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STUDY TO EXPLORE THE ANTI-TUMOR POTENTIAL OF WHOLE WHITE SHRIMP *LITOPENAEUS VANNAMEI* EXTRACT COLLECTED FROM GWADAR WATER FRONT

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Abstract

Cancer is a collection of illnesses marked by unchecked cell division and proliferation. Genetic, environmental, and lifestyle factors are among the several causes of this condition, which can impact different body areas. Apart from this, Cancer is one of the leading causes of death globally; hence, the discovery of novel therapeutic medications is essential. The treatments which are now being used for cancer, like chemotherapy and radiotherapy, can cause disruption to many physiological processes, due to which the healthy cells and hair are damaged. The fauna of the sea contains low molecular weight peptides, so researchers around the world are working to utilize these natural faunas instead of the chemical pharmaceutical products, which have high side effects. Additionally, bioactive peptides from plants and animals have gained increased interest in recent years. Their modes of action and potential uses in the prevention and treatment of cancer, heart disease, and infectious diseases have drawn the attention of researchers. In this study, the anti-cancer potential of *Litopenaeus vannamei* extracts from Gwadar against human cancer cell lines was investigated. White shrimp extracts' cytotoxic effects on cancer cell lines were evaluated using the MTT assay. Our results demonstrated a dose-dependent increase in apoptosis and a significant reduction in cell viability when white shrimp extracts were administered to cancer cells. Based on the range of IC50 values, the extracts showed potential anti-cancer properties. The results imply that compounds found in white shrimp may have therapeutic uses in the management of cancer. This study contributes to the growing body of research indicating that compounds obtained from marine sources hold a promising source of anticancer medications.

Keywords: Anticancer activity, Bioactive peptides, Cell viability, Cytotoxic effect, Gwadar, *Litopenaeus vannamei*, MTT assays (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

INTRODUCTION

Cancer is becoming increasingly common worldwide, with an estimated 20 million new cases occurring annually. The Global Cancer Observatory (GLOBOCAN) reported that in 2012, there were 14.1 million new cancer cases and approximately 12.2 million cancer-related deaths. In Europe, the most prevalent cancer types include lung, colon, prostate, and breast cancer. Lung cancer is considered the world's leading cause of cancer-related death (1). According to GLOBOCAN statistics for 2022, there were approximately 20 million new cancer cases, including nonmelanoma skin cancers (NMSCs), and 9.7 million cancer-related deaths (2). Furthermore, currently employed cancer treatments such as chemotherapy and radiation therapy can disrupt numerous physiological processes, as they damage healthy cells and hair follicles (3).

Interest in bioactive peptides derived from plants and animals has grown recently. Researchers are particularly interested in their mechanisms of action and potential applications in the treatment and prevention of infectious diseases, cancer, and cardiovascular diseases. In clinical trials, bioactive peptides derived from meat, fish, milk, and plants have demonstrated promising lipid-lowering and antihypertensive effects. While certain bioactive peptides possess immunomodulatory and antibacterial properties, many



others exhibit specific cytotoxic activity against various cancer cell lines in both in vitro and in vivo settings (4). Natural compounds derived from marine organisms exhibit a range of pharmacological effects through their bioactive secondary metabolites. Apoptosis, endoplasmic reticulum (ER) stress, reactive oxygen species (ROS) generation, cell survival, and proliferation are among the key biochemical and cellular processes affected in most malignancies. Consequently, several bioactive compounds from marine species warrant more thorough investigation into their biochemical and anticancer properties (5).

Litopenaeus vannamei is one such species that offers benefits to human physiological health, as both the readily available white shrimp and its derivatives contain valuable components. These components are crucial for the synthesis of nitrogenated compounds, including hormones and neurotransmitters. They contain high-value proteins with functional peptides and essential amino acids (6). Bioactive peptides (BPs) are organic substances formed through covalent interactions between peptides and amino acids. Since the parent protein is typically inactive, most BPs are generated during enzymatic activity. Chemical synthesis represents another method for producing certain BPs (7). The lipid fraction of shrimp muscle, comprising glycolipids, phospholipids, carotenoids, and neutral lipids such as cholesterol, triglycerides, free fatty acids, diglycerides, and monoglycerides, accounts for 1% to 2% of its dry weight. Carotenoids, the primary pigment group found in aquatic organisms, have been investigated for their chemopreventive properties (8). Moreover, recent food science literature has thoroughly investigated high-intensity ultrasound (HIUS) as an extraction method, with a focus on various bioactive compounds, including fatty acids, pectin, carotenoids, polysaccharides, peptides, and polyphenols. These studies cover diverse sources such as fruits, vegetables, and microorganisms (6). Peptide bonds can be broken through processes such as enzymatic hydrolysis, fermentation, or food processing, resulting in the formation of bioactive peptides (9). Owing to the discovery of novel active compounds, aquaculture is becoming an increasingly important source for producing bioactive peptides. White shrimp contributes approximately 80% of global aquaculture production. These compounds play an important biological role in the bloodstream by crossing the epithelial barrier (10).

