ANTHELMINTIC EFFECT OF HERBAL COMPOUNDS (BIO-DEWORMER) AGAINST GASTROINTESTINAL PARASITES IN CAMELS

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ABSTRACT: Parasitic infections particularly gastrointestinal helminths are the leading cause of production losses in all dairy animals and in camels as well. Synthetic anthelmintics have been used for many years for the control of helminths. However, helminths have developed resistance against most of these synthetic anthelmintic agents including Levamisole, Oxfendazole, Albendazole, Piprazine, and Triclabendazole. Ancient herbs still have excellent therapeutic effects. While facing emerging synthetic anthelminthic resistance, there is a need to focus on plant-based alternative medicines. Phytochemical findings revealed the presence of various biologically active compounds in plants or their extracts that may have anthelmintic effects. This research aimed to study the effect of herbal combinations on 20 mature camels in District Layyah. The 20 camels were split into four groups: C1, C2, C3, and C4, each group contained five camels. The research trial was continued for four weeks. The treatment was given in different concentrations from week 1 to week 4 (Group C1 350 mg/kg, Group C2 400 mg/kg, Group C3 450 mg/kg, and Group C4 as a control one). During research, Blood, serum, and fecal samples were collected to analyze their hematological parameters, serum biochemistry, and fecal egg count. The result revealed that herbal compound gives concentration-related responses, as groups C1, C2 and C3 showed 33.76, 46.60, and 70.23% fecal egg reduction, respectively. In the control group, C4 fecal egg count increased by 0.03%. HPLC analysis of a methanolic extract of an herbal compound showed that the levels of gallic acid, quercitin, and cinnamic acid were 2.37, 3.28, and 0.46 ppm, respectively. Likewise, serum biochemistry of all groups of camels showed no toxicity and adverse effects of herbal compounds. Hematological parameters showed very positive results of herbal compounds on RBCs, WBCs, PCV, and hemoglobin values.

Keywords: Anthelmintic; bio-dewormers; helminths, Hematology, camels.

INTRODUCTION

Livestock contributes 11.22% in GDP and 60.54% in agriculture (GOP.2020). Pakistan's camel population has grown to 1.2 million. Similarly, 0.6 million tons of camel milk and 539,100 Tons of meat were consumed (GOP. 2019-20). Like all other animals, the camel has to face various diseases and threats especially gastrointestinal parasites like helminths. Helminthes cause loss of appetite, decreased food and mineral absorption, protein metabolism abnormalities, protein loss in the gastrointestinal tract, sloughing of gastrointestinal tract epithelial cells, and enhanced muco-protein secretion (Coop and Kyriazakis, 2001; Abbas et al., 2016). Helminths have developed resistance against most of these synthetic anthelmintic agents including Levamisole, Oxfendazole, Albendazole, Piprazine, and Triclabendazole.

Many traditional plants contain unique chemical components that are even more effective and safer than chemical derivatives (Muthee *et al*., 2011). Alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and a variety of other compounds are being used against helminths. The mode of action of these biologically active substances derived from plants is unique (Abbas, 2020). However, the mode of action of certain compounds is yet uncertain. Similarly, saponins cause helminths' cell membranes to lyse, resulting in their death (Maestrini *et al*., 2020).

Camels are commonly raised for meat in the eastern and central provinces. (Yakhchali and Athari 2010) and can be found in arid and semi-arid regions. Its milk production is also comparable to other livestock even under poor management conditions (Sazmand *et al*., 2011)

According to a prior study, *Eimeria Rajasthani, Eimeria cameli, and Eimeria droemdarii* are infective species to newly born camel calves (Yakhchali and Cheraghi 2007). There are a variety of geographical areas where gastrointestinal helminths should be tested in livestock populations (Iqbal *et al*., 2005; Wahba *et al*., 2003). Although camels are well-known to tolerate a variety of parasitic infestations that are economically significant among domestic animals (Al Haj *et al*., 2010, Retwatker *et al.*, 2009), however, internal parasites are known to contribute a significant loss of production and productivity in severe cases in animals (Rahman et al., 2016; Borji *et al*., 2010, Bathiae *et al*., 2010; Parsani *et al*., 2008) and some of these parasitic helminths have zoonotic potential as well. (Worboys*et al*., 2010, McCarthy *et al*., 2000).

Helminthes are becoming resistant to a variety of anthelminthic drugs, and many anthelmintics (including levamisole, ivermectin, thiabendazole, and refoxanideetc.) now lose their effect against parasites (Mckenna *et al*., 1990; Chagas *et al*., 2013). However, medicinal plants have the potential to be used as veterinary anthelmintics, but the safety and effectiveness of the plant or its extract must be proven by numerous *in vivo* and *in vitro* trials (Rates, 2001). Because of their bioactive substances, many plants have been confirmed to be anthelmintic by researchers. Although plants have a wide range of effects against GIT helminths, little is known about their synergistic and combination effects. As a result, there are no studies that prove the combined effects of Pakistani herbs against GIT parasites in camels. This trial aims to close the gap by assessing the combined effect of previously tested proven herbs for their antihelmintic properties. Therefore the present study was planned to check the efficacy of herbal compounds as an anthelmintic in camels.

MATERIALS AND METHODS

Study Area: The research trial was conducted in the District of Layyah during the year 2021 on camels selected from different age groups. Plant materials were collected in dry form from Layyah's local market and identified by experts (See Table 1). All of these materials were kept at 4°C until they were needed.

