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Hydra as an alternative model organism for toxicity testing: Study using the endocrine disrupting chemical Bisphenol A

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ABSTRACT

In the recent times use of vertebrate animal models in the toxicity testing of chemical entities has come under criticism and surveillance in view of lack of relevance as surrogates to humans and also ethical considerations. Though *in vitro* and *in silico* tools can obviate animal models in toxicity testing, use of some simple organisms, the sentience level of which is not a big issue, as animal model is also considered pertinent. *Hydra*, a simple freshwater cnidarian is already a model organism for studies in developmental biology, and is also thought to be suitable for environmental toxicity assessments. Our group has conducted studies along this direction and found it appropriate for testing a few bulk as well as nano-materials. In the present study, we have tested Bisphenol A (BPA), an environmental chemical which is also an endocrine disruptor, in *Hydra* towards furtherance of biotechnological application of this simple organism. We worked out the LC_{50} of BPA adopting the morphological assay. Effective sub-lethal doses were fixed and tested for toxicity adopting regeneration and feeding assays. Since the anatomy of *Hydra* is such that all the cells in its two body layers can potentially be continuously exposed to the toxicant present in the water, the present study justifies another of its application *viz.*, environmental risk assessment.

1. Introduction

Russell and Burch's concept of 3Rs has revolutionized animal experimentation in drug development and chemical risk assessment scenarios globally (Rowan and Goldberg, 1985; National Research Council, 1988). Caught up in the uncertainties of relevance of data generated in mammalian animal models to humans, and in the context of ethical considerations of sentience, scientists and regulatory authorities have come to encourage cell culture models and computational tools in the place of animal models (Krewski et al., 2010). Yet, there is also an impression that at some level, there must be whole body testing however much less the complexity of the organism is. Thus, zebra fish, *Drosophila*, and *Caenorhabditis elegans* are being developed as alternative model organisms of lesser complexity and sentience (Lagadic and Caquet, 1998). Along these lines, there is an impression that there is scope for introduction of still simpler invertebrate organisms into this domain. It has been hypothesized that *Hydra*, a freshwater cnidarian, simple in organization and biology, and of little if any of sentience, would be an appropriate alternative model organism for toxicity testing of environmental chemicals (Yum et al., 2014; Prouse et al., 2015; Murugadas et al., 2016; Zeeshan et al., 2016, 2017; Blaise et al., 2018; Murphy and Quinn, 2018). This is relevant in the context that this organism has been in use as model in developmental biology research, particularly pattern formation and regeneration (Sarras, 2012; Galliot, 2012). *Hydra* dwells in slow running waters, but easy to maintain and culture under controlled conditions in the laboratory. This fresh water polyp has a long history as model organism in view of its remarkable plasticity in differentiation capability and high ability to regenerate the amputated body regions (Bosch and Fujisawa, 2001; Steele, 2002; Bosch and Khalturin, 2002; Bode, 2003). Owing to its high sensitivity and robust regeneration potential, *Hydra* is adopted as one of the suitable test organisms for the evaluation of pharmaceutical drugs (Pascoe

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et al., 2003; Quinn et al., 2009). The major relevance of *Hydra* as model for toxicity testing of water-borne chemicals is its diploblastic organization whereupon all its cells are in close proximity to the medium.

In the present study we introduced the novelty of testing the environmental chemical Bisphenol A, which is also an endocrine disruptor, in *Hydra*. Polycarbonate plastics are used in various home appliances like kitchenware, electrical and electronic devices, and even in medical appliances. The monomer for polycarbonate plastic, Bisphenol A (BPA), has been identified as one of the potential endocrine disrupting chemicals (EDCs). BPA is also used in the production of epoxy resin which is a lining material in food packaging and water pipelines. As an additive, BPA is found in the PET containers and can leach into the water and cause serious health hazard to the living organisms including human (MubarakAli et al., 2019). Particularly, children are vulnerable to this compound, since the vast majority of baby feeding bottles and toys are made using polycarbonate plastics that contain BPA. Besides causing health risks, BPA can reach the aquatic environment from the sources like PVC water pipels coated with plastic or epoxy resins, industrial discharges, solid wastes in aquatic bodies, and so on. As a result many attempts have been made to degrade BPA in the liquid phase using various methods (MubarakAli et al., 2019). There are reports of presence of BPA in river and coastal water samples (Selvaraj et al., 2014; Careghini et al., 2015; Staniszewska et al., 2015; Yamazaki et al., 2015). Yet, its toxic effects on aquatic organisms are only poorly understood and so need to be investigated. In this study, we determined the LC_{50} of BPA in *H. magnipapillata* 105, a wild species, and investigated its toxic effects at sub-lethal doses adopting regeneration and feeding assays.

