Original Research Article

Revisiting the Rewarding Properties and Abuse Potential of Natural Compound Sinomenine Using Conditioned Place Preference (CPP) Model in Mice

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ABSTRACT

Sinomenine, derived from *Sinomenium acutum*, has a chemical structure similar to morphine and shows potential for treating opioid addiction in mice. Despite claims of being non-addictive, its safety and abuse potential remain underexplored. Conditioned place preference (CPP) approach was used to evaluate sinomenine's rewarding properties. ICR mice were divided into five groups, receiving either saline, morphine (8 mg/kg), or sinomenine (50, 100, or 200 mg/kg) where each group was confined to the drug-paired compartments during conditioning phase. Alternately, mice received saline when confined in the saline-paired compartment. During pre-conditioning and post-conditioning phases, the time spent in the drug-paired compartment, the number of entries to the drug-paired compartment, and rearing behaviours to assess the rewarding properties. Mice conditioned with morphine showed a significant increase in time spent in the drug-paired compartment from pre-conditioning to post-conditioning, but no significant changes in the number of entries and rearing behaviours. For saline and all sinomenine groups, there were no significant differences in the time spent, number of entries, and rearing behaviours except for sinomenine 200 mg/kg group, which showed a significant increase in rearing behaviours indicating an increase in exploratory behaviours. This study suggests that sinomenine, at various doses, is less likely to produce rewarding effects, indicating low abuse potential. The observed increase in exploratory behaviours during post-conditioning with sinomenine 200 mg/kg warrants further investigation.

Keywords: sinomenine, abuse potential, conditioned place preference, drug addiction

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1.0 Introduction

Chinese herbal medicines have been shown to be useful and effective in treating various types of diseases (1). They contain natural active compounds as the principal components that exert the therapeutic properties. Until recently, the rising number of advancements in preclinical and clinical research on Chinese herbal medicines and their neurochemical action have shown their effectiveness in the treatment of the relapse of addiction and withdrawal syndromes (2). Some Chinese herbal medicines that showed promising beneficial effects in treating drug addiction include *ginseng* (3), *Corydalis* (4), *Salvia miltiorrhiza* (5), *Radix Pueraiae* (6), *Caulis sinomenii* (7) and *Stephania intermedia* (8). These traditional Chinese herbal medicines may be used as a complementary to the existing treatments for drug addiction, including relapse and withdrawal (2).

Sinomenine is one of the natural compounds from Chinese herbal medicines which showed potential in the treatment of drug addiction (7, 9). It is the main active ingredient of *Sinomenium acutum* or also can be known as Fang-ji or Qing-teng. The chemical structure of sinomenine is closely related to morphine but was reported to produce no addictive properties (10, 11). Sinomenine has phenanthrene nucleus and ethylamine bridge which is highly similar to morphine where drugs or compounds that have highly similar chemical structures usually bind to the same receptors and produce the same effects (12). These high structural similarities are likely responsible for sinomenine's ability to bind and activate opioid receptor particularly μ-opioid receptor (MOR) (13, 14). The interaction between sinomenine and MOR could be the mechanism where sinomenine can attenuate opioid addiction (9). Apart from that particular interaction, some studies also reported that the effects could be attributed to the interaction with other targets such as tyrosine hydroxylase (TH) or N-methyl-d-aspartate (NMDA) receptor (15). In terms of the pharmacokinetic properties, following intravenous administration in animals, sinomenine was detectable up to 6 hours after dosing, with a half-life of 4.48 hours and a volume of distribution of 35.94 L/kg (16).

Since sinomenine could interact with MOR, it's worth noting that one of the most frequent adverse effects of MOR agonists is the potential for abuse and addiction (17). MOR agonist drugs are not only misused but also result in significant illness and fatality from overdose (18). To evaluate potential abuse liability, Conditioned place preference (CPP) method can be used (19). CPP is a method to measure the rewarding and aversive effects of a drug where the procedure relies on the association between the effects of a drug and environmental cues. The amount of time the animal spends in the drug-paired compartment during the post-conditioning phase, compared to its initial time in that compartment during the pre-conditioning phase, indicates the rewarding (or aversive) effects of the drug. A significant increase in time indicates that the drug has reinforcing properties, making it likely to induce a "rewarding" experience. A more comprehensive examination of the reinforcing effects related to drug abuse can be conducted through drug self-administration procedures (20). There is a strong correlation between drugs that induce place preferences and those that are self-administered by rodents and non-human primates, which are also commonly abused by humans.

