Author’s response to reviews

Title: Sedative and hypnotic effects of Compound Anshen essential oil inhalation for insomnia

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Version: 2 Date: 05 Sep 2019

Author’s response to reviews:

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled “Sedative and hypnotic effects of Compound Anshen essential oil inhalation for insomnia” (ID: BCAM-D-19-00899R1). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as
Responds to the Editor comments:

María Celina Elissondo (Editor)

1. Response to comment: Both experts brought forward some minor and major concerns (see comments below) that very carefully have to be addressed by the authors. The authors should more detail the novelty of the study.
Response: We have made correction according to the Editors’ comments.

(e.g. Background section, line 7, page 5) Multi-herb therapy is one of the most important characteristics of traditional Chinese medicine. Herb pairs, the most fundamental and the simplest form of multi-herb formulae, are a centralized representative of Chinese herbal compatibility[21]. Many diseases are regulated by multiple pathogenic factors and thus they are hard to deal with. In these cases, the potency of a single herb is modest and cannot address the complicated and multivariate conditions of patients. In order to achieve better curative effect, several herbs are combined according to their properties so as to extend their abilities[21, 22]. As insomniacs are usually accompanied by symptoms of depression and anxiety of varying degrees, based on the theory of aromatherapy of traditional Chinese medicine, this experiment is to study a Compound Anshen essential oil that is compatible with lavender essential oil [23, 24], sweet orange essential oil [25, 26], sandalwood essential oil [27] and other aromatic medicine essential oils with sedative and hypnotic effects, anti-anxiety and anti-depression effects. Lavender has sedative-hypnotic effects and anti-anxiety effects, Sandalwood and Agarwood[28] have sedative-hypnotic effects, Sweet orange, Rose[29], Frankincense[30] and Orange Blossom all have anti-depression effects, among which Sweet orange (Orange Peel) and Agarwood are the classic Herb pairs. Traditional Chinese medicine believed that insomnia can cause by Liver depression and qi stagnation, stomach enranges disaccord. Therefore, it is possible to treat insomnia from the perspective of dispersing stagnated liver qi to relieve depression, and relieving qi stagnancy in stomach[31]. In traditional Chinese medicine, sweet orange, sandalwood, rose, frankincense, agarwood, orange blossom are qi-regulating drugs[32]. Qi-regulating drugs have the effect of dispersing stagnated liver qi to relieve depression, and the effect of relieving qi stagnancy in stomach [33]. Therefore, the inhalation of Compound Anshen essential oil may become an effective treatment or adjuvant treatment for insomnia. The sedative and hypnotic effects of inhaling Compound Anshen essential oil were compared and analyzed with the pharmacodynamics of diazepam, a commonly used drug for insomnia. The sedative and hypnotic effects of the Compound Anshen essential oil were evaluated by autonomous activity test and pentobarbital-induced sleep latency and sleep time experiments, and the changes of neurotransmitter 5-HT and GABA concentrations in the brain were observed. Neurotransmitter dysfunction such as 5-HT and GABA is closely related to insomnia. It has been reported that commonly used drugs for treating insomnia induce sedative and hypnotic effects by regulating neurotransmission, such as the serotonergic system in the central nervous system or GABAergic[34]. Therefore, the analysis of 5-HT and GABA content in the brain is of great significance for the detection of the effect of Compound Anshen essential oil in the treatment of insomnia[35]. To explore the mechanism of action of compound Anshen essential oil in the treatment of insomnia, aiming to provide a theoretical basis for the treatment of Compound Anshen essential oil through aromatherapy and improvement of insomnia.

