ASSESSMENT OF IN VITRO ANTICANCER ACTIVITY OF THE MARINE GASTROPOD CYPRAEA ARABICA (L.1758)

P.Subavathy, A.Uma Maheswari, R.D.Thilaga, Jemma Hermelin Jesy Diaz

PG and Research Department of Zoology, St.Mary’s College (Autonomous), Thoothukudi, Tamil Nadu, India

Corresponding author mail ID: Subavathy.P89@gmail.com

ABSTRACT

Nature is an attractive source of new therapeutic candidate compounds as a tremendous chemical diversity is found in millions of plants and animals species. Cancer is one of the most lethal diseases in human beings. After taking into consideration the immense side effects of synthetic anticancer drugs, many researchers are making concerted efforts to find novel natural anticancer compounds. Marine molluscs are very promising source for wide range of bioactive compounds. The present study aims to evaluate the antitumor activity of the gastropod extract Cypraea arabica. The cell viability was measured using MTT assay on HepG2 cancer cell line. The percentage of cell viability was decreased with increasing concentration i.e 74.14% at 250 μg/ml, 56.93% at 500 μg/ml and 45.34% at 750 μg/ml concentrations of the substance and the percentage of toxicity was observed as 25.86% of inhibition at 250 μg/ml, 43.06% at 500 μg/ml and 54.65% at 750 μg/ml concentrations of F3 fraction of Cypraea arabica respectively.

KEYWORDS: Gastropod, Cypraea arabica, anticancer activity, MTT assay, HepG2 cell line
INTRODUCTION

Marine ecosystem covers more than 70% of the earth’s surface but represents 95% of the biosphere (Ellis, 2001). Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species and more than double that of the land (Anand et al., 1997). Mollusca is one of the largest phyla of invertebrate animals with around 1,60,000 species recorded all over the world. The research on marine natural products in the last three decades has also brought to the discoveries of many chemically and biologically interesting molecules, that have become indispensable tools in biochemical research and played significant roles in the recent advancement of life sciences (Umaya Parvathi et al., 2012). In recent years, more and more researchers have come to the realization that marine organisms hold immense potential as a source of novel molecules and new anticancer agents. Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million case per year (Sarfaraj Hussain et al., 2012). In cancer, cells divide and grow uncontrollably, forming malignant tumours, which may invade nearby body parts. The causes of cancer are diverse, complex and only partially understood. Many things are known to increase the risk of cancer, including tobacco use, dietary factors, certain infections, exposure to radiation, lack of physical activity, obesity and environmental pollutants (Anand et al., 2008). Molluscs represent good candidates for anti-cancer natural products research considering their evolutionary and ecological significance. Currently, natural products isolated from molluscs and their structural analogues are particularly well represented in the anticancer compounds in clinical trials (Simmons et al., 2005). Visualizing gastropods as the treasure house of bioactive substances, presently an attempt has been made to screen the gastropod Cypraea arabica for antitumor property.

MATERIAL AND METHODS

Collection of specimen

Specimens of Cypraea arabica used in the present study were collected from Gulf of Mannar coastal region which is situated on the South-east coast of India. This coast contains a rich biological diversity of flora and fauna largely due to diversified microhabitats such as mangroves, corals, seaweed beds, sea grasses, sandy, rocky and muddy shore etc. The faunal diversity is also well pronounced with reference to different molluscan groups. In the present
study whole body tissue extract of *Cypraea arabica* was used for the anti-cancer assay. The freshly collected samples were cleaned and washed with fresh sea water to remove all impurities. The shells were broken, tissues were removed and then dried in hot air oven at 56°C for 48 hours and used for further studies.

