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Anti-Cancer Activity of Brachyuran Crab *Dromia dehaani* (Rathbun, 1923)

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ABSTRACT

Marine chemotherapy is well recognized now a days and profound development has been achieved by researchers to deal with different molecular pathways of tumors. In the present study, anticancer activity of the hemolymph of brachyuran crab *D. dehaani* was assayed with standard MTT colorimetric procedure against a range of human cell lines viz., HepG2, HT-29, rhabdomyosarcoma and A549. Cell viability was evaluated by 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cytotoxicity was assessed by the morphological characteristics were analyzed through phase contrast microscope. The results exhibited remarkable cytotoxicity against HepG2 cells. The results obtained from the MTT assay showed that the tested samples were significantly reduced the viability of all the tested cell lines in a dose-dependent manner upon 24 h of exposure, the results were recorded in IC₅₀. The present results revealed that the brachyuran crab *D. dehaani* could be a potential alternative agent for human cancer therapy.

Key words: Brachyuran crabs, human cell lines, anticancer compounds

INTRODUCTION

The chemical and biological diversity of the marine environment is immeasurable and therefore is an extraordinary resource for the discovery of new anticancer drugs (Pomponi, 1999). An exciting “Marine pipeline” of new anticancer clinical and preclinical agents has emerged from intense efforts over the past decade to more effectively explore the rich chemical diversity offered by marine life. There are plenty of works related to antitumor (Natarajan *et al.*, 2010a) and antimicrobial activity (Natarajan *et al.*, 2010b) carried out in ascidians, which made the chemist to isolate the active principle responsible for the actions as they are the viable source for drug discovery process. Anticancer compounds have characteristics of multi-function, high sensitivity, stability and so on (Leng *et al.*, 2005). It was reported that the denbinobin obtained from *Ephemerantha lonchophylla* was found to reduce the cell viability of human colorectal cancer HCT-116 and HT-29 cells in a concentration-dependent manner as measured by MTT assay (Chen *et al.*, 2008). From the literature cited it is undoubtedly revealed that the sponge crab *D. dehaani* has not been explored for its biomedical especially *in vitro* anti-cancer potential. Hence this present research effort made an attempt to evaluate the anti-cancer properties of the hemolymph of the crab *D. dehaani* against the cell lines viz., HepG2, HT-29, rhabdomyosarcoma and A549.

MATERIALS AND METHODS

Animal and hemolymph collection: Sponge crabs *D. dehaani* were collected from the Pazhayar landing center during the year of 2014. Hemolymph was collected by cutting walking legs of the

crab *D. dehaani* with a fine sterile scissor. To avoid hemocyte degranulation and coagulation, the hemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, V/V). Equal volume of physiological saline (0.85%, NaCl, w/v) was added to it. To remove hemocytes from the hemolymph it was centrifuged at 2000 rpm for 15 min at 4°C. Supernatant were collected by aspirating and stored at 4°C until use.

Cell line and culture: The HepG2, HT-29, rhabdomyosarcoma and A549 cell lines purchased from the National Center for Cell Science (NCCS, Pune) were grown as monolayer in Minimal Essential Medium (MEM) (Himedia) supplement with 10% FCS, 3% glutamine penicillin (100 U mL⁻¹) and streptomycin (100 µg mL⁻¹) at 37°C for 5% CO₂ atmosphere. Stocks were maintained in 25 cm² tissue culture flasks. The *in vitro* cytotoxicity's of the sample on cell lines were examined using a modified MTT assay (Plumb *et al.*, 1989). The trypan blue exclusion test was used for detection of cell viability (Morgan and Darling, 1992).

DNA fragmentation assay: To confirm morphological changes in the nuclei, cells were seeded in 16-mm cover slips placed in 6-well plates at 2×10⁶ cells. Cells were treated 1 day after seeding with hemolymph at different concentrations for 24 h. The HOECHST 33258 solutions was added and the cells were incubated for 30 min before being examined by fluorescence microscopy.

Data analysis: All data were analyzed by the software of SPSS (SPSS Science Inc.).

