

## EFFICACY OF CURCUMIN AGAINST GASTRO-INTESTINAL PARASITES IN GOAT

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Article Received on 13/06/2016

Article Revised on 03/07/2016

Article Accepted on 23/07/2016

### ABSTRACT

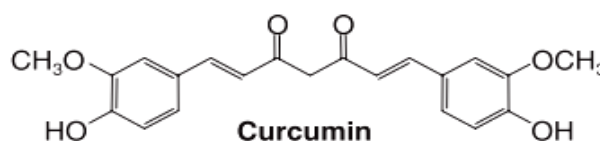
A total of 30 Black Bengal goats weighing (11-19 ± 2.6) kg irrespective of sex infested with gastrointestinal parasites were selected for this experiment and were randomly assigned into one of three equal groups (n =10) A(control), B (0.2% curcumin with normal ration) and C (0.6% curcumin with normal ration) with free access to food and water for 60 days. The body weight increased in some cases by the treatment with the advancement of age but statistically insignificant. The highest body weight was found in the group C. The eosinophil count of group B and C decreased significantly (p<0.05) compared to control. At the end of experiment the lowest eosinophil count was observed in the group C but at the beginning of the experiment, it was the highest. The fecal egg count of *Paramphistomum* worm in group C was significantly higher than others (p<0.05). In control group the rate of infestation were increased (Akanda *et al.*, 2012) but at the 60<sup>th</sup> day of the experiment the fecal egg count in all group is significantly lower (p<0.05). The egg count of *Haemonchus sp.* and *Strongyles* was insignificant throughout the experiment. No characteristic lesion has been identified in the gastro-intestinal mucosa but gastritis with infiltration of inflammatory cells in the sub mucosa and in the liver parenchyma showing coagulation necrosis in the hepatocytes around the central vein and slightly swollen hepatocytes of the control animals as well as 0.6 % curcumin fed animals.

**KEYWORDS:** Curcumin, *Paramphistomum sp.*, *Fasciola sp.*, *Haemonchus sp.*, *Strongyles*.

### INTRODUCTION

Helminth infections are a major cause for reducing the productivity in livestock, particularly in poor world (Githiori *et al.*, 2006). The anthelmintic resistance in parasitism, particularly in small ruminants, is becoming an urgent problem for organic livestock producers to protect their animals from the parasite infection which affects their productivity. These major changes in livestock production systems may potentially result in re-emergence (or emergence) of parasitic infections (Thamsborg, S. M. and Roepstorff, A. 2014; Lindgren, K *et al.*, 2014). The use of grazing management strategies, combined with anthelmintic treatment, may provide good result in better parasite control at less cost, but may not reduce significantly (Barger, I. A. 1997) owing to the frequent movement of animals from one region to another (*J Am Vet Med Assoc* 2008; 233:1913–1919). Now-a-days, it is a crying need for alternative control measures of anthelmintic-resistant parasitic infestation. Turmeric, the bright yellow spice extracted from the

tuberous rhizome of the plant *Curcuma longa*, has been used as a medicine for centuries to treat a variety of ailments, including jaundice, hepatic disorders, rheumatism, anorexia, diabetic wounds and menstrual difficulties. Here we study the biological mechanisms and possible clinical effects of curcumin treatment on goat against gastro-intestinal parasites (Salvioli *et al.*, 2007).



**Fig. a. Chemical structure of curcumin (Pandit *et al.*, 2009)**

Black Bengal goat rearing is very popular in Bangladesh though it is going with struggle against parasitism. It is considered as one of the vital limitation of livestock