**Anticancer activity of *Cypraea arabica***

The anticancer activity of methanolic extract of *Cypraea arabica* was performed on HepG2 cancer cell lines obtained from National Centre for Cell Science, Pune, India. The cell viability was measured using MTT assay (Kang et al., 2004). MTT stands for (3[4, 5-dimethylthiazol – 2-y] – 2, 5 – diphenyltetrazolium bromide) by mitochondrial succinate dehydrogenase the MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (Dark purple) formazan product. The cells were then solubilized with an organic solvent (Isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. The cells were grown in a 96 well plate in DMEM medium (Delbucco’s Minimum Essential Medium) (Hi media) supplemented with 10% foetal bovine serum (Gibco Laboratories) and antibiotics (Streptomycin, Penicillin G, Canamycin, Amphoteracin B). About 1ml cell suspensions (1.2 × 10⁴ cells/ ml) was seeded in each well and incubated at 37°C for 48hrs in 5% CO₂ for the formation of confluent monolayer. The monolayer of the cells in the plate was exposed to various dilutions (100 µg/ml, 250 µg/ml, 500µg/ml). The cell viability was measured using MTT assay after 4 hrs the medium was discarded and 100 µl of DMSO was added to dissolve the formazan crystals. The tetrazoliun salt is metabolically reduced by viable cells to yield a blue insoluble formazan products and was measured at 570nm spectrophotometrically. Controls were maintained throughout the experiment (untreated well as cell control) the assay was performed in triplicate of the methanolic extract. The mean cell viability values was compared to the control to determine the effect of extract on cells and the percentage of cell viability was plotted against concentration of extract. Percentage of viability was calculated by the following formula

\[
\% \ of \ viability = \frac{\text{Test OD}}{\text{Control OD}} \times 100
\]

\[
\% \ of \ toxicity = 100 - \% \ of \ viability
\]
Morphological studies using a normal inverted microscope were carried out to observe the cell death in the drug (methanolic extract of *Cypraea arabica*) treated Hep G2 liver cancer cells. Concentrations of 250 µg/ml, 500 µg/ml and 750 µg/ml of *Cypraea arabica* extract were used for morphological cells. Untreated cells were used as control.

**RESULT**

In recent years, great attention has been paid to study the bioactivity of natural products, because of their potential pharmacological utilization. In the present study, 3 concentrations of methanolic extract of *Cypraea arabica* were tested for anticancer activity on HepG2 cell line. The percentage of cell viability was decreased with increasing concentration (i.e 74.14% at 250µg/ml, 56.93% at 500µg/ml and 45.34% at 750µg/ml concentrations) of the substance (Table.1) and the percentage of toxicity was observed as 25.86% of inhibition at 250µg/ml, 43.06% at 500µg/ml and 54.65% at 750µg/ml concentrations of methanolic extract of *Cypraea arabica* (Table.2). The MTT assay of methanol extract of *Cypraea arabica* revealed a dose dependent cell toxicity in HepG2 cell line (Fig.1).

**Table.1** Anti-cancer activity of methanolic extract of *Cypraea arabica* on HepG2 cell line (Absorbance at 570nm)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>sample</th>
<th>Average</th>
<th>% of viability</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>250µg</td>
<td>1.752</td>
<td>1.297</td>
<td>1.301</td>
<td>1.299</td>
<td>74.02968</td>
</tr>
<tr>
<td>500µg</td>
<td>1.752</td>
<td>0.975</td>
<td>1.02</td>
<td>0.9975</td>
<td>55.65068</td>
</tr>
<tr>
<td>750µg</td>
<td>1.752</td>
<td>0.791</td>
<td>0.798</td>
<td>0.7945</td>
<td>45.1484</td>
</tr>
</tbody>
</table>

**Table.2** Toxicity analysis of methanolic extract of *Cypraea arabica* on HepG2 cell line

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract</th>
<th>Concentration</th>
<th>% of inhibition</th>
<th>Average</th>
<th>S.D</th>
<th>IC50 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>250µg</td>
<td>25.97032</td>
<td>25.74201</td>
<td>25.85616</td>
<td>0.16144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500µg</td>
<td>44.34932</td>
<td>41.78082</td>
<td>43.06507</td>
<td>1.816199</td>
</tr>
<tr>
<td></td>
<td></td>
<td>750µg</td>
<td>54.8516</td>
<td>54.45205</td>
<td>54.65183</td>
<td>0.28252</td>
</tr>
</tbody>
</table>
DISCUSSION

Marine organisms are taxonomically diverse, each and every thousand new compounds are derived from marine natural source and they enter into clinical trials for human welfare. Marine invertebrates, which develop in a different environment from terrestrial animals, are the source of a broad range of pharmacological substances. They either express constitutively or the expression is induced by exposure to pathogens (Sri kumaran et al., 2011). By the virtue of such excellent property marine organisms are of interest in terms of pharmaceutical potentials particularly invertebrates (Keivan et al., 2007). Especially in a marine environment, the phylum mollusca produce a large number of therapeutic drugs including antibiotic, antiviral, antiphrastic, analgesic and anticancer activities (Sugesh et al., 2014).

