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IN-VITRO CYTOTOXIC ACTIVITY OF SQUID AND CUTTLEFISH BONE EXTRACT ON HEP G2 CELL LINE

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ABSTRACT: The present study was carried out to assess the anticancer activity of methanol extracts of internal bone of *L.duvauceli* and *S. pharaonis* for cell viability and toxicity on HepG2 liver cancer cells. The bone extracts had significant cytotoxicity on HepG2 cell in different concentrations (100,500,1000 µg/ml). Methanol fraction of squid bone powder showed cell death at a higher concentration of 1000µg. 27% toxicity was observed at 100µg/ml and 37% at 1000µg/ml. The viability of cuttlefish bone powder ranged from 58% (1000µg/ml) to 68% (100µg/ml). Hep G2 cells experienced a significant decrease in viability at low concentration with an eventual decline at the highest concentration. The percentage of toxicity of cuttle bone extracts varied from 32 to 42. Minimum toxicity of 32% was observed at 100µg and maximum of 42% was observed at 1000µg concentration and IC 50 at a concentration of >1000µg. The percentage of toxicity showed increasing trend with increasing concentration of the extract. The result suggested that the cephalopods bone is a source to be considered in the discovery of drug development for cancer.

INTRODUCTION: Liver cancer is one of the leading causes of malignancy- related death worldwide because of tumour heterogeneity and the development of multidrug resistance phenotypes [1]. Till now the availability of treatments for liver cancer remains unsatisfactory [2]. Hence, the search for active drugs from alternative sources including marine environment, obviously becomes imperative. Cephalopods are the largest single group of biotoxic invertebrates, which may be largely useful in the biomedical arena. The physical activities of squid and cuttlefish ink such as antimicrobial [3-13], antioxidant [14-16], antiradiation [17], anti-tumour [18-24], immunity promotion [25, 26] induction of many cytokines [27, 28] have been widely studied in recent years.

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The cuttlebone refers to the internal cartilaginous shell of cuttlefish, squid and octopus. Traditionally cuttlebone powder is used as a medicine for some ear ailments, stop bleeding and improve kidney efficiency [29, 30]. Antibacterial and antifungal activity of internal bone of cephalopods was studied in different species [31, 32]. But only a few cephalopods have been tested for anticancer activities, especially in India. Therefore, the aim of the present study was to assess the potential anticancer activity of the methanolic extracts from the internal bone of *L.duvauceli* (squid) and *S. pharaonis* (cuttle fish) using cell viability and toxicity assay (MTT assay) on human HepG2 liver cancer cell line.

MATERIALS AND METHODS:

Collection and Preparation of extract:

In the present study the animals (*L. duvauceli* and *S.pharaonis*) were collected from Gulf of Mannar, Thoothukudi coastal region (Long 78^0 8" to 79^0 30" E and Lat 8^0 35" to 9^0 25" N) by trawl catch, brought to the laboratory, cleaned and washed with

fresh sea water to remove all impurities. The internal bones were dissected, washed, air dried and pulverized. 10g of pulverized bone powder was mixed with 100 ml of methanol solvent and kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and concentrated to dryness at 40^0c, lyophilized and stored at 4^0c until further use.

Anticancer activity on liver cancer cell lines:

The antitumor assay was performed on Liver Cancer Cell lines (Hep-G2) obtained from National centre for cell science, Punae, India. The cell viability was measured using MTT assay [33] as described.

The cells were grown in a 96-well plate in Delbucco's Minimum Essential Medium (DMEM) (HiMedia, Mumbai)) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B).

About 1mL cell suspension (10^5cells/ml) was seeded in each well and incubated at 37^0 C for 48 hour in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of methanolic extract (1000µg, 500µg and100µg). The cell viability was measured using MTT assay with MTT (5 mg/ml) and DMSO. The tetrazolium salt is metabolically reduced by viable cells to yield a blue insoluble Formozan product measured at 570nm spectrophotometerically.

Controls were maintained throughout the experiment (untreated wells as cell control). The assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells and % of cell viability was plotted against concentration of the extract. The minimum concentration of the extract that was toxic to liver cancer cells was recorded as the effective drug concentration compared to positive control (PC-Cyclophosphamide).

Percentage of viability = Absorbance of the sample/Absorbance of control

Percentage of toxicity = 100 – percentage of viability

Morphological studies using a normal inverted microscope were carried out to observe the cell death treated with sample on HepG2 liver cancer cells.