METHODOLOGY

SAMPLE COLLECTION

A total of five kilograms of white shrimp samples were collected from Gwadar water front. Frozen samples were transported to the Center for Advanced Studies in Vaccinology & Biotechnology for further procedure.

EXTRACTION METHOD

In order to get extract, 500g of white shrimp were minced and then 500 mL of distilled water was added and the material was homogenized for two minutes at a high speed using a homogenizer. The hydrolysis process approach is the one that is used; it started at a pH of about 7.1. Over three hours, progressively the temperature was raised between 40 and 60 °C. Through the use of an overhead stirrer, the autolysis was continuously stirred. For ten minutes, the mixture obtained was then placed in a boiling water bath at 95°C to stop the process. Following cheesecloth filtering, the mixtures were centrifuged at 4000 × g for 20 minutes at 4 °C. The supernatant was collected and the samples were placed in an incubator to evaporate liquid content. The dried material was collected and stored at -20°C for later use.

INOCULATION OF CELLS WITH SHRIMP EXTRACT IN 96 WELL PLATES

In order to inoculate cancerous cells with shrimp extract 75mg of the white shrimp extract was dissolved in 3 milliliters of phosphate buffer saline (PBS) and stirred for five minutes to create the stock solution. After that, the mixture was sterilized using a 0.2 micron filter. The stock solution was diluted with cell culture media supplemented with 10% fetal bovine serum to get the final concentration. In 96-well plates, cancerous cell MDA-MB-231, human breast cancer cell lines were seeded and incubated at 37°C to get 70 to 80% confluent cell monolayer. These cells were then inoculated with already prepared concentrations of white shrimp extract as follows 0.3, 0.6, 1.2, and 2.4 mg/ml.

ASSESSMENT OF CELL VIABILITY USING MTT ASSAY

The MDA-MB-231 cells were grown in 96 well plates which were then inoculated with extract as previously described. The cell viability was assessed using quick colorimetric test utilizing the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT assay. After the completion of incubation post inoculation, MTT was added to the 96 well plate at the concentration of 5mg/ml and again incubated at 37°C for four hours. Dimethyl sulfoxide (DMSO) was added to convert formazine into formazan which result in color change. Finally absorbance was measured at 630 nm using micro plate reader. Blank and negative controls were added to the plate in order to calculate the percent viability using following formula:

$$\% \text{viability} = \frac{(\text{OD Sample} - \text{OD Blank})}{(\text{OD Control} - \text{OD Blank})} \times 100$$

RESULTS

The data was analyzed by constructing a standard curve and % viability was conducted against increasing concentrations of the extract, the concentration required to attain 50% inhibition (IC₅₀) was also determined. To find the IC₅₀ value, linear regression was used to fit the dose-response record. The IC₅₀ value was calculated using the Hill coefficient formula. Results showed that white shrimp extract exhibited considerable cytotoxic effect on MDA-MB-231 breast cancer cells and the cells showed decreasing viability with increase in concentration of the extract. These results also suggest the potential dose dependent effect of white shrimp extract against breast cancer cells. These results are depicted in Fig. 1.

$$Y = \text{Min} + \frac{\text{Max} - \text{Min}}{1 + \left(\frac{x}{\text{IC}_{50}}\right)^{\text{Hill coefficient}}}$$

$$Y = 0.1676 + \frac{1.0381 - 0.1676}{1 + \left(\frac{x}{0.5079}\right)^{5.8582}}$$

$$\text{IC}_{50} = 0.5079$$

Chart

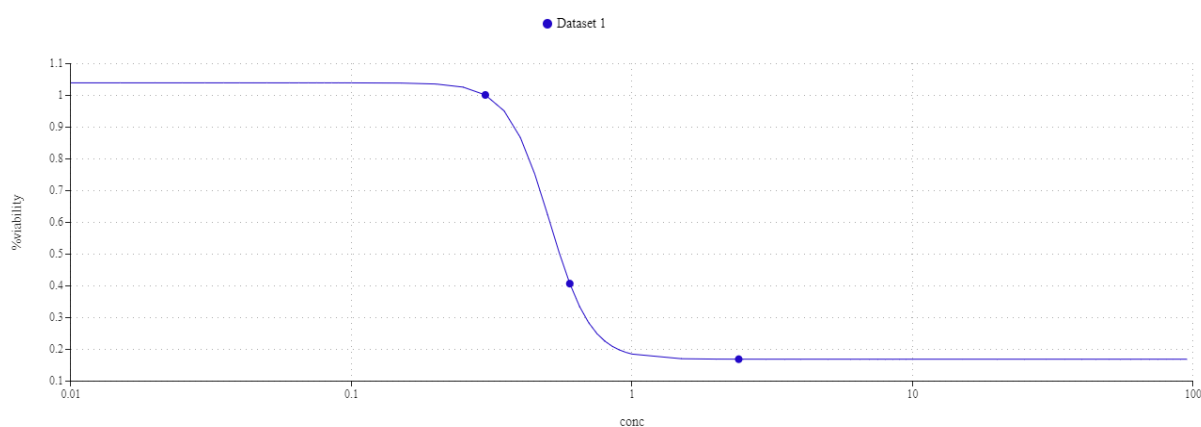


Fig. 1. Graph results demonstrated a dose -dependent reduction in cell viability when white shrimp extracts were administered to cancer cells in increasing concentrations.