RESULTS

The crab's hemolymph was tested to evaluate their cytotoxic potential against the cancer cells. Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. Cytotoxicity was assessed by the morphological characteristics of the cells such as rounding of the cells, shrinkage, aggregation and cell death was observed through Phase Contrast Microscope. The sample was screened using MTT assay. This sample showed remarkable cytotoxicity against almost all the tested cells in a dose dependent manner.

Cytotoxicity of vero cell line: The cytotoxicity of the hemolymph in Vero cell line is represented graphically in Fig. 1. The tested samples showed high cytotoxicity on vero cells at concentrations viz. 600, 500, 400, 300, 200, 100 and 50-10 µg mL⁻¹. The 50% of cytotoxicity (IC₅₀) was observed at the concentration of 200 µg mL⁻¹. The photographical confirmation of the Vero cell line has presented in Fig. 2.

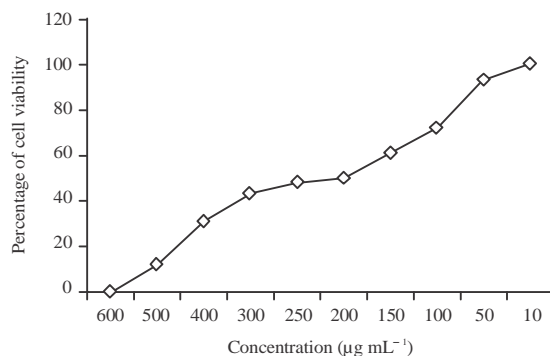


Fig. 1: Cytotoxicity of Vero cells after treating with various concentration of hemolymph

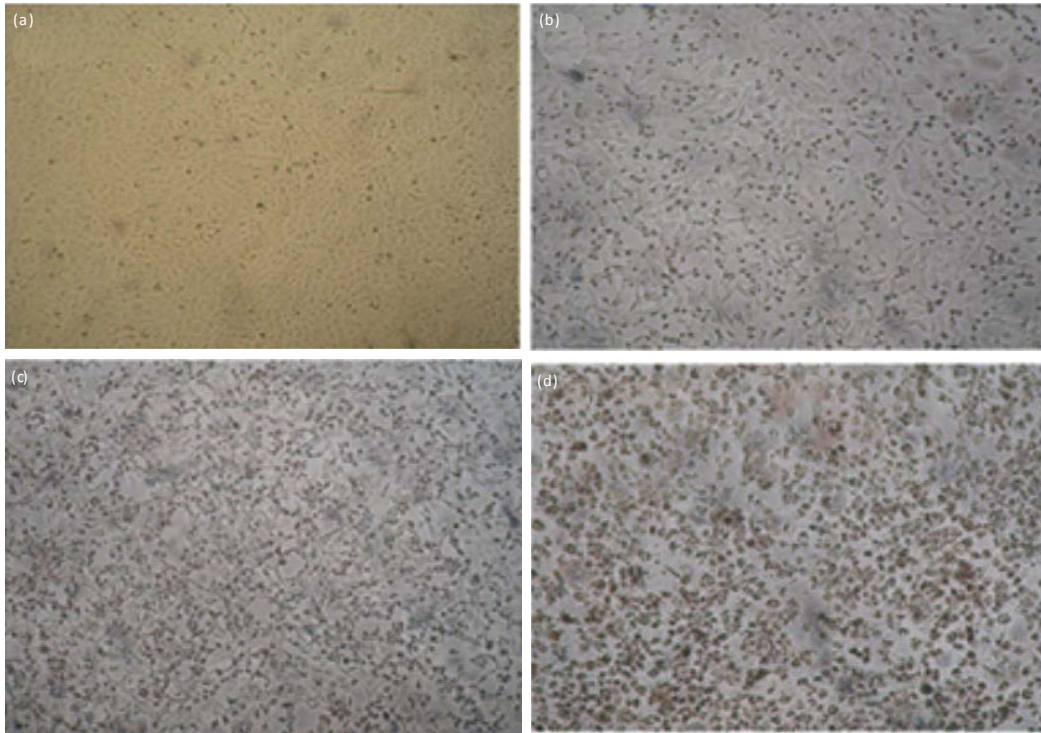


Fig. 2(a-d): *In vitro* cytotoxicity assay of *D. dehaani* in Vero cell line, Vero cells (a) Control, (b) 24 h, (c) 48 h and (d) 72 h

