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# Amalgamated effect of garlic extract and cadmium chloride on the histoarchitecture liver of a freshwater catfish, *Clarias batrachus*

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The water pollution is thus no longer considered to be an aesthetic problem, but a serious economic and public health problem as well (Shanthi *et al.,* 2005). Unfortunately, raw or inadequately treated sewage of millions of people still flow into our lakes and rivers, creating several major kinds of disorders (Chukwuma, 1995). The release of discharge of large number of pollutants, especially heavy metals and pesticides, posed a threat to human life (Saikia, 1988). Pollution of aquatic environment by heavy metals is an extremely important and serious problem and has attracted the attention of the scientists all over the world. During last few decades, industrialization has added ample quantity of various pollutants in the environment. In such situation, it becomes highly imperative to alleviate heavy metal pollution in aquatic ecosystem and make its amelioration through easily available and low cost antidotes to minimize the level of heavy metal accumulation in aquatic food chain which may save the fish and human consumers from acute heavy metal intoxication.

Garlic has played an important dietary and medicinal role throughout the history of mankind. For over 5000 years, garlic has been consumed both as food and used for medicine by ancient scholars. Garlic, *Allium sativum* is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. It is a remarkable plant, which has multiple beneficial effects such as antimicrobial, cardiovascular protectant, platelets aggregation inhibitors, antiasthematic, antineoplastic, fibrinolytic and antioxidant (Augusti, 1996) and hence a proved antidote and a useful agent against various ailments of heavy metal toxicity.

Present investigation has been carried out under controlled conditions in the Department of Zoology and Applied Aquaculture, Barkatullah University Bhopal, during 2010. Indian catfish, *Clarias batrachus* was selected for the purpose of present study as it is one of the best experimental animal by being hardy and bottom feeder. Specimens of this fish were collected from the fish markets of Bhopal. Disease free and healthy fishes were selected for experimentation. Immediately after bringing into laboratory, they were treated with 0.01% $KMnO_4$ solution for 15 minutes and then transferred to plastic pools of 500 *l* capacity containing non-chlorinated water where they were kept for fifteen days for acclimatization. With the pH range of 6.95 to 7.60 and temperature ranging from 16 to 24 °C for 15 days.

***Experimental design***

Three groups of fish were maintained for a maximum period of 60 days. Group (I) was kept unexposed as

control. Group (II) was exposed to 4 ppm of cadmium chloride and group (III) was exposed to 8 ppm cadmium chloride. Group (IV) was exposed to 4 ppm of cadmium chloride and simultaneously treated with 4 ppm of garlic extract. Group (v) was exposed to 8 ppm of cadmium chloride and simultaneously treated with 4 ppm of garlic extract. Exposure concentrations were decided on the basis of 96 h $LC_{50}$ value of $CdCl_2$ which was determined to be 103 ppm. The exposure medium was changed every alternate day to maintain the desired concentration of $CdCl_2$. The water in control group was also changed at the same time. On completion of one month of exposure, three fish randomly selected from control as well as exposed groups were sacrificed. The rest of the fish were sacrificed after two months of exposure.

***Preparation of garlic extract***

Fresh garlic (*Allium Sativum*) bulbs were purchased from a retail food store (Bhopal, India). Then, the garlic bulbs were peeled, weighed (100g), and cleaned garlic were taken and surface sterilized with ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber and the garlic was homogenized aseptically using a sterile mortar and pestle. The homogenized mixture was filtered through sterile cheese cloth. This extract was considered as the 100% concentration of the extract. There after, 4ppm of the extract was prepared as and when required for the experiment.

***Histopathological study***

Liver of the test animals (control and treated) were removed aseptically and preserved in 10% buffered formalin solution. Preserved tissues were washed under tap water for 24 hrs to remove formalin, dehydrated, clarified with xylene and embedded in paraffin blocks. They were cut at 4-5µ thickness by using rotatory microtome and stained routinely with haematoxylin and eosin (H&E) for histopathological examination (Luna, 1968). Stained histopathological sections were examined under Olumpus research microscope. Histopathological changes observed were photographed and interpreted in comparison to the work of others.