Sample collection

Fecal samples: Samples of feces were taken using plastic gloves safely from the rectum, placed in fecal pots, and labeled before being transported to the local veterinary investigation laboratory. The presence of gastrointestinal eggs in fecal samples was detected using the sedimentation and floatation technique (Cebra, 2008).

Blood samples: Vacutainer tubes were used to collect whole blood samples from each examined camel's jugular vein and transported to the laboratory for complete blood count and serum analysis. According to (Solusby, 1986).

Nutritive and Phyto-chemical Analysis: Random samples from the herbal compounds were processed to check out its neutral detergent fiber (NDF), Crude protein (CP), acid detergent fiber (ADF), dry matter and Organic matter (AOAC, 1990). These samples were analyzed at the Animal Nutrition Laboratory, University of Agriculture Faisalabad. Phytochemical analysis (qualitative) of the crude extract of the herbal compound was performed to check the presence of tannins, phenols, carbohydrates, and cardiac glycosides by the described method (Ruskin *et al*., 2014). Quantitative analysis of herbal compounds was done by high Performance Liquid Chromatography (HPLC), a technique defined by (Coskin, 2016). Through these procedures, bioactive substances of herbal compounds were identified, purified, and then their quantity evaluated. The technique described by (Qayyum *et al*., 2016), was used for the estimation of secondary metabolites. The quercitin, cenamic acid, and gallic acid were identified in the herbal compounds and their concentrations were also calculated.

Animals: Non-pregnant, adult camels were selected randomly from the District Layyah. The trial was conducted on 20 camels. Camels were divided into four groups C1, C2, C3, and C4. The trial was continued for 4 weeks. During the trial, treatment was given to animals for 4 weeks. From week 0 to week 4 fecal samples and blood samples were collected in polythene bags and EDTA tubes respectively and transported to the laboratory for the diagnosis of animal diseases in Layyah.

***In vivo* examination:** Non-pregnant, non-lactating 20 adult camels of non-specific type having an age of 3 to 10 years were selected randomly in District Layyah. The animals were divided into four groups at random (C1, C2, C3 and C4). Each group comprises 5 camels and all groups were kept separately in the whole trial. Fecal and blood samples were collected and transported to the laboratory for further analysis from week 0 to week 4. Fecal samples were collected in polythene bags. EDTA (Ethylene diamine tetra acetic acid) tubes were used to collect the blood for hematological parameters. For serum analysis Gel- clot vacutainers were used to collect blood for serum analysis.

Group C1: 350mg/kg of herbal compound per animal for four weeks at an interval of seven days. Group C2: 400mg/kg of herbal compound per animal for 4 weeks with an interval of 7 days. Group C3: 450mg/kg of herbal compound per animal for 4 weeks at an interval of 7 days. Group C4: As a control group.

Parameters used for the evaluation of in *vivo* anthelmintic activity

Effect of Herbal compound on fecal egg count: Fecal samples were collected in polythene bags, labeled according to the animal group, from week 0 to week 4, and transported to the laboratory for the analysis of animal diseases in District Layyah. Fecal samples were analyzed for fecal egg counting, a procedure described by (Soulsby, 1982).

Table 1: Plant names which were used in the Herbal Compound.

Sr. No.	Urdu name	Scientific (Botanical) name	English name
1.	Alessi	*Linum usitatissimum*	Linseed
2.	Sonef	*Foeniculum vulgare*	Fennel
3.	Harad	*Terminalia chebula*	Chebulic myrobalan
4.	Malathi	*Combretum indicum*	Chinese honeysuckle
5.	Podina	*Mentha spicata*	Mint
6.	Soya	*Glycine soja*	Soybean
7.	Mathery	*Sansevieria trifasciata*	Vipers bowstring hemp
8.	Chairrata	*Swertia L.*	Felworts
9.	Harmel	*Peganum harmala*	Harmel
10.	Dodi	*Leptadenia colocynythis*	Beaumont root
11.	Korr tumma	*Citrullus colocynthis*	Bitter apple
12.	Amal Tas	*Casia fistula*	Golden rain tree
13.	Haloon	*Lepidium sativum*	Garden cress
14	Gulab	*Rosa sericea*	Rose
15.	Zeera sofaid	*Cumininum cyminum*	Cumin
16.	Ajwain	*Trachyspermum ammi*	Bishop weed
17.	Kamala	*Camellia sinensis*	Camilla

Table 2: Components of Herbal Compound.

Sr#	Urdu name	Scientific name	English name	weight	(%)
1	Sheera		Molasses	20	2
2	Alssi	*Linum usitatissimum*	Linseed	20	2
3	Sonef	*Foeniculum vulgare*	Fennel	180	18
4	Harad	*Terminalia chebula*	Chebulic myrobalan	20	2
5	Malathi	*Combretum indicum*	Chinese honeysuckle	20	2
6	Podina	*Mentha spicata*	Mint	20	2
7	Soya	*Glycine soja*	Soybean	100	10
8	Mathery	*Sansevieria trifasciata*	Vipers bowstring hemp	100	10
9	Chairrata	*Swertia L.*	Felworts	10	1
10	Harmel	*Peganum harmala*	Harmel	20	2
11	Dodi	*Leptadenia colocynythis*	Beaumont root	30	3
12	Korr tumma	*Citrullus colocynthis*	Bitter apple	20	2
13	Amal Tas	*Casia fistula*	Golden rain tree	20	2
14	Haloon	*Lepidium sativum*	Garden cress	10	1
15	Gulab	*Rosa sericea*	Rose	20	2
16	Zeera sofaid	*Cumininum cyminum*	Cumin	30	3
17	Ajwain	*Trachyspermum ammi*	Bishop weed	180	18
18	Kamala	*Camellia sinensis*	Camilla	40	4
19	Common salt	Sodium chloride	Common salt	100	10
20	Kala namak	Black salt	Black salt	20	2
21	Toxin binder		Toxin binder	10	1

