Reviewer's report

Title: In vivo analgesic, anti-inflammatory, and sedative activity and a molecular docking study of dinaphthodiospyrol G isolated from Diospyros lotus

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The authors report on a series of animal experiments intended to evaluate anti-inflammatory, analgesic and sedative effect of a chloroform extract of Diospyros lotus, as well as of one specific (extracted) compounds - DDG. There is a reasonable background that supports such an effort - considering the traditional use of various preparations of the plant for similar conditions in humans.

I have several comments.

1. English is not my native language, but thorough language editing is needed by English-proficient person acquainted with the biomedical terms (in addition to quite some typos across the manuscript).

2. Materials and methods. Extraction and isolation. The authors refer to several previous publications considering the process of extraction (chloroform), which is OK. But, at the end - no particular specification of the extract is available. Since DDG (seemingly) is the main component, it would be at least needed to state the estimated % content of DDG in the extract, and at least (qualitatively state) - what would other constituents include. DDG is referred to as a "single extracted" compound (that is - there were two "test" items - the extract, and "purified"? DDG?) - but it should be stated what is meant by "DDG". Extract "enriched in DDG"?..Extract with...e.g. 97% w/w DDG? "Pure DDG"?. WHen things are being tested, then the item(s) tested should be well described.

3. Materials and methods. Animals. There is some confusion in this section. The last sentence of this paragraph states that n=8 animals were assigned across each of the exp. group (in several experiments), but then - under the next sub-heading on the anti-inflammatory screening it is stated that n=6 per group were used. Etc.

So - it should be clear (by stating it in the methods, or under each table/figure reporting on a particular experiment) what was the exact number of animals per (each) group.

In one place, the authors state that animals were treated with "six compounds" - but actually, there were 2 tested compounds (extract and DDG), but each applied at 3 different dose-levels. This should be corrected.

4. Materials and methods. Anti-inflammatory screening. Authors refer to their previous publication to more detailed description of the procedure. In this respect, I would like to point-out: a) the fact that something, some procedure or a protocol was already published does not automatically mean that it is valid; b) I agree that it is not really needed to always repeat all the details about a particular procedure. But, it could be stated "In brief,..." and key points of the procedure need to be outlined. It is not really practical for anyone reading this or any other paper, to search and try to acquire the published paper in order to be able to figure-out whether the methodology was appropriate or not. In this section, DDG is addressed as "compound 1"...you should use the same term for the tested item..always. So, it is either "compound 1" or DDG.

Loratadine dose is missing. The formula for "%inhibition" is not completely clear: what is "A" -
the mean value for the "negative" control group? (saline?) or? And the value for each animal from
the "tested group" is then "converted" into a "percent inhibition" using this mean as 100%? Or?.
This should be explained. Also - when reporting results (e.g., Figure 2) - the Figure legend should
contain numerical values from the plethysmometry measurements: "mean baseline volume" (intact
animals prior to carrageenan/histamin); saline-treated animals at 1 hours: xyz (represents 100%),
at 2 hours;...etc. ). So that it is clear what "100%" actually means. at certain time-points. Figure 1
and Figure 2- WHAT was the DOSE of the "extract"? and what was the DOSE of DDG to which
the data in these figure refer?

5. Materials and methods. Hot plate. Again, the authors refer to "previously published method".
But, many points remain unclear (unless a reader is willing to search for and acquire "previous
publication"). The unknown points: 1. each treatment group was tested repeatedly over 120
minutes, or at each time point a new group with the same treatment was used? 2. table 1 shows
results. Without any units. What are the numbers? Seconds of latency time? index of analgesia?.
The method should be at least to some level explained. For mice included into hot-plate testing, it
is accustomed to a) have "conditioning" a day before; b) that all animals (groups) are tested at
baseline (before treatment), in triplicate; c) that they are re-tested after received treatment.d) that
index of analgesia is calculated for each animal based on "baseline" and tested value. Baseline
"runs" are needed to ascertain that all animals show comparable reactivity. Triplicates are needed
to "smooth" the response, because there are intra- and inter- individual variabilities in latency
times that can really be huge. The procedure needs to be explained. The meaning of "percent
effect" needs to be explained.