Histological studies revealed that liver sections from control fishes show normal histoarchitecture, characterized by polygonal shaped hepatocytes with granular cytoplasm and centrally placed round nuclei. Hepatocytes were arranged in well-organized hepatic cords and separated by narrow blood sinusoids. Liver of fishes exposed to 4 ppm cadmium chloride for 30 and 60 days exhibited deshaping of hepatocytes, eccentric position of nuclei, enucleation, development of vacuoles in cell cytoplasm and necrosis of hepatic tissue(Figs. 2, 3,6 & 7).. More or less similar results are also observed by Rani and Ramanmurthi (1989) in *Tilapia mossambica (Oreochromis mossambicus)* exposed to cadmium chloride at 5 and 50 ppm for 1, 7, 15 and 30 days. Dyk *et al.* (2007) in *Oreochromis mossambicus* exposed to cadmium and zinc. Ashish and Banalata (2008) in liver of *Channa punctatus* exposed to hexavalent chromium. Nunitya *et al.* (2008) in *Clarias gariepinus* exposed to cadmium. Thopan *et al.* (2002) in White Sea bass, *Lates calcarifer* exposed to 5mg/l cadmium chloride for 3 weeks. These findings lend support to the observations of present author.

The histological structure of the liver of fishes exposed to 4 ppm and 8 ppm cadmium chloride alongwith 4 ppm garlic extract for 30 and 60 days exhibited slight improvement over that of exclusively 4 ppm and 8 ppm cadmium chloride exposed

fishes. Simultaneous treatment of fishes exposed to cadmium chloride with garlic extract showed slightly less severity in comparison to exclusively cadmium chloride exposed groups (Figs.4, 5, 8 & 9). The magnitude of changes increased in proportion to increased dose and time period. Under present investigation, addition of garlic extract @ 4 ppm has been found to be beneficial to mitigate the cadmium chloride induced histo-pathological disorders in liver. The lesions were less severe in the liver when compared to the fishes exposed to exclusively cadmium chloride. It explicates that the garlic has the capacity of mending the damage caused by cadmium toxicity. More or less similar results are also observed by Shaaraway *et al.* (2009) in male albino rats exposed to N-nitrosodiethylamine and carbon tetrachloride and Sharma *et al.* (2010) in Swiss albino mice exposed to lead nitrate have also reported the same findings.

Results of present investigation also revealed that the use of garlic extract along with cadmium chloride can reduce cadmium accumulation in tissue. The efficiency of garlic was perhaps due to the presence of sulfur-containing amino acids and compounds having free carboxyl (C=O) and amino ($NH_2$) groups in their structures. These biologically active compounds might have chelated cadmium and enhanced its excretion from the body, resulting in reduced cadmium accumulation in tissues. The mechanism of *Allium sativum* mediated chelation of cadmium might include formation of ionic bonds between sulfur-containing compounds and cadmium.

## CONCLUSION

Exposure to sublethal concentrations of cadmium chloride, thus, caused dose and duration-dependent histopathological alterations in the liver of *Clarias batrachus*. The lesions in these vital organs might have resulted in physiological and metabolic dysregulations, which further led to behavioral alterations and growth impairment. In the long-run, therefore, Cadmium chloride exposures to even sublethal concentrations may pose serious threat to fish health and affect their population. Simultaneous addition of garlic extract has been found to be beneficial to mitigate the cadmium chloride induced histopathological disorders in liver. The lesions were less severe in the liver when compared to the fishes exposed to exclusively cadmium chloride. The efficiency of garlic was perhaps due to the presence of sulfur-containing amino acids and compounds having free carboxyl (C=O) and amino ($NH_2$) groups in their structures. These biologically active compounds might have chelated cadmium and enhanced its excretion from the body, resulting in reduced cadmium accumulation in tissues. The mechanism of *Allium sativum* mediated chelation of cadmium might include formation of ionic bonds between sulfur-containing compounds and cadmium.

