

REVIEW

Glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation as biomarkers of oxidative stress in snails: A review**J Bhagat, BS Ingole, N Singh***Biological Oceanographic Division, CSIR-National Institute of Oceanography, Dona Paula, Goa, India**Accepted October 17, 2016***Abstract**

Antioxidant defense plays a crucial role in the response of an organism to pollutants. Several processes stimulate the production of free radicals or deplete the antioxidant defense, which if not regulated properly, may cause oxidative stress in the organisms, leading to damage in DNA, proteins or lipids. Free radicals are also beneficial as it plays an important role in defense against infectious agents, and signal transduction. Hence a delicate balance between antioxidants and free radicals is required. Oxidative stress biomarkers are very useful in disease etiology and environmental toxicological studies. The increase in anthropogenic activities and environmental awareness has resulted in an explosive increase of research in the field of oxidative stress. Snails are excellent organisms for environmental biomonitoring and contribute a major proportion of the invertebrate biomass. In our article, we have summarized the research carried out using glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation (LPO) in snails exposed to various toxicants and their implication in the environmental monitoring programs. In the end, we have discussed different factors affecting the variations in oxidative biomarkers response for a better understanding of the phenomenon.

Key Words: antioxidant defense system; gastropods; oxidative stress; reactive oxygen species**Introduction**

Gastropods are ubiquitous invertebrates in the aquatic environment and are commonly studied as a suitable bioindicator for contaminants (Itziou and Dimitriadis, 2011; Abdel-Halim *et al.*, 2013). They are widely studied for their ability to accumulate higher amount of heavy metal and other toxic pollutants (Baurand *et al.*, 2014; Bo *et al.*, 2015). Contaminants entering into the aquatic bodies from several sources are taken up by these organisms, and may disturb the free radical process. Reactive oxygen species (ROS) are potentially dangerous because of their highly reactive nature and hence are neutralized by several antioxidant defense systems. ROS are also involved in hormonal responses, signal transduction, and several others physiological processes including heart's pumping, aging, and disease (Andersson *et al.*, 2011). There is usually a balance between production of free radical and antioxidant defense. Oxidative stress occurs when there are excess amount of free radical due to faulty or lower antioxidant defense

system in the body. Antioxidant defense system helps protect the cell from damages caused by free radical by restoring their level. Antioxidant defense system involves both enzymatic and non-enzymatic free radical inactivation and scavenging processes. Changes in antioxidant systems of aquatic organisms can serve as indicators for a variety of pollutant exposures related to oxidative stress. Thus, it provides sensitive biochemical markers for exposure and toxicity in environmental monitoring. Among antioxidant defense, [superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases (GST), glutathione peroxidase (GPx) and Lipid peroxidation (LPO) are extensively studied (Radwan *et al.*, 2010; El-Shenawy *et al.*, 2012; Abdel-Halim *et al.*, 2013; Zheng *et al.*, 2013; Wang *et al.*, 2014).

Glutathione-S-transferases are phase II multifunctional enzymes, which play a critical role in conjugation of electrophilic compounds (phase I metabolites) on one hand, and in the defense against oxidative damage and peroxidative products of DNA or lipids (Oost *et al.*, 2003) on the other hand. Superoxide dismutases are a class of naturally occurring enzymes that repairs and prevent the oxygen metabolizing cells from the harmful effects of free radicals mainly superoxide.

*Corresponding author:*Jacky Bhagat
CSIR-National Institute of Oceanography
Dona Paula, Goa-403004, India
E-mail: bhagatjack@gmail.com

SOD catalyze the dismutation of superoxide radicals to oxygen and hydrogen peroxide. Catalase plays a key role in antioxidant mechanism by converting hydrogen peroxide (H₂O₂) to water. Glutathione peroxidase plays major role in reductive detoxification process by catalyzing the reduction of hydrogen peroxide, organic hydroperoxide, and lipid peroxides using reduced glutathione. LPO can affect membrane fluidity, association of biomolecules (membrane bound proteins or cholesterol) with membrane, organelle function, and cell health (Halliwell and Gutteridge, 1999). Oxidative stress biomarkers are commonly used by aquatic toxicologists, where multiple

contaminants are involved. Oxidative stress may cause damage to macromolecules in cell including DNA, proteins, and lipids (Di Giulio *et al.*, 2008). Gastropods are widely studied as bioindicator species because of their sedentary lifestyle, easy availability, high sensitivity, and higher bioaccumulation. In this review, we have summarized the current research on oxidative stress biomarkers in gastropods and their usefulness in environmental monitoring program (Table 1). In the end, we have discussed different factors affecting the variations in oxidative biomarkers response for a better understanding of the phenomenon.

Table 1 Glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation (LPO) in gastropod

