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EVALUATION OF ANTI INFLAMMATORY AND ANTI PROLIFERATIVE PROPERTIES OF HAEMOLYMPH OF *SCYLLA SERRATA* (FORSSKAL, 1775)

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ABSTRACT

Marine organisms comprise approximately half of the total biodiversity on the earth and the marine ecosystem is the greatest source to discover useful therapeutics. The potential of marine crabs as source of biologically active products is largely unexplored. The present study was aimed to explore the in vitro ant proliferative and anti-inflammatory activities of haemolymph of *Scylla serrata*.(Forsskal,1775). Preliminary zoo chemical screening of the haemolymph was performed for the identification of constituents and the extract revealed the presence of phenolic compounds, flavanoids, steroids, proreins and amino acids. *In vitro* anti-inflammatory activity was evaluated using HRBC membrane stabilisation method and protein denaturation method and confirmed that haemolymph extract showed anti-inflammatory activity. *In vitro* antiproliferative study of the haemolymph of *Scylla serrata* was evaluated by brine shrimp lethality assay, MTT assay and alamar blue assay in MCF-7 cells. The preliminary screening for anti mitotic activity of the extract was done in Allium *cepa* roots. Results indicated that the haemolymph of *Scylla serrata* possessed good antiproliferative property when compared to the standard drug doxorubicin.

Key words: *Scylla serrata*, *Allium cepa* roots, Anti- inflammatory, Ant proliferative.

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INTRODUCTION

Marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural or chemical features not found in terrestrial natural products. The development of molecular biology field and recent progress of new technologies such as genetic engineering and bioactivity screening offer a unique opportunity to establish marine natural products as drug leads. Whenever a cell's internal environment is perturbed by infections, disease, toxins or nutritional and increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins and radiation. (Siju EN *etal*., 2017). Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. The therapeutic use of plants certainly goes back to the Sumerian and the Akkadian civilizations in about the third millenium BC. Hippocrates (ca. 460–377 BC), one of the ancient authors who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes(Siju EN *etal*., 2014).

Inflammation is protective response involving host cells, blood vessels and proteins and other mediators

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that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues resulting from the original insult, and to initiate the process of repair. Inflammation is a dynamic process which includes the proinflammatory cytokines like tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and vascular endothelial growth factor (VEGF). Prostaglandins play a key role in the generation of the inflammatory response. Inflammation is the tissue reaction to infection, irritation or foreign substance. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The development of non-steroids in overcoming human sufferings such as Rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property(Siju EN *etal*., 2012).. Inflammation is the protective mechanism of the local microcirculation to tissue injury which caused by physical trauma, noxious stimuli, by heat, chemical agent, microbial effect, and antigen –antibody reaction (Siju EN *etal*., 2014).. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury (Siju EN *etal*., 2014).. Because inflammation involves many inflammatory mediators and pathways that lead to a wide range of changes in pathology, it is difficult to target the desired area when treating inflammation (Siju EN *etal*., 2014).

Cancer has been one of the major causes of death in the world, particularly developing countries for the past few decades. It is reported that nine million deaths result from cancer each year, which may rise to twenty million by 2020 as anticipated by WHO. Loss of control of the cell cycle is one of the critical steps in the development of cancer. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled. These altered cells divide and grow in the presence of signals that normally inhibit cell growth; therefore, they no longer require special signals to induce cell growth and division. As these cells grow they develop new characteristics, including changes in cell structure, decreased cell adhesion, and production of new enzymes. These heritable changes allow the cell and its progeny to divide and grow, even in the presence of normal cells that typically inhibit the growth of nearby cells. Such changes allow the cancer cells to spread and invade other tissues. The abnormalities in cancer cells usually result from mutations in protein- encoding genes that regulate cell division. Cancer is a growing public problem which remains the second major cause of death after cardiovascular diseases. Cancers begin as the result of an abnormality in the genes of one or more cells in the body and abnormality may either be inherited; the faulty gene being passed from one generation to the next, or acquired; a normal gene being damaged or mutating for some reason. (EN Siju *etal*., 2017). Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. Cancer treatment depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and additional personal characteristics. Medicinal plants have long played vital roles in the treatment of diseases all over the world (Siju EN *etal*., 2019). Cancer is a cluster of diseases that are produced due to loss of cell cycle control or abnormal/uncontrolled cell growth. It may be caused due to several changes in the expression of genes which results in dysregulation of balance between cell proliferation and cell death (Siju EN *etal*.,2020). Oxidative stress then targets the lipids, proteins, DNA/RNA ultimately causing changes in these molecules and may induce somatic mutation and neoplastic transformation (Siju EN *etal*., 2020). Over the past decade, several new experimental anti cancer agents derived from marine sources have entered preclinical and clinical trials. Present study address anti cancer drug discovery from an evolutionary perspective and present a series of case study that demonstrate that the rate of anti cancer drug discovery can be increased greatly by targeted screening of natural compounds from marine source (Sarfaraj Hussain *etal*.,2012). Some marine crabs have shown pronounced activities and are useful in biomedical research (M.Suja, 2014).

