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A study on Epidemiology and Haematological changes of Fascioliasis in Cattle and its Therapeutic Management with Indigenous Medicinal Plants

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ABSTRACT

The present investigation has been carried out to study the epidemiology of fascioliasis in cattle and its therapeutic management with indigenous medicinal plants. Under the prevailing agro-climatic condition of four locations of Kamrup district of Assam, out of 551 nos. of randomly selected cattle, 46 number of animals were found positive for fascioliasis. The overall occurrence of fascioliasis was 8.35% out of which 8.28% local male, 8.23% local female, 8.33% crossbred male and 8.59% crossbred female cattle were found to be affected. In the present study, efficacy of *Entada phaseoloides* was found to be 87.50% and that of *Azadirachta indica* was 81.25%. However, triclabendazole showed 100% efficacy. Various blood parameters viz., haemoglobin, TEC and albumin level were found to be elevated following treatment with methanolic extract of *E. phaseoloides*, *A. indica* as well as triclabendazole in comparison to the Fasciola positive untreated group. The declining levels of TLC, eosinophil, monocyte count, total serum bilirubin, ALT, AST, ALP and GGT could be observed after treatment with these drugs. However, the percentage of PCV did not increase significantly after the administration of all these drugs, singly. Also, no significant difference could be observed in basophil, neutrophil, lymphocyte count and ESR level following treatment with triclabendazole, *E. phaseoloides* and/or *A. indica*. Based on clinical recovery and improvement in haemato-biochemical parameters, the methanolic extracts of the indigenous plants were found to be highly effective against fascioliasis in cattle through their efficacy were not at par with that of standard commercial drug, triclabendazole.

HIGHLIGHTS

- *E. phaseoloides* and *A. indica* plants are effective against treating fascioliasis in cattle.
- There was no effect of breed and sex on incidence of fascioliasis in cattle

Keywords: Fascioliasis, Epidemiology, Indigenous medicinal plants, Cattle, Haematology, Biochemical

India is an agricultural based developing country where animal husbandry is the backbone of agriculture. The productivity of the domesticated animals is poor due to complex multiple infections and environmental factors. Among the parasitic diseases, fascioliasis is one of the most important disease of ruminants, having world-wide distribution. The disease causes great economic losses to the livestock industries due to high mortality and morbidity, decrease in milk and meat yield, decrease in weight gain, infertility and liver condemnation (Siddiki *et al*., 2010). Fascioliasis is mainly caused by two species: *Fasciola gigantica* and *F. hepatica* and commonly known as liver fluke. In India the disease is mostly caused by Fasciola gigantica (Yadav *et al*., 2007). And it is found in most continents, primarily in tropical regions. Both species are transmitted by the snails of the family Lymnaeidae. The fluke infects the liver and bile ducts of the ruminants such

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as cattle, sheep, goat, buffaloes etc (Choubisa and Jaroli, 2013) and infections are usually associated with grazing wetland and drinking places contaminated by the infective metacercariae. Fasciola can also infect human and has been classified as Neglected Tropical Disease (NTD). The recent epidemiological picture of human fascioliasis has been changed. Since 1980, cases of human fascioliasis have been increased significantly and their distributions into several geographical areas make fascioliasis an important disease with public health concern. According to WHO (2006) report of human fascioliasis, atleast 2.4 million people are infected with and more than 180 million are at risk of infection. To formulate the control strategies, epidemiological study is very much essential criterion. In recent years, the worm population has developed resistance to modern anthelmintics. The long-term regular use of such drugs for the treatment of various diseases leads to loss of an animal's natural resistance. The search for indigenous medicinal plants against parasitic disease may overcome some of the problems associated with synthetic drug. Therefore, the objective of this study was to assess the epidemiology of fascioliasis in cattle in Chunsali, Palashbari, Halogaon and Khetri areas of Kamrup district of Assam to determine the prevalence and haemato-biochemical changes for better and appropriate control strategies.

MATERIALS AND METHODS

Study area

The study was carried out in 4 areas of Kamrup district from April to March for a period of one year for epidemiological study of fascioliasis in cattle. The animals of four selected locations were kept in open grazing system. All four locations were flood prone, low lying and marshy land areas. Chunsali, Palashbari and Halogaon area are very nearer to the mighty Brahmaputra river. The average temperature of these areas during the study period in summer was 30ºC and in winter 22ºC. Little to moderate rainfall (average 0.14 cm) occured during the year of the study period. The epidemiological parameters were taken on the basis of breed and sex wise incidence of fasioliasis. Distribution of faecal sample from four selected places was shown below (Table 1).

Table 1: Distribution of faecal sample

Place	Local		Crossbred	
	Male	Female	Male	Female
Chunsali	36	39	33	30
Palashbari	36	40	29	32
Halogaon	37	39	28	34
Khetri	36	40	30	32

Animal source and screening

Cattle of different sex and breed were selected at random for preliminary screening in different locations of Kamrup district of Assam. A total of 551 nos. of faecal samples were collected manually by back racking through rectum at about mid day (Bhatia *et al*., 2006) and examined for the presence of Fasciola egg using standard technique, the sedimentation method as per Coffin, (1953). The details of the animal and human source, and sample distribution have been presented in Table 2.

Grouping of animals

On the basis of faecal examination, cattle were grouped as "Fasciola positive" (FP) and "Fasciola negative control Group" (FNC Group).

