In Vitro Cytotoxic Activity of *Isostichopus badionotus,* a Sea Cucumber from Yucatan Peninsula Coast

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Abstract: The *in vitro* cytotoxic activity of hexane, ethyl acetate and butanol extracts of the sea-cucumber *Isostichopus badionotus* (Holothuroidea) was tested against normal cells (Vero), human cervical carcinoma (HeLa) and breast adenocarcinoma (MCF-7 and MDA-MB-231) ATCC cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Hexane extracts from body walls and viscera showed high cytotoxic activity against HeLa cells (IC_{50}'s = 48.5 and 42.5 µg mL^{-1}, respectively), while the ethyl acetate extract of body walls was considered low active (IC_{50} = 98.3 µg mL^{-1}). In addition, the body walls hexane extract showed a good selectivity index value of 12.0.

Keywords: Holothurian, organic extracts, selectivity index, HeLa cells.

INTRODUCTION

Cancer is a leading cause of death around the world with 12.7 million new cases and 7.6 million deaths worldwide reported in 2008, and it is estimated that the death toll will be 13.1 million deaths in 2030 [1]. Presently, more than 100 types of cancer have been identified, and despite enormous advances in treatment of cancer patients, a growing body of clinical and experimental evidence has revealed a strong impact of drug resistance on clinical outcomes in cancer chemotherapy. Therefore, the need for finding novel agents to improve anticancer drug therapy is imperative [2,3].

Natural products, defined as small-molecule secondary metabolites produced for organisms, have proved to be very useful in development of anticancer drugs over last five decades. Currently, a significant numbers of compounds obtained from marine environment are in clinical trials; hence marine organisms are considered valuable sources of cytotoxic, antiproliferative, antitumor or anticancer compounds [4-7]. Therefore, several sea cucumbers species collected worldwide have been studied in order to determine its potential as sources of anticancer compounds.

Sea cucumbers species are commercially exploited fresh or in dehydrated form (*bêche de mer, trepang, gamat*) in Asian markets, mainly in China, Korea, Indonesia and Japan as functional foods [8-11] because of their high-protein content and their putative aphrodisiac, tonical and medicinal properties [12,13]. However, some species have been overexploited; which may result in a population collapse and the loss of significant potential source of anticancer drugs for the future.

Isostichopus badionotus (Selenka, 1867), is distributed mainly in Gulf of Mexico, Caribbean Sea, and the joining of these two outstanding marine ecosystems, the Yucatan Channel [14, 15]. It is one of four species of sea cucumbers overexploited in Yucatan, Mexico, due to illegal fishing. A literature survey indicated that there is too little information about the metabolites of this species and their biological activity, since only one study about citotoxicity of a hydrolysate of *I. badionotus* against colorectal HT-29 cancer cells (IC_{50} = 1450 µg mL^{-1}) is reported [16].

In consequence, the present study was conducted to test the activity of body walls and visceral organ extracts from *I. badionotus* against Vero, HeLa, and

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breast adenocarcinoma (MCF-7 and MDA-MB-231) ATCC cells.

MATERIALS AND METHODS

Animal Material and Extraction Procedure

Specimens of *Isostichopus badionotus* (27.8 kg wet weight) were collected by scuba diving at depths ranging from 14.5 to 16 m in the coast of Progreso (N 21° 27.3' – W 89° 42.7'), Yucatan (Mexico) in June and November 2010. The specimens were collected and authenticated by M. Sc. Carlos Zetina Moguel and voucher specimen (YUC-CC-250-11/CAR/293) was deposited in the collection of Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, in Mérida, Yucatán, México.

The animals were immediately placed in ice water in sealed plastic bags and transported to the laboratory where they were dissected. Body walls (21.6 kg wet weight) and visceral organ (6.2 kg wet weight, gut content included) were stored separately at –17 °C.

Both body walls and visceral organ samples underwent the same analytical procedure. The frozen tissues were homogenized, chopped and extracted exhaustively with methanol at room temperature. The extracts were subsequently evaporated at reduce pressure for removal of the solvent. The syrup was successively extracted three times each with hexane (Hx), ethyl acetate (EtOAc) and n-butanol (BuOH).

Cytotoxicity Assay

Breast adenocarcinoma (MCF-7 and MDA-MB-231), human cervical carcinoma (HeLa), and African green monkey kidney (Vero) ATCC cells were used. The cells were cultivated in D-MEM (Invitrogen, México), supplemented by 10% fetal calf serum and antibiotics (100 units mL^{-1} penicillin and 100 pg mL^{-1} streptomycin), at 37 °C in a humid atmosphere containing 5% CO_2.

The cytotoxicity test [17] was conducted by using 96-well microtiter plates containing 2,500 cells per well. The cell suspensions were incubated for 24 h under the conditions mentioned above. After incubation 18.75, 37.5, 75, 150 and 300 µg mL^{-1} of each extract were added per well. The microplates were further incubated for 48 h and then 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich, USA) solution (5 mg mL^{-1}) was added to each well. After 4 h additional incubation, the liquid was discarded and replaced with 100 µL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) and the absorbance measured in a microplate reader (Multiskan Asent) at 550 nm. Each assay was carried out in three independent experiments. The cell viability in response to treatment was calculated using Origin 8.6 software. The selectivity index (SI) was calculated with the equation: SI = IC_{50}normal cells / IC_{50}cancer cells.