A lot of structurally and pharmacologically important substances have been isolated from marine gastropods with novel antimicrobial, antitumour and anti-inflammatory properties (Bhadury and Wright, 2004). Chemotherapy is currently the primary treatment modality in many tumors. However, the development of multidrug resistance (MDR) to chemotherapeutic drugs is a main obstacle for the successful treatment of malignant tumors (Tao et al., 2010). Therefore the development of novel chemotherapeutic agents would play a key role in the treatment of refractory or relapsing cancer patients. Marine organisms are rich source for natural products. Many compounds that are derived from these organisms have generated interest both as
challenging problems for structure elucidation and synthesis as well as for their cytotoxicity (Schwartsmann, 2000; Schwartsmann et al., 2001). It is believed that, a rich source of anticancer drug candidates could be obtained from marine organisms or their metabolites.

In the present study, the gastropod *Cypraea arabica* has been screened for cytotoxic property on HepG2 cell line MTT assay. The methanolic extract of *Cypraea arabica* showed 54.65% cytotoxic effect in hepatocarcinoma cell lines at 750μl/ml concentration. Eventhough such effect is about 54.65% (at 750μl/ml) it is presumed that the activity would be increased if the purified fraction of the extract is tested. Therefore it could be concluded that body tissue of *Cypraea arabica* is having substance that could be capable of inhibiting the proliferation of cancer cells by cellular lysis or at molecular level in Hepatocarcinoma G-2 cell line. There are several reports on the possession of anticancer properties of molluscs in general and gastropods in particular. To cite few of those; cytotoxic glycoprotein (antitumour) aplysianin E, isolated from *Aplysia kurodai* (Kisugi et al., 1989b and Yamazaki et al., 1990), Julianins (Kamiya et al., 1989) from *A. julianin* and *A. dolabellanin* from *Dolabella auricularia* (Kisugi et al., 1992 b; Takamatsu et al., 1995; Turner et al., 1998 and Wessels, 2000) have also observed similar type of anti-proliferative property in *A.dactylomela* and *D.auricularia*. Trindoleninone 6 – bromoisatin with anticancer properties have been isolated from gastropod *Dicathais orbita* (Westley et al., 2010 and Benkendorff et al., 2011). Gastropods with antitumour property have been reported by Shanthi et al., (2011), Jemma Hermalin Jesy Diaz (2012), George et al., (2012) and Krishnakumar and Yogamoorthi (2013). Sugesh et al., 2014 analysed the cytotoxic effects of two edible bivalves *Meretrix meretrix* and *Meretrix casta*. Both molluscan extracts treated HepG2 cells showed significant inhibition of cell viability at (IC_{50}) 50μg/ml concentration in the trypan blue exclusion assay. On observation, it can be concluded that *Meretrix meretrix* extract has been a highly selective and effective anticancer drug for human welfare. In view of the previous reports, it is felt that the gastropod *Cypraea arabica* might possess such pharmaceutically important anticancer principle.

**CONCLUSION**

The marine environment is a huge source for discovering many novel drugs. Apart from the food that is derived from the marine environment, wide varieties of drugs are being isolated and characterized with great promise for the treatment of human diseases. It is also understood
that, the rich diversity of marine biota with its unique physiological adaptations to the harsh marine environment provides a fruitful source for the discovery of life saving drugs. Each year, several new anticancer molecules were either isolated or synthesized, however the majority of these molecules remain in preclinical investigation stages while people are waiting for an alternative treatment option without the disadvantages or severe side effects of chemotherapy.

ACKNOWLEDGEMENT

The authors are grateful to UGC, for providing financial assistance under which the project was undertaken. They also express their sincere thanks to the Principal of St.Mary’s College (Autonomous), Thoothukudi for the facilities provided to pursue the research project.

REFERENCES