FNC Group: Out of 505 preliminary screened Fasciola negative animals, 10 cattle were selected randomly from four locations and were kept as healthy FNC Group. Animals of Fasciola positive groups were also selected randomly from four different locations. Out of 46 preliminary screened FP cattle, 40 were divided into following 4 groups consisting of 10 cattle in each group, and were referred as:

- ❒ 'Fasciola Positive Control Group' (FPC Group) Untreated infected group.
- ❒ 'Fasciola Positive Group A' (FP Group A) Treatment group.
- ❒ 'Fasciola Positive Group B' (FP Group B) Treatment group.
- ❒ 'Fasciola Positive Group C' (FP Group C) Treatment group.

Table 2: Epidemiological status of fascioliasis in cattle

Month	No of cattle examined	Local		Crossbred		Fasciola positive cases			
						Local		Crossbred	
		Male	Female	Male	Female	Male positive	Female positive	Male positive	Female positive
April	47	12	15	9	11	1	0	1	1
May	47	13	11	11	10	0	1	1	0
June	42	11	14	8	9	1	1	0	1
July	42	13	10	10	9	1	0	1	1
August	45	12	9	11	13	1	1	1	2
September	46	10	14	10	12	1	2	1	1
October	48	12	16	8	12	2	3	2	2
November	48	10	12	11	13	1	1	1	0
December	45	13	16	9	9	1	1	1	1
January	47	12	8	13	13	1	1	0	1
February	50	15	17	9	8	1	1	1	0
March	44	12	16	11	9	1	1	0	1
Total	**551**	**145**	**158**	**120**	**128**	**12(8.28%)**	**13(8.23%)**	**10(8.33%)**	**11(8.59%)**

Estimation of egg per gram (EPG)

Faecal samples of 50 animals from FNC and FP groups were examined on '0' day, 7 day, 14 day and 21" day at an interval of 7 days, by using standard sedimentation method to find the presence or absence of Fasciola egg. Determinations of egg per gram (EPG) of all the 50 selected animals were carried out by Modified Mc Master Method described by HMSO (1979) for 4 occasions at 7 days interval. For screening human fascioliasis, a total of 120 stool samples comprising of 10 stool samples in each month were collected randomly from the same locations as for cattle faecal samples and examined by standard sedimentation method.

Collection of blood and serum for analysis

The collection of blood and serum was done as per the methods described by Benjamin (1985).

The following haematological parameters were carried out: Estimation of haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), done as per the standard method. Each of the haematological parameter was studied for four occasions (viz. '0', 7^{th}, 14^{th} and 21^{th} day) at an interval of 7 days. Biochemical parameters studied were as follows: Estimation of total serum bilirubin, serum albumin, alanine aminotransferase (ALT), aspertate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT), done as per standard method as described below. Each of the biochemical parameter was studied for four occasions ('0', 7^{th}, 14^{th} and 21 day) at an interval of 7 days using Ultra Violet Single Beam Spectrophotometer (UV 2100, Chemito, Germany).

Collection and identification of plants

The seeds of locally available plant *E. phaseoloides* were collected from local market of Guwahati and the leaves of *A. indica* were collected from Khanapara, Guwahati, in the district of Kamrup, Assam. The plants were sent for identification to Botanical Survey of India, Meghalaya, Shillong. The plant materials were cleaned and shade dried. Then these were powdered and stored in a tightly closed container at room temperature.

Preparation of methanolic extracts and Phytochemical screening

Methanolic extract of *E. phaseoloides* and *A. indica* were prepared as per standard method described by Harbone (1973). Phytochemical investigation of the methanolic

extracts were carried out for the presence of the phyto-constituents by using standard technique described by Harborne (1973).

Determination of lethal dose (LD_{50})

The 50 % lethal dose of the *E. phaseoloides* and *A. indica* were estimated by the employment of up and ~down stair case method in mice as per Bruce (1985). Doses were adjusted by a constant multiplicative factor viz. 4, for this experiment. The dose for each successive animal was adjusted up and down depending on the previous outcome, The acute toxicity and gross effect of crude methanolic extract of *E. phaseoloides* and *A. indica* were studied in albino mice by using 1/2 LDsp dose. A total of six numbers of male albino mice were selected for the experiment. Animals were observed initially at 1 hour interval for 6 hours and at 24 hr interval thereafter. The parameters for motor activity and gross effect were determined after administration of *E. phaseoloides* and *A. indica* orally at a dose rate of 2.5 g /kg b. wt.

Therapeutic management

FNC Group: All the animals of this group were dewormed prior to sample collection. Faecal sample was collected from each animal and examined to rule out fascioliasis in this group:

- **FPC Group:** Faecal sample was examined for the presence of Fasciola egg using standard procedure.
- **FP Group A:** Prior to collection of blood and faecal samples, each animal of this group received oral single dose of triclabendazole (Fasinex® y› at the dose rate of 12 mg/kg b.wt.
- **FP Group B:** Before collection of blood and faecal samples, each animal of this group was administered with an oral single dose of the methanolic extract of *E. phaseoloides* (500 mg/kg b.wt) as treatment measure against fascioliasis. Therapeutic efficacy of the plant extract was evaluated by analyzing the blood and faecal sample of each animal. The time schedule for examination of blood and faecal sample were similar to that of other preceding groups.
- **FP Group C:** Each animal of this group received an oral single dose of the methanolic extract of *A. indica* (500 mg/kg b.wt) as treatment measure against fascioliasis. Evaluation of therapeutic efficacy for this group of animals was similar to that of FP Group B.

