

Review of: "Cadmium Toxicity Induced Changes on Antioxidative Enzymes Level in Fresh Water Catfish *Channa Punctatus* (Bloch)"

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Potential competing interests: No potential competing interests to declare.

From my point of view, this work needs a profound revision. Some suggestions, presented NOT in order of importance:

1. The authors sometimes write cadmium, other times Cd. I suggest always writing Cd; in any case, the important thing is that there is a criterion.
2. In the introduction, the authors state that Cd produces dose- and time-dependent increases in intracellular GSH concentration based on data from a single article (Yusu et al., 2008).
3. I would rearrange the introduction: first, describe where Cd comes from, where it is found in nature, and what the sources of contamination are. Then describe the effects of Cd and how organisms can take it.
4. The bibliographic citations in the text do not have an order: they must be either in alphabetical or chronological order.
5. Report in the materials and methods how many individuals were used.
6. In the paragraph "Experimental animals and chemicals," the authors write: "A part of this homogenate was used for biochemical estimations, and the other part was centrifuged at 9,000 rpm for 30 minutes at 4°C. Supernatants were taken for analysis of SOD, CAT, GPx, GST, and protein estimations." It is not clear what they mean by biochemical estimates carried out on the homogenate. The analyses carried out on the supernatant are also biochemical analyses. LPO, GSH, SOD, CAT, GPX, GST, and total proteins were carried out on the homogenate or supernatant? Furthermore, it is not sufficient to report the method with which the authors carried out these analyses, but they must briefly report the method, making it clear what they determined, the principle of that method. For example, LPO: the absorbance of what was determined at 530 nm? GSH: to determine this tripeptide, it is normally homogenized with 5' sulfosalicylic acid which deproteinizes, so how is it possible to normalize the GSH content to the total protein content? To the enzymatic activities of SOD, CAT, GPx, and GST, it must be defined what 1 enzymatic unit corresponds to.
7. In table 1, I would write the dose of Cd used instead of "Low" and "High."
8. Catalase (CAT) Results: The authors write that the CAT level increased. From the table and the figure, it appears that it decreased!
9. In the results, the authors write: "The livers of the exposed groups at lower After 60 days of exposure, the cellular architecture was almost lost due to degenerative change." There is no figure in this regard that shows necrosis, vacuolization, hypertrophy, etc. Where are the methods for these analyses?
10. Where is figure 3?

11. And where are the results of GPX and GST activities? I can't find the SOD, GPX, and GST figures.
12. If the total protein content decreases following Cd treatment, it makes no sense to normalize other assays to protein. Normalization is carried out with a parameter that is assumed to remain constant beyond the treatment carried out.
13. The discussion needs to be profoundly revised: for example, what SOD, CAT, GPx, and GST do and where they are located should not be written here. It is often unclear whether the authors are referring to their own results or those of the literature. Authors cannot cite other works in isolation, without contextualizing them. A critical discussion of your results and what they mean is necessary.
14. The authors write: "In the present study, lipid peroxidation was increased after cadmium exposure in the kidney. Increased LPO levels in the kidney caused.....". Subsequently: "The present study showed decreased levels of GSH in the liver and kidneys of *Channa punctatus* after exposure to cadmium". Again: "Increase in ROS and LPO resulted in the depletion of GSH in the liver and ovary of exposed fish." In this work, the authors only present data relating to the liver, not to the kidney nor to the ovaries! And again: "In the present study, SOD, CAT, and GST levels increased significantly in the liver and ovary with increased concentration of fluoride and exposure duration." "In the present findings, SOD and CAT levels decreased in the kidney with increased concentration of cadmium."
15. I do not understand "An indirect antioxidant effect could be responsible for the lower kidney CAT activity; once formed, GSH radicals can further react with other thiol molecules in oxygenated tissues to provide O₂"
16. "It is an enzyme with selenium, and glutathione S-transferase works together with glutathione in the decomposition of H₂O₂ or other organic peroxides to nontoxic products at the expense of reduced glutathione" It seems to me that there is some confusion about the functions of GPX and that of GST.
17. Several bibliographic citations are missing, and it is not clear in what order they are written.