It was reported that sinomenine did not cause psychological or physical addiction or dependence in mice and rats, suggested that it can be further developed as anti-addictive agents. However, previous studies lack comprehensive data on the abuse potential of

sinomenine. For example, two studies investigated the development of physical dependence in mice based on the behavioural symptoms but only low doses of sinomenine were evaluated (below 60 mg/kg) (10, 11). Another study that utilized CPP produced the same result but the doses used in the experiment was not clearly stated (21). These previous studies came into conclusions that sinomenine will not produce addiction and less likely to be abused but further study is necessary to evaluate the abuse potential and possible rewarding effects of sinomenine at much higher doses.

Therefore, this study was conducted to evaluate sinomenine abuse potential at three different doses (50 mg/kg, 100 mg/kg and 200 mg/kg). CPP method was used where the time spent of the mice in the drug-paired compartment were calculated and analyzed. Additionally, other parameters such as the number of mice entries to the drug-paired compartment and also rearing behaviours were analyzed as well.

2.0 Materials and methods

2.1 Animals

Thirty-five male ICR mice (30-40 gram) were obtained from the Laboratory Animal Facility and Management (LAFAM), Universiti Teknologi MARA (UiTM) Puncak Alam Campus. The ICR mice used for this experiment were 9 to 12 weeks old. Before the experiment was carried out, the mice were habituated to the laboratory environment with temperature of 20±2ºC, humidity of 55±5% and 12-hour dark/light cycle, in which the lights were switched on from 7:00 AM to 7:00 PM for a week. All mice had free access to water and food. Animal care procedures were strictly followed based on the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the ethics application was approved by the UiTM Ethics Committee. The ethical approval number is UiTM CARE: 407/2023.

2.2 Drugs and reagents

Sinomenine hydrochloride (No. S487500, purity 98%) was purchased from Toronto Research Chemical. Morphine sulphate 10 mg/ml injection drug was purchased from Pharmaniaga Sdn Bhd. All drugs were dissolved in normal saline.

2.3 CPP apparatus

The CPP apparatus is a box consist of two equally sized compartments (30cm x 30cm x 30cm), namely compartment A and compartment B and also a small middle compartment (10cm x 10cm x 10cm) namely compartment C located in between. In order to differentiate between the two larger compartments, some visual and tactile clues were used. Compartment A has dotted wall with smooth floor, meanwhile compartment B has vertical striped wall and equipped with rough floor. The compartments were joined together with a guillotine-style doors which were manually operated by the experimenter. The illustration of CPP box is shown in Figure 1. All compartments received the same lighting condition throughout the experiment. A camera was mounted on top of the CPP box to record the movement of the mice during pre-conditioning and post-conditioning phase. The experiment was carried out in a location with minimum noise level to prevent any bias or external factors affecting the experiment. The mice were randomly divided into five groups. Morphine and saline group were used as control groups in this experiment. The mice were weighed and the dose for drugs were calculated according to mice weight before starting the CPP procedure.

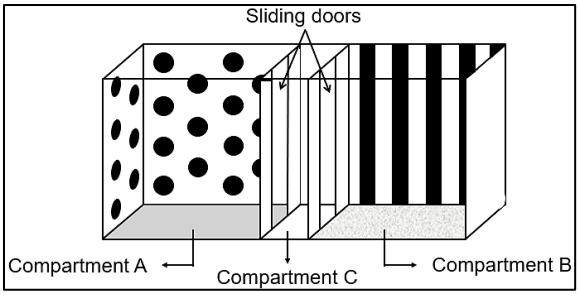

Figure 1: The illustration of CPP box used for this experiment

In the initial pre-conditioning phase (day 1-3), the mice were allowed to have free access to all compartments for 15 minutes. On the day 3 of pre-conditioning phase, the time spent (in seconds) of the mice in compartment A and B were calculated as well as the number of entries to the drug-paired compartment and the number of rearing behaviours. The mice that spent more than 75% of their total time in any compartment during pre-conditioning test was considered to develop initial bias and was excluded from the experiment. In this experiment, the drug-paired compartment for the conditioning phase was chosen based on the least preferred compartments during pre-conditioning phase.