MATERIALS AND METHODS

Collection and authentification

Healthy crabs at two different stages of moulting (pre-moulting stage i.e. hard shell crab and post-moulting stage i.e. soft shell crab) were collected from the Muzhapilangad Landing Centre, Kannur, Kerala and were authenticated. A voucher specimen (No.213/2, APSC) deposited in Dept of Pharmacology, College of Pharmaceutical Sciences, Govt Medical College, Kannur, Kerala.

Preparation of extract

Haemolymph was collected by cutting each walking legs with a fine sterile scissor. The haemolymph was collected by using a 23-guage needle and 1.0 ml syringe contained 300 µl (4°C) pre cooled 10% sodium citrate solution as anticoagulant in glass distilled water. To remove haemocytes from the haemolymph it was centrifuged at 2000 rpm for 15 min at 4°C. The supernatant was collected and used for the following experiments (Zachariassen, K.E, 1999).

Zoo chemical screening

For primary zoochemical screening freshly prepared extract were tested for the presence and absence

of phytoconstituents such as carbohydrates, alkaloids, glycosides, tannins flavonoids, phenolic compounds, steroids by using standard methods (Evan WC., 1998).

***In vitro* determination of anti inflammatory activity**

The human red blood cell (HRBC) membrane stabilization method

As the erythrocyte membrane is analogous to the lysosomal membrane, the method of HRBC membrane stabilisation is used to study the *invitro* anti-inflammatory action(Sangita C *etal*., 2012) (Vijayakumar *etal*., 2013).

Methodology

Blood was collected from healthy volunteers who were not taking NSAID'S for the past two week. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, .8%sodium citrate, .05% citric acid and .42% sodium chloride in water) and centrifuged at 3000 rpm.The packed cells were washed three times with isosaline (.85%, p^H 7.4) and a 10%v/v suspension was made with isosaline. The assay mixture contains .5 ml of various concentration of the extract (62.5, 125,250,500,1000µg/ml),or standard drug diclofenac sodium(50 and 100µg/ml), 1ml phosphate buffer (.15M,p^H 7.4), 2ml of hypo saline (.36%) and 5ml of HRBC suspension. Instead of hypo saline, 2ml of dist. Water was used in control. All the assay mixtures were incubated at 37^o c for 30 minute and centrifuged. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage of hemolysis was calculated by assuming the hemolysis produced in the presence of dist water as100%. The percentage of HRBC membrane stabilization or protection was calculated by using the formula:

Percentage protection = 1- (optical density of test /optical density of control) x 100

Protein denaturation method

Denaturation of tissue protein is one of the well documented cause of inflammatory and arthritic diseases. Therefore method of protein denaturation may be used for evaluating the efficacy of anti- inflammatory agents. (Sangita C *etal*., 2012).

Methodology

The reaction mixture (5ml) consisted of .2ml of egg albumin(from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, p^H 6.4) and 2ml of varying concentration of extract so that the final conc. Became31.25, 62.5, 125, 250, 500,1000µg/ml. Similar volume of double- distilled water served as control. Incubate the mixtures at 37+/-2^{o}c in a BOD incubator for 15 min. Heated at 70^o c for 5 min. After cooling, their absorbance was measured at 660nm by using vehicle as blank. Diclofenac sodium (31.25, 62.5, 125, 250, 500, 1000µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the formula:

$$\% \text{ Inhibition} = \left\{ \frac{\text{Absorbance of test}}{\text{Absorbance of contr}} - 1 \right\} \times 100$$

Determination of lethal dose (LD_{50})

Brine shrimp lethality

Methodology (Imran Khan *etal*., 2012).

