Author’s response to reviews

Title: Protective and restorative effects of the traditional Chinese medicine Jitai tablet against methamphetamine-induced dopaminergic neurotoxicity

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Reviewer reports:

Sung-Ting Chuang (Reviewer 1): This manuscript entitled "Protective and restorative effects of the traditional Chinese medicine Jitai tablet against methamphetamine-induced dopaminergic neurotoxicity" used immunohistochemical staining and autoradiography staining and stereotyped behavior rating scale to investigate the effects of JTT on methamphetamine-induced dopaminergic neurotoxicity in rats. The results showed that pre-treatment with JTT or post-treatment with high dose JTT could attenuate and reverse METH-induced neurotoxicity. Some comments are listed below:

1. The authors indicated that the 0.290kg/g of JTT was converted from clinical dose. Did you test the doses of JTT on 0.029kg/g and 0.290kg/g with pre-treatment? Why you just tested these doses with post-treatment? It would be better to explain in detail in materials and methods section.
We designed the two schemes of JTT to evaluate the protective effect by pre-treatment and the restorative effect by post-treatment against METH-induced dopaminergic neurotoxicity. We tested three doses of JTT (0.029g/kg, 0.087g/kg and 0.290g/kg) with post-treatment, and one dose of JTT (0.087g/kg) with pre-treatment.

The post-treatment of JTT was scheduled to examine its therapeutic ability to interdict the METH-induced decreases in DAT, D2R and TH expression. Usually, a drug is evaluated with three or more doses [1-3]. To confirm whether there is a dose-dependent effect, we designed three doses of JTT in the post-treatment regimen with ratio 10: 3: 1 and the high dose was directly converted from the clinical dose, namely 0.290 g/kg.

Pre-treatment could provide us another perspective on the mechanism of JTT, through examination of its possible inhibition of behavior response and neurobiological disturbance in the nervous system. With this scheme, we investigated whether JTT could attenuate METH-induced stereotyped responses, and interdicted METH-induced changes in the levels of DAT, D2R and TH expression. To avoid unnecessary animal use in consideration of the international guidelines for care and use of laboratory animals, we set only one dosage for pre-treatment in our experiments.

All changes are shown in red in the revised manuscript.

Reference:


2. Although authors provided some staining evidence to support their conclusion, it would be more powerful to investigate what mechanism is involved in this effect of JTT?

Reply: We appreciate the reviewer’s comments. The present study is the first to indicate that JTT protects against METH-induced neurotoxicity and restores the dopaminergic function, and thus might be a potential treatment for the dopaminergic deficits associated with METH abuse. Repeated exposure to moderate to high levels of methamphetamine has been related to neurotoxic effects on the dopaminergic systems. Although the exact molecular mechanisms of neurotoxicity remain unclear, there is evidence that the oxidative stress, mitochondrial
dysfunction, apoptosis, neuro-inflammation and excitotoxicity are mechanisms commonly implicated in dopaminergic neurotoxicity[1, 2]. Therefore, further studies could proceed from these aspects to further investigate the mechanism of JTT’s effects.

Reference:


3. Please add the scale bar in all photographs and describe the original magnification of photographs in figure legends.

Reply: We have added the scale bar in the photographs and also described the magnification of photographs in figure legends.

4. Please check the statistical symbol correctly in figure 3a. Authors wrote "post-treatment with JTT attenuated D2R reduction in a dose-dependent manner, with D2R at 95.3% ± 5.1 of the control level for the group post-treated with medium dose of JTT (JTT-M vs. METH, p<0.05)" in result section. According to this statement, the

Reply: We apologize for this omit. We have changed the symbol as "*" in the bar chart of JTT-M in figure 3a.

Hongtao Bi, Ph.D. (Reviewer 2): JTT has been proved to be very safe and effective in the inhibition of protracted withdrawal symptoms with less harmful side effects, which have been already approved for the treatment of opiate addiction by SFDA. The present study aimed to evaluate the protective and restorative effects of JTT on METH-induced dopaminergic impairment. The experimental design is straightforward, and data are clearly presented. These findings could give insights into a possible treatment for METH addiction using JTT and may form the basis for the new therapeutic strategy using phytochemicals. So, because JTT is prepared from fifteen Chinese herbal medicines, and contains an abundance of bioactive components, it is suggested to further discuss the results based on the published pharmacochemistry data.

Reply: Thanks very much for the reviewer’s comment and suggestion. The JTT is a mixture of 15 different herbs with 101 compounds tentatively identified in a previous study. It is difficult to pinpoint how and which of these compounds modulates the dopaminergic activity in the striatum though the normalization of the activity seen in this study may be attributed to the combined effect of the complex mixture of the tablet. We have added the published data of JTT’s bioactive components to explain the possible mechanism underlying the protective and restorative effects of JTT on the METH-induced dopaminergic changes. Further studies are still needed to
investigate which and how these bioactive components play the major role in its therapeutic effects.

All changes are shown in red in the revised manuscript.