Excretory/Secretory (E/S) antigen of adult Fasciola gigantica was prepared and purified by two step alcoholic fractionation and anion exchange chromatography methods. Adult flukes were collected from bile ducts of cattle and buffaloes in normal saline solution from Slaughter houses, Chennai.

E/S antigen was prepared by incubating live adult flukes in RPMI-1640 medium at 37°C for 24 hours and the culture supernatant was used as antigen. Viability of flukes were assessed by their motility. Alcoholic fractionation (75 %) and DEAE-Sephadex A-25 anion exchange chromatography were used for the purification of E/S antigen. A total of 6.4 mg of E/S antigen was obtained from the 60 flukes collected from slaughterhouses. SDS-PAGE analysis of E/S antigen revealed 7 polypeptide bands at 23, 25, 28, 43, 47, 52 and 66 kDa for total E/S antigen, 3 protein bands at 28, 43 and 47 kDa for E/S antigen by 75 per cent alcoholic fractionation and a single band at 28 kDa size was detected in anion exchange chromatography purified fraction. Excretory/secretory antigen can be used for serodiagnosis of fasciolosis in ruminants.

Key words: Fasciola gigantica, Excretory/Secretory antigen, Cattle, Buffaloes

INTRODUCTION

Fasciolosis is one of the major parasitic diseases caused by digenetic trematodes Fasciola gigantica, tropical liver fluke (Linnaeus, 1758) and Fasciola hepatica, temperate liver fluke (Cobbold, 1885) in a wide range of hosts including cattle, buffaloes, sheep and goats which leads to a significant economic loss (FAO, 1994). F. hepatica has a more cosmopolitan distribution and F. gigantica tends to be restricted to tropical regions (Soulsby, 1982).

Tropical fasciolosis caused by Fasciola gigantica is the most economically important helminthic infection of ruminants in Asia and Africa (Spithill et al., 1997). Chowdhuri (1994) documented that 14 Indian states and Union territories are affected by the disease. Prevalence of bovine fasciolosis in different parts of India include, 14.71 % in Punjab (Maqbool et al., 2002), 20.43 % in Uttar Pradesh (Bhatia et al., 1989), 39.61 % in Haryana (Gupta et al., 1986), 52.9 % in Uttarakhand (Yadav et al., 2007), 53.2 % in Meghalaya (Roy and Tandon, 1989), 85.1 % in Jammu and Kashmir (Sharma et al., 1990).
Traditional diagnosis of liver fluke infections is by detection of eggs in faeces or flukes in the liver and in bile ducts during post-mortem. But the methods are cumbersome and labour intensive, and sensitivity can be as low as 30% in animals shedding small number of eggs in faeces (Happich and Boary, 1969). Faecal examination technique does not allow the detection of early-stage prepatent infection, which lasts approximately 13 weeks (Gupta and Yadav, 1992). As no eggs are passed in immature fascioli, serological tests namely Enzyme Linked Immuno Sorbent Assay and Dot-Enzyme Immuno Assay (Dot-EIA) have been used to diagnose the disease during early stage of the infection as early as two weeks after infection (Fagbemi and Guobadia, 1995). Serodiagnosis of bovine fascioli has not been tried to elucidate the prevalence of acute fascioli in Tamil Nadu. Hence, the study was undertaken to prepare and purify excretory/secretory antigen of *Fasciola gigantica* which can be used in serological tests for early diagnosis of fascioli in ruminants.

**MATERIALS AND METHODS**

**Collection of liver flukes**

Adult *Fasciola gigantica* worms were collected from bile duct of cattle and buffaloes slaughtered at Corporation Slaughter house, Perambur and Saidapet Slaughter house, Chennai using normal saline. The collected worms were washed in normal saline and in phosphate buffered saline (PBS, pH 7.4).

**Preparation of Excretory/Secretory Antigen from liver fluke**

Excretory/Secretory (E/S) antigen was prepared following the method of Coles and Rubano et al. (1988). This procedure was repeated several times to obtain sufficient amount of antigen. The antigen material was stored at -20°C till further use. The protein concentration of the E/S antigen was determined using bicinchoninic acid method using protein estimation kit (Genei, Bangalore).

**Purification of E/S antigen from liver fluke by two step Alcoholic fractionation**

Alcoholic fractionation of E/S antigen was done as per procedure described by Coles and Rubano et al. (1988). The supernatant which was collected from the liver flues was thawed and then cold ethanol was added drop by drop until final ethanol concentration of 60 per cent (V/V) was achieved. E/S antigen was aliquoted and stored at -20°C until further use.

**Purification of E/S Antigen by DEAE-Sephadex A-25 Anion Exchange Chromatography**

The excretory/secretory antigen was further purified by using anion-exchange chromatography described by Sriveny et al. (2006). The bound proteins were eluted using elution buffer 0.5M NaCl as 1ml fractions in microfuge tubes. OD values were taken using spectrophotometer at 260 nm and 280 nm and the peak fractions were pooled. The protein concentration was determined by spectrophotometer using the formula;

\[
(\text{OD at } 280\text{nm} \times 1.55) - (\text{OD at } 260\text{nm} \times 0.77) = \text{mg/ml.}
\]

The purified antigen was then stored at -20°C till further use.

