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Clinico-therapeutic Studies on Haemonchosis in Goats

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ABSTRACT

Background: Haemonchosis is one of the major parasitic infestations in small ruminants. *Haemonchus contortus* is a highly pathogenic parasite that localises in the abomasum of affected animals and exerts its pathogenicity by blood-sucking activity, adversely affecting the health and productivity of animals. In recent years, there is widespread development of anthelmintic resistance against *Haemonchus spp.* Therefore, a sustainable strategy needs to be implemented to effectively control haemonchosis in goats. The aim of the present study was to find out the efficacy of herbal anthelmintic drug *(Carica papaya* aqueous seed extract) and haemato-biochemical alterations in *Haemonchus* infection.

Methods: In this study, goats were screened for *Haemonchus spp.* by the FAMACHA technique. Hemato-biochemical examination was done before and after the therapeutic trial. Herbal anthelmintic drug was prepared from Papaya seed. *In-vitro* and *in-vivo* trial were carried out to test the efficacy of prepared herbal anthelmintic drug.

Result: The present study showed that FAMACHA can be used for the early screening of *Haemonchus spp.* in a herd. Significant improvement in haemato-biochemical examination was found in the group treated with the prepared herbal anthelmintic drug. Thus, the herbal anthelmintic (*Carica papaya* seed extract) can be a good alternative for controlling Haemonchosis in goats.

Key words: Anaemia, FAMACHA, Haemonchus, Herbal anthelmintic.

INTRODUCTION

Haemonchosis spp. infection is most common and economically important strongyle nematode infection in sheep and goats (Brahma *et al.,* 2015). The disease is more rampant and has been reported from all over the world where sheep and goats are reared (Odeniran *et al.,* 2016). Goats may become carriers once they acquire the infection, therefore, act as a potential source of pasture contamination (Shrivastava *et al.*, 2018). In goats and sheep, the prevalence may be as high as 100 per cent over a period in monsoon season (Futagbi *et al.,* 2015). Acute haemonchosis is characterized by dark-coloured faeces with blood and sudden death of affected animals (Kandasamy *et al.,* 2013). Diagnosis is mainly based on clinical signs, faecal examination and molecular techniques. A novel system called FAMACHA was developed in South Africa for screening of anaemia in sheep and goat. This technique can be applied at farm level to mitigate the problems related to treatment like, drug resistance in *Haemonchus contortus* (Kaplan *et al*., 2004). There are several classes of anthelmintic drugs effective against Haemonchus *spp.* like benzimidazoles (*e.g*., albendazole), imidazothiazoles, macrocyclic lactones, salicylanides (*e.g*., closantel) *etc*. but resistance to all classes of anthelmintic drugs have been seen (Sager *et al*., 2009). Thus, the treatment should be carried out with some recent anthelmintic because they have advantages over traditional anthelmintic, also a new indigenous anthelmintic should be tried with the idea that it will be cheaper easily available and less anthelmintic resistant.

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MATERIALS AND METHODS

In-vitro evaluation of anthelmintic activity of plant extract residues

Aqueous herbal extract of papaya seed was prepared and the dilution of extract was made in different concentrations *viz*. 2.5 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml in NSS for evaluation of their anthelmintic activity (Sharma *et al.,* 1971). Adult Haemonchus worms were collected from the abomasum of freshly slaughtered goats and transferred in beaker containing NSS at 37°C (Bhatnagar *et al.,* 1961). Anthelmintic efficacy was observed by keeping 30 worms

in different dilutions of test extract in NSS. Number of live and dead adult worms were counted at 1 hr, 2 hr and 3 hr. intervals. The corrected mortality for each extract was calculated by considering the mortality of worms, if any, in the NSS.

Corrected mortality was calculated as per the formula given by Sangwan and Sangwan (1988).