Preparation of sea water

38gm of sodium chloride was weighed and dissolved in 1000ml of dist water and filtered to obtain clear solution.

Hatching of brine shrimp

Sea water and shrimp eggs were placed in a tank and hatched for 1 day matured as nauplii. The hatched shrimps were attracted to the lamp through the perforation in the tank. These nauplii were taken into the bioassay.

Preparation of sample and standard solution

100mg of the test sample were dissolved in 10ml DMSO to prepare a stock solution of 10mg/ml from this different concentrations of the extract (10µg/ml-600µg/ml) were prepared. From this stock solution 1mg/ml of the doxorubicin different concentration (10µg/ml-600µg/ml) were prepared.

Application of test solution and nauplii to the test tube

5ml of sea water were added to each test tube containing 10 brine shrimp larve. Different conc of the test sample were applied to the test tubes containing nauplii.

Preparation of control

Control tubes were prepared without the addition of extract.

Counting of nauplii

After 24 hrs the test tubes were observed and the number of surviving nauplii in each test tubes were counted using magnifying glass and recorded. From the record % of lethality of brine shrimp were calculated for each concentration of sample.

Evaluation in mcf-7 cells by MTT assay

Cell line

The human breast cancer cell lines (MCF7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The

cells were maintained at 37°C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passages weekly, and the culture medium was changed twice a week (S Lorenzon, 2005).

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) to make single cell suspensions. Viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x105 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity.The medium containing without samples were served as control and triplicate was maintained for all concentrations (Rhama S *etal*.,2012).

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate- dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purpleformazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h.The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Measured the absorbance at 570 nm using micro plate reader. The % cell viability was determined using the following formula. Farzaneh Fesahat *etal*.,2014

$$\text{Cell viability} = \frac{\text{Absorbance of treated cell}}{\text{Absorbance of control}} \times 100$$

The linear regression graphis plotted between % viability and concentration. IC_{50} was determined using Graph Pad Prism software.

Evaluation by alamar blue assay

Alamar blue is a non-fluorescent dye that is reduced to pink coloured, highly fluorescent resorufin by mitochondrial reductases. Resazurin solution is highly dichromatic and act as an intermediate electron acceptor in the electron transport chain without interference of the normal transfer of electrons. The increase in dead cells reduces the ability of cells to convert resazurin to resorufin which is corelated from decrease in fluorescent intensity. Fluorescence is measured at wavelength at 530-560nm and emission wavelength at 590 nm and correlated with untreated control cells. (Saudat Adamson Fadeyi *etal*.,2013)

Preparation of Cells for Testing

Harvest MCF-7 cell line by trypsinization and subsequent trypsin inhibitor treatment.Centrifuge cells, re-suspend in growth medium and count. Calculate the total cell number and adjust to 1 x 10^4 cell/ml. This is a suggested cell density with cancer cell line. Add 250µl of cell suspension to each well. Incubate at 37°C in 5% CO_2 atmosphere for the number of days required for the particular cell line to be in log phase (usually 3 days).

Exposing Cells to Test Agents

Prepare appropriate dilutions of test agent in growth media. Aspirate spent growth medium from the wells and add 250µl of each dilution of test agent to the wells. Cover, then return to the incubator for 2 days. After incubation, add 25µl of the indicator to each well. Incubate panels for an additional 3 hours. Panels may then be read spectrophotometrically (absorbance at 570nm and 600nm) or spectrofluorometrically (excitation, 530-560nm; emission, 590nm).

Anti mitotic activity

***Allium cepa* root tip meristem model**

Locally available onion bulbs (*Allium cepa* 50 ± 10 g) were grown in the dark over 100 ml tap water at ambient temperature until the roots have grown to approximately 2-3 cm length.The base of each of the bulbs were suspended on the extract inside 100 ml beakers, root length (newly appearing roots not included). Root number at 0, 48, 96 hrs for each concentration of extract and control was measured. The percentage root growth inhibition after treating with crab haemolymph at 48 and 96 hrs was determined. Doxorubicin (standard) as well as haemolymph was used at 10 mg/ml concentration (Renu Sankar *etal*.,2013)

RESULTS

Preliminary zoo chemical screening

ANTI-INFLAMMATORY ACTIVITY

The human red blood cell (HRBC) membrane stabilization method

The haemolymph showed dose dependent anti-inflammatory activity. The results are tabulated in table 2 and depicted in fig. 1.

