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Effect of Ascorbic Acid on Bioaccumulation of Lead nitrate in Fresh Water Bivalve Lamellidenscorrianus

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Abstract: *Heavy metal pollution can have detrimental effects on the growth rate and biochemical reserves of organisms. Bivalves, known bioindicators of pollutants, can be used as experimental models to screen the effects of toxicants and their detoxifying drugs. In this study, we aimed to investigate the impact of lead toxicity induced by ascorbic acid on the whole body of freshwater bivalves (Lamellidenscorrianus) through chronic exposure to PbNo3 (6.810 ppm) for 20 days. After exposure, we analyzed the samples using an atomic absorption spectrophotometer. Our results showed that the bioaccumulation of lead in the soft tissue of the bivalves was high (276.077 µg/Kg) after exposure. However, the rate of bioaccumulation was lower in bivalves exposed to lead nitrate with ascorbic acid (146.714 µg/Kg). Furthermore, exposure to 50 mg of ascorbic acid resulted in faster recovery in bivalves than those in normal water. This suggests that ascorbic acid (50mg) provides protection against Pb due to its antioxidant activity, which scavenges reactive oxygen species generated by heavy metals. Additionally, ascorbic acid plays a role in collagen synthesis, which rapidly replaces the basement membrane, and binds to metal ions. In conclusion, our study highlights the potential of ascorbic acid as a detoxifying drug against heavy metal pollution. By using bivalves as experimental models, we can better understand the effects of toxicants and develop effective strategies to mitigate their impact on the environment.*

Keywords: Toxicity, Heavy Metals, Ascorbic Acid, Bivalve.

I. INTRODUCTION

Heavy metals build up in living things when they are absorbed and stored more quickly than they can be broken down or eliminated. Freshwater fish and shellfish from rivers have been examined by Liu et al. (2018), Bean et al. (2018), Jia et al. (2018), Mason et al. (2000), and Winger et al. for the distribution, pollution, and accumulation of heavy metals (2000).

The presence of lead in the environment is a growing concern, as it continues to increase due to the discharge of industrial waste, petroleum industry waste, and agricultural contaminants. Heavy metals, including lead, are highly toxic and have a worldwide distribution in the aquatic environment. They tend to accumulate in sediments and are bioconcentrated in molluscs and fishes, even in small quantities.

Metals can enter an organism's body through the consumption of nutrients, food, and water. This can result in the accumulation of metals in the organism's tissues, which can impair physiological functions and potentially lead to health issues. In some cases, the concentration of metals in an organism can become greater than that in the surrounding water or soil. This is known as bioaccumulation, and it can occur when plants and animals are exposed to relatively low concentrations of metals over an extended period of time. It is important to monitor and regulate the levels of metals in our environment to prevent harmful bioaccumulation.

Bivalves are known to accumulate a diverse range of metals in certain tissues (Peter et al., 2018 and Islam et al., 2021) valuable indicator organisms for monitoring the contamination of marine and estuarine environments on a global scale (Feldstein et al., 2003; Copat et al., 2013 and Bogdanovic et al., 2014). These metals can include both essential and toxic elements, and their accumulation can have significant implications for both the health of the bivalves themselves and the ecosystems in which they reside. Mussels are equipped with a range of highly effective detoxification mechanisms that work to reduce the toxicity of any metals they may absorb.

These mechanisms are essential for the survival of mussels in their natural habitat, where they are constantly exposed to a variety of potentially harmful substances. (Byrne and Vesk, 2000).

One particular species of edible bivalve, Venerupis decussata, has been found to accumulate various heavy metals such as iron, zinc, lead, copper, cadmium, and cobalt in their tissues, regardless of their size. (Sherif etal. 2017). It is important to note the potential health risks associated with consuming bivalves that have accumulated high levels of heavy metals. Therefore, further research and monitoring of these populations is necessary to ensure the safety of seafood consumption.

Usero et al., (1997), Buschd, et al., (1998) and 2005, Munir et al. (2005) studied the bioavailability and bioaccumulation of various heavy metals in mussels and oysters. Since then, further research has revealed that heavy metals are distributed, stored, and bound in varying ways within different tissues of mollusks. This information is crucial for understanding the potential impact of heavy metal pollution on aquatic ecosystems and the organisms that inhabit them.

