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Shift in aggregation, ROS generation, antioxidative defense, lysozyme and acetylcholinesterase activities in the cells of an Indian freshwater sponge exposed to washing soda (sodium carbonate)

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ABSTRACT

Washing soda, chemically identified as anhydrous sodium carbonate, is a popular cleaning agent among the rural and urban populations of India which often contaminates the freshwater ponds and lakes, the natural habitat of sponge *Eunapius carteri*. Present investigation deals with estimation of cellular aggregation, generation of ROS and activities of antioxidant enzymes, lysozyme and acetylcholinesterase in the cells of *E. carteri* under the environmentally realistic concentrations of washing soda. Prolonged treatment of washing soda inhibited the degree of cellular aggregation. Experimental exposure of 8 and 16 mg/l of sodium carbonate for 48 h elevated the physiological level of reactive oxygen species (ROS) generation in the agranulocytes, semigranulocytes and granulocytes of *E. carteri*, whereas, treatment of 192 h inhibited the ROS generation in three cellular morphotypes. Activities of superoxide dismutase, catalase and glutathione-*S*-transferase were recorded to be inhibited under prolonged exposure of washing soda. Washing soda mediated inhibition of ROS generation and depletion in the activities of antioxidant enzymes were indicative to an undesirable shift in cytotoxic status and antioxidative defense in *E. carteri*. Inhibition in the activity of lysozyme under the treatment of sodium carbonate was suggestive to a severe impairment of the innate immunological efficiency of *E. carteri* distributed in the washing soda contaminated habitat. Washing soda mediated inhibition in the activity of acetylcholinesterase indicated its neurotoxicity in *E. carteri*. Washing soda, a reported environmental contaminant, affected adversely the immunophysiological status of *E. carteri* with reference to cellular aggregation, oxidative stress, antioxidative defense, lysozyme and acetylcholinesterase activity.

1. Introduction

Freshwater sponge, *Eunapius carteri* (Porifera: Demospongiae: Spongillidae) is distributed in the perennial ponds and lakes of India with inadequate scientific information regarding its toxicological response against environmental xenobiotics. *E. carteri*, which is considered as an important component of the biota (Mukherjee et al., 2015a) and source of bioactive and biomimetic molecules (Manconi et al., 2013), bears the potential to act as an effective biomonitoring agent of environmental pollution (Kakavipure and Yeragi, 2008). An advanced grade of canal system and nonselective filter feeding mode of digestion (Duckworth and Pomponi, 2005) enabled sponges to circulate a large volume of water inside their body following a specific route. Sponges, due to their structural porosity and cellular dynamism, are continuously exposed to diverse groups of xenobiotics and environmental pathogens of the ambient water.

For the last two to three decades, the freshwater ecosystem of India has been experiencing the toxic exposure of new generations of environmental xenobiotics including different commercial brands of detergents (Ray et al., 2011) and allied chemical compounds. Field survey carried out by the authors in the ponds and lakes in and around Kolkata metropolis (22° 56′ N, 88° 36′ E) of the state of West Bengal, India revealed that the natural habitat of *E. carteri* is being contaminated alarmingly by anhydrous sodium carbonate (CAS number: 497-19-8), commonly known as "washing soda". Washing soda is a component of laundry detergent (Warne and Schifko, 1999) and acts as a water softening precipitating builder (Bajpai and Tyagi, 2007) which bears the potential to increase the alkalinity of water (HERA, 2005). Washing soda has been identified as a popular brand of cleaning agent used by the rural and urban human populations of India for the purposes of cleaning of clothes, bathing of cattles and washing of utensils (Mukherjee et al., 2015b). Household cleaning agents containing sodium carbonate are generally disposed through drain (HERA, 2005) which has been

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identified as a major route of washing soda contamination of freshwater aquifers. Industries like glass, metal, mining and paper have been reported as sources of sodium carbonate which contaminates the global environment (UNEP, 2003). However, report of toxicity of sodium carbonate in freshwater invertebrates is limited in the current scientific literature (McKee and Wolf, 1963; Warne and Schifko, 1999). Washing soda has been reported as a precarious aquatic contaminant which adversely affected the various immunological parameters in *E. carteri* (Mukherjee et al., 2015b,c). According to them, sublethal treatment of washing soda yielded an alteration in the phagocytic and apoptotic response, cytotoxic status, activities of phosphatases and lysosomal membrane integrity in the cells of *E. carteri*. According to HERA (2005), the permissible concentration of sodium carbonate in aquatic ecosystem ranges from 2 to 20 mg/l. Following this report, the experimental concentrations of 2, 4, 8 and 16 mg/l of sodium carbonate are appeared to be environmentally realistic ones.

Poriferans largely consist of a cellular grade of structural organization, depend mostly on innate immunological system (Wiens et al., 2007; Mukherjee et al., 2016) for the purposes of killing and inactivation of invading pathogens and toxins. Sponges, in general, exhibit morphofunctional diversities of heterogenous populations of cells (Smith and Hildemann, 1991; Mukherjee et al., 2015a) for the purposes of different physiological functioning. Cell–cell attachments and selective cellular contacts had long been a moot point of debate among the scientists working in the fields of growth, development and differentiation in metazoans (Moscona, 1963). Wilson (1907) reported that dissociated cells of sponge usually reaggregate and reorganize into a functional specimen with developed water channels under a controlled physiological environment. For their developmental characteristics, these simple structured organisms are often considered as candidate species in evaluating the toxicity of aquatic ecosystem (Goh, 2008). Modulation of cellular aggregation under the face of environmental toxins is in report in invertebrates (Auffret and Oubella, 1997) including sponges (Philip, 2001).