production (Jabbar and Green, 1983). Helminthiasis especially, GI nematodiasis overwhelming a severe havoc on health and production (Rehman *et al.*, 2009) throughout the world that has also economic impacts, is a vital factor for lower productivity of Black Bengal goats (Silvestre *et al.*, 2000). Gastrointestinal nematodes pretense serious loss to goat health and production in tropical areas (Perry *et al.*, 2002; Sahlu *et al.*, 2009) with a high rate of anthelmintic resistance prevalence (Howell *et al.*, 2008; Kaplan *et al.*, 2004). The use of sustainable, integrated parasite control systems, using scientifically proven non-chemical methods and limited use of drugs is being considered to ensure animal health and food safety (Waller, 2006). We can prevent and control the parasitic diseases by using a routine prophylactic anthelmintics measurement. *Hemonchus contortus* is a blood feeding nematodes which are found in the abomasum of sheep and hampers on sheep production. Curcumin has an antioxidant and anti-inflammatory activity (Tohda *et al.*, 2006 & Punitha *et al.*, 2005 & Salvioli *et al.*, 2007). Very little study has found about the effect of curcumin on the development of immune responses (Gautam *et al.*, 2007 & Yadav *et al.*, 2005) and against gastro-intestinal parasites in goats (Gautam *et al.*, 2007). This is why, the present research work was undertaken to find out the scientific phenomena in this regards which is not yet studied in South Bengal. Based on the above consideration, the research was carried out with the following objectives:

- Control of parasitic infection in goat by changing nutritional status.
- Reduced use of anthelmintic drugs.
- Improvement in meat and milk production in goat.

## MATERIALS AND METHODS

### Study placement and duration:

Thirty (30) goats of 24-26 months age and weighing ( $11-19 \pm 2.6$ ) kg were selected in Khanpura, Babuganj Upazila of Barisal district of Bangladesh. Tenure of the study was June, 2012 to December, 2012. Age of goat was determined by asking the owner and farm attendant and by visual inspection and also by dentition whenever possible. Animals were housed during the experiment and for 15 days before the start of the study. Animals were fed on a commercial balanced concentrate diet and water was provided ad libitum.

### Hematological studies

#### Blood collection for Eosinophil count

Blood samples from all 30 selected animals were collected especially from the jugular vein using sterile syringe and needle maintaining aseptic condition, 5 ml of blood sample was collected from Jugular vein of each goat and kept in vials containing anticoagulant (Sodium citrate) and this was done on 1, 15, 30, 45 and 60 days after administration of 0.2% curcumin and 0.6% curcumin along with normal ration. During the experimental period hematological studies performed following the methods were described by Coffin 1955) for eosinophil count.

### EPG counting

EPG was recorded just prior to treatment from each group. EPG of experimental goat was determined by McMaster method. In this method a known volume of feces (5gm) was thoroughly suspended in a known volume of (50ml) saturated salt solution. The suspension was stirred through a 150 mm mesh sieve to remove the coarse particles. A portion of the suspension was withdrawn with the help of Pasteur pipette and allowed to run into the chambers of the McMaster slide. The slide was allowed to stand for 5 minutes to allow the eggs to float. The eggs in the two chambers were counted using low power objectives (X10). The number of eggs per gram of feces was calculated by using the following formula;

$$\text{Number in one gm} = \frac{\text{Number in two chambers}}{0.3} \times \text{dilution factor}$$

$$\text{Dilution factor} = \frac{\text{Total volume of suspension in ml}}{\text{Total volume of feces}}$$

EPG count was also done on 1, 15, 30, 45 and 60 days post treatments by using Stoll's ova counting technique.

### Histopathology

At the end of the experiment, the animals were sacrificed and examined for gross pathological lesions. At necropsy, gross tissue changes were observed, recorded carefully and representative tissue samples were preserved in 10% buffered formalin for histopathological studies as per standard procedure.

### Statistical analysis

Mean values of both the hematological and EPG counts and EPG of different body scores were analyzed by Duncan's Multiple Range Test procedure following the SPSS software version 17 and the graphical presentation was done by the Sigma Plot version 12.