**Characterization of E/S Antigen by SDS-PAGE**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to observe the polypeptide patterns of E/S antigen of *F. gigantica*. Both total E/S antigen and purified fractions were electrophoretically resolved on 1 mm thick gel using a discontinuous system. The stacking gel constituted of 5% acrylamide in 0.5 M Tris, pH 6.8 with 0.4% SDS and resolving gel was
RESULTS AND DISCUSSION

A total of 60 live adult Fasciola gigantica worms were collected from slaughter houses for preparation of excretory/secretory antigen. During the incubation period, flukes remained viable for 24 hours as assessed by their motility. The protein content of the E/S antigen of F. gigantica was 1.5, 1.7 and 1.8 mg/ml in trials I, II and III, respectively. A total of 6.4 mg of E/S antigen was obtained from the 60 flukes collected from slaughter houses.

The E/S antigen of F. gigantica purified by 75 per cent alcoholic fractionation was having a protein concentration of 3.2 mg/ml and DEAE-Sephadex A-25 anion exchange chromatography purified fraction was having a protein concentration of 2.1 mg/ml, respectively. Fractions 14, 15 and 16 from anion exchange chromatography method which showed major peak were pooled before use. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 12 % resolving gel) analysis of total E/S antigen revealed 7 polypeptide bands at 23, 25, 28, 43, 47, 52 and 66 kDa molecular weights. The E/S antigen by 75 per cent alcoholic fractionation revealed 3 protein bands at 28, 43 and 47 kDa. A single band at 28 kDa size was detected in anion exchange chromatography purified fraction which is similar to the findings of Fagbemi and Hillyer (1992) who observed molecular weight between 20 to 100 kDa in SDS-PAGE with peak proteolytic fraction obtained from adult F. gigantica revealed 28.3 kDa protein under reducing and non-reducing SDS-PAGE.

Sriveny et al. (2006) observed doublet 27-29 kDa cathepsin L cysteine proteinase antigen from F. gigantica in 15 % SDS-PAGE on purification with anion exchange chromatography and Dixit et al. (2003) characterized 28 kDa cysteine proteinase from bubalian liver flukes using 15 % SDS-PAGE.

In the present study, the E/S antigen from F. gigantica was prepared by incubating the flukes in RPMI-1640 medium at 37°C for 24 hours which is similar to Coles and Rubano (1988). The protein concentration of total E/S antigen varied from 1.5 to 1.8 mg/ml and a total of 6.4 mg of E/S antigen was obtained from the 60 flukes. On the contrary, Vongpakorn et al. (2001) reported 3.57 mg/ml of protein concentration in E/S antigen while Dixit et al. (2003) reported 66.71 mg of total cysteine proteinase antigen from 580 adult flukes.

SDS-PAGE analysis (12 % resolving gel) of total E/S antigen revealed 7 polypeptide bands at 23, 25, 28, 43, 47, 52 and 66 kDa molecular weights. On the contrary, four proteins bands of size 17, 21, 57 and 69 kDa were observed for E/S antigen of F. gigantica in 12.5 % SDS-PAGE by Goubadia and Fagbemi (1997). El Ridi et al. (2007) also observed prominent bands at 62-60, 40, 30 and 28 kDa for excretory/secretory products from F. gigantica by 12 % SDS-PAGE. The present study revealed 7 polypeptide bands for total E/S antigen in 12 % SDS-PAGE whereas others reported only 4 to 6 bands with more or less similar molecular weights. This may be due to the dilution of resolving gel SDS-PAGE or may be the variation of strains of F. gigantica in different parts of the world.

E/S antigen from F. gigantica by 75 % alcoholic fractionation revealed 3 protein bands at 28, 43 and 47 kDa. A single band at 28 kDa size was detected in anion exchange chromatography purified fraction (peak fractions 14, 15 and 16) which is similar to the findings of Fagbemi and Hillyer (1992) who observed molecular weight between 20 to 100 kDa in SDS-PAGE with peak proteolytic fraction obtained from adult F. gigantica revealed 28.3 kDa protein under reducing and non-reducing SDS-PAGE.

Purification of F. gigantica functional antigens by chromatography method is expected to remove host components, which if present are liable to cross react with the conjugate and elicit false results in ELISA and Dot-ELISA. Fagbemi et
al. (1997) reported that chromatography methods could be used as very effective tools for isolation of candidate diagnostic molecule from the parasites.

Excretory/secretory antigen may be used as easily available, safe and inexpensive antigen in serodiagnosis / seroepidemiological surveys of Fasciola gigantica infection in ruminants.

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REFERENCES


**SDS - PAGE (12%) ANALYSIS OF EXCRETORY/SECRETORY ANTIGEN OF FASCIOLOA GIGANTICA**

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