Glutathione S-transferase (GST)				
Organism	Organ studied	Exposure	Biomarker effects	References
<i>Bellamya purificata</i>	Gills and digestive glands	Landfill leachate effluent and BPA	Increase	Li <i>et al.</i> , 2008
<i>Bellamya aeruginosa</i>	Hepatopancreas	Ethylbenzene	Increase	Zheng <i>et al.</i> , 2013
<i>Bellamya aeruginosa</i>	Hepatopancreas	Toxic cyanobacterium (<i>Microcystis aeruginosa</i>) and toxic cyanobacterial cells mixed with a non-toxic green alga (<i>Scenedesmus quadricauda</i>)	Increase	Zhu <i>et al.</i> , 2011
<i>Cantareus apertus</i>	Digestive gland	Carbamate pesticide Carbaryl	Increase	Leomanni <i>et al.</i> , 2015
<i>Chilina gibbosa</i>		Azinphos-methyl	No observed effect	Bianco <i>et al.</i> , 2013
<i>Eobania vermiculata</i>	Digestive glands	Sites contaminated with heavy metals	Increase	El-Shenawy <i>et al.</i> , 2012
<i>Gibbula umbilicalis</i>	Whole tissue	Mercury chloride	Increase	Cabecinhas <i>et al.</i> , 2014
<i>Helix aspersa</i>	Digestive gland, kidney and mantle cavity forming tissue (MCFT)	Napthalene saturated atmosphere	No observed effect	Ismert <i>et al.</i> , 2002
<i>Helix aspersa</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Abdel-Halim <i>et al.</i> , 2013
<i>Hexaplex (Murex) trunculus</i>	Digestive gland, gill, muscle	Cadmium, copper, carbofuran and lindane	Increase/decrease	Romeo <i>et al.</i> , 2006
<i>Helix aspersa</i>	Digestive glands	Imidacloprid	Increase	Radwan and Mohamed, 2013
<i>Lymnaea luteola L</i>	Hepatopancreas	Single walled carbon nanotubes	Decreases	Ali <i>et al.</i> , 2015
<i>Lymnaea luteola</i>	Digestive gland	Zinc oxide nanoparticles	Decrease	Ali <i>et al.</i> , 2012
<i>Lymnaea luteola</i>	Hepatopancrease gland	Single walled carbon nanotubes	Decrease	Ali <i>et al.</i> , 2014
<i>Lymnaea stagnalis</i>	Digestive gland		Cytosolic as well as microsomal GST activity of the digestive gland of <i>Lymnaea stagnalis</i> has been examined	Wilbrink <i>et al.</i> , 1991
<i>Monodonta lineata</i>	Gill	Cd ²⁺ , Cu ²⁺	No observed effect	Cunha <i>et al.</i> , 2007
<i>Nucella lapillus</i>	Gill	Cd ²⁺ , Cu ²⁺	Increase/decrease	Cunha <i>et al.</i> , 2007
<i>Physa acuta</i>		Abamectin	Increase/decrease	Ma <i>et al.</i> , 2014

<i>Physa acuta</i>		Imidazolium ionic liquids (ils)	Decrease	Ma <i>et al.</i> , 2014
<i>Planorbarius corneus</i>	Soft tissue and gonads	Chlorpyrifos	No effect	Rivadeneira <i>et al.</i> , 2013
<i>Theba pisana</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Radwan <i>et al.</i> , 2010
<i>Theba pisana</i>	Digestive gland	Copper (Cu), lead (Pb), and zinc (Zn)	Increase/decrease	Radwan <i>et al.</i> , 2010b
<i>Lanistes carinatus</i>		Chlorpyrifos for 28 days	Increase till 21 day and then Decrease	Khalil <i>et al.</i> , 2015
<i>Theba pisana</i>	Digestive gland	Copper-based pesticides; copper oxychloride, copper hydroxide and copper sulphate	Increase	El-Gendy <i>et al.</i> , 2009

Catalase

Organism	Organ studied	Exposure	Biomarker effects	References
<i>Achatina fulica</i>	Kidneys and digestive gland	CdCl ₂ and ZnSO ₄	Decrease at both exposure	Chandran <i>et al.</i> , 2005
<i>Biomphalaria glabrata</i>	Whole body soft tissue	Azinphos- methyl	Decrease in pigmented snail whereas non-pigmented snails are unaffected	Kristoff <i>et al.</i> , 2008
<i>Biomphalaria arabica</i>	Whole tissue	Plant molluscicide <i>Solanum nigrum</i>	Increase	Al-Daihan <i>et al.</i> , 2010
<i>Biomphalaria alexandrina</i>	Soft tissue	Atrazine and Roundup	Decrease	Barky <i>et al.</i> , 2012
<i>Biomphalaria glabrata</i>	Whole tissue	Paraquat	No significant change	Cochon <i>et al.</i> , 2007
<i>Bellamya aeruginosa</i>	Hepatopancreas	Cu-spiked sediment	Increase	Ma <i>et al.</i> , 2010
<i>Bellamya aeruginosa</i>	Hepatopancreas	Ethylbenzene	Increase	Zheng <i>et al.</i> , 2013
<i>Cantareus apertus</i>	Digestive gland	Carbamate pesticide Carbaryl	Increase	Leomanni <i>et al.</i> , 2015
<i>Chilina gibbosa</i>		Azinphos-methyl	Increase	Bianco <i>et al.</i> , 2013
<i>Eubania vermiculata</i>		Methomyl and thiodicarb	Increase	El-Wakil <i>et al.</i> , 1991
<i>Eobania vermiculata</i>	Digestive glands	Sites contaminated with heavy metals	Increase	El-Shenawy <i>et al.</i> , 2012
<i>Gibbula umbilicalis</i>		Mercury chloride	No significant change	Cabecinhas <i>et al.</i> , 2014
<i>Hexaplex (Murex) trunculus</i>	Digestive gland, gill, muscle	Cadmium, copper, carbofuran and lindane	Increase/decrease	Romeo <i>et al.</i> , 2006
<i>Helix aspersa</i>	Digestive glands	Imidacloprid	Increase	Radwan and Mohamed, 2013
<i>Helix aspersa</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Abdel-Halim <i>et al.</i> , 2013
<i>Lymnaea natalensis</i>	Whole tissue	Sediment and water from metal polluted sites	Increase	Siwela <i>et al.</i> , 2010
<i>Lymnaea luteola</i>	Hepatopancrease gland	Single walled carbon nanotubes	Increase	Ali <i>et al.</i> , 2014
<i>Lymnaea luteola</i>	Digestive gland	Zinc oxide nanoparticles	Increase	Ali <i>et al.</i> , 2012
<i>Onchidium struma</i>	Hepatopancreas and muscle	Cu ²⁺	Increase/decrease	Li <i>et al.</i> , 2009
<i>Physa acuta</i>	Soft tissues	Abamectin	Increase	Ma <i>et al.</i> , 2014
<i>Physa acuta</i>	Viscera	Imidazolium ionic liquids (ils)	Increase	Ma <i>et al.</i> , 2014
<i>Theba pisana</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Radwan <i>et al.</i> , 2010
<i>Theba pisana</i>	Digestive gland	[copper (Cu), lead (Pb), and zinc (Zn)	Increases	Radwan <i>et al.</i> , 2010b
<i>Theba pisana</i>	Digestive gland	Copper-based pesticides; copper oxychloride, copper hydroxide and copper sulphate	Increase	El-Gendy <i>et al.</i> , 2009
<i>Lanistes carinatus</i>		Chlorpyrifos for 28 days	Increase till 21 day and then Decrease	Khalil <i>et al.</i> , 2015