Data obtained in the study were analysed by following the standard method as described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Fascioliasis is one of the major factors limiting production, weight gain, fertility in cattle due to its high mortality and morbidity. It leads to a number of characteristic changes in blood constituents. Acute fascioliasis is less common than the chronic entity (Souslby, 1982). High prevalence of fascioliasis in human does not necessarily occur in area where fascioliasis is a major veterinary problem (Mas-Coma *et al*., 1999).

Prevalence of fascioliasis and EPG count

In the present study, the overall occurrence of fascioliasis in four selected locations was 8.35 per cent and it correlated with the findings reported by Bhattacharyya and Ahmed, (2005). Cattle of either sexes were found to be equally affected to fascioliasis which coincides with the findings of Maqbool *et al*., (2002). There was no difference in breed wise incidence of fascioliasis which are linear with the reports of Ahmed *et al*. (2008). No cases of human fascioliasis were found in the present study which might be due to hygienic food habit of the people of those four selected locations. There was no significant difference in EPG of FPC Group because they were not treated with any anthelmintic. In case of FP Group A, on day '0' the EPG was 95.00 ± 8.12 which gradually decreased and finally on 21st day no Fasciola egg was observed. This might be due to fasciolicidal effect of triclabendazole. Triclabendazole produced its effect by inhibiting mitochondrial fumerate reductase, thus reducing the glucose uptake of parasite (Upadhyay and Kumar, 2005). Similar finding of 100% efficacy of triclabendazole (12 mg/kg b. wt) was also observed by Agrawal *et al*. (2004). In FP Group B, treated with *E. phaseoloides*, the initial EPG count was 120.00± 8.41 on day '0' and finally at the end of the

Table 3a: Haematological changes in FNC & FPC groups

	FNC					FPC				
	0 day	**7th day**	**14th day**	**21st day**	**F Value**	**0 day**	**7th day**	**14th day**	**21st day**	**F Value**
Hb	10.97 ± 0.30	11.23 ± 0.34	11.26 ± 0.33	11.32 ± 0.31	<1.00^{NS}	8.97 ± 0.17	8.86 ± 0.18	8.79 ± 0.19	8.59 ± 0.22	<1.00^{NS}
TEC	6.42 ± 0.17	6.45 ± 0.17	6.42 ± 0.21	6.46 ±0.18	<1.00^{NS}	5.51 ± 0.18	5.42 ± 0.19	5.48 ± 0.19	5.54 ± 0.17	<1.00^{NS}
TLC	6.96 ± 0.06	7.03 ± 0.10	7.13 ± 0.08	7.08 ± 0.14	<1.00^{NS}	6.62 ± 0.27	6.65 ± 0.30	6.71 ± 0.32	6.74 ± 0.30	<1.00^{NS}
Eosin-ophil (%)	3.30 ± 0.26	3.20 ± 0.25	3.30 ± 0.15	2.90 ± 0.18	<1.00^{NS}	6.10 ± 0.41	6.10 ± 0.38	6.40 ± 0.34	6.60 ± 0.31	<1.00^{NS}
Basop-hil (%)	0.00	0.00	0.00	0.00	<1.00^{NS}	0.40 ± 0.22	0.30 ± 0.22	0.10 ± 0.10	0.00 ± 0.00	<1.00^{NS}
Neutr-ophil (%)	36.90 ± 0.53	36.30 ± 0.73	36.60 ± 0.64	36.40 ± 0.54	<1.00^{NS}	35.80 ± 0.94	35.70 ± 1.14	36.00 ± 1.26	37.20 ±1.16	<1.00^{NS}
Lymphocyte (%)	55.60 ± 0.60	54.80 ± 0.87	55.50 ± 0.65	54.50 ± 0.76	<1.00^{NS}	52.20 ± 1.40	50.70 ± 1.82	50.10 ± 1.64	49.20 ± 1.78	<1.00^{NS}
Monocyte (%)	3.41 ± 0.32	3.00 ± 0.21	2.80 ± 0.25	3.40 ± 0.31	1.23^{NS}	5.10 ± 0.38	4.20 ± 0.33	4.10 ± 0.23	5.12 ± 0.28	3.17*
PCV (%)	31.87 ± 0.59	32.02 ± 0.64	32.14 ± 0.54	32.42 ± 0.60	<1.00^{NS}	27.88 ± 0.66	26.66 ± 0.66	26.69 ± 0.64	97.42 ± 0.63	<1.00^{NS}
ESR (mm/hr)	1.61 ± 0.06	1.57 ± 0.05	1.57 ± 0.05	1.56 ± 0.05	<1.00^{NS}	1.73 ± 0.09	1.75 ± 0.10	1.78 ± 0.09	1.79 ± 0.10	<1.00^{NS}

*Indicates significant at p<0.05; ** Indicates significant at p<0.01; NS indicates Not Significant.