## RESULTS AND DISCUSSION

### The effects of curcumin (0.6% curcumin and 0.2% curcumin) on body weight of goat

The effects of body weight by curcumin (0.2% curcumin and 0.6% curcumin) were presented in the table-1 and figure 1. With the advance of age the body weight of 0.2% curcumin and 0.6% curcumin treated animals showed significant differences but comparing to control 0.6% curcumin pretreatment animals were statistically similar. Overall, result did not significantly affect growth performance comparing to control. The body weight was almost similar to their pre-treatment ('0' day) values. The result is in conformity with the earlier researchers Guha *et al.*, 1986. Findings of the present study reasonably agreed with the findings of the above mentioned authors. The body weights by the curcumin treated goats were supported by previous reports (Pandit *et al.*, 2009). The parasitic infestation might be responsible to arrest the growth. The body weight was increased that might be due to removal of parasitic load

and might have had fascilitate the weight regain through proper digestion, absorption and metabolism of feed nutrient in the parasite free gastrointestinal tract.

#### The effects of curcumin (0.6% curcumin and 0.2% curcumin) on eosinophil count of goat

Hematological studies of the treated goats (0.2% curcumin and 0.6% curcumin) showed a decrease in eosinophil count ( $p < 0.05$ ) which is presented in the table 2 and fig. 2. Group A showed a greater absolute number of eosinophil count than did in the groups B or C. Peripheral eosinophilia during the elimination of parasites has been proposed as a marker of host response against gastrointestinal worms, but this finding does not appear to be universal. However, the decrease in eosinophil count was significant only in goats infested with *Fasciola* and *Haemonchus* spp. (Siddiqua *et al.*, 1990). There is a close relationship between natural resistance and the mechanisms that control the parasite load which allows animals to be productive in spite of low levels of parasitism. This observation, combined with the relative values of blood components, especially the absence of eosinophilia in the more susceptible group (group C), may indicate that the leucopaenia found in most infected groups can be a result of bone marrow depletion, but since no bone marrow analysis was made, this cannot be proved by the present data, furthers studies are required under natural conditions to evaluate bone marrow analysis with more accuracy.

#### Effects of curcumin (0.2% curcumin and 0.6% curcumin) on parasitic egg count

The effects of curcumin (0.2% curcumin and 0.6% curcumin) on parasitic egg count are presented in the

table 3 and fig. 3-4. The eggs found throughout the experiment are *Paramphistomum*, *Fasciola*, Stomach worm and *Strongyles*. Cestodes were not detected in any samples examined but mixed infections (nematodes and trematodes respectively) were observed in most cases (Bhutto *et al.*, 2002). At the 45<sup>th</sup> day of the experiment, the fecal eggs count of *Paramphistomum*, *Fasciola*, Stomach worm and eggs count of *Strongyles* among control, 0.2% curcumin and 0.6% curcumin treatments were significant ( $p < 0.05$ ) compared to the control. In untreated naturally parasitized control group the rate of infestation were increased (Akanda *et al.* 2012) but at the 60<sup>th</sup> day of the experiment the fecal egg count in all group is significantly lower ( $p < 0.05$ ). Investigations of young animals indicated that no reinfection can occur during the dry season. The findings are discussed with regard to their relevance for strategic control of gastrointestinal nematodes in small ruminants (Fritsche T, Kaufmann J, Pfister K. 1993).

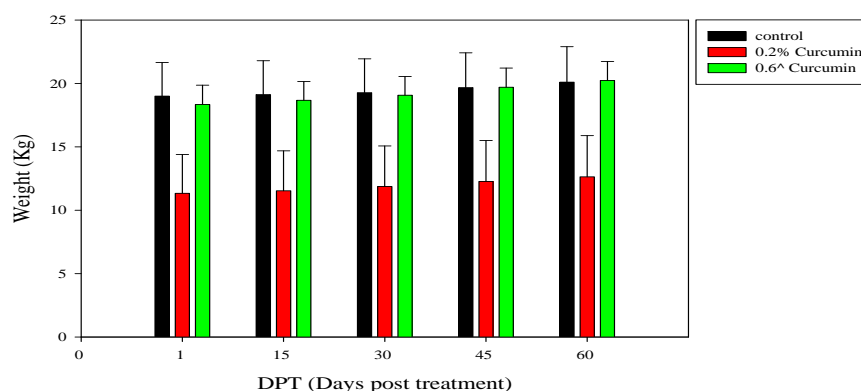
#### Gross and histological observation of tissues

