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Editorial

Metal Stress in the Environment: Molluskan Strategy

Mukherjee S and Bhattacharya S*
Department of Zoology, Visva-Bharati University, India

***Corresponding author:** Bhattacharya S, Environmental Toxicology Laboratory, Department of Zoology, Visva-Bharati University, Santiniketan-731235, India

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Editorial

The increasing load of environmental pollutants, majorly the heavy metals poses a serious threat to the environment. Position of lead nitrate as a severe toxicant compromising the status of human health especially children is well documented [1,2]. Alteration in lead induced pro-oxidant/ anti-oxidant balance could accelerate tissue injury via oxidative damage to critical biomolecules such as lipids, proteins and DNA [3]. Consequently epidemic occurrence of global lead poisoning has elicited the need for preventive action, and a number of preventive measures have been introduced in several countries [4]. Various technologies have been implemented to treat lead-contaminated soils. Research and evaluation of these technologies is still on an experimental stage and are somewhat limited in scope. Most importantly, these analyses do not account for interactions among different chemicals and validation of these data with *in situ* biological experiments results appears to be controversial [5].

Taking all these into account, a desperate need was felt to develop a bio product to put a ceiling on lead induced toxic insults to the ecosystem. A research group from the Environmental Toxicology Laboratory (ETL), Department of Zoology, Centre for Advanced Studies, of Visva-Bharati University, India initiated research on C-Reactive Protein (CRP) in rat [6] and mollusk [7,8]. CRP is a prototypic acute phase reactant, which is a phylogenetically conserved protein expressed in invertebrates [9,10] and also in all vertebrates [11-13]. Interestingly, CRP is inducible in vertebrates but constitutively expressed in invertebrates [9]. It was envisioned that CRP in the invertebrates probably allow the arthropods and mollusks to be evolutionary successful.

The ETL research group has demonstrated that the endogenous level of CRP in the hemolymph of *A.fulica*, a gastropod mollusk, remains significantly high (2-4 mg/ml) [8] as compared to rat [6]. Considering the evolutionary success of *Achatina* and their falling into disrepute as a major agricultural pest in India, it was contemplated that the high endogenous level of CRP is the key to their evolutionary success in the environment. Although there are many reports on properties of CRP in a wide range of *in vitro* and *in vivo* model systems, a clear understanding of definite biological functions of this phylogenetically ancient and highly conserved molecule remains elusive. Recently this group [14] tested anti-bacterial activity of *Achatina* CRP (ACRP) against several human pathogenic bacteria, where they delineated bacterial death through inhibition of salient metabolic enzymes and induction of oxidative stress and apoptosis-like phenotypes in bacterial cells on administration of ACRP.

The anti-stress property of ACRP was investigated in mice, known to have a very low level of endogenous CRP (~2µg/mL) [15]. In order to prove this hypothesis, lead nitrate [$Pb(NO_3)_2$], was administered intraperitoneally at an environmentally relevant dose [14.8 mg/kg body weight (1/10 of LD_{50})] in mice [16]. Lead induced oxidative stress in the experimental mice was counteracted by exogenous administration of ACRP prior to Pb treatment. Further confirmation was obtained from *in vitro* studies on rat hepatocytes where the native protein and its subunits were tested for reversal of lead induced hepatotoxicity [16]. In addition, mice administered (i.p) with $Pb(NO_3)_2$ (100 µg/mouse) showed a marked elevation of liver function indices (SGPT, SGOT) which was reversed by administration of ACRP. Administration of $Pb(NO_3)$ in mice resulted significant depletion in liver GSH and induction of Lipid Peroxides (LPO). Concomitant activities of the antioxidant enzymes, GST and catalase, decreased in treated mice against control. Interestingly, ACRP co-treatment not only reversed liver glutathione and LPO levels but also restored catalase and GST activities. Thus, it has been clearly demonstrated that lead induced oxidative damage was reversed by ACRP.

The mechanism of ACRP induced amelioration of lead toxicity was further studied through *in vitro* experiments in rat hepatocytes where ACRP and its subunits significantly inhibited generation of superoxides when rat hepatocytes were pre-incubated with ACRP and its subunits prior to $Pb(NO_3)_2$ treatment. A sharp depletion in GSH level was noted in the lead treated rat hepatocytes accompanied by a time dependent increase in Malondialdehyde (MDA) production and increased generation of superoxides. Interestingly, ACRP reversed this effect which proves beyond doubt that ACRP is a highly potent anti stress agent and can counter the Pb-induced oxidative stress. Furthermore, they also wondered whether ACRP as a total protein or its degraded fragments are responsible for reducing stress in these rodent models. Therefore they studied the localization of ACRP in the cytoplasm of rat hepatocytes through FITC labeling which clearly demonstrates that ACRP might be degraded into small peptides leading to typical receptor mediated endocytosis alleviating Pb induced stress.

It is probable that a dramatic drop in GSH level in $Pb(NO_3)_2$ treated hepatocytes triggered the cells towards oxidative damage accelerating apoptosis. The depletion in GSH level, being one of the indices of oxidative stress, indicates profound scavenging of superoxides; however, utilization of GSH beyond a critical level could induce apoptosis in cells. Interestingly, their findings clearly showed that ACRP suppressed $Pb(NO_3)_2$ induced extrinsic and intrinsic pathways of apoptosis effectively. ACRP was found to significantly retard nuclear degradation and DNA ladder formation in $Pb(NO_3)_2$ treated cells. In addition, Annexin V-Cy3/CFDA dual staining showed

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increased number of apoptotic cells in $Pb(NO_3)_2$ treated hepatocytes against control or ACRP plus Pb treated cells, where lesser number of apoptotic cells and higher number of CFDA positive cells (live cells) were observed. Changes in the mitochondrial membrane potential were determined by JC1 staining. ACRP pre-treatment maintained the membrane integrity as demonstrated by enhanced number of red fluorescent cells compared to the hepatocytes treated with Pb alone. Moreover, $Pb(NO_3)_2$ induced activation of FasL, caspase 8, Bid, caspase 9 and caspase 3 in rat hepatocytes was significantly inhibited by ACRP treatment.

The major contribution of this work [16] is that a new functional role of ACRP crossing the species barrier as a cytoprotective agent for lead nitrate toxicity in rodents is proposed. Moreover, adequate availability of this protein, in *Achatina*, may be exploited further to use ACRP as an anti-stress agent in mammals. Overall, these studies on *A.fulica* will provide a useful indication on the biologic reactivity and toxicologic effects of lead under different conditions and also to assess the validity of *Achatina* as a model for human disease outcomes. Further research is warranted which may elucidate the possibility of integrating a multi marker toxicological approach in the management programs in rural and urban areas.

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