Table 3b: Haematological changes in FP Group A, FP Group B & FP Group C

	FP Group A					FP Group B					FP Group C				
	0 day	**7th day**	**14th day**	**21st day**	**F Value**	**0 day**	**7th day**	**14th day**	**21st day**	**F Value**	**0 day**	**7th day**	**14th day**	**21st day**	**F Value**
Hb	8.90 ± 0.31	9.30 ± 0.37	9.91 ± 0.39	10.62 ± 0.37	4.36**	8.76 ± 0.21	9.16 ± 0.27	9.64 ± 0.29	10.13 ± 0.38	4.11**	8.91 ± 0.27	9.28 ±0.28	9.71 ± 0.34	10.29 ± 0.37	3.49*
TEC	5.84 ± 0.20	6.10 ± 0.23	6.85 ± 0.18	7.45 ± 0.10	15.77**	5.44 ± 0.21	5.58 ± 0.23	5.92 ± 0.25	6.09 ± 0.26	1.58^{NS}	5.85 ± 0.20	5.97 ±0.18	6.13 ± 0.18	6.44 ± 0.17	1.96^{NS}
TLC	5.84 ± 0.24	5.79 ± 0.19	5.72 ± 0.16	5.80 ± 0.16	<1.00^{NS}	5.97 ± 0.14	5.87 ± 0.14	5.78 ± 0.13	5.74± 0.11	<1.00^{NS}	5.95 ± 0.10	5.77 ± 0.10	5.66 ± 0.09	5.54 ± 0.09	3.23*
Eosin-ophil (%)	5.60 ± 0.27	5.00 ± 0.30	4.80 ± 0.39	3.50 ± 0.34	7.32**	5.50 ± 0.22	5.30 ± 0.26	4.60 ± 0.27	3.50 ± 0.27	12.5**	5.30 4 0.26	5.00 ± 0.33	4.50 ± 0.22	3.50 ± 0.27	8.27**
Basop-hil (%)	0.30 ± 0.15	0.20 ± 0.21	0.10 ±0.21	0.00 ± 0.00	<1.00^{NS}	0.20 ± 0.13	0.30 ± 0.27	0.10 ±0.10	0.00 ± 0.00	2.80^{NS}	0.10 ± 0.10	0.30 ± 0.22	0.20 ± 0.20	0.00 ± 0.00	1.87^{NS}
Neutr-ophil (%)	33.10 ± 0.55	34.70 ± 0.50	35.00 ± 0.49	34.80 ± 0.47	<1.00^{NS}	34.40 ±0.40	33.50 ± 0.54	34.70 ± 0.37	35.70 ± 0.26	<1.00^{NS}	35.40 ± 0.73	35.50 ± 0.65	35.10 ± 0.96	35.70 ± 0.78	<1.00^{NS}
Lymphocyte (%)	55.90 ± 0.46	54.10 ± 0.64	54.20 ± 0.36	53.30 ± 0.73	<1.00^{NS}	55.00 ± 0.45	54.50 ± 0.40	53.20 ± 0.80	52.20 ± 0.51	2.67^{NS}	54.60 ± 0.86	54.10 ± 0.67	53.30 ± 0.75	53.40 ± 0.88	<1.00^{NS}
Monocyte (%)	4.80 ± 0.20	3.50 ± 0.31	3.90 ± 0.31	4.30 ± 0.15	4.82**	5.10 ± 0.18	3.90 ± 0.43	3.90 ± 0.18	4.00 ± 0.26	4.30**	4.60 ± 0.27	3.40± 0.45	3.10 ±0.35	4.00 ± 0.26	3.82*
PCV (%)	29.24 ± 0.71	29.51 ± 0.70	30.22 ± 0.64	30.66 ± 0.65	<1.00^{NS}	28.49 ± 0.71	29.75 ± 0.72	30.32 ± 0.73	30.92 ± 0.81	1.96^{NS}	29.55 ± 0.77	30.04 ± 0.77	30.77 ± 0.82	31.26 ± 0.80	<1.00^{NS}
ESR (mm/ hr)	1.60 ± 0.08	1.54 ± 0.08	1.51 ± 0.08	1.49 ± 0.07	<1.00^{NS}	1.45 ± 0.08	1.43 ± 0.08	1.41 ± 0.08	1.39 ± 0.07	<1.00^{NS}	1.61 ± 0.09	1.60 ± 0.09	1.58 ± 0.09	1.57 ± 0.08	<1.00^{NS}

*Indicates significant at p<0.05; ** Indicates significant at p<0.01; NS indicates Not Significant.

experiment on 21th day it was 15.00 ± 1.50 which shows an efficacy of 87.50 per cent. This shows effectiveness of methanolic extract of *E. phaseoloides* against fascioliasis. Though there are limited scientific reports regarding the effectiveness of *E. phaseoloides* against fascioliasis, there are few reports on its traditional folklore claim as an anthelmintic (Sur *et al*., 2003). In FP Group C, treated with *A. indica*, the EPG count was 80.00±7.27 on '0' day, which decreased gradually and finally on 21st day it was 15.00±1.42 showing 81.25 per cent efficacy of the treated drug. Similar finding was also reported by Chandrawathani *et al*., (2000).

