STM JOURNALS

Research & Reviews: A Journal of Life Sciences
ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print)
Volume 8, Issue 2
www.stmjournals.com

Brine Shrimp Cytotoxicity was not Warrant for Teratogenicity: Comparative Analysis of Seaweed Methanolic Extracts Collected from Tamil Nadu Coastal Region on Brine Shrimp and Zebra Fish Embryos

Meenakshi Sundaram K.[1,2], Nagarajan M.[1], Sundararaman M.[1,*]
[1]Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
[2]Centre for Environment Research and Development, Loyola Institute of Frontier Energy, Loyola College, Chennai, Tamil Nadu, India

Abstract

Brine shrimps have been served as valuable toxicological model systems. Besides, they are being used as preliminary models to identify the effective bioactive drug candidates against different diseases including cancer in recent decades. In the present study, we comparatively analyzed twenty-eight seaweed methanolic extracts for their cytotoxicity and teratogenicity using brine shrimp nauplii and zebra fish embryos (ZFEs). All of the seaweed extracts killed brine shrimps, but, only, Ulva lactuca showed hatchability inhibition on developing ZFEs. The delayed-hatched nauplii were similar to controls in body length and heartbeat. The U. lactuca extract showed no acute toxicity on the hatched nauplii up to five days (240 hpf). The chromatographical fractionation of the U. lactuca extract resulted in neither hatching delay nor deformities. This study also implied that there was no warranty for the teratogenicity even they may possess the cytotoxicity. Recent studies showed that the embryos could be valuable models for cancer drug discovery and further selective analysis on U. lactuca could give a potential drug candidate for cancer therapy since, it showed selective inhibition on hatching.

Keywords: *Cytotoxicity, teratogenicity, hatching delay, Ulva lactuca, brine shrimps*

****Author for Correspondence*** E-mail: sundarbdu1@gmail.com

INTRODUCTION

The plant-based secondary metabolites serve as antipredating agents on targeted organisms and eventually, could be explored as the valuable therapeutic drugs in human [1]. Due to multiple target ability, they are termed as 'gentle medicines' with minimal required concentration due to pleiotropic effects [2]. Watson *et al*., reviewed the significance of plant polyhydroxylated alkaloids against various diseases such as cancer, Alzheimers', anti-diabetic, antimalarial etc. [3]. The recent advances in phytochemistry and pharmacognosy have enhanced the chances to analyze their action at molecular level scientifically [4, 5]. In addition to terrestrial plants, the seaweeds are also consumed in large proportion worldwide; but their toxic nature is scarcely found [6]. Mayer and Gustafson discussed the teratogenic role of *Sargassum* sp., *Dictyota* sp., *Turbinaria* sp., and *Padina* sp. on *Nasa lituratus* [7]. The role of seaweed secondary metabolites in the protection against UV radiation in zebra fish embryos (ZFEs) is was also reported due to their tyrosine inhibitory activity [8].

Many model organisms have been developed for analyzing their bioactive potential [9–11]. Besides others, *A. salina* nauplii (*Artemia* sp.,) are being used popularly for cytotoxic analysis and the ZFEs (*Danio rerio*) are used for teratogenicity studies in the present research advancement [12]. Since teratogenic effects of seaweed extracts on ZFEs are not much explored, the present study aims to comparatively analyse the methanolic extracts of seaweeds (28 nos.) commonly available in Tamil Nadu coastal region, Indian subcontinent. The present study is of its first kind to correlate the brine shrimp cytotoxicity with the teratogenicity of the seaweeds collected from the Tamil Nadu coastal region.

COLLECTION OF PLANT LEAVES AND STORAGE

The seaweeds were collected from different coastal regions of Tamil Nadu (Mandapam 9.28 °N 79.12 °E, Dhanushkodi (9.15 °N 79.44 °E), Pudumadam (9.28 °N 78.99 °E) in India (Table 1). They were carefully brought to the laboratory and washed thoroughly with running tap water followed by a brief wash in distilled water for removing all the debris. The algal samples were shade dried, powdered, extracted with 80% methanol and evaporated using vacuum. The crude alcoholic extracts were stored at 4 °C for analysis.