Superoxide dismutase (SOD)

Organism	Organ studied	Exposure	Biomarker effects	References
<i>Achatina fulica</i>	Kidneys and digestive gland	CdCl ₂ and ZnSO ₄	Decrease at both exposure	Chandran <i>et al.</i> , 2005
<i>Achatina fulica</i>	Viscera (digestive gland)	Triclosan	Increases	Wang <i>et al.</i> , 2014
<i>Biomphalaria glabrata</i>	Whole body soft tissue	Azinphos- methyl	Decrease in pigmented snails whereas non-pigmented snails showed biphasic effect	Kristoff <i>et al.</i> , 2008
<i>Biomphalaria alexandrina</i>	Whole tissue and hemolymph	Schistosoma mansoni	Increase/decrease	Mahmoud and Rizk, 2004
<i>Biomphalaria alexandrina</i>	Soft tissue	Atrazine and Roundup (glyphosate)	Decrease	Barky <i>et al.</i> , 2012
<i>Biomphalaria glabrata</i>	Whole tissue	Paraquat	Decrease	Cochon <i>et al.</i> , 2007
<i>Bellamya aeruginosa</i>	Hepatopancreas	Ethylbenzene	Increase	Zheng <i>et al.</i> , 2013
<i>Bellamya aeruginosa</i>	Hepatopancreas	Cu-spiked sediment	Increase	Ma <i>et al.</i> , 2010
<i>Cantareus apertus</i>	Digestive gland	Carbamate pesticide Carbaryl	Increase	Leomanni <i>et al.</i> , 2015
<i>Chilina gibbosa</i>		Azinphos-methyl	No observed effect	Bianco <i>et al.</i> , 2013
<i>Gibbula umbilicalis</i>		Mercury	No significant change	Cabecinhas <i>et al.</i> , 2014
<i>Lymnaea stagnalis</i>	Hemocyte	Particulate agents (latex, Escherichia coli, Staphylococcus saprophyticus, zymosan and with phorbol myristate acetate	Phagocytic stimulation of the hemocytes resulted in a superoxide dismutase induction	Dikkeboom <i>et al.</i> , 1987
<i>Lymnaea natalensis</i>	Whole tissue	Sediment and water from metal polluted sites	Decrease	Siwela <i>et al.</i> , 2010
<i>Onchidium struma</i>	Hepatopancreas and muscle	Cu ²⁺	Increase/decrease	Li <i>et al.</i> , 2009
<i>Oncomelania hupensis</i>	Liver tissue	<i>Arisaema erubescens</i> and <i>Nerium indicum</i> extracts	Increase	Zhang <i>et al.</i> , 2009
<i>Oncomelania hupensis</i>	Liver	Sanguinarine (50 Oncomelania hupensis per 500 ml solution)	Increase	Sun <i>et al.</i> , 2011
<i>Oncomelania hupensis</i>	Cephalopodium and liver	Extracts of <i>Arisaema erubescens</i> tubers by acetic acetal (AAE), benzinum (BZE), n-butanol (NBE) and chloroform (CFE) were	Increase	Ke <i>et al.</i> , 2008
<i>Physa acuta</i>		Abamectin	Increases at earlier exposure and later declines	Ma <i>et al.</i> , 2014
<i>Physa acuta</i>		Imidazolium ionic liquids (ILS)	Decrease	Ma <i>et al.</i> , 2014

Glutathione peroxidase (GPx)

Organism	Organ studied	Exposure	Biomarker effects	References
<i>Austrocochlea porcata</i>	Soft tissues	Crude oil	Increase	Reid <i>et al.</i> , 2003
<i>Achatina fulica</i>	Kidneys and digestive gland	CdCl ₂ and ZnSO ₄	Decrease at both exposure	Chandran <i>et al.</i> , 2005
<i>Bellamya purificata</i>		Landfill leachate effluent and bisphenol A (BPA)	No observed effect	Li <i>et al.</i> , 2008
<i>Cantareus apertus</i>	Digestive gland	Carbamate pesticide Carbaryl	Increases	Leomanni <i>et al.</i> , 2015
<i>Eobania vermiculata</i>	Digestive glands	Sites contaminated with heavy metals	Increase	El-Shenawy <i>et al.</i> , 2012
<i>Helix aspersa</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Abdel-Halim <i>et al.</i> , 2013
<i>Lanistes carinatus</i>		Chlorpyrifos	Increases	Khalil <i>et al.</i> , 2015
<i>Lymnaea luteola L</i>	Hepatopancrease gland	Silver nanoparticles	Increases	Ali <i>et al.</i> , 2014

<i>Lymnaea natalensis</i>		Polluted sediment and water	Increases	Siwela <i>et al.</i> , 2010
<i>Lymnaea luteola L</i>	Hepatopancreas	Single walled carbon nanotubes	Decreases	Ali <i>et al.</i> , 2015
<i>Lymnaea luteola L</i>	Digestive gland	Zinc oxide nanoparticles	Increases	Ali <i>et al.</i> , 2012
<i>Theba pisana</i>	Digestive gland	Metal polluted sites	Increases	Radwan <i>et al.</i> , 2010
<i>Theba pisana</i>	Digestive gland	Copper-based pesticides; copper oxychloride, copper hydroxide and copper sulphate	Increase	El-Gendy <i>et al.</i> , 2009