Haematological changes

Haemoglobin

Analysis of variance showed that the effect of treatment between days was highly significant (P < 0.01) for FP Group A and FP Group B and significant (P < 0.05) in FP Group C. In present study, the haemoglobin levels were 10.97±0.30 g/dl on '0' day and finally on 21st day in FNC Group it was 11.32±0.31 g/dl. There was no significant difference in FNC group. The values were within normal range and coincided with the report of Radostits *et al*. (2000). The haemoglobin level in FPC Group on '0' day was 8.97 ± 0.17 g/dl which gradually decreased and became 8.59 ± 0.22 g/dl on 21st day. The low level of haemoglobin might be due to Fasciola parasites (Souslby, 1982). Similar report was also observed by Howlader *et al*. (2004). In FP Group A, the haemoglobin levels were found to be increased from 8.90 ± 031 g/dl on '0' day to 10.62 ± 0.37 g/dl on 21st day. The increased values of haemoglobin were highly significant (P<0.01) in comparision to '0' day. This might be due to increased activity of erythropoiesis after the treatment of triclabendazole. Similar findings were also reported by Agrawal *et al*. (2004), Sharma *et al*. (2005) and Barua *et al*. (2008).The haemoglobin levels in FP Group B were 8.76 ± 0.21 g/dl on '0' day and 10.13 ± 0.38 g/dl on 21st day which was highly significant (P<0.01). This might be attributed due to anthelmintic effect of *E. phaseoloides*.

In present study, the haemoglobin levels of FP Group C were 8.91 ± 0.27 g/dl on '0' day and 10.29 ± 0.37 g/dl on 21st day which was significant (P<0.05). This might be possibly due to 'anthelmintic effect of *A. indica*. Upadhyay and Kumar, (2005) reported that there was significant increase in haemoglobin levels after treatment with single oral dose of powdered fruits of *Mallotus phillipinensis* (Kamala) and powdered seeds of *Butea frondosa* (Palasa) against fascioliasis in cows.

Total erythrocyte count (TEC)

Analysis of variance showed that the effect of treatment between days was highly significant (P < 0.01) for FP Group A. The TEC levels in FNC Group were 6.42 ± 0.17 million/mm' on '0' day and 6.46 ± 0.18 million/mm^3 on 21st day. There was no significant difference in this group. The values were within normal range and correlated with the report of Radostits *et al*. (2000). In FPC Group, the TEC levels were 5.51 ± 0.18 million/mm^3 on '0' day and 5.54 ± 0.17 million/mm^3 on 21st day and no significant difference was observed in this group. The low level of TEC might be due to blood sucking activity of the parasites. Haroun and Hussein, (1975) and Kumar *et al*. (1982) also reported reduction in red blood cell count in fascioliasis in cattle and buffaloes respectively. The TEC levels in FP Group A ranged from 5.84 ± 0.20 million/mm^3 on '0' day to 7.45 ± 0.10 million/mm^3 on 21st day. There was gradual increase in TEC level which was highly significant (P<0.01). This might be due to increased activity of erythropoiesis after treatment with triclabendazole. Similar findings were also reported by Sharma *et al*. (2005), Pal and Dasgupta, (2006) and Barua *et al*. (2008). The TEC levels in FP Group B were 5.44±0.21 million/mm^3 on '0' day and 5.09±0.26 million/mm^3 on 21st day. There was gradual increase in TEC level, although no significant difference could be observed in this group. The TEC levels in FP Group C were 5.85 ± 0.20 million/mm^3 on '0' day which increased upto 6.44 ± 0.17 million/mm^3 on 21st day. Although there was gradual increase in TEC level, but no significant difference could be observed in this group. Upadhyay and Kumar, (2005) reported that there was significant increase in TEC levels after treatment with single oral dose of powdered fruits of Mallotus phillipinensis (Kamala) and powdered seeds of Butea frondosa (Palasa) against fascioliasis in cows. In our study also, anthelmintic property of the plant extract may exert its action on the flukes.

Total leucocyte count (TLC)

Analysis of variance showed that the effect of treatment

Table 4a: Biochemical changes in FNC & FPC groups

	FNC					FPC				
	0 day	**7th day**	**14th day**	**21st day**	**F Value**	**0 day**	**7th day**	**14th day**	**21st day**	**F Value**
TSB (mg/dl)	0.40 ±0.08	0.38 ± 0.07	0.31 ± 0.05	0.27 ± 0.04	<1.00^{NS}	0.59 ± 0.09	0.54 ± 0.09	0.46 ± 0.07	0.44 ± 0.04	<1.00^{NS}
SA (g/dl)	3.59± 0.07	3.65 ±0.09	3.74± 0.12	3.64 ±0.07	<1.00^{NS}	2.57 ±0.13	2.59 ±0.14	2.46 ±0.13	2.53 ±0.15	<1.00^{NS}
ALT (U/L)	31.27 ±0.89	30.89 ±1.17	31.06± 1.02	31.70 ±0.78	<1.00^{NS}	44.24 ±2.52	44.70 ±2.80	45.02± 2.69	45.38 ±2.65	<1.00^{NS}
AST (U/L)	79.04 ±1.47	78.36± 1.26	79.27 ±1.23	81.06 ±1.25	<1.00^{NS}	92.24 ±3.45	93.36± 4.18	95.25 ±4.66	95.65 ±4.76	<1.00^{NS}
ALP (U/L)	127.65 ±2.61	125.88 ±3.01	126.71± 2.55	126.17 ±2.45	<1.00^{NS}	162.44± 6.74	165.34 ±7.87	169.61 ±9.70	172.28 ±8.79	<1.00^{NS}
GGT	13.82± 0.48	13.49± 0.43	13.05 ±0.38	13.46 ±0.31	<1.00^{NS}	18.00± 0.85	18.23± 0.86	18.04 ±0.75	18.40± 0.94	<1.00^{NS}

*Indicates significant at p<0.05; ** Indicates significant at p<0.01; NS indicates Not Significant.