Effect of Herbal compound on Hematological parameters: Blood samples were taken in Ethylene diamine tetra acetic acid (EDTA) vacutainers from week 0 to week 4. All the vacutainers were tagged according to animal group and number. The hematological parameters that were observed are Hemoglobin, Erythocyte and leukocyte counts, and Pack cell volume (PCV %). Red blood cell (RBCs) and White blood cell (WBCs) counts were performed by hemocytometer by using Natt and Herrick solution for the dilution of blood. For Pack cell

volume (PCV) determination, the Micro-hematocrit technique was used. Hematocrit was used in this technique to determine the volume of red blood cells as a percentage of total blood volume. Micro- hematocrit technique is an authentic and simple technique for the determination of packed cell volume (PCV). MCH was calculated using the formula given below

MCH= Hemoglobin (g/dl) / Red blood cells (106/µl) x 10.

Mean Corpuscular Volume (MCV) was calculated by formula MCV=Packed cell volume (%)/Red blood cells (10^6/µl) x 10 whereas, mean corpuscular hemoglobin concentration (MCHC) was calculated by MCHC= Hemoglobin (g/dl)/Packed cell volume (%) x 10.

Effect of Herbal Compound on Serum Biochemistry: Gel-clot-activated vacutainers were used for serum separation. The collection of blood samples was done for four weeks from week 0 to week 4. All the Gel-clot activated vacutainers were marked according to animal number and group. All the samples were stored at 4°C until these samples were safely transferred to the laboratory. The serum biochemistry was done by using standard kits and results. The following tests were performed on the serum of camels, Total protein, Aspartate Aminotransferase (AST), Blood urea nitrogen (BUN), Total Bilirubin, Alkaline phosphate, Alanine Aminotransferase (ALT), Creatinine, Gamma-glutamyl transferase (GGT) Lactate Dehydrogenase (LDH) was determined as described by Akhtar, (2000).

Fig 1: Microscopic view of the slide for parasites eggs

Statistical Analysis: Anthelminthic activity data were submitted to probit analysis using software anthelminthic activity was analyzed by hierarchical design. Other Data obtained from all the above methods were analyzed by using two ways of variance and Turkey's test for means comparison of statistical analysis. Data collection was in percentages and the Chi-square test was used for survival percentage analysis.

RESULTS

Phytochemical and nutritive analysis of herbal extract: Herbal compounds' nutritive and phytochemical analysis showed that the mixture is rich in carbohydrates, quinines, tannins, flavonoids, proteins, cardiac glycosides, and phenols. The presence of these phytochemicals like flavonoids, tannins phenol compounds shows that the herbal compounds have good potential for anthelminthic effects (See tables 3 and 4).

Table 3 Qualitative analysis of herbal extract.

Phytochemical	Present/ absent
Flavonoids	+
Saponins	-
Tannins	+
Terpenoids	-
Alkaloids	-
Carbohydrates	+
Quinones	+
Proteins	+
Phenols	+
Cardiac glycosides	+

(+) = present (-) = Absent

Nutritive analysis results

Table 4: Nutritive analysis of herbal compound.

Component	Percentage (%)
Acid detergent fiber	04.00
Dry matter	91.82
Nitrogen	01.928
Organic matter	64.64
Neutral detergent fiber	21.00

High-performance Liquid Chromatography (HPLC): HPLC results show the presence of gallic acid, quercetin, and cinnamic acid in concentrations of 2.37, 3.28, and 0.46 ppm, respectively (See Figure 2).

Results of *in vivo* trial

Effect of the herbal compound on the eggs per gram (EPG): In *vivo*, the trial was conducted to assess the effect of the herbal compound on the gastrointestinal helminths of camels. Non-pregnant, non-lactating adult 20 camels of non-specific type having ages of 3 to 10 years were selected randomly in District Layyah. All the selected animals were divided into 4 groups (C1, C2, C3 and C4). Each group was comprised of 5 camels and all groups were kept separately in the whole trial. From week 0 to week 4 blood and fecal samples were collected while weight gain or loss was observed. Different concentrations of the herbal compound were offered to each group as in the C1, C2, and C3 group doses of the herbal compound were 350, 400, and 450mg/kg, respectively, and the C4 group was kept as a control group. These results show that 86.76% decrease in eggs per gram (EPG) after four weeks in the C3 group while 61.93, 50.12, and 0.9% decrease in EPG in groups C1, C2, and C4, respectively (See table 6).

Effect of herbal compound on fecal egg count reduction test (FECRT) of camels: This figure shows the results of the fecal egg count reduction test (FECRT), which represents the data from week 0 to week 4 in all groups. FECRT of groups C1, C2, C3, and C4 was 33.76, 41.60, 72.23, and -0.05, respectively. The results show that the highest FECRT was in group C3 and lowest in C4 which was a negative control (See Table 7).