No characteristic gross lesions has been identified in the gastro-intestinal mucosa of treated animals (0.2% curcumin) but gastritis with mild infiltration of inflammatory cells in the sub mucosa and in the liver parenchyma showing coagulation necrosis in the hepatocytes around the central vein and slightly swollen hepatocytes of the parasitized animals (control) as well as fed on 0.6% curcumin mixed diet. This is may be due to the anti- inflammatory effects of curcumin. (Fig-5-13).

**Table 1. Effects of curcumin on body weight of goat**

Treatment	Body weight (Kg)				
	1 <sup>st</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
Control	19 ± 2.65 <sup>a</sup>	19.12 ± 2.67 <sup>a</sup>	19.27 ± 2.67 <sup>a</sup>	19.67 ± 2.74 <sup>a</sup>	20.1 ± 2.80 <sup>a</sup>
0.2% Curcumin	11.33 ± 3.06 <sup>b</sup>	11.53 ± 3.15 <sup>b</sup>	11.87 ± 3.20 <sup>b</sup>	12.27 ± 3.23 <sup>b</sup>	12.63 ± 3.25 <sup>b</sup>
0.6% curcumin	18.33 ± 1.53 <sup>a</sup>	18.67 ± 1.48 <sup>a</sup>	19.07 ± 1.48 <sup>a</sup>	19.7 ± 1.51 <sup>a</sup>	20.23 ± 1.50 <sup>a</sup>

Means ± standard deviation followed by different letters indicate statistically significant differences (SPSS, DMRT test,  $p < 0.05$ ) from each other. 30 samples for each treatment maintained by three replicates were conducted.

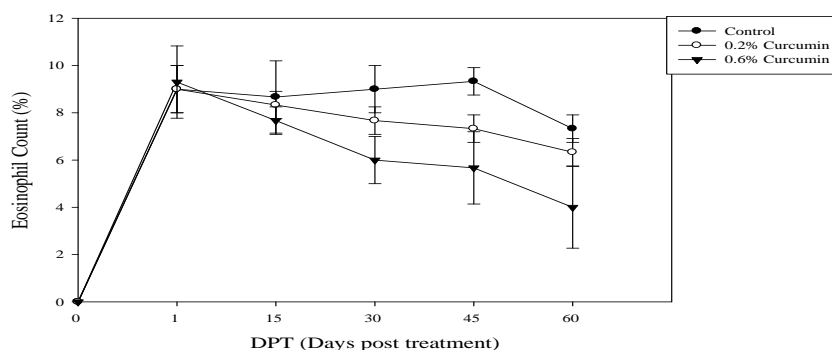


**Fig.1: Effects of curcumin on the body weight**

**Table 2. Effects of curcumin on Eosinophil count**

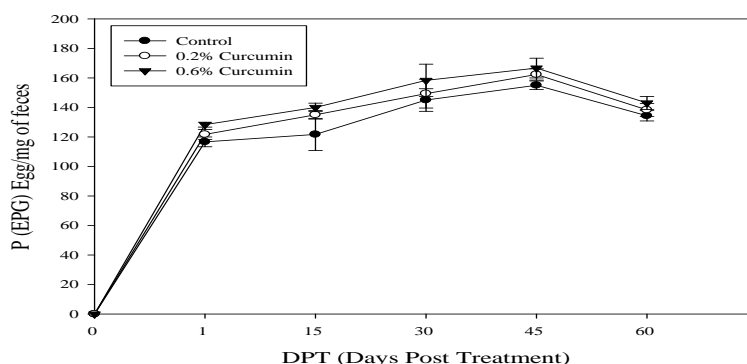
Treatment	Eosinophil count (%)									
	1 <sup>st</sup> day		15th day		30th day		45th day		60 <sup>th</sup> day	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	9a	± 1	8.67a	± 1.53	9a	± 1	9.33a	± 0.58	7.33a	± 0.58
0.2% curcumin	9a	± 1	8.33a	± 0.58	7.67ab	± 0.58	7.33b	± 0.58	6.33a	± 0.58
0.6% curcumin	9.3a	± 1.53	7.67a	± 0.58	6b	± 1	5.667b	± 1.53	4b	± 1.73