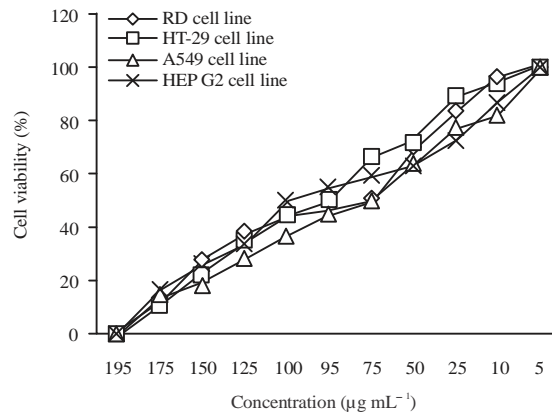


Fig. 3: *In vitro* anticancer activity of *D. dehaani* after treating with various concentration of hemolymph

The hemolymph was tested for its cytotoxicity on various cells at concentrations viz., 195, 175, 150, 125, 100, 95, 75, 50, 25 and 10-5 $\mu\text{g mL}^{-1}$. The cells displayed dose dependent decrease in viability visible as early as 24 h. The hemolymph that produced half Inhibitory Concentration (IC_{50}) at 100 $\mu\text{g mL}^{-1}$ against HepG2 cells. In the HT-29 cell lines the hemolymph that produced the half inhibitory concentration was 95 $\mu\text{g mL}^{-1}$. In rhabdomyo sarcoma cell lines the hemolymph produced the half Inhibitory Concentration (IC_{50}) was 75 $\mu\text{g mL}^{-1}$. In the A549 cell lines hemolymph produced the IC_{50} was 75 $\mu\text{g mL}^{-1}$ (Fig. 3).

