Reviewer’s report

Title: Experimental study of Delphinium denudatum Wall. on the abstinence signs in morphine dependent rats.

Authors:

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Reviewer: Dr shadab zafar

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

February, 2002

Comments:

Title: ‘Experimental study of Delphinium denudatum Wall. on the abstinence signs in morphine dependent rats.’

Rehman et al.

This paper presents interesting results but the following points need to be addressed:

Discretionary revisions:
Calculate, if possible, all the data by one way analysis of variance (ANOVA) followed by Student's 't' test, with multiple comparisons (to find out the difference between group 2 and 3 with group 4, 5 and 6).

Compulsory revisions:

Page 2
Summary:
Methods: (Lines 8-9): c) multiple doses (350 mg/kg) twice a day for 7 days?. This sentence does not specify regime, whether with morphine or without morphine?.

Conclusion: The conclusion drawn “that the D.d. extract reduces the aggregate score for all parameters in morphine withdrawal syndrome by central action” does not find adequate support from the data shown, because the drug (D.d extract) appears to act by dual action, on opioid receptors and by central action.
Introduction:

Para 2:
Several pharmacological studies have appeared on Delphinium denudatum Wall., but no other relevant pharmacological properties of the plant, except one (8), have been mentioned.

Some pharmacological reports are as follows:
Anticonvulsive activities:
Hepatoprotective activity:
Cardioprotective activities:
Antibacterial activity:
Antifungal activity:
Immuno-modulating activity:
De-addiction properties of a Compound Unani Formulation containing Delphinium denudatum Wall.:
De-addiction property on Delphinium denudatum:
Anti-stress activity of Jawahar Mohra, a compound formulation of Unani medicine containing Delphinium denudatum Wall.:

Therefore, the second para of "Introduction" needs to be re-written so as to include the related studies/other pharmacological studies (in brief).

Materials and methods:
It should be mentioned whether the animals used were procured from market or provided by JNMC animal house facility?.

Drugs:
Indicate whether the roots used were fresh or dried?
Also, indicate month or at least season of plant collection and whether the yield of the extract (7.43% w/w) was in terms of dried starting material or fresh starting material.

Page 4:
Para 2:
What was the dose vehicle with test drug (D.d. extract)?
Simplify the treatment schedule and don’t write “as per 7-d schedule” respectively. Do not write "Control" and "Experimental" here because it is obvious as to what treatment is being given.

It can be written as follows:
The rats were divided into 6 groups of 8 animals each. All the animals were treated with 0.9% saline (ml/kg, p.o.), D.d. extract (mg/kg, p.o.) using ? as the dose vehicle and morphine (mg/kg, i.p.) as per 7-d schedule (Table 1) for 7 days.
Group 1: Saline (1.0) X twice daily.
Group 2: D.d. extract (350) X twice daily.
Group 3: Morphine (10-100) X twice daily.
Group 4: D.d. extract (350) and morphine (10-100) X twice daily.
Group 5: D.d. extract (700) X single dose 10h after the last dose of morphine.
Group 6: D.d. extract (700) X single dose 10h prior to the first dose of morphine.

The following information may be furnished about the recording of the signs of spontaneous abstinence syndrome:
Whether the transparent glass box was used for observation?
If yes, what were its dimensions?
If no, what technique was then followed?

Page 5
Line 1: Missed 1973 with Blasig et al?
Statistical analysis:
P value, which was considered significant, is not mentioned?. Also adjust with multiple comparisons to find out the difference between group 2 with groups 4, 5 and 6.

Results:
Line 10-11: sentence "twice daily x 7 day" should not be repeated.
Line 14: "p.o.", to be deleted.
Line 17: (Table 3)- No need here, write down on page 6 in line 1, after the end of the sentence, because Table 3 is showing the comparison of group 3 to Groups 4, 5 and 6.

Page 6
Line 2-4: The mean aggregate score is mentioned as 12.9, 7.19, 5.8 and 10.67 for the group 3, 4, 5 and 6, respectively. Write down their standard errors also, as drawn in Fig. 1, otherwise it (Fig.1) cannot represent the data presented for the aggregate score.

Discussion:
Para 2:
Line 5: "p.o." and "injections" to be deleted.

Page 7
Line 3: write "squeaking on touch" in place of "response to touch".
Line 4: write P value after 'jumping fell to zero?.

Para 2
Line 5: The statement that "the drug is neither acting as opioid agonist nor opioid antagonist" does not make sense with these data. Though the drug (D.d. extract) seems to act centrally independent, yet it also ameliorates to some extent the signs of abstinence syndrome (in group 4) as compared to group 3, although it did more in group 5. Therefore it seems to be opioid antagonist also.
Moreover, the entire data of Group 2 could not be incorporated in Table 3 and Fig.1 to prove the above statement.

From line 6- The following information about the principle alkaloid (methyllycaconitine) and series of C-19 deterpenoid alkaloids. Were these alkaloids isolated from Delphinium denudatum roots? If yes, give their references (to specify the particular species). If no, from the same species and root, then your support of the interpretation "as reference (13), (14), (15) and (16)" does not seem to be justify the claim (as de-addiction) of your objective. So you cannot presume or emphasize the site of action of the drug (D.d. extract). Therefore, discussion needs to re-written, and justified from the data presented. Another way of interpretation may be the support of the reported claim of classical literature (4-5) and the reported de-addiction properties of the same.

Page 10-11
References:
All references (3, 5, 6, 7, 8, 10, 11, 13 and 14) may be checked and kept strictly in accordance with the format prescribed. Also incorporate names of all the authors in reference 14.

Table 2:
Spelling of Rhinorrhoea may be corrected.

Table 3:
Legend under the table creates confusion. Single astrix (*) may be used for P0.01 and double astrix (**) for P0.001, like *P0.01 and **P0.001.
The scores of group 1 and group 2 may be added for better presentation.
The mean aggregate score of each group may be indicated with standard errors.

Fig.1:
"Mean aggregate of parameters/signs" on Y-axis looks odd, when the heading is showing mean + S.E.? The standard errors of aggregate score of each group have not been mentioned in "Results" but are shown in Fig.1, why?

**Competing interests:**
I am also giving my consent that you can pass my signed report to the authors, and if the paper is accepted for publication, then you can pass my signed report together with paper as a part of the pre-publication history.

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