Lipid Peroxidation

Organism	Organ studied	Exposure	Biomarker effects	References
<i>Achatina fulica</i>	Viscera (digestive gland)	Triclosan	Dose dependent Increase	Wang <i>et al.</i> , 2014
<i>Achatina fulica</i>	Kidneys and digestive gland	CdCl ₂ and ZnSO ₄	Increase at both exposure	Chandran <i>et al.</i> , 2005
<i>Biomphalaria glabrata</i>	Whole tissue	Paraquat	Increase	Cochon <i>et al.</i> , 2007
<i>Biomphalaria arabica</i>	Whole tissue	Molluscicide Solanum nigrum	Increase	Al-Daihan <i>et al.</i> , 2010
<i>Biomphalaria alexandrina</i>	Soft tissue	Exposure to pesticides Atrazine and Roundup (glyphosate)	Increase	Barky <i>et al.</i> , 2012
<i>Bellamyia aeruginosa</i>	Hepatopancreas	Ethylbenzene	No observed effect	Zheng <i>et al.</i> , 2013
<i>Eobania vermiculata</i>	Digestive glands	Sites contaminated with heavy metals	Increase	El-Shenawy <i>et al.</i> , 2012
<i>Gibbula umbilicalis</i>		Mercury	No significant change	Cabecinhas <i>et al.</i> , 2014
<i>Helix aspersa</i>		Extremely low frequency (ELF) 50-Hz magnetic fields	Increase	Regoli <i>et al.</i> , 2005
<i>Helix aspersa</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Abdel-Halim <i>et al.</i> , 2013
<i>Lymnaea luteola</i>	Hepatopancreas gland	Single walled carbon nanotubes	Increase	Ali <i>et al.</i> , 2014
<i>Lymnaea natalensis</i>	Whole tissue	Sediment and water from metal polluted sites	Increase	Siwela <i>et al.</i> , 2010
<i>Lymnaea luteola L</i>	Hepatopancreas	Carbon nanotubes	Increase	Ali <i>et al.</i> , 2015
<i>Lymnaea luteola</i>	Digestive gland	Zinc oxide nanoparticles	Increase	Ali <i>et al.</i> , 2012
<i>Lanistes carinatus</i>		Chlorpyrifos	Increase	Khalil <i>et al.</i> , 2015
<i>Oncomelania hupensis</i>	Liver	Sanguinarine (50 Oncomelania hupensis per 500 ml solution)	Increased but not significant	Sun <i>et al.</i> , 2011
<i>Physa acuta</i>	Soft tissue	Abamectin	Increase	Ma <i>et al.</i> , 2014
<i>Physa acuta</i>	Viscera	Oxicities of imidazolium ionic liquids (ils)	Increase	Ma <i>et al.</i> , 2014
<i>Theba pisana</i>	Digestive gland	Sites contaminated with heavy metals	Increase	Radwan <i>et al.</i> , 2010a
<i>Theba pisana</i>	Digestive gland	Copper (Cu), lead (Pb), and zinc (Zn)	Increases for all exposures	Radwan <i>et al.</i> , 2010b
<i>Theba pisana</i>	Digestive gland	Copper-based pesticides; copper oxychloride, copper hydroxide and copper sulphate	Increase	El-Gendy <i>et al.</i> , 2009

Reactive Oxygen Species (ROS)

Oxygen is essential to life and also toxic at the same time due to formation of free radicals. The production of ROS is a natural phenomenon, which is triggered by various external factors. Oxidative stress is caused due to imbalance between cell's oxidative defense and the production of excess

ROS during the impaired oxidant defense mechanism (Fig. 1). ROS can be radical (Superoxide, $\cdot\text{O}_2^-$; Hydroxyl, $\cdot\text{OH}$; Peroxyl, $\cdot\text{RO}_2$; Alkoxy, $\cdot\text{RO}$; Hydroperoxyl, $\cdot\text{HO}_2$) or non-radical (Hydrogen peroxide, H_2O_2 ; Hypochlorous acid, HOCl ; Ozone, O_3 ; Singlet oxygen, $^1\text{O}_2$; Peroxynitrite, ONOO^-). Radicals are very reactive