(e.g. Discussion section, line 49, page 11) Therefore, the effect of Compound Anshen essential oil on the levels of 5-HT and GABA in the brain is of great significance for evaluating the pharmacodynamics of Compound Anshen essential oil for the treatment of insomnia. In addition to the above reasons, this experiment uses the PCPA insomnia model, because PCPA is a tryptophan hydroxylase inhibitor, consumes 5-HT, leading to insomnia, detection of brain 5-HT levels can reflect the successful establishment of insomnia model. In this study, the levels of 5-HT and GABA in the brain were
measured by enzyme-linked immunosorbent assay. The results showed that the low-dose, middle-dose and high-dose groups of the Compound Anshen oil showed different degrees of 5-HT up-regulation and GABA up-regulation in a dose-dependent manner. The levels of 5-HT and GABA in the middle-dose and high-dose groups were similar to those in the control group and higher than those in the diazepam group. Therefore, the inhalation administration of the Compound Anshen essential oil can significantly increase the levels of 5-HT and GABA in the brain. From the experiment of weight change and the analysis of brain neurotransmitter level, the effect of Compound Anshen essential oil inhalation on insomnia is better than that of diazepam. It can be seen that inhalation of Compound Anshen essential oil may be a safe and effective treatment for or adjuvant treatment of insomnia, which may reduce the excessive use of prescription drugs such as diazepam and reduce the risk of short-term or long-term effects on sleep health. Its deeper sedative and hypnotic mechanism needs further study.

Special thanks to you for your good comments.

Responds to the reviewer’s comments:

Tadaaki Satou (Reviewer 1)

1. Response to comment: Please clarify the novelty of this paper. The results obtained this time are the results already reported for each essential oil. Show the importance or further effect of blending essential oils. As such, novelty is not recognized in this paper.

Response: We have made correction according to the Reviewer’s comments.

(e.g. Background section, line 7, page 5) Multi-herb therapy is one of the most important characteristics of traditional Chinese medicine. Herb pairs, the most fundamental and the simplest form of multi-herb formulae, are a centralized representative of Chinese herbal compatibility[21]. Many diseases are regulated by multiple pathogenic factors and thus they are hard to deal with. In these cases, the potency of a single herb is modest and cannot address the complicated and multivariate conditions of patients. In order to achieve better curative effect, several herbs are combined according to their properties so as to extend their abilities[21, 22]. As insomniacs are usually accompanied by symptoms of depression and anxiety of varying degrees, based on the theory of aromatherapy of traditional Chinese medicine, this experiment is to study a Compound Anshen essential oil that is compatible with lavender essential oil [23, 24], sweet orange essential oil [25, 26], sandalwood essential oil [27] and other aromatic medicine essential oils with sedative and hypnotic effects, anti-anxiety and anti-depression effects. Lavender has sedative-hypnotic effects and anti-anxiety effects, Sandalwood and Agarwood [28] have sedative-hypnotic effects , Sweet orange, Rose [29], Frankincense [30] and Orange Blossom all have anti-depression effects, among which Sweet orange (Orange Peel) and Agarwood are the classic Herb pairs. Traditional Chinese medicine believed that insomnia can cause by Liver depression and qi stagnation , stomach enranges disaccord. Therefore, it is possible to treat insomnia from the perspective of dispersing stagnated liver qi to relieve depression, and relieving qi stagnancy in stomach[31]. In traditional Chinese medicine, sweet orange, sandalwood, rose, frankincense, agarwood, orange blossom are qi-regulating drugs[32]. Qi-regulating drugs have the effect of dispersing stagnated liver qi to relieve depression, and the effect of relieving qi stagnancy in stomach [33]. Therefore, the inhalation of Compound Anshen essential oil may become an effective treatment or adjuvant treatment for insomnia. The sedative and hypnotic effects of inhaling Compound Anshen essential oil were compared and analyzed with the pharmacodynamics of diazepam, a commonly used drug for insomnia. The sedative and hypnotic effects of the Compound Anshen essential oil were evaluated by autonomous activity test and pentobarbital-induced sleep latency and sleep time experiments, and the changes of neurotransmitter 5-HT and GABA concentrations in the brain were observed. Neurotransmitter dysfunction such as 5-HT and GABA is closely related to insomnia. It has been reported that commonly used drugs for treating insomnia induce sedative and hypnotic effects by
regulating neurotransmission, such as the serotonergic system in the central nervous system or GABAergic[34]. Therefore, the analysis of 5-HT and GABA content in the brain is of great significance for the detection of the effect of Compound Anshen essential oil in the treatment of insomnia[35]. To explore the mechanism of action of compound Anshen essential oil in the treatment of insomnia, aiming to provide a theoretical basis for the treatment of Compound Anshen essential oil through aromatherapy and improvement of insomnia.

