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Immunotoxicity of washing soda in a freshwater sponge of India

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ABSTRACT

The natural habitat of sponge, *Eunapius carteri* faces an ecotoxicological threat of contamination by washing soda, a common household cleaning agent of India. Washing soda is chemically known as sodium carbonate and is reported to be toxic to aquatic organisms. Domestic effluent, drain water and various human activities in ponds and lakes have been identified as the major routes of washing soda contamination of water. Phagocytosis and generation of cytotoxic molecules are important immunological responses offered by the cells of sponges against environmental toxins and pathogens. Present study involves estimation of phagocytic response and generation of cytotoxic molecules like superoxide anion, nitric oxide and phenoloxidase in *E. carteri* under the environmentally realistic concentrations of washing soda. Sodium carbonate exposure resulted in a significant decrease in the phagocytic response of sponge cells under 4, 8, 16 mg/l of the toxin for 96 h and all experimental concentrations of the toxin for 192 h. Washing soda exposure yielded an initial increase in the generation of the superoxide anion and nitric oxide followed by a significant decrease in generation of these cytotoxic agents. Sponge cell generated a high degree of phenoloxidase activity under the experimental exposure of 2, 4, 8, 16 mg/l of sodium carbonate for 96 and 192 h. Washing soda induced alteration of phagocytic and cytotoxic responses of *E. carteri* was indicative to an undesirable shift in their immune status leading to the possible crises of survival and propagation of sponges in their natural habitat.

1. Introduction

Sponges are considered as the earliest extant multicellular animals (Adamska et al., 2011). They are filter feeders and bear ecological and evolutionary significance. During filter feeding, the sponges are physically exposed to a wide array of environmental contaminants of the ambient water and accumulate various pollutants in their body. They bear the potentiality to function as biomonitoring organisms of aquatic pollution (Cebrian et al., 2007) and are considered as promising indicators of water quality monitoring (Mahaut et al., 2013). High concentrations of pollutants including hydrocarbons (Zahn et al., 1981), organochlorinated compounds (Perez et al., 2003) and heavy metals (Hansen et al., 1995; Venkateswara et al., 2009) were reported to be accumulated in marine sponges. Zahn et al. (1981) reported an increased activity of ornithine decarboxylase in the marine sponge, *Tethya lyncurium* under the exposure of benzo[a]pyrene, an environmental toxin. Sponge, *Haliclona tenuiramosa* is reported to act as a bioindicator to monitor heavy metal pollution in the coasts of Gulf of Mannar of India (Venkateswara et al., 2009). Mediterranean sponge, *Crambe crambe* can accumulate high amounts of lead, copper and vanadium (Cebrian et al., 2003). According to them, habitat contamination by metal pollution leads to inhibition in the physiological growth, fecundity and survival of the test species. Different sublethal concentrations of rogar and endosulfan were reported to increase the protein content of the freshwater sponge, *Spongilla lacustris* (Ingle et al., 2003). However, these pesticides resulted in depletion in the activities of peroxidase, carbonic anhydrase and total content of carbohydrate in the same species. Saby et al. (2009b) reported alteration in the immune status of selected species of marine sponges in response to metal pollution. Authors reported species specific inhibition of 2',5'-oligoadenylate synthetase in the Mediterranean sponges, *Geodia cydonium*, *Crella elegans* and *Chondrosia reniformis.* This enzyme is actively involved in the immune system of vertebrates with insufficient information in invertebrates. Furthermore, due to high level of tolerance of aquatic contaminants, a special "sponge watch programme" was launched (Mahaut et al., 2013) for water quality monitoring.

Eunapius carteri (Porifera: Demospongiae: Spongillidae) is a common variety of sponge distributed in the freshwater ecosystem of India with limited scientific information on its ecology, cell biology and immunity. Freshwater ecosystem of India is being contaminated by the diverse groups of environmental toxins including different commercial brands of detergents (Ray et al.,

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2011) and allied chemical toxins. Anhydrous sodium carbonate (CAS number: 497-19-8), commonly known as “washing soda” in India, is a component of laundry detergent (Warne and Schifko, 1999). Glass industry has been reported as another source of sodium carbonate which contaminates the global environment (HERA, 2005). However, sodium carbonate or washing soda itself is a popular brand of cleaning agent among the rural human populations of India and is reported to be toxic for the freshwater aquatic organisms including fish (Van Horn et al., 1949; Wallen et al., 1957), *Daphnia magna* (Mckee and Wolf, 1963) and cladocera (Warne and Schifko, 1999). The extensive use of the detergents and their components are reported to cause a serious pollution of water of rivers and lakes (Saouter et al., 2001). Washing soda acts as a water softening “precipitating builder” (Bajpai and Tyagi, 2007) present in the laundry detergent and is capable of increasing the alkalinity of water. The contaminated bottom deposits or precipitates act as the major stressors of aquatic ecosystem which presents a negative impact on the aquatic organisms (Burton, 1992). Detergent exposure yielded an undesirable alteration in the protein content of freshwater gastropod (*Bellamya bengalensis*) of southern region of India (Kumari, 2013). Contamination of water by detergent is reported to increase the oxygen consumption of an Indian freshwater fish, *Mystus montanus* (Chandanshive, 2014). “Soda ash”, synonymous to washing soda, is reported to contaminate the coastal region of state of Gujarat of India (Jadeja and Tewari, 2009). However, the contamination of the aquifers by detergent occurs in the states of West Bengal (Ghose et al., 2009), Gujarat (Jadeja and Tewari, 2009), Maharashtra (Chandanshive, 2014), Andhra Pradesh (Kumari, 2013) and Kerala (Mathew et al., 2013) of India. On the basis of the buffering capacity of the aquatic ecosystem, the acceptable concentration of sodium carbonate in the natural pond water ranges between 2 and 20 mg/l (HERA, 2005). In this present study, the experimental concentrations of 2, 4, 8 and 16 mg/l of sodium carbonate are thus appeared to be rational and environmentally realistic. The report of toxicity of sodium carbonate in spongillid *E. carteri* is almost absent in the current scientific literature. Present investigation is aimed to analyse the immunotoxicity of sodium carbonate in *E. carteri* with reference to the phagocytic potential and generation of cytotoxic molecules like superoxide anion, nitric oxide and phenoloxidase at cellular level.

