Reviewer's report

Title: An-jun-ning, a traditional herbal formula, attenuates spontaneous withdrawal symptoms via modulation of the dopamine system in morphine-dependent rats

Version: 2
Date: 28 December 2013
Reviewer: Reinaldo Takahashi

Reviewer’s report:

The present work describes the effects of An-jun-ning (AJN), a commercial traditional Chinese medicine on the dopaminergic system in morphine-dependent rats. ANJ reduced the wet dog shakes or the episodes of writhing induced by morphine and post-treatment with AJN restored the levels of TH, DAT and D2R in the striatum. The authors conclude that the mechanism of the effects of AJN on withdrawal signs may be related to the modulation of the “dopamine” system by AJN. In general the study appears to address an interesting issue. However, I have some major concerns with the study and its interpretations as it stands.

Major concerns:

The crucial problem with this paper is the author’s attempt to examine without clear criteria, 2 schemes of AJN, pre-treatment with one dose and post-treatment using 3 doses and different duration of treatment, on morphine-induced signs of abstinence in rats. Besides the authors rely on dosages of AJN converted from those used clinically, on behavioral signs dependent on locomotor performance of rats without an adequate control response of the treatment on this matter.

- The main argument of the authors for a reduced behavioral response of treatment is based on the assumption that AJN reverted the number of wet dog shakes or episodes of writhing in rats. In my view, one critical issue is that the authors rely on writhing responses showing mean around 1.1 episodes for morphine group and did not bother to discuss the bias of affecting such low level of responses. It is unlikely that the selected “dependence” condition will detect meaningful protective effects of AJN treatments.

- The authors should include some more details concerning the main constituents of the commercial AJN selected for this study. The omitted information calls for a great deal of caution in interpreting the data obtained here.

- Authors should provide in the text how the behavioral responses were recorded, e.g., is the observer blind to the treatment of the groups, were the experiments video monitored.

Minor

- Legends, Figs. 1 – 4, please provide the meaning of NS, JTT, also should include more details, e.g., the effects of AJN are pre or post-treatments (Figs 3-4).
-Fig. 1, the results depicted in Panel A and B are for wet dog-shakes only in pre-treated groups?. Authors should explain why post-treatment of AJN, panel C-D, only for writhing episodes and the convenience to maintain both panels.

-The manuscript should be carefully examined for typos, grammatical and spelling errors.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.