(e.g. Discussion section, line 49, page 11) Therefore, the effect of Compound Anshen essential oil on the levels of 5-HT and GABA in the brain is of great significance for evaluating the pharmacodynamics of Compound Anshen essential oil for the treatment of insomnia. In addition to the above reasons, this experiment uses the PCPA insomnia model, because PCPA is a tryptophan hydroxylase inhibitor, consumes 5-HT, leading to insomnia, detection of brain 5-HT levels can reflect the successful establishment of insomnia model. In this study, the levels of 5-HT and GABA in the brain were measured by enzyme-linked immunosorbent assay. The results showed that the low-dose, middle-dose and high-dose groups of the Compound Anshen oil showed different degrees of 5-HT up-regulation and GABA up-regulation in a dose-dependent manner. The levels of 5-HT and GABA in the middle-dose and high-dose groups were similar to those in the control group and higher than those in the diazepam group. Therefore, the inhalation administration of the Compound Anshen essential oil can significantly increase the levels of 5-HT and GABA in the brain. From the experiment of weight change and the analysis of brain neurotransmitter level, the effect of Compound Anshen essential oil inhalation on insomnia is better than that of diazepam. It can be seen that inhalation of Compound Anshen essential oil may be a safe and effective treatment for or adjuvant treatment of insomnia, which may reduce the excessive use of prescription drugs such as diazepam and reduce the risk of short-term or long-term effects on sleep health. Its deeper sedative and hypnotic mechanism needs further study.

2. Response to comment: Examining essential oils and their blends is not a scientific understanding of aromatherapy. The effects of essential oils, which are mixtures, are thought to be elucidated scientifically by conducting experiments and discussions on single chemical components. Response: Multi-herb therapy is one of the most important characteristics of traditional Chinese medicine. Herb pairs, the most fundamental and the simplest form of multi-herb formulae, are a centralized representative of Chinese herbal compatibility. Many diseases are regulated by multiple pathogenic factors and thus they are hard to deal with. In these cases, the potency of a single herb is modest and cannot address the complicated and multivariate conditions of patients. In order to achieve better curative effect, several herbs are combined according to their properties so as to extend their abilities. As insomniacs are usually accompanied by symptoms of depression and anxiety of varying degrees, based on the theory of aromatherapy of traditional Chinese medicine, this experiment is to study a Compound Anshen essential oil that is compatible with lavender essential oil, sweet orange essential oil, sandalwood essential oil and other aromatic medicine essential oils with sedative and hypnictic effects, anti-anxiety and anti-depression effects. Lavender has sedative-hypnotic effects and anti-anxiety effects, Sandalwood and Agarwood have sedative-hypnotic effects, Sweet orange, Rose, Frankincense and Orange Blossom all have anti-depression effects, among which Sweet orange (Orange Peel) and Agarwood are the classic Herb pairs. Traditional Chinese medicine believed that insomnia can cause by Liver depression and qi stagnation, stomach enrages disaccord. Therefore, it is possible to treat insomnia from the perspective of dispersing stagnated liver qi to relieve depression, and relieving qi stagnancy in stomach. In traditional Chinese medicine, sweet orange, sandalwood, rose, frankincense, agarwood, orange blossom are qi-regulating drugs. Qi-regulating drugs have the effect of dispersing stagnated liver qi to relieve depression, and the effect of relieving qi stagnancy in stomach. Therefore, the inhalation of Compound Anshen essential oil may become an effective treatment or adjuvant treatment for insomnia. The sedative and hypnotic effects of inhaling Compound Anshen essential oil were compared and analyzed with the pharmacodynamics of diazepam, a
commonly used drug for insomnia. To explore the mechanism of action of compound Anshen essential oil in the treatment of insomnia, aiming to provide a theoretical basis for the treatment of Compound Anshen essential oil through aromatherapy and improvement of insomnia.