2. Materials and methods

2.1. Culture and maintenance of Hydra

The test organism *Hydra magnipapillata* 105 was cultured in standard *Hydra* medium [$CaCl_2$ (1 mM), NaCl (1 mM), $MgSO_4$ (0.1 mM), KCl (0.1 mM), and Tris-Cl (1 mM), pH 7.4] (Lenhoff and Brown, 1970). Temperature of the medium was maintained at 18 ± 1 °C to avoid batch-to-batch variation, under 12 h dark-light cycle (Murugadas et al., 2016). The animal was fed *ad libitum* with freshly hatched *Artemia salina* nauplii.

2.2. Chemicals and treatment conditions

Stock solution of BPA (CAS No. 80–05-07; 99% purity; Sigma-Aldrich, USA) was prepared by dissolving the solid BPA in *Hydra* medium and stirred continuously for overnight to obtain the stock concentration (100 mg/L). The test concentrations were obtained every time of use from the stock solution. The medium was changed every day without harming the animals. Animals were starved for 24 h prior to commencement of the experiments.

2.3. Morphological alterations and acute toxicity testing

Acute toxicity testing in *Hydra* was conducted according to Murugadas et al. (2016). Briefly, 10 polyps without bud were placed in small petri-dishes containing 10 mL of *Hydra* medium and incubated as described above. The polyps were continuously exposed to varying concentrations of BPA ranging from 10 µM (1.14 mg/L) to 45 µM (10.27 mg/L) for 96 h and the progressive changes in morphology were recorded at every 24 h interval using a stereo-zoom dissecting microscope (Carl Zeiss, Jena, Germany) enabled with a camera (ERc5 s). A score of 10 was assigned to healthy polyps and 0 to the animals that had undergone disintegration; animals with scores 9–1 indicated altered morphology to different levels, in that order (Table 1). Median lethal concentration 50 (LC_{50}) was analyzed for each time point based on the observed median score and the values were calculated adopting PROBIT analysis. LC_{50} determination enabled fixing the sub-lethal doses to assess the low-dose level toxicity of BPA in respect of regeneration and feeding behavior of *Hydra*.

The experiments were always conducted in triplicates.

Table 1
Scoring system devised by Wilby (1988) for assessing morphological alterations in *Hydra*.

Score	Morphology of polyp
10	Extended tentacles; body reactive
9	Partially contracted; slow reactions
8	Clubbed tentacles; body slightly contracted
7	Shortened tentacles; body slightly contracted
6	Tentacles and body shortened
5	Totally contracted; tentacles visible
4	Totally contracted; no visible tentacles
3	Expanded; tentacles visible
2	Expanded; no visible tentacles
1	Dead but intact
0	Disintegrated

2.4. Regeneration assay

To assess the impact of BPA exposure on the potential for regeneration, two sub-lethal concentrations (5 µM and 15 µM), which did not induce much an adverse effect on *Hydra's* morphology, were chosen based on the 96 h LC_{50} value. A group of 15 animals was bisected using a sterile medical scalpel and the lower portion of body column was allowed to regenerate the missing upper parts in the presence of sub-lethal concentrations of BPA. The amputated body parts were kept in 15 mL of *Hydra* medium and observed for 72 h. The progress of regeneration was monitored using the stereo-zoom dissecting microscope, and score was assigned according to Ambrosone et al. (2012). The scores for regeneration assay are shown in Table 2.

2.5. Feeding assay

The ability of untreated and BPA (sub-lethal concentrations)-treated animals to catch and ingest the prey was recorded according to Ambrosone et al. (2014). After the treatment period (24 h exposure), freshly hatched *Artemia salina* nauplii or 10 mM reduced glutathione (GSH) was challenged against *Hydra* and *Hydra's* ability to catch the prey, and writhing of tentacles in response to reduced glutathione were recorded using the stereo-zoom dissecting microscope equipped with an Axiocam ERc5s camera. (Table 3)

3. Results and discussion

3.1. Morphological assessment and determination of LC_{50}

As stated earlier, *Hydra* is a diploblastic animal and responds quickly to changes in the immediate aquatic environment. The surface contaminants in fresh water significantly influence the morphology and physiology of the organism (Tarrant, 2007). Since no BPA toxicity has been reported for *Hydra* with regard to Indian surface water, an attempt was made to calculate 96 h LC_{50} along with morpho-physiological

Table 2
Scoring system devised by Ambrosone et al. (2012) for assessing regeneration capacity in *Hydra*.