Ascorbic acid, also known as Vitamin C, is a crucial component for normal development in most species. It is produced endogenously, which gives it a hormone-like quality. Glucose and other hexoses can be converted to glucose, which serves as the starting material for the biosynthesis of ascorbic acid. Ascorbic acid plays a significant role in tissue synthesis, growth processes and rapid tissue repair in trauma or disease conditions. Ascorbic acid plays a crucial role in the formation of tissue collagen and the maturation of red blood cells (Talwar, 1980). Additionally, Chatterjee et al. (1995) found that ascorbic acid provides protection against oxidative damage in mammalian tissues, both at the intra and extra cellular levels. The crucial nutrient, vitamin C, is significantly impacted and modified by a range of environmental pollutants, such as pesticides (Ali etal.1993; Bhusari,1987; and Jadhav et al.1996).

Numerous references suggest that bivalves are the optimal experimental model for investigating the mechanisms of detoxification and the effects of detoxifying agents. Perusal of literature shows that the ascorbic acid content in the tissue of freshwater bivalve molluscs has received little attention. Given the various roles of ascorbic acid and the lack of information regarding its concentration in freshwater bivalve tissues and its potential role in mitigating lead toxicity, the present research was undertaken.

II. MATERIALS AND METHODS

Lamellidenscorrianus, a type of bivalve, were collected from Jayakwadi dam in Paithan, located 60 km away from Aurangabad in Maharashtra State. To ensure optimal conditions for experimentation, the bivalves were acclimatized in dechlorinated tap water for five days in a laboratory setting.

After acclimatization, the healthy and active bivalves were divided into three groups: A, B, and C. Group A served as the control, while group B was exposed to a chronic concentration of PbNO3 (6.810 ppm) with an LC50 value of 96 hr/10. Group C was exposed to a chronic concentration of PbNO3 (LC50 value of 96/hr/10) with 50 mg/L of ascorbic acid for 20 days. After being exposed to heavy metals for a period of 20 days, the bivalves in group B were divided into two subgroups, D and E The entire body mass of bivalves from groups A, B, and C was collected after 10 and 20 days, while that of groups D and E was collected after 3, 6, and 9 days. T While the bivalves in group E were exposed to 50 mg/L of ascorbic acid for up to 9 days, the bivalves in group D were permitted to self-cure spontaneously in normal water. The collected samples were then dried at 80°C in an oven until a constant weight was obtained. Finally, the samples were stored in air-tight specimen bottles, with the cork outside waxed to ensure maximum preservation.

The 500 mg sample of whole-body tissue was digested in 10 ml of acid mixture (HCl: HNO3 in 3:1 ratio) on hot plate till dryness. After digestion, the samples were mixed with double distilled water and cooled in a water bath for 6-7 hours. Once cooled, the samples were filtered using Whatman grade 541 filter paper. The resulting volume was then diluted to 50 ml using double glass-distilled water in a volumetric flask.

To determine the lead content, the prepared samples were analyzed using an Atomic Absorption Spectrophotometer (Chemito). It is important to note that proper filtration and dilution techniques were employed to ensure accurate and reliable results. The use of high-quality filter paper and double distilled water also helped to minimize any potential sources of contamination.

Overall, this method provides a precise and efficient means of analyzing lead content in various samples

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III. OBSEREVATION AND RESULTS

Table 1 summarizes the PbNO3 content in Lamellidenscorrianus after exposure to lead nitrate (6.810 ppm) with and without ascorbic acid, as well as during recovery. The results indicate that the concentration of bioaccumulated lead in the soft tissue of the bivalves was high in the presence of PbNO3 (6.810 ppm).

After 10 days of exposure, the bioaccumulated lead in the presence of PbNO3 was 115.658µg/kg, which increased to 276.077µg/kg after 20 days. The rate of bioaccumulation was lower in bivalves exposed to lead nitrate with ascorbic acid compared to those exposed to only PbNO3. After 10 days, the bioaccumulated lead was 49.827µg/kg, and after 20 days, it was 146.174 µg/kg.