Protein denaturation method

Concentration dependent inhibition of protein (albumin) denaturation was observed in haemolymph which is tabulated in table 3 and depicted in fig. 2. At 1000µg/ml haemolymph showed 168% inhibition whereas diclofenac showed an inhibition of 213.49% at 1000µg/ml.

Evaluation of cytotoxicity

Preliminary screening by brine shrimp lethality assay

The haemolymph showed concentration dependent mortality with an LC50 value 197.89µg/ml and of doxorubicin was found to have an LC_{50} value of 59.92 µg/ml.

Cytotoxicity evaluation in MCF -7 Human breast cancer cell lines by Alamar blue

The haemolymph showed concentration dependent cytotoxicity on MCF-7 cells with an IC50 value 73.97µg/ml. Doxorubicin was found to have an IC50 value of 20µg/ml. The findings are tabulated in the table 5 and depicted in fig.4.

Cytotoxicity evaluation of MCF-7 cell lines by MTT assay

The haemolymph showed concentration dependent cytotoxicity on MCF-7 cells with an IC50 value of 64.04µg/ml. Doxorubicin was found to have an IC50 value 19.7µg/ml. The findings are tabulated in table 6 and depicted in fig.5.

Table 1: Zoo chemical constituents

Si. No	Constituents	Haemolymph of *Scylla serrata*
1.	Alkaloids	-
2.	Carbohydrate	+
3.	Phenolic compound	+
4.	Proteins and amino acids	+
5.	Flavanoids	+
6.	Steroids	+
7.	Glycosides	-

(+):PRESENT (-): ABSENT

Table 2: *In vitro* Anti- Inflammatory Activity of Haemolymph Membrane Stabilisatio Method

Groups	Concentration (µg/ml)	Absorbance (560nm)	% Protection
Control	-	0.516±0.003	0
Haemolymph	25	0.4293±0.003***	16.80
	50	0.4117±0.001***	20.21
	100	0.2461±0.002***	52.37
	200	0.1816±0.002***	64.80
Diclofenac sodium	50	0.162±0.002***	68.7
	100	0.140±0.003***	72.7

Values are in mean±SEM, n=3 ,*** significant at $p<0.0001$

Table 3: *In vitro* anti- inflammatory activity of haemolymph by protein denaturation method

Groups	Concentration (µg/ml)	Absorbance (660 nm)	% inhibition
control	-	0.126±0.001	0
Haemlymph	31.25	0.1436±0.001***	14.20
	62.5	0.1612±0.001***	28.10
	125	0.1978±0.003***	56.90
	250	0.2305±0.001***	83.00
	500	0.2772±0.002***	120.00
	1000	1.386±0.001***	168.00
Diclofenac sodium	31.25	0.174±0.001***	38.09
	62.5	0.212±0.001***	68.25
	125	0.245±0.002***	94.40
	250	0.312±0.002***	147.60
	500	0.342±0.002***	171.42
	1000	0.365±0.001***	213.49

Values are in mean±SEM, n=3 ,*** significant at p<0.0001

Table 4: Evaluation of Cytotoxicity by Brine Shrimp Lethality Assay

Groups	Concentration (µg/ml)	No. of death	%mortality	LC_{50} (µg/ml)
Control	-	0		
Haemolymph Extract	20	0	0	197.89
	50	2.333±0.333***	20	
	100	3.667±0.333***	40	
	200	7.000±0.000***	70	
	500	7.667±0.333***	80	
Doxorubicin	20	6.33±0.333***	60	59.92
	50	7.333±0.333***	70	
	100	8±0.000***	80	
	200	9.667±0.333***	100	
	500	10±0.000***	100	