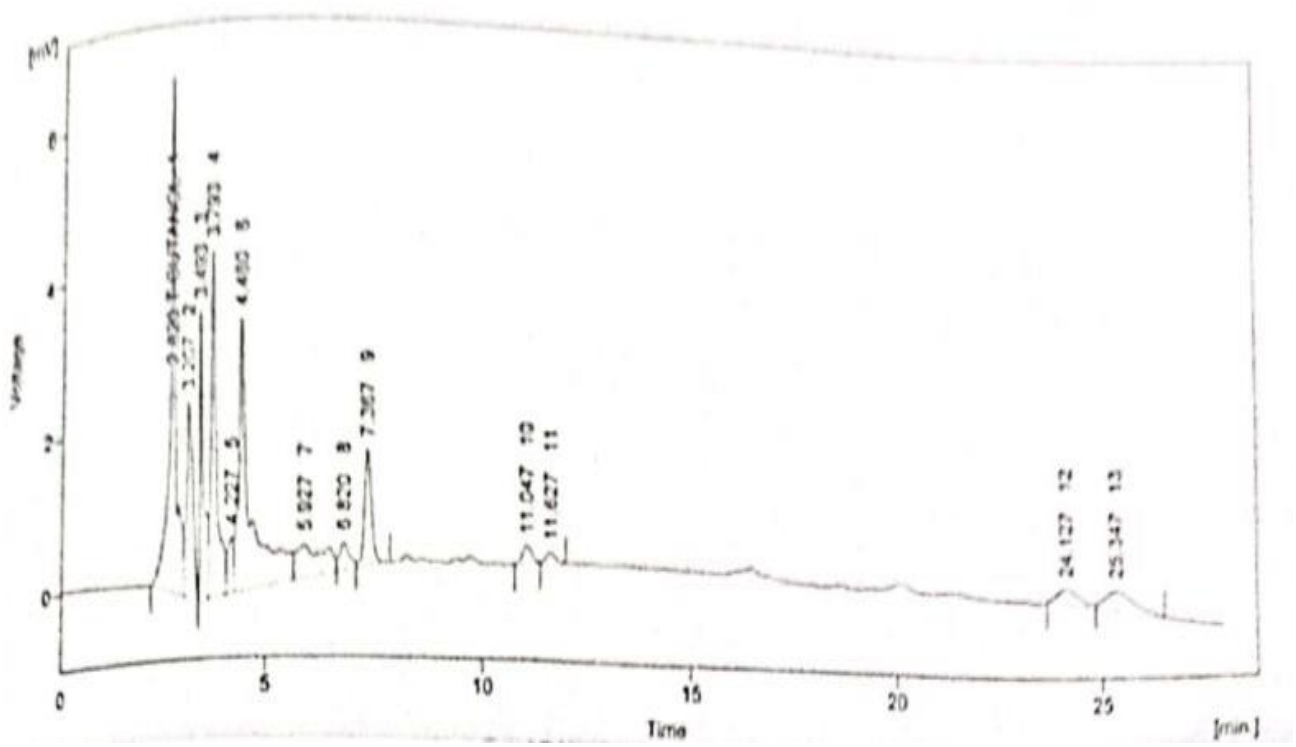

Fig 2: High-Performance Liquid Chromatography (HPLC) of herbal compound

Table 5: High-Performance Liquid Chromatography (HPLC) results of herbal compound.

Compound name	Retention time	Area (%)	Area (mv.s)	Concentration(ppm)
Gallic acid	4.48	28.6	65.928	2.37
Quercitin	2.82	26.6	61.21	3.28
Cenamic acid	25.347	5.8	25.347	0.46

Table 6: Effect of herbal compound on eggs per gram (EPG) of different groups of camels concerning weeks.

Weeks	Group			
	C1	C2	C3	C4
W0	1240.60±27.81	1237.40±19.64	1258.00±33.08	1266.40±23.34
W1	1160.20±27.30	913.20±22.60	768.00±28.52	1258.20±19.19
W2	924.80±24.04	796.60±16.94	581.40±20.96	1270.40±15.24
W3	800.20±17.81	727.60±12.93	487.40±23.32	1280.40±14.51
W4	768.00±15.76	620.02±17.3	235.00±15.76	1286±17.51

Statistically non –non-significant (p>0.05) means the sharing of common alphabets in a column or a row. Small alphabets show the comparison among interaction means.

Table 7: Effect of herbal compound on fecal egg count reduction test (FECRT) of camel

FECR	Group			
	C1	C2	C3	C4
FECR1	22.76±0.35	36.89±0.56	49.78±0.71	0.79±1.22
FECR2	13.34±0.70	18.60±0.45	45.23±1.67	-0.80±1.80
FECR3	33.76±0.45	41.60±0.56	72.23±0.67	-0.05±1.32

Statistically non-significant (p>0.05) means the sharing of common alphabets in a column or a row. Small alphabets show the comparison among interaction means.

Effect of the herbal compound on hematological parameters of camels

Table 8: Effect of the herbal mixture on mean PCVs (%) values of camel

Week	Group			
	C1	C2	C3	C4
W0	24.45±0.20	24.63±0.17	24.57±0.11	24.62±0.12
W1	24.51±0.20	24.67±0.16	24.64±0.11	24.67±0.12
W2	24.53±0.20	24.78±0.17	24.76±0.11	24.54±0.12
W3	24.59±0.20	24.84±0.17	24.67±0.09	24.50±0.12
W4	24.63±0.19	24,94±0.17	24.92±0.10	24.46±0.11

Statistically non-significant (p>0.05) means the sharing of common alphabets in a column or a row. Small alphabets show the comparison among interaction means.