Interestingly, bivalves pre-exposed to PbNO3 showed faster recovery in the presence of ascorbic acid than those allowed to cure naturally. After 3 days of recovery, the bioaccumulated lead was reduced to 185.236 µg/kg in normal water and to 167.178 µg/kg in the presence of ascorbic acid. After 6 days, the levels reduced to 117.679 µg/kg and 97.017 µg/kg, respectively, and after 9 days, they were further reduced to 95.432 µg/kg and 76.077 µg/kg in normal water and ascorbic acid, respectively.

In conclusion, the results suggest that the presence of ascorbic acid can aid in the recovery of bivalves exposed to lead nitrate. Additionally, the rate of bioaccumulation of lead is lower in bivalves exposed to lead nitrate with ascorbic acid. These findings have important implications for the management of aquatic ecosystems and the protection of aquatic organisms from the metal toxicity.

IV. DISCUSSION

One of the unique characteristics of heavy metals is their ability to strongly bind with biological molecules. This trait has piqued the interest of many biologists, as heavy metals are known to be stable in the environment and can induce toxic effects as long as they are present. For example, lead is a cumulative toxicant that can accumulate in the tissues of a wide variety of freshwater organisms (Mason and Simkiss, 1983). Understanding the distribution and accumulation of lead within various animal tissues is crucial for ensuring environmental safety. In recent years, there has been significant concern about the bioaccumulation of lead by mollusks, as high levels of this heavy metal may have detrimental effects on these aquatic organisms. Therefore, studying the effects of heavy metals on biological systems is an important area of research for both environmental and health reasons.

Metal ions are absorbed into the body through semi-permeable membranes found in various organs, including the gill, mantle, lining of the mouth, and gastrointestinal tract. In aquatic organisms, the uptake of metals is directly proportional to the concentration of metals in the water. However, accumulation of these chemicals can only occur if the rate of uptake exceeds the rate of elimination. The extent to which various organs accumulate toxicants can vary, and the distribution of toxic chemicals in the body can change over time. Factors such as physiology and the environment can influence the concentration of lead in mollusk tissue, highlighting the importance of understanding the complex interactions between organisms and their surroundings (El-Gendy et al., 2021).

Recent studies have revealed that heavy metals are distributed, stored, and bound differently in various tissues of organisms exposed to field and laboratory conditions. The order of distribution, from least to most affected, was found to be muscle, visceral mass, mantle, gills, and labial palps (MarasingheWadige et al., 2014c). Additionally, Labrot et al. (1999) discovered differences in lead concentrations between tissues of C. fluminea under laboratory conditions, with the foot being the least affected and the remaining tissues being the most affected.

Furthermore, Passow et al. (1961) reported that lead can induce the synthesis of specific proteins that selectively bind to it. The inhibition of enzyme activities by heavy metals can occur due to direct binding with enzyme proteins, damage to cell organelles, or a poor rate of enzyme synthesis (Simkiss and Manson, 1984 and RissodeFaverney et al., 2000).

These findings highlight the importance of understanding the differential distribution and binding of heavy metals in various tissues of organisms. Such knowledge can aid in the development of effective strategies for mitigating the harmful effects of heavy metal exposure on both human and environmental health. The metal content found in mollusks is regulated by either an active mechanism that is directly linked to the organism's metabolism or by the surface-to-volume ratio of the animal (Boyden, 1974; 1977). It is important to note that the distribution of toxic chemicals within the body can change over time. The initial site where a chemical is localized is dependent on various factors, including the flow to the area, the permeability of membranes to the toxicant, and the availability of binding sites.

It is crucial to understand the mechanisms that control the metal content and distribution of toxic chemicals in mollusks, as these organisms are often consumed by humans and can pose a risk to human health. By studying these mechanisms, we can better understand how to mitigate the potential risks associated with consuming mollusks. Additionally, this research can inform policies and regulations aimed at protecting both human health and the health of our marine ecosystems.

