Reviewer’s report

Title: Antidepressant-like effects of the aqueous macerate of the bulb of Gladiolus dalenii Van Geel (Iridaceae) in a rat model of epilepsy-associated depression.

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Reviewer: Hans-Peter Lipp

Reviewer’s report:

This is a nice and well-written study assessing the impact of a widely distributed African plant (Gladiolus dalenii), used frequently in traditional herb medicine in the sub-Saharan Africa, on epilepsy-associated depression. Using rats made epileptic by previous pilocarpine treatment, the authors show that plant extracts reduce stress markers (corticosterone/ACTH), increase the levels of BDNF (brain derived neurotrophic factors) and show behavioral improvement in two tests believed to measure rodent depression. These data compare favorably with the effects of fluoxetine, a typical antidepressant drug used in humans.

The rationale of the study is described well (one wonders a bit on the emphasis on Cameroon), and the methods are sound. The strength of the study are the biochemical measurements, the weaker part is the behavioral assessment. Although both tests (open field activity and forced swimming task) are widely used in the field, they represent the weakest possible combination. A large number of conditions can affect locomotion of rodents in an open field, and the so-called passive floating in the Porsolt test became a marker for depression only because it is reduced by antidepressants used for humans. Thus we have a classic case of circular argumentation. Quite often, the Porsolt test correlates poorly with other measures of rodent depression, therefore one should include tests that are independent of locomotion, for examples reduced sucrose consumption as a marker for anhedonia. A second concern is the so-far non-documented and pretty massive pretreatment to obtain a rat model of epilepsy-associated depression, whose effects as compared to normal non-treated rats cannot be judged satisfactorily from the data.

Nonetheless, the paper contains valid information deserving to be made known.

Major essential revisions should include:

1. The nature of the controls is not clear. Fig. 1 speaks of non-handled rats, while the method section tells that experiments were conducted with animals selected for occurrence of seizures. Please provide a small table indicating the number of rats according to treatments, and also indicating how many rats underwent sequential testing with final assessment of biochemical parameters.
2. Perhaps the authors have normative data from normal non-handled and non-treated rats for BDNF, ACTH and corticosterone levels. If so, inclusion would
be helpful.

3. Calculating correlation coefficients between the behavioral and biochemical scores within the treatment groups, possibly across groups with preserving the identity of the treatment groups. Sample sizes are probably too small to provide statistically valid differences, but the graphic plots will quickly indicate whether there appears a meaningful relationship between open field activity, Porsolt test, BNDF levels and corticosterone/ACTH levels. Such plots might be given under additional information. A pilot factor analysis may help. In case that correlations emerge, it would be justified to assume some causality. If not, one might face one of the many cases in medicine in which a treatment provides cure for the symptoms but not through the suspected mechanisms. In my view, this information is essential to guide further investigation of the effects of the Gladiolus treatment, even if it might not support the suspected relations.

Minor essential revisions should include:

4. The way of oral administration of drugs must be indicated (e.g., gavage).

5. In the discussion, the emphasis on good face validity and good construct validity should be toned down to “some face validity” etc. Likewise, for interpreting the relations between BDNF levels and depression and animal tests, the authors cite supporting information only. In reality, the relation between these variables is often contradictory. A sentence mentioning contradictory findings should be added.

6. The additional graph appears out of context and explanations are not provided with the URL.

7. Table 1 partially relates to numbers of mice. Have there been other experiments with mice? Please correct.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests