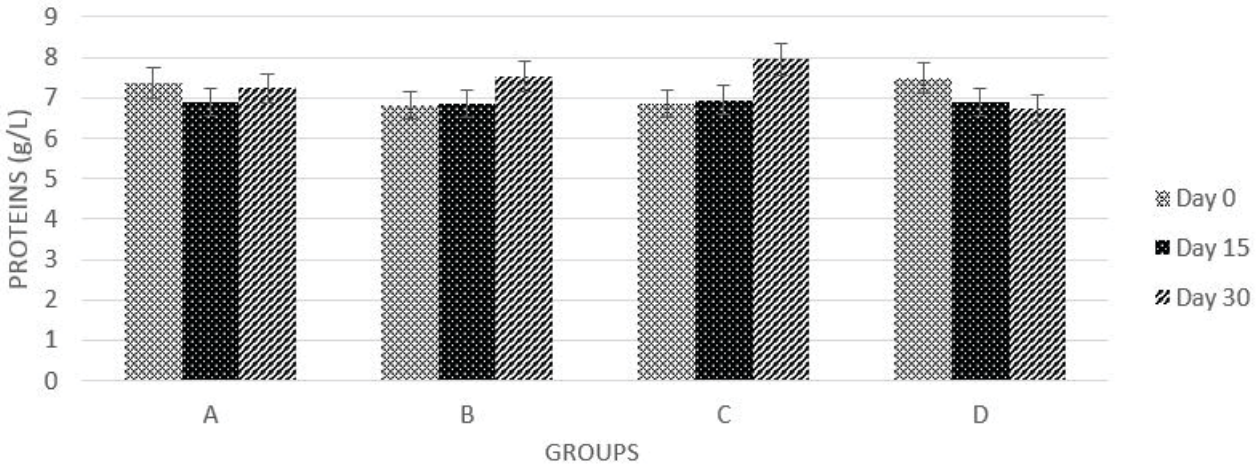

Figure 5: Total serum Protein values of Sahiwal cattle on days 0, 15 and 30 of trial

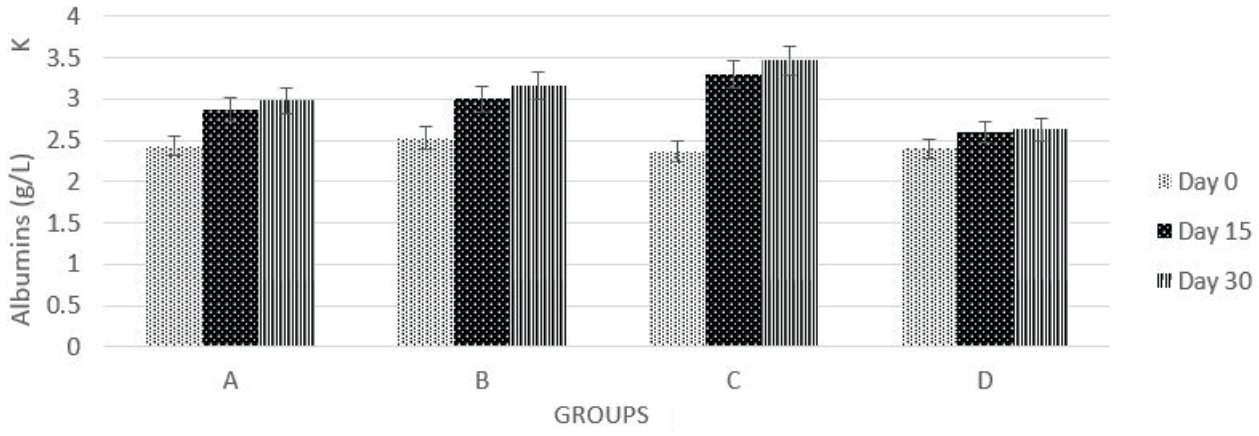

Figure 6: Serum Albumin values of Sahiwal cattle on days 0, 15 and 30 of trial

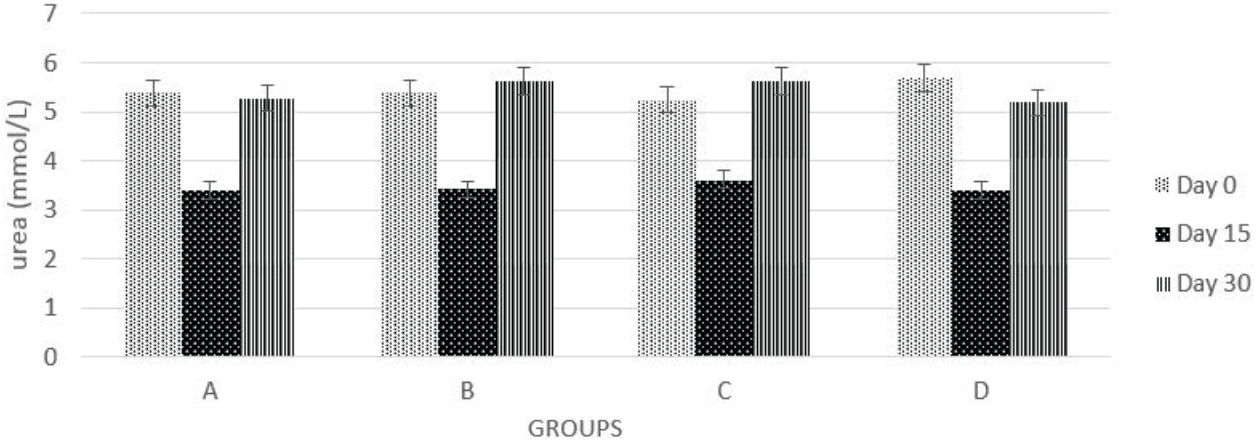

Figure 7: serum Urea values of Sahiwal cattle on days 0, 15 and 30 of trial

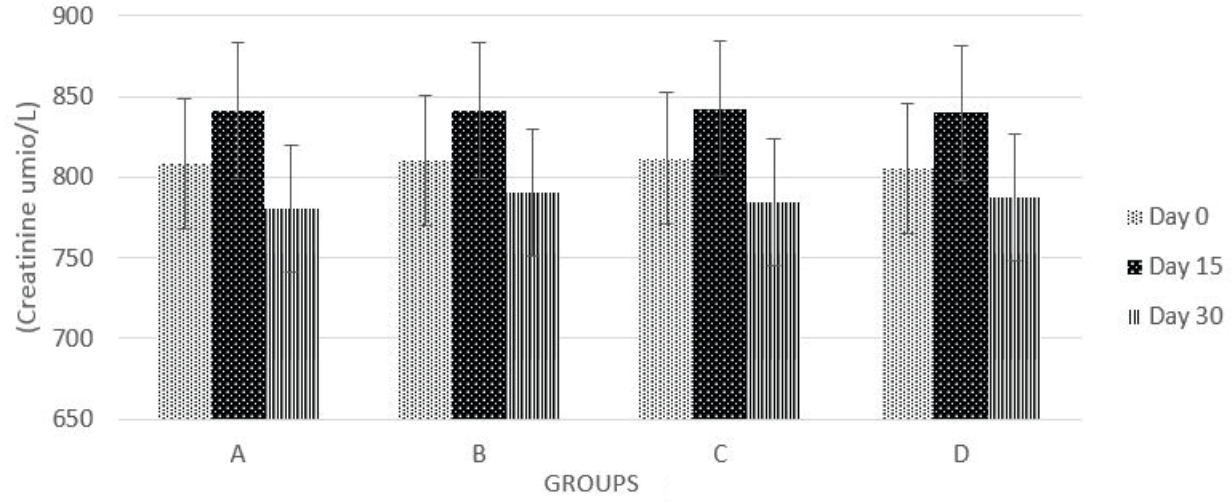

Figure 8: Creatinine values of Sahiwal cattle on days 0, 15 and 30 of trial

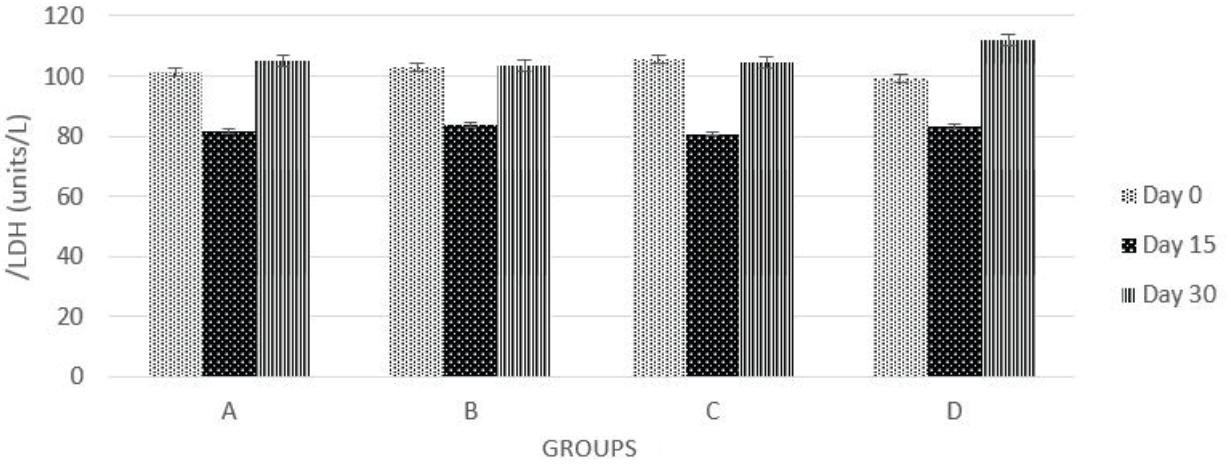

Figure 9: Lactate Dehydrogenase (LDH) values of Sahiwal cattle on days 0, 15 and 30 of trial

Table 5: Weight gain, Average daily weight Gain and Percent weight gain of Sahiwal cows during experiment

	Initial weights (Kg)	Final weights (Kg)	Weight Gain (Kg)	Average daily weight gain (grams)	Percent Weight Gain (%)
A	223.66 ± 1.6	238.66 ± 5.5	15 ± 3.9	500 ± 130.17	6.26 ± 1.47
B	217.83 ± 2.51	238.33 ± 3.05	20.5 ± 3	683.33 ± 100	8.59 ± 1.19
C	225 ± 2.29	249.16 ± 2.02	24.16 ± 0.28	805.55 ± 9.62	9.7 ± 0.19
D	215.66 ± 2.75	226.83 ± 3.17	11.16 ± 1.44	372.22 ± 48.11	4.92 ± 0.6

Discussion

In this research, Sahiwal heifers were used for evaluation of the anthelmintic activity of the herbals belonging to various families of plants the herbal dewormer showed a great reduction in the fecal egg counts. The herbal dewormer showed excellent efficiencies in reducing the egg counts and they reduced the egg count of both *Trichostrongylus* and *Paramphistomum* spp. efficiently. Hematological profile was also efficiently improved by the herbal dewormer. Herbal dewormer showed 80.97% reduction in total helminth egg counts, 83.8% reduction in nemathelminths (*Trichostrongylus*), and

81.43% reduction in platyhelminths (*Paramphistomum*) eggs.

Many researchers have conducted anthelmintic trials of the herbals and reported that the herbals were efficient in reducing the egg counts and reducing the helminths in the ruminants (3, 25-28). Similar results have been reported by Abbas et al. (3) who conducted a trial to evaluate the anthelmintic effects of same herbal dewormer in the goat species**.** They concluded that the herbal dewormer was efficient in reducing the egg counts reduced the egg counts efficiently. It improved hematological profile and had a positive effect on the egg counts. Results of our study were in line with Kaitay et al. (28). They proved that the extract of pomegranate peel was efficient in controlling the parasite members of phylum nemathelminths in the goat, cattle and sheep efficiently. These studies suggest that the plants have efficient role in controlling the gastrointestinal helminths. Our dewormer was beneficial for reducing total helminths including nemathelminths (*Trichostrongylus*) and platyhelminths (*Paramphistomum*). These activities have been reported by other researchers which represent that the plants have ability to reduce the nemathelminths as well as platyhelminths (29).

Anthelmintic activities of the plants are mainly attributed because of the presence of active compounds in the plants. These herbals contain various bioactive compounds including phenolics, flavonoids, thymols and terpenoids etc. (30). These compounds are in varying concentrations and have altering concentrations in different plants. These compounds can control the various types of helminths because of diverse mode of actions of their constituents (30). Many phytochemicals i.e., thymols usually inhibit the reproductive efficiency of the helminths and restrict oocyst shedding (31). While some phytochemicals like nicotinic compounds cause paralysis of the worms leading to their inability to attach to the body hence direct removal from the body (32, 33). There were multiple plants that contained a variety of compounds belonging to these groups. Many researchers have reported that the plants in combination show the higher effects than the single plant because there is increased diversity of bioactive compounds, and their mode of actions (34).

In current research the herbal dewormer improved the hematological profile of the cattle. Increased red blood cells, PCV and Hemoglobin contents can directly be correlated with the reduced number of worms (35). Parasites feed upon blood and have drastic effects on circulatory blood cells leading to anemia and lead to low circulatory blood cells, lowered hemoglobin and decreased packed cell volume (36). Our herbal dewormer was efficient in reducing the worms hence causing less blood and serum metabolites loss. Reduction in WBCs is observed when the infectious agents are reduced, leading to lower circulatory immune cells which was shown in current research (37). Likewise, the improvement in serum proteins and albumins was observed in the Sahiwal cattle being given herbal dewormer. Parasites cause serum proteins loss when they are higher in number (38, 39), our herbal dewormer reduced worms efficiently, so there was significant ($P<0.05$) increase in the serum proteins and albumin counts in the treated animals. Improved weight gains observed in this research can be attributed to reduction in worm burden which improves nutrient availability and feed uptake of the animals (3, 40).

There are some limitations in this research as many researchers (41) have reported improvement in ALT, AST and creatinine values, but in present research no significant effect was observed. All the serum values including ALT, AST, ALKP, urea, LDH and creatinine were in normal ranges, that may be a reason that the animals remained in normal physiological states (42). Along with it these differences can be affected by multiple other parameters which were not considered in this research i.e., feed intake, type and amount of disease, other diseases which were not caused by the helminths.

Conclusion

The results of this research depict that the food animals are always at a threat of helminths and their safe and economical control can be achieved by the herbal mixtures. Herbal mixtures are safe, effective, and economical alternatives for control of parasites of livestock. multiple other species should be included in the research, so that the herbal formulations for all the species may be formulated. Long term studies of these herbals should be conducted to estimate any hazardous effect on prolonged status.

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