EXTRACTION AND CYTOTOXICITY ANALYSIS

Extraction of collected seaweed samples were carried out by previous methods using 80% methanol [13]. The *A. salina* (Ocean Star International Inc., USA) toxicity assay was adopted with minor modifications [14]. Different concentrations of test substances (100–500 $\mu g.ml^{-1}$), positive and vehicle controls treated nauplii were kept in tubes for 24 h at 28 °C. The percentage mortality was calculated using probit method. The percentage mortality between 1% and 99% was calculated from average number of died nauplii per concentration in 24 h. The 50% lethal concentration (LC_{50}) was plotted using probit method for each extract to analyze the effects of cytotoxicity.

TERATOGENICITY ASSAY BY USING ZEBRA FISH EMBRYO (ZFE)

Matured and healthy male and female Zebra fishes (*Danio rerio*) were bought locally and used for the embryo toxicity assay as previously mentioned [15–18]. The fertilized ZFEs were tested using Embryo medium (E3). The healthy ones were selected by discarding the nonfertilized, coagulated, eggs with ruptured chorion. The selected eggs were incubated in E3 medium with respective test substances (200, 400, 600, 800, 1000 and 2000 $\mu g.l^{-1}$) at 27°C. The study was designed with five replicates containing twenty ZFE each and DMSO served as a vehicle control (maximum concentration, 0.01%). The viability of the treated embryos was evaluated up to 120 hour postfertilization (hpf), i.e. up to early larval stage from the commencement of the experiment. The treated embryos were observed under a microscope (Olympus, 4–10X) to analyze the end points *viz*. yolk material coagulation, tail nondetached and somites undifferentiation. Randomly ten embryos from each replicate were photographed once in 24 h using Magnus MIPS software (http://www.magnusanalytics.com/downloads/MIPS%20Manual.pdf) to observe its morphological changes. The mortality, % hatchability, the % body (total) length reduction were calculated and the heart beat rate of each embryo was calculated by converting the time as 30 beats per minute (bpm) as previously reported method. The *U. lactuca* treated embryos were analyzed up to 144 hpf for all the above said parameters with the intervals of 96 and 120 hpf.

Purification and Identification of Active Compounds from *U. lactuca*

Crude extract of *U.lactuca* was dissolved in (1:10 ratio) distilled water and separated with petroleum ether, ethylacetate, butanol using 1:1 ratio, respectively. Then the individual fractions were concentrated and tested for bioactivity using *Artemia* and ZFE toxicity assays.

RESULTS

Cytotoxicity of Seaweed Extracts on *A. salina* Nauplii

A total of 28 seaweed extracts were tested for their cytotoxic nature on *A. salina* nauplii belonging to Chlorophyceae (eleven species), Phaeophyceae (ten species) and Rhodophyceae (seven species) families. Cytotoxicity of the selected extracts was varied and few of them showed comparatively lesser mortality on *A. salina*. But, most of them showed dose-dependent mortality with the following toxic symptoms such as losing balance, failed or felt difficulty in swimming and sinking to the bottom before dying or none of them (in case of nontoxic extracts) as compared to controls. The cytotoxic activity was considered to be low, if the LC_{50} was between $500<LC_{50}>1$ $mg.ml^{-1}$; moderate, if LC_{50} was between 500 and 100 $\mu g.ml^{-1}$; strong if LC_{50} ranged between 0 to 100 $\mu g.ml^{-1}$; and

designated as nontoxic when the LC_{50} value was greater than 1 $mg.ml^{-1}$. All seaweed extracts affected the nauplii on various levels from mild to severe; the mortality (%) was calculated on 24 h incubation and represented in Figures 1(a) and (b), by using their IC_{50}, respectively. The IC_{50} values for all seaweeds were calculated using probit analysis.

Developmental Toxicity of Seaweeds on Zebra Fish Embryos

The ZFEs are gaining popularity and creditability as a model for cancer and teratogenic research. In the present study, they were used to analyse the teratogenic effects of respective seaweed extracts from initiation of gastrula (3 hpf) to termination of hatching (72 hpf). The experiments were extended up to 120 hpf based on the requirements (in case of *U. lactuca*). The twenty-eight (28) seaweed extracts were tested by using different concentrations (*viz*., 50, 100, 150, 200, 250, 300, 400, 500, 1000 and 2000 $\mu g.ml^{-1}$; in case of *U. lactuca* 3000 $\mu g.ml^{-1}$). All the embryos were treated separately with negative and vehicle (DMSO) controls and they fulfilled the acceptance criteria, *viz*., ≥ 90% hatching rate and ≤ 0% teratogenic effects.