In this experiment, we aimed to study the sedative and hypnotic effects of Compound Anshen essential oil inhalation in the treatment of insomnia. In the process of further exploring the mechanism of sedation and hypnosis, we will conduct experiments and discussions on a single chemical component.

We have made correction according to the Reviewer’s comments.

Due to the errors described in the previous paper's conclusions, based on the revision of the conclusions of the paper, the authors discuss the GC-MS analysis of the Compound Anshen essential oil and the network pharmacology analysis results of Dr. Ren.

(e.g. Conclusion section, line 18, page 13)

Conclusion
The study found that the inhalation of compound Anshen essential oil has sedative and hypnotic effect, which can significantly reduce autonomic nervous activity, shorten latency of sleeping time and prolong duration of sleeping time. The main chemical components of Compound Anshen essential oil contain many kinds of chemical components which have the sedative and hypnotic effect on the nerve center, anti-anxiety effect and anti-depression effect. (e.g. Discussion section, line 57, page 11) On this basis, the composition of Compound Anshen essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 30 chemical constituents were identified, accounting for 93.39% of total volatile oil. The main components of Compound Anshen essential oil are esters, alcohols, alkenes, alkyls and other compounds, with the highest content of D-limonene(24.07%), Linalool (21.98%), Linalyl acetate (15.37%), α-Pinene (5.39%) and α-Santalol (4.8%). D-limonene [45], Linalool [46], Linalyl acetate [47] and α-Santalol [48] have been reported to have central nervous sedative effect. In addition, D-limonene has anti-anxiety and soothing effects [49], Linalool has anti-anxiety and depression-like effects [50] and α-Pinene has anti-anxiety [51] and stress-relieving effects [52]. It is speculated that the sedative and hypnotic effect of Compound Anshen essential oil is related to its main chemical composition and activity. Based on the network pharmacology, the active components, targets and pathways of the Compound Anshen essential oil were predicted. The results of the network pharmacology analysis were published in the paper of the laboratory peer Dr. Ren [53]. Among the volatile chemical constituents of the compound anshen essential oil traced by GC-MS, the active ingredients related to insomnia were screened and the relevant targets and pathways were predicted. The study found that Dibutyl phthalate, Caryophyllene, Geranyl acetate, Linalool, α-Terpineol, and Terpinen-4-ol played a key role in the treatment of insomnia in the Compound Anshen essential oil. The chemical components with the highest content tracked by GC-MS were D-Limonene, Linalyl acetate, α-Pinene, which were also correlated with treatment of insomnia. It is predicted that the compound anshen essential oil mainly exerts pharmacodynamic effects through related target proteins such as ESR1, GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, NR1H4, CHRM1, SLC6A2, SLC6A3, SLC6A4, CYP3A4, DRD1, DRD2, OPRD1, OPRM1, HCRTR1, HTR2A,etc. It is mainly related to Calcium signaling pathway, Neuroactive ligand-receptor interaction, Cholinergic synapse, GABAergic synapse and other pathways. Based on the network pharmacology method, the active ingredients of the Compound Anshen essential oil have sedative, hypnotic, anti-anxiety and anti-depression effects, and the correlation between the active ingredients and the target and pathway. To some extent, the reliability of the study on the sedative and hypnotic effects of the Compound Anshen essential oil and the reliability of the GC-MS analysis of the chemical components were confirmed.

Special thanks to you for your good comments.

Thomas Heinbockel (Reviewer 2)
1. Response to comment: In the abstract, the results section and conclusions are duplicated. The conclusions should be conclusions and not a repeat of the results.
Response: We are very sorry for our negligence of the results section and conclusions are duplicated. At the same time, we are very sorry for our incorrect writing in conclusions. Therefore, we revised the results and conclusions in the abstract.