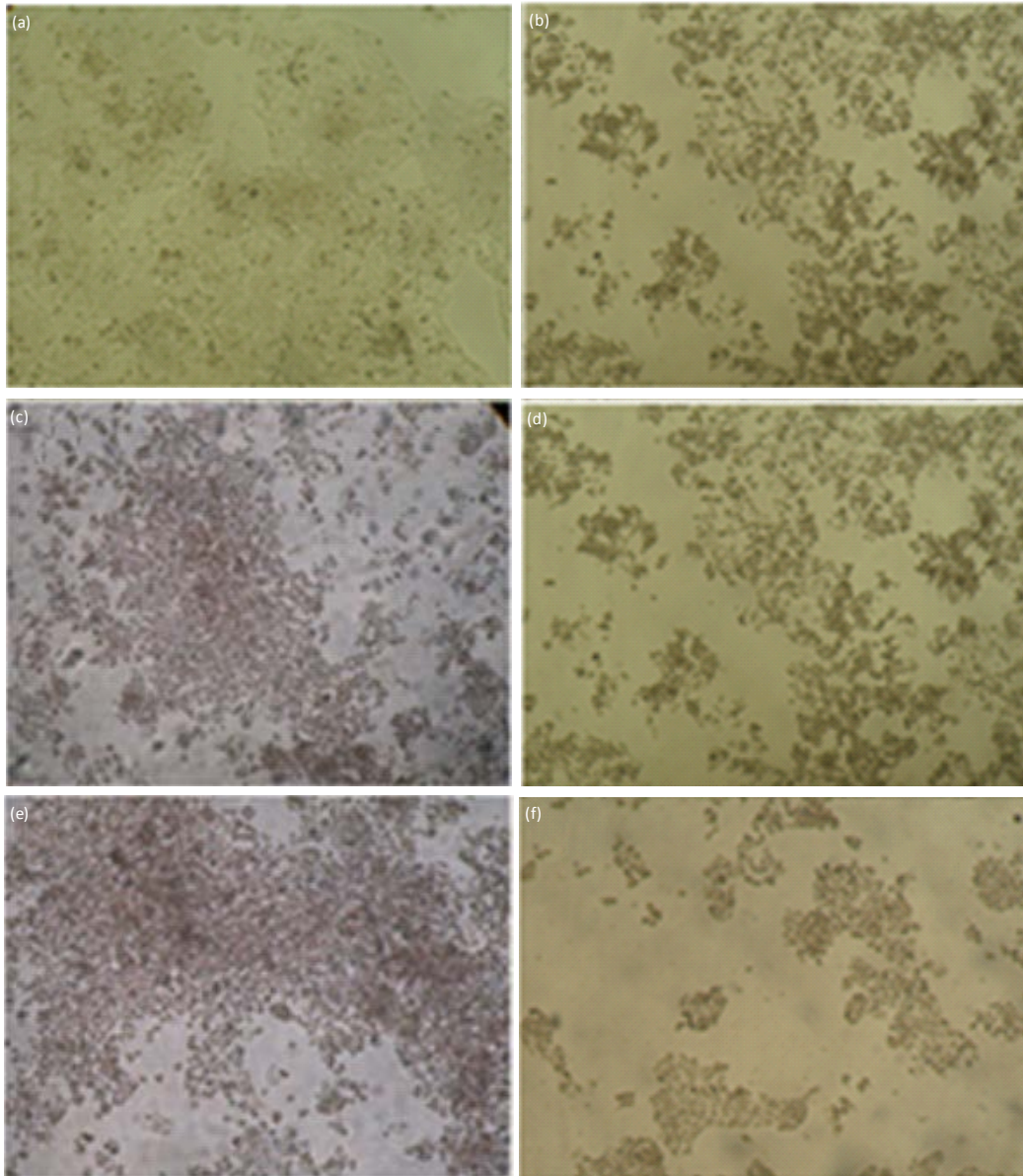


Fig. 4(a-f): Morphological changes on HepG2 cell line after treating with various concentrations of hemolymph, (a) Cell control, (b) $195 \mu\text{g mL}^{-1}$, (c) $125 \mu\text{g mL}^{-1}$, (d) $95 \mu\text{g mL}^{-1}$, (e) $75 \mu\text{g mL}^{-1}$ and (f) $10 \mu\text{g mL}^{-1}$

The morphological changes on HepG2 cell line after treating with various concentrations of hemolymph has presented in Fig. 4. The Morphological changes on HT-29 cell line has presented in Fig. 5. The morphological changes on RD cell line has displayed in Fig. 6. The morphological changes on A549 cell line have shown in Fig. 7.

DNA fragmentation: Among the four cell line tested HepG2 showed pronounced activity hence it has been taken further for the DNA fragmentation studies. DNA was isolated from HepG2 cell