Values are in mean±SEM, n=3, *** significant at p<0.0001

Table 5: Evaluation of cytotoxicity by alamar blue assay

Groups	Concentration (µg/ml)	% viability	IC_{50} (µg/ml)
Control	-		
Haemolymph	6.25	70.4±0.002***	73.2
	12.5	61.7±0.001***	
	25	55.4±0.004***	
	50	50.0±0.002***	
	100	38.3±0.001***	
Doxorubicin	6.25	61.0±0.003***	20.0
	12.5	56.0±0.004***	
	25	46.5±0.002***	
	50	24.0±0.003***	
	100	20.0±0.001***	

Values are in mean ±SEM, n=3, * significant at p<0.0001**

Table 6: Evaluation of cytotoxicity by MTT assay

Groups	Concentration (µg/ml)	% viability	IC_{50} (µg/ml)
Control	-		
Haemolymph	6.25 12.5 25 50 100	80.13±0.001*** 70.45±0.003*** 61.18±0.001*** 47.63±0.002*** 40.35±0.004***	64.0
Doxorubicin	6.25 12.5 25 50 100	48.7±0.003*** 31.51±0.001*** 27.57±0.004*** 22.6±0.002*** 16.92±0.002***	20.0

Values are in mean ±SEM, n=3,***significant at p<0.0001

Table 7: *Allium cepa* bulbs showing the effect of different extracts on root length

Groups	Concentration mg/ml	Roots length in cm		
		0hr	48hr	96hr
Control	-	0.33 (n=16)	4.12 (n=22)	4.63 (n=28)
Haemlymph	10	2.56 (n =18)	2.33 (n =14)	2.23 (n=11)
Doxorubicin	10	2.32 (n=13)	2.29 (n=17)	2.15 (n=21)

Fig.1, *In vitro* anti- inflammatory activity by HRBC method

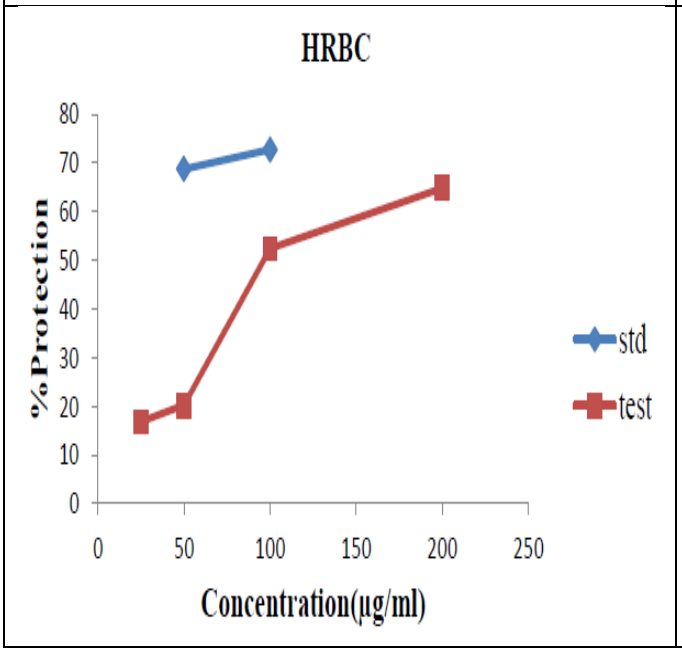

Fig. 2. *In vitro* anti-inflammatory activity of haemolymph by protein denaturation method