(e.g. Abstract section, line 26, page 2) Results: Inhalation of Compound Anshen essential Oil can significantly reduce the spontaneous activity of mice, reduce latency of sleeping time and prolong duration of sleeping time. The results of enzyme-linked immunosorbent assay showed that Compound Anshen essential oil can increase the content of 5-HT and GABA in mouse brain. The main volatile chemical constituents of the Compound Anshen essential oil are D-limonene (24.07%), Linalool (21.98%), Linalyl acetate (15.37%), α-Pinene (5.39%), and α-Santalol (4.8%).

(e.g. Abstract section, line 40 page 2) Conclusion: The study found that the inhalation of compound Anshen essential oil has sedative and hypnotic effect, which can significantly reduce autonomic nervous activity, shorten latency of sleeping time and prolong duration of sleeping time. The main chemical components of Compound Anshen essential oil contain many kinds of chemical components which have the sedative and hypnotic effect on the nerve center, anti-anxiety effect and anti-depression effect. This study provides a theoretical basis for further research and development of the sedative and hypnotic effects of Compound Anshen essential oil based on the theory of aromatherapy.

2. Response to comment: Page 5, line 13: do you mean 'compatible' or 'comparable'?
Response: The meaning here is compatible, because Compound Anshen essential oil is composed of lavender essential oil, sweet orange essential oil, sandalwood essential oil and other essential oils.

3. Response to comment: P5, l32: you should give a rationale why you observed Response: 5-HT and GABA levels in the brain rather than any other neurotransmitter.
Response: It is really true as Reviewer suggested that we should give a rationale why observed 5-HT and GABA levels in the brain rather than any other neurotransmitter. Therefore, we supplement and explain in the article the reasons for observing 5-HT and GABA levels in the brain.

(e.g. Background section, line 32 page 5) Neurotransmitter dysfunction such as 5-HT and GABA is closely related to insomnia. It has been reported that commonly used drugs for treating insomnia induce sedative and hypnotic effects by regulating neurotransmission, such as the serotonergic system in the central nervous system or GABAergic[34]. Therefore, the analysis of 5-HT and GABA content in the brain is of great significance for the detection of the effect of Compound Anshen essential oil in the treatment of insomnia[35].

4. Response to comment: P5, l49: what do you call modeling? This needs more explanation.
Response: We have made correction according to the Reviewer’s comments. We added and explained the modeling in the paper.

(e.g. Results section, line 48 page 5) Animals were weighed before the establishment of the PCPA insomnia model and at the end of the experiment, and the weight gain was calculated.

5. Response to comment: P5, l46: You do not refer to a figure for the data and you do not provide any numbers for your observations. If you do not want to include a figure reference, you need to give the data plus SEM.
Response: We list the experimental data in the "Figure Legends" module, which shows the mean and SD values. In addition, we added the statistic P value in "Results". (e.g. Figure Legends section, line 23 page 25)
We have made correction according to the Reviewer’s comments.

(e.g. Results section, line 46 page 5)
Results
Measurement results of body weight changes in mice
Animals were weighed before the establishment of the PCPA insomnia model and at the end of the experiment, and the weight gain was calculated. The effect of Compound Anshen essential oil on animal body weight was studied by weight change experiment and compared with the diazepam group. Compared with the control group, the weight gain of the model group (P=0.000) was extremely significantly reduced, the weight gain of the low-dose group (P=0.14) and high-dose group (P=0.14) was significantly increased, and the weight gain of the medium-dose group (P=0.370) was not significantly different; Compared with the model group (P=0.237), the weight gain of the other groups (P=0.000) was extremely significantly increased except for the diazepam group.

