



SDI Review Form 1.6

PART 1:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	MS: 2012/BMRJ/2091
Title of the Manuscript:	Antimicrobial Profile of Selected Snake Venoms and their associated enzymatic activities.

General guideline: Reviewers are requested to follow these guidelines during review: *(Note: Title of different sections as proposed below may differ in case of review paper / case reports)*

- Introduction *(Is the problem/objective of this study original, important and well defined?)*
- Materials & methods *(Kindly comment on the suitability of the methods. Sufficient details of the methods should be provided to allow peers to evaluate and/or replicate the work)*
- Results & discussion *(Kindly comment on: 1. Are the data well controlled and robust? 2. Authors should provide relevant references during discussion. 3. Discussion and conclusions should be based on actual facts and figures. Biased claims should be pointed out. 4. Are statistical analyses must for this paper? If yes, have sufficient and appropriate statistical analyses been carried out?)*
- Conclusion *(Is the conclusion supported by the data, discussed inside the manuscript? Conclusions should not be biased and should be based on the data, presented inside the manuscript only)*
- Are all the references cited relevant, adequate? Are there any other suitable current references authors need to cite?
- This form has total 9 parts. Kindly note that you should use all the parts of this review form.



SDI Review Form 1.6

PART 2: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part and write here 'Corrected'/ if not agreed, give suitable justifications)
<u>COMPULSORY REVISION</u> comments	1. The study did not find a clear correlation between the two studied enzymatic activities and the antiviral or antibacterial potencies of the examined snake venoms. Thus the title "Antimicrobial Profile of Selected Snake Venoms and their associated enzymatic activities", especially the use of the word "associated" is misleading. The title should be changed. 2. In order to better understand the correlation or association between the enzymatic activities of the venoms and their antiviral or antibacterial efficacies, the use of specific inhibitors of the studied enzymatic activities should be used as long as it is demonstrated that these inhibitors do not affect the viruses or bacteria themselves. Performing experiments in which the enzymatic activities tested are neutralized with specific inhibitors, and the biocidal properties are shown to be neutralized too, or the other way, not to be neutralized, would strengthen the manuscript very significantly.	1-Thanks a lot, actually the title is misleading and it is now changed to the following: Experimental evaluation of antiviral and antibacterial potentials of selected snake venoms against viral and bacterial models 2- We agree with you, but according to the changed title the main aim of the present study was to not to detect the biological activities of the enzymes but just to evaluate the antiviral and antibacterial potentials of test venoms. Thus further studies were recommended for more characterization of the biologically active enzymatic components.



SDI Review Form 1.6

	3. Separate the cytotoxicity and antiviral activities into two separate sections. Not clear why they are under the same subtitle, as these are two separate issues. 4. Regarding the venom pre-treated cells and the antiviral tests – after the 24 hour incubation of the venom with the cells - has the cells been washed to remove extracellular venom present in the media? Apparently not. Such a control should be performed to understand if the venom is “removing” or “damaging” a cell component that allows better viral infection. It may be that the venom is not affecting the cells, but when the virus is added to the mixture, the venom present in the culture damages the virions directly, reducing their infectivity. Thus, these tests should be performed - venom washed and unwashed cells should be exposed to the viruses, and the infectivity compared.	3-Titles were separated as you kindly recommended, the non toxic concentration of venom (determined using cytotoxicity assay) must 1st be determined to be used in the evaluation of the antiviral activity of tested venoms. 4-We concerned the antiviral potential of test venoms as some antiviral agents promote the expression of some genes like MX gene that can inhibit viral replication or viral capsid formation etc.... In addition we used the safe concentration of the venom that will not affect cell components as if it can affect cells the virus can't integrate inside the damaged cell to show cytopathic effect (CPE) of which we evaluated the virus activity and titer post treatment. If the venom damaged a cell component and this would allowed better viral infection (as you kindly reported), the virus titer in venom treated cells will not decreased compared to its titer in control untreated cells as observed in the results.
--	--	--



SDI Review Form 1.6

	5. The discussion regarding the antiviral mechanism in lines 183-185 should be revised according to the results of the experiment suggested in point 4 above. It seems the venom was present during the infection step, therefore, it may be that the venom affected the virus directly by e.g. digesting some of its components, interacting with them, etc.	This part was revised according to your kind recommendation where this test can reflect the viricidal activity, as venom is in direct contact to the already adsorbed virus that resemble the direct contact of venom with the virus suspension in a test tube and we can evaluate its viricidal activity by collecting samples at time intervals and determine the virus titer relative to time.
--	--	--



SDI Review Form 1.6

Minor REVISION comments	1. In the attached pdf file several revisions (and comments) in the text are suggested. Please revise accordingly.	Revised according to reviewer comment, except for changing antimicrobial activity to biocidal activity in the conclusion because we didn't investigated the exact mechanism of action of venoms.
Optional/General comments	1. Results and Discussion: line 134: The 100% cellular viability is mentioned as, for example for <i>N. nigricollis</i>, 2 ug/0.1 ml, which is equivalent to 20 ug/ml, and a reference to Figure 1 is made. However, in Figure 1 the data is shown, for <i>N. nigricollis</i>, as 2 ug/ml. So either it is a "typo" mistake, and then change the figure or the text accordingly, or the calculation is wrong. 2. Based on line 77, the non-cytotoxic concentrations are referred to as 10 and 20 ug/ml. These values seem high; do not concur with Figure 1 of the manuscript and with some of what has been published in the literature. For example, the minimal cytotoxic dose of <i>N. nigricollis</i> venom was 2.5 ug/ml (Chaim-Matyas et al, 1991) (although not for VERO cells). Thus, please check if there is not a mistake of 1 order of magnitude.	1-Thanks a lot , the figure was corrected according to the recorded results. 2- We already got the non toxic concentrations (10 and 20 ug/ml) in a right way but the variation in the cytotoxicity concentration range may be attributed to the venom source, purification level and different cell line susceptibility and passage no used