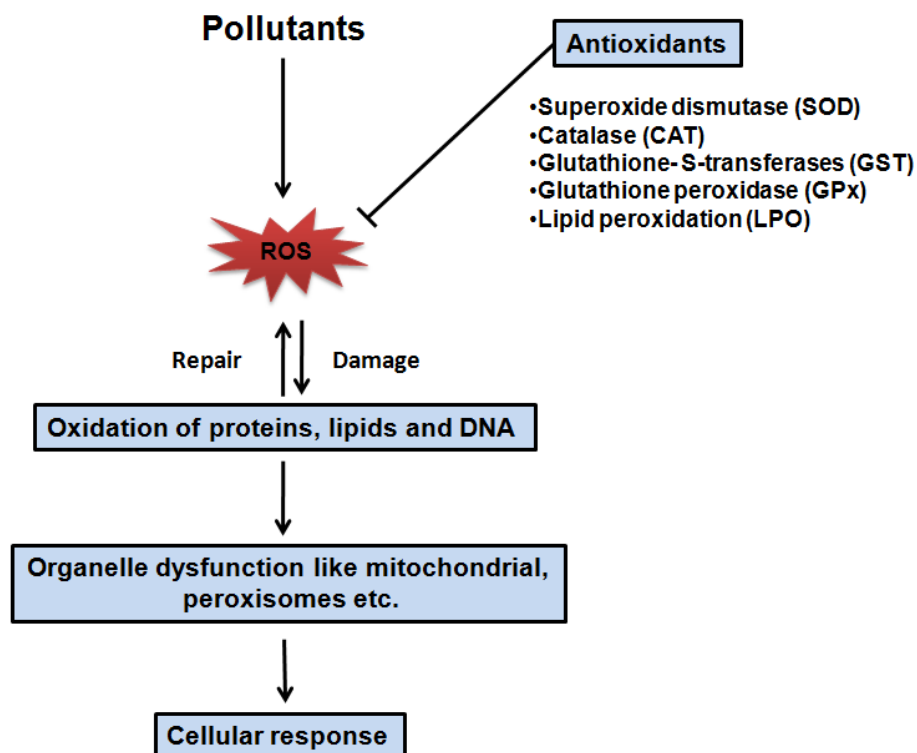


Fig. 1 Overview of reactive oxygen species and oxidative stress

that carry one or more unpaired electron, and is important in lipid peroxidation and DNA damage (Halliwell and Gutteridge, 1999). The non-radicals are mainly responsible for causing oxidative stress.

Some chlorinated compounds can generate hydrogen radical from H_2O_2 by direct metal ion-independent reactions (Halliwell and Gutteridge, 2007). Hydroxyl radical can be produced as a result of conjugation of transition metal ions and H_2O_2 during fenton reaction and hemolytic fission of O-O bond in H_2O induced by UV radiation (Halliwell and Gutteridge, 1999). Hydroxyl radical is strongly reactive and can cause more biological damage than any other ROS. Highly reactive $\cdot OH$ can abstract H atom from sugars, purines, and pyrimidines in DNA bases and form variety of products (Breen and Murphy 1995). $\cdot OH$ can also react with protein or lipid molecule to form oxidative damage products. $\cdot OH$ can be enzymatically metabolized to oxygen and water or converted to extremely reactive hydrogen peroxide.

Organic compounds are well-known pro-oxidants and accelerate the production of ROS and also increase lipid peroxidation. During the metabolic pathway of oxidation of organic compounds, oxygen undergoes stepwise reduction coupled with generation of ATP during electron transport, leading to production of ROS. Transfer of one, two or three electrons to oxygen result in production of $O_2\cdot^-$ (superoxide radical), H_2O_2 (hydrogen peroxide) and $OH\cdot$ (Hydroxyl radical) respectively. ROS are product of normal metabolism and plays an important role in cell signaling and

pathogen defense. The superoxide anion can also be produced due to action of xanthin and hypoxanthin oxidase enzyme. It doesn't readily cross cell membrane, although it can pass through anion exchange protein. Superoxide anions are readily converted to oxygen and hydrogen peroxide by the action of superoxide dismutase. However, due to imbalance between the production of ROS and biological system's ability of detoxification, ROS get accumulated into the cell and causes deleterious effects. The ROS formed can react with macromolecules (e.g. lipids, proteins, nucleic acids) and provoke protein denaturation, lipid peroxidation, DNA damage and others.

Hydrogen peroxide can penetrate biological membrane and act as an intermediate in the production of other highly reactive ROS species (eg. hydroxyl radical). Hydrogen peroxide can be removed from the cell by several enzymes like catalase, glutathione peroxidase and peroxiredoxins.

Consequently, cells have evolved with several defense mechanisms to neutralize the damage caused by ROS and their reactive products. The non-enzymatic mechanism involves quenching of ROS by molecules like GSH, carotenoids or ascorbate. Antioxidant enzyme is the major mechanism in scavenging the free radical produced as a result of various metabolic activities.

Snails in environmental monitoring

Molluscs have been used extensively for environmental monitoring programs. Gastropods

account for eighty percent of all living molluscs, and have recently attracted attention because of their potential as sentinel organisms, thanks to the discovery of imposex due to TBT pollution (Lima *et al.*, 2011). Gastropods perform an important role in marine food chain and are used as a source of food for many fishes and birds. They are omnipresent in the aquatic environment and are identified as a suitable bioindicator for heavy metal pollutions (Selgrade, 1999, 2005; Gundacker, 2000; Galloway and Depledge, 2001; Galloway and Handy, 2003; Auffret, 2005; Woolhiser *et al.*, 2005; Itziou and Dimitriadis, 2011; Abdel-Halim *et al.*, 2013). They transfer toxicants from lower to higher trophic level organism through food chain. They have limited ability to metabolize xenobiotics and thus they are prone to accumulate high concentrations of hydrocarbons (Zheng *et al.*, 2012). The studies involving the genotoxic and cytotoxic damage in snails exposed to pollutants are well documented (Sarkar *et al.*, 2008, 2011; An *et al.*, 2012; Bhagat *et al.*, 2012; Ma *et al.*, 2014).

Oxidative stress biomarkers

Oxygen is indispensable for all the aerobic species but the reactive forms of oxygen such as the superoxide (oxygen with an extra electron) can lead to certain disasters. To combat these phenomenon, the organism has several mechanisms which keep these reactive species in control as they are also important in several beneficial processes. However, there are several processes which are interdependent on each other because they share many metabolites and products. Antioxidant enzyme can be induced under slight oxidative stress but severe oxidative stress can lead to suppression of these enzymes (Xu *et al.*, 2009).

GST

Glutathione S-Transferase is a phase II multifunctional enzyme and plays a critical role in conjugation of electrophilic compounds (phase I metabolites) on one hand and in the defense against oxidative damage and peroxidative products of DNA and lipids (Oost *et al.*, 2003) on the other hand. The GST activity in marine species is greatly induced by the presence of xenobiotic contaminants in the environment. Thus an increase in the GST activity in the marine organisms clearly indicates their exposure to such toxic contaminants. An increasing concentration of ROS causes oxidative stress which may lead to increase in the GST activity. Therefore, an increase in GST activity shows the high concentration of xenobiotic compounds present in the environment.