6. Response to comment: P5, l51: you need to explain the purpose of the model group and the diazepam group. Why did you include them and what do you expect to find?
Response: Considering the Reviewer’s suggestion, we have supplemented and explained the purpose of the model group and the diazepam group in the article.
The purpose of the PCPA model group: (e.g. Discussion section, line 18 page 10) In order to study the sedative and hypnotic effects of Compound Anshen essential oil inhalation in the treatment of insomnia, animals need to establish a PCPA insomnia model. The control group and the PCPA model group did not implement insomnia treatment intervention, and other groups performed drug therapy intervention. Comparing and analyzing the experimental parameters of other groups and model groups, in order to explore the pharmacodynamics of Compound Anshen essential oil inhalation for insomnia.
The purpose of the diazepam group: (e.g. Discussion section, line 18 page 10) By comparing and analyzing the pharmacodynamics of Compound Anshen essential oil and diazepam, the difference between the sedative and hypnotic effects of Compound Anshen essential oil and diazepam was explored. Therefore, the sedative and hypnotic effects of Compound Anshen essential oil on animals were studied experimentally and compared with the diazepam group.

7. Response to comment: P6, l5: again, you do not refer to a figure but just state P values. You should include the data and refer to the corresponding figure.
Response: We list the experimental data in the "Figure Legends" module, which shows the mean and SD values. In addition, we added the statistic P value in "Results". (e.g. Figure Legends section, line 23 page 25)
We have re-written this part according to the Reviewer’s suggestion (e.g. Results section, line 5 page 6)
Open field test results
From the perspective of mice movement distance, compared with the control group, the movement distance of the model group (P=0.002) was extremely significantly increased, and the movement distance of the diazepam group (P=0.039) was significantly decreased. Compared with the model group, there was no significant difference in the movement distance in the low-dose group (P=0.062), and the movement distance of the control group (P=0.002), the diazepam group (P=0.000) and the medium-dose group (P=0.000) was extremely significantly reduced, and the movement distance of the high-dose group (P=0.020) was significantly increased. From the perspective of average velocity of mice, the average velocity of the model group (P=0.002) increased extremely significantly, and the average velocity of the diazepam group diazepam group (P=0.039) decreased significantly. Compared with the model group, there was no significant difference in the average velocity of the low-dose group (P=0.062), the average velocity of the control group (P=0.002) decreased extremely significantly, the average velocity of the diazepam group (P=0.000) and the medium-dose group (P=0.000) decreased extremely significantly, and the average velocity of the high-dose group (P=0.021) decreased extremely
significantly. From the point of view of the maximum velocity of mice, compared with the control group, the maximum velocity of the model group (P=0.000) was extremely significantly increased, and there was no significant difference in the other groups (P>0.05). Compared with the model group, the maximum velocity of the control group (P=0.000), the diazepam group (P=0.000) and the medium-dose group (P=0.002) was extremely decreased extremely significantly, and the maximum velocity of the low-dose group (P=0.017) and the high-dose group (P=0.015) was decreased significantly. From the resting time of the mice, compared with the control group, the model group (P=0.004) had extremely significant difference, which showed an increase in resting time, and there was no significant difference in other groups (P>0.05); Compared with the model group, the other groups had extremely significant differences (P<0.05); Compared with the model group, the other groups had extremely significant differences (P<0.05); Compared with the model group, the other groups had extremely significant differences (P<0.05).

Pentobarbital-Induced Sleeping

From the perspective of latency of sleeping time, compared with the control group, the latency of sleeping time of the diazepam group (P=0.000) and the medium-dose group (P=0.000) was extremely significantly shortened, and the latency of sleeping time of the model group (P=0.000) was extremely significantly increased. Compared with the control group, the latency of sleeping time of the model group (P=0.000) was extremely significantly increased. Compared with the model group, the latency of sleeping time of the other groups was significantly different (P<0.01), which was manifested as decreased latency of sleeping time. From the perspective of duration of sleeping time, compared with the control group, the sleep time of the diazepam group (P=0.000), the low-dose group (P=0.000), the medium-dose group (P=0.000), and the high-dose group (P=0.001) was extremely significantly increased, and the sleep time of the model group (P=0.027) was significantly reduced. Compared with the model group, the duration of sleeping time of the other groups was significantly different (P<0.01), which was manifested as increased duration of sleeping time.