Corrected mortality= Total mortality - Control mortality × 100

Therapeutic trials

Therapeutic trials were conducted to determine the efficacy of herbal anthelmintic (*Carica papaya*) in comparison to chemical anthelmintic to control the nematode worms of goats. FAMACHA technique was applied for the screening of the cases for faecal, haematological and biochemical examination by FAMACHA score card. Adult goats naturally infected with nematodal worms without any previous history of use of anthelmintic was randomly selected for this study.

Collection and examination of faecal sample

Faecal samples were collected from the animals showing clinical signs and symptoms of haemonchosis. The samples were examined by using direct and indirect methods as described by Soulsby (1982). The speciation of parasites was done as per egg and larval morphology.

Experimental protocol

Following faecal examination, 12 positive goats were selected and were divided into two groups- T1 and T2. Another Group T was kept as positive control. The group T1 was treated by closantel (10 mg/kg body wt.) as single dose and group T2 was treated by Aq. Extract of *Carica papaya* seed extract. All the clinical parameters were studied at 0-, 7- and 14-day interval periods.

Collection of blood samples for hematology and biochemical study

Blood samples were collected for the study of different haemato-biochemical parameters from all the goats on day 0, 7 and 14. The parameters studied were Hb, TEC, TLC, total protein, albumin, globulin, serum glucose, ALT, GGT, Na, K, calcium and phosphorus.

Efficacy of the drugs

It was assessed based on percent reduction in faecal egg count (EPG), restoration of haemogram and different bio-chemical parameters, absence of clinical signs and improvement in general condition after treatment.

Statistical analysis

Statistical analysis was conducted to determine the difference among the groups at the same sampling time by using ANOVA, post- hoc Tukey's test with general linear models in SPSS 16.

RESULTS AND DISCUSSION

***In vitro* trial of anthelmintic activity of *Carica papaya* extract and closantel drug**

Corrected mortality of adult worms with Closantel and aq. Extract of *Carica papaya* (2.5, 5, 10, 15 mg/ml) has been shown in the Table 1. *In vitro* trial revealed that closantel @ 10 mg/ml had corrected mortality 26, 28 and 25 at 1 hr, 2 hr, and 3 hrs respectively with respect to control 0, 2 and 5 in the same interval period. Among different concentration the corrected mortality of aq. Extract 15 mg/ml were found to be 29, 27 and 25 at 1 hr, 2 hrs and 3 hrs of trial with respect to control. Thus, based on *in vitro* trial and corrected mortality percentage the herbal extract of 15 mg/ml concentration was selected for further *in vivo* study along with closantel @ 10 mg/ml b.wt. Similar *in vivo* and *in vitro* studies on the efficacy of anthelmintic against *Haemonchus* in goats were done by Akhter *et al*. (2014). Unlike corrected mortality of adult worm of *Haemonchus contortus,* they used Egg Hatch Assay (EHA). Present findings were in accordance with Hoste *et al*. (2006) who reported the effect of tannin-rich plants on internal nematodes in ruminants.

Haematological observations

The present study revealed that goats suffering from Haemonchosis had significantly ($p<0.05$) lower Haemoglobin, PCV and TEC (Table 2). Rapid blood loss in Haemonchosis affected goats was due to blood sucking nature of the worms (Besier *et al*., 2016). Significant increase in Hb, PCV and TEC in both groups T_1 and T_2 indicated *Carica papaya* extract efficacy in treatment and increase in haematological parameters was comparable to closantel in this study. Significant decrease in total leucocytic count (TLC) was found on 7^{th} and 14^{th} day and it might have occurred due to antioxidant property of *Carica papaya* (Farida and Iswahyani, 2018). Similar observation was also made by (Qamar and Makbool, 2012).

Table 1: Corrected mortality of adult worm with Closantel and aq. Extract of *Carica papaya* (2.5, 5, 10, 15 mg/ml).