Means ± standard deviation followed by different letters indicate statistically significant differences (SPSS, DMRT test,  $p < 0.05$ ) from each other. 30 samples for each treatment maintained by three replicates were conducted.

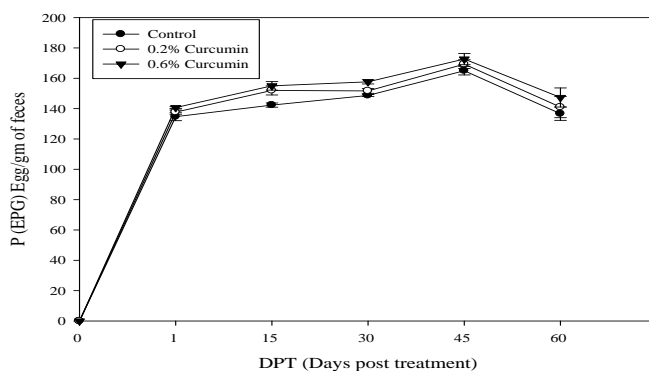
**Fig.2: Effects of curcumin on Eosinophil count****Table 3. Effects of curcumin (0.2% curcumin and 0.6% curcumin) on fecal egg count**

Treatment	DPT	P (EPG)	F (EPG)	S (EPG)	St (EPG)
Control	1	116.67 ± 3.3 <sup>b</sup>	134.67 ± 2.6 <sup>c</sup>	122.33 ± 1.4 <sup>bc</sup>	68.33 ± 4.4 <sup>b</sup>
	15	121.67 ± 10.9 <sup>b</sup>	142.33 ± 1.4 <sup>bc</sup>	111.67 ± 7.2 <sup>c</sup>	60 ± 10 <sup>b</sup>
	30	145 ± 7.6 a	148.67 ± 0.6 b	129.33 ± 2.3 b	77.67 ± 1.4 b
	45	155 ± 2.8 a	165 ± 2.8 a	150 ± 2.8 a	117.67 ± 2.6 a
	60	134.33 ± 3.4 ab	136.67 ± 4.4 c	131 ± 3.0 b	68.33 ± 9.2 b
0.2% Curcumin	1	121.67 ± 3.3 c	137.67 ± 1.4 c	127.33 ± 2.6 b	105 ± 2.8 ab
	15	135 ± 2.8 bc	152 ± 3 b	121.67 ± 6 b	78.33 ± 4.4 d
	30	149.33 ± 9.7 ab	151.67 ± 1.6 b	134.33 ± 0.6 ab	96 ± 7.0 c
	45	162.33 ± 3.8 a	169.33 ± 2.9 a	145 ± 7.6 a	116.67 ± 4.4 a
	60	138.33 ± 4.4 bc	141 ± 7 bc	133.67 ± 1.8 ab	88.33 ± 1.6 cd
0.6% Curcumin	1	128.33 ± 1.6 c	140.67 ± 0.6 c	133.33 ± 1.6 b	111.67 ± 1.6 ab
	15	140 ± 2.8 bc	155 ± 2.8 b	130 ± 2.8 b	86.33 ± 6.9 c
	30	158 ± 10.9 ab	157.67 ± 1.4 b	138.67 ± 2 ab	98.33 ± 6.1 bc
	45	166.67 ± 6.6 a	172.67 ± 3.7 a	150 ± 7.6 a	120.67 ± 4.7 a
	60	143 ± 4.4 bc	147.33 ± 6.3 bc	138 ± 1.6 ab	95.33 ± 3.18 c