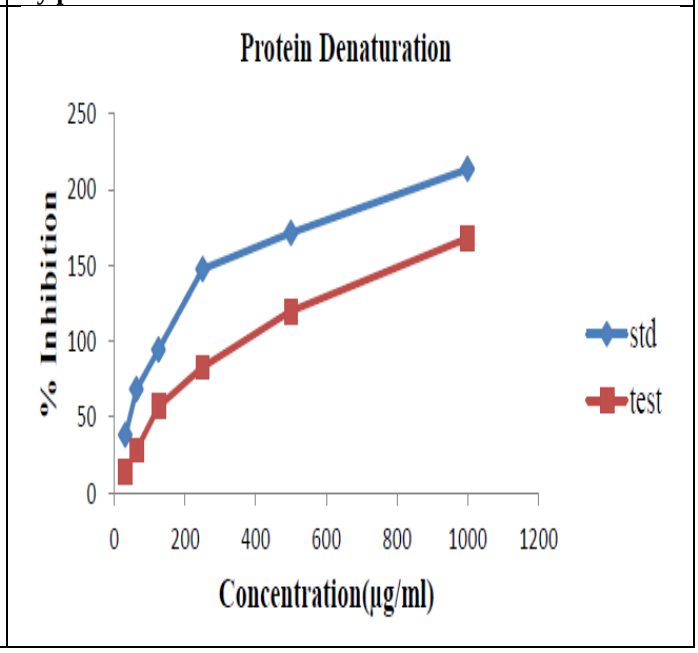

Fig. 3, Evaluation of Cytotoxicity using Brine Shrimp Lethality Assay

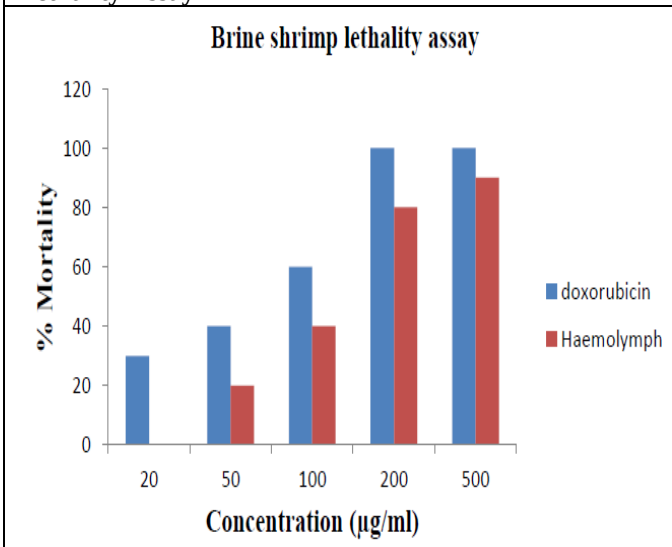

Fig. 4, Evaluation of cytotoxicity by alamar blue assay

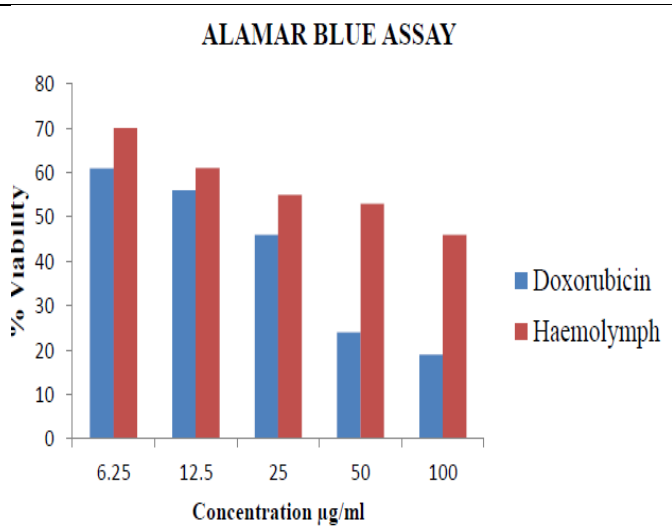

Fig.5, *In vitro* cytotoxicity activity of haemolymph by MTT assay

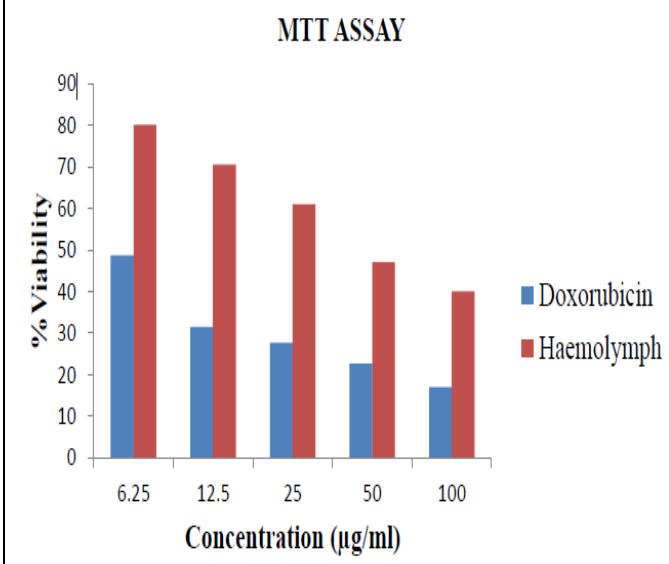

Fig. 6, Cell death in haemolymph by MTT assay at various concentrations

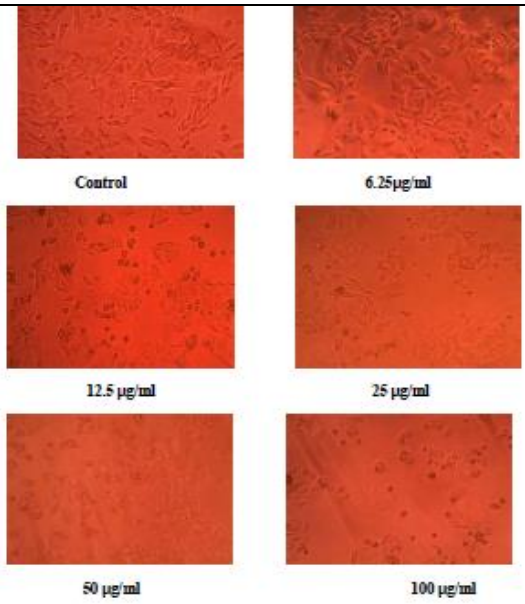

Fig. 9, Cell death in doxorubicin by MTT assay at various concentrations

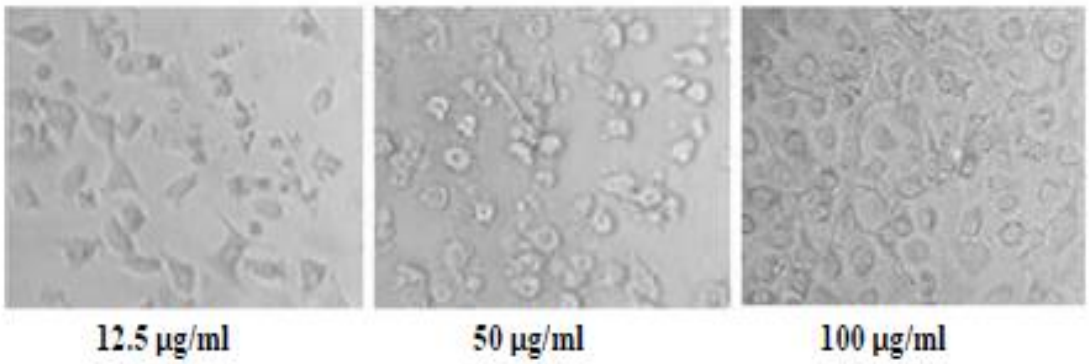

Fig.07 Picture shows anti mitotic activity on A*llium cepa* roots

A. CONTROL(water)

B. TEST (Haemolymph)

C.STANDARD (Doxorubicin)

ANTI MITOTIC ACTIVITY

The extract produced dose and time dependent growth inhibition. Incubation of bulbs in different concentrations of extract and standard produced a growth retarding effect that was associated with a decrease in the root number.

DISCUSSION

Natural products are extremely an important source of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development (Jagetia C *etal* .,2006). A number of researchers have focused on identifying novel marine natural product as anticancer drugs.

Anticancer compounds have characteristics of multi-function, high sensitivity, stability and so on. Nowadays, usage of natural products as medicine for curing human diseases has received much attention because of its potential biological activity with few side effects compared to synthetic molecules. The present study was undertaken to investigate the anticancer mechanisms of the marine crab *Scylla serrata* against human cancer cell lines. In the present study the hemolymph of the crabs were tested to evaluate their anti proliferative and antioxidant potential.

The haemolymph of *Scylla serrata* was collected and stored at 4^{o}C. The haemolymph showed positive for phenolic compound, amino acids, flavonoids, carbohydrate and steroids.

The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG). Non-steroidal anti-inflammatory drugs (NSAIDs) act either by inhibiting these lysosomal enzymes (cyclooxygenase) or by stabilizing the lysosomal membrane (Chippada SC., 2006). Stabilization of the HRBC membrane by hypo tonicity induced membrane lysis and inhibition of protein denaturation were used to establish the anti-inflammatory action of haemolymph extract of *Scylla serrata*.

