Review Form 1.7

Journal Name:	**Asian Journal of Biochemistry, Genetics and Molecular Biology**
Manuscript Number:	**Ms_AJBGMB_114837**
Title of the Manuscript:	**GENOTOXICITY AND CYTOTOXICITY ACTIVITIES OF STEMBARK EXTRACT OF Mammea africana**
Type of the Article	

Review Form 1.7

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)*
Compulsory REVISION comments 1. **Is the manuscript important for scientific community?** (Please write few sentences on this manuscript) 2. **Is the title of the article suitable?** (If not please suggest an alternative title) 3. **Is the abstract of the article comprehensive?** 4. **Are subsections and structure of the manuscript appropriate?** 5. **Do you think the manuscript is scientifically correct?** 6. **Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form.** **(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)**	**Yes**. The authors tested 50% methanol extract of *Mammea africana* Sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.) used in traditional medicine in parts of Nigeria for Physiological (root number and root length) and cytogenetic (Mitotic Index and chromosome and cells aberration) effects on 5 onion bulbs each at three different concentrations of the extract. **No.** Physiological and cytogenetic effects of stembark extract of *Mammea Africana on* onion bulbs **MATERIALS AND METHODS:** Should be re-arranged and better described at the section on *Allium cepa* test, after the sentence, "After 24 hours, the test samples were changed in the controls and all test concentrations and photographs of the growing *A. cep*a roots were captured. This continued for 72 hours. On page 3, correct 1. "5 per test concentration---" change to "5 beakers per test concentration." 2. "--with the root primordia downward toward the liquid." Change to "with the root primordia SUBMERGED IN THE TEST SOLUTION." 3. **Delete from "**the bulbs ~~at 8:30 am~~, and ~~respectively~~ 4. **"---**before putting them in sample bottles---." Change to "----before putting them in SPECIMEN bottles—" **PHYSIOLOGICAL ANALYSES** and not **Physicochemical Characterization:** Describe the method used to mease root number and root length here. How many roots from each of the five bulbs? The lengths of how Many roots were measured from each of the five onion bulbs? Re-do Tables 1 with extra columns of the measurements before the mean ($\bar{x} \pm SD$). Formula for determination of inhibition of root growth and root length compared with control groups should be stated here. Tables should be in the Results section. **CYTOGENETIC STUDIES**: **ROOT HARVEST, AND SLIDE PREPARATION**: Several root tips were cut at a length of 10 mm from the bulbs at 8:30 am, and respectively fixed in 3:1 (v/v) ethanol: glacial acetic acid and 1N HCL before putting them in sample bottles and storing in a refrigerator until use. State how many root tip (s) used per slide and how many slides prepared per treatment group and from each of the five onions per treatment group? **SCORING OF SLIDES:** You must score a minimum of 1000 cells and not 100 cells per slide and using the 100x magnification not x40. Cells were classified in Mitotic cells (Prophase, metaphase, Anaphase and Telophase) and the types of aberrant cells observed (Name the different types of chromosomal aberrations and aberrant cells observed here). **CYTOTOXICITY TEST**: Describe how you determined the MI and give the formula here. The formula, MI (%) = [number of cells in mitosis] / [total number of cells] x 100 should be MI (%) = [number of cells in mitosis] / [total number of cells scored] x 100. **GENOTOXICITY TEST**: Describe how you determined the frequency of aberrant cells and give the formula here. % Aberrant cells = [Number of Aberrant cells] /[Total number of dividing cells] X 100 and NOT Number of Aberrant cells /Total number of Cells X 100. A VERY SERIOUS ERROR OF DESIGN AND THEREFORE RESULT THAT RENDERS THE	

DISCUSSION, ABSTRACT AND CONCLUSION UNTENABLE. No where in the literature have authors scored only 100 cells/ slide. Consequently, the figure of 57.60% IN Table 2 for the mitotic Index (MI) for the negative control group is wrong. No where in the literature has it been recorded that of 57 of 100 cells counted were in division stages!!!!! The normal range is between 4 to 8 dividing cells per 1000 cells scored.

Refer to the following papers identification and classification of aberrations and correct your Tables.

1. Asita Okorie Asita and Matebesi L.P (2010). Genotoxicity of hormoban and seven other pesticides to onion root tip meristematic cells. *African Journal of Biotechnology*, 9 (27): 4225-4232. DOI: 10.5897/AJB09.1496

2. Asita Okorie Asita, Sello Moramang, Thabang Rants'o & Sibusisiwe Magama (2017): Modulation of mutagen-induced genotoxicity by vitamin C and medicinal plants in Allium cepa L., Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 2017 VOL. 70, NO. 2, 151–165. Taylor & Francis Group. http://dx.doi.org/10.1080/00087114.2017.1311166.

Table 2 should be something like the one below.

TC (µM)	Statistics	Total cells scored	Cells in interphase	Dividing cells	MI	Cells with aberrations							
						MNs	Sticky chrom.	Vagrant Chrom.	Fragment	Bridges	Vacuolated nucleus	C-Anaphase	Multipolarity
Group 1	Mean												
	SD												
Group 2	Mean												
	SD												

DISCUSSION: In the materials and Methods section, "each slide was examined using a Light Microscope at a magnification of x40. Microphotographs were taken to show chromosomal aberrations."

1. **The chromosome aberrations that are mentioned in the discussion section cannot be identified and distinguished clearly under** a Light Microscope at a magnification of x40. You need a 1000X magnification!!!!
2. **Consequently, t**he figures are of very poor quality because of the magnification used to observe and capture the images. It is doubtful that the authors actually saw the different types of aberrations in the Discussion Section under 40X magnification.
3. **Table 3 has too few aberration types compared with the ones mentioned in the discussion.**
4. **What is the difference between Aberrant cells and chromosome breaks, stickiness etc? in table 3?**
5. **The study was not designed to determine some of the issues discussed:**
   a. Page 12: The extract was found to not only interfere with the cell cycle, but also affect chromatin organization or DNA replication?
   b. **Page 13:** In this study, membrane damage cells were observed in groups treated with 5 mg/mL and 10 mg/mL of the extract especially???
   c. **Page 13:** Multinucleated and binucleated cells have been observed in extract treated groups. No photographs of them!!!
   d. **Page 14: "**Some ghost cells were observed in various frequencies" No photographs and not in the Results section or Tables!!!
   e. **Page 14: "**the extract also induced DNA damage and cell death and/or apoptosis in various frequencies in this study." No photographs and not in the Results section or Tables!!!
   f. **Page 14: "**The results of this study show that stembark extract of *M. africana* can

	induced cytogenetic alterations (cytoplasmic shrinkage, nuclear condensation, DNA fragmentation, membrane blebbing, cytoskeleton alterations and appearance of apoptotic bodies) and cell death in root tips of *A. cepa* (Figures 1(a), 1(b), 1(c), and 1(d)), The damages mentioned are not distinguishable in the figures!!!	
Minor REVISION comments **1. Is language/English quality of the article suitable for scholarly communications?**	Yes	
Optional/General comments	The design of the experiment is faulty. The figures are of very poor quality because of the magnification used to observe and capture the images. Instead of 1000X magnification, they used 40X. The discussions, conclusions and abstracts have been based on the wrong MI and are therefore untenable. The discussion is rather superfluous as it includes issues that the experiments and their design are unable to elucidate.	

PART 2:

	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)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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