The teratogenicity endpoints (malformation of head, tail, or heart, scoliosis, deformity of yolk, growth retardation, coagulation, tail extension, spontaneous movement, heartbeat, pigmentation, eye development and pericardial edema) were evaluated under a microscope at subsequent intervals up to 72 hpf. Of the 28 seaweed extracts, except *U. lactuca*, all other extracts did not show any malformation or delayed hatching in the tested ZFE (Tables 2 and 3). These results implied that those methanolic extracts might not contain teratogenic compounds to render the teratogenicity in the given concentration (50–2000$\mu g.\ ml^{-1}$).

Malformation Effects of *U. lactuca* Extract on the Developing Zebra Fish Embryos

(i) Effects on Hatchability

The ZFEs were evaluated for finding out the teratogenic effects of *U. lactuca* (50–3000 $\mu g.\ ml^{-1}$) at 28 °C for 3–144 hpf. DMSO was used as vehicle to make aliquots concentrations and it showed neither mortality nor developmental toxicity on ZFE for 120 hpf (the highest concentration [0.01%] in the study) and they were hatched at 72–78 hpf. The embryos at 50– 250 $\mu g.ml^{-1}$ of *U. lactuca* extract hatched similarly as control. The extract delayed the ZFE hatchability 500 $\mu g.\ ml^{-1}$onwards. As a result many of the *U. lactuca* treated embryos were unable to shed off their chorion at 72 hpf (Figure 2). The hatchability delay (or inhibition) was calculated (%) as compared to the control and found to be increased with the increasing concentrations. At 2000 $\mu g.\ ml^{-1}$ the embryos had their respective chorion even up to 144 hpf (Figure 3).

(ii) Effects on Lethal or Sublethal End Points

U. lactuca extract showed no significant lethal or sublethal effects (*viz*. pericardial edema, hemorrhage etc.,) between 100 and 3000 $\mu g.ml^{-1}$ as compared to the control (Table 3).

(iii) Effects on Body Curvature

The body length (mm) of the treated and control (vehicle) embryos were measured at 120 hpf. The controls and treated embryos did not show any prominent body length variations. DMSO-treated embryos (control) exhibited normal morphology with straight trunk and tail; similarly, the embryos treated with the above mentioned *U. lactuca* extract showed delayed pigmentation as compared to control (Data not shown). The photographs of selected individual embryos are displayed in Figure 2.

(iv) Effects on Heart Beat

Heart beat rates (HBRs) were videographed and counted on the treated ZFE on 96 h and 120 h in treated *U. lactuca* extract in ZFEs and control embryos. There was no variation or reduction in HBR in treated embryos (Table 2).

Purification and Identification of Active Compounds from *U. lactuca*

Among the tested 28 seaweeds, *U. lactuca* extract showed cytotoxicity on BST and hatchability inhibition on ZFE. Further, *U. lactuca* was partitioned with the petroleum ether (PE), ethylacetate (EtoAc), butanol (BU) and aqueous (Aq). The fractions were concentrated using vacuum evaporator and tested for the bioactivities by using *Artemia*

and ZFE bioassay. The fractions were tested by different concentration, i.e., 0, 100, 150, 200 and 250 µg.ml^{-1} on *Artemia.* All of the tested fractions showed toxicity on *Artemia*. EtoAC fraction showed highest activity on *Artemia.* Interestingly, none of them showed any toxicity on *D. rerio* (Table 4). Since, this study aimed to isolate the teratogenic compounds from the extracts, the *U. lactuca* fractions had not been carried for further analysis, due to the absence of bioactivity on *D. rerio*.

DISCUSSION

In the present study twenty-eight seaweeds were collected from different site of Tamil Nadu coastal region and screened for their cytotoxicity and teratogenicity using brine shrimp and ZFEs, respectively. Our results indicated that the cytotoxicity on brine shrimp was not warranty for the teratogenicity in the ZFEs. These methods allow us to use minimal extract quantities and to process maximum samples number within a short time.

