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RESEARCH ARTICLE

Anticancer Activity of Zinc Oxide Nanoparticles from the Ink of *Uroteuthis duvaucelii* on HeLa (Human Cervix Adenocarcinoma Cell Line) Cells

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Abstract

Objectives: The present study was carried out to assess the anticancer activity of zinc oxide nanoparticles synthesized from the ink of *U. duvaucelii* for cell proliferation and cytotoxicity on HeLa (Human cervix adenocarcinoma cell line) cells. **Methods:** MTT Assay was carried out to evaluate the anticancer activity of zinc oxide nanoparticles from the ink of *Uroteuthis duvaucelii*. **Findings:** The zinc-oxide nanoparticles had significant cytotoxicity on HeLa cells in different concentrations (25, 50, 100, 200, 500 μg/ml). A maximum of 37% toxicity was observed at 500μg/ml and a minimum of 1% at 25μg/ml. The cell viability of squid ink varied from 63% (500μg/ml) to 99% (25μg/ml). The percentage of toxicity increased with increasing concentration of zinc-oxide nanoparticles. The result proved that the zinc-oxide nanoparticle from the ink is a source to be considered as a promising agent in cancer treatments. **Novelty :** At present, there is a growing need to develop environmentally benign nanoparticle synthesis without using any toxic chemicals and the preparation of uniform nanosized drug particles with specific requirements in terms of size, shape, and physical-chemical properties is of great interest in the formation of new pharmaceutical products. Zinc oxide nanoparticles have excellent biocompatibility, low toxicity, and destroy cancer cells specifically. So, the synthesized nanoparticles from the ink of *Uroteuthis duvaucelii* can be used for targeted chemotherapy in the future as they have anticancer properties.
Keywords: *Uroteuthis duvaucelii*; Zinc-oxide nanoparticles; Squid ink; Anti-cancer activity; HeLa cells.

1 Introduction

Cancer is one of the diseases that spreads worldwide, causing morbidity, death, and the new cases of cancer increased during the last two decades [(1)]. Cancer may affect

people of all ages, even the fetus, but the risk for cancer increases with age. Cancer causes about 25% of human deaths and is a major public health problem in many parts of the world [2]. Cancer deaths are due to tobacco smoking, alcohol consumption, and diets low in nutrition. In developed countries overweight, and obesity are the leading cause of cancer, and in low- and -middle-income countries sexual transmission of the human papillomavirus is a leading risk factor for cervical cancer [3]. About 200 different types of cancer were reported among which cervical cancer is considered the second leading cause of death in females. Cervical cancer occurs in the cervix, the narrow opening into the uterus from the vagina. About 80 to 90% of cervical cancers are squamous cell cancers, while remaining the 20% of cases are adenocarcinoma type [3–5]. Recently metal nanoparticles have received much attraction in cancer treatment due to their unique physiochemical properties [6,7]. Newer anticancer drugs act directly against abnormal proteins in cancer cells and kill them this is termed targeted therapy. Majority of chemotherapeutic drugs can be divided into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other anti-tumor agents. All of these drugs affect cell division or DNA synthesis and function [8]. The anti-cancer effects of squid and cuttlefish ink occur through the initiation of apoptosis and its associated different chemicals of ink [9]. Therefore, the aim of the study is to investigate the action of zinc oxide nanoparticles against HeLa cells.

2 Methodology

The methodology given by Kang *et al*., 2004 was used to carry out MTT assay [10].

2.1 Maintenance of cell lines

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°C temperature in the CO_2 incubator and sub-cultured for every 2 days.

2.2 Anticancer activity on Human Cervix Adenocarcinoma cell lines

Seed 200μl cell suspension in a 96-well plate at the required cell density (20000 cells per well), without the test agent. Allow the cells to grow for about 24 hours. Add appropriate concentrations of the test agent. Incubate the plate for 24 hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, take out the plates from the incubator, and remove spent media and MTT reagent to a final concentration of 0.5mg/ml of total volume. Wrap the plate with aluminum foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. Remove the MTT reagent and then add 100μl of solubilization solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer at 570nm and 630nm. % Cell viability is calculated using the below formula

% Cell viability = [Mean abs of treated cells / Mean abs of untreated cells] $\times$ 100

% Cell toxicity = 100 - percentage of cell viability

The IC_{50} value was determined by using the linear regression equation Y = Mx + C. Here, Y=50, M, and C values were derived from the viability graph.

3 Results and Discussion

Squid and cuttlefish ink has the potential to act as anticancer agents based on *in vitro* studies on cancer cell lines [11]. Natural products have long been used to prevent and treat many diseases, including cancer, and thus they are good as applicant for the development of anticancer drugs [12].