For the following conditioning phase (day 4-9), the sliding doors were placed to separate the compartments. In this phase, mice were confined only to a specific compartment either saline-paired compartment or drug-paired compartment. The mice received intraperitoneal injection of either normal saline or drugs such as morphine 8 mg/kg or sinomenine (50, 100 and 200 mg/kg) and immediately being confined to saline-paired or drug-paired compartment for 1 hour. The administration of saline and drugs were carried out alternately for 6 days, except for saline group as the mice in this group received saline every day. In this study, morphine at dose of 8 mg/kg was chosen to induce place preference in the control group. This particular dose was chosen based on the previous studies that showed morphine from 5 mg/kg to 10 mg/kg could induce place preference in mice and rats (22-25). For sinomenine, the doses of 50, 100 and 200 mg/kg were chosen by considering the effective and median lethal dose (LD_{50}) of sinomenine reported from the previous works (9, 26). Since sinomenine 80 mg/kg has been showed to be effective in attenuating morphine's addictive effects while sinomenine 453.54 mg/kg was determined to be the LD_{50} for sinomenine (which could result in the death of the mice), this study has chosen these three different doses representing low, moderate and high doses which are close to the effective dose but lower than the LD_{50} so that the dose-effect relationships can be investigated as well.

Finally, for the post-conditioning phase, the mice were allowed to have free access to all compartments for 15 minutes without any administration of drugs or normal saline. The time spent of the mice in each compartment were calculated as well as the number of entries into the drug-paired compartment and the number of rearing behaviours. All experimental procedures were conducted between 8:00 AM and 6:00 PM to minimize any interferences with the sleep-wake cycle of the mice and all compartments were cleaned thoroughly with 70% alcohol before each session. After the completion of all procedures, all mice were sacrificed using carbon dioxide gas euthanasia method. The summary of CPP procedure is illustrated in Figure 2.

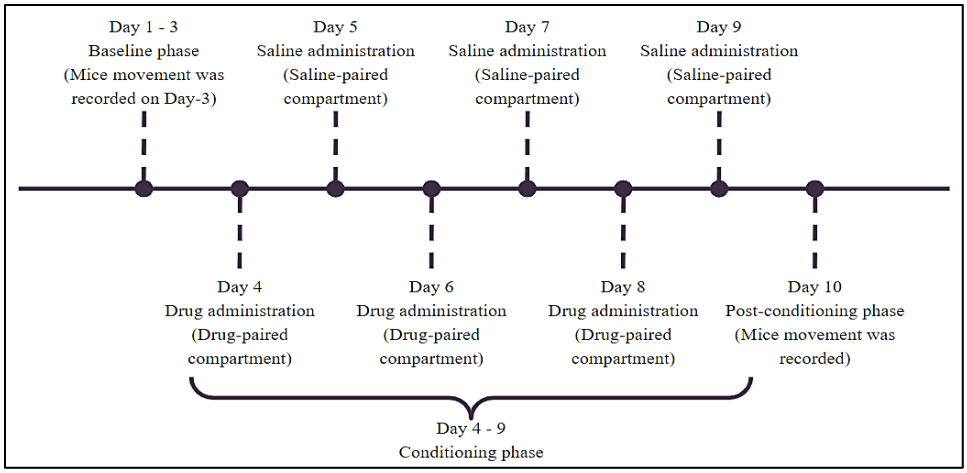

Figure 2: The summary of CPP procedure

2.4 Statistical analysis

The time spent of the mice in the drug-paired compartment, the number of entries into the drug-paired compartment and the number of rearing behaviours during pre-conditioning and post-conditioning phases were compared using either paired t-test or Wilcoxon signed-ranked test following the normality test (Shapiro–Wilk test). All data were expressed as mean and standard error of the mean (± SEM). The differences were considered significant when the p-value is below than 0.05 ($p < 0.05$). The GraphPad Prism version 9.0.0 (GraphPad Software, Inc., San Diego, California, U.S.A.) was used to analyze all data obtained from this experiment.

3.0 Results

3.1 Time spent in the drug-paired compartment during pre-conditioning and post-conditioning phases

There is a significant increase in the time spent in drug-paired compartment from pre-conditioning test to post-conditioning phase for morphine group (control) receiving morphine 8 mg/kg indicating successful place preference (paired t-test, $p = 0.024$; Figure 3). Another control group (saline) showed no significant difference between the time spent in the drug-paired compartment from pre-conditioning to post-conditioning (paired t-test, $p = 0.462$). All sinomenine groups showed no significant difference when compared between the time spent in drug-paired compartment from pre-conditioning to post-conditioning phases (paired t-test: $p = 0.480$ for sinomenine 50mg/kg, $p > 0.999$ for sinomenine 100mg/kg, $p = 0.267$ for sinomenine 200mg/kg). Sinomenine produced neither CPP nor conditioned place aversion at doses of 50 mg/kg, 100 mg/kg or 200 mg/kg.