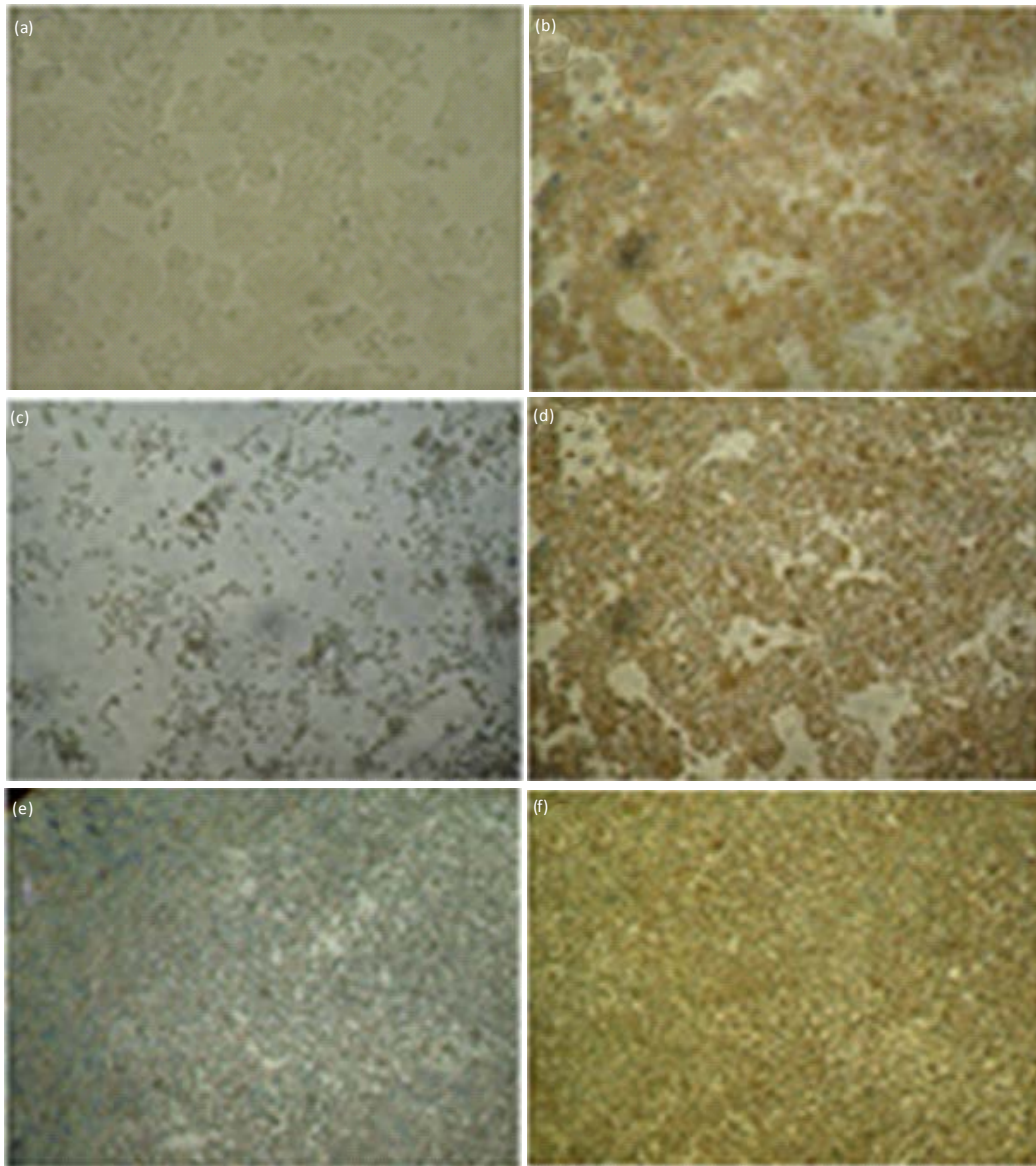


Fig. 5(a-f): Morphological changes on HT-29 cell line after treating with various concentrations of hemolymph, (a) Cell control, (b) $195 \mu\text{g mL}^{-1}$, (c) $125 \mu\text{g mL}^{-1}$, (d) $95 \mu\text{g mL}^{-1}$, (e) $75 \mu\text{g mL}^{-1}$ and (f) $10 \mu\text{g mL}^{-1}$

line treated with different concentration of hemolymph from normal cell line for control. The result of the DNA fragmentation test is presented in Fig. 8. The results indicate a clear DNA damage to HepG2 cells after the treatment of hemolymph. These results suggest that the crab's hemolymph had a dose dependent deleterious effect on HepG2 cell viability.

DISCUSSION

A number of researchers have focused on identifying novel marine natural product as anticancer drugs (Simmons *et al.*, 2005). Anticancer compounds have characteristics of

