

Immunostimulating Activities of Tunicate-Phallusia Nigra Savigny, 1816 on Mda-Mb-231 Tumor Bearing Mice

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Abstract: - The present study was carried out to assess the immunostimulating activities of ethanolic extract of simple tunicate *Phallusia nigra* on MDA-MB-231 tumor bearing Swiss albino mice. 100% toxicity was observed in 0.60 mg/ml concentration. Since the tunicates are sedentary organisms, they are rich in secondary metabolites as chemical defence. Standard methodology has been adopted to assess the various parameters. The results showed highly significant and dose related increase in quantitative hemolysis of sheep red blood cells, lymphocyte proliferation, NK cytotoxic activity and phagocytosis rate. The results obtained for group IV treated with highest dose of the extract was near to the group treated with the standard drug vincristin. It is concluded that the bioactive compounds present in *Phallusia nigra* may modulate immune functions and play a vital role in cancer prevention and treatment.

Keywords

Phallusia nigra, MDA-MB-231, Immunostimulating, Hemolysis.

1. INTRODUCTION

Breast cancer is one of the most prevalent malignancies among women worldwide as stated by Ferlay et al., 2010. Devasagayam and Sainis, 2002 noticed that chemotherapy and radiation cause severe adverse effects, such as bone marrow suppression resulting in cytopoenia, and subsequent devastation of the immune responses. Hence there is an urgent need for the development of a drug which can enhance the immune system. Number of natural products is being used as therapeutic agents. Tunicates are marine sedentary organisms have been largely studied since it has been shown that many extracted compounds display potent antitumoral activities. The extracts of *Cystodytes dellechiaiei* showed remarkably high antiproliferative activity in human breast carcinoma SKBR3 cell lines (Garcia et al., 2007). Isogranulatimide from *Didemnum granulatatum*, Aplidin from *Aplidium albicans*, clavaminols A-F from *Clavelina phlegraea*, aplidinone A from *Aplidium conicum*, Cephalostatin from *Cephalodiscus gilchristi*, ritterazine G from *Ritterella tokioka* and Styelin D from *Styela clava* showed antitumor activity against various cell lines (Berlinck et al., 1998; Fernandez, 2002; Aiello et al., 2007; Aiello et al., 2010; Wojtkielewicz et al., 2003; and Taylor et al., 2000.) A significant antiproliferative and immunomodulatory activity to DLA, EAC, S-180 and HLCA-549 cells was obtained with the ethanolic extract of *Phallusia nigra* (Meenakshi et al., 2012a, b, 2013, 2014a, 2014b; Paripooranaselvi et al., 2015; Paripooranaselvi and Meenakshi., 2015, 2016). As tunicates are available in plenty along the Tuticorin coast an attempt has been made

to assess their immunomodulatory aspect to MDA-MB-231.

II. MATERIALS AND METHODS

Specimen collection and identification

Samples of *Phallusia nigra* were collected from the under surface of the barges of Tuticorin harbour. Identification up to the species level was carried out based on the key to identification of Indian ascidians (Meenakshi, 1997). A voucher specimen AS 2083 has been submitted to the museum, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002, Tamilnadu, India.

Systematic position

Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Suborder: Phlebobranchia; Family: Ascidiidae; Genus: *Phallusia*; Species: *nigra*

Animal material

Phallusia nigra is a simple ascidian covered by a thick black envelope (tunic) which contains cellulose like material. An adult *Phallusia nigra* may be 10 cm long. The sac-shaped body has two siphons for water entrance and exit. It is a marine, sessile, filter feeding animal.

Experimental animals

Adult Swiss Albino mice weighing 20-25 g were obtained from the Breeding section, Central Animal House, Dr. Raja Muthiah Medical College, Annamalai University, Chidambaram, Tamilnadu.

Preparation of powder and extract

The animal was dried at 45°C and powdered. Ten grams of the powder was soaked overnight in 100 ml of 70 percent

ethanol and filtered. The filtrate was centrifuged at 10,000 rpm at 4 °C for 10 minutes. The supernatant was collected and evaporated to get a residue, which was used for in vitro studies. For in vivo animal experiments it was resuspended in 1% gum acacia blended with vanillin and administered orally at different concentrations.

In vitro cytotoxic activity

MDA-MB-231 cells (1x10⁶ cells) were incubated with various concentrations of extract in a final volume of 1ml for 3hr at 37o C. The viability of the cells was confirmed by trypan blue dye exclusion method (Ebada et al., 2010).

Effect of Phallusia nigra extract on MDA-MB-231 bearing mice

The effect of the extract was determined by evaluating cytotoxicity and immune function. MDA-MB-231 cells were procured from Adayar Cancer Institute, Chennai, India and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated calf serum, 100 U/ml penicillin G, 100 U/ml streptomycin, pH 7.4 in a Water Jacketed CO₂ incubator with a humidified atmosphere of 5% CO₂ at 37o C.