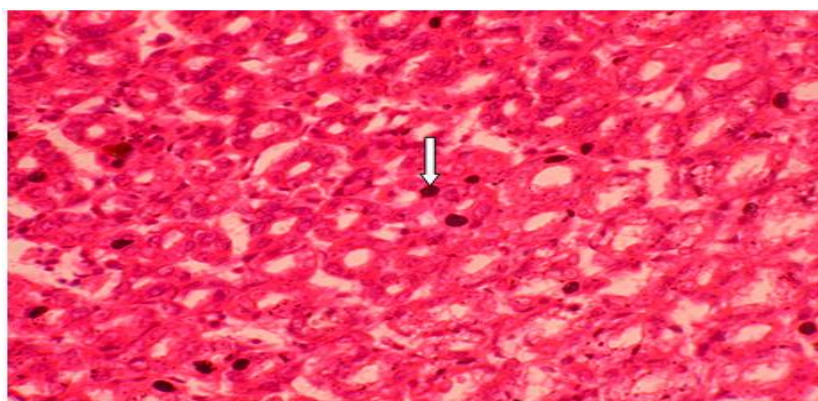
Means ± standard error followed by different letters indicate statistically significant differences (SPSS test,  $p < 0.05$ ) from each other. 30 samples for each treatment maintained by three replicates were conducted. Here P= Eggs of *Paramphistomum*, F= Eggs of *Fasciola*, S= Eggs of *Stomach worm*, St= Eggs of *Strongyles*, EPG= Egg per gram of feces and DPT= Days Post Treatment.



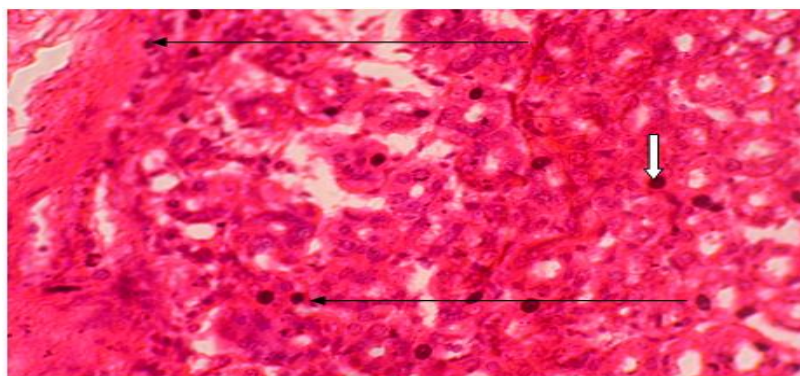




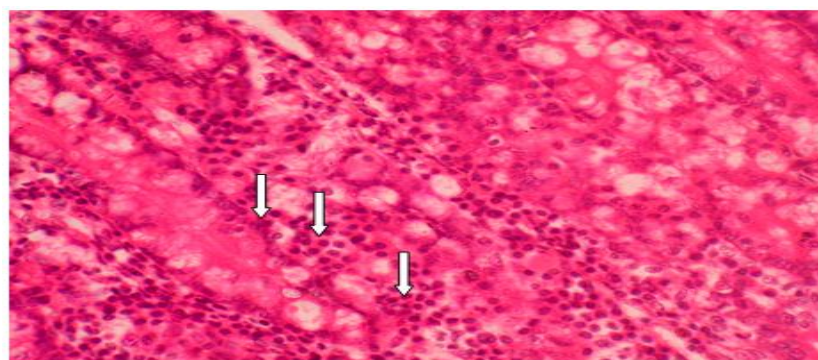
**Fig.3-4:** Effects of curcumin (0.2% curcumin and 0.6% curcumin) on fecal egg count