Superoxide dismutase and catalase are classical antioxidant enzymes that play an important role in protecting the host cell from oxidative damage induced by environmental xenobiotics (Ahmad, 1995). Determination of antioxidative defense response is suggested as an early "warning tool" to monitor environmental pollution (Markad et al., 2012). Exposure to environmental xenobiotics in the aquatic ecosystem can lead to the generation of reactive oxygen species (ROS) which induce oxidative damage in biological system (Vlahogianni et al., 2007). ROS consisted of hydrogen peroxide and other oxidant molecules including superoxide anion, peroxyl radical and peroxynitrite anion (Wang et al., 2012). Glutathione-*S*-transferase is biochemically involved in xenometabolic and general detoxification reactions (Manduzio et al., 2004). It is also involved in the process of antioxidative defense in invertebrates and is functionally associated with the reduction of oxidative stress generated in the immunocytes. Studies on generation of ROS, oxidative damage and antioxidant defense have been demonstrated in invertebrates (Donaghy et al., 2012; Singaram et al., 2013) with insufficient information available in sponge (Peskin et al., 1998; Marques et al., 2008; Aktas et al., 2013) exposed to xenobiotics. According to Peskin et al. (1998), sea sponge *Sycon* sp. generates a high degree of superoxide radical in absence of any added stimulus. The rate of outflow of superoxide anion from sponges to their ambient water was recorded to be 0.5 nmol/min per sponge at pH 6.5. It is known that sponges consumed environmental bacteria for the purpose of nutrition. Therefore, it was assumed that superoxide anion generated during phagotrophy might act as an antibacterial agent.

Lysozyme, a bactericidal hydrolytic enzyme, acts as a first line of defense in invertebrates (Bathige et al., 2013) against the invasion of microbial pathogens (Yin et al., 2014). Besides its antibacterial activities, lysozyme exhibited fungistatic or fungicidal properties (Fiołka et al., 2005). Nappi and Ottaviani (2000) reported lysozyme as a potential "killing agent" of invertebrates against pathogens and parasites. The effect of environmental toxins on lysozyme activity of sponge is less studied with inadequate scientific report.

Freshwater sponges contract their canals rhythmically and exhibit occasional sneezing (Ludeman et al., 2014). The genomic analyses indicated the existence of a primitive pre-nervous system that became extinct in the course of evolution (Elliott and Leys, 2010). Acetylcholine, a neurotransmitter, is functionally involved in the regulation of body extension formation and retraction in sponge (Ellwanger and Nickel, 2006). Acetylcholinesterase catalyses the hydrolysis of acetylcholine into acetic acid and choline during neural transmission. Being an important mediator of neural communication and coordination, acetylcholinesterase plays a significant role in maintenance of cellular and physiological homeostasis. Acetylcholinesterase activity in sponge, *Cliona celata* has been reported as a biomarker of environmental pollution along the Western coast of Iberian Peninsula (Marques et al., 2007). However, report of activity of acetylcholinesterase is absent in Indian freshwater sponge with reference to environmental toxicity.

In this present investigation, the cellular aggregation response, generation of ROS, activities of antioxidant enzymes, lysozyme and acetylcholinesterase were estimated in the dissociated cells of *E. carteri* under the sublethal and environmentally realistic concentrations of washing soda. The current analyses would provide an important ecotoxicological information regarding the toxicity of sodium carbonate in *E. carteri*, a common but less studied poriferan of Indian freshwater ecosystem. Furthermore, the selected experimental parameters of *E. carteri* may bear the potentiality to act as efficient biomarkers of washing soda toxicity in water.

2. Materials and methods

2.1. Collection, transportation and laboratory acclimation of experimental *E. carteri*

Live specimens of *E. carteri* were collected from the specific waterbodies (22° 86′ N, 88° 40′ E) of the state of West Bengal of India without the history of pisciculture and toxin contamination. During collection of specimens, the dissolved oxygen, pH and temperature of the pond water were estimated as 14 mg/l, 7.6 and 25 °C respectively. Body masses of freshwater sponge were manually dissected from the submerged plant twigs by sterile scalpel and were immediately transported to the laboratory with adequate volume of freshly collected water procured from their natural habitat. Sponges were acclimated in well aerated glass aquaria for 7 d and temperature, pH and dissolved oxygen of the water of the sponge aquaria were maintained at 24–26 °C, 7.6 and 14.5 mg/l respectively (Mukherjee et al., 2015b). Proper and uniform illumination of 12:12 h dark–light cycle was maintained throughout the experiment. The water of the experimental glass aquaria was replenished routinely at every 24 h with freshly collected pond water to supplement suspended food and for avoidance of toxicity due to accumulation of excretory products (Mukherjee et al., 2015b). The experiment on sponge specimens was designed in accordance to the guidelines of institutional norms of animal handling and care of the University of Calcutta. The West Bengal Biodiversity Board of the Department of Environment of Government of West Bengal, India permitted us to collect the specimens of *E. carteri* from their natural habitat.

2.2. Experimental treatment of *E. carteri* with washing soda

Body mass of *E. carteri* was dissected into pieces each with an approximate size of 8 cm^3 bearing at least one osculum (Hansen et al., 1995). The acclimation of the sponge specimen was carried out in aerated glass aquaria in controlled laboratory conditions for 7 d to minimize the physiological stress and to reorganize their canal system (Duckworth and Pomponi, 2005). A set of 5 replicates of dissected *E. carteri* was treated with sublethal and environmentally realistic concentrations of 2, 4, 8 and 16 mg/l of washing soda along with the