Score	Polyp Morphology
2	Fully regenerated
1	Emergence of tentacles
0	No regeneration
	Lethal

Table 3
Median lethal concentrations (LC50) of BPA for *H.magnipapillata,* calculated by Probit analysis at different time points.

Time (h)	95% confidence limits for concentrations (mg/L)		
	Estimate	Lower Bound	Upper Bound
24	8.664	8.392	8.931
48	6.751	6.527	6.979
72	5.345	5.113	5.546
96	5.137	4.924	5.356

amputated body parts in the presence of sub-lethal concentrations of BPA for 72 h. Head regeneration in *Hydra* was retarded mostly at 48 h post-amputation and only a few polyps were able to regenerate completely even after 72 h following exposure to 15 µM BPA, whereas on exposure to 5 µM BPA tentacles emerged at 48 h and complete regeneration occurred within 72 h of amputation (Fig. 3). These data clearly show that BPA causes impairment of regeneration in a concentration-dependent manner. This result is in good agreement with the earlier finding of Pascoe et al. (2002) that BPA exposure to *H. vulgaris* at concentration > 460 µg/L significantly affects the regeneration of gut

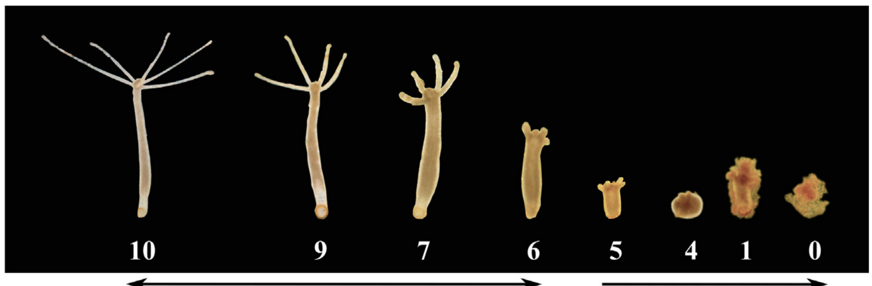

Fig. 1. Morphological changes of *Hydra* following BPA exposure.

changes in *Hydra* following BPA exposure. Fig. 1 depicts the representative images of *Hydra* showing progressive morphological changes following exposure to BPA. From the Fig. 2, it is observed that till 96 h there is no prominent change in polyp's morphology at concentrations up to 10 µM BPA. However, with further increase in concentration, the scores significantly decreased as the body became contracted and the animals started to retract and/or disintegrate. The LC_{50} value of BPA for 96 h treatment as calculated in this study is 5.137 mg/L. This data is close to the finding of Park and Yeo (2012) that *H. magnipapillata* does not show any mortality till 5 mg/L of BPA tested for 96 h. Similarly, Pascoe et al. (2002) calculated 6.9 mg/L as 96 h LC_{50} value for *H. vulgaris* which is slightly lesser toxic than the value for *H. magnipapillata* in our study. The difference in the LC_{50} would reflect species-specific response. However, the common factor identified from these two species is the robust nature and reliability as indicator of BPA toxicity.

3.2. Regeneration assay

Hydra is known for its remarkable property of regeneration. This dynamic process can be altered on exposure to toxic chemical entities. In our experiment, after bisection, *Hydra* was allowed to regenerate its

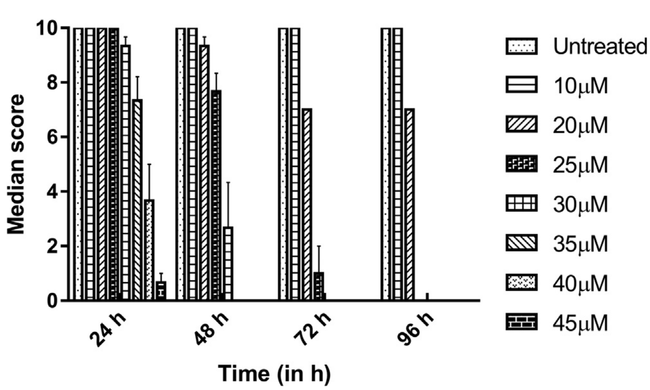

Fig. 2. Median score of polyps exposed to different concentrations of BPA at various time points. Scores ≥ 6 were reversible and sub-lethal, while scores ≤ 5 were considered irreversible and lethal.

region. Park and Yeo (2012) have also observed that BPA-exposed *Hydra* population was slower in tentacle regeneration. Though BPA is found to inhibit regeneration in *Hydra*, this normally occurs at relatively higher concentrations. This is probably due to the cytotoxic effect of BPA at higher doses as suggested by Jalal et al. (2018), or increased ROS generation by BPA at the amputated region which promotes DNA damage and cell death as suggested by Gassman (2017).

