Reviewer's report

Title: Anti-stress and neuronal cell differentiation induction effects of Rosmarinus officinalis L. essential oil

Version: 0 Date: 29 Sep 2017

Reviewer: Ephrem Engidawork

Reviewer's report:

This is a very interesting study that included both in vivo and cell line studies, used to substantiate findings obtained from animal studies. Here are some of my comments.

* How is the volume related to the amount of EO inhaled by the animal? The MTT assay revealed that the EO was toxic at 50 and 100 ug/ml. How can the authors prove that the amount inhaled by the animal was not toxic?

* Statistical analysis: t-test could produce a false positive result if the groups within the experiment are more than two. Please, use One way ANOVA followed by an appropriate post hoc test.

* Treatment was done for 14 days and TST was performed every other day. What was the rationale for doing the test every other day? Why not daily or at the end of treatment? What does the value presented in Figure 1 represent? I am asking this because various measurements had been taken. I think if time is taken as a factor, ANOVA with repeated measure should be done.

* Noradrenergic input from the Locus ceruleus to the hypothalamus controls the production of corticosteroids. Depression is characterized by decreased noradrenaline, which is believed to contribute to enhanced production of corticosterone. I presume this notion is the basis for determination of both the steroid and the transmitter. However, amelioration of corticosterone was not accompanied by increase in noradrenaline. How do the authors explain these seemingly conflicting findings? Moreover, how does the dopamine story fits into the stress induced depression-like activity?

* Page 18, Line 291-291: "ACh is produced from ingested choline.." I do not think that's an appropriate statement. In fact, much of the choline used for ACh synthesis comes from the recycling of choline from metabolized ACh. Another source is the breakdown of the phospholipid, phosphatidylcholine. One of the strategies to increase ACh neurotransmission is the administration of choline in the diet. However, this has not been effective, probably because the administration of choline does not increase the availability of choline in the CNS.
AChE activity is similar in both undifferentiated (Figure 2) and differentiated (Figure 4) PC12 cells. It is known that differentiation of PC12 cells with NGF or other agent is associated with an increase in the cholinergic system.

Is the value given for Gap-43 mRNA expression a normalized value?

What was the basis for inferring that ROEO regulates the sympathetic nervous system?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
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