The cell viability ranges from 63% (500μg/ml) to 99% (25μg/ml). The viability of zinc-oxide nanoparticles is shown in Figure 1 morphological changes of drug-treated cells were examined using an inverted microscope Figure 2. As the concentration of the extracts increases from 25μg to 500μg the number of HeLa cancer cells decreases.

In this study, the test compound is evaluated for cytotoxicity against HeLa cells. 37% of toxicity was observed at 500μg and 1% was observed at 25μg. 18%, 11%, and 4% toxicity were observed at 200μg, 100μg & 50μg respectively Figure 3.

In the present study, zinc-oxide nanoparticles from the ink of squid were tested for cytotoxicity and cell viability against HeLa cells. Results reveal that the extract decreases the viability as the concentration increases and indicate zinc-oxide nanoparticles from the ink exhibit anticarcinogenic activities. Similar to the present study Chen [13] *et al*., 2010 reported the anti-cancer properties of a sulfated polysaccharide isolated from the ink of the squid *Ommastrephes bartrami* using *in vitro* and *in vivo* models. The six sulfated samples of squid ink polysaccharide were used to determine the activity. The results revealed that

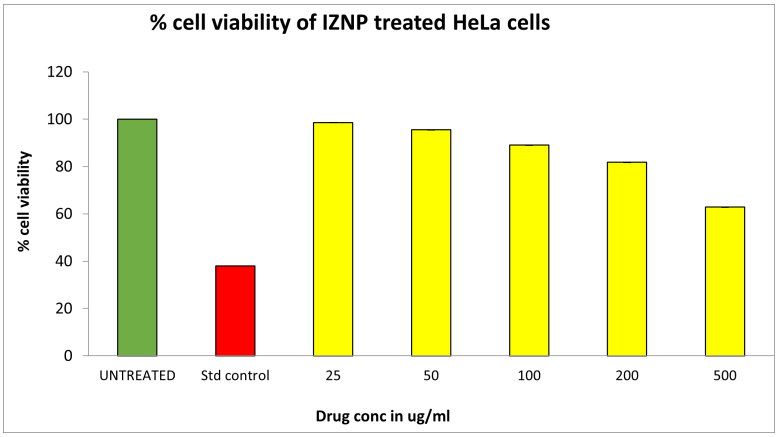

Fig 1. Percentage of cell viability

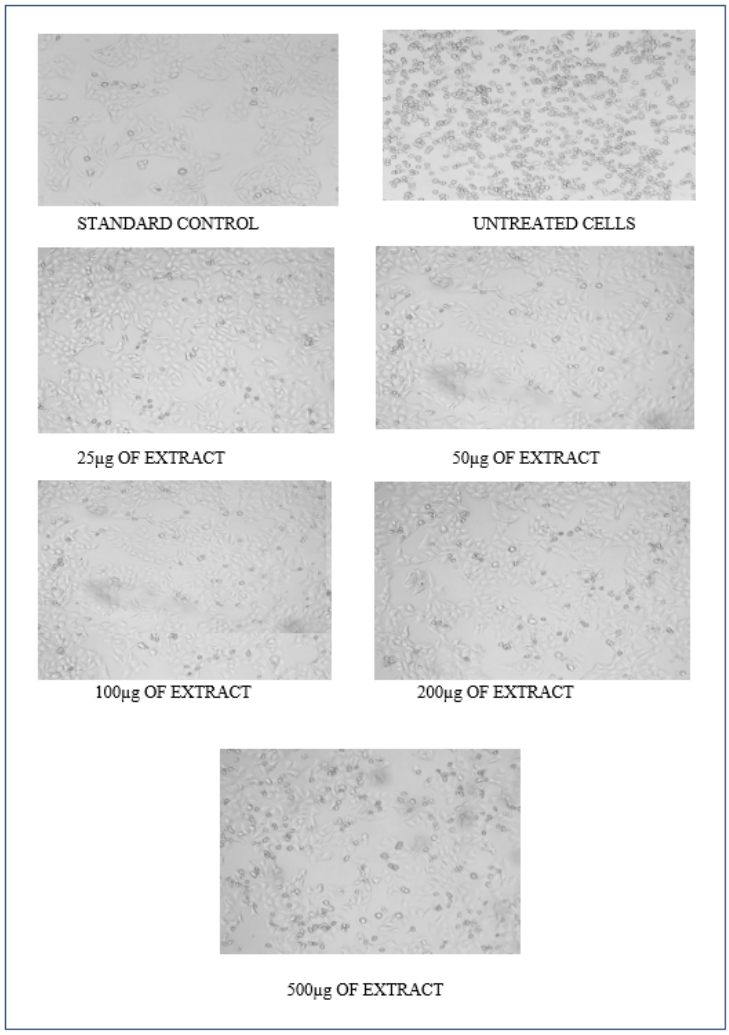

Fig 2. MTT assay- ink zinc-oxide nanoparticles from *Uroteuthis duvaucelii*

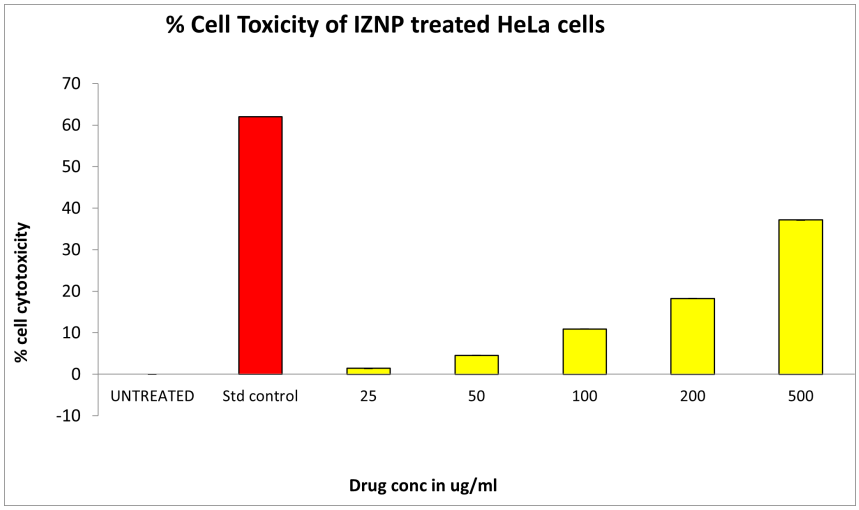

Fig 3. Percentage of cell toxicity

TBA-1 inhibited HepG2 in a dose-dependent manner. The rate of inhibition ranges from 38.4% to 78.2%. In the present study also the concentration of the extracts increases from 25μg/ml to 500μg/ml the number of HeLa cells decreased from 99 % to 63%.

In the present study, zinc-oxide nanoparticles on HeLa cells revealed high cell viability (99%) at lower concentrations (25μg/ml) and 63% at 500μg/ml. Concentration and cell viability are inversely proportional. Similarly, Selvakumari[14] *et al.