3.3. Feeding assay

Hydra goes through a series of processes to capture the prey and engulf it. The nematocysts and the associated neurons in the tentacles of *Hydra* are directly concerned with feeding and defense. Initially, it approaches the prey (or the prey would hit the *Hydra* inadvertently) (Media file, M1). The physical contact of prey stimulates the nematocysts lining the tentacles. Once this happens, the tentacle strikes the prey when the nematocysts paralyze the prey with the prey remaining adherent to the nematocyst/tentacle. Once the prey is captured, the mouth opens and the tentacle bends towards the mouth to insert the prey into the mouth. Reduced glutathione (GSH) was used as copycat of *Hydra*-feed which stimulates all these processes (M2). The study revealed that polyps exposed to BPA at 15 µM concentration were either unable to catch the prey or those few captured slipped-off the tentacles (M3). Interestingly, the time taken to respond to GSH was also reduced in polyps treated with BPA at higher concentrations as revealed in tentacle writhing ability (M4). In contrast, the polyps treated with BPA at 5 µM concentration showed no perceptible alteration in the prey capture process or tentacle writhing ability (M5 and M6, respectively). This observation is in line with the earlier finding of Ambrosone et al. (2014) and Murugadas et al. (2016) who standardized feeding as an indicator of toxicity. Reduced entanglement of prey, inability to ingest the prey, and the incidence of prey falling-off from the tentacles at the higher sub-lethal concentration may be due to failure of nematocyst discharge for purpose of paralyzing the prey and inability to open the mouth due to BPA toxicity. Since the actual mechanism underlying the inhibition of glutathione response is unknown, we speculate that BPA interaction with the glutathione receptor may inhibit feeding response, which needs further investigation.

Supplementary material related to this article can be found online at doi:10.1016/j.bcab.2019.01.009.

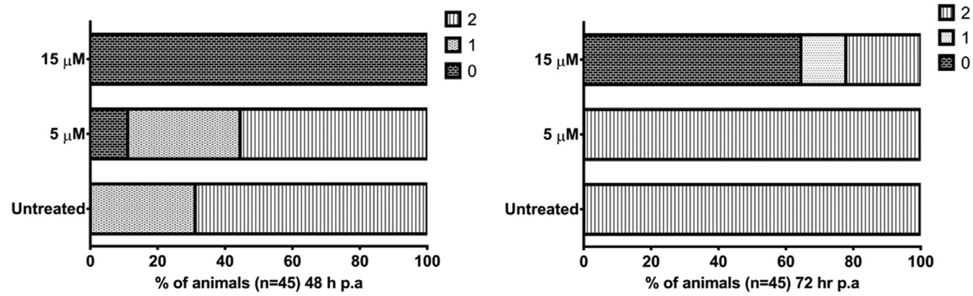

Fig. 3. Impact of BPA on regeneration of *H.magnipapillata.* (a) regeneration at 48 h post-amputation; (b) regeneration at 72 h post-amputation.

4. Conclusion

The present study examined if *Hydra* would be an appropriate organism, in all its simplicity, to test the toxicity of BPA in freshwater bodies so as to further support its projection as an alternative model organism for toxicity testing of chemical entities in the freshwater environment. The result revealed that not much of morphological changes occurred with concentrations of BPA that would generally be present in water bodies. But at the test concentrations, 96 h $LC_{50,}$ which itself were based on the regressive morphological changes, or one closer to which *Hydra* underwent prominent morphological changes. At sub-lethal concentrations of BPA, impairments were recorded in feeding behavior and regeneration. Otherwise, the lower-end sub-lethal doses produced minimal or no impairment. Thus, from this study, it is revealed that the biology of *Hydra* is significantly affected by BPA in a concentration-and time-dependent manner. Therefore, the observations in this study support the idea that *Hydra* has the potential to be developed into an alternative model organism for purpose of whole body testing of environmental chemicals. It would be possible to take to advantage the cellular organization, especially the stem cell lineages, and the consequent 'stemness" and the potential immortal nature so as to find newer targets and end points in the context of environmental risk assessment.

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Competing financial interests

The authors declare no competing financial interests.

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