3.2 Number of entries into the drug-paired compartment during pre-conditioning and post-conditioning phases

Number of mice entries to the drug-paired compartment during pre-conditioning and post-conditioning phase were compared for each group (Figure 4). All groups of mice showed no significant difference in the number of entries to the drug-paired compartment between pre-conditioning and post-conditioning phases (paired t-test: $p = 0.631$ for saline, $p = 0.219$ for morphine, $p = 0.552$ for sinomenine 50mg/kg, $p = 0.792$ for sinomenine 200mg/kg; Wilcoxon signed-ranked test: $p = 0.635$ for sinomenine 100mg/kg).

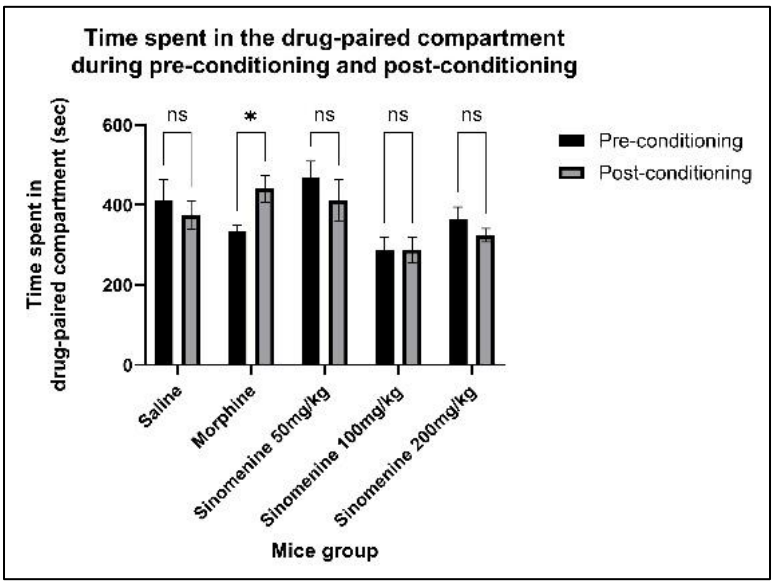

Figure 3: The time spent of the mice in drug-paired compartment at pre-conditioning and post-conditioning phases (n = 5-6 for each group). "ns" indicates not significant ($p > 0.05$), whereas the asterisk indicates significant difference ($p < 0.05$) between pre-conditioning and post-conditioning groups (paired t-test).

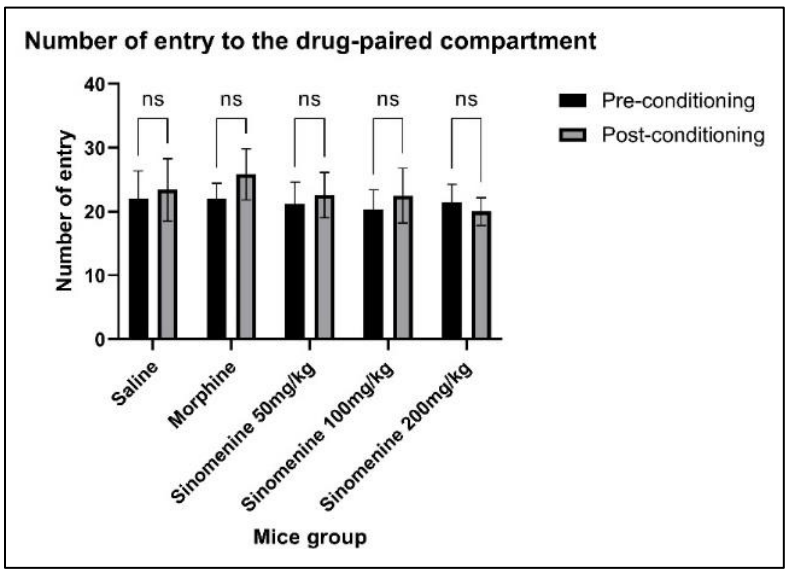

Figure 4: Number of mice entries to the drug paired compartment during pre-conditioning and post-conditioning phases (n = 5-6 for each group). "ns" indicates not significant ($p > 0.05$) between pre-conditioning and post-conditioning groups (paired t-test or Wilcoxon signed-ranked test).

