PURIFICATION AND IMMUNO-BIOCHEMICAL CHARACTERIZATION OF CATTLE HYDATID CYST FLUID ANTIGEN

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ABSTRACT : In the present study cattle crude hydatid cyst fluid antigen (CCHCFA) was prepared by ammonium sulfate precipitation, centrifugation and dialysis. Proteins of CCHCFA showed two peaks as PI and P2 when resolved by gel filtration chromatography on Sephacryl S 200. SDS-PAGE of CCHCFA showed four major polypeptides of 68 kDa, 22.3 kDa, 15.8 kDa and 8.4 kDa along with two minor polypeptides of 104 kDa and 31.2 kDa. The initial part of the ascending loop of the first peak (PI) when resolved by 12.5% SDS-PAGE depicted the well defined polypeptide of molecular weight of 68 kDa and 61.4 kDa. Double immunodiffusion test, indirect ELISA and western blot analysis demonstrated that the 68 kDa and 61.4 kDa polypeptides were immunoreactive when treated against hyperimmune sera and known positive sera.

INTRODUCTION

Hydatidosis is a silent, cyclozoonotic and oldest infection of man and domestic animals and is caused by larvae of the cestode, Echinococcus (Bhatia and Pathak, 1990). The infected human beings and animals loose their normal functional capacities leading to huge economic losses. The diagnosis of hydatid disease is based on clinical, radiological, microscopical and immunological methods of which the last one is most sensitive. The crude or fractionated sterile hydatid cyst fluid obtained from cattle may be used as antigen in a variety of immunoassays. For improving the efficacy of immunodiagnosis of hydatid diseasis in cattles, the purification and characterization of hydatid cyst fluid is essential. Moreover researches so far were performed mostly on diagnosis of Echinococcus granulosus in human beings only; relatively less research work hasbeen performed for the early diagnosis of hydatidosis in cattles. Considering the above, the present study is carried out with the objectives of purification and Immunobiochemical characterization of hydatid cyst fluid antigen of cattle.

MATERIALS AND METHODS

COLLECTION OF SERA AND PREPARATION OF CRUDE CATTLE HYDATID CYST FLUID ANTIGEN (CCHCFA)

During the study sera of five positive cases of cattle with presence of visible cysts in their visceral organs and five negetive cases of cattle with the absence of visible cyst in their visceral organs were collected and preserved at -20^{0}C.

CCHCFA (Crude Cattle Hydatid Cyst Fluid Antigen) were prepared from fertile hydatid cysts by centrifugation and dialysis. Phenyl Methyl Sulfonyl Fluoride - PMSF(0.03mM) was added. The globulinic antigen of the hydatid fluid was obtained by precipitating with ammonium sulfate at half saturation. The protein concentrations of

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CCHCFA were determined by the method of Lowry et al. (1951). The antigens thus prepared were stored at -20^{0}C.

PREPARATION OF PURIFIED CATTLE HYDATID CYST FLUID ANTIGEN (PCHCFA) BY GEL FILTRATION CHROMATOGRAPHY.

A sample of CCHCFA was purified by gel filtration chromatography in a column on Sephacryl S 200 (1.5 cm diameter and 60 cm in length) in Phosphate Buffered Saline (pH 7.2), PMSF (0.03mM) and 0.02% sodium azide at a flow rate of 20 ml per hour. The elutes were collected in 65 fractions of 3 ml each. The distribution of protein was monitored by taking the absorbance at 280 nm in a UV/VIS spectrophotometer (Systronics - 119).

IMMUNOBIOCHEMICAL CHARACTERIZATION OF PCHCFA.

(a) Analytical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The CCHCFA and PCHCFA were analyzed by SDS-PAGE, (12.5%) according to Laemmli (1970), with some modification. Vertical mini slab gel electrophoresis system (Bangalore Genei) was used. The gel was then stained with monochromatic silver stain.

(b) Determination of molecular weight by SDS Page.

Molecular weights were determined by standard protein markers (PMW-M, bangalore Genei). Phosphorylase (97.4 kDa), BSA (66 kDa), Ova albumin (43 kDa), Carbonic anhydrase (29 kDa), Soyabean trypsin inhibitor (20.1 kDa) and Lysozyme (14.3 kDa) were electrophoresed with CCHCFA and PCHCFA.

(c) Indirect ELISA.