Table 4b: Biochemical changes in FP Group A, FP Group B & FP Group C

	FP Group A					FP Group B					FP Group C				
	0 day	**7th day**	**14th day**	**21st day**	**F Value**	**0 day**	**7th day**	**14th day**	**21st day**	**F Value**	**0 day**	**7th day**	**14th day**	**21st day**	**F Value**
TSB (mg/dl)	0.66 ± 0.10	0.58 ± 0.12	0.40 ± 0.08	0.32 ± 0.04	2.96*	0.63 ± 0.08	0.50 ± 0.08	0.40 ± 0.06	0.31 ± 0.05	4.27**	0.63 ± 0.06	0.46 ± 0.06	0.35 ± 0.04	0.32 ± 0.03	7.65**
SA(g/dl)	2.53 ± 0.15	2.83 ± 0.12	3.07 ± 0.11	3.51 ± 0.08	12.31**	2.54 ±0.17	2.86 ± 0.14	3.21 ± 0.15	3.40 ± 0.14	6.30**	2.54 ± 0.12	2.76 ±0.11	2.98 ± 0.08	3.21 ± 0.08	8.48**
ALT(U/L)	41.07 ± 0.17	36.61 ± 0.14	35.04 ± 0.15	33.28 ± 0.14	11.99**	42.61 ± 1.01	38.42 ± 0.93	35.47 ± 0.91	32.66 ± 0.77	21.97**	42.82 ± 1.11	39.50 ±0.95	37.17 ±0.87	34.16 ± 0.60	16.44**
AST(U/L)	89.60 ± 3.67	84.86 ± 2.77	80.94 ± 1.97	78.54 ± 0,87	3.63*	88.42 ± 2.14	83.08 ± 2.38	79.53 ± 2.61	78.73 ±2.55	4.31**	91.18 ± 3.33	87.89 ± 3.15	84.80 ± 3.14	81.84 ± 2.77	1.68^{ns}
ALP(U/L)	157.79 ± 4.80	154.75 ± 4.63	151.98 ± 4.52	147.83 ± 4.34	<1.00^{NS}	164.62 ± 4.71	163.14 ± 4.69	162.05 ± 4.86	161.49 ± 4.69	<1.00^{NS}	162.99 ±5.43	161.77 ±5.34	160.09 ± 5.27	159.21 ± 5.22	<1.00^{NS}
GGT	18.40 ± 0.75	17.74 ± 0.69	16.24 ± 0.61	14.95 ± 0.64	5.22**	15.31 ± 0.50	14.94 ± 0.41	14.37 ± 0.51	13.61 ±0.50	2.37^{ns}	15.56 ± 0.61	14.70 ± 0.51	13.58 ± 0.65	12.05 ± 1.38	3.13*

*Indicates significant at p<0.05; ** Indicates significant at p<0.01; NS indicates Not Significant.

between days was significant (P<0.05) in FP Group C. In FNC Group, the TLC levels were 6.96 ± 0.06 thousand/mm^3 on '0' day and at the end of the experiment the recorded TLC level was 7.08 ± 0.14 thousand/mm^3 on 21st day. The values were within normal range and correlated with the report of Radostits *et al*. (2000). The TLC levels in FPC Group were 6.62 ± 0.27 thousand/mm^3 on '0' day and was elevated upto 6.74 ± 0.30 thousand/mm^3 on 21st day. There was slight increase in TLC levels which might be due to reduction in plasma volume (Rajkhowa, 1997) and this elevation in TLC level was not significant. In FP Group A, the TLC levels were 5.84 ± 0.24 thousand/mm^3 on '0' day and decreased to 5.80 ± 0.16 thousand/mm^3 on 21st day. This finding contradicts the earlier report of Pal and Dasgupta, (2006) where they reported increase in TLC levels after treatment with triclabendazole in *F. gigantica* infection in buffaloes. The TLC levels in FP Group B were 5.97±0.14 thousand/mm^3 on '0' day and 5.74±0.11 thousand/mm^3 on 21st day. The slight decrease in TLC levels albeit not significant might be due to the anthelmintic effect of *E. phaseoloides*. In FP Group C, the levels of TLC on '0' day 5.95 ± 0.10 thousand/mm^3

decreased to 5.54 ± 0.09 thousand/mm^3 on 21st day. The slight decrease in TLC levels might be due to the effect of *A. indica*. The decreased TLC level of this group on 21st day was significant (P<0.05) in comparison to '0' day level. Upadhyay and Kumar, (2005) reported that there was reduction in TLC levels after treatment with single oral dose of powdered fruits of Mallotus phillipinensis (Kamala) and powdered seeds of Butea frondosa (Palasa) against fascioliasis in cows. Our plant extracts also showed similar trend.