6. Materials and methods. Sedative activity. Sedative effect is typically assessed by observing
spontaneous locomotor activity. E.g., in the open-field test. The employed test certainly has a
name (i.e., the employed paradigm). it should be hence termed like that. The result of the number
of "crossed lines" - but this is given within the certain time-frame. Method should be explained.

7. Statistics. 1. All raw data are summarized as means (SEM). This is not appropriate. SEM is NOT
a measure of data scatter - it is a measure of precision. Hence, if mean is the measure of central
tendency that fits the data - it should be given with SD. 2. One-way ANOVA is appropriate (if
residuals were not skewed) for the "sedation test" - since the only factor was "treatment". For the
hot-plate and inflammation paradigms -one-way anova is appropriate ONLY if at each time point
there was a different animal group by treatment. E.g., positive control, negative control, 3 doses of
an extract, 3 doses of DDG. That is 8 independent groups a each time-point. Then, at each time
point one uses one-way ANOVA with 1 factor - "treatment" with 8 levels. But, if the same animals
(per treatment) were repeatedly evaluated at different time-points, then TWO-WAY anova is
needed: because there are two factors (1) treatment and (2) time. And the model needs to include
the "treatment*time interaction". and differences between treatments at different times are
generated from the interaction term.

EXACT P-values with clearly depicted comparisons to which they pertain - need to be presented
for each experiment.

8. Results. Anti-inflammation (Figure 1 and Figure 2). The two figures were already commented-
on. BUT: i. what are points? individual animals? what are horizontal lines? If you want to show
data scatter- show all individual data, or mean(SD). Figure legend should declare numerical values
which served as "100%". Textual part: it is not really obvious form the figure when and what "had
an effect". Data should be reorganized and more clearly shown. There is no reason not to use a
Table (like for other data).
9. Results. Pain. Table 1. As already mentioned - what are the data? Seconds? percentages...of what? How determined? To which comparisons do p-values refer? Use SD to illustrate data scatter. I have done quite some hot plate testing..I have never seen such consistent means...eg., for saline group - always around "9.0" (something) at different times of testing. This is an unclear experiment. Should be better explained (methodology etc.). And - two-way ANOVA should be used (see above).

10. Results. Sedation. Table 2. Again..completely unclear...values for treatments are compared..to what? where is the "saline group"?...use SD for data scatter.

11. DESIGN of EXPERIMENTS. The "sedative" effect (e.g., diazepam) in the used paradigm is evidenced by reduced spontaneous locomotion. Hot-plate test actually quantifies a COMPLEX response to a thermal stimulus (i.e, a complex behavior, not only "reaction to pain") - if a compound reduces spontaneous locomotion..could it influence the behavioral response in hot-plate?...A diazepam-treated group in the hot-plate test would help to "separate" the true analgesic and behavioral effects.

12. Results - computer simulations.- I am not qualified to assess this.

13. Discussion - there is a lot of speculation there. The experiments WERE NOT designed in a way that would allow you to claim ANYTHING about the potential mechanisms of the DDG effect. You DO NOT HAVE "material" to speculate that DDG might be affecting "central opioid system". The hot plate experiment would need to include e.g., opioid antagonists and you would need to show that e.g. naloxone could abolish the anti-nociceptive effect of DDG - in order to imply the possible "opioid mechanism". The same for sedation and GABA. You did not use, e.g., flumazenil to "remove" the sedating effect. So - there is no grounds for any speculation about the mechanisms. You can only state- that data are suggestive, and deserve further investigation of the involved mechanisms.

14. Discussion -in line with this and the limitations of the experiments and the fact that there was only one paradigm used for pain and sedation - the entire Discussion should be less "enthusiastic" about what was actually shown.

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

No

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

No

Are the conclusions drawn adequately supported by the data shown?
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Yes

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I am able to assess the statistics

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