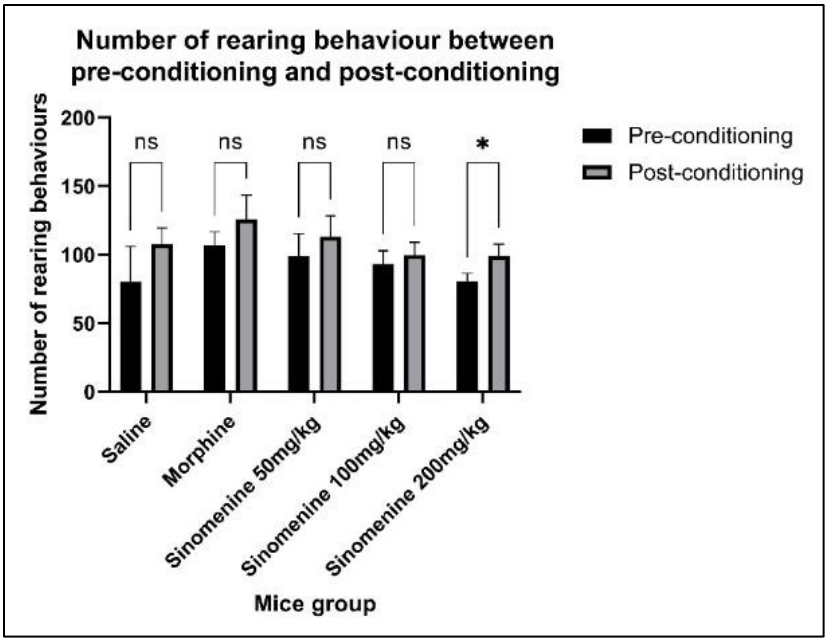

Figure 5: Number of rearing behaviours between pre-conditioning and post-conditioning phase (n = 5-6 for each group). ''ns'' indicates not significant ($p > 0.05$), whereas the asterisk indicates significant difference ($p < 0.05$) between pre-conditioning and post-conditioning groups (paired t-test).

3.3 Number of rearing behaviours

The number of rearing behaviours of the mice for each group during pre-conditioning and post-conditioning phases were compared (Figure 5). Sinomenine 200 mg/kg group is the only group that showed a significant increase in the number of rearing behaviours between pre-conditioning and post-conditioning (paired t-test, $p = 0.027$). Meanwhile, there are no significant differences in number of rearing behaviours from pre-conditioning to post-conditioning for saline group, morphine group, sinomenine 50 mg/kg group and sinomenine 100 mg/kg group (paired t-test: $p = 0.212$ for saline, $p = 0.174$ for morphine, $p = 0.363$ for sinomenine 50mg/kg, $p = 0.555$ for sinomenine 100mg/kg).

4.0 Discussion

In this study, the rewarding and abuse potential of sinomenine at three different doses were studied and compared to morphine 8 mg/kg using CPP experimental approach following the conditioning session of the mice with either saline, morphine or sinomenine at specific compartment namely drug-paired compartment. Generally, addictive drugs will increase the time spent of the mice in the drug-paired compartment during post-conditioning session indicating an increase in preferences for that compartment due to the rewarding effects of the drugs (19). Despite other studies have investigated and confirmed the non-addictive property of sinomenine pointing to its safety to be further developed as treatments for various diseases, these studies did not determine the possibility of the rewarding effects at much higher doses (10, 11). Therefore, in this study, not only the time spent of the mice in the CPP box compartments were observed but also the number of entries into the compartments as well as the number of rearing behaviours. Based on our analysis, there are a few

interesting findings that can be interpreted from our data and results.