Table 9: Effect of the herbal mixture on mean HB (g/dl) values of camels.

Week	Group			
	C1	C2	C3	C4
W0	12.3±0.009	14.4±0.050	11.7±0.089	11.3±0.126
W1	12.9±0.121	14.5±0.011	11.9±0.024	11.0±0.136
W2	13.1±o.116	14.6±0,005	12.2±0.032	10.9±0.130
W3	13.3±0.112	14.7±0.014	12.5±0.026	10.7±0.145
W4	13.4±0.119	14.8±0,012	12.9±0.031	10.5±0,134

Statistically non-significant (p>0.05) means sharing common alphabets in a column or a row. Small alphabets show the interaction between the values.

Table 10: Effect of the herbal mixture on mean WBCs (10^9/mm^3) values of camels.

week	Group			
	C1	C2	C3	C4
W0	12.9±0.34	24.6±0.37	19.2±0.43	15.1±0.49
W1	12.7±0,38	23.7±0.33	18.5±0.45	15.8±0.45
W2	12.3±0.42	22.9±0.28	17.8±0.56	16.4±0.37
W3	11.8±0.45	15.8±0.25	16.4±0.50	17.5±0.39
W4	10.6±0.36	13.4±0.17	14.7±0.38	18.9±0.55

Statistically non-significant (p>0.05) means sharing common alphabets in a column or a row. Small alphabets show the interaction between the values.

Table 11: Effect of herbal mixture on mean MCH (pg/l) values of camels.

Week	Group			
	C1	C2	C3	C4
W0	22.7±0.221	31.3±0.145	25.1±0.165	25.4±0.024
W1	22.8±0.258	31.6±0.199	25.2±0.167	25.9±0.046
W2	22.9±0.270	33.5±0.166	25.40.145	25.10±0.057
W3	22.10±0.269	34.4±0.145	25.50.160	24.9±0.079
W4	21.3	34.2±0.171	25.7±0.147	24.9±0.062

Statistically non-significant (p>0.05) means sharing common alphabets in a column or a row. Small alphabets show the interaction between the values.

Table 12: Effect of the herbal mixture on mean MCHC (g/dl) values of camels

Week	Group			
	C1	C2	C3	C4
W0	49.70±0.22	71.01±0.36	55.90±0.38	56.6±0.40
W1	49.81±0.32	72.30±0.26	55.95±0.18	56.4±0.44
W2	49.67±0.36	72.32±0.24	56.20±0.17	56.76±0.28
W3	49.80±0.31	72.65±0.45	56..42±0.21	56.80±0.46
W4	49.85±0.18	72.80±0.24	57.34±0.34	56.90±0.50

Statistically non-significant (p>0.05) means sharing common alphabets in a column or a row. Small alphabets show the interaction between the values.

Table 13: Effect of the herbal mixture on mean MCV (fl) values of camels.

Week	Group			
	C1	C2	C3	C4
W0	45.80±0.56	44.2±0.52	45.1±0.56	44.78±0.33
W1	45.81±0.48	43.42±0.52	44.91±0.56	44.85±0.34
W2	45.86±0.47	43.28±0.44	44.81±0.60	44.90±0.34
W3	45.78±0.48	43.20±0.40	44.71±0.58	44.92±0.37
W4	45.70±0.45	43.12±0.43	45.1±0.57	44.95±0.35

Statistically non-significant (p>0.05) means sharing common alphabets in a column or a row. Small alphabets show the interaction between the values.

Table 14: Effect of the herbal mixture on mean RBCs ($x10^6$/pl) values of camels.

Week	Group			
	C1	C2	C3	C4
W0	5.41±0.102	3.61±0.165	4.67±0.135	5.66±0.122
W1	5.42±0.084	3.69±0.171	4.77±0.136	5.64±0.121
W2	5.54±0.084	3.76±0.157	4.97±0.156	5.55±0.122
W3	5.57±0.084	3.81±0.148	5.20±0.157	5.49±0.129
W4	5.81±0.080	3.97±0.158	5.40±0.158	5.45±0.124

Statistically non-significant (p>0.05) means sharing common alphabets in a column or a row. Small alphabets show the interaction between the values.

Effect of Herbal Compound on serum biochemistry of camels

A) Effect of the herbal compound on the mean Total bilirubin, (mg/dl) value of camels naturally infected by gastrointestinal helminths: Total bilirubin values decrease as the dose of herbal mixture increases from the group C1 to C3 (See figure 3).

B) Effect of the herbal compound on the mean alkaline phosphatase, ALP (U/L) value of camels naturally infected by gastrointestinal helminths: Alkaline phosphate values decrease as the dose of herbal mixture increases from the group C1 to C3 (See figure 4).

C) Effect of the herbal compound on the mean Aspartate aminotransferase AST (U/L) value of camels naturally infected by gastrointestinal

helminthes: Aspartate aminotransferase values decrease as the dose of herbal mixture increases from the group C1 to C3. Figure 5 shows that means is between groups C1, C2, C3, and C4.