According to HoseobLihm's research in 2013, lead toxicity can cause an imbalance in antioxidants in mammalian tissue due to the production of reactive oxygen species (ROS). These ROS can react with tissue, causing damage and leading to the bioaccumulation of lead. Our study has shown that long-term exposure to lead can result in excess accumulation of this toxic substance.

It is important to note that even small amounts of lead can be harmful, which is why detoxification is necessary. This has been emphasized by Angelo et al. in 2007, Bollhöfer in 2012, and Krause-Nehring et al. in 2012.

To prevent the harmful effects of lead toxicity, it is crucial to take steps towards detoxification. This can include reducing exposure to lead and implementing measures to remove it from the body. By doing so, we can protect ourselves and our environment from the damaging effects of lead. Ascorbic acid, also known as vitamin C, is a vital nutrient that plays a crucial role in the distribution and excretion of trace minerals and toxic metals. Numerous researchers have studied the effects of ascorbic acid on these elements.

Hughes (1974) reported that ascorbic acid acts as a diffusible biological antioxidant when present in appropriate concentrations, contributing to the maintenance of the -SH group of proteins. This group is primarily responsible for metal interaction. L-ascorbic acid is a potent antioxidant that can extend its protective effects by chelating metals or reacting with free radicals and removing them from the system (TajmirRiahi, 1991).

Ascorbic acid also acts as a detoxifying agent by forming poorly ionized but soluble compounds with lead (Pilemer, 1940). It is generally considered an excellent reducing agent that can serve as a donor antioxidant in free radical-mediated oxidant processes and reduce metals such as copper and iron (Beuttner and Jurkiewicz, 1996). Ascorbate can serve as both a pro-oxidant and an antioxidant, and it is proposed that the position of this crossover effect is a function of catalytic metal concentration. Therefore, ascorbic acid can protect against the effects of other toxic metals (Bhattacharje et al., 2003).

Furthermore, ascorbic acid can neutralize lead in a less toxic form through its interaction with the metal (Bhattacharje et al., 2003). In summary, ascorbic acid is a powerful nutrient that can play a crucial role in protecting against the harmful effects of toxic metals and trace minerals. There are a variety of detoxification agents that can be utilized to mobilize lead and other stored toxins within the body. One such agent is ascorbic acid, which has been shown to cause lead to transform from its immobilized state to a mobilized form. This transformation leads to an increase in excretion through the lymphatic system, bloodstream, and bile (HoseobLihm et al., 2013).

It is important to note that the decrease in lead excretion on the 10th and 20th day of treatment may be attributed to the fact that a significant amount of lead had already been excreted from the body of the bivalve. As a result, only a small amount of lead remained within the body.

Overall, the use of detoxification agents such as ascorbic acid can be an effective method for mobilizing and eliminating stored toxins from the body. It is important to consult with a healthcare professional before beginning any detoxification regimen to ensure safety and effectiveness.

The results of this study demonstrate that the use of ascorbic acid can significantly reduce the bioaccumulation of lead in bivalves. This is likely due to the chelating activity of ascorbic acid, which binds to the metal or free radical and causes them to precipitate. Once these free radicals have been precipitated, they can be excreted by the bivalve.

When ascorbic acid was externally supplemented to lead-exposed bivalves, the bioaccumulation of Pb decreased from 167.178 µg/kg to 76.077 µg/kg over a period of 3 to 9 days. This suggests that some of the ascorbic acid was absorbed by various tissues in the bivalve, including the gills, gonads, foot, digestive gland, and whole body. As a result, the level of ascorbic acid in the bivalve increased, which helped to minimize the toxic load of the toxicant.

Interestingly, the vitamin C group excreted more lead than the non-treated group, as the bioaccumulation was higher in the non-treated group and excretion was limited due to the toxicity of Pb to various tissues. Overall, these findings suggest that the use of ascorbic acid may be a promising strategy for reducing the bioaccumulation of lead in bivalves and minimizing the potential health risks associated with this toxicant.

V. CONCLUSION

The current study has found that the administration of ascorbic acid can effectively reduce the bioaccumulation of Pb. This is due to the chelating activity of ascorbic acid, which binds to the metal or free radical and causes them to precipitate. Once these free radicals have been precipitated, they are excreted by the bivalve.

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