Journal Name:	Journal of Advances in Medical and Pharmaceutical Sciences
Manuscript Number:	**Ms_JAMPS_42129**
Title of the Manuscript:	**EFFECTS OF CHLOROTOXIN ON THE MATRIX METALLOPROTEINASE-2 (MMP-2) EXPRESSION IN BREAST CANCER AND MELANOMA CANCER**
Type of the Article	**Original Research Article**

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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)*
Compulsory REVISION comments	**1) The overall result from this manuscript is 'Pro-MMP detected from A375 cell lines is inhibited by chlorotoxin".** **Thus, in Abstract section, "The catalytic cleavage of the gelatin in gel zymography indicated pro-MMP-2 at72kDa and activated MMP-2 at 68kDa. In addition, there was no active response of MMP-2 in the presence of chlorotoxin recorded on pre-made zymogram gel, which highlights 25 inhibitory action of chlorotoxin on MMP-2." should be rewritten.** **Pro-MMP-2 and activated MMP-2 are detected in zymogram gel of collagenase enzyme, not in gelatin zymogram.** **The conclusion is totally wrong, should be rewritten.** **As well as in Conclusion section, "This study provides evidence that expression of MMP-2 is significantly increased in melanoma cell line compared to breast cancer cell line." It is totally not, the research didn't show the expression level of MMP-2 in melanoma and breast cancer cell. Even the MMP-2 (either Pro- or activated form) was not detected in breast cancer cell line. It should be rewritten.** **"Therefore, MMP-2 can be used as a good therapeutic option for both types of cancer." It cannot be concluded like this. Only Pro-MMP is detected in A375 cell lines and inhibitory action of chlorotoxin on it seen in this cell lines. Not in MDA-MB-463 cell line. The authors should seriously revise the conclusion section again.** **2) Figure 4 should be caption with how much sample/which sample was loaded in respective lane. The abbreviation of molecular weight should be kDa, not kda.** **3) Figure 5, cell line number should be corrected to MDA-MB-436. The caption should be included amount of sample, type of sample loaded and label the name of 56 kDa band as Akt.** **4) Figure 7, author should re-examine the test again with molecular weight marker in both conditions.** **5) The references should be revised carefully again.** **Line 29-32, in Ref 14, facts are not seen in the corresponding reference.** **Line 56-58, the fact is not seen in Ref 25.** **Line 62-64, the data are not seen in Ref 17. The right reference is Cancer facts & figures, 2003. Atlauta: American Cancer Society, 2003.** **Line 66, "Patient can survive up to 10 years if diagnosed early and surgical excision." is not found in both Ref 15 & 17.** **Line 68-70. The facts are not seen in both Ref 15 & 17.** **6) Figure 6 is missing.** **7) Authors should revise some references carefully again in Discussion section as follow.** **Line 289, Ref 4 does not tell about MMP-2.** **Line 312, Ref 18 does not contain the mentioned facts. Ref 25 is talking about breast cancer, not melanoma.** **Line 316-318, "phosphatase and tension homolog (PTEN) protein negatively regulates phosphorylation of AKT" is found in Ref 33, neither in 5 nor 20. "upregulates activity of MMP-2, a transcription factor (SP1) is upregulated" not found in any Ref mention above. Please revise them again.** **Line 319-328, Ref number should be 20. The primary references should be cited: Ref 167 & 168 from paper of Ref 20.** **Line 329-330, should add Ref 110 & 181 from Ref 20.** **Line 331-335, should be Ref 187 from Ref 20.** **Line 336, should correct as ' because collagen is a substrate of MMP-2'.** **Line 339, should add Ref 22 & 23 more from Ref 8.**	

	Line 339, should correct 'Sontheimer et al' as the paper cited was only 2 authors involved. **Line 341, should be 'ClC-3'.**	
Minor REVISION comments	Abbreviation and spelling correction should be done as follows. 1) Line 73, matrix metalloproteinase-2. Small letter 'm' instead of 'M' 2) Line 92, Fetal Calf Serum should be mentioned uniformly through the whole manuscript. Company name should be in capital letter 'Sigma Aldrich'. 3) Line 96, Long form of BMRC should be mentioned. 4) Line 97-105, the materials should not be listed. It should be written in full sentences. 5) Line 110, agreement of subject and verb, should be 'are added', not 'is added'. 6) The author should be careful the tense written in the whole section of Material and Methods. 7) Line 187, 211, 212, 'was pipetted'. 8) Line 214, Viva spin, V should be in capital. 9) Line 239, 'Their images were …..' should be started as next sentence. 10) Line 254, Kurzepa et al 2014 is not found in Reference section. 11) Line 270-272, should be mentioned after Figure 7. 12) Line 278, missing word "weight" in molecular weight of 72 kDa. 13) Line 286, FCS (abbreviation) is not mention anywhere before it. 14) Line 305-318, Akt should be used uniformly. Protein is written in Akt, not AKT. 15) Line 308, 'which' should be deleted. 16) Line 345, opened and closed bracket should be the same. [6, 10] 17) Line 351, Frankowski et al used 42 hours for renaturation, not 48 hours. 18) Line 359, Should be 'Cltx-binding protein', not CITX.	
Optional/General comments	The Abstract and Conclusion section should be re-written again according to the findings from the research work. The author should also carefully revise the references in Discussion section.	

Reviewer Details:

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