D) Effect of the herbal compound on the mean blood urea nitrogen, BUN (mg/dl) value of camels naturally infected by gastrointestinal helminths: Blood urea nitrogen values decrease as the dose of herbal mixture increases from group C1 to C3. Figure 6 represents the effect of the herbal compound on the mean blood urea nitrogen, BUN (mg/dl) value of camels naturally infected by gastrointestinal helminths.

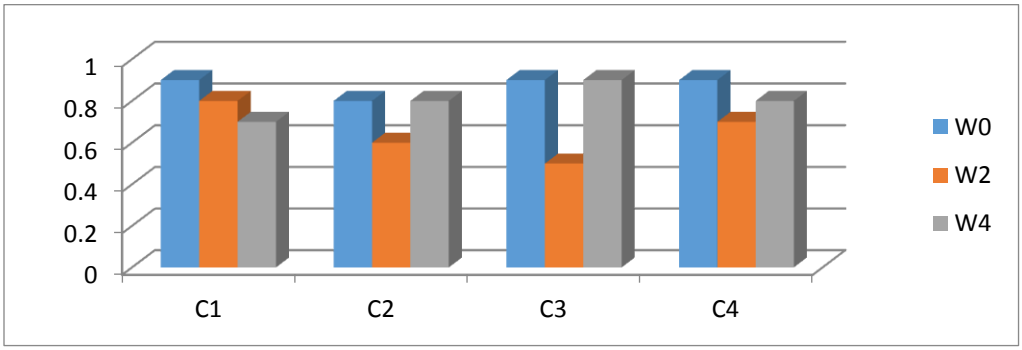

Fig 3 Showing mean serum total bilirubin values of camel infected by gastrointestinal helminths and then treated with an herbal mixture

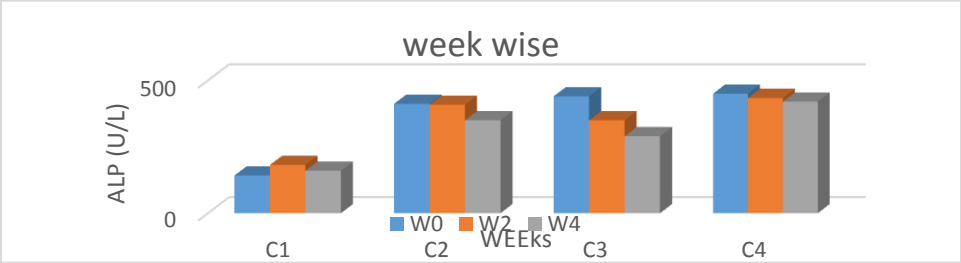

Fig 4: Showing mean serum alkaline phosphatase values of camel infected by gastrointestinal helminths and then treated with an herbal mixture

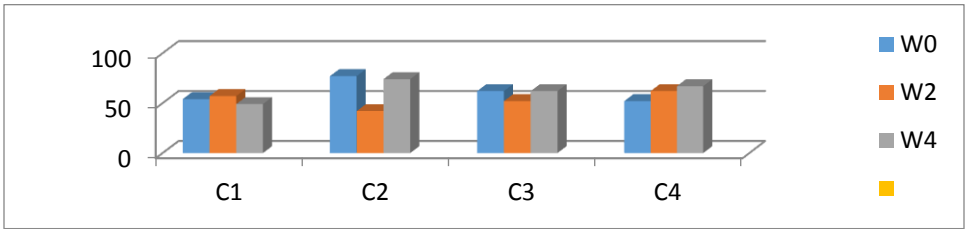

Fig 5 Showing mean serum Aspartate Aminotransferase values of camel infected by gastrointestinal helminths and then treated with an herbal mixture.

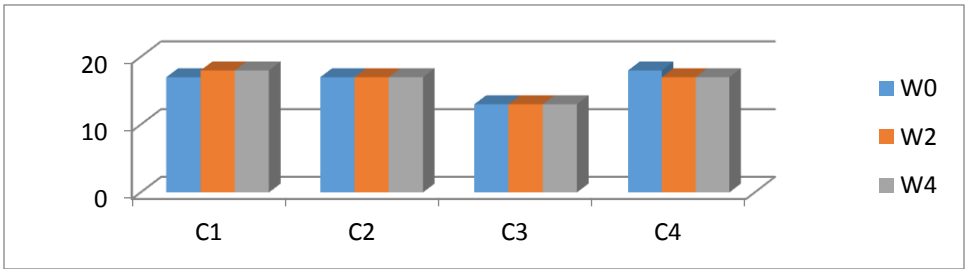

Fig 6 Showing mean serum Blood urea nitrogen values of camel infected by gastrointestinal helminths and then treated with an herbal mixture.

E) Effect of the herbal compound on the mean Creatinine (mg/dl) value of camels naturally infected by gastrointestinal helminths: Blood urea nitrogen values decrease as the dose of herbal mixture increases

from group C1 to C3. Figure 7 indicates that means are between groups C1, C2, C3 and C4. Similarly, 350mg/kg, 400mg/kg, and 450mg/kg doses of the herbal compound were given to groups C1, C2, and C3 respectively. Group C4 was a negative control.

F) Effect of the herbal compound on the mean Alanine Aminotransferase, ALT (U/L) value of camels naturally infected by gastrointestinal helminths: Alkaline phosphate values decrease as the dose of herbal mixture increases from the group C1 to C3. Figure 8 shows the effect of the herbal compound on the mean Alanine Aminotransferase, ALT (U/L) value of camels naturally infected by gastrointestinal helminths.