Drugs	No. of adult worm in different time intervals.			
concentrations	0 hr.	1 hr.	2 hrs.	3 hrs.
Control	0	0	2	5
Closantel	0	26	28	25
Carica papaya 2.5 mg/ml	0	0	4	15
Carica papaya 5 mg/ml	0	2	7	21
Carica papaya 10 mg/ml	0	25	25	24
Carica papaya 15 mg/ml	0	29	27	25

Table 2: Mean±S.E of Hb (gm%), PCV (%), TEC (10^6/ µL), and TLC (10^3/µL) in different treatment groups.

Days	Hb (g%)			PCV (%)			TEC (10^6/µL)			TLC (10^3/µL)		
	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2
0	11.06±0.388^{ax}	4.083 ±0.597^{bx}	5.833 ±0.247^{cx}	33.200 ±1.164^{ax}	15.250 ±1.055^{bx}	17.500 ±0.742^{cbx}	5.533±0.194^{ax}	2.467±0.189^{bx}	3.417±0.531^{cx}	9.150±0.198^{ax}	10.767±0.049^{bx}	10.733±0.067^{cbx}
7	11.217±0.394^{ax}	5.862±0.320^{by}	8.000±0.289^{cy}	33.650± 650^{ax}	18.000±0.866^{bx}	24.000±0.866^{cy}	5.600±0.197^{ax}	2.300±0.163^{bx}	3.917±0.139^{cay}	9.383±0.130^{ax}	10.033±0.061^{by}	10.000±0.063^{cby}
14	11.350±0.357^{ax}	7.500±0.365^{bz}	9.333± 0.333^{cz}	34.050±1.070^{ax}	22.500±1.095^{by}	28.000±1.000^{cz}	5.658±0.172^{ax}	3.750±0.183^{by}	4.667±0.167^{cy}	9.483±0.095^{ax}	9.533±0.067^{az}	9.417±0.130^{az}

Mean with different row wise superscripts (a, b, c) differ significantly (p<0.05) Mean with different column wise superscripts (x, y, z) differ significantly (p<0.05).

Biochemical observations

Decreased total serum protein (Table 3) in the infected goats in present study may be attributed to haemodilution, a compensatory mechanism for the abomasal haemorrhages. The mean albumin levels were also significantly lower that might be due to abomasal damage, selective loss of albumin which is smaller in size and osmotic sensitivity to fluid movement (Tanwar and Mishra, 2001). After treatment with closantel and *Carica papaya* extract non- significant improvement in total protein level while significant improvement in albumin level was observed (Bandyopadhya and Dasgupta, 2000).

Lower blood glucose (Table 3) finding in Haemonchosis affected goats was in accordance with Arora *et al*. (2001). This might be due to reduction in food intake and absorption from injured gut and due to elevation of blood gastrin level (Nicholls *et al*., 1988). Non-significant improvement was noticed in treatment group on 14th day post treatment.

Serum enzyme ALT concentration in goats varied indifferently while GGT had significant variation (Table 4). Findings of Kumar *et al*. (2013) supported increased levels of ALT in the present study. Non - significant lowering of mean ALT level was observed in both treatment group on 7th and 14th days post treatment. Significant decreases in the mean GGT levels were also observed in group T2 on 14th day of treatment. The elevation of serum ALT and AST level indicated some disruptive activities in organs or altered membrane permeability or may be due to lack of excretion (Bordoloi *et al*., 2012). High level of ALT and AST also occur due to traumatic damage to the abomasal mucosa caused by larvae and adult (Al-Zubaidy *et al.,* 1987).

Major electrolytes Na and K and macro minerals Ca and P (Table 5) were significantly decreased from the unaffected control level. Murad *et al*. (2018) also reported 7.2% drop in calcium level in blood during experimental Haemonchus *spp*. infection. Gilani, (1981) reported hypophosphatemia after conducting the trial on haemonchosis. Significant improvement in mean Na, K, Ca, and P post treatment in both Closantel and *Carica papaya* group might be due to elimination of parasites, improvement in digestion and absorption.