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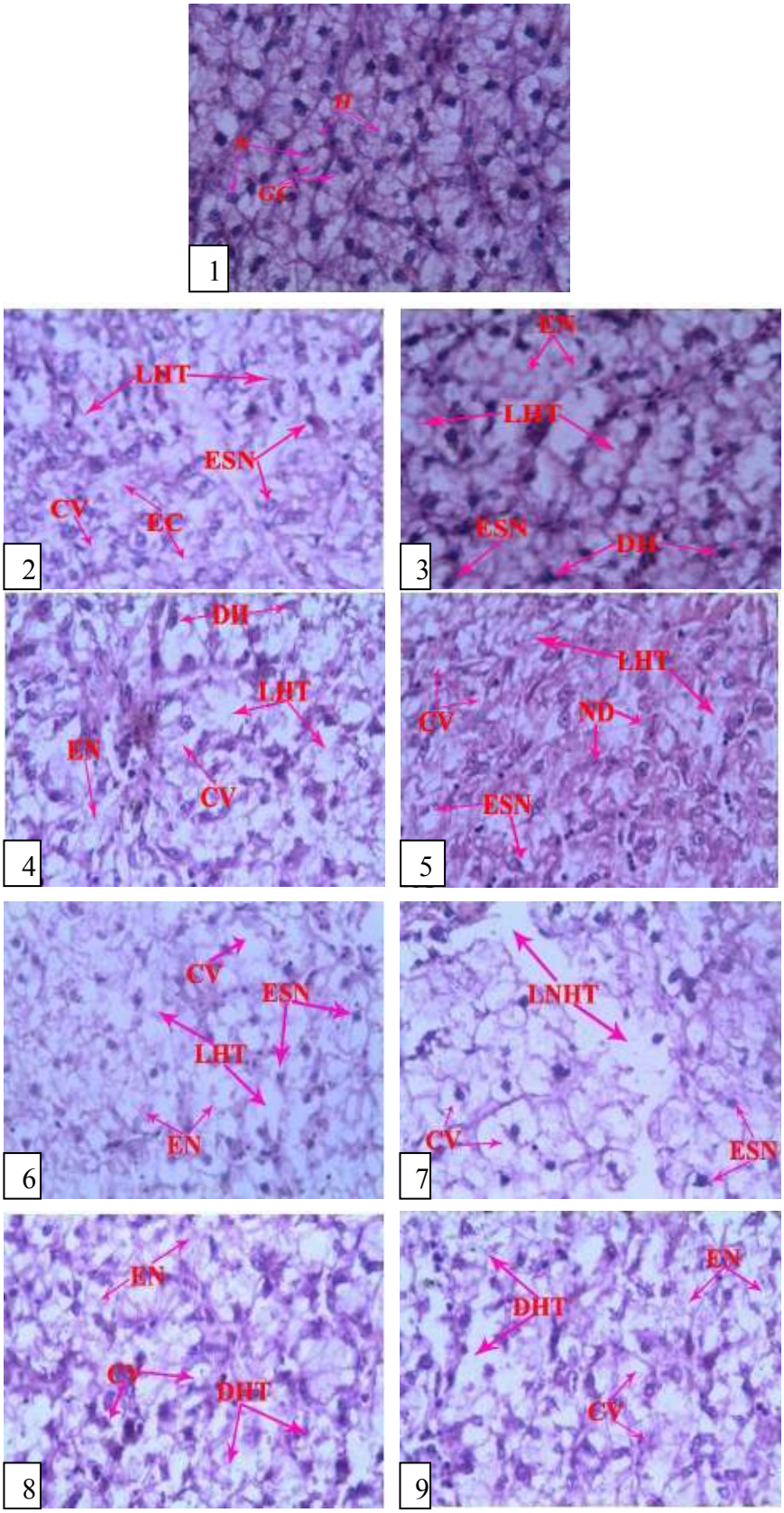
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Figs.1–5 (1) Liver structure of control fish: showing hepatocytes (H) with granular cytoplasm (GC) and centrally placed round nuclei (CPN). H&E, X 400. (2) Liver of exposed fish to 4 ppm $CdCl_2$ for 30 days loosening of hepatic tissue, vacuolated cytoplasm, enucleation and eccentric nuclei (Fig.2). H&E, X 400. (3) Liver of exposed fish to 4 ppm $Cdcl_2$ for 60 days excessive loosening of hepatic tissue, distended hepatocytes, enucleation, vacuolated cytoplasm and eccentric nuclei (Fig.3). H&E, X 400. (4) Liver of exposed fish to 4 ppm $Cdcl_2$ alongwith 4 garlic extract for 30 days hepatic tissue exhibited slight compact appearance and hepatocytes regained more or less, normal polygonal shape with centrally placed nuclei (Fig.4). H&E, X 400. (5) Liver of exposed fish to 4 ppm $Cdcl_2$ alongwith 4 ppm garlic extract for 60 days hepatic tissue gained less compactness and hepatocytes regained, more or less, normal shape and size, but the process of enucleation remained the same (Fig.5). H&E, X 400. (6) Liver of exposed fish to 8 ppm $Cdcl_2$ for 30 days pronounced loosening of hepatic tissue, distended hepatic cells, enucleation and eccentric nuclei (Fig.6). H&E, X 400. (7) Liver tissue of exposed fish to 8 ppm $Cdcl_2$ for 60 days loosening and necrosis of hepatic tissue, distended hepatocytes, vacuolation, enucleation and eccentric nuclei (Fig.7). H&E, X 400. (8) Liver of exposed fish to 8 ppm $Cdcl_2$ alongwith 4 ppm garlic extract for 30 days hepatic tissue has regained somewhat compact structure and reduced cell size (Fig.8). (9) Liver of exposed fish to 8 ppm $Cdcl_2$ alongwith 4 ppm garlic extract for 60 days hepatic tissue showed some compactness in its structure, reduced cell size has got, enucleation and vacuolization (Fig.9).

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