



SDI Review Form 1.6

Journal Name:	European Journal of Nutrition & Food Safety
Manuscript Number:	Ms_EJNFS_39438
Title of the Manuscript:	Effects of higher doses of vitamin E on toxicity and inflammation.
Type of the Article	

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/journal/30/editorial-policy>)

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. In mice/animal study, I guessed you cited your previous works (ref # 12 & 31). I was wondering why you changed the administration route from feeding to intra-peritoneal injection. This change would have enhanced the bioavailability and effect of vitamin E or caused a bias of the possible results. 2. Number of mice per group (test & control) was 8 which was too small, thus, it was impossible to have a normal distribution. Small error could change your conclusion. This was a reason of your unexpected or unexplained results such as no anti-oxidation or low BAP. It is too soon to conclude that the biomarker showed a U-shaped relation with the dose of vitamin E, unless large number of subjects is studied. 3. Enzyme activities are possibly used to determine tissue injury, not to investigate enzyme induction. In human, AST, ALT, and ALP are used to investigate hepatobiliary toxicity. These enzymes are normally not much in kidney and brain. I was surprised you found almost equal activities of these enzymes in kidney and brain. I guessed they were from blood cells presenting in these organs and their results meant nothing. To investigate kidney toxicity, BUN and Creatinine are relevant. 4. The given doses were not high and the study duration was quite short, only 14 days. This might be another reason of your inconsistent results. Are there ethical issues in this manuscript? Yes. There is an animal research ethical issue but no approved licence was indicated. The message "This study was performed according to the Republic of Lithuania Law on the Care, Keeping 283 and Use of Animals (License of State Veterinary Service for working with laboratory animals 284 No 0200)" might not protect the right of animals in a certain project.	1. the reason that we changed the route of exposure was that we wanted to see whether ip application would gave the same effects as oral exposure. In general we can conclude now that this is the case and that we have confirmed the unexpected effects of vitamin E. 2. The number of mice per group was 8. This number per group we always have used in our previous study and the reviewers never discussed this number of 8 animals. In addition we tested the data for equal variances and all data passed this test. We will add a text in the Discussion about this number and the U-shaped relation (in yellow). 3. The enzyme activities represent tissue damage in general. We agree that ALT and AST are used mainly for liver toxicity, but also in kidney and brain they are present and can reflect tissue damage of these organs. We do not agree that the enzyme activities are almost equal in all tissues. For instance, ALT is about 1000 IU in liver, but only about 100 IU in kidney and only 60 IU in brain. The same is valid for ALP. Only AST show similar activities. We agree that de concentrations of BUN and creatinine should be useful, but since we had only a limited amount of sample, we could not perform there analyses in addition. 4. We cannot comment on this, but we will make a note on this in the discussion. 5. To our opinion the ethical aspects were sufficient covered in this study and the animals did not suffer any inconvenience.
Minor REVISION comments		
Optional/General comments		