EVALUATION OF BIOACTIVE COMPOUNDS FROM NEWLY ISOLATED *Haematococcus* sp. BY IN-VITRO CELL CYTOTOXICITY ASSAY WITH FIBROBLAST AND NEUROBLASTOMA CELL LINES

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Abstract: Present study deals with extraction and isolation of bioactive compounds from indigenous green microalgae *Haematococcus* sp. isolated from Karnataka, Dharwad. Study objectives mainly focused on investigate the efficacy of compound in crude form. Isolated strain cultivated in nutrient stress conditions in laboratory using mixotrophic cultivation. In stress condition isolate synthesized secondary carotenoids, and these carotenoids extracted using organic solvent . extract separated by TLC and used as crude drug to examine cytotoxic effects on fibroblast and cancer cell lines. Two cell lines NIH/3T3 and SKNSH used for in vitro cell viability study by MTT assay. Results indicated that the carotenoid rich crude drug have potential cytotoxic effect on SKNSH cell lines at concentration 800 and 1000µg/ml compared with cytotoxic effect of standard anticancer drug Cisplatin at concentration 15µg/ml. It found that the crude drug is non-cytotoxic to normal fibroblast at low concentration 200µg/ml cell lines but cytotoxic to neuroblastoma SKNH cell lines at low concentration 200-400 µg/ml. This property makes the crude drug to be used as potential bioactive anticancer natural supplement.

Keywords: Crude drug ,Carotenoids, *Haematococcus* sp., MTT, NIH/3T3 and SKNSH .

1. Introduction

Microalgae are known as an abundant reservoir of natural bioactive compounds having industrial importance and application in human health (Shah *et al.,* 2016). Microalgae *Haematococcus* is one the important fresh water microalgae known to produce carotenoid pigments as secondary metabolites with response to environmental stress(Wan *el a.,* t 2014). It produces red color pigment in cyst stage and pigment is known to have bioactive properties . In present conditions though many species of *Haematococcus* are reported to synthesize important metabolites, yet exploration of native microalgae for cultivation and production of commercially viable compounds holds great importance (Pokharel *et al.,* 2018).

In present study objectives focused on extraction and evaluation of bioactive compounds from isolated species of green microalgae belongs to genera *Haematococcus*. In vitro cytotoxic effects of the carotenoid rich extract studied with respect to cell proliferation properties of NIH/3T3 and SKNSH cell line. Determination of crude formulation as drug mainly emphasize on formulating dietary neutraceutical compound from isolated strain having bioactive properties.

2. Materials and Methods

2.1 Preparation of crude drug

Test sample prepared by extraction of pigment from *Haematococcus* sp. isolated from local fresh water lake from Dharwad, Karnatak. Carotenogenesis in isolated *strain* induced by nutrient stress, culture grown by mixotrophic cultivation using BBM media estimation of carotenoid done by following method described by (Marxen *et al.,* 2007). Carotenoid pigment then separated by preparative thin layer chromatography (Harrera , *et al.,* 2015).Extracted drug dissolved in 1% DMSO [v/v] prepared in serum - free DMEM medium and filtered using 0.28µ syringe filter and stored at cold temperature.

2.1.1. Cell lines and culture maintenance

Fibroblast cell line - NIH /3T3 and neuroblastoma cell line SKNSH procured from National Center for Cell Science (NCCS), Pune, India. Cells were grown as monolayer in MEM and DMEM media formulated with addition of 10% FBS and incubate under controlled environment at 37°C, 5% CO_2 .

2.2. MTT assay using crude drug with NIH/3T3 and SKNSH cell lines

The fibroblast cell line NIH/3T3 grown using DMEM media and SKNSH grown using MEM media separately. Trypsinised cells aspirated into 15 ml centrifuge tube and cell pellet was obtained by centrifugation at 300_X g. The cell count was adjusted using DMEM and MEM medium such that 200µl of suspension contained approximately 10,000 cells. Cells transferred to 96 well microtitre well and plate incubated at 37°C and 5% CO_2 for 24h .