EGG Per gram (EPG)

The mean value of EPG (Table 6) showed variable results. Al Omar *et al*. (2016) also reported variability in faecal egg count of *Haemonchus contortus* infection in their study. This might be due to individual animal's genetic makeup and the environment to which the animals are exposed (Gadahi *et al*., 2009). It was found that significant improvement in faecal egg count was reported in both treatment groups on 7th and 14th day post treatment with slightly better Faecal egg count Reduction Test (FECRT) in group T2 in comparison to T1. This signifies that the *Carica papaya* seed extract works satisfactorily in reducing the faecal egg count and can be a good alternative to chemical anthelmintic.

Table 3: Mean±S.E of total protein (g/dl), abumin (g/dl), globulin (g/dl) and glucose (mg/dl) in different treatment groups.

Days	Total protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)			Glucose (mg/dl)		
	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2
	7.933±0.295^{ax}	5.462±0.783^{bx}	7.572 + 0.285^{ax}	3.683±0.125^{ax}	1.950±0.230^{bcx}	1.950±0.230^{bcx}	4.983±0.162^{ax}	3.805±0.368^{ax}	4.815±0.536^{ax}	55.167± 2.227^{ax}	35.743± 2.864^{bx}	45.000±1.915^{cx}
7	7.933± 0.309^{ax}	6.992± 0.564^{ax}	7.710+ 0.577^{ax}	3.717± 0.135^{ax}	2.750±0.395^{bcxz}	3.570±0.331^{acyz}	5.100±0.234^{ax}	4.242 ±0.657^{ax}	4.442 ±0.735^{ax}	55.667± 1.820^{ax}	38.318± 2.695^{bcx}	44.195±0.926^{cx}
14	7.883±0.324^{ax}	7.710±0.577^{ay}	8.973 + 0.520^{ax}	3.617± 0.178^{ax}	3.490±0.403^{ayz}	3.501±0.222^{az}	4.983±0.172^{ax}	4.220±0.635^{ax}	5.120±0.538^{ax}	54.000± 1.932^{ax}	44.403±2.648^{bx}	46.207±1.702^{bx}

Table 4: Mean±S.E of SGPT (U/L), GGT (U/L) in different treatment groups.

Days	SGPT (U/L)			GGT (U/L)		
	T	T_1	T_2	T	T_1	T_2
0	41.167±3.293^{ax}	43.167±2.845^{ax}	52.333±3.23^{bx}	20.667±2.201^{ax}	38.000±1.932^{bcx}	38.618±2.130^{cx}
7	41.667±3.293^{ax}	42.000±2.745^{ax}	46.500±2.184^{axz}	22.000±1.880^{ax}	24.482±1.403^{ayz}	15.508±1.780^{byz}
14	40.500±2.975^{ax}	35.920±1.683^{ax}	31.238±0.678^{ay}	22.333±0.919^{ax}	23.337±0.682^{bz}	15.928±1.132^{bz}

Table 5: Mean±S.E of Sodium (meq/L), Potassium (meq/L), Calcium (mg/dl), and Phosphorus (mg/dl) in different treatment groups.

Days	Days Sodium (meq/L)			Potassium (meq/L)			Calcium (mg/dl)			Phosphorus (mg/dl)		
	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2	T	T_1	T_2
0	144.500±4.113^{ax}	122.000±0.577^{bx}	125. 755 ±9.842^{bx}	4.983± 0.162^{ax}	3.805±0.368^{abx}	3.293± 0.089^{cx}	8.752±0.563^{ax}	6.985±0.279^{bx}	4.484±0.384^{bcx}	4.983±0.162^{ax}	3.805±0.368^{bx}	4.495± 0.173^{abx}
7	144.750±4.554^{ax}	141.833±0.601^{y}	132.167±0.833^{axy}	5.100±0.234^{ax}	4.785± 0.202^{cxy}	4.863±0.103^{cxy}	9.112±0.450^{ax}	7.083 ±0.337^{bx}	7.682±0.571^{aby}	5.100±0.234^{ax}	4.075± 0.272^{bx}	4.822±0.079^{ax}
14	143.050±3.519^{ax}	142.167±0.792^{ay}	140.667±0.494^{ay}	4.983±0.176^{ax}	4.817 ±0.136^{bz}	4.892±0.388^{cxz}	9.262±0.471^{ax}	7.700±0.285^{bx}	8.332± 0.561^{aby}	4.983±0.176^{ax}	4.857±0.214^{ay}	4.927± 0.060^{ax}