GST has been proposed as a promising biomarker in snails exposed to aquatic pollutants (Li *et al.*, 2008). Induction of GST activity has been reported in several studies on gastropods exposed to variety of pollutants. The studies concerning seasonal and developmental differences in GST in snails are limited. GSTs are involved in the metabolic activation and deactivation of PAH metabolites. An increased GST activity was observed in experiments with freshwater snail *Bellamya aeruginosa* exposed to ethylbenzene for 7 days (Zheng *et al.*, 2013). However, GST activity at

higher concentrations (450 and 1,000 µg/L) showed progressive decrease after 14 or 21 day of exposure which may be due to the poisoning effect on GST by extra ROS (Cunha *et al.*, 2007). Ismert *et al.*, (2002) reported no change in GST activity in digestive gland, kidney and mantle cavity forming tissue (MCFT) in snail *Helix aspersa* exposed to naphthalene. The author reported 1.5 fold decreases in GPx activity in digestive gland in exposed snails.

Exposure to pesticides has shown induction or inhibition of enzyme activities. Copper-based pesticides have been shown to cause decline in GSH content in digestive gland of land snail, *Theba pisana* (El-Gendy *et al.*, 2009). Rivadeneira *et al.* (2013) showed no effects on GST activity in freshwater snail *Planorbarius corneus* exposed to organophosphate insecticide chlorpyrifos. In another study azinphos-methyl also failed to induce any effect on GST in *B. glabrata* (Kristoff *et al.*, 2008). In contrast, azinphos-methyl showed no change in GST activity in freshwater gastropod *Chilina gibbosa* (Bianco *et al.*, 2013). More research in this context is needed to understand the detoxification mechanism of pesticides involving GSTs.

Li *et al.* (2008) has investigated the effects of landfill leachate effluent and bisphenol A in *B. purificata* and reported decrease in the GSH content and increase in the GST content in treated snails. Ma *et al.* (2014) has studied the toxic effects of abamectin (ABM), in *Physa acuta* and reported promotion of GST activity at the earlier periods of treatment (12 - 48 h) in exposed snails, and inhibition at the end of test. Accumulations of heavy metal in shells and whole tissues of *Lymnaea luteola* exposed to contaminated sediments have been studied by Siwela *et al.* (2010). The author reported significantly higher concentrations of Pb, Cd, Zn and Ni in soft tissues and Zn, Cu and Cd in shells of exposed snails. An increase in GST activity in *H. aspersa* was observed in the metal contaminated sites then in reference sites (Abdel-Halim *et al.*, 2013). *H. trunculus* has been shown to accumulate heavy metals like cadmium (Bouquegneau *et al.*, 1988; Dallinger *et al.*, 1989), copper (Romeo *et al.*, 2006), mercury (Catsiki and Arnoux, 1987). Increased activity of GST with increasing Hg concentrations was observed in sea snail *Gibbula umbilicalis* (Cabecinhas *et al.*, 2014). Some studies have shown conflicting results with unaltered or lower GST activities in snails exposed to heavy metals. *In vivo* exposure to cadmium chloride showed no significant change on GST activity snails (*Monodonta lineata* and *Nucella lapillus*) (Cunha *et al.*, 2007). The author reported decrease in GST activity when *N. lapillus* was exposed to copper sulphate pentahydrate. Snail *H. trunculus* has shown a decrease in GST activity when exposed to cadmium. Decrease in GST activity after exposure to heavy metals can be due to direct action of metals on the enzyme or inhibition of GST by extra ROS (Shumilla *et al.*, 1998). GSH tend to chelate with heavy metal before the heavy metal reacts with metallothioneins, which can also indirectly cause decrease in the GST activity. Metals can also cause depletion of the GSH substrate by binding or oxidizing it (Canesi *et al.*, 1999). Lower

reduced glutathione (GSH) content was observed in snail *T. pisana* collected from sites polluted with heavy metals (Radwan *et al.*, 2010a). Negative correlation of GSH and heavy metal concentrations in digestive gland has been reported by the author. Various factors can also influence the down-regulation of the GST gene (Roling and Baldwin, 2006). Ali *et al.* (2012) have also reported decrease in GST activity in digestive glands of *L. luteola* exposed to zinc oxide nanoparticles. Seasonal variation in GST activity has also been reported in snails. Highest activity of GST was observed in summer close to polluted sites with heavy metal in *H. aspersa* (Larba and Soltani, 2014). Oxidative stress responses were correlated with increasing metal concentrations in soil samples in *H. aspersa* (Larba and Soltani, 2014). Romeo *et al.* (2006) has investigated the effect of toxic metals (cadmium and copper) along with two organics (carbofuran and lindane) to snail *H. trunculus*. The author found that digestive gland and gill cells have been shown to accumulate more metals than muscle. This observation indicates that GSTs are a suitable biomarker in snails exposed to heavy metals.

CAT

Catalase plays an important role in enzymatic oxidant defense by protecting the cell from hydrogen peroxide by converting them into oxygen and water. Niyogi *et al.* (2001) has also reported positive correlation of CAT activity with PAH content in oyster *S. cucullata*. Such a relationship has also been reported for different bivalve species exposed to hydrocarbons that suggest that oxidative stress may be induced by hydrocarbons. *B. aeruginosa* (Reeve), exposed to ethylbenzene (5 - 1,000 µg/L) for 21 days has reported an increase in CAT activity (Zheng *et al.*, 2013). Increase in CAT activity were also reported in snails exposed to imidacloprid (Radwan and Mohamed, 2013), zinc oxide (Ali *et al.*, 2012), carbon nanotubes (Ali *et al.*, 2015), abamectin (Ma *et al.*, 2014), imidazolium ionic liquids (Ma *et al.*, 2014).

