



## Short Communication

# Cytotoxic Potential of Fertility Booster Medicinal Plants as Evident through Brine Shrimp Toxicity Assay

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### ABSTRACT

The study was designed to check the toxicity of five fertility enhancing medicinal plants viz root of sweet flag, seed of radish, root of land-calotrops, leaves of peppermint and flower of red cabbage through brine shrimp toxicity assay. Brine shrimp eggs hatching was optimized by applying three temperature levels (25°C, 27°C and 29°C), three salinities (10 ppt, 30 ppt and 35 ppt), two food demands (0.8 g and 1.6 g) and two levels of shrimp egg (1 g/l and 1.5 g/l). Five concentrations (1200, 2500, 5000, 10000 and 15000 µg/ml) of aqueous extracts of five fertility enhancing plants were tested for 24 h incubation to check mortality of brine shrimp nauplii. Mean comparison of brine shrimp cytotoxicity assay revealed noticeable deviations from the both positive and negative controls. Alive percentage of nauplii observed in the aqueous extract of sweet flag root and radish seed ranged from 0 to 10%, land-calotrops root extract from 0 to 22.5%, peppermint leaves extract from 3 to 47.5% and for red cabbage extract ranged from 0 to 15%. Dose dependent death rate was only observed in red cabbage extract while other plant extracts given random death rate of nauplii as compare to +ve control (Ethyle methane sulphonate). Therefore, it can be finally concluded that all tested plants were toxic to brine shrimp, however comparatively higher toxicity was observed by flag root extract.

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### Authors' Contribution

AN Soomro planned and managed the experiments. AN Saddar conducted the research. STQ helped in writing the manuscript. SSH helped in material identification. MAM supported for statistical analysis.

### Key words

Cytotoxicity, Fertility booster, Brine shrimp, Mortality

Approximately 48.5 million couples are affected with infertility worldwide (Mascarenhas *et al.*, 2012; Agarwal *et al.*, 2015). The frequency of infertility in Pakistan is approximately 22%, where primary infertility is 4% and secondary infertility is 18% (Ali *et al.*, 2011). To overcome this major problem, both male and female use usually plant based medicines (Jaradat and Zaid, 2019). In Pakistan, especially in Sindh the mostly used fertility enhancers are sweet flag, radish, land-calotrops, peppermint and red cabbage (Rahman *et al.*, 2011). These plants are known to possess fertility enhancing substances, but some of their compounds like eugenol, sitosterol, saponin, menthol and glucosinolate are reported cytotoxic to normal and human cancer cell lines (Chauhan *et al.*, 1992; Burel *et al.*, 2001; <https://www.poisson.org/>; Kijpornyongpan *et al.*, 2014). Most often cytotoxicity of these therapeutic herbs is not evaluated before use. It is therefore, extremely essential to screen herbs for cytotoxicity.

Brine shrimp cytotoxicity assay is preferred among other animal cytotoxicity assays because of its ease

handling, quickness and inexpensiveness (Mayorga *et al.*, 2010; Insanu *et al.*, 2012; Veni and Pushpanathan, 2014; Elyana *et al.*, 2016; Kosasih *et al.*, 2019). The most crucial hatching parameters of brine shrimp eggs are temperature, salinity, feed and shrimp egg quantities that drastically delay hatching process. Quantity of optimized parameters not only play major role during hatching stage but also during the assay (Vanhaecke and Sorgeloos, 1989; Wasonga and Olendi, 2014; <https://www.oceannutrition.com/>). Present study is designed to predict prospective cytotoxicity of five fertility enhancing medicinal plant parts viz. root of sweet flag, seed of radish, root of land-calotrops, leaves of peppermint and flower of red cabbage using brine shrimp toxicity assay.

### Materials and methods

Three temperatures (25°C, 27°C and 29°C), three levels of salinity (10 ppt, 30 ppt and 35 ppt), two food demands (0.8 g and 1.6 g) and two shrimp egg quantities (1 g/l and 1.5 g/l) were tested. All the mentioned hatching parameters were applied in combinations.

