

Cytotoxic effect of coelomic fluid of earthworm *Eudrilus eugeniae*

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ABSTRACT

Cytotoxic effect of coelomic fluid of earthworm *Eudrilus eugeniae* was studied on Baby Hamster Kidney 21 (BHK₂₁) Cells. Coelomic fluid from *Eudrilus eugeniae* was extracted by cold shock treatment. BHK₂₁ cells were cultured according to standard cell culture method. BHK₂₁ cells were treated with the various protein concentrations of coelomic fluid of *Eudrilus eugeniae* to study the cytotoxic effect. The result revealed that the coelomic fluid of *Eudrilus eugeniae* induced cell death and the activity was concentration dependent, confirming the presence of cytotoxic molecule/s.

Key words: Coelomic fluid, cytotoxic activity, *Eudrilus eugeniae*.

INTRODUCTION

Coelomic fluid (CF) of earthworms contain cytolytic, antibacterial and/or agglutinating components¹⁻⁵ (Roch et al., 1989; Valembois et al., 1982; Lassegues et al., 1989 Mohrig et al., 1996). The primary function of this cytolytic system may be to destroy membranes of foreign cells which causes cell death by cytosol release. Cytotoxicity of CF of *Eisenia fetida* is studied by a number of workers⁶⁻⁹ (Suzuki and Cooper, 1995; Cossarizza et al., 1996; Quaglino et al., 1996). Lange et al., 1999.) Many peptide molecules from the CF of *Eisenia fetida* were identified, purified and characterized¹⁰⁻¹¹ (Yamaji-Hasegawa et al., 2003; Konig et al., 2004.). This is a preliminary report on the cytotoxic effect of coelomic fluid of *Eudrilus eugeniae*. The cytotoxicity was checked using BHK₂₁ cells.

MATERIAL AND METHODS

Earthworms *Eudrilus eugeniae* were obtained from GKVK (Gandhi Krishi Vignyan Kendra) Bangalore, and were cultured. BHK₂₁ cells were procured from NCCS, courtesy IAH & VB, Bangalore. TBP, NBBS & cell culture media were from Sigma – Aldrich fine chemicals. All other chemicals were of analytical grade purchased from S.D. fine chemicals, Mumbai, India.

Culturing of earthworms

Earthworms *Eudrilus eugeniae* were cultured on suitable bedding in plastic trays. Under ideal conditions, they were fed with organic substances from plant and animal origin. The feed stock chosen were dairy and beef manures which is considered as best natural food for the Earthworms¹². 75%- 80% moisture content were

maintained so that the average worm weight and the reproduction rate increased¹¹. As the worms are aerobic, sufficient aeration was provided.

Extraction of coelomic fluid

Extraction of Coelomic fluid from chosen earthworm species was performed according to the method of Kale R., (1991)¹³. The earthworms were washed with cold water 3-4 times at room temperature and their body surface were dried on filter paper. Then they were subjected to cold shock. Cold shock was performed using an icepack at a temperature of 0-2°C. The icepack was slowly rubbed against the body of the earthworms. The procedure is repeated for 3- 4 times. The coelomic fluid obtained from the body of the earthworms was then collected and stored in vials.

Estimation of protein

Protein was measured by the method of Lowry *et al.*, (1951)¹⁴ using bovine serum albumin standard (0-75µg).

Culture of BHK₂₁ cells

BHK₂₁ cells were first grown MD bottles using Sigma media supplemented with 7% New Born Calf Serum (NBCS) and 10% Tryptose Phosphate Broth (TBP). The cultures were incubated in CO₂ incubator for 48hr at 37°C. The monolayer formed was further trypsinized and cultures were transferred to different plates.

Assay of cytotoxic activity:

445µl of the medium containing BHK₂₁ cells were taken in two different microtitre plates and 5µl containing 10µg protein of sample (CF of *Eudrilus eugeniae*) was added and serially diluted. The plates were observed after three days under invert microscope at 5x magnification. Control was maintained without adding the sample. The experiments were performed in triplicates.