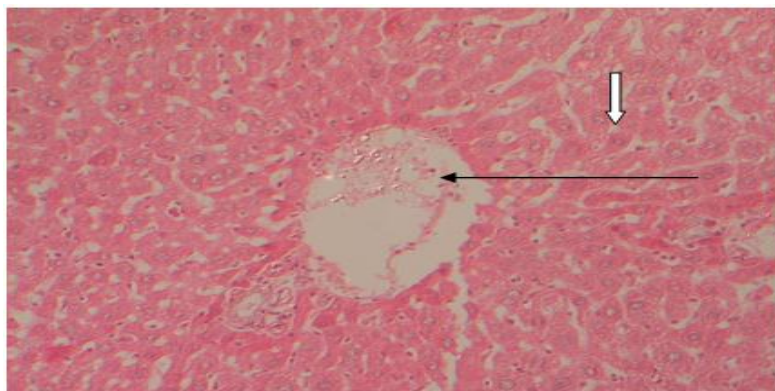
**Fig.5:** Histopathology of glandular stomach of group B (H&E x100) showing mild gastritis with little infiltration of inflammatory cells in the sub mucosa



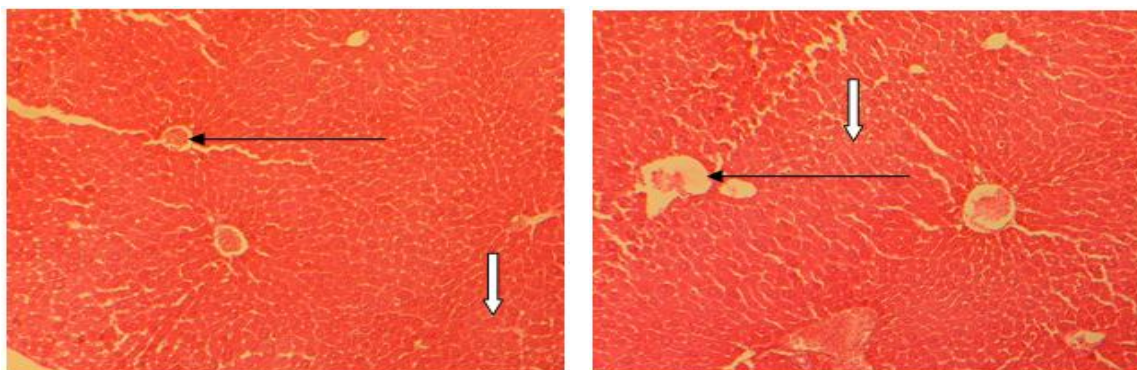
**Fig. 6:** Histopathology of glandular stomach of group C (H&E x100) showing gastritis with moderate infiltration of inflammatory cells in the sub mucosa and muscularis mucosa



