The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(1): 787-794, 2026 www.thebioscan.com

In-vitro anticancer activity in the gonad extract of *Stomopneusres variolaris* on HeLa Cells

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DOI: 10.63001/tbs.2026.v21.i01.pp787-794

Keywords

Stomopneustes variolaris, gonad extract, HeLa cells, toxicity, therapeutic.

Received on:

17-11-2025

Accepted on:

15-12-2025

Published on:

31-01-2026

ABSTRACT

The present study was carried out to assess the anticancer activity of gonad extract of *Stomopneustes variolaris* for cell proliferation and cytotoxicity on HeLa (Human cervix adenocarcinoma cell line) cells at different concentration (10, 25, 50, 100, 200, 500µg/ml). A maximum of 60% toxicity was observed at 500µg/ml and a minimum of 3% at 10 µg. The cell viability of gonad extract ranges from 40% (500µg/ml) to 97% (10µg/ml). The percentage of toxicity increased with increasing concentration of gonad extract. The result suggest that the extract of sea urchin gonad can be considered as a valuable source as therapeutic agents against human cervical cancer.

Introduction

Marine ecosystems represent one of the most diverse and chemically rich habitats on the planet, offering a vast reservoir of unique natural compounds with promising pharmacological properties. Over the past decades, several marine-derived metabolites have been identified as potential candidates for drug development, and a number of compounds have advanced into preclinical and clinical evaluation as anticancer agents [1]. Despite this progress, only a fraction of marine biodiversity has been explored, leaving substantial untapped potential for the discovery of novel chemotherapeutic molecules.

Cervical carcinoma remains a major global health burden and ranks among the most prevalent gynecological malignancies in women. Persistent infection with high-risk human papillomavirus (HPV) types is the primary etiological factor in cervical carcinogenesis, with the disease disproportionately affecting women in low- and middle-income countries owing to insufficient screening and limited access to treatment facilities [2]. Current therapeutic interventions including surgery, radiotherapy, and chemotherapy have improved patient outcomes; however, challenges such as drug resistance, systemic toxicity, and low complete response rates underscore the need for safer and more effective therapeutic alternatives [3].

In this context, natural products have attracted considerable interest in oncology research as potential sources of novel anticancer agents or complementary therapeutics. Marine invertebrates, particularly echinoids, have garnered attention due to their rich biochemical composition. The gonadal tissues of sea urchins such as *Stomopneustes variolaris* are known to contain a high concentration of bioactive constituents, including lipids, polyunsaturated fatty acids (PUFAs), carotenoids, sterols, and various glycosides [4]. These compounds play critical roles in inflammation, infection control, and cellular homeostasis, and several have been shown to modulate pathways implicated in cancer development and progression.

Emerging evidence suggests that echinoid-derived compounds exert anticancer activity through multiple mechanisms, including disruption of mitochondrial membrane integrity, induction of reactive oxygen species (ROS), downregulation of anti-apoptotic proteins such as Bcl-2, and activation of caspase-dependent apoptotic pathways [5]. Moreover, diets rich in natural antioxidants have been associated with decreased cancer incidence and mortality, potentially through the regression of premalignant lesions and suppression of oxidative stress an important contributor to tumorigenesis [6].

In view of the growing interest in marine natural products and the urgent need for alternative therapeutic strategies for cervical cancer, further investigation of bioactive compounds derived from echinoid species is strongly warranted. Therefore, the present study aims to investigate the anticancer potential of gonad extract derived from the sea urchin *S.variolaris* against the human cervical cancer

Materials and Methods

The methodology given by Kang *et at.,* (2004) was used to carried out MTT assay [7].

Maintenance of Cell Line

The HeLa (Human cervix adenocarcinoma cell lines) is purchased from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2days.

Anticancer activity on Human Cervix Adenocarcinoma Cell Line

HeLa cells were seeded into 96-well plates at a density of 20,000 cells per well and incubated for 24 hours. Following attachment, cells were treated with various concentrations of the gonadal extract (10, 25, 50, 100, 200, and 500 µg/ml) and incubated for another 24 hours.

After treatment, the medium was removed, and 20 µL of MTT solution (0.5 mg/mL in PBS) was added to each well. Plates were incubated for 3 hours at 37°C in the dark. The formazan crystals formed were dissolved in 100 µL of DMSO, and the absorbance was read at 570 nm using a microplate ELISA reader.

% Cell viability is calculated using below formula:

% of cell viability = (OD of treated cells / OD of control cells) × 100

The IC_{50} value was determined by using liner regression equation Y=Mx+C.

Here, Y=50, M and C values were derived from the viability graph.

Result

The effect of the gonad extract of ***S.variolaris*** on cell viability and cytotoxicity are shown in Table 1, Fig 1&2. The cell viability ranged from 40 % (500µg/ml) to 97% (10µg/ml). The viability of 84% was observed at 25µg/ml; 73% at 50µg/ml; 62 % at 100µg/ml; and 49% at 200µg/ml.