High-throughput toxicity analyses gathering great attention towards medicinally valuable secondary metabolites using *in vitro* and *in vivo* based assays for biological characterization [19]. Among them, the brine shrimp are easy and reliable models for analyzing the cytotoxicity of the given drug candidates [9]. The zebra fishes combine the relevance of a vertebrate with the scalability of an invertebrate; and can be considered as whole mammalian organism due to its higher relevancy in cellular nature [20]. Anticancer activities of natural products and brine shrimp cytotoxicity have been discussed in a number of publications [21–23] and we tried to correlate with teratogenicity on ZFEs with artemial cytotoxicity. In this study, we assessed 28 seaweeds methanolic extracts toxicity by using two models viz., brine shrimp nauplii and ZFEs.

The aquatic organisms have more tendencies to detoxify most of the toxicants on moderate concentrations. The failure may cause direct damage to epithelial cells of gills or possible destruction of liver (in case of higher invertebrates and vertebrates, but gills in lower phyla) and internal asphyxiation may account for the rapid mortality [24]. *A. salina* is used as better toxicological models. Meyer *et al.*, reported three different classes of toxicity such as highly toxic (0.1 mg. ml^{-1} > LC_{50} < 0.5 mg. ml^{-1}), moderate toxic (0.5 mg. ml^{-1} < LC_{50} > 1 mg. ml^{-1}) and nontoxic (LC_{50}> 1 mg. ml^{-1}), respectively [25]. In the present study, the seaweed extracts showed higher toxicity to *Artemia* as their IC_{50} values were less than 500 µg.ml^{-1} (Table 4). Potassium dichromate was used as positive control (LC_{50} -54.5 µg.ml^{-1}). Previous studies also reported the brine shrimps cytotoxicity on seaweed extracts from Mandapam region [26, 27].

Zebra fishes genome have 90% resemblance to the human genome and could be used as a valuable model system for mammalians [20]. In the previous studies [28, 29], ZFEs were used without eliminating their chorion membranes for the teratogenicity analysis of cyanobacterial and *Ecklonia cava* extracts, respectively. In accordance with their studies, we can conclude that the unknown compound(s) of *U. lactuca* methanolic extract can penetrate the chorions and render the hatchability inhibition in the incubated developing ZFEs.

This result also emphasized that the presence of active compound(s) in *U. lactuca* extract more specifically affected the developing embryos at lower concentrations than the nauplii without any deformity in post larva. Similarly, Sun *et al.* reported that tamoxifen affected the hatching rate, but not rendered any morphological deformalities in Japanese medaka (*Oryzias latipes*) eggs [29].

In our study, except *U. lactuca,* other seaweeds had not shown any deformity or hatching delay on the developing ZFEs. In the mean time, all of the seaweed extracts showed severe cytotoxicity on brine shrimps. These results attributed that there is no warranty for the teratogenicity of seaweed extracts though they had shown higher cytotoxicity on the brine shrimp nauplii.