DISCUSSION

This study tested the anticancer properties of white shrimp from the waterfront in Gwadar. As cancer has become a major cause of concern in Pakistan in recent years due to its increasing burden. Pakistan's cancer incidence has been steadily rising, according to the World Health Organization. Breast cancer is 24.1%, oral cavity 9.6%, colorectum 4.9%, and esophageal 4.2%, according to the current study, according to the reports 9.6 million Deaths before the age of 70 occur worldwide each year, while 18.1 million new cases of cancer are diagnosed. 16.3 million deaths from cancer in 2040, and 29.5 million new cases are predicted in study. New issues related to cancer are becoming more prevalent worldwide and are caused by factors like air pollution, unhealthy lifestyles, increasing population, inactivity, and fast urbanization (11). Due to high ratio in level of breast cancer in Pakistan breast cancer cell lines was selected in this study, namely MDA-MB-231. Furthermore, researchers are focusing on natural compounds to tackle critical issue like cancer as its prevalence is on the rise. Nevertheless, for the natural compounds usage this

study focus on marine specie white leg shrimp *Litopenaeus vannamei* from the water front of Gwadar. Research shows that physiologically active chemicals are primarily found in marine organisms. *Litopenaeus vannamei* muscle organic extracts have demonstrated an Antiproliferative action on malignant cells, including breast adenocarcinoma. The use of omega-3 polyunsaturated fatty acids from marine sources, such as eicosapentaenoic acid and docosahexaenoic acid, is recommended in a therapeutic context for patients undergoing treatment for a variety of cancer types. In recent years, marine lipids and lipid classes with anti-tumor properties have been identified (12). The compounds made from *Litopenaeus vannamei*, or white shrimp opens a new avenue for researchers. The white shrimp bioactive peptide and potential benefit make them more viable alternative to current methods used for treatment. In the past few decades, some 3,000 novel natural compounds have been identified and their anticancer potential has been evaluated. Human clinical trials are currently being conducted on a number of marine anticancer bioactive compounds that have recently been discovered, characterized, and preclinically assessed (13). Additionally, the white shrimp is one of the most effective natural products for the treatment of cancer. The white shrimp are an excellent source of proteins, vitamins, minerals, astaxanthin, essential amino acids, and other nutrients. They may also fill in dietary shortages and greatly improve human health. In terms of the amino acid makeup of all seafood proteins, shrimp protein is the most well-known because of its high lysine content. It is also highly popular due to its unique flavor and taste. Since its introduction to China in the early 1990s, the South American white shrimp has risen to become one of the top three shrimp species with the highest aquaculture production worldwide (14). In this study *Litopenaeus vannamei* or white shrimp species were tested for anticancer activity on MDA-MB-231, human breast cancer cell lines and the MTT assay was used to determine the outcome. The MTT technique is primarily based on mitochondrial activity. In living cells, mitochondrial activity is constant. (15). Lastly, the results demonstrated a dose-dependent increase in apoptosis and a significant reduction in cell viability when white shrimp extracts were administered to cancer cells. The result was calculated through IC_{50} calculation which was about 0.5079. different other studies shows different values likewise in addition to the *Litopenaeus vannamei* shells' ethyl acetate extract's antioxidant activity using the DPPH test, and the sample's IC_{50} values were 15.03 $\mu\text{g/ml}$ and the standards were 22.6 $\mu\text{g/ml}$ (16). The outcomes of this study could offer a positive effect to cancer patient and the society.

CONCLUSION

The study explored the anticancer effects in an extract of whole white shrimp (*Litopenaeus Vannamei*) from the water front of Gwadar. The white shrimp found in the marine environment, a natural source to combat the global threat demonstrated promising results in present study showing decrease in the rate of cell viability and ascertain the dosage response on malignant cell line with increasing concentration of the extract employed.

Recommendation:

To identify the precise compound responsible for the anticancer activity in white shrimp extract, this study suggests new opportunities for researchers to purify and analyze the bio-active chemicals. In vivo studies to ascertain the safety and effectiveness of white shrimp extract in animal models and dose-response tests to determine the optimal concentration of white shrimp extract for its anticancer activities could be helpful in the development of treatment against cancer.

Conflict of interest:

The authors report no conflicts of interest

Author's contributions:

SAB Conducted the experimental work and data collection; AU Supervised and conceptualized the study; ZT Performed data analysis and interpretation; ZB Assisted in laboratory assays; MMR Participated in manuscript drafting and editing.



Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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