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Evaluation of impairment of DNA integrity in marine gastropods (*Cronia contracta*) as a biomarker of genotoxic contaminants in coastal water around Goa, West coast of India ☆

A. Sarkar[a,1], Dipak C.S. Gaitonde[b,*], Amit Sarkar[a,1], D. Vashistha[a], Classy D'Silva[a], S.G. Dalal[a]

[a] *Marine Pollution Assessment and Ecotoxicology Laboratory, National Institute of Oceanography, Dona Paula, Goa 403004, India*
[b] *Environmental Science Laboratory, Government Polytechnic, Bicholim, Goa 403504, India*

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Abstract

The measurement of the impairment of DNA in marine gastropod (*Cronia contracta*) provides an insight into the genotoxic effects of contaminants on marine organisms along the Goa coast. The impact of genotoxic contaminants on Goan coastal environment was evaluated in terms of the loss of DNA integrity (expressed as the value of '*I*') in marine snails with respect to those from the reference site (Palolem) over a period from April 2004 to May 2005 using the technique of alkaline unwinding assay. The DNA integrity in marine snails was found to be significantly damaged at Dona Paula (58%), Vasco (73.5%), and Velsao (48.5%) during the monsoon period (July–August 2004). Similar trend in the loss of DNA integrity in marine gastropods was also detected during the post-monsoon (November–December 2004) and the pre-monsoon (April–May 2005) periods. The low integrities of DNA in marine gastropods at these sites can be attributed to exposure to genotoxic contaminants especially polycyclic aromatic hydrocarbons (PAHs) and toxic heavy metals (Pb, Cd, Cu, Fe, and Mn) prevalent in the marine environment as evident by their accumulation in the tissues of the marine snails inhabiting different sites along the Goa coast. The contaminant-induced DNA strand breaks in marine snails increased significantly at Dona Paula, Vasco, and Velsao clearly indicating the levels of contamination of the sites by genotoxic compounds in those regions. The genotoxic effects of contaminants were further substantiated by detection of the impairment (39%) of DNA integrity in marine snails in a field experiment in which the same species of marine snails (*C. contracta*) collected from the reference site, Palolem, were deployed at Dona Paula and caged for 25 days for exposure to ambient marine pollutants. The impairment of DNA integrity in marine gastropods along the Goa coast can thus act as a biomarker for marine pollution monitoring of genotoxic contaminants.

Keywords: Marine gastropods; DNA integrity; Impairment; Genotoxic contaminants; PAHs

1. Introduction

The environmental risk of human cancer is greatly increased due to exposure to genotoxic contaminants such as polycyclic aromatic hydrocarbons (PAHs) and the heavy metals through the food chain. The carcinogenicity of PAHs is attributed to their ability of formation of DNA adduct resulting into mutations that ultimately lead to carcinogenesis (IARC, 1980, 1990, 1991, 1993). Extensive studies on environmental carcinogenesis all over the world indicate that the risk of cancer increased alarmingly due to occurrence of carcinogenic compounds in the environment (Ruddon, 1995; Vogelstein and Kinzler, 1998). In order to understand the mechanisms and to characterise high-risk toxicants, the application of biomarkers is of immense significance. In this regard, DNA strand breaks as well as

☆It is stated that this study involving marine snails was conducted in accordance with national and institutional guidelines for the *protection of human subjects and animal welfare*.

*Corresponding author.
E-mail addresses: asarkar@nio.org (A. Sarkar), dgaitonde@rediffmail.com (D.C.S. Gaitonde), samit@nio.org (A. Sarkar).
[1]Fax: +91 832 2464258.

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markers of early damage, including mutagenicity, other endpoints of genotoxicity, and molecular biomarkers of cancer are of great significance. Numerous experimental studies *in vitro* and *in vivo* have provided unambiguous evidence for genotoxicity of environmental pollution. In addition, various PAHs induce oxidative damage to DNA (Vineis and Husgafvel-Pursiainen, 2005). Genomic injury induced by environmental carcinogens, such as PAHs and other aromatic compounds, is the primary step that can trigger mutagenesis and carcinogenesis. In addition to the physico-chemical properties of DNA damaging agents, several important factors such as primary sequence, chromatin structure, methylation, protein association, and transcriptional activity can affect not only the initial level and distribution of DNA damage but also the efficiency of repair (Tang et al., 2002).