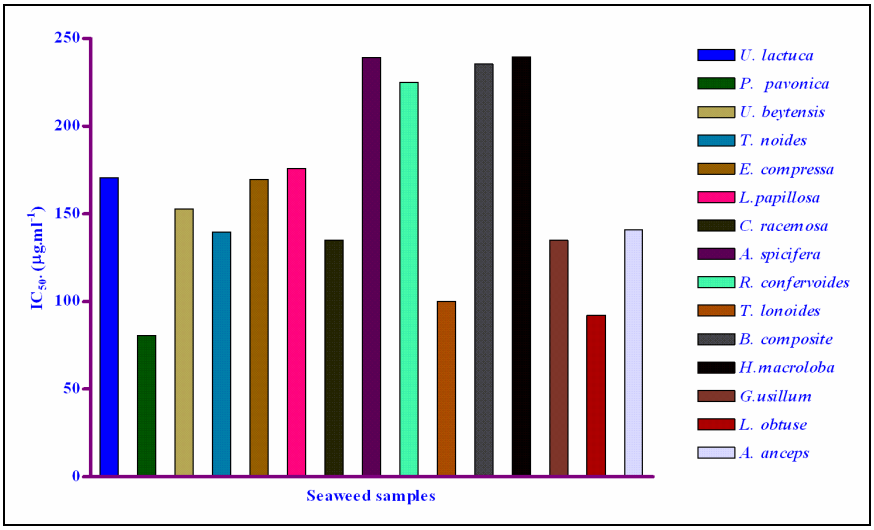

Fig. 1: *(a) Cytotoxic Effect of the Seaweed Extracts on A. salina Nauplii.*

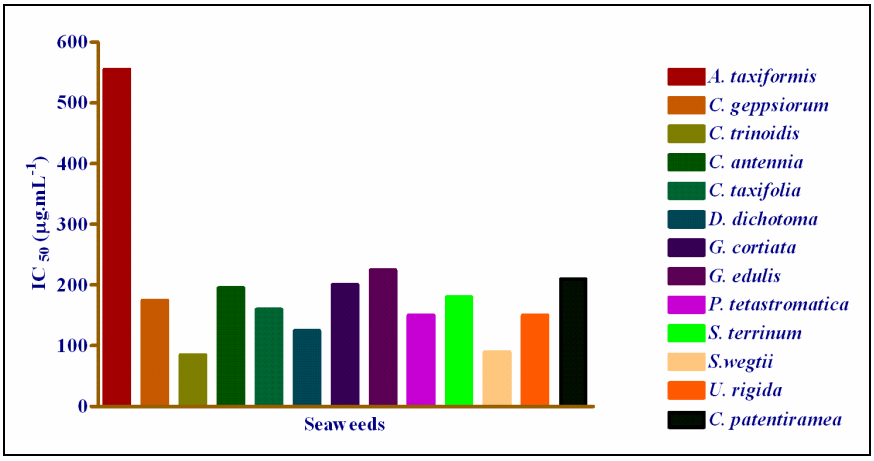

Fig. 1: *(b) Cytotoxic Effect of the Seaweed Extracts on A. salina Nauplii.*

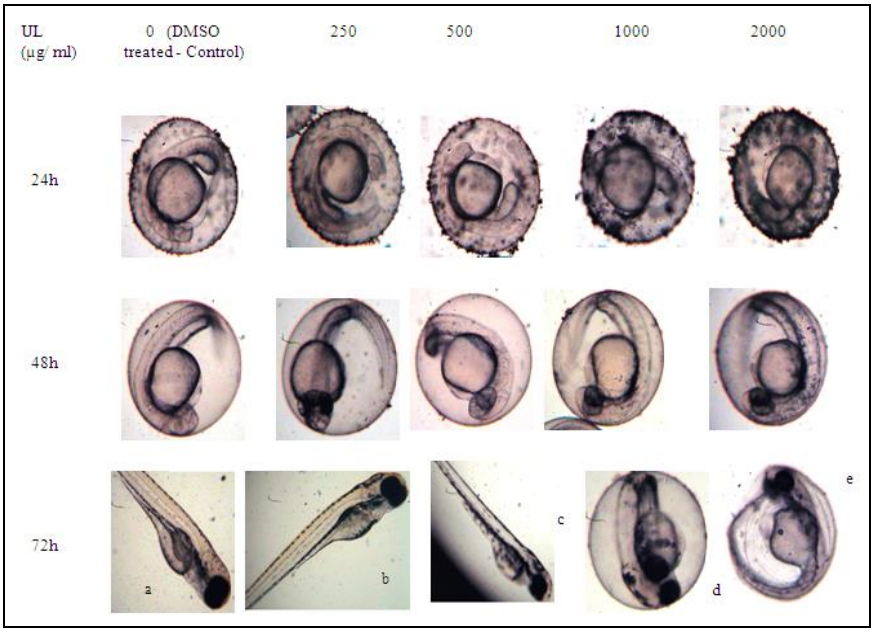

Fig. 2: *Hatching Delay Effect of U. lactuca on the Treated Zebra Fish Embryos (Since, other Extracts did not show any Toxic Symptom on ZFE, they are not Shown in Photographs).*