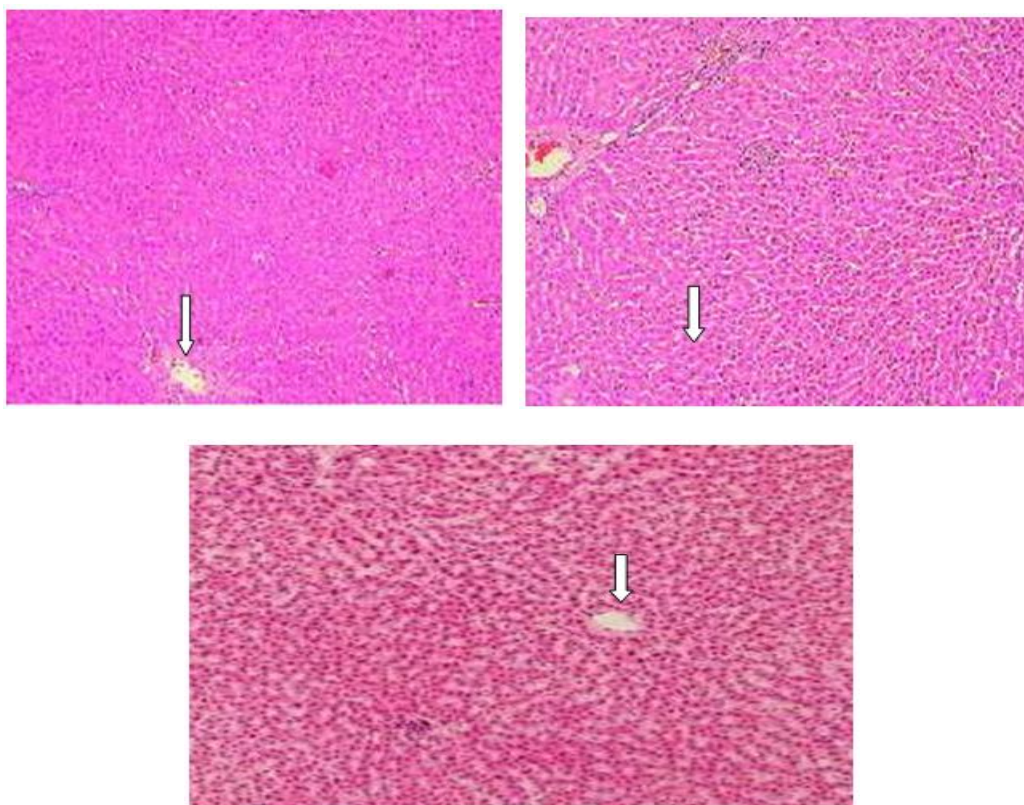
**Fig. 7:** Histopathology of glandular stomach of group A (H&E x100) showing gastritis with heavy infiltration of inflammatory cells in the sub mucosa



**Fig. 8:** Histopathology of liver section of a sample of group A (H&E x100) showing mild coagulation necrosis in the hepatocytes around the central vein and slightly swollen hepatocytes



**Fig. 9-10:** Histopathology of liver section of a sample of group C (H&E x40) showing central vein congestion and necrosis in the liver parenchyma, coagulation necrosis with mild inflammatory cell around the central vein and slightly swollen hepatocytes



**Fig. 11-13:** Histopathology of liver section of a sample of Group B having almost normal appearance (H&E, X10).



## CONCLUSION

Our data demonstrated that, the differential effects of curcumin on the ability of goat to improve the immune response to counter the gastro-intestinal parasitism. The fecal egg count and worm population has been given an idea about the effect of curcumin treatment on the parasite population. The animals receiving curcumin did not show increased parasitic egg count. Again, in some of the cases, parasitic egg count in curcumin treated animals was much lower. It is difficult to differentiate by their morphological study of their ova in fecal sample examination. The findings of the present study may assist the future researchers to explore the details pharmacokinetic and toxic effects for wide therapeutic uses for the treatment and control of parasitic infestation in goats, as those were found more effective in live weight gain of goats comparing with non treated ones. No characteristic lesion has been identified in the gastro-intestinal mucosa but gastritis with little infiltration of inflammatory cells in the sub mucosa and in the liver parenchyma showing coagulation necrosis around the central vein and slightly swollen hepatocytes of the parasitized animals as well as the animals fed on 0.6 % curcumin mixed diet. Further study is proposed to clarify the efficacy of curcumin supplementation against the anthelmintics that is widely used in different agro-ecologies and always remain the cornerstone of effective parasite control programmes. Detailed investigation should be performed on the distribution of special immune cells such as globule leukocytes and mast cells in the gastrointestinal tract through immunohistochemistry.

## Conflict of interest statement

The authors have no competing interests.

## ACKNOWLEDGMENTS

The author is grateful to the Post graduate studies faculty of Patuakhali Science and Technology University for financial assistance in the research work, Dept of Pathology and Parasitology, Patuakhali Science and Technology University and Dept of Physiology, Bangladesh Agricultural University for their cooperation in conducting the research work.

## Author's contribution

Fahmida Afrin performed the experiment, M. M. R. Chowdhury supervises and designed the experiment, S. S. Saha helps in histopathology of the samples, Md. Ataur Rahman analyze the data, Md. Ali Asgar helps in hematological studies and Professor M. K. Islam PhD revise and check the manuscript.

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