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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Author’s response to reviews:

Dear Editor:

Thank you very much for inviting us to revise our manuscript. We appreciate the comments and made point-to-point response. All changes are marked with a different font color (red color). We hope that you find the revised manuscript acceptable for publication in BMC Complementary & Alternative Medicine.

Responses to the Editor’s Comments:

1. In experimental research, it is controversial that repeated METH alters D2R (Shishido et al, Neurosci let 1997, 222(3), 175-8)

Reply: Different studies of the dopamine D2 receptor have reported either no change or a decrease in dopamine D2 receptor density. The results of the study from Shishido et al, revealed...
that there was no significant change in the D2R mRNA levels in the striatum [1]. However, a recent study reported a significant decrease in D2 receptor mRNA expression after the METH administration [2]. In addition, one study conducted by Segal et al, also reported a significant reduction in D2 receptor after intravenous METH administration, which is consistent with our results [3]. As previously reported [4], the amphetamine-sensitized animals showed a 31% lower number of D2 receptors in conventional binding. However, with the addition of guanilylimidodiphosphate there was an equalization of both amphetamine and control groups. The results showed that while in vivo binding shows a decrease in the specific/nonspecific binding, suggesting a decrease in receptor density, in vitro experiments show that there is no loss of receptors, but that the receptors are bound to endogenous dopamine in a noncompetitive fashion [4]. It has been described, that both D1 and D2 receptors exist in high- and low-affinity states. High-affinity states of dopamine D1 (D1 High) and D2 (D2 High) receptors have much higher affinity for dopamine than D1 and D2 receptors in low-affinity states. Dopamine D1 High and D2 High receptors are considered to be the functional state of dopamine receptors [5] and Seeman et al. suggested that the proportion of D2 High receptors was increased in the striatum of amphetamine-sensitized rats, despite of no changes in the density of D2 receptors [4].

Therefore, this discrepancy between previous results and our result may result from the different measuring methods. The immunohistochemical and autoradiography staining methods may just reveal the levels of high-affinity states of D2 receptors, not the absolute D2 receptors. In addition, the question also remains whether PCR method or in situ hybridization histochemistry is capable to detect mRNA expression of D2 receptors in both states (high- and low-affinity states). Further studies are required to elucidate these difference among these different methods for D2 receptor measuring.

References:


2. Acute administration of METH acts on the dopamine transporter (DAT), resulting in increased release of dopamine (DA) [2], and leading to the stereotyped behavior. Present reference to prove that acute METH generates stereotyped behavior.

Reply: Thanks for the editor’s advice, we have added the references to prove that acute METH generates stereotyped behavior [1, 2].

References:


3. The fingerprint of the JTT used in this study was presented in the supplement material of our previously published article [19]. But, no fingerprint data are seen in reference #19.

Reply: We apologize for this omission. The fingerprint of the JTT used in this study was presented in the supplement material of our previously published article, as presented in reference #18 (previous #16) not the previous reference #19 (now #21). The correct reference citation is now included in our revised manuscript.

4. “the high dose was directly converted from the clinical dose, namely 0.290 g/kg.”. Provide supporting reference.

Reply: The clinical usage of JTT is 3-5 tablets per day, and every tablet (0.4 g/table) contains 0.188 g crude drug powder [1, 2]. Therefore, 15 tablets for an adult weighing 70 kg are equal to the dose 0.047 g/kg/day for humans. The equivalent dose of rats was 6.25 times that of the patients by the unit weight dose. Therefore, converting to the dosages of JTT for rats was 0.047 g/kg × 6.25 = 0.294 g/kg/day. Hence, we used the dosages of JTT as 0.29 g/kg/day.

In our revised manuscript, we have provided the supporting references.

References:

5. “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.” It is not reasonable justification for trial of one dosage in pretreatment experiment. Why did you choose the dosage?

Reply: The post-treatment of JTT was scheduled to examine its therapeutic ability to interdict the METH-induced decreases in DAT, D2R and TH expression. 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 (0.290, 0.087 and 0.029 g/kg). The high dose (0.290 g/kg) was directly converted from the clinical dose. Therefore, we hypothesize that the high dose would have apparent therapeutic effects on METH model, the middle dose (0.087 g/kg) may have a slight effect, and the low dose (0.029 g/kg) may have no effects on METH-induced neurotoxicity. As the pre-treatment of JTT was scheduled to investigate whether JTT could attenuate METH-induced stereotyped responses, and interdicted METH-induced changes in the levels of DAT, D2R and TH expression. Thus, the high dose of JTT (0.290 g/kg) for pre-treatment may confound the results that whether the obvious inhibition effect of JTT was partially resulted from the therapeutic effect of JTT. Therefore, we chose the middle dose of JTT (0.087 g/kg) to investigate its inhibition effects on METH-induced neurotoxicity. Our results showed that the middle dose of JTT (0.087 g/kg) in post-treatment regimen have really slight effects on METH-induced decreases in DAT, D2R and TH expression. However, the pre-treatment with middle dose of JTT (0.087 g/kg) could significantly attenuate METH-induced stereotyped responses, and completely interdict METH-induced changes in the levels of DAT, D2R and TH expression.

Therefore, considering the above issue, the dose we choose in the pre-treatment regimen was appropriate and enough to illustrate its effects, and to avoid unnecessary animal use of laboratory animals.

6. Stereotyped behavior used in the study is too subjective. As commended previously, provide more objective data to show whether JiTai tablet can inhibit METH-induced behaviors, such as methamphetamine enhanced locomotor activity (video tracking system) or self-administration behaviors.