G) Effect of the herbal compound on the mean Lactic Dehydrogenase LDH (U/L) value of camels naturally infected by gastrointestinal helminths: Lactic Dehydrogenase values decrease as the dose of herbal mixture increases from the group C1 to C3. Figure 9 shows the effect of the herbal compound on the mean Lactic Dehydrogenase LDH (U/L) value of camels naturally infected by gastrointestinal helminths.

H) Effect of herbal compound on the mean Gamma-Glut amyl Transferees GGT (U/L) value of camels naturally infected by gastrointestinal helminths: Gamma-Glut amyl Transferees values decrease as the dose of herbal mixture increases from group C1 to C3 (See figure 10).

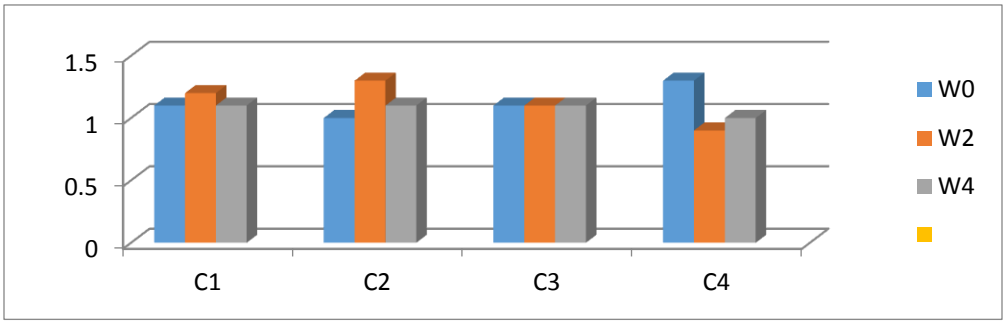

Fig 7 Showing mean serum Creatinine values of camel infected by gastrointestinal helminths and then treated with an herbal mixture.

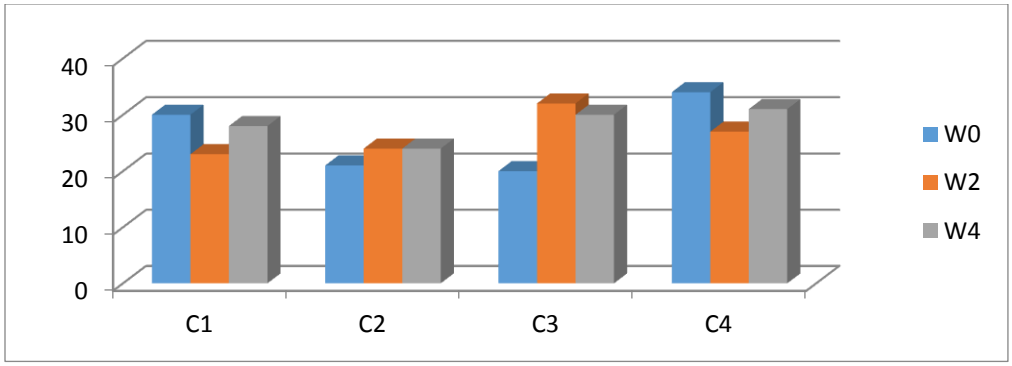

Fig 8 Showing mean serum Alanine aminotransferase values of camel infected by gastrointestinal helminths and then treated with an herbal mixture

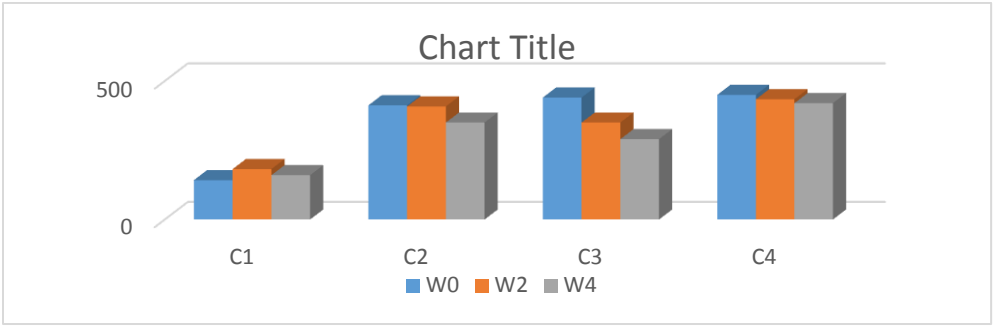

Fig 9 Showing mean serum lactic dehydrogenase values of camel infected by gastrointestinal helminths and then treated with an herbal mixture

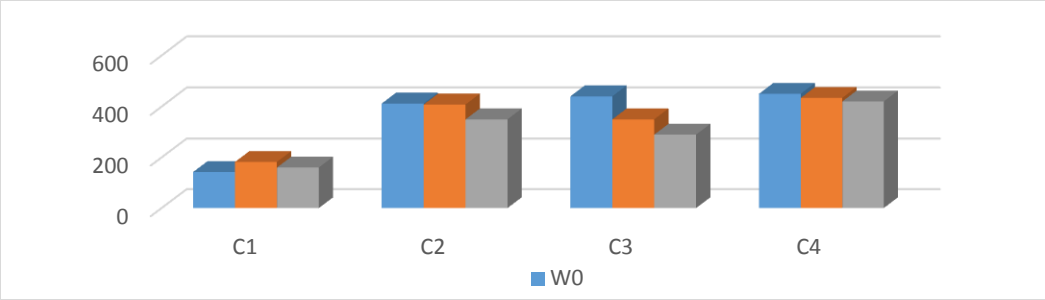

Fig 10 Showing mean serum Gamma glutamyl transferase values of camel infected by gastrointestinal helminths and then treated with an herbal mixture.