Firstly, it was found that when the time spent of the mice in the drug-paired compartment at pre-conditioning and post-conditioning phases were compared, there are no significant differences for all sinomenine groups (50 mg/kg, 100 mg/kg and 200 mg/kg). This indicates that sinomenine at the studied doses (from low to high) did not induce any place preference or place aversion in mice. Although the structure of sinomenine is closely related to morphine and sinomenine was reported to interact with opioid receptor, our study showed that sinomenine did not produce any rewarding effects (13, 14). These findings are consistent with the previous studies that showed sinomenine did not cause any physical or psychological dependence in mice and rats (10, 11, 21). On another note, it can be observed that sinomenine did not produce aversive effects in mice as well where the aversive effect can be observed if the time spent in the drug-paired compartment were significantly reduced. From the experiment, morphine is the only drug that significantly increased the time spent of the mice from pre-conditioning to post-conditioning since morphine is widely known as an addictive drug. In addition to that, the number of entries to drug-paired compartment during pre-conditioning and post-conditioning were assessed as well since the number of entries could reflect the reinforcing conditioned responses in the CPP experiments, despite this parameter was not widely used (27). For example, increased number of entries has been previously associated with the reward memory and a phenomenon known as the incubation of drug craving (28, 29). However, our findings showed that the number of entries might not be a reliable parameter to compare the rewarding effects between morphine and sinomenine since there were no significant changes in the number of entries to the drug-paired compartment from pre-conditioning to post-conditioning phase for all mice groups. We interpreted that compared to the number of entries to the drug-paired compartment, the time spent might provide a better indicator to evaluate the rewarding effects of the drug in this specific study.

Secondly, the lack of rewarding effects shown in our studies despite the high dose of sinomenine (200 mg/kg) given to the mice indicates that sinomenine might be involved in more complex pathways and receptors interactions not limited to opioid receptor only, unlike morphine. Initially, we assumed that sinomenine did not induce rewarding effects based on the previous studies was due to the low doses of sinomenine given to the mice (10, 11). Therefore, we hypothesized that at much higher doses, the interactions between sinomenine and the opioid receptors especially MOR will be more significant to cause rewarding effects and increase the time spent in the drug-paired compartment. However, the findings from our study showed that most likely the interactions between sinomenine and opioid receptors might be a minor interaction compared to the other interaction. With regards to the neuroprotective effects of sinomenine, studies have shown that sinomenine could inhibit oxidative stress, neuroinflammation and neuronal apoptosis via multiple mechanisms (30). Not only that, sinomenine can interfere in pathways related to addiction or dependence by modulating NMDA receptor and γ-aminobutyric acid type A ($GABA_A$). The interactions between sinomenine and these targets might be more dominant causing sinomenine to lack any rewarding effects. Not only that, these targets might be playing an important role in countering the development of addiction in the mice brain.

Thirdly, it was found that sinomenine at high dose (200 mg/kg) might affect the

exploratory activities of the mice based on the increase in number of rearing behaviours during post-conditioning phase. Rearing behaviours in mice can be defined as episodes of standing or using the wall to support themselves to stand erect which could be associated with their exploratory activities and also mental conditions (31, 32). For example, previously, it was suggested that reduction in the rearing behaviours could indicate that the animal is experiencing generalized anxiety and depressive symptoms associated with biochemical changes in the brain (31). In our study, sinomenine 200 mg/kg group is the only group that showed a significant increase in the number of rearing behaviours when compared between pre-conditioning and post-conditioning phases where the notable increase in the number of rearing behaviours in this group could be interpreted as elevated mice exploratory activities to investigate their environment and other stimulus after being treated with a particular drug. It is important to note also that in our study, sinomenine 200 mg/kg was given during conditioning but the increase in rearing behaviours were observed during post-conditioning. Given that previous studies have indicated that sinomenine reduces locomotor activity in mice, we hypothesized that the increased rearing behaviours observed in our study might be attributed to withdrawal effects following the suppression of locomotor activity caused by sinomenine during the conditioning phase (33, 34). This finding could be linked to the modulation of the gamma-aminobutyric acidergic (GABAergic) pathway (33). This particular finding was only evident for sinomenine 200 mg/kg group indicating that neither saline, morphine nor other sinomenine produce the same effects.

5.0 Conclusion

In conclusion, sinomenine at different doses did not produce rewarding effects thus having no abuse potential. This finding has important implications in the development of sinomenine as a therapeutic agent in the treatment of drug addiction. On another note, at higher dose, sinomenine might heighten exploratory activity which suggest that the absence of the drug triggered a behavioural response that requires further investigations. Future studies to determine any biochemical changes in the mice brain such as neurotransmitter levels and gene expressions are necessary to further understand the effects of sinomenine.

Authorship contribution statement

NSM: Carried out the experiments, performed the analysis and drafted the manuscript; **AAA & HSJC**: Participated in the design and served as principal investigators throughout its execution and coordination of the study and helped to revise the manuscript; **MHZ**: Supervision, funding acquisition, writing – review & editing.

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Conflict of Interest

The authors declared that they have no conflicts of interest to disclose.

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