The highest toxicity of 60% was observed at a concentration of 500µg/ml and the lowest toxicity of 3% at a concentration of 10µg. 16%, 27%, 38%, and 51% toxicity were observed at 25µg/ml, 50µg/ml, 100µg/ml, and 200µg/ml respectively shown in (Table 1).

IC50 value was observed at a concentration of 200µg/ml. As the concentration of the extract increased toxicity also increased. Morphological alterations of gonad extract treated cell were examined using an inverted microscope (Figure 2). As the concentration of the gonad extracts increases from 10µg/ml to 500µg/ml the number of HeLa cancer cells decreased.

TABLE 1: Percentage of Viability and Toxicity of Gonad Extract *S.variolaris*

Concentration	Control	10µg/ml	25µg/ml	50µg/ml	100µg/ml	200µg/ml	500µg/ml
Viability (%)	38	97	84	73	62	49	40
Cytotoxicity (%)	62	3	16	27	38	51	60

Figure. 1: Analysis of Cell Viability- Gonad Extract from *S.variolaris*

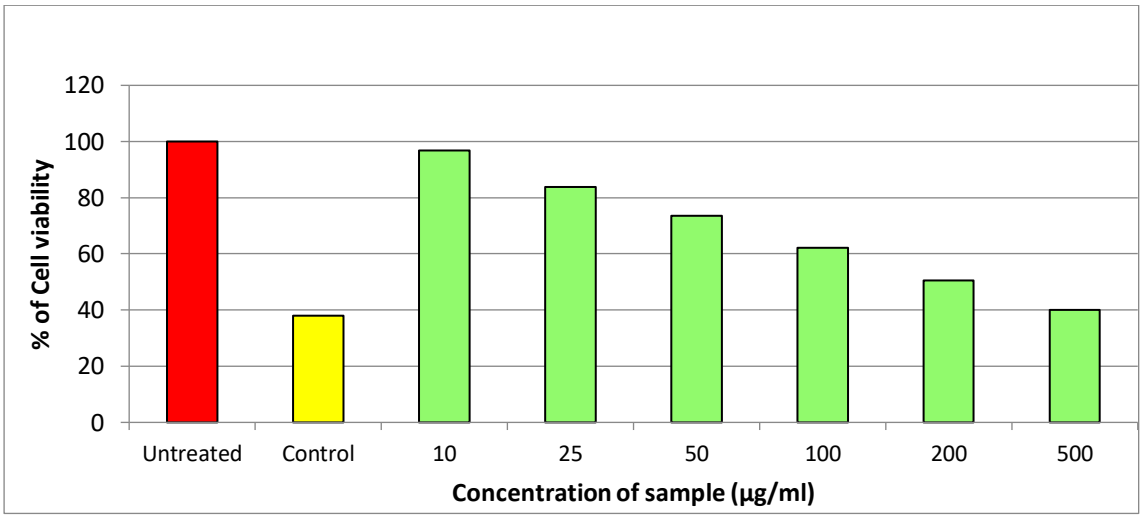
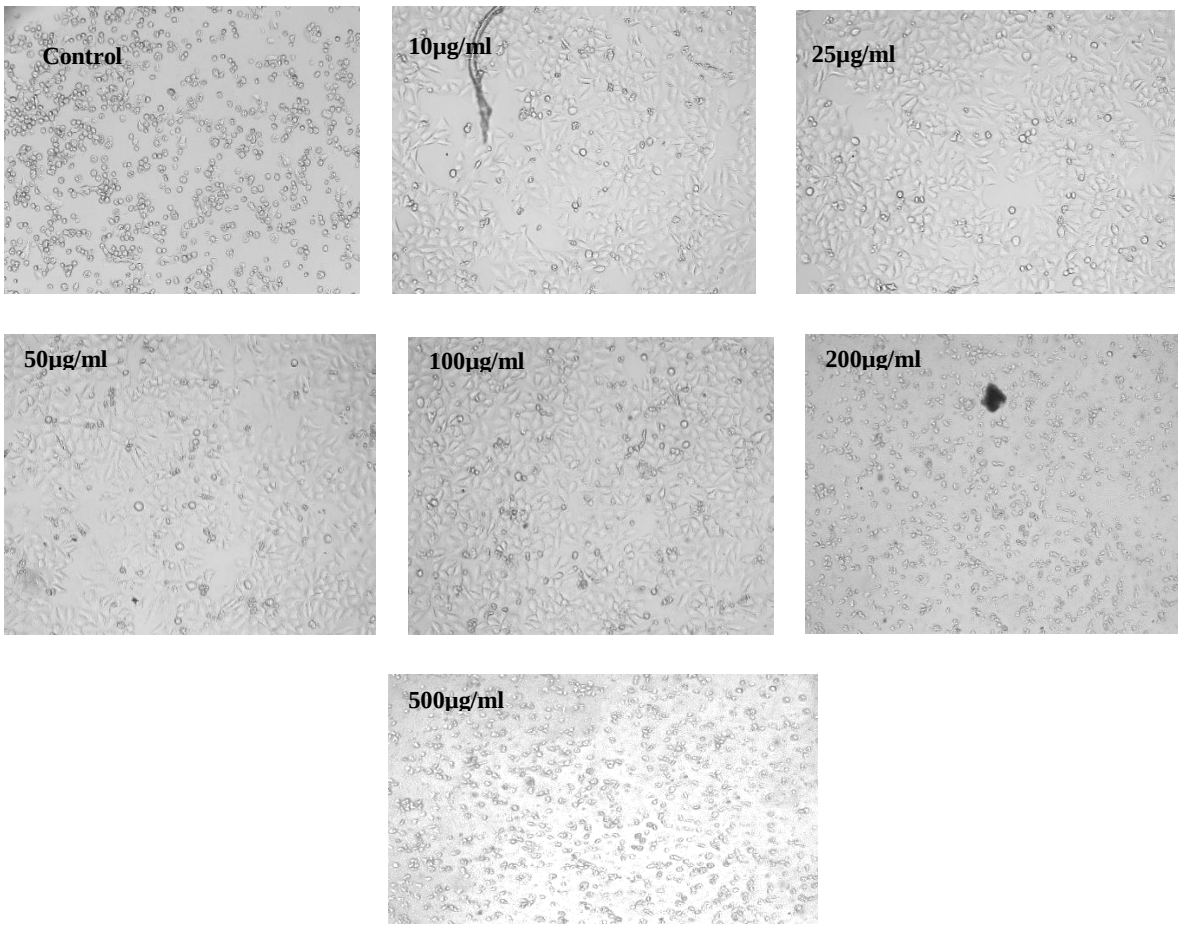