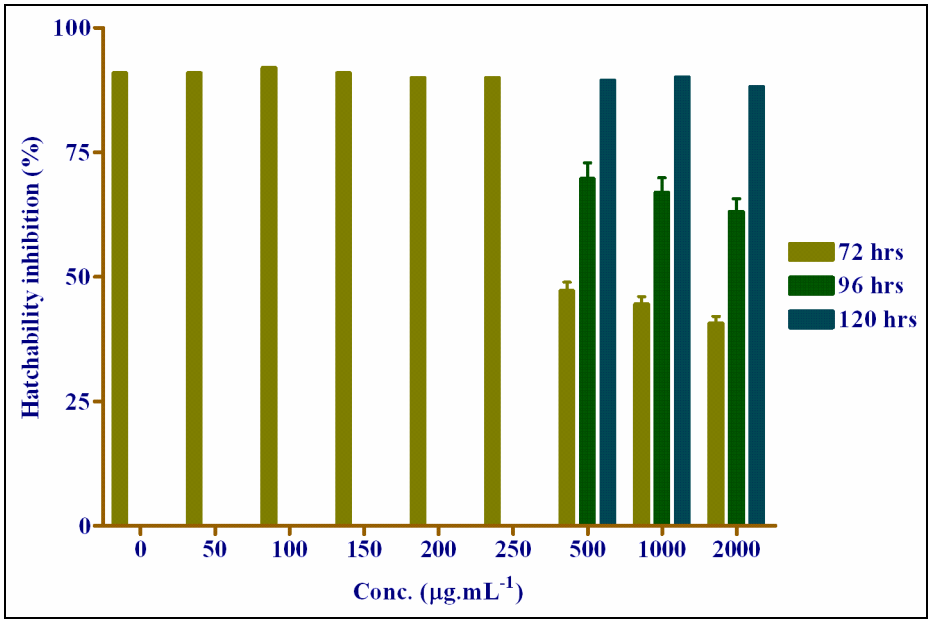

Fig. 3: *Hatching Delay Effect of U. lactuca on the Treated Zebra Fish Embryos (Legend: Number of Eggs (n) = 100).*

Table 1: *The Selected Seaweeds Collected along Tamil Nadu Coastal Region for Analyzing Their Toxic Properties.*

S. N.	Name of the seaweed	Food property	Place of collection
1.	*Acanthophora spicifera*	Edible	Pudumadam, Ramanathapuram District, Tamil Nadu
2.	*Amphiroa anceps*	Edible	Pudumadam
3.	*Asparagopsis taxiformis*	Edible	Mandapam, Ramanathapuram District, Tamil Nadu
4.	*Boodlea composite*	Nonedible	Pudumadam
5.	*Caulerpa racemosa*	Edible	Mandapam
6.	*C. taxifolia*	Edible	Mandapam
7.	*Chaetomorpha antennia*	Edible	Mandapam
8.	*Cladophora patentiramea*	Nonedible	Mandapam
9.	*Codium geppsiorum*	Edible	Mandapam
10.	*Cystoseira trinodis*	Nonedible	Pudumadam
11.	*Dictyota dichotama*	Edible	Mandapam
12.	*Entreomorpha compressa*	Edible	Dhanushkodi, Ramanathapuram District, Tamil Nadu
13.	*Gelidium pusillum*	Edible	Mandapam
14.	*Gracilaria cortiata*	Edible	Mandapam
15.	*G. edulis*	Edible	Mandapam
16.	*Helimeda macroloba*	Edible	Pudumadam
17.	*Laurencia obtuse*	Nonedible	Mandapam
18.	*L. papillosa*	Nonedible	Mandapam
19.	*Padina tetrastromatica*	Nonedible	Mandapam
20.	*P. pavonica*	Nonedible	Mandapam
21.	*Rhodomela confervoides*	Edible	Mandapam
22.	*Sargassum wegtii*	Edible	Mandapam
23.	*S. terrinum*	Edible	Mandapam
24.	*Turbinaria canoides*	Nonedible	Mandapam
25.	*T. lonoides*	Nonedible	Pudumadam
26.	*Ulva beytensis*	Edible	Mandapam
27.	*U. rigida*	Edible	Mandapam
28.	*U. lactuca*	Edible	Mandapam,

Table 2: *Malformation Effects of Methanolic Extracts of Seaweeds Except U. lactuca.*

S. No.	End points	Conc. (mg. mL^{-1})	
		Control	**Seaweed extracts***
1.	Coagulation (within 15 hpf) *	Absent	Absent
2.	Tail extension*	Normal	Normal
3.	Spontaneous movement*	Normal	Normal
4.	Heart beat*	Normal	Normal
5.	Pigmentation*	Normal	Normal
6.	Eye development*	Normal	Normal
7.	Pericardial edema*	Absent	Absent
8.	Hatchability (%)*	Normal to control	
9.	Body length (%)*	Normal to control	

Legend: **Extracts denoted all extracts except, U. lactuca in plant and seaweed, respectively and their effects were observed under light microscope (4X). The control indicates vehicle control (DMSO, 0.01–0.1%) and the water control, since, no teratogenic effects were observed on them.*