DNA strand breaks are believed to induce carcinogenesis. DNA base oxidation is a continuous process. Severe or prolonged exposure to oxidative stress can elicit all stages of carcinogenesis (Guyton and Kensler, 1993). The metal-induced genotoxicity is predominantly due to inhibition of DNA repair process. Moreover, most carcinogenic metal compounds have been shown to increase cytotoxicity, mutagenicity, and clastogenicity in mammalian cells when combined with different types of DNA damaging agents (Hartwig, 2000). Some metals such as chromium, nickel, arsenic, cadmium, and colbalt have long been recognised as human and/or animal carcinogens (Hartwig et al., 2002). Two modes of action, namely, the induction of oxidative damage and the interaction with DNA repairs processes are predominant for enhancement of genotoxicity in combination with a variety of DNA damaging agents (Hartwig, 2000). Chemical carcinogenesis is closely associated with planar molecules which may intercalate with DNA; with oxidative activation of the carcinogens by cytochromes P450 to reactive intermediates, which are electrophilic and bind covalently to DNA, thus greatly damaging the genetic material and activating oncogenes; with the production of reactive oxygen species (ROS), which may directly damage the DNA or activate carcinogens to reactive intermediates (Dennis, 1994; Guengerich, 1992a, b).

In this context, the occurrence of xenobiotic contaminants, such as PCBs and PAHs, in the marine environment is thus highly detrimental to the biologic integrity as well as physiologic functions of marine organisms. Uptake of xenobiotic contaminants occurs from the sediment, water column, and other biota and increases with increasing bioavailability, lipophilicity/hydrophobicity, and concentration of the chemicals (Sarkar, 1994, 2006a, b; Livingstone and Pipe, 1992; Sethi et al., 1999; Sarkar and Everaarts, 1998; Everaarts and Sarkar, 1996). Many of these pollutants are chemical carcinogens and mutagens with the capacity to cause various types of DNA damage. Benzo(*a*)pyrene, a representative PAH is reported to be converted at cellular level to the ROS, diol-epoxide (BaPDE), which can form stable adduct with DNA resulting into DNA strand breaks (Pisoni et al., 2004; Bihari and Fafanđel, 2004). The occurrence of single strand breaks can be induced in various ways such as chemical induction during excision repair, interaction with DNA-intercalating agents, degradation due to autolysis or disruption, formation of alkali labile sites, interstrand cross links, DNA–protein cross (DPC) links. Moreover, significant DNA damage can occur due to interaction of alkylating agents with DNA at multiple sites.

Several biomarker studies have shown the impact of genotoxic contaminants in various species of marine organisms such as marine mussels, *Mytilus galloprovincialis* (Accomando et al., 1991; Bolognesi et al., 1996; Jakšić and Batel, 2003; Steinert et al., 1998), eelpout (*Zoarces viviparus*) (Frenzilli et al., 2004), mollusk (*Scapharca inaequivalvis*) (Gabbianelli et al., 2006), marine mollusks (*Mytilus edulis*) (Hagger et al., 2005; Jha et al., 2005), Calico scallop (*Argopecten gibbus*) (Quinn et al., 2005), rainbow trout (*Oncorhynchus mykiss*) (Cabrita et al., 2005), sea anemone (*Anthopleura elegantissima*) (Mitchelmore and Hyatt, 2004), crabs (Pan and Zhang, 2006), carp (*Cyprius carpio*) (Kammann et al., 2000), green-lipped mussels (*Perna viridis*) (Siu et al., 2003), sea urchins (*Strongylocentrotus droebachiensis*) and mussels (*M. edulis L.*) (Taban et al., 2004; Wilson et al., 1998), fathead minnows (*Pimephales promelas*) (Shugart et al., 1989). A few data have been observed on the occurrence of DNA damage in gastropods (Hagger et al., 2006; Regoli et al., 2006; Benton et al., 2002).

In view of the growing global concern for the occurrence of genotoxic contaminants in the marine eco-system, it is of prime importance to evaluate the impact of these compounds on the marine organisms at molecular levels. The aim of this study is to assess the impairment of DNA integrity in marine snails (*Cronia contracta*) in terms of the extent of DNA strand breaks as an early warning signal for detection of environmental carcinogenesis. Marine snails are being used as delicious seafood in different parts of the world. Thus, contamination of the snails by genetoxic pollutants can eventually lead to cause serious damage to human health through the food chain.

2. Material and methods

The marine snails being intertidal and euryhaline organisms are very good indicator organisms for evaluation of environmental stress of varying mixture of contaminants. They can accumulate within its tissues many of the contaminants (persistent chlorinated hydrocarbons, PAHs, toxic metals, etc.) present in the seawater (Krishna Kumari et al., 2006; Sarkar and Everaarts, 1998; Sarkar, 1994). Among the various types of marine snails, *C. contracta* are known as oyster drill snails grazing on the rough surface of the rocks and are mostly found in damp habitat (Gaitonde et al., 2006). Exposure of marine snails to environmental contaminants give rise to DNA damage (strand breaks) (Sarkar et al., 2006). The marine snails (*C. contracta*) collected from different sampling stations along the Goa coast were in abundance mainly in the intertidal and sub-tidal zones on rocky shores. They were adult and most probably carnivores and scavengers. They feed on a variety of invertebrates by immobilising the prey with secretions from the proboscis gland. They use a