RESULTS:

The effect of methanolic extract of *L.duvauceli* bone on cell viability and toxicity are shown in Table 1, Fig 1&2. The cell viability ranged from 63% (1000µg/ml) to 73% (100µg/ml) and the toxicity was 37% for 1000µg/ml and 27% for 100µg/ml. The minimum effective concentration that was toxic to HepG2 liver cancer cell was recorded at a concentration greater than 1000µg. The viability and toxicity of methanolic extract of *S. pharaonis* bone on Hep G2 cell line was shown in Table -2, Fig 3 & 4 and the toxicity was found to be 32% for 100µg/ml and 42% for 1000µg/ml and the IC 50 at a concentration of >1000µg.

Morphological changes of drug treated cells were examined using an inverted microscope. (**Plate 1**). As the concentration of the extracts increases from 100µg to 1000µg the number of Hep G2 cancer cells decreased.

TABLE 1: PERCENTAGE OF VIABILITY AND TOXICITY - METHANOL EXTRACT FROM *L. DUVAUCELI* BONE POWDER

Concentrations	Control	1000 µg	500 µg	100 µg	Cyclo- 90
Viability (%)	100	63.16196	71.04755	73.36683	39.19598
Cytotoxicity (%)	o	36.84	28.96	26.64	60.805

TABLE 2: PERCENTAGE OF VIABILITY AND TOXICITY OF METHANOL EXTRACT FROM *S.PHARAONIS* BONE POWDER

Concentrations	Control	1000 µg	500 µg	100 µg	Cyclo-90
Viability (%)	100	58.36877	59.21917	68.38036	39.19598
Cytotoxicity (%)	0	41.632	40.781	31.62	60.805