Reply: In our manuscript, to avoid the subjective effects, a blind manner was used to rate stereotype behaviors. In general, the stereotype behaviors are closely associated with the psychosis induced by amphetamine-type stimulants including methamphetamine, whereas hyperlocomotion is involved in their stimulative effects and the self-administration of drug in their addiction. Our present study focused the effects of JTT on METH-induced neurotoxicity on dopaminergic system, not on the METH addiction. In addition, this acute METH neurotoxicity model is not appropriate for the METH addiction study, therefore, we just chose the stereotyped behavior evaluation, not involve the locomotor activity or self-administration. Certainly, we
really appreciate the editor’s good suggestions and advice. In the next investigation, we attempt to establish the METH addiction model and pay attention to the effects of JTT on the hyperactivity and self-administration of METH.

7. Provide error and DF values in F values.

Reply: We appreciate the editor’s advice. We have provided error and DF values in F values in the Results section.

8. Typos: “Two-way repeated measures ANOVA with time as a repeated measure (time × treatment) followed by LSD as post hoc test was used to analyzed the data on..”

Reply: We apologize for this omission, and thank the editor’s reminder. We have corrected this sentence in our revised manuscript as follows: “Two-factor repeated measure analysis of variance (ANOVA) with time as a repeated measure (time × treatment) followed by LSD as post hoc test was used to analyze the data on stereotyped behaviors.”

9. Mark statistical results (i.e., asterisk) on data analyzed every 5 min in Fig 1.

Reply: Thanks for the editor’s suggestion, we have marked statistical results on data analyzed every 5 min in Fig 1.

10. In results, “previous studies have documented that the chronic use of stimulants results in decreased DAT levels in the striatum.” Where is reference?

Reply: Thanks, we have added the reference in our revised manuscript.

Reference:


11. “TH is a key enzyme in DA synthesis and generally used as a marker for dopaminergic neurons.” where is reference?

Reply: We appreciate the editor’s reminding, we have added the reference in our revised manuscript.

References:


12. “METH administration induced a marked loss of TH positive neurons”. However, authors did not count the numbers of DA neurons in Fig 4b.

Reply: We apologize for this error. Immunohistochemical staining was used to determine the concentration of TH-positive neurons and fibers in the striatum and SN. The optical densities of TH in the striatum and SN were calculated by Image-Pro Plus software. We have modified the relevant statements to be more appropriate in our revised manuscript.

13. Their findings are superficial. There is no mechanism study. Authors should perform mechanism study.

Reply: We thank the editor for this suggestion. Clear mechanism(s) of action can hardly be attributed to a prescription constituted of a complex herbal mixture. On the other hand, traditional Chinese medicines (TCMs) are more likely to exert mild and multi-target action with few side-effects. The Jitai tablets (JTT) are approved for the opioid addiction in clinical. Our recent clinical and animal studies have shown that the JTT has the ability to modulate the dopaminergic function both in heroin addicts and in morphine-dependent rats. As the METH also has neurotoxicity on dopaminergic system. Hence, this preliminary study was designed to evaluate the JTT’s effects on METH-induced neurotoxicity. Our data have indicated the integrative effect of JTT on methamphetamine-induced neurotoxicity of dopaminergic system in rats, which may be significant for studying traditional Chinese medicine. As the positive results of JTT’s therapeutic and inhibition effects on METH model, further studies are required to establish the METH addiction model to make further exploration on its effects, and more importantly to perform the investigation to illustrate the mechanism of JTT’s effects on METH-induced dopaminergic neurotoxicity.

14. Many parts of discussion are just duplication of results. i.e., “Multiple smaller doses of METH….both in the striatum and SN”.

Reply: Thanks for the editor’s excellent comments. We have made adjustments in the discussion section.

15. …”Therefore, the attenuation effect of JTT on behavioral responses, as well as the protective effect of JTT on METH-induced neurotoxicity in the nigrostriatal dopaminergic system, may result from its antioxidant effect.” There are many ways
which JTT decreased METH neurotoxicity. It is difficult to conclude it with only limited data.

Reply: We agree with the editor’s comments. The pharmacological mechanisms underlying JTT’s ability to regulate the behavior response and dopaminergic system in the METH model rats are likely due to multiple targeting as a result of the combined effects of its active ingredients. Therefore, the results of our present preliminary study are difficult to come to this conclusion. The last paragraph in Discussion has illustrated the probably pharmacological mechanisms of JTT underlying its protective effect to dopaminergic neurotoxicity, therefore we have deleted this immature and redundant paragraph in our revised manuscript.

16. “The present study is the first to evaluate the traditional Chinese medicine Jitai tablet as a novel treatment for METH-induced dopaminergic neurotoxicity. However, there are lots of papers using ginseng (one component of JTT) in METH toxicity.

Reply: We agreed with the editor’s comments that ginseng has the protective effects in METH-induced toxicity [1-3]. Our present study wants to emphasize the first study to use Jitai tablets for METH-induced neurotoxicity treatment. As the Jitai tablets is approved for the opioid dependence treatment in clinical. Previous clinical and preclinical studies just focused on the its therapeutic effects on opioid addiction. No study was conducted to explore its potential therapeutic effects on METH-induced neurotoxicity. Therefore, our present study is the first to evaluate Jitai tablet as a novel treatment for METH-induced dopaminergic neurotoxicity.

We thank the editor’s comments, and have modified this confounding statement in our revised manuscript.

References:


We hope that our revisions are sufficient for publication in BMC Complementary & Alternative Medicine.

Thank you very much for your consideration.
Sincerely yours

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