Differential leucocyte count (DLC)

Eosinophil

Analysis of variance showed that the effect of treatment between days was highly significant (P < 0.01) for FP Group A, FP Group B and FP Group C. The eosinophil counts in FNC Group were 3.30 ± 0.26 per cent on '0' day which was declined to 2.90 ± 0.18 per cent on 21st day which was not significant. The values were within normal range which correlated with the reports of Benjamin, (1985). In FPC Group, the eosinophil counts were 6.10 ± 0.41 per cent on '0' day and 6.60 ± 0.31 per cent on 21st day. Increase in eosinophil counts might be due to parasitic infestation releasing histamine as a result of allergic condition (Benjamin, 1985). However there was no significant difference in this group. The eosinophil counts in FP Group A were 5.60 ± 0.27 percent on '0' day which declined to 3.50 ± 0.31 percent on 21st day. The gradual decrease in eosinophil level after treatment in FP Group A was highly significant (P≤0.01). Decrease in eosinophil count could be due to adverse affect of releasing histamine or histamine like substances after the treatment of triclabendazole. Present finding corroborated with the findings of Pal and Dasgupta, (2006). The eosinophil percent in FP Group B, 5.50 ± 0.22 percent on '0' day declined to 3.50 ± 0.27 percent on 21st day. This gradual decrease in eosinophil count after treatment with FP Group B was highly significant (P<0.01). This might be due to the effect of *E. phaseoloides* extract in the infected animals. Similarly, in FP Group C, the eosinophil counts were reduced from 5.30 ± 0.26 percent on '0' day to 3.50 ± 0.27 percent on 21st day. The gradual decrease is eosinophil level after treatment in FP Group C was highly significant (P<0.01) and might be due to administration of *A. indica* plant extract. Upadhyay and Kumar, (2005) reported that there was reduction in eosinophil count after treatment with Mallotus phillipinensis (Kamala) and Butea frondosa (Palasa) against fascioliasis in cows. However, our literature survey could not find such report with *A. indica*.

Basophil

In the present study, the basophil counts did not show any significant difference in FNC, FPC Group, FP Group A, FP Group B and FP Group C.

Neutrophil

Neutrophil counts also did not show any significant difference in FNC, FPC Group, FP Group A, FP Group B and FP Group C.

Lymphocyte

Similarly, lymphocyte counts also did not show any significant difference in FNC, FPC Group, FP Group A, FP Group B and FP Group C.

Monocyte

Analysis of variance showed that the effect of treatment between days was highly significant (P < 0.01) for FP Group A and FP Group B and significant (P< 0.05) for FPC and FP Group C. The monocyte counts in FNC Group were 3.41 ± 0.32 per cent on '0' day and 3.40± 0.31 per cent on 21st day. There was no significant difference in this group. The values were within normal range and correlated with the finding of Benjamin, (1985). In FPC Group, the monocyte counts were 5.10±0.38 % on '0' day and 5.12 ±9.28 per cent on 21st day. The values were significant (P<0.05). The slight increase in monocyte counts might be due to stress factor in late phase of acute and chronic infection (Benjamin, 1985). Furthermore, the monocyte counts in FP Group A were reduced from 4.80 ± 0.20 per cent on '0' day to 4.30 ± 0.15 per cent on 21st day. The values were highly significant (P<0.01). The variation in monocyte counts might be due to stress factor in late phase of acute and chronic infection (Benjamin, 1985). In FP Group B, the monocyte counts were 5.10 ± 0.18 per cent on '0' day and 4.00 ± 0.26 per cent on 21st day,

which were highly significant (P<0.01). The variation of monocyte counts could be due to stress factor in late phase of acute and chronic infection (Benjamin, 1985). In FP Group C, the monocyte counts were declined from 4.60 ± 0.27 per cent on '0' day to 4.00 ± 0.26 per cent on 21st day. The values were significant (P<0.05) and the slight variation in the values might be due to the stress in late phase of acute and chronic infection (Benjamin, 1985).

Packed cell volume (PCV)

In present study, the PCV levels did not show any significant difference in ENC, FPC Group, FP Group A, FP Group B and FP Group C. The PCV levels in FNC Group were in normal range. Low levels of PCV in FPC Group might be due to blood sucking activity of the parasites (Souslby, 1982). The PCV levels of FP Group A, FP Group B and FP Group C were increased after treatment. Similar findings with triclabendazole was reported by Pal and Dasgupta (2006). Upadhyay and Kumar, (2005) reported that the PCV levels were increased significantly after treatment with single oral dose of powdered fruits of Mallotus phillipinensis (Kamala) and powdered seeds of Butea frondosa (Palasa) against fascioliasis in cows. Hence, *E. phaseoloides* and *A. indica* might also exert their anthelmintic effect in a similar manner in the infected animals.

Erythrocyte sedimentation rate (ESR)

In present study, the ESR levels did not show any significant difference in FNC, FPC Group, FP Group A, FP Group B and FP Group C.

Biochemical changes

Total serum bilirubin

In present study, the total serum bilirubin levels in FNC group were found to be within the normal range which correlated with the report of Benjamin, (1985). Increase total serum bilirubin levels in FPC group might be due to Fasciola infection leading to hepatic dysfunction. In FP Group A, the total serum bilirubin levels were 0.66 ± 0.10 mg/dl on '0' day and 0.32 ± 0.04 mg/dl on 21st day which were significant (P<0.05). The increase level of total serum bilirubin before treatment could be due to impairment of bile flow which might be intrahepatic or extrahepatic. The values were decreased after treatment with triclabendazole. Similar findings were reported by Pal and Dasgupta, (2006). In FP Group B, the total serum bilirubin levels were 0.63 ± 0.08 mg/dl on '0' day and 0.31 ±0.05 mg/dl on 21st day. The values of total serum bilirubin in this group were highly significant (P<0.01). This gradual decline in the level of total serum bilirubin might be due to the effect of *E. phaseoloides* plant extract. In FP Group C, the total serum bilirubin levels were 0.63±0.06 mg/dl on '0' day and 0.32 ± 0.03 mg/dl on 21st day. The values of total serum bilirubin in this group were highly significant (P<0.01) in comparison to the values at '0' day. The gradual decrease in the level of total serum bilirubin could be due to treatment with *A. indica* extract.