Exposure to metal increases the production of ROS including H₂O₂ which in turn induces the CAT activity in the body. Increased activity of CAT was shown in *H. aspersa* exposed to metal dust containing Cu, Zn, Pb, Cr, Ni and Fe (Nedjoud *et al.*, 2009). Snail *H. trunculus* when exposed to cadmium, carbofuran, and lindane, has shown increase in CAT activity (Romeo *et al.*, 2006). Radwan *et al.* (2010b) has reported increased level of CAT in *Theba pisana* exposed to copper (Cu), lead (Pb), and zinc (Zn). CdCl₂ and ZnSO₄ have also shown to induce CAT activity in *Achatina fulica* (Chandran *et al.*, 2005). Chronic exposure to Cu-spiked sediment has shown to induce CAT activity in *B. aeruginosa* (Ma *et al.*, 2010). There was no significant increase in CAT activity in *Gibbula umbilicalis* when exposed to mercury for 96 h (Cabecinhas *et al.*, 2014). However, there are also reports that CAT activity decreases in the digestive gland of the *H. aspersa* exposed to Ni (Zawisza-Raszka *et al.*, 2010).

Evidences from laboratory exposure of pesticides and insecticides to snails have shown conflicting results. Significant increase in CAT

activity was reported in *A. fulica* exposed to 40, 69, and 118 mg/kg of triclosan but high concentrations of 200 and 340 mg/kg showed inhibition in the enzyme activity (Wang *et al.*, 2014). In *C. gibbosa*, significant increase in CAT activity was reported in snails exposed to azinphos-methyl (Bianco *et al.*, 2013). In another study el-Wakil *et al.* (1991) also reported significant increase in CAT activity in *Eubania vermiculata* exposed to methomyl and thiodicarb whereas a decrease in CAT activity was observed when the snails were exposed to metaldehyde. *Theba pisana* exposed to sublethal doses (40 % and 80 % of LD₅₀) after 48 h) of copper-based pesticides; copper oxychloride, copper hydroxide and copper sulphate have shown increase in CAT activity (El-Gendy *et al.*, 2009). In another study, Khalil *et al.* (2015) reported increase in CAT activity in *Lanistes carinatus* exposed to Chlorpyrifos till 21 days and then a decline in enzyme activity was observed. *Biomphalaria alexandrina* exposed to Atrazine and Roundup (glyphosate) has shown decrease in CAT activity (Barky *et al.*, 2012). In another study Cochon *et al.* (2007) has reported no significant change in *Biomphalaria glabrata* exposed for either 4 or 48 h to 0.5mg/l of paraquat.

SOD

Significant increase in SOD activity in hepatopancreas of *Onchidium struma* was observed after one week exposure to Cu²⁺ (range 1.35 to 4.20 mg/L), however in the muscle the increase in SOD activity was not consistent. Cadmium has shown to decrease SOD activity in snail *A. fulica* (Chandran *et al.*, 2005). Exposure to heavy metal results in increased production of ROS, which is controlled by enzymatic and non-enzymatic defense in the cell. GSH act as a quencher of O₂^{*}, decrease in GSH level can lead to production of high amount of O₂^{*}, which in turn can inhibit SOD activity in the cell. Inhibition of SOD activity was reported in *A. fulica* exposed to CdCl₂ and ZnSO₄ (Chandran *et al.*, 2005). In snail (*L. natalensis*), exposed to sediment and water from metal-polluted sites, decrease in SOD was observed (Siwela *et al.*, 2010).

Exposure to triclosan resulted in significant increase in SOD activity in *A. fulica* (Wang *et al.*, 2014). Similar results were also reported in the digestive gland of *Cantareus apertus* exposed to carbamate pesticide carbaryl (Leomanni *et al.*, 2015). Barky *et al.* (2012) found exposure to pesticides Atrazine and Roundup (glyphosate) tends to decrease SOD in *B. alexandrina*. Cochon *et al.* (2007) also observed SOD activity was significantly decreased (27 %) in *B. glabrata* snails after 48 h of treatment with paraquat. A decrease in SOD activity in pigmented *B. glabrata* was observed, after exposure to 5 mg/l azinphos- methyl for 48 or 96 h, however in the non-pigmented exposed snails the enzyme activity was found to be biphasic. Bianco *et al.* (2013) reported no change in SOD activity in *C. gibbosa* exposed to exposure to azinphos-methyl (0.2 - 20 µg/l).

Ke *et al.* (2008) observed an increase in SOD activity in cephalopodium and liver in *Oncomelania hupensis* exposed to four extracts of *Arisaema erubescens* tubers by acetic acetal (AAE),

benzium (BZE), n-butanol (NBE) and chloroform (CFE). The snail *Physa acuta* exposed to toxicities of imidazolium ionic liquids (ILs) showed decrease in SOD activity (Ma *et al.*, 2014). It has been found that SOD is inactivated by dismutation product, H₂O₂ over time (Rhodes, 2000).

Glutathione peroxidase (GPx)

GPx catalyzes the reduction of hydroperoxides including H₂O₂, using reduced glutathione. The level of H₂O₂ is dependent on the SOD, which produces it and also on CAT which uses H₂O₂ as a substrate. GPx activity also depends on the intake of dietary selenium and the level of reduced glutathione (GSH), which is involved in the reaction catalyzed by GST. Hence a battery of biomarker is involved in detoxification of ROS and these biomarkers are inter-dependent on each other. The increase in GPx activity is often observed in snails collected from sites contaminated by heavy metals (Radwan *et al.* 2010; Abdel-Halim *et al.*, 2013). Snails exposed to water from metal-polluted area have also been reported to induce GPx activity (Siwela *et al.*, 2010). In another study, Li *et al.* (2008) has observed no change in GPx activity in snail *B. purificata* exposed to landfill leachate effluent and bisphenol A (BPA). Exposure to zinc oxide nanoparticles resulted in increase in GPx activity in snail *L. luteola*.