Plate 1: Viability of Hela Cells Treated with *S.variolaris* Gonad Extract



Discussion

In the present investigation the cell viability of *Stomopneustes variolaris* gonad extract was studied against HeLa cervical cancer cells at 10 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 500 µg/ml. The cell viability ranged from 40 % (500µg/ml) to 97% (10 µg/ml). As the concentration of the sample increases the viability of the cancer cells decreases. Gonad extract of *Stomopneustes variolaris* shows the highest toxicity of 60% at a concentration of 500µg/ml and the lowest toxicity of 3% at a concentration of 10 µg/ml. IC50 value was observed at a concentration of 200µg/ml. As the concentration of the extract increased toxicity also increases. Similar study was carried by Nehal Shawky Nagy *et al*., (2024)[8]. They investigated the bioactivity of the gonadal extract of the sea urchin *Paracentrotus*

lividus (*P. lividus*) against three different breast cancer cell lines, namely MDA-MB-231, MDA-MB-453, and HCC-1954. The extract exerted concentration-dependent cytotoxic effects on the cell line tested. The IC50 value of the extract in each cell line was estimated to be 360.8 µg/ml for MDA-MB-231, 379.8 µg/ml for MDA-MB-453 and 371.37 µg/ml for HCC 1954 indicating significant cytotoxic activity against all cell lines.

Glu-e-Saba Chaudhry *et al*., (2020) [9] studied four fractions of *Acanthaster planci* (sea star) and *Diadema setosum* (sea urchin) at seven concentrations. DS-9 fraction of *Diadema setosum* had an IC50 value of 6.13µg/ml at 24hr and 6.89µg/ml at 72hr, indicating higher cytotoxic activity against HeLa cells. Similarly, in the present study gonad extract of *Stompneustes variolaris* were tested for cytotoxicity against HeLa cells. At the concentration of 500µg/ml, 60% toxicity of observed against HeLa cell line where as 3% was observed at 10 µg/ml. As a concentration decrease the percentage of toxicity also decreases.

In contrast to the present study Ahmed Faisal Mutee *et al*., (2012)[10] evaluated the cytotoxic activity of *A. planci* extracts acquired by different methods of extraction on MCF-7 and HCT-116, human breast and colon cancer cell lines, respectively. Results of the cell proliferation assay showed that PBS extract exhibited very potent cytotoxic activity against both MCF-7 and HCT-116 cell lines with IC(50) of 13.48 µg/mL and 28.78 µg/mL, respectively, while the extracts prepared by Bligh and Dyer method showed moderate cytotoxicity effect against MCF-7 and HCT-116 cell lines, for chloroform extract, IC(50) = 121.37 µg/mL (MCF-7) and 77.65 µg/mL (HCT-116), and for methanol extract, IC(50) = 46.11 µg/mL (MCF-7) and 59.29 µg/mL (HCT-116). However, the extracts prepared by sequential extraction procedure from dried starfish found to be ineffective.

Conclusion

This study highlights the potential of *Stomopneustes variolaris* gonad extracts as a valuable source of novel therapeutic agents against cancer. These findings provide a foundation for further research, including isolation and characterization of bioactive compounds, and evaluation of their efficacy and safety. Ultimately, this research may contribute to the development of innovative cancer therapies, offering new hope for patients and advancing our understanding of marine-derived compounds in cancer treatment.

Acknowledgements

Authors are grateful to the Principal and Head of the Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, Tamil Nadu, India for providing all the facilities and support used to carry out the work.

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