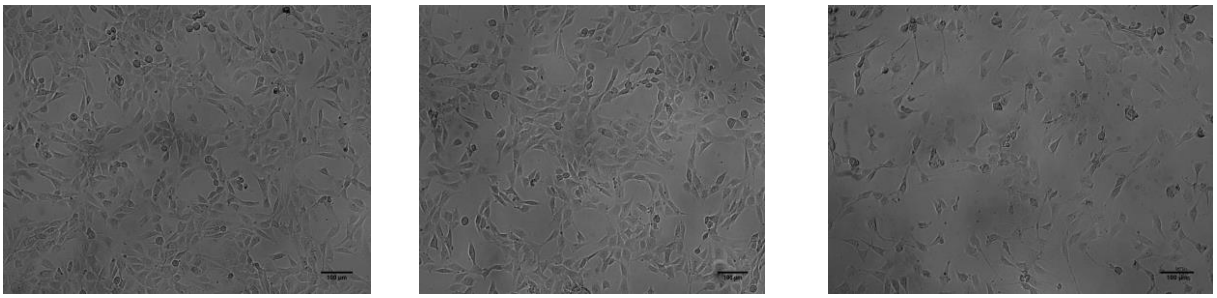
Different aliquots of test sample 200, 400, 600,800 and 1000µg/ml stock added to the wells and plates incubate at 37°C and 5% CO_2 for 24h. After incubation drug containing media aspirated and 10% of 200µl MTT added to each well to get the final concentration of 0.5 mg/ml and the plates were incubated again at 37°C and 5% CO_2 for 24 h. After final incubation culture medium removed and 100 µl/ml of DMSO added to the medium and shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength 570nm and also at 630nm. The percent growth inhibition was calculated, after substracting the background and the blank, and concentration of test drugs needed to inhibit cell growth . IC_{50} generated from dose – response curve for the cell lines.

2.2.1. Statistical Analysis

All experiments carried out in triplicates and values expressed as ± mean SD using SPSS software .

3. Result and discussion:

Isolated microalgae *Haematococcus* sp. is an indigenous species from Dharwad region, it synthesizes bio active metabolites proved by MMT cell cytotoxicity assay on SKNSH cell line indicates that this microalgae holds great economic importance towards commercial production of algae based natural drugs. In this report isolation and cultivation techniques used for growth of isolated green microalgae *Haematococcus* sp. is not discussed in detail because data related to its morphological and molecular properties and identification of isolated microalgal species is already being communicated for publication. Present experimental results determines that carotenoid rich crude pigment extract of *Haematococcus* sp. having strong cell cytotoxicity activity on SKNSH human neuroblastoma cell line similar as reported by (Santocono *et al* ., 2007). However the test sample observed to have very less cytotoxicity at higher concentration on NIH/3T3 normal fibroblast cell line(Awad *et al.*, 2005). The IC_{50} values measured using MTT assays shows potential efficacy of pigment extract, determined values are as IC_{50} - 1715.0µg/ml and for NIH/3T3 cell line while IC_{50} - 8885.0µg/ml for SKNSH cancer cell line results are significant compared with earlier report by(Murphy et el 2014 and Pallozza *et al.*, 2009). Crude formulation of drug lethal to SKNSH cell lines and inhibits cell proliferation at centration 800-1000 µg/ml as compared to results of cell cytotoxicity with cell lines treated with standard anticancer drug Cisplatin at concentration 15µg/ml. Epithelial cell morphology observed by using inverted phase contrast microscope while cell viability measured using MTT assay (Kowshik *et al*., 2014). Results of MTT assay reveals that test sample is cytotoxic to cancer cell lines while non-cytotoxic to normal fibroblast cell lines hence the crude formulation of carotenoid extract is effective against SKNSH cell lines to treat cancer (Wang et al., 2014 and Rao *et al*., 2013). Data generated in present study helpful for conducting in vivo anticancer activity using animal models. As results of present study indicates that the pigment extract is having strong anti-proliferation activity against cancer cell lines hence we aim to use *Haematococcus* sp. pigment extract as therapeutic natural bioactive compound.



