



**SDI Review Form 1.6**

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Journal Name:	<a href="#">British Journal of Medicine and Medical Research</a>
Manuscript Number:	<b>Ms_BJMMR_27687</b>
Title of the Manuscript:	<b>Oxidative Stress Pathway Mechanisms Induced by Four Individual Heavy Metals (As, Hg, Cd and Pb) and their Quaternary on MCF-7 Breast cancer cells</b>
Type of the Article	<b>Original Research Article</b>

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<p>It has been well established that cell death induced by the production of reactive oxygen species (ROS) has largely been associated with the activation of oxidative stress pathway, but the direct mechanism(s) involved are still unknown. Current study attempted to evaluates the oxidative stress pathways by which four heavy metals (As, Hg, Cd and Pb) administered singly and as a quaternary mixture induce their cytotoxic effects on MCF-7 breast cancer cells, in the presence and absence of cellular antioxidant, glutathione (GSH). Cells lines were exposed to 21.7<math>\mu</math>g/ml of the individual metals and mixture and assayed after 5 hr. Cellular levels of non specific ROS, superoxide anion (<math>O_2^-</math>), mitochondria membrane potential (MMP), and GSH were assayed using the FACS calibur equipped with cell quest pro for data collection. Results showed that in the presence of cellular GSH, As and Pb induced cytotoxicity by reducing the MMP while Cd, and Hg were cytotoxic by the production of mostly superoxide anions and Non specific ROS. The mixture exhibited cytotoxicity by decreasing the cellular</p>	



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	<p>MMP as well as producing ROS and O<sub>2</sub> . When the synthesis of cellular glutathione was inhibited, all five treatments damaged the mitochondria membrane and depleted basal GSH. Cd, As, and Pb also elicited the production of ROS.</p> <p>I am very impressed with the authors' hypothesis and goal of this study. Unfortunately, at the present conditions in my judgment, this manuscript has several key weaknesses. Consequently, validating authors' observations and significance is difficult without addressing some of the following issues:</p> <ol style="list-style-type: none"> <li>1. Study design and experimental tasks utilized by the authors have some poor points. Authors' should describe pattern of the tumor cells growth and malignancy with and without treatment.</li> <li>2. Authors' should provide cytological pattern of the changes seen in mitochondrial complex I-V overexpression and potential quantitative results from this experiments that would be necessary for the interpretation of their results and validation of the changes that seen in an different experimental conditions.</li> <li>3. Major problems with this manuscript are that it is very difficult to make judgment the regarding the conclusion that based on the authors' observation. Authors' should describe detailed features of the above mentioned markers as well as other pathways (for example energy crisis et c) that involved in this effects. It has been already demonstrated that heavy metal and oxidative stress has direct effects on the mitochondrial ability to provide energy in cancer as well as other diseases conditions.</li> </ol>	<p>This is a continuation of a previous study where cell growth patterns were described. Please see Egiebor et. al., 2013</p> <p>Mitochondria membrane changes can be studied using the dye that we used in this study and we have some references to back it up in the paper.</p> <p>This paper studies the possible oxidative stress pathway induced by four heavy metals and their mixture. We studied four biomarkers of oxidative stress; ROS, superoxide anion, GSH and MMP and our conclusion was based on our results. We cannot draw conclusions on</p>
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	<p>4. Based on the literature evidence toxic action of the free radicals most likely plays pivotal roles on this type pathology. Therefore authors should search literature evidence regarding the results observed in this current study.</p> <p>5. Several very strength statement was seen throughout in the results section of this manuscript.</p> <p>Only providing glutathione data it is enough to make such as statement? Did authors' provide any measurement several key oxidative stress markers , if yes, any significance differences? Any immunocytochemical staining differences?</p> <p>6. Terse but effective language should be used in order to make much clear the results and discussion without removing content central to the investigation.</p> <p>7. Literature search also very weak and often does not make any sense. I strongly recommend pubmed.com search by using key words.</p> <p>8. Based on my impression this manuscript has to be rewritten. Moreover, this manuscript contains large scale of error such as typo and stylistic misspelling. Therefore, Authors' should check their manuscript very carefully before resubmission.</p>	<p>other pathways that were not studied.</p> <p>Literature evidence were highlighted in a whole paragraph of the paper</p> <p>We measured four markers and we found differences in cytochemical staining. Differences in our results are represented in the bar chart</p> <p>The paper was edited again</p> <p>More literature from pubmed was included</p> <p>The paper was edited for grammatical errors</p>
<b><u>Minor</u></b> REVISION comments		
<b><u>Optional/General</u></b> comments	See the above section	