*, 2015 studied the anticancer activity of Zinc-oxide nanoparticles and Commercial zinc-oxide nanoparticles against MCF7 and A549 cell lines. The exposure of MCF7 and A549 cells to ZnO nanoparticles at various concentrations (1000μg/ml, 500μg/ml, 250μg/ml, 125μg/ml, 62.5μg/ml, 31.2μg/ml, 15.6μg/ml, 7.8μg/ml) for 72h. ZnO nanoparticles reduce cell viability in a concentration-dependent manner. As the concentration increases from 7.8μg/ml to 31.2μg/ml the percentage of cell viability significantly decreases from 78.7 to 54.2 (A549) and 82.8 to 50.8 (MCF7).

Diaz[15] *et al.*, 2014 evaluated the anticancer activity of the crude and partially purified protein fractionated ink of *Loligo duvauceli* on the Hep G2 cell line using cell viability and cell proliferation assay (MTT assay) at three different concentrations (125μg, 250μg, 500μg). Crude ink of *L. duvauceli* at the concentration of 500 μg/ml was found to inhibit the viability of the cell effectively and the percentage of viability ranged between 33 – 41. Cell viability decreased with increasing concentrations of the samples. HepG2 cells treated with protein fractionated ink, decreased the percentage of viability and the percentage ranged from 30-49. This study is in corroboration with the present work. The number of HeLa cancer cells decreased as the concentration of the extracts increased from 25μg to 500μg.

Priya Senan[16] *et al.*, 2013 studied the cytotoxic effects of squid (*Loligo duvauceli* [female]) and cuttlefish ink (*Sepia pharaonis* [female and male], *Sepiella inermis* [female], *Sepia aculeata* on chick embryo fibroblasts. The ink extract from different species of squid and cuttlefish inhibited cell proliferation and induced cell death in a dose dependent manner, which is in agreement with the present work.

Ahmed Bakr Mousa *et al.*, 2023[17] studied the cytotoxicity of zinc-oxide nanoparticles using sulforhodamine B (SRB) assay against the SKOV3 cell line in five concentrations (0.008,0.08,0.80,8 and 80μg/mL). The synthesized ZnO nps demonstrated concentration-dependent cytotoxic behavior in ovarian cancer cells. The concentration-dependent activity demonstrated by Ahmed *et al.* was consistent with the present study.