Pesticides cause generation of free radical which increases the oxidative stress in the body. Several investigations have reported increase in GPx activity in snails exposed to pesticides and insecticides. Increase in GPx has been reported in *Cantareus apertus* (Leomanni *et al.*, 2015), *Lanistes carinatus* (Khalil *et al.*, 2015), and *T. pisana* (El-Gendy *et al.*, 2009) exposed to various pesticides. In contrast, few studies have also shown decrease or no effects on GSH in organism exposed to pesticides. Cacciatore *et al.* (2015) has reported significantly decreased glutathione (GSH) in *Planorbarius corneus* exposed to Azinphos-methyl and chlorpyrifos. In a study carried out by Liu *et al.* (2015), decrease in GSH content were reported in goldfish *Carassius auratus* exposed to dichlorvos. As GPx uses GSH as the reducing factor for the detoxification of hydroperoxide, unavailability of GSH might lead to decrease value of GPx.

Lipid peroxidation (LPO)

Lipid peroxidation has been studied extensively in environmental monitoring programs. Malondialdehyde (MDA) is a low molecular weight end product of lipid peroxidation and is widely used as a biomarker of oxidative stress. Increased level of oxidative damage in terms of lipid peroxidation has been reported in various snail species exposed to laboratory or environmental contaminants (Al-Daihan *et al.*, 2010; Barky *et al.*, 2012; El-Shenawy *et al.*, 2012; Ali *et al.*, 2015; Ma *et al.*, 2014).

A widely used pesticides paraquat has shown a significant increase in LPO in *B. glabrata* (Cochon *et al.*, 2007). In another study Barky *et al.* (2012) reported induction of LPO in *B. alexandrina* exposed to Atrazine and Roundup pesticides. *Lanistes carinatus* when exposed to Chlorpyrifos for 28 day has also shown induction of LPO (Khalil *et al.*, 2015). Carbon nanotubes have shown to induce

LPO in *L. luteola* (Ali *et al.*, 2015). Some studies have shown no increase in LPO with exposure to contaminants e.g. Zheng *et al.* (2013) has shown no change in LPO in *B. aeruginosa* (Reeve), exposed to ethylbenzene. Dose dependent increase in LPO was reported in digestive gland of *Achatina fulica* exposed to broad-spectrum antimicrobial agent, triclosan (Wang *et al.*, 2014).

In the field, sites contaminated with heavy metal have shown to induce LPO in snails (Radwan *et al.*, 2010a; Abdel-Halim *et al.*, 2013). Cu, Pb, Zn have also shown to increase LPO in *T. pisana* (Radwan *et al.*, 2010b). Increased lipid peroxidation occurred in *L. natalensis* exposed to sediment and water from metal-polluted sites (Siwela *et al.*, 2010). Higher values of LPO were reported in digestive glands of *H. aspersa* collected from metal contaminated sites by Abdel-Halim *et al.* (2013). Elevated level of LPO was reported in kidneys and digestive gland in *A. fulica* exposed to CdCl₂ and ZnSO₄ (Chandran *et al.*, 2005). Cadmium doesn't generate ROS directly, but can alter the GSH and MT level in the cell, which can lead to LPO of cell membrane (Mahboob *et al.*, 2013). It has been known that LPO products are complex hydroperoxides that exerts cytotoxic and genotoxic damage. It has been demonstrated that CAT and SOD inhibit lipid peroxidation (Packer and Fuchs, 1992). Exposure of contaminants results in increase production of hydrogen peroxide which is destroyed by CAT. Increase activity of CAT under these condition can effectively counteract LPO.

Increase in LPO was reported in Oysters exposed to Cu but Cu in combination with GSH inhibitor (buthionone sulfoximine) has shown lower LPO values, suggesting that GSH has protective role to Cu toxicity (Connors and Ringwood, 2000). Exposure to Cu in oysters (resulted in increased lipid peroxidation (LPO) (Connors and Ringwood, 2000) but at the same time, when exposed to Cu and a GSH inhibitor, a lower LPO was reported suggesting a protective role of GSH to Cu toxicity. Collectively these data suggests that LPO along with other oxidative stress biomarkers such as GST, CAT, and SOD can help in better understanding of the oxidative stress phenomenon.

Conclusion and perspective

Variations in oxidative stress biomarkers also depend on food availability, spawning period and exposure to pollutants and water temperature (Sheehan and Power, 1999; Hagger *et al.*, 2010). However, several other factors such as mode of life, reproductive cycle, and feeding habit can also influence the oxidative stress biomarkers (Sheehan and Power, 1999). Abiotic factors such as temperature, salinity and dissolved oxygen content also influence the levels of antioxidant enzymes. Temperature and salinity significantly affect the biomarker response in an organism (Cailleaud *et al.*, 2007). Weak seasonal changes in environmental parameters can result in lack of seasonal variations in the biomarker. Higher temperature induces the oxygen consumption in the cell leading to elevated production of ROS, which are compensated antioxidant defense (Ronisz *et al.*, 1999). Increase in LPO activity at higher temperature is due to

increase in the polyunsaturation in mitochondrial membrane due to higher mitochondrial respiration, with increase in the formation of ROS. Use of multi-biomarkers has become an increasingly popular approach to study environmental parameters and organismal health. Combination of biomarkers gives a comprehensive picture and provides better insights to study the effects of pollutants. Hence the appropriate combination of biomarkers is recommended for the study of oxidative stress.

Acknowledgement

We would like to thank the Director, CSIR-NIO for all the facilities and encouragement and CSIR New Delhi for financial support under project No PSC0206. Department of Biotechnology (Govt. of India), New Delhi is acknowledged for Senior Research Fellowship (SRF) to Jacky Bhagat. This is contribution No 5955 of CSIR-NIO, Goa.

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