Table 6: Mean±S.E of EPG in different treatment groups.

Days	T (Control)	T1 (Drug)	T2 (Herbal)
0	250.000±22.362^{ax}	1000.000±57.735^{bx}	950.000±111.803^{cx}
7	1.333±0.211ax	2.500±0.224aby	2.333±0.211ac
14	233.333±21.082^{ax}	383.333±47.726^{axz}	283333±30.732^{az}

Table 7: Mean±S.E of FAMACHA score in different groups.

Days	T (Control)	T1 (Drug)	T2 (Herbal)
0	1.667±0.211^{ax}	3.333±0.211^{aby}	3.167±0.307^{acz}
7	233.333±21.082^{ax}	533.333±33.333^{by}	483.333±60.093^{cyz}
14	1.333±0.211^{ax}	2.000±0.258^{bay}	1.833±0.307^{caz}

Table 8: Haematological correlation with FAMACHA score and EPG.

	FAMACHA score	Hb%	EPG	PCV	TEC	TLC
FAMACHA score	1					
Hb %	-7.18	1				
EPG	.860	-.799	1			
PCV	-.718	.999	-.799	1		
TEC	-.714	.999	-.796	.999	1	
TLC	-.324	.264	-.272	.267	.264	1

FAMACHA

Mean±S.E of FAMACHA score in different groups have been shown in Table 7. FAMACHA score system measuring from 1 to 5 was used to assess the degree of anaemia in goats in all three groups. The post treatment values with each of the drug decreased significantly on 7th and 14th day of treatment with lowest FAMACHA score found in T2 group (*Carica papaya* group). As per Pugh and Baired (2012) plants that contains tannin have been found to reduce Faecal Egg Count and worm burden in goats. Jaiswal *et al*. (2008) and Iliev *et al*. (2017) also reported the anthelmintic property of papaya seed extract. Reduction in FAMACHA Score in both the treatment group suggest that closantel is a preferred choice of anthelmintic for Haemonchosis in goats with rotational use of *Carica papaya* seed extract is alternative and economical choice for the treatment.

FAMACHA score, EPG and haematological parameters

Haematological correlation with FAMACHA Score and EPG has been shown in Table 8. Strongly negative correlation was found between FAMACHA vs Hb, PCV and TEC. Highly significant negative correlation between EPG and PCV, EPG and Hb and positive correlation between FAMACHA score and EPG was observed. These findings are in accordance with the findings of Bala *et al*. (2015) and Baihaqi *et al*. (2020).

CONCLUSION

The present study concluded that FAMACHA technique can be used for detecting the severity of anaemia in *Haemonchus* infected goats. Strong negative correlation was found between Hb and EPG, FAMACHA score and Hb and strong positive correlation was found between FAMACHA score and EPG. Closantel and *Carica papaya* seed extract (aq.) were found to be effective in *in vitro* and *in vivo* trial. Strong negative correlation was found between Hb and EPG, F.S and Hb and strong positive correlation was found between F.S and EPG. Based on biochemical and haematological examination, it was found that herbal anthelmintic (*Carica papaya* seed extract) can be a good alternative for controlling Haemonchosis in goats. Planned grazing, high plain of nutrition, early diagnosis by FAMACHA technique and use of anthelmintic can prevent drug resistance and help in preventing mortality in goats from *Haemonchus.*

Conflict of interest: None.

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