Analysis of Brain Neurotransmitters

From the perspective of the concentration of 5-HT in the brain in mice, compared with the control group, the 5-HT concentration in the model group (P=0.001) was extremely significantly reduced, the 5-HT concentration in the low-dose group (P=0.031) and the high-dose group (P=0.012) was significantly increased ; Compared with the model group, the 5-HT concentration in the diazepam group (P=0.002), the medium-dose group (P=0.000), and the high-dose group (P=0.000) was extremely significantly increased, and the 5-HT concentration of the low-dose (P=0.193) group was not significantly different. From the perspective of the concentration of GABA in the brain in mice, compared with the control group, the GABA concentration in the model group (P=0.000), the diazepam group (P=0.000) and the low-dose group (P=0.000) was extremely significantly reduced, while there was no significant difference in the medium-dose group (P=0.434) and the high-dose group (P=0.114). Compared with the model group, except the low-dose group (P=0.614), all the other groups showed significant differences (P<0.01), which showed that the concentration of GABA in the brain increased.
8. Response to comment: P7, l38: I find the description in the text confusing. The authors should state that the aromatherapy had no significant effect on pentobarbital-induced sleeping. 
Response: Considering the Reviewer’s suggestion, we have revised the title of the experiment and added explanations to the results.
(e.g. Results section, line 38 page 7) The title has been changed to “Pentobarbital-Induced Sleeping”. The study found that the Compound Anshen essential oil can significantly shorten latency of sleeping time and prolong duration of sleeping time.