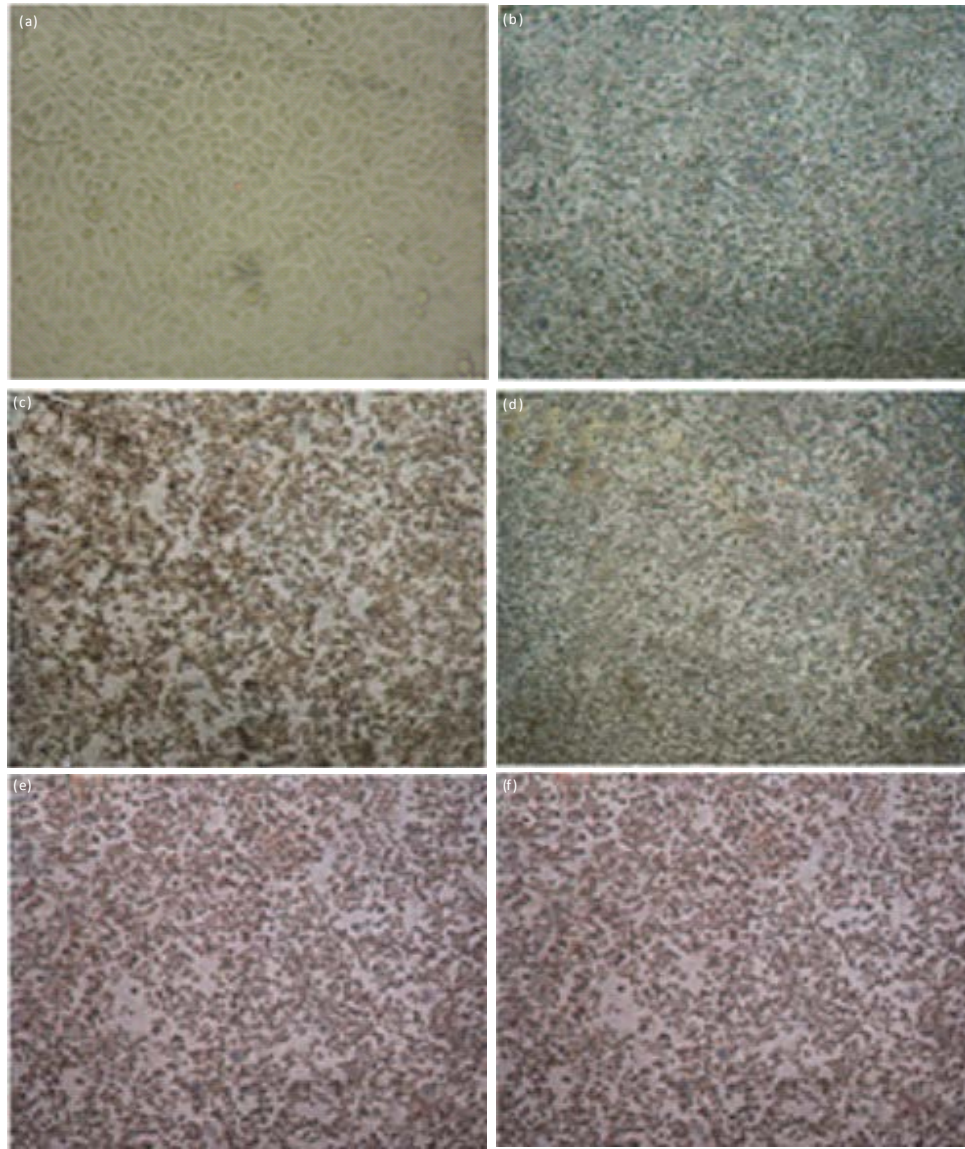


Fig. 6(a-f): Morphological changes on rhabdomyosarcoma cell line after treating with various concentrations of hemolymph. (a) Cell control, (b) $195 \mu\text{g mL}^{-1}$, (c) $25 \mu\text{g mL}^{-1}$, (d) $995 \mu\text{g mL}^{-1}$, (e) $75 \mu\text{g mL}^{-1}$ and (f) $10 \mu\text{g mL}^{-1}$

multi-function, high sensitivity, stability and so on (Leng *et al.*, 2005). The present study was undertaken to investigate the anticancer mechanisms of the marine crab *D. dehaani* against several human cancer lines. There have been several researches to get new cytotoxic agents. In this regard antimicrobial compounds isolated from marine organisms showed considerable promises. In the present study the hemolymph of the crabs were tested to evaluate their cytotoxic and anticancer potential.

Wu *et al.* (2001) observed an enhanced scavenging effect at the low concentration of combined selenium and Ge-132. Antitumor mechanisms of carboxyethyl-germanium sesquioxide (Ge-132) in

