Author's response to reviews

Title: Protective and restorative potency of Vitamin D on persistent biochemical autistic features induced in propionic acid -intoxicated rat pups

Authors:

Hanan A Alfawaz Dr (halfawaz@KSU.EDU.SA)
Ramesa Shafi Bhat Mrs (ramesa.aftab@gmail.com)
Laila Al-Ayadhi Prof (ayadh2@gmail.com)
Afaf El-Ansary Prof (elansary@ksu.edu.sa)

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Author's response to reviews: see over
Point by point response to reviewer’s comments

Reviewer’s report

# Reviewer 1

Interesting study. It would have been more interesting had the authors measured plasma and stool and if possible urinary levels of propionic acid in patients with autism in support of their animal model of autism.

- The suggested point is very interesting and we will consider it in our future clinical studies. We had already measured plasma levels of propionic acid in Saudi autistics and our work is published as El-Ansary et al, lipids in health and disease, 2011, 10:62

If vitamin D has a role in autism, then why that the incidence of autism seems to be increasing in USA and other countries even though vitamin D is supplemented in milk and other foods and given even to pregnant women?

Vitamin D could be just a bystander in autism!

- As suggested in this manuscript higher plasmatic vitamin D could reflect lower brain concentration which could be due to lower vitamin D receptors (VDRs) numbers in autistic brain. This was supported by other studies in which impairments in working memory or anxiety and behavioural impairment were observed in the VDR-KO model mouse. (see references 30,31 &32).

- The authors could have measured plasma, stool and urinary levels of propionic acid in their animal model and also measured propionic acid in various tissues such as liver, gut, and various regions of brain to know how it is distributed and to know how long it will remain in various tissues. It is important to note that autism
is also characterized by gut, and involvement of other organs as well.

- The suggested point is very interesting and we will consider it in our future clinical studies.

Is propionic acid treatment (model of autism studied by the authors) also produces changes in IL-6 and TNF and alters gut function?

- Yes both cytokines were significantly elevated in propionic acid-model of autism. Please see El-Ansary et al: Etiology of autistic features: the persisting neurotoxic effects of propionic acid. J Neuroinflammation. 2012 Apr 24; 9:74.

# Reviewer 2: Title: Protective and restorative potency of Vitamin D on persistent biochemical

Major compulsory revision:

Page 2 and page 6

It is not clear in methods the difference between protective group and therapeutic group. The therapeutic group must have gotten PPA first and then 1,25 but it does not say that. As it reads now there is not difference between the two groups.

- Thanks a lot for this point. Actually the difference between group 3 & 4 was not clear. Description of group 4 was rewritten to be clear and highlighted in yellow.

# Reviewer 3:

The manuscript was done in a comprehensive manner. However, there are some errors in the text;

missing figure captions; the fourth slide of Figure 4 brings the number VI. After appropriate revision is suitable for publication
- For Biomed Central Journals, Figure Captions are usually written at the end of the manuscript after the references. Below You can see after copied it from the submitted manuscript.

- **Figure’s legends:**

  - Fig. 1: Percentage change of vitamin D and calcium in plasma of PPA, protected (PPA+D), and therapeutic (D+PPA) groups compared to control.
  - Fig. 2: Percentage change in serotonin, IFγ and GST in the three treated groups compared to control.
  - Fig. 3: Percentage change in tail length, tail DNA% and tail moment in the three treated groups compared to control.
  - Fig. 4: Photograph showing comet tailing in PPA treated (II) together with the protective and therapeutic effects of vitamin D (III &IV) in rat brains compared to control (I).
  - Fig. 5: (A-G): Pearson correlations between plasmatic levels of vitamin D, calcium and different measured brain parameters.

Abstract

Line 11 neurotoxic

-Done

Background

Line 7 aetiological

-Done

Page 6 line 4 GST (Glutathione-S-transferase)

-Done
MATERIALS AND METHODS

List all the reagents used for the analyzes indicating Supplier

List all equipment used for the analyzes and Suppliers

- Although I never asked to do this but reagents and equipments used were listed.

Delete Experimental procedure because the subdivision of the rats into 4 groups and their preparation is not an experimental process, but it is the organic matter on which you are running the experiment.

- Done and highlighted in yellow

Page 7 The brain was removed from the skull and................. is repeated in the same way twice

- Done

Line 13 of

The analysis related to Serotonin, Glutathione-S-transferase, Interferon gamma, are described in a superficial way, without any indication of the amount of sample and reagents used, without specifying the product obtained, nor the color change due to the reaction.

- Done and highlighted in yellow

Page 9 Line 1 0.075 M NaCl

- Done

Page 15 Line 1 aetiology

- Done

Fig 4 The fourth slide brings the number VI.

- Corrected

The conclusions are poorly developed. For people with autism who have known allergies to foods containing calcium (milk and dairy products) must resort to mineral supplements, but the
current pharmaceutical form (pills, tablets) are unwelcome because of the difficulty in swallowing. Were tested other solutions?

- Conclusion was rewritten to suggest other protective solutions

References 18 and 29 are not written correctly

-Corrected