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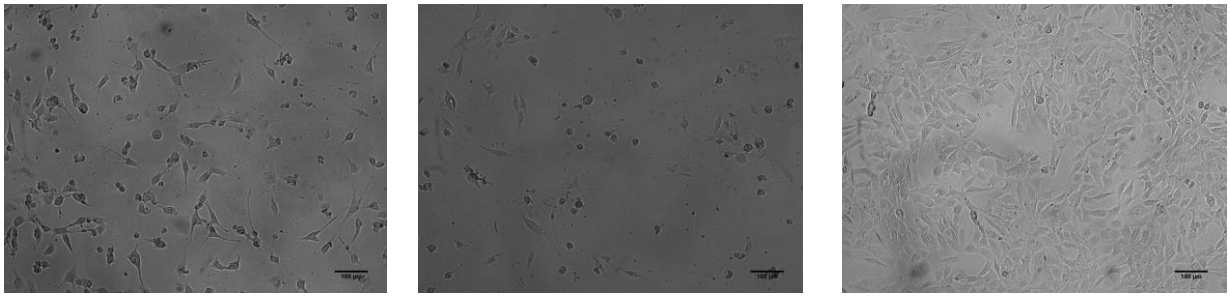

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Fig: Morphological changes observed using inverted phase contrast microscope in NIH3T3/ and SKNSH cell line treated with crude drug and Cisplatin compared with untreated cells of SKNSH .Image : a, and b microscopic image of crude drug showing less cytotoxicity treated with crude drug at concentration 200µg/ml 600µg/ml ,Image c and d : Cytotoxic effect and cell viability image SKSNH cell lines treated with of crude drug at concentration 800 µg/ml and 1000 µg/ml ,Image e: cells treated with Cisplatin 15 µg/ml, f :untreated cell SKNSH ,scale bar represents size 100 µm.

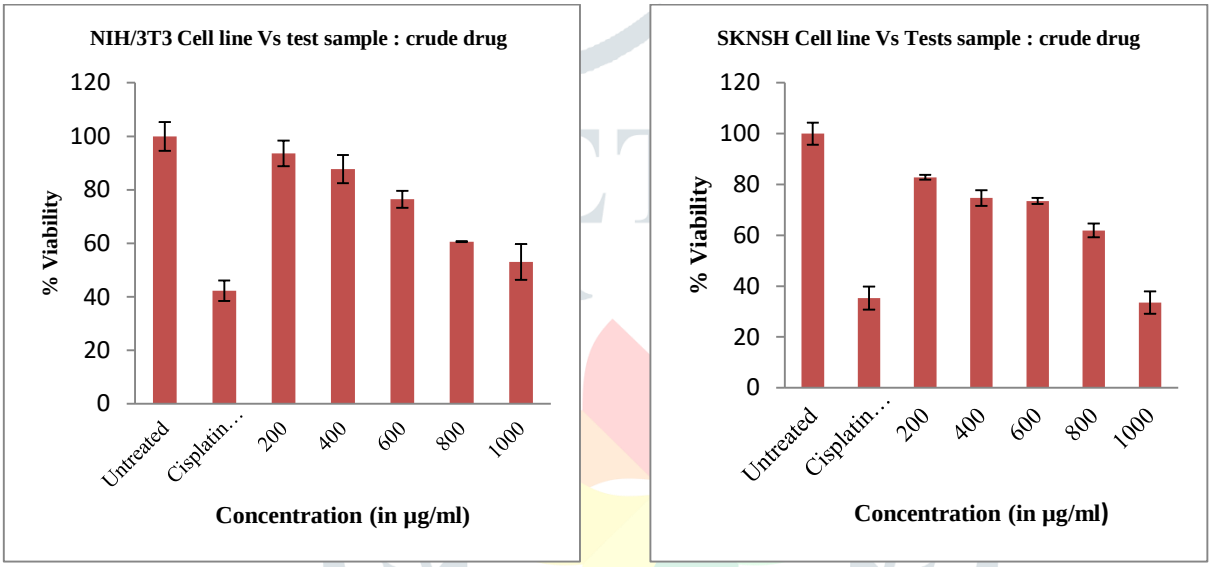

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Fig:2 a: Cell viability analysis using NIH/3T3 fibroblast cell line ,b: cell viability assesment using SKSNH Neurocblastoma cell line treated with Standard anticancer drug Cispltin and test sample.

4.Conclusion

In present study proves that isolate microalgae *Haematococcus* sp. synthesizes potential bioactive compounds, bioactive metabolite in crude form also exhibits antiproliferation activity against cancer cell lines confirmed by MTT assay. The crude drug can be used as potential neutraceutical dietary therapeutic compound.

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Conflict of interest

Authors declare no Conflict of Interest.

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