Experimental protocol

Adult Swiss albino mice were divided into five groups of six animals each. Tumor was induced by injecting 0.1 ml of 1x10⁶ MDA-MB-231 cells per 10 gram body weight of animals intraperitoneally on day zero. A day of incubation was allowed for multiplication of cells. Group I acted as control and was given normal saline. Group II, III and IV and V were treated with ethanolic extract of Phallusia nigra at 50, 100, 150 and standard drug Vincristin at 80 mg/kg bw respectively for 9 days. The extract was blended with 1% gum acacia and vanillin solution and administered intra gastrically.

Immunomodulatory assays

Quantitative hemolysis of sheep red blood cells assay, Lymphocyte proliferation rate, NK cell cytotoxicity and Phagocytic activity as per the standard procedures suggested by Thirunavukkarasu et al., 2011; Aravind et al., 2012; Sachs et al., 1999 etc.

Statistical Analysis

Values are expressed as mean ± SEM. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett s test. P-values less than 0.05 were considered to be significant.

III. RESULTS AND DISCUSSION

Cytotoxic activity to MDA-MB-231 cells

The extract at a concentration of 0.05, 0.10, 0.20, 0.40 and 0.60 mg/ml produced 21, 40, 78, 91 and 100 percent cytotoxicity to MDA-MB-231 cells respectively (Figure - 1). A dose dependent increase in percentage cytotoxicity was observed.

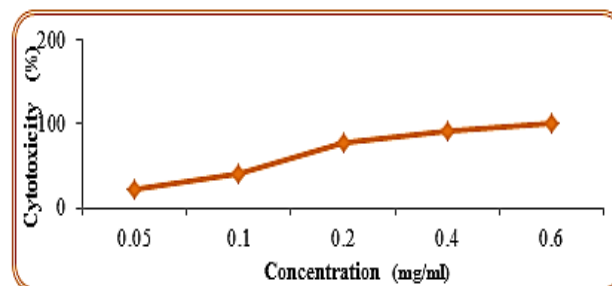


Figure - 1. Cytotoxicity of ethanolic extract of Phallusia nigra to MDA-MB- 231

Effect on Immune Function

The quantitative hemolysis (HC₅₀) of sheep red blood cells (68.15±8.14; 118.65±10.54 and 141.16±8.54) and lymphocytes (3420±1340; 4740±1120 and 4850±960) increased significantly in all the extract treated in a dose dependent manner when compared to control (Table-1). NK cytotoxic activity and the percentage of phagocytosis increased significantly in the experimental groups with the increase in concentration of the extract (Figure - 2). Studies on humoral, cellular and nonspecific immune function by quantitative hemolysis of SRBC, lymphocyte proliferation, NK cytotoxic activity and phagocytosis rate showed a significant increase in the extract administered groups.

Table-1: Effect on Immune function

Group & Dose (mg/kg bw)	Quantitative hemolysis of sheep red blood cells (HC ₅₀)	Lymphocyte proliferation (cpm)
I- T. Control	43.13±5.91	3160±980
II- 50	68.15±8.14*	3420±1340*
III- 100	118.65±10.54**	4740±1120**
IV- 150	141.16±8.54**	4850±960**
V- Vincristin (80)	194.55±7.94***	5660±1015***

Data represented as mean ± SEM, (N=6). Significance between MDA-MB-231 control and extract treated groups. *p < 0.05; **p < 0.01; ***p < 0.001.

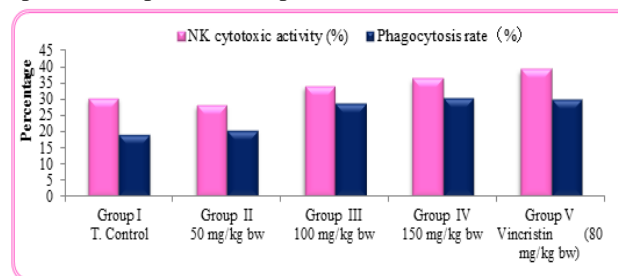


Figure - 2. Effect on NK Cytotoxicity and Phagocytosis

Flavonoids produce several biological effects and have been shown to have antitumor actions (Shen et al., 2003 and Ren et al., 2003). Presence of flavonoids has been reported in Phallusia nigra and this might be a reason for cytotoxicity (Gopalakrishnan et al., 2013). Administration of the extract of Phallusia nigra also showed an increase in lymphocyte proliferation which can be considered as an indication of

the activation of cellular and humoral immunity. It is suggested that the polysaccharides present in the test of sedentary *Phallusia nigra* also might play a similar role in stimulating immune function. NK cells are a type of lymphocytes that form part of the first line of innate defense against cancer cells and virus infected cells (Moretta et al., 2001). In the tumor control a reduction in the percentage of phagocytosis rate was noted whereas on treatment with the extract, there was a significant increase. This observation is supported by the increased production of lymphocytes and NK cytotoxic activity which is an indication of the stimulation of cell mediated immunity. A preliminary GC-MS study of the ethanolic extract has shown the presence of anticancer, cancer preventive and antioxidant compounds (Meenakshi, 2012c). Further detailed work on isolation, purification and structure determination using spectroscopic methods is suggested to conclude the compounds responsible and mechanism of action.

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