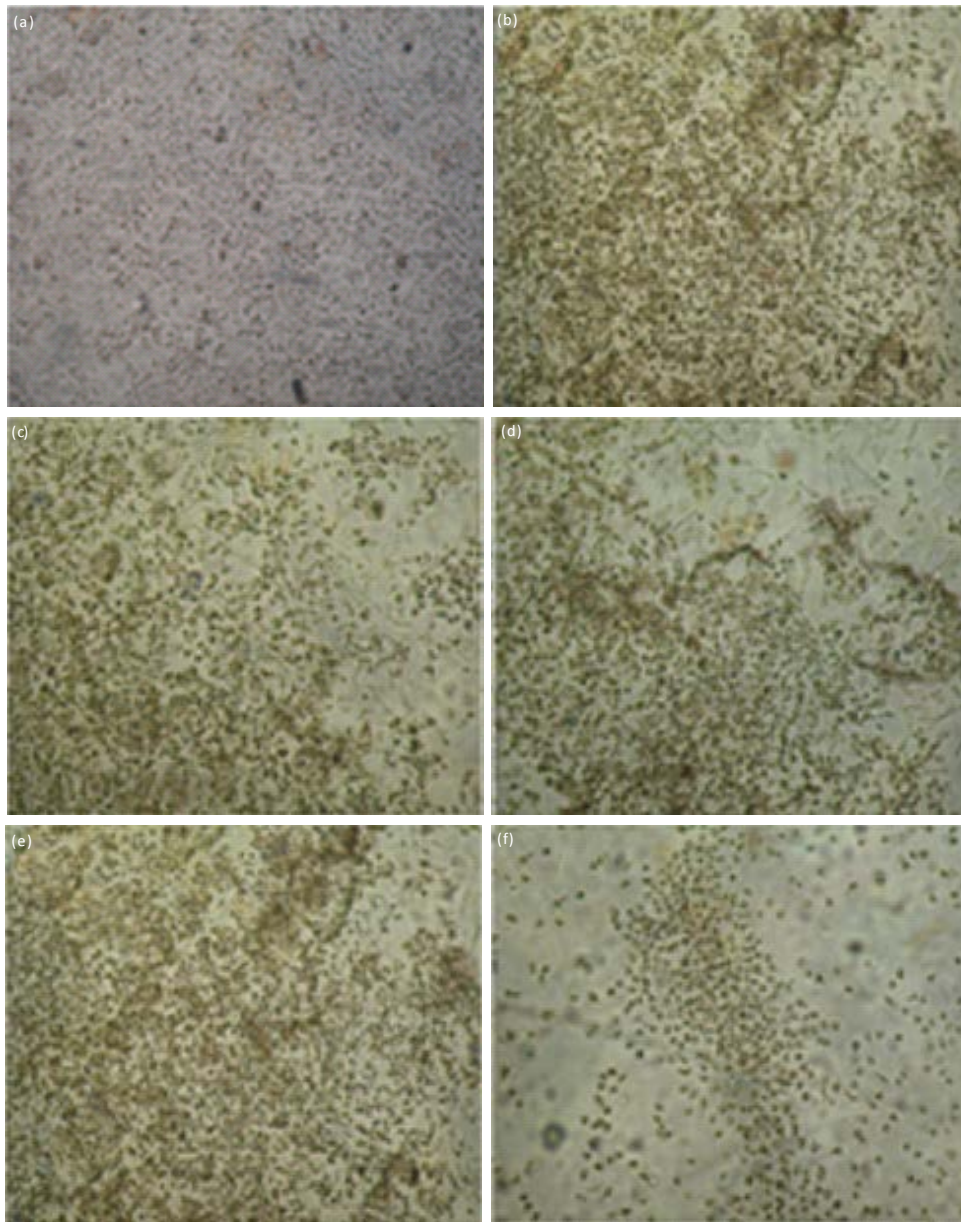


Fig. 7(a-f): Morphological changes on A549 cell line after treating with various concentrations of hemolymph. (a) Cell control, (b) $195 \mu\text{g mL}^{-1}$, (c) $125 \mu\text{g mL}^{-1}$, (d) $95 \mu\text{g mL}^{-1}$, (e) $75 \mu\text{g mL}^{-1}$ and (f) $10 \mu\text{g mL}^{-1}$

mice bearing ehrlich ascites tumors were reported. Ge-132 showed its *in vivo* antitumor effect partly due to its inducing the antitumor immunity of the host (Zhang *et al.*, 2009). In the present study the results showed remarkable cytotoxicity against the cells in a dose dependent manner. In the cytotoxicity of Vero cell line the IC_{50} value was found in the concentrations of $200 \mu\text{g mL}^{-1}$.