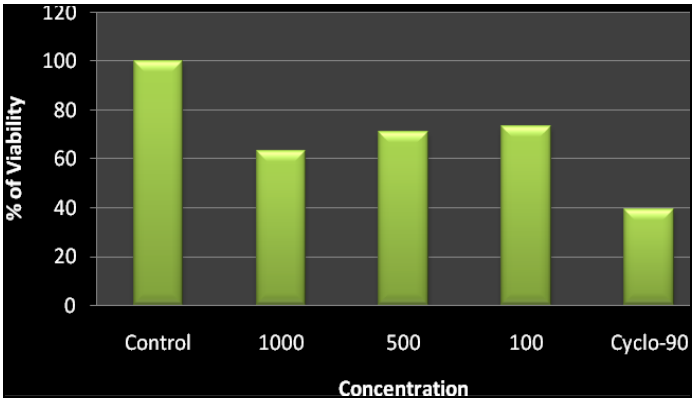

FIG. 1: ANALYSIS OF CELL VIABILITY - METHANOL EXTRACT FROM *L.DUVAUCELI* BONE POWDER

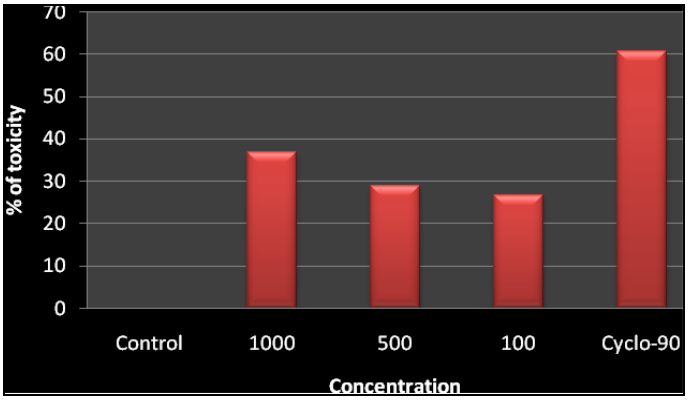

FIG. 2: ANALYSIS OF CELL TOXICITY – METHANOL EXTRACT FROM *L. DUVAUCELI* BONE POWDER

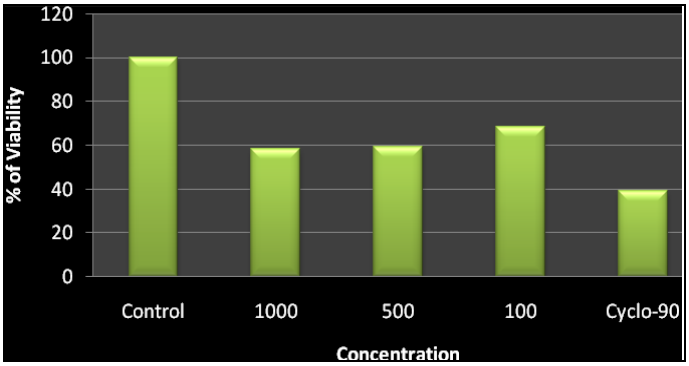

FIG.3: ANALYSIS OF CELL VIABILITY – METHANOL EXTRACT FROM *S.PHARAONIS* BONE POWDER

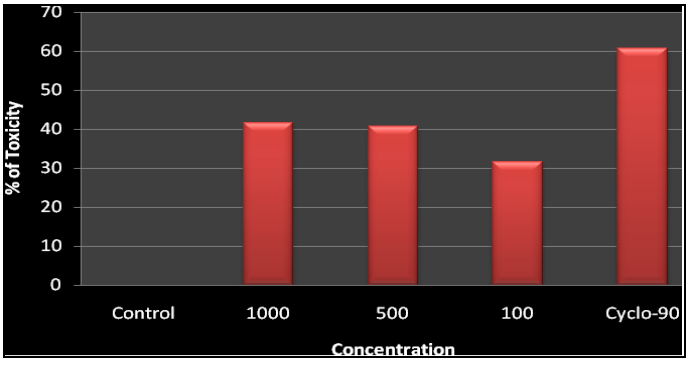

FIG. 4: ANALYSIS OF CELL TOXICITY – METHANOL EXTRACT FROM *S.PHARAONIS* BONE POWDER

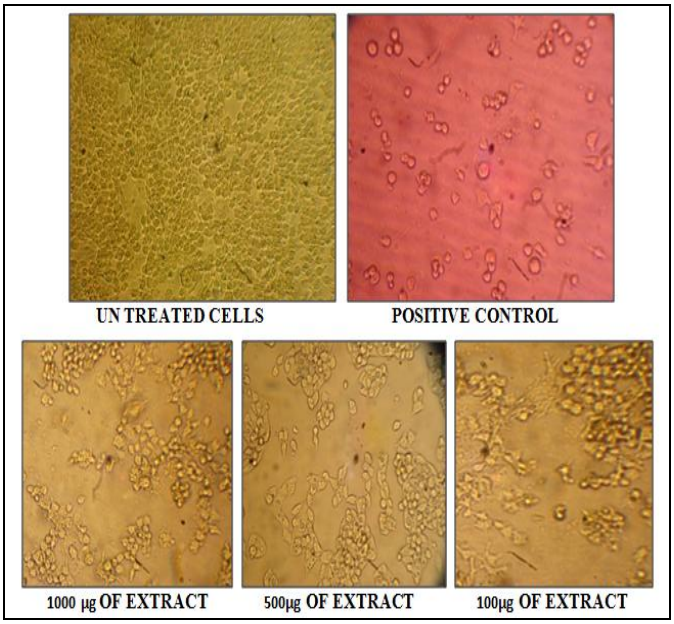

PLATE 1: MTT ASSAY - METANOL EXTRACT FROM *S. PHARAONIS* BONE POWDER

DISCUSSION:

Cephalopods play an important role in marine ecosystem and are valuable to man as food, in biomedical research and proven to be a very rich source of extremely potent compounds that have significant anticancer activity [34-36]. The cephalopod ink extract have protective effect towards hemopoietic injuries from chemotherapeutics [37]. In the present study methanolic extract from squid and cuttle bone powder was tested for anticancer activity against HepG2 liver cancer cells and the cells experienced a significant decrease in viability as the concentration increases. The crude extract obtained from the internal shell of squid contains polysaccharides showed an antitumor activity against mouse sarcoma -180 was reported [37].

The anticancer potential of salivary gland extracts (AGE, PGE) of *Octopus ageina in vitro* (COLO 205 cells) and *in vivo* (Albinio Wistar rats). PGE showed promising effects by reduced tumor occurrence [38]. There are previous report on the cytotoxic activity of the methanolic extract obtained from flesh of *Sepia brevimana* and *Sepiella inermis* using Dalton's ascites and the cytotoxic acitivity was found to be *S.brevimana* >*S. innermis* [16] . The results of this study revealed the presence of bioactive compounds in the bone

extract of *L.duvauceli and S.pharaonis* so it can be used as a source of drug in the treatment of cancer.

CONCLUSION: The results of this study shows that **a**mong the two extracts tested here methanol fraction from cuttlebone of *S.pharaonis* has more toxicity towards Hep G2 cell line than the *L.duvauceli* and hence cuttlefish are the best targets for cancer therapy. If their nature, structure and mechanism of action are explored they would be a better drugs for site-specific chemotherapy.

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