Serum albumin

The values of albumin in FNC Group were found to be within normal range which correlated with the finding of Benjamin, (1985). The low levels of albumin in FPC Group might be due to liver insufficiency to anabolise amino acid and protein (Radostits *et al*., 2000). In FP Group A, the albumin level was 2.53 ± 0.15 g/dl on '0' day which became 3.51 ± 0.08 g/dl on 21st day. Increased albumin level in this group was highly significant (P<0.01). The gradual increase in albumin level after treatment with triclabendazole was also reported by Sharma *et al*. (2005) and Pal and Dasgupta, (2006), corroborating our finding. In FP Group B, the albumin levels were 2.54 ± 0.17 g/dl on '0' day which became 3.40 ± 0.14 g/dl on 21st day and this increased trend was highly significant (P<0.01). The gradual increase in albumin level after treatment with *E. phaseoloides* might be due to the anthelmintic activity of the plant extract. In FP Group C also albumin level was increased from 2.54 ± 0.12 g/dl on '0' day to 3.21 ± 0.08 g/dl on 21st day and was highly significant (P<0.01). The gradual increase in albumin level after treatment with *A. indica* might be due to its anthelmintic activity.

Alanine aminotransferase (ALT)

The values of ALT in FNC Group were found to be within normal range which correlated with the report of Radostits *et al*. (2000). The increased level of ALT in FPC Group might be due to liver impairment and bile duct

injuries associated with chronic infection in fascioliasis Lotfollahzadeh *et al*. (2008). In FP Group A, the ALT level was gradually decreased and became 33.28±0.14 U/L on 21st day. The decreased trend in ALT level of this group were highly significant (P< 0.01) with triclabendazole treatment and was also reported by Pal and Dasgupta,(2006). In FP Group B, the ALT level was 42.61 ± 1.01 U/L on '0' day which decreased significantly (P< 0.01) to 32.66 ± 0.77 U/L on 21st day. The decreased values of ALT found after treatment with *E. phaseoloides* shows correction of liver impairment by the extract. In FP Group C also, the ALT level was declined significantly to 34.16±0.60U/L on 21st day. The decreased value of ALT found after treatment with *A. indica* could be due to hepatoprotective effect of the plant extract.

Aspartate aminotransferase (AST)

The values of AST in FNC Group were found to be within normal range which correlated with the report of Radostits *et al*. (2000). The increased level of AST in FPC Group might be due to liver impairment and bile duct injuries associated with chronic infection in fascioliasis (Lotfollahzadeh *et al*., 2008). In FP Group A, the AST levels were 89.60 ± 3.67 U/L on '0' day and 78.54 ± 0.87 U/L on 21st day. The value was significant (P<0.05) in comparison to '0' day. The decteased value of AST found after treatment with triclabendazole was also reported by Pal and Dasgupta, (2006). In FP Group B, the AST level was 88.42 ± 2.14 U/L on '0' day which was decreased to 78.73 ± 2.55 U/L on 21st day. The value of AST in this group was highly Significant (P< 0.01). The decreased value of AST found after treatment with *E. Phaseoloides* could be attributed to its hepatoprotective activity, which was not studied in the present investigation. The AST values in FP Group C were decreased, but not significantly after the treatment with *A. indica* which might be due to hepatoprotective effect of the plant extract.

Alkaline phosphatase (ALP)

The values of ALP in FNC Group was found to be within normal range which correlated with the finding of Radostits *et al*. (2000). The increase level of ALP in FPC Group might be due to liver impairment and bile duct injuries associated with chronic infection in fascioliasis. Similar findings were reported by Kumar *et al*. (1982), Swarup *et al*. (1986) and Ahmed *et al*. (2006). There was gradual decrease in ALP values after treatment in FP Group A, FP Group and FP Group C. Similar treatment effect of triclabendazole in FP Group A was reported by Pal and Dasgupta, (2006). However no such report was available in case of *E. phaseoloides* and *A. indica.*

Gamma glutamyl transpeptidase (GGT)

The values of GGT in FNC Group were found to be within normal range which correlated with the report of Radostits *et al*. (2000). The increase level of GGT in FPC Group might be due to liver impairment and bile duct injuries associated with chronic infection in fascioliasis. The present finding of increased values of GGT in FPC Group was correlated with Matanovic *et al*. (2007), Lotfollahzadeh *et al*. (2008) and Kamla and El-Shafie (2008). In FP Group A, the GGT levels were 18.40 ± 0.75 U/L on '0' day which declined to 14.95 ± 0.64 on 21st day and the value was highly significant (P<0.01) in comparison to '0' day. In FP Group C, the GGT value was declined from 15.56 ± 0.61 U/L on '0' day to 12.05 ± 1.38 U/L on 21st day which was significant (P<0.05). The GGT values were decreased gradually after treatment in FP Group A, FP Group B and FP Group C indicating the hepatoprotective activity of the drugs.

CONCLUSION

Based on the reduced faecal egg count and improvement in haemato-biochemical parameters, it may be concluded that the methanolic extracts of the indigenous plants, *E. Phaseoloides* and *A. indica* were found to be highly effective against fascioliasis in cattle though their efficacy were not at par with that of the standard commercially available drug, triclabendazole.

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