9. Response to comment: P8, l16: the authors need to better justify the reason for testing neurotransmitter levels. They also need to justify why 5-HT and GABA. Finally, I do not believe that there is a significant difference in neurotransmitter levels and their control animals.
Response: We have re-written this part according to the Reviewer’s suggestion (e.g. Discussion section, line 54 page 10) Dysfunction of 5-HT, GABA and other neurotransmitters is closely related to insomnia. It has been reported that therapeutic drugs induce sedation and hypnosis by regulating neurotransmission, such as the 5-HTergic system or GABAergic system in the central nervous system [34]. Therefore, the analysis of 5-HT and GABA levels in the brain is of great significance for the detection of drugs for the treatment of insomnia [35]. Serotonin (5-HT) plays a key role in sleep-wake regulation [36]. Serotonin is thought to be a sleep neurotransmitter that produces sleep by inhibiting the midbrain's reticular activating system or the norepinephrine component of the blue spot [37]. Conversely, PCPA, a tryptophan hydroxylase inhibitor, consumes 5-HT, leading to insomnia. After 24-36 hours, PCPA had a strong inhibitory effect on 5-HT synthesis [38]. GABA is a major inhibitory neurotransmitter in the central nervous system (CNS). GABA receptor system plays a major inhibitory role in the brain and plays a crucial role in regulating the overall balance between neuron excitation and inhibition [39]. Neurons in the front of the hypothalamus release GABA, which inhibits areas of the hypothalamus and brainstem that promote wakefulness [40]. Dysfunction or deficits in the GABAergic system are associated with epilepsy, pain and anxiety [41]. The GABAergic neurotransmitter plays a key role in sleep regulation. The BZD binding site on GABAA receptor is the target of most sedative and hypnotic drugs [42]. Barbiturates, such as pentobarbital, act on ion group complexes of GABA receptors, which are favorable for GABA binding. Benzodiazepines, such as diazepam, increase the affinity of GABA to its receptors, thereby increasing the duration of pentobarbital induced sleep [43]. It has been suggested that essential oil components play a sleep promoting role by regulating the 5-HTergic system or GABAergic system [44]. Therefore, the effect of Compound Anshen essential oil on the levels of 5-HT and GABA in the brain is of great significance for evaluating the pharmacodynamics of Compound Anshen essential oil for the treatment of insomnia. In addition to the above reasons, this experiment uses the PCPA insomnia model, because PCPA is a tryptophan hydroxylase inhibitor, consumes 5-HT, leading to insomnia, detection of brain 5-HT levels can reflect the successful establishment of insomnia model. In this study, the levels of 5-HT and GABA in the brain were measured by enzyme-linked immunosorbent assay. The results showed that the low-dose, middle-dose and high-dose groups of the Compound Anshen oil showed different degrees of 5-HT up-regulation and GABA up-regulation in a dose-dependent manner. The levels of 5-HT and GABA in the middle-dose and high-dose groups were similar to those in the control group and higher than those in the diazepam group. Therefore, the inhalation administration of the Compound Anshen essential oil can significantly increase the levels of 5-HT and GABA in the brain. From the experiment of weight change and the analysis of brain neurotransmitter level, the effect of Compound Anshen essential oil inhalation on insomnia is better than that of diazepam. It can be seen that inhalation of Compound Anshen essential oil may be a safe and effective treatment for or adjuvant treatment of insomnia, which may reduce the excessive use of prescription drugs such as diazepam and reduce the risk of short-term or long-term effects on sleep health. Its deeper sedative and hypnotic mechanism
10. Response to comment: P9, l1: It is not clear how the GC-MS data help with the interpretation of the behavioral data. What is the reader supposed to learn from knowing the quality and quantities of the various essential oils in CAE?
Response: We have made correction according to the Reviewer’s comments. 
Due to the errors described in the previous paper’s conclusions, based on the revision of the conclusions of the paper, the authors discuss the GC-MS analysis of the Compound Anshen essential oil and the network pharmacology analysis results of Dr. Ren.
(e.g. Discussion section, line 57 page 11) On this basis, the composition of Compound Anshen essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 30 chemical constituents were identified, accounting for 93.39% of total volatile oil. The main components of Compound Anshen essential oil are esters, alcohols, alkenes, alkyls and other compounds, with the highest content of D-limonene (24.07%), Linalool (21.98%), Linalyl acetate (15.37%), α-Pinene (5.39%) and α-Santalol (4.8%). D-limonene [45], Linalool [46], Linalyl acetate [47] and α-Santalol [48] have been reported to have central nervous sedative effect. In addition, D-limonene has anti-anxiety and soothing effects [49], Linalool has anti-anxiety and depression-like effects [50] and α-Pinene has anti-anxiety [51] and stress-relieving effects [52]. It is speculated that the sedative and hypnotic effect of Compound Anshen essential oil is related to its main chemical composition and activity. Based on the network pharmacology, the active components, targets and pathways of the Compound Anshen essential oil were predicted. The results of the network pharmacology analysis were published in the paper of the laboratory peer Dr. Ren [53]. Among the volatile chemical constituents of the compound anshen essential oil traced by GC-MS, the active ingredients related to insomnia were screened and the relevant targets and pathways were predicted. The study found that Dibutyl phthalate, Caryophyllene, Geranyl acetate, Linalool, α-Terpineol, and Terpinen-4-ol played a key role in the treatment of insomnia in the Compound Anshen essential oil. The chemical components with the highest content tracked by GC-MS were D-Limonene, Linalyl acetate, α-Pinene, which were also correlated with treatment of insomnia. It is predicted that the compound anshen essential oil mainly exerts pharmacodynamic effects through related target proteins such as ESR1, GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, NR1H4, CHRM1, SLC6A2, SLC6A3, SLC6A4, CYP3A4, DRD1, DRD2, OPRD1, OPRM1, HCRTR1, HTR2A, etc. It is mainly related to Calcium signaling pathway, Neuroactive ligand-receptor interaction, Cholinergic synapse, GABAergic synapse and other pathways. Based on the network pharmacology method, the active ingredients of the Compound Anshen essential oil have sedative, hypnotic, anti-anxiety and anti-depression effects, and the correlation between the active ingredients and the target and pathway. To some extent, the reliability of the study on the sedative and hypnotic effects of the Compound Anshen essential oil and the reliability of the GC-MS analysis of the chemical components were confirmed.

Special thanks to you for your good comments.

Other changes: We have studied reviewer’s comments carefully and have made revision which marked in red in the paper.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper.

We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval.
Once again, thank you very much for your comments and suggestions.

Thank you and best regards!

Yours sincerely,
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