



SDI Review Form 1.5

PART A:

Journal Name:	International Blood Research & Reviews
Manuscript Number:	2012/IBRR/1812
Title of the Manuscript:	ERYTHROPOIETIC AND BONE MARROW STIMULATING ACTIVITY OF TERMINALIA CATAPPA EXTRACT: POSSIBLE ROLE OF NITRIC OXIDE SIGNALING

(Note: Title of different sections as proposed below may differ in case of review paper)



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PART B: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part and write here 'Corrected'/ if not agreed, give suitable justifications)</i>
General comment:	This is a descriptive study investigating the effects of terminalia catappa (TC) extracts on erythropoiesis in a murine model of anemia. Although the study addresses a significant issue, there are a number of serious concerns that detract from the manuscript. In particular, a statistical section must be included to confirm that appropriate analysis was undertaken. In addition, the study provides minimal mechanistic insight into the molecular mechanism by which TC extracts influence erythropoiesis. The conclusion that NO is involved in the actions of TC extracts is not supported by the data since NO was never measured. Finally, some of the stated effects of TC are not supported by the data presented in the figures.	I quite disagree that the molecular mechanistic evidence in this paper is minimal. We investigated the ability of the plant extract to induce erythropoiesis in anemic mice models using an untreated control in order to compare any erythropoiesis induced by the anemic stress induced by phenylhydrazine with the effects observed to be due to the plant extracts and a positive control HU which is a known inducer of erythropoiesis. In addition we investigated the ability of the plant extract to induce erythroid proliferation by measuring the emergence of bone marrow carbonic anhydrase (a known molecular biomarker of the emergence of erythroid cell lineage) of the mice. We found that the plant extract were quite active in improving the carbonic anhydrase signal in the anemic mice models in addition to other observed results. It is pertinent to point out that studies have shown that HU improves erythropoiesis through a nitric oxide pathway, hence its use as a control erythropoietic drug also shows that the observed results which were similar to the control is safe to infer that the extract and the control drug work through a similar pathway though probably not the same but in the light of the available data.
Specific comments:		
Title and abstract	The units are missing from the values provided in the Abstract.	Corrected



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Introduction (<i>Is the problem/objective of this study original, important and well defined?</i>)	The objective of this study is important but somewhat incremental to previous work which has already established that TC extracts promote erythropoiesis in Balb C mice. SCA should be defined when it first appears in the Introduction.	We have shown previously that TC extracts promote erythropoiesis in Balb C mice (Aimola, 2011). This study further improves the understanding of a likely mechanism by which TC plant extract functions, and also attempted to identify an active plant fraction which makes it significant understanding the possibility of a possible novel plant based therapeutic component for SCA Comment on SCA corrected
Are all the references cited relevant, adequate? Are there any other suitable current references authors need to cite?	References appear appropriate	Acknowledged
Materials & methods (<i>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</i>)	The methodology appears appropriate. However, a major concern is the lack of a statistical analysis section. PCV should be defined.	PCV defined Method of statistical analysis spelt out
Results & discussion (<i>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?</i>)	The Figures should be numbered in the order that they are first mentioned in the text of the Results. The data is not adequately presented in the figures. Statistically significant changes should be noted in all the figures. The "n" number should be stated in all the figure legends. It is unclear whether appropriate statistical analyses have been performed since the statistical tests that were used are never stated. The stated increase in PCV in anemic mice treated with different fractions of TC compared to untreated phenylhydrazine treated mice is not observed in Figure 5. The stated increase in intracellular haemoglobin by TC above the phenylhydrazine induced anemic mice is not observed in Figure 1 for most of the TC extracts	Figure legends altered to include n values and statistically significant differences. Figures also properly numbered in the way they appear mentioned in the text The increase in intracellular haemoglobin by TC in Figure1 now renamed Figure 4 is clearly observable
Are there any ethical or competing interest issues?	No apparent ethical issues.	Acknowledged
Conclusion (<i>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</i>)	The conclusion that TC induces erythropoiesis in bone marrow through a NO signalling pathway is not supported by the data since NO was not measured in the study.	Nitric oxide synthase and arginase activities are modulated by the amount of L-arginine in the system are metabolized through a competitive pathway. Hence arginase activity in vivo has been shown to modulate the level of NO in vivo inversely. Hence we used the activity of arginase as a biomarker for the level of NO due to the high sensitivity of the enzyme assay.



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References	Appropriate	Acknowledged
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