I) Effect of the herbal compound on the mean Total albumin (g/dl) value of camels naturally infected by gastrointestinal helminths: Total albumin values increase as the dose of herbal mixture increases from the group C1 to C3 (See figure 11).

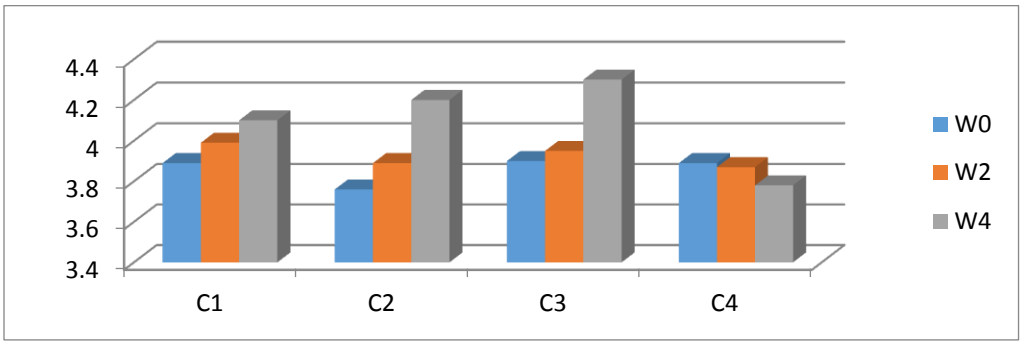

Fig 11 Showing mean serum Total albumin values of camel infected by gastrointestinal helminths and then treated with an herbal mixture.

DISCUSSION

This study was designed to evaluate the effects of the herbal compound against the gastrointestinal helminths of camels and to assess the effect of methanolic and aqueous extracts *in vivo*. The herbal compound was prepared from different proportions of seventeen plants and contained minerals, molasses, toxin binder, black salt, and common salt. Twenty nonpregnant, adult camels were selected from District Layyah. The trial was conducted on 20 camels. Camels were divided into 4 groups. The trial was conducted for 4 weeks. Treatment was given to camels for 4 weeks. Blood samples were collected in EDTA (Ethylene diamine tetra acetic acid) tubes and transported to the department of CMS, Faculty of Veterinary Science, University of Agriculture Faisalabad. Fecal samples were collected in polythene bags and transported to a laboratory for the diagnosis of animal diseases from week 0 to week 4, in Layyah. The presence and identification of secondary metabolites were determined using high-performance liquid chromatography. Phyto-chemical and nutritive analyses were also conducted.

There were only a few animals available for sampling. Farmers' refusal to allow their animals to be sampled did not appear to be a factor, though this has been reported as a problem in other areas.

Gastrointestinal helminth infection and economic losses in Pakistan livestock are among the major problems being faced by livestock owners of the country. Dried leaves of *Albizia lebbeck L.* and Crude ethanolic extract of the whole plant of *Camellia sinensis were evaluated against Hemoncus multicips.* Extracts of plants exhibited a remarkable inhibitory effect on *Hemonchus* species. The anthelmintic activity of the herbal compound was tested by using the eggs per gram (EPG) method. These results showed that plants contain bioactive compounds that can replace chemically prepared anthelmintic or can be a source to develop new anthelmintic. Different parts of plants and their extract have been used for many years for the treatment of different diseases and are a part of ancient science. This source of treatment and knowledge is transferred orally from one generation to the next generation. This type of treatment is very common in developing countries. It is a cheap source of treatment, can overcome the problem of drug resistance, has no side effects or resistance in milk and meat, and is safe for both animals and the environment. These products are now named

nutraceutical or ethnoveterinary medicine (Zaheer *et al.*, 2019).

An in vitro trial was conducted to investigate the potential of *Piper cubeba* fruit alcoholic extract on the gastrointestinal nematodes (eggs and larvae) of camels. A series of tests was performed to analyze its potential against eggs and larvae of nematodes. Results reveal that it can halt 100%.

Conclusion: The infection of gastrointestinal helminths is one of the major causes of losses in terms of meat, nutrition, milk production, and general health. Development of resistance in gastrointestinal helminths against chemical drugs which results in the development of multidrug resistant parasites. This scenario produces an urge for alternative medicine. These factors have directed the attention of scientists towards alternatives including medicinal plants as sustainable alternatives. In this scenario, plants, extracts, and essential oils are alternatives to natural dewormers. Scientists have confirmed that many traditional plants contain several unique chemical compounds. These compounds mainly include tannins, terpenoids, steroids, flavonoids, alkaloids, saponins, and a lot of others which are effective against helminths. The mode of action of these biologically active compounds has a unique action, even the mode of action of some compounds is still unknown. All over the world, many trials have proven various plants for their actives against gastrointestinal helminths. Biological active compounds are responsible for all the anthelminthic effects shown by plants and their extract that is present in these plants. The plants that were used in herbal compounds have already been proven for their anti-parasitic effects during various studies. Seventeen plants with different percentages were used to prepare the herbal compounds. In this mixture, along with these plants, toxin binder, black salt, common salt, and molasses were also used to make the herbal compound.

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