The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which further cause tissue inflammation and damage extra cellular release. The extract showed maximum inhibition of 64.8% at concentration of 200 µg/ml .Diclofenac sodium (standard) showed an inhibition of 72.7% at 100 µg/ml. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. Inhibition of protein (albumin) denaturation by haemolymph extract of *Scylla serrata* gave a maximum value of 168.06% at a concentration of 1000 µg/ml and for diclofenac sodium it was found to be 213.49% at a concentration of 1000 µg/ml.

The present study clearly indicated that haemolymph extract of crab have appreciable in vitro

cytotoxic potential against MCF-7 breast cancer cells in MTT assay and Alamar blue assay. The presence of flavanoids and polyphenols in the extract might be responsible for exhibiting anticancer effect. There are reports indicating biological interactions of flavanoids, polyphenols, or phenolic compounds with proteins, enzymes, and other biological processes in the cells that make them toxic to the cell or serve as growth inhibitors (Murray B.*etal*,2003). Flavanoids have been extensively studied because of their numerous biological activities and have shown to have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis.

The measure of potency of an extract using the brine shrimp (*Artemia salina*) has become increasingly common since the early 1980's, when the assay was first proposed by a group of chemists in the United States as a useful way to flag a plants effects. The ease of using brine shrimp is that it is easy to handle, economic and easy to dispose of unlike in experimants involving rodents where their handling, withdrawal of body fluids are needed for estimating different parameters. Brine shrimp is much closer to humans than bacteria, in evolutionary and structural terms (Hayden C *etal*.,2003). The haemolymph at concentration ranging from 20µg/ml to 500µg/ml was compared with standard cytotoxic drug doxorubicin. The LC_{50} of haemolymph of *Scylla serrata* by brine shrimp lethality assay was found to be 197.89 µg/ml against the LC_{50} value of 59.92 µg/ml by doxorubicin, the percentage mortality was found to be directly proportional to the concentration of extract. Maximum mortality was found to be at 500µg/ml. The lethal concentration is within the range, so the extract could be screened for cytotoxic activity.

The colorimetric assay of MTT measures the reduction of 3-[4,5- dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT that enters the cells and passes into mitochondria, gets reduced to an insoluble, coloured (purple) formazan product. Since redcution of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Monks A *etal*.,1999). The MTT assay showed a concentration dependent increase in the growth inhibition of MCF-7cells by the haemolymph of crab *Scylla serrata* which is evident in the photographs provided. The viability of cells decreased with increasing concentration. Doxorubicin had an IC_{50} value of 19.75µg/ml, whereas haemolymph extract possessed an IC_{50} value of 64.04µg/ml.

Alamar blue monitors the reducing environment of the living cell. It is a blue non-fluorescent dye that is reduced to pink- coloured, highly fluorescent resorufin by mitochondrial reductases. Increase in dead cells reduces the ability of cells to convert resazurin to resorufin which is correlated from decrease in fluorescent intensity. Fluorescent signals are measured at an excitation wavelength at 530-560nm and an emission wavelength at 590nm and correlated with untreated control. The haemolymph extract showed concentration dependent cytotoxicity on MCF-7 cells with an IC_{50} value 73.97µg/ml. Doxorubicin was found to have an IC_{50} value of 20µg/ml.

Preliminary anti mitotic screening was done in *Allium cepa* roots .The extract produced dose and time dependent growth inhibition. Incubation of bulbs in different concentrations of extract and standard produced a growth retarding effect that was associated with a decrease in the root number.

CONCLUSION

The present investigation was mainly centered to evaluate the in vitro cytotoxic activity of haemolymph of Scylla *serrata* using cell proliferation assay methods on cancer cell lines. *In vitro* studies were also conducted to purport the anti- inflammatory activity. The preliminary screening for anti mitotic activity of the extract was done in *Allium cepa* roots. The observation from the assay demonstrated dose dependent increase in anti proliferative activity. The exhibited cytotoxic effect of the extract in various models may be attributed to the presence of poly phenols and phenolic compound. Since the study revealed the significant anti proliferative property further investigation is required to isolate the active constituent responsible for the activity and to find the out the molecular mechanism.

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