Dried plant parts were purchased from local market of Hyderabad, dried in electric oven and then ground with pestle and mortar and electric grinder. Different

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concentrations (1200, 2500, 5000, 10000 and 15000 µg/ml) of plant aqueous extracts were prepared in vials. Ten alive nauplii was added and then the final volume was raised up to 5 ml by adding artificial sea water (35 ppt) according to the described procedure followed by Memita *et al.* (2018) and Braguini *et al.* (2018). Artificial sea water (5 ml) vial was used as negative control and 1000 µg/ml EMS (ethyl methane sulphonate) in sea water was used as positive control. After 24 h all vials were inspected with torch/ magnifying glass and surviving nauplii number were counted. Toxicity of plant aqueous extract was checked by mean percentage of alive and dead brine shrimp nauplii. Experiment was repeated thrice and the data represented as Mean±SD. Death and alive rate of brine shrimp nauplii was calculated by using following formulae (Arulvasu *et al.*, 2015).

$$\text{Death \%} = \frac{\text{No. of dead brine shrimps}}{\text{Total no. of brine shrimps}} \times 100$$

$$\text{Alive \%} = \frac{\text{No. of alive brine shrimps}}{\text{Total no. of brine shrimps}} \times 100$$

### Results and discussion

In optimization highest hatching percentages (95 and 91) were found in 35ppt salinity with 27°C having 1 and 1.5 g egg of brine shrimp with 0.8 g food, lowest hatching % (8 and 14) were observed in 35 and 10 ppt on 25°C with 0.8 g and 1.6 g food, respectively though 35 ppt on 29°C hatched eggs moderately (Table I). Mean alive percentage of brine shrimp nauplii is compiled in Table II plant wise. Total alive nauplii observed in sweet flag root and radish seed extract were ranged from 0 to 100%, it means both plants induced nauplii death percentage from 90 to 100. Alive nauplii percentage witnessed in land-calotrops root aqueous extract ranged from 0 to 22.5% with death rate from 77.5 to 100%. In peppermint leaves aqueous extract 3 to 47.5% of alive nauplii observed with 52.5 to 97% death rate. In red cabbage flower aqueous extract alive percentage of brine shrimp nauplii was observed from 0 to 15 with 85 to 100% death rate. Out of all tested fertility enhancing plants sweet flag and radish seed were more cytotoxic. Dose dependent death effects were found in red cabbage, in its minimum concentration death rate was 85% and as the concentration increases the death percentage was about 90, 92.5 and 100%. While other tested plants effect was random, this may be due to high toxic potential of these extracts. Negligible death rate was observed in negative control indicating cytotoxic potential of different chemicals present in plant extracts.

Sweet flag root medium concentration (5000µg/ml) given lowest death rate, curve fluctuates randomly (Table II). Results supported by the work of number of researchers (Akeem *et al.*, 2017). These scientists observed brine shrimp random mortality mediated by

anti-malarial plants and random mitotic inhibition by anti typhoid in *Allium cepa* root tip cells, indicates low cytotoxic potentials of plant extracts. Sweet flag induced cytotoxicity may be because of eugenol and sitosterol as reported in independent studies on human cancer cell lines. Eugenol is proved hepatotoxic in previous studies as it exhausts cytoprotective thiols and interference in thiol-dependent processes to maintain lipid-based membrane integrity. Furthermore, the toxic effects of eugenol are more pronounced when it is converted to quinine methide metabolite (Thompson *et al.*, 1998). Membrane damages by eugenol are also reported in human studies as a causative agent of stomatitis that is inflammatory process affecting oral mucous membranes sometimes conversion in to oral cancer (Deshpande *et al.*, 2014). Exposure to 0.7 mmol l<sup>-1</sup> of sitosterol for 72 h can cause contraction of the

**Table I. Brine shrimp eggs hatching percentage by different temperatures, salinities, food levels and eggs concentration.**

Temperature	Salinity (ppt)	Food (g)	Brine shrimp eggs (g/l)	Hatching %	
25°C	10 ppt	0.8	1	24	
			1.5	43	
		1.6	1	14	
			1.5	32	
		30 ppt	0.8	1	41
				1.5	52
	35 ppt	1.6	1	50	
			1.5	51	
		0.8	1	8	
			1.5	25	
		1.6	1	60	
			1.5	63	
27°C	10 ppt	0.8	1	54	
			1.5	49	
		1.6	1	28	
			1.5	19	
		30 ppt	0.8	1	55
				1.5	47
	35 ppt	1.6	1	48	
			1.5	51	
		0.8	1	95	
			1.5	91	
		1.6	1	60	
			1.5	50	
29°C	10 ppt	0.8	1	40	
			1.5	17	
		1.6	1	44	
			1.5	32	
		30 ppt	0.8	1	31
				1.5	45
	35 ppt	1.6	1	55	
			1.5	56	
		0.8	1	73	
			1.5	69	
		1.6	1	65	
			1.5	55	

endothelial cells and increase the release of intracellular lactate dehydrogenase type A (LDHA) in human being (Boberg *et al.*, 1991). Elevated level of LDHA is molecular indicator of carcinogenesis as evident in many malignant tumors and a strong suspect behind tumor proliferation and malignant growth (Miao *et al.*, 2013).