4 Conclusion

The present study reveals the anticancer activity of zinc-oxide nanoparticles in the ink of Indian squid (*Uroteuthis duvaucelii*). The results of the present study suggest that the zinc-oxide nanoparticles synthesized from the ink of *Uroteuthis duvaucelii* exhibited strong anticancer activity. It can be used for therapeutic applications and in the development of new drugs.

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References

1) Wild C. World cancer report . *World Health Organization*. 2014. Available from: https://doi.org/10.3945/an.116.012211.
2) Who J, Consultation FE. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser*. 2003;916:1–49. Available from: https://iris.who.int/handle/10665/42665.
3) Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. . *The Lancet*. 2005;366:11403–11409. Available from: https://doi.org/10.1016/S0140-6736(02.
4) Soumya D, Kumar RA. A brief assessment on cervical cancer. *J Cancer Sci Ther S*. 2011;17. Available from: https://doi.org/10.4172/1948-5956.S17-007.
5) Myles Z, Rice K, Degroff A, Miller J. A profile of the national breast and cervical cancer early detection program provider network: Results from the year 1 survey of program implementation. *Quality in primary care*. 2015;23(6):315–315. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4912227/pdf/nihms793025.pdf.
6) Pugazhendhi A, Edison TN, Karuppusamy I, Kathirvel B. Inorganic nanoparticles: a potential cancer therapy for human welfare. *International journal of pharmaceutics*. 2018;539(1-2):104–115. Available from: https://doi.org/10.1016/j.ijpharm.2018.01.034.
7) Oves M, Aslam M, Rauf MA, Qayyum S, Qari HA, Khan MS, et al. Antimicrobial and anticancer activities of silver nanoparticles synthesized from the root hair extract of Phoenix dactylifera. *Materials Science and Engineering: C*. 2018;89(1):429–472. Available from: https://doi.org/10.1016/j.msec.2018.03.035.
8) Takimoto CH, Calvo E. Cancer management: a multidisciplinary approach . *Principles of oncologic pharmacotherapy*. 2008;11:1–9. Available from: https://www.cancernetwork.com/view/principles-oncologic-pharmacotherapy.
9) Derby CD. Cephalopod ink: production, chemistry, functions and applications. . *Marine drugs*. 2014;12:2700–2730. Available from: https://doi.org/10.3390/md12052700.
10) Kang BK, Chon SK, Kim SH, Jeong SY, Kim MS, Cho SH, et al. Controlled release of paclitaxel from microemulsion containing PLGA and evaluation of anti-tumor activity in vitro and in vivo. *International journal of pharmaceutics*. 2004;286(1-2):147–56. Available from: https://doi.org/10.1016/j.ijpharm.2004.08.008.
11) Hossain MP, Rabeta MS, Azan TH. Medicinal and therapeutic properties of cephalopod ink: a short review. *Food Research*. 2018;3(3):188–198. Available from: https://doi.org/10.26656/fr.2017.3(3).20.
12) Smith-Warner SA, Elmer PJ, Tharp TM, Fosdick L, Randall B, Gross M, et al. Increasing vegetable and fruit intake: randomized intervention and monitoring in an at-risk population. *Cancer Epidemiology Biomarkers & Prevention*. 2000;9(3):307–324. Available from: https://pubmed.ncbi.nlm.nih.gov/10750670/.
13) Chen S, Wang J, Xue C, Li H, Sun B, Xue Y, et al. Sulfation of a squid ink polysaccharide and its inhibitory effect on tumor cell metastasis. . *Carbohydrate Polymers*. 2010;81:560–566. Available from: https://doi.org/10.1016/j.carbpol.2010.03.009.
14) Selvakumari D, Deepa R, Mahalakshmi V, Subhashini P, Lakshminarayan N. Anti cancer activity of ZnO nanoparticles on MCF7 (breast cancer cell) and A549 (lung cancer cell). *ARPN J Eng Appl Sci*. 2015;10(12):5418–5439. Available from: https://www.researchgate.net/publication/282918208.
15) Diaz JH, Thilaga RD, Sivakumar V. Cytotoxic activity of crude and partially purified ink of L. duvauceli towards HepG2cell line. *International Journal of Pharma Research*. 2014;3(6):19–23. Available from: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=2231b3e4755d72762fb6db9bf4984c7550a5c1d2.
16) Senan VP, Sherief PM, Nair JR. Cytotoxic effect of ink extracts of cuttlefish and squid on chick embryo fibroblasts. *International Journal Of Pharmaceutical Sciences And Research*. 2013;33:1893–96. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(5).1893-96.
17) Mousa AB, Moawad R, Abdallah Y, Abdel-Rasheed M, Zaher AM. Zinc oxide nanoparticles promise anticancer and antibacterial activity in ovarian cancer. *Pharmaceutical Research*. 2023;40(10):2281–90. Available from: https://doi.org/10.1007/s11095-023-03505-0.