PCHCFA was used as coating antigen and kept for overnight at 40C. Blocking buffer (2% BSA in PBS) was added and kept for 2 hours followed by washing with PBS-T (0.05% Tween 20 in PBS). After that serial dilution of hyperimmune and normal rabbit sera (1:100, 1:200 and 1:300) were added accordingly and kept for 2 hours followed by washing. Conjugate solution containing anti-rabbit horse radish peroxidase conjugate (sigma) was added and kept for 2 hours. After washing with PBS-T, Substrate buffer (3 µl H_2O_2, 0.025 gm of O-Phenylene diamine in 25 ml. citrate buffer) was added and kept for 30 min. in dark. After the development of colour 2N H_2SO_4 solution was added and the reading was taken in an ELISA reader at 492 nm (Multiskan, Labsystem, Model - 355).

(d) Western blot.

The PCHCFA was characterized by western blotting technique according to Towbin et al. (1979) with some modifications. The antigen was separated by SDS-PAGE (Laemmli, 1970) and then the resultant proteins were electroblotted to nitrocellulose filter paper from gel to a mini western blot apparatus (Bangalore Genei).

IMMUNOCHEMICAL ANALYSIS

(a) Raising of antisera.

The antibody against PCHCFA was raised in healthy New Zealand white rabbits. The antigen was mixed with equal volume of Freud's complete adjuvant (FCA) and freund's incomplete adjuvant (FIA). Sera were collected from these rabbits 7 days after the last booster dose and were stored at -20^{0}C.

(b) Double immunodiffusion test (DID).

The DID test was performed to detect the specific antibody raised against purified CHCFA.

RESULTS AND DISCUSSION

Protein fractions of CCHCFA showed mainly 2 peaks and were pooled together into 5 parts and named as PI (fractions of initial part of ascending loop of first peak), P2 (fractions of rest part of ascending loop first peak), P3 (fractions of descending loop of first peak), P4 (fractions between first and second peak) and P5 (fractions of second peak), Shown in Figure 1. The 5-pooled fractions were concentrated and preserved at -20^{0}C. 12.5% SDS-PAGE (Laemmli, 1970)

of the fraction PI showed two polypeptide of CCHCFA and this was considered as purified CHCFA.

The protein concentration of CCHCFA determined (Lowry et al. 1951) was 2.8 mg/ml. The protein concentration of the part PI determined (Lowry et al. 1951) was 2.4 mg/ml. The resolution of CCHCFA in 12.5% SDS-PAGE revealed polypeptides of MW of 104, 68, 31.2, 22.3 15.8 kDa and 8.4 kDa (Fig. 2). The result was fairly similar to the findings of Jiang et al. (1998).

standard error values has been expressed in table No. 1. The mean O.D. value ranged from 0.67 ± 0.003 to 0.48 ± 0.008 for 1:100 to 1:300 hyperimmune sera dilution whereas the mean O.D. value ranged from 0.26 ± 0.01 to 0.24 ± 0.08 for the same range of normal sera dilution. The present study of isolation, purification and characterization of hydatid cyst fluid antigen of cattle showed that the PCHCFA as 68 kDA & 61.4 kDa polypeptides are inimunoreactive which may be used for immunodiagnosis of echinococcosis in cattle.

Table 1 : Sero-reactivity expressed as mean O.D. along with standard error values of PCHCFA against normal and Hyperimmune Sera as assessed by indirect ELISA

	Sera dilution (1:100)		Sera dilution (1:200)		Sera dilution (1:300)	
Antigen	Hyperim-mune sera	Normal sera	Hyperim-mune sera	Normal sera	Hyperimmune sera	Normal sera
PCHCFA	0.67±0.003	0.26±0.01	0.51±0.005	0.25±0.01	0.48±0.008	0.24±0.008

Values are expressed in mean of three observations ± standard error

The bands of 66.5 kDa, 41 kDa and 19 kDa of the present study were almost similar to 66, 41 and 19 kDa bands as mentioned by Jiang et. al (1998). PCHCFA when resolved by gel filtration chromatography showed two polypeptide bands of 68 kDa and 61.4 kDa in 12.5% SDS-PAGE (Fig.2) and it was found to be immunoreactive when analyzed by western blot (Fig.3). The result was found to be similar to the findings of Felice et. al (1986). Single precipitin line was observed in double immunodiffusion test when the PCHCFA was treated with the hyperimmune serum. No precipitin line or band was found against the normal control sera .

Indirect ELISA using hyperimmune and normal rabbit sera at different dilutions assessed the seroreactivity, expressed as mean O.D. of PCHCFA. The mean O.D. along with their

CONCLUSION

From the above study , it can be concluded that these polypeptides (purified cattle hydatid cyst fluid antigen) might prove to be a promising tool for the diagnosis of hydatid disease.

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