RESULTS AND DISCUSSIONS

The CF treated BHK₂₁ cells shows cytopathic effect on the 3rd day, upto a concentration

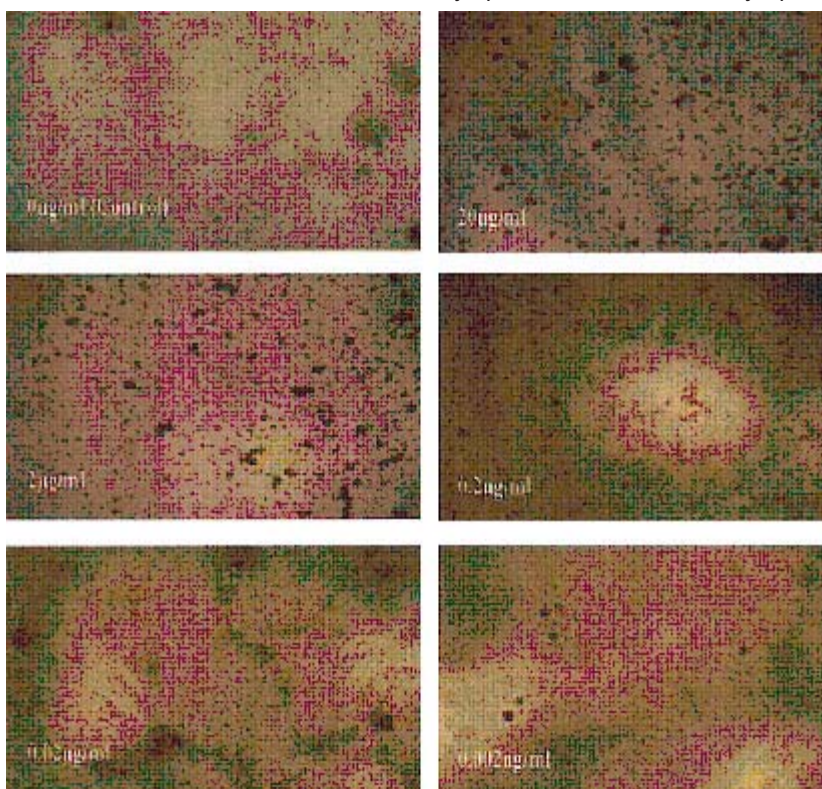


Fig. 1: Invert Microscopic photographs of BHK₂₁ cells treated with CF of *Eudrilus eugeniae* under 5x magnification.

of 0.2µg/ml (Fig.1). The effect was dependent on concentration as evidenced in the figure. The monolayer appeared from well-5 containing protein concentration 0.02µg/ml. These experimental results were reproducible.

The mechanism of action of cytolytic proteins from CF of other genus of earthworm *Eisenia fetida* are worked out in other laboratories. The cytolytic protein 'Eiseniapore', a 38 kDa protein from coelomic fluid of the earthworm *Eisenia fetida* induces cytolysis by forming pores, which is confirmed by electron microscopy of erythrocyte membranes treated with the protein⁹ (Lange *et al.*, 1999). 'Lysenin', from the coelomic fluid of the earthworm *Eisenia foetida*, binds to the plasma membrane of target cells via sphingomyelin¹⁰

(Yamaji-Hasegawa *et al.*, 2003). When earthworm coelomocytes from *Eisenia fetida* are incubated with the tumor target cells - K562, damage to target membranes has been observed by scanning electron microscopy and transmission electron microscopy⁶⁻⁸ (Suzuki and Cooper, 1995; Quaglino *et al.*, 1996; Cossarizza *et al.*, 1996;). Hemolytic proteins both from coelomocytes (CL_{39,41}) and coelomic fluid (H_{1,3}) of wildtype *E. fetida* have been isolated and assigned to fetidin and lysenin using mass spectrometry and bioinformatic tools¹¹ (Konig *et al.*, 2004). Efforts are being made to purify the molecule/s responsible for the cytotoxicity from CF of *Eudrilus eugeniae*. Further studies on their mechanism of action may throw light on drug designing.

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