Table 3: *Effect of Seaweed Methanolic Extract of U. lactuca on the Hatchability of the Zebra Fish Embryos.*

S. No.	Lethal end point				Conc. (µg.ml^{-1})		
	200	400	600	800	1000	2000	3000
Tail extension	N	N	N	N	N	N	N
Spontaneous movement	N	N	N	N	N	N	N
Pigmentation	N	N	N	N	N	N	N
Eye development	N	N	N	N	N	N	N
Pericardial edema	A	A	A	A	A	A	A
Spinal deformation	A	A	A	A	A	A	A
Lordosis	A	A	A	A	A	A	A
Cyphosis	A	A	A	A	A	A	A

Table 4: *Purification and Identification of Active Compounds from U. lactuca.*

S. No.	Fraction	Retrieval of compound (g)	Tested conc. (µg.ml^{-1})		IC_{50} (µg.ml^{-1})	
			A. salina	*D. rerio*	*A. salina*	*D. rerio*
1.	Crude extract (methanolic)	35	-	-		4355.36
2.	PE	18	-	-	153.7	-
3.	EtoAc	8	50	50	76.4	-
4.	BU	4	50	-	52.0	-
5.	Aq	3	-	-	6104.5	-

CONCLUSION

This preliminary study revealed that the respected seaweed extracts showed any teratogenic activity on developing ZFEs except, *U. lactuca*. Our preliminary analysis showed that the brine shrimp toxicity could not be correlated with the teratogenicity of those selected seaweed extracts. Further study need to be conducted to confirm these preliminary results.

ACKNOWLEDGMENTS

The authors acknowledge the generous funding from the University Grant Commission (UGC), Government of India as Fellowship for Meritorious Scholar (UGC Non

SAP.4-1/2008(BSR)/11. 55/2008(BSR)). The authors thank the Bharathidasan University, Tiruchirappalli, India, for providing the lab facilities. We also remember and thank the Management of Loyola College, Chennai, India, for giving the clerical facilities.

REFERENCES

1. Konno K. Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. *Phytochemistry*. 2011; 72: 1510–30p.
2. Efferth T, Li PC, Konkimalla VS, *et al*. From traditional Chinese medicine to rational cancer therapy. *Trends Mol Med*. 2007: 13: 353–61p.
3. Watson AA, Fleet GW, Asano N, *et al*. Polyhydroxylated alkaloids—natural occurrence and therapeutic app*lications. Phytochemistry*. 2001; 56(3): 265–95p.
4. Albuquerque UP, Ramos MA, Melo JG. Newstrategies for drug discovery in tropical forests based on et*hnobotanical a*nd *chemical ecol*ogical studies. *J Ethnopharmacol*. 2012; 140: 197–201p.
5. Bienert MD, Siegmund SE, Drozak A, *et al*. A pleiotropic drug resistance transporter in *Nicotianatabacum* is involved *in defense again*st the herbivo*re Manduca sexta. Plant J*. 2012; 72: 745–57p.
6. Jeeva K, Sagayaraj. Nymphicidal and ovipositional efficacy of seaweed *Sargassum tenerrimum (J. Agard*h) against *Dysdercus cingulatus* (F*ab.) (Pyrrhocoridae). Chilean JAR*. 2012; 72: 152–6p.
7. Mayer A, Gustafson KR. Marine pharmacology in 2000: Antitumor and Cytotoxic compounds. *Int J Cancer*. 2003; 105: 291–9p.
8. Cha SH, Ko CI, Kim D, *et al*. Protective ef*fects of* phlorotannins against ultraviolet B radiation in zebrafish (*Danio rerio*). *Vet Dermatol*. 2012; 23: 51–e12p.
9. Castillo G, Schäfer L. Evaluation of a bioassay battery for water toxicity testing: A Chilean experience. *Environ Toxicol*. 2000; 15: 331–7p.
10. Fochtman P, Raszka A, Nierzedska E. The use of conventional bioassays, microbiotests, and some “rapid” methods in the selection of an optimal test battery for the assessment of pesticides toxicity. *Environ Toxicol*. 2000; 15: 376–84p.
11. Wu C. An important player in brine shrimp lethality bioassay: The solvent. *J Adv Pharm Technol Res*. 2014; 5: 57–8p.
12. Brannen KC, Panzica-Kelly JM, Danberry TL, *et al*. Development of a zebrafish embryo teratogenicity assay and quantitative prediction model. *Birth Defects Res B Dev Reprod Toxicol*. 2010; 89: 66–77p.
13. Harborne J. Methods of plant analysis. In: *Phytochemical methods*. Germany: Springer; 1973.
14. Carballo JL, Hernández-Inda ZL, Pérez P, *et al*. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnology*. 2002; 2: 17p.
15. Cerda GA, Thomas JE, Allende ML, *et al*. Electroporation of DNA, RNA, and morpholinos into zebrafish embryos. *Methods*. 2006; 39: 207–11p.
16. Lam KS. New aspects of natural products in drug discovery. *Trends Microbiol*. 2007; 15: 279–89p.
17. Lee JH, Park S, Hossain M, *et al*. 2,3,6-Tribromo- 4,5- dihydroxybenzyl Methyl Ether Induces Growth inhibition and apoptosis in MCF-7 human breast cancer cells. *Arch Pharm Res*. 2007; 30: 1132–7p.
18. Weigt S, Huebler N, Strecker R, *et al*. Zebrafish (*Danio rerio*) embryos as a model for testing proteratogens. *Toxicology*. 2011; 281: 25–36p.
19. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol Adv*. 2015; 33(8): 1582–614p.
20. Goldsmith P. Zebrafish as a pharmacological tool: the how, why and when. *Curr Opin Pharmacol*. 2004; 4: 504–12p.
21. Glinma B, Kpoviessi SD, Gbaguidi FA, *et al*. Trypanocidal and cytotoxic evaluation of synthesized thiosemicarbazones as potential drug leads against sleeping sickness. *Mol Biol Rep*. 2014; 41: 1617–22p.