In the current investigation anticancer activity half inhibitory concentration (IC_{50}) was $75 \mu\text{g mL}^{-1}$ against HepG2 cell lines. Similarly Erythrazole B from *Erythrobacter* sp. was found to

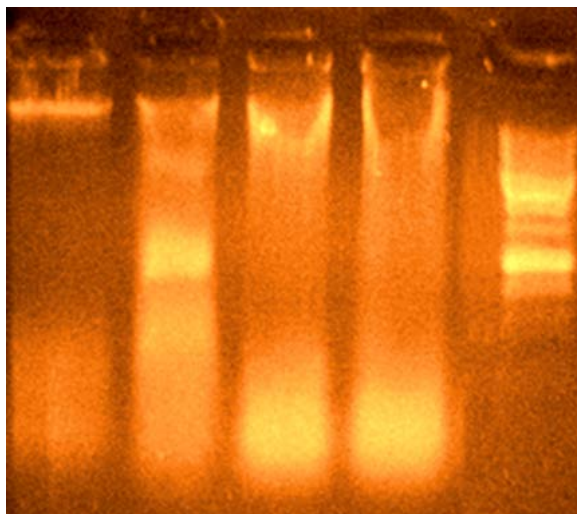


Fig. 8: DNA fragmentation of HepG2 cell line after treating with various concentrations of hemolymph (L1: Control, L2: $62.5 \mu\text{g mL}^{-1}$, L3: $125 \mu\text{g mL}^{-1}$, L4: $31.6 \mu\text{g mL}^{-1}$, M: Marker-100 bp DNA ladder)

be highly toxic against H1395, H2122 and HCC366 cell lines with IC_{50} values of 1.5, 2.5 and $6.8 \mu\text{M}$, respectively (Hu and MacMillan, 2011). In the HT-29 cell lines the IC_{50} was $95 \mu\text{g mL}^{-1}$. In the rhabdomyosarcoma cell lines the IC_{50} was $75 \mu\text{g mL}^{-1}$, this result was supported by the findings of (Chen *et al.*, 2008). Methyl spongoate displays potent toxicity against six hepatocellular carcinoma cell lines, with IC_{50} values ranging from 1.7-9 μM . It is known that advanced hepatocellular carcinomas are generally resistant to anticancer drugs because of the multidrug resistant (MDR) phenomena (Thomas, 2009; Papatheodoridis *et al.*, 2010). In the A549 cell lines the IC_{50} was $75 \mu\text{g mL}^{-1}$. Similar results were found with keenamide A which exhibited significant activity against the P-388, A-549, MEL-20 and HT-29 tumor cell lines (Wesson and Hamann, 1996). An anticancer glycol peptides was reported from *Meretrix meretrix* and its inhibitory rate affecting the KB (human Caucasian/epidermal carcinoma) cell line was 69% at $200 \mu\text{g mL}^{-1}$ (Zhang and Wu, 2006). Relatively strong anticancer peptides were also found from *M. meretrix* with IC_{50} of $10 \mu\text{g mL}^{-1}$ (Liu *et al.*, 2012).

Dalastatin-10, extracted from the sea hare *D. auricularia* has entered into clinical trials. Dolastatin-10 is a penta peptide with four of the residues being structurally unique. It is the most potent anti-proliferative agent known with an ED_{50} of $4.6 \times 10^{-5} \mu\text{g mL}^{-1}$ against murine PS leukemia cells (Pettit *et al.*, 1987). In the present study the half Inhibitory Concentration (IC_{50}) was $75 \mu\text{g mL}^{-1}$ on HepG2. Similarly studies were carried out by Ariffin *et al.* (2009) and Waiyaput *et al.* (2012). In the present study the hemolymph showed pronounced activity against HepG2 cell lines. Likewise the dolastatins isolated from *Dolabella auricularia* inhibit cell proliferation and induce apoptosis in numerous malignant cell lines (Haldar *et al.*, 1998; McElroy *et al.*, 1997; Tran *et al.*, 1997; Bagniewski *et al.*, 1997; Garteiz *et al.*, 1998). In this regard anticancer compound isolated from marine organisms showed considerable promises. In the present study, MTT assay was used for evaluation of cytotoxic activity of the crab hemolymph and showed cytotoxic effects. The findings of the present showed that the hemolymph of the crab *D. dehani*

might be a good source to inhibit the HepG2 cells and it would be a great source for anticancer compounds which can be useful for human welfare.

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