Radish seed aqueous extract induced toxicity pattern was low to high against brine shrimp nauplii but its two concentrations given similar death rate (Table II). It may be because of the presence of glucosinolates. Glucosinolate is natural compound found in radish and red cabbage. It is more toxicant after converting into secondary metabolites (isothiocyanate followed by thycinates, oxazolidinethiones and nitriles (Burel *et al.*, 2001). Effect of land-calotrops aqueous extract on brine shrimp toxicity varies by different concentration; medium concentration (5000 µg/ml) was less toxic to brine shrimp. Saponin is main compound of land-calotrops, its direct and indirect action may be related with toxicity of different organisms. Toxicity of saponins to warm blooded animals varies with type of plant species as each contains different amounts, proportion of different saponins in mixture and of course frequency of intake (George, 1965; Okenfull and Sidhu, 1990). Fishes and cold-blooded animals also affected by saponins toxicity (Leistner and Drewke, 2010). Saponins (steroid glycosides) although are only absorbed in traces and their toxic effect is confined to the intestinal tract but its latent effects generate secondary metabolites with zinc and iron molecules, thus limiting their bioavailability (Chauhan *et al.*, 1992). In addition, this plant extract also contains alkaloids like  $\alpha$  and  $\beta$ -asarone that may contribute final cytotoxicity. Peppermint aqueous extract exerted indirect effect on brine shrimp toxicity, low concentration was more toxic and high concentration was less except (5000 µg/ml) concentration that given more lethal percent than 10000 µg/ml concentration (Table II). It may be due to activation of secondary metabolites. The cytotoxic effects of peppermint can be correlated with menthol that can cause cell death by unbalancing cytosolic Ca<sup>2+</sup> levels (Kijpornyongpan *et al.*, 2014). Menthol possesses ability to integrate and disrupt membrane structure and function through calcium channels according to a bacterial study (Landau and Shapira, 2012). It can cause systemic toxicity to humans after swallowing or inhaling concentrated amounts. Its serious effects include seizures, coma, and death (<https://www.poison.org/>).

Hepatotoxicity and nephrotoxicity in rats were described by *Mentha piperita* and *mentha spicata* due to imbalance hormone concentration; high level changes in germ cell captured meiosis (Akdogan *et al.*, 2004). Cytotoxic effects of flavonoids found in peppermint in various cancer cells are well accepted (Park *et al.*, 2015; Sak and Everaus, 2015).

**Table II. Brine shrimp cytotoxicity induced by fertility booster medicinal plants aqueous extract.**

Treatment with aqueous extract	Concentration (µg/ml)	% of alive nauplii (mean±SD)
-ve Control (Artificial seawater)	0	95±0.5
Sweet flag root	1200	5±0.5
	2500	3±0.5
	5000	10±0.8
	10000	00±0
	15000	00±0
Radish seed	1200	10±0
	2500	7.5±0.9
	5000	2.5±0.5
	10000	2.5±0.5
	15000	00±0
Land-calotrops root	1200	5±0.5
	2500	5±0.8
	5000	22.5±2.87
	10000	7.5±1.5
	15000	00±0
Peppermint leaves	1200	3±0.5
	2500	10±1.41
	5000	42.5±2.06
	10000	47.5±0.95
	15000	50±1.4
Red cabbage flower	1200	15±1.29
	2500	10±0.81
	5000	7.5±1.5
	10000	00±0
	15000	00±0
+ve control (EMS)	1000	00±0

No, Number; SD, Standard deviation.

Red cabbage aqueous extracts induced cytotoxicity to test organism may be due to presence of cadmium common heavy metal of brassicaceae family that is reported to cause hepatotoxicity in animal tissue leading to nephrotoxicity. The probable reason of liver injury in rats was lipid peroxidation that consequently caused membrane damage altering permeability of membrane (Renugadevi and Prabu, 2010). Siddiqui (2010) revealed 30 days regular oral intake of CdCl<sub>2</sub> solution (in drinking water) causes the secretion of low and high molecular proteins which cause damages in renal cortex, proximal tubules membrane, cell nuclei and blood vessels. Observed results are supported by previous test of ethanolic extract of *Moringa olifera* (drumstick tree) leaves, methanolic extract of *Barringtonia acutangula* L. (Hijal) leaves (Assaduzaman *et al.*, 2015).

### Conclusion

Temperature is the most sensitive parameter for brine shrimp eggs hatching among others. Brine shrimp larvae were most sensitive to sweet flag root and least to peppermint. Red cabbage flower aqueous extracts induced

concentration dependent cytotoxicity to test organism. All tested plant parts were highly toxic to brine shrimp larvae; therefore, it is essential to test plants at lower concentration by modern techniques to get semi lethal concentration.

#### Statement of conflict of interest

The authors have declared no conflict of interest.

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