22. Krishnan GS, Sebastian D, Savarimuthu I, *et al*. *In vitro* and *in silico* anticancer effect of combined crude acetone extracts of *Plumbago zeylanica* L., *Limonia acidissima* L. and *Artocarpus* heterophyllus Lam. *Synergy*. 2017; 5: 15–23p.
23. Muthukrishnana S, Senthil T, Rao KMV. Anticancer activity of biogenic nanosilver and its toxicity assessment on *Artemia salina*—evaluation of mortality, accumulation and elimination: An experimental report. *J Environ Chem Eng*. 2017; 5(2): 1685–95p.
24. Valerio E, Chaves S, Tenreiro R. Diversity and impact of prokaryotic toxins on aquatic environments: a review. *Toxins* (*Basel*). 2010; 2: 2359–410p.
25. Meyer BN, Ferrigni NR, Putnam JE, *et al*. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*. 1982; 45: 31–4p.
26. Ragupathi RKR, Arumugam R, Iyapparaj P, *et al*. *In vitro* antibacterial, cytotoxicity and haemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India, *Food Chem*. 2013; 15136(3–4): 1484–9p.
27. Murugan K, Iyer VV. Antioxidant and Antiproliferative Activities of Extracts of Selected Red and Brown Seaweeds from the Mandapam Coast of Tamil Nadu. *Food Chem*. 2017; 38(1): 92–101p.
28. Wright AD, Papendorf O, Konig GM, *et al*. Effects of cyanobacterium *Fischerella ambigua* isolates and cell free culture media on zebrafish (*Danio rerio*) embryo development. *Chemosphere*. 1006; 65: 604–8p.
29. Wijesinghe W, Kim EA, Kang MC, *et al*. Assessment of anti-inflammatory effect of 5β-hydroxypalisadin B isolated from red seaweed *Laurencia snackeyi* in zebrafish embryo *in vivo* model. *Environ Toxicol Pharmacol*. 2014; 37: 110–17p.
30. Sun L, Zha J, Spear PA, *et al*. Tamoxifen effects on the early life stages and reproduction of Japanese medaka (*Oryzias latipes*). *Environ Toxicol Pharmacol.* 2007; 24: 23–9p.

Cite this Article
Meenakshi SK, Nagarajan M, Sundararaman M. Brine shrimp cytotoxicity was not warrant for teratogenicity: Comparative analysis of seaweed methanolic extracts collected from Tamil Nadu coastal region on brine shrimp and zebra fish embryos. *Research & Reviews: A Journal of Life Sciences*. 2018; 8(2): 24–32p.