Physiological defense of *E. carteri* is conceived to be dependent on the activities of heterogenous populations of cells. Evolutionarily, sponges overcame diverse environmental challenges and stressors (Saby et al., 2009a). This suggests the existence of a highly developed immune system in sponges, which is yet to be investigated in *E. carteri*. Poriferans mostly rely on innate immunological system for their biological defense (Wiens et al., 2007). Phagocytosis is reported as a prime innate immune response of sponges (Saby et al., 2009a) against possible invasion and exposure of pathogenic microorganisms and toxins respectively. In sponge, phagocytosis plays a dual physiological attribute namely feeding and cell mediated immune response. Superoxide anion and nitric oxide were reported as the prevalent cytotoxic molecules involved in the innate immune response of sponge (Mukherjee et al., in press) and played a significant role in the destruction and deactivation of microorganisms (Nappi and Ottaviani, 2000). Generation of reactive oxygen species is associated with the intracellular production of superoxide anion in the immunoeffector cells of sponge and acts as a potent cytotoxic agent (Peskin et al., 1998). Nitric oxide is reported as a ubiquitous signaling molecule involved in a variety of physiological processes, including immune defense in invertebrates (Colasanti et al., 2010). Like superoxide anion, nitric oxide is also involved in generating cytotoxicity in the immunocytes of invertebrates for the purpose of pathogen destruction. Enzyme phenoloxidase is an established component of innate immune system of invertebrates (Müller et al., 1999a) with no information available in *E. carteri*. Pathogen invasion or injury leads to the activation of phenoloxidase cascade and synthesis of melanin in sponges (Müller et al., 1999b). Wiens et al. (1998) reported the involvement of melanin during allorecognition in sponge tissues.

In this paper, the phagocytic potential and the generation of cytotoxic molecules like superoxide anion, nitric oxide and phenoloxidase were estimated in the dissociated cells of *E. carteri* under the sublethal and environmentally realistic concentrations of sodium carbonate in controlled laboratory conditions. Exposure of sodium carbonate yielded a significant alteration in the phagocytic index and cytotoxic potential in the cells of *E. carteri* indicating a state of physiological stress. The present investigation would provide a significant information on the cell biology of *E. carteri* and toxicity of sodium carbonate in the same specimen, a common but less studied poriferan species of Indian freshwater ecosystem. Current findings suggest that the cells of *E. carteri* are physiologically responsive to varying concentrations of washing soda. Primary goal of the investigation is to establish the studied immunological parameters of *E. carteri* as an effective tool of monitoring the health of the freshwater environment.

2. Materials and methods

2.1. Collection, transportation and laboratory acclimation of freshwater sponge

Live and healthy specimens of *E. carteri* were manually sampled from the selected freshwater aquifers located in the district of north twenty four parganas (22° 86'N, 88° 40'E) of the state of West Bengal of India. Sponges, attached over the rocky substratum or live submerged twigs of ponds, were collected from a depth of 4–6 cm below the surface of water. During collection of *E. carteri* from their natural habitat, the dissolved oxygen, pH and temperature of the pond water were estimated *in situ* as 14 mg/l, 7.4 and 25 °C respectively. The field stations from where specimens were collected did not bear any history of sodium carbonate contamination. The sponges were transported to the laboratory within 2 h of collection and acclimated in the controlled static water environment at 24–26 °C for 7 d in well aerated tanks. During acclimation, dissolved oxygen and pH of the water of the sponge aquaria were maintained at 14.5 mg/l and 7.2 respectively. Proper illumination and uniform light rationing were maintained throughout the experiment. The water of the glass aquaria was replenished with fresh supply of pond water at every 24 h to supplement suspended food and for avoidance of toxicity due to accumulation of excretory products. The experiment on sponge species was designed according to the guidelines of the institutional norms of animal handling and care of the University of Calcutta.

2.2. Treatment of E. carteri with sodium carbonate

Body mass of *E. carteri* was dissected into five small pieces with an approximate dimension of 8 cm^3 and each small dissected mass contained at least one osculum (Hansen et al., 1995). After dissection, the sponges were acclimated in aerated glass aquaria in controlled laboratory conditions for 7 d to minimize the mechanical stress and to reorganize their canal system (Duckworth and Pomponi, 2005) prior to experimentation. Aqueous solutions of sodium carbonate bearing the sublethal and environmentally relevant concentrations of 2, 4, 8 and 16 mg/l were prepared using detergent free water as solvent. The highest experimental concentration was less than the one third of the median lethal