Author's response to reviews

Title: Vanda roxburghii: An experimental evaluation of antinociceptive properties of a traditional epiphytic medicinal orchid in animal models

Authors:
M Josim Uddin (josim_84@yahoo.com)
M Masudur Rahman (mamun2001@hotmail.com)
M Abdullah-Al-Mamun (mamunabdullah808@yahoo.com)
Golam Sadik (gsadik2@yahoo.com)

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From
Md. Josim Uddin
Lecturer, Dept. of Pharmacy
International Islamic University Chittagong
Chittagong, Bangladesh

To
The Editor-in-Chief
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Sub: Resubmission of Revised Manuscript

Article Type: Original research article

Dear Editor,
I am pleased to submit a research article entitled “Vanda roxburghii: An experimental evaluation of antinociceptive properties of a traditional epiphytic medicinal orchid in animal models” for possible publications in your esteemed journal. On the basis of three reviewer’s comments, I have made some changes in my manuscript. Here all of the answers and corrections recommended by reviewers are given below-

Referee 1
a.
1. Number of animals used is n=5 why not n=6.
   Reply: Extensive literature review reflects that most of the Acetic Acid Induced writhing test has been performed with 5 or more mice. Based on our availability of mice, we have chosen 5 mice in each group.

2. V. roxburghii methanol extract percentage of inhibition was mentioned for 25 mg/kg as 15.97% , the values for 12.5 and 50 mg/kg was not shown.
   Reply: Percentage of inhibition for 12.5 and 50 mg/kg has been included.

3. Line 180 - 183 values for maximum percentage of inhibition (50 mg/kg) for all extracts was shown similarly values for lower dose (12.5 mg/kg) could be mentioned in results.
   Reply: Values for lower dose (12.5 mg/kg) have been included.

4. ED_{50} values are not mentioned for all extracts.
Reply: In our preliminary study we have determined percentage of inhibition. ED50 will be observed in our ongoing study for isolated lead compound.

b.
1. Standard drug diclofenac sodium value for early phase?
Reply: Since intraperitoneal route was used and it showed minor analgesic activity for diclofenac-Na and that was represented in our result.

2. Standard drug morphine value for early and late phase - % of inhibition not shown for comparison with all extracts.
Reply: Percentage of inhibition of morphine for early and late phase for all extracts has been added.

3. Extracts of VRA and VRM values for early and late phase was not mentioned in results.
Reply: VRA and VRM results have been included.

4. The extracts VRP and VRE early phase inhibition of 44.59 and 43.5 % is in comparison with morphine or diclofenac sodium. Similarly for late phase 42.4 and 50.9 % is it in comparison with diclofenac sodium or morphine was not mentioned.
Reply: VRP and VRE results in comparison with diclofenac-Na or morphine for late phase have been included.

Discussion: Line 259 - 260 (Formalin assay)
If late phase is in comparison with diclofenac sodium then compound (extracts) suppress inflammatory pain. Early phase with morphine then suppression of neurogenic pain.
Reply: Since the plant extracts are effective in both phases it suppress both neurogenic and inflammatory pains. The mentioned line has been corrected.

c.
1. Morphine (10 mg/kg) latency time is given but the percentage of inhibition was not mentioned for all observation period (30, 60,90,120 min).
Reply: Percentage of inhibition of morphine for all observation periods has been included.

2. The increase in latency period values could have been shown in table as numerical values instead of graphical representation.
Reply: We have observed antinociceptive activities of five different fractions of V. roxburghii. Graphical representation is more convenient to understand and compare to each other at a glance. For this reason we have chosen the graphical representation of hot plate data. If you recommend strongly changing the graph into table, please let us know, we will convert it into table later.
Referee 2

1. In the acetic acid-induced writhing test, five mice were randomly divided each groups. I known the variation is greater in this test. How do you have excellent results by five mice? Reply: Since the plant is traditionally widely used for pain, we have observed the experiment very carefully. Variations of result among five mice is lower, it might be for potent activity of the extract.

2. In your paper, the mice are overweight (30-50 g). How old the mice are used in the animal model? Will weight affect the animal’s response in your tests? Reply: In paper page-5, line-99 mice weight (30-35) g was shown. We did not mention (30-50) g in the article.

3. Both sex mice were used in the hot plate test. Why? Reply: In general same doses are used for both genders (male/female). In some cases the effect of drug/extract may vary for different gender. It is our preliminary study, that’s why we have used both genders of mice randomly. Furthermore, we will continue our study to identify the effect of isolated compound of the extract on both genders specifically.

5. When the extracts reach its peak in blood? Why the author selected 30 and 60 min in different models? Reply: We did not analyze blood peak plasma level to measure the maximum effect of the extract. We have observed the latency period for licking/flicking hind paw or jumping. Based on that parameter we have identified the maximum antinociceptive effect of the extract including hot plate experiment at 30 to 60 min.

6. The concentration of the extracts seems pretty low in treatment of animals. How did the author get those doses? Please provide justification and references. Reply: previously we have observed the activity of methanol extract of leaf of this plant and found promising activity at 100 and 200 mg/kg doses on mice (Ref: doi: 10.1186/1472-6882-14-464). Further, we collected the roots and extracted with fractions using different solvents. Therefore, we selected the three consecutive doses from 12.5 to 50 mg/kg.
7. The result about chemical constituents was weak. Please determine the content of
different constituents and discuss these results.
Reply: In our study we have ascertained the phytochemical constituents qualitatively,
where (+++) indicates presence of that constituent intensely. We did not perform any
quantitative analysis in this study. In response to your query, we have updated our
phytochemical screening data that reflects presence or absence only.

8. Please check all figures. There are some obvious mistakes.
Reply: We have checked the graph thoroughly and corrected some minor anomalies in
hot plate graph. If you identify any graphical errors or anomalies, please notify us. We
will rectify later.

9. What statistical software the author used for the analysis? Are you sure is GraphPad
software?
Reply: Obviously we have analyzed our data using GraphPad Prism Software (version
6.0).

Referee 3

1. Page 4 – line 74 – compounds
Reply: Corrected

2. Line 79 – Vanda Tesselata [19].
Reply: Corrected

3. Page 5 – Line 91 is 500 gm or 500 mg.
Reply: 500 gm

4. Line 113 – Which tests carried out for each phytochemical class?
Reply: The presence of tannins, alkaloids, saponins, flavonoids, phenols, steroids and
glycosides are determined by the methods of gelatin test, Mayer’s test, froth test, lead
acetate test, ferric chloride test, Libermann burchard’s test and legals’s test respectively.

Reply: Corrected

6. Page 8 – Line 181 – highest activity with 80.23% in which concentration?
Reply: At 50 mg/kg (Corrected)

Reply: Rewritten

8. Page 10 – Line 209 – The major value in Table 5 is 400 µg/mL.
Reply: The major value in Figure-5 is 400 µg/ml. The value has been substituted in paper.

9. Line 212 – which is the cutoff value?
Reply: According to Logarto, the LC50 value 10 µg/ml is considered as the cutoff value for cytotoxicity.

10. Page 19 – Add in legend of Table 1, the test carried out in the phytochemical screening.
Reply: In the chapter Materials and Methods ‘phytochemical screening’ all of the tests carried out in the phytochemical screening have been included.

11. Some correlation can be made among phytochemical screening of extract and fractions and antinociceptive properties.
Reply: Some correlations among phytochemicals and antinociceptive properties have been included with reference.

I am looking forward to hear from you.

Thanking you

Sincerely yours,

Md. Josim Uddin
Lecturer, Department of Pharmacy
International Islamic University Chittagong
Chittagong-4203, Bangladesh.