RESULTS AND DISCUSSION

The cytotoxic activity of hexane, ethyl acetate and n-butanol extracts from body walls and viscera of *Isostichopus badionotus* collected at the Yucatan Channel were tested *in vitro* against human tumor cell lines of cervix (HeLa) and breast adenocarcinoma (MCF-7 and MDA-MB-231). In order to evaluate the level of harmfulness of the extracts, the selectivity index was determined on African green monkey kidney normal cells (Vero). Results are showed in Table **1**.

Based on Manosroi *et al*. [18] screening, in the present study the activity of extract was ranked in two levels: high (CI_{50} ≤50 µg mL^{-1}), and low (CI_{50} 51-100 µg mL^{-1}), and the selectivity according to Vonthron-Sénécheau *et al*. [19] who consider that an SI ≥10 indicated that biological efficacy of the extract is not due to cytotoxicity.

The HeLa cell line was the most susceptible to non-polar extracts of body walls and viscera of *I. badionotus*, while MCF-7 and MDA-MB231 were not affected by any of the tested extracts with exception of the EtOAc extract, which inhibited the cellular growth at 105.4 and 101.2 µg mL^{-1}, respectively. The hexanic extract of body walls exhibited cytotoxic activity at 48.5 µg mL^{-1} and the one from viscera at 42.5 µg mL^{-1}. The polar extract (BuOH) did not show cytotoxic activity against the three cancer cell lines (HeLa, MCF-7, and MDA-MB231), while the normal cell line (Vero) was not susceptible to any extract. Most of the extracts showed selectivity indexes in the range of 1.0 – 3.8 with exception of hexane extracts, which exhibited selectivity indexes of 12 and 5.2 against HeLa cells, respectively.

According to the ranks described above, the cytotoxic activity of hexane extracts of body walls (48.2 µg mL^{-1}) and viscera (42.5 µg mL^{-1}) of *I. badionotus* against HeLa cells were considered high, while the cytotoxic activity of EtOAc extract of body walls was low (98.3 µg mL^{-1}).

Table 1: Cytotoxic Activity and Selectivity Index of Extracts from *Isostichopus badionotus*

Animal part	Extract	Cell lines IC_{50}[a] µg mL^{-1} ± SD[b] (*SI*[c])			
		HeLa	MCF-7	MDA-MB231	Vero
Body walls	Hex	48.5 ± 1.9 (12.0)	197.5 ± 11.5 (3.0)	152.5 ± 4.8 (3.8)	582.8 ± 21.5
	EtOAc	98.3 ± 5.0 (1.6)	105.4 ± 12.0 (1.5)	101.2 ± 12.1 (1.6)	158.2 ± 21.3
	BuOH	> 600	> 600	> 600	> 600
Viscera	Hex	42.5 ± 2.0 (5.2)	167.1 ± 10.0 (1.3)	152.8 ± 11.0 (1.4)	218.9 ± 13.7
	EtOAc	166.7 ± 8.9 (1.3)	204.7 ± 7.9 (1.0)	117.7 ± 7.9 (1.8)	209.3 ± 2.3
	BuOH	> 600	> 600	> 600	> 600
	Paclitaxel	0.08 ± 0.01 (15.5)	0.07 ± 0.02 (17.7)	0.03 ± 0.01 (41.3)	1.24 ± 0.01

[a]Inhibitory Concentration 50.
[b]Standard Deviation.
[c]Selectivity Index.

A study of the less polar lipid fraction of the chloroform–methanol extract from body walls of *Cucumaria frondosa*, a North Atlantic commercially harvested sea cucumber, resulted in the isolation of cerebrosides, which exhibited an antiproliferative effect on Caco-2 colon cancer cells (IC_{50} = 4.13 µg mL^{-1}) [20]. Probably, the cytotoxic activity of hexane extracts of viscera and body walls of *I. badionotus* could be explained by the presence of cytotoxic cerebrosides.

Although, the cytotoxic activity of non-polar extract of *I. badionotus* body walls was classified as high, its SI value was 12.0 unlike the other extracts which showed poor selectivity (1.0-5.2). Besides, it is remarkable that the selectivity showed by the hexane extract of body walls of *I. badionotus* was close to the SI of paclitaxel (15.5), the commercial anticancer drug used as positive control.

On the other hand, the lack of cytotoxic activity of BuOH extracts was unexpected because it is known that triterpene glycosides (saponins), commonly isolated from polar extracts of holothurians, shown cytotoxic activity which is related to membranotropic action due to the presence of an 18(20)-γ-lactone in the aglycon moiety in most cases, as well as a linear tetrasaccharide fragment. Moreover, oligoglycosides containing quinovose instead of glucose or xylose as second monosaccharide unit usually exhibited a high bioactivity [21].

Regarding the presence of saponins in *I. badionotus*, up today, only a mixture of D-xylosides of 3β-hydroxy sterols of the cholestane series has been isolated from the body walls of this species [22]. Therefore, the non-cytotoxic activity of the BuOH extracts of *I. badionotus* might be related with absence of saponins that fulfill the characteristics above described.

CONCLUSIONS

Only the non polar extracts from *I. badionotus* were considered significantly active and selective against human tumor cell lines of cervix (HeLa). The good selectivity index exhibited by the hexane extract of the body walls of *I. badionotus* encouraging further research on the isolation of the cytotoxic compounds.

On the other hand, the lack of cytotoxic activity of butanolic extracts might be related with absence of holostan-type triterpene glycosides.

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