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ORIGINAL ARTICLE

Effect of hydro-alcoholic extract of Melissa Officinal is (Lemon balm) on morphine state dependent learning in mice

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Abstract: Backgrounds and Objectives: Melissa officinalis has a variety of effects, including sedation and antioxidant .M. Officinalis has also terpenoides that is useful in memory and learning. Opioid agonists have destructive effects on memory andthese drugs are injected pre- training decrease memory. Moreover morphine can effect on memory and learning by opioid receptors of hippocampus opioidergic system. The present study was conducted the effects of M.Officinalis extract on SDL in mice. Materials and methods: 66 male mice were selected and allocated to 11 groups (n=6).To study memory in mice using passive avoidance method to measure step-down latency. Results: The injection of pre- training of morphine (3mg/kg) Cause memory impairment But injection of pre–testing of morphine(3mg/kg) can improve memory. The obtained results showed that morphine was caused state dependent learning. injection of pre- training of M.Officinalis extract 25 mg/kg and pre – testing of saline intraperitoneally can increase memory. injection of pre- training of morphine (3mg/kg) and pre – testing of M.Officinalis extract 25 mg/kg can decrease memory. Conclusion: Interaction effect of morphine and Varangboo decrease memory.

Key words: Melissaofficinalis extract (Varangboo), steady state- dependent learning, morphine, mice

Introduction

Melissa officinalis is one of the eldest and most commonly used medicinal plants which belongs to the family lamiaceae. Man y studies have been performed which confirm the various benefits of this plant (1 & 2). Analysing the effect of M.Officinalis on the nervous system revealed that this plant relieves the symptoms of nervous complications such as stress, anxiety and irritability (3). In addition, M.Officinalis protects neurons and eliminates free reactive agents (4). It has been proven that the ethanolic hydro-alcoholic extract derived from M.Officinalis has anti-choline-esterase properties in human brain tissue (5).

Opioids and their antagonists are able to affect the memory and learning ability of laboratory animals (6). Studies show that treating subjects with beta- endorphins before training them, disrupts their memory (7 & 8). In other words, the memory of the subjects only improves if they are treated with a similar dose of opioids 24 hours prior to their training. This state, in which the subjects are capable of retaining new information only under certain physiologic and emotional background, is referred to as State Dependent Learning (SDL) (9). Same results were obtained in mice after admission of relevant doses of morphine (5-10 mg).

Materials and methods

Animals

This experimental study used Syrian male mice of the NMRI species, with a weight range of 20-30 grams, obtained from Pasteur institute, Karaj, as subjects. The subjects were transferred to the laboratory animal house of Qom branch of Islamic Azad University and stored under standard water, food, temperature and moisture conditions. Every subject was used only once and was removed after examination.

Apparatus

The inhibitory avoidance learning system (passive), step down model, is a wooden box with the following dimensions: 30*30*40 centimetres. The bottom of the machine is surfaced with 29 steel rods with a diameter of 0.3 cm, with a gap of 1 cm separating the rods. A wooden cubic seen (4*4*4 cm) has been provided in the centre of the rods. These rods are attached to the pulse inducer mechanism, which sends electric shocks via these rods to the subjects placed within this box. When activated, a direct current of electricity with a frequency of 1Hz and 50 mv flows for a duration of 0.5 seconds. The testing room should be relatively dark and soundproof.

Drugs

In this study, morphine (opioid receptor agonist) and Melissa officinalis (varangboo) extract were used.

Subjects were divided to eleven groups, each made up of 6 mice. Morphine and varangboo were both administratedvia intra-peritoneal injection.

Morphine was administrated in doses of 1 & 3 mg/kg and varangboo was injected with a dosage of 25 mg/kg. All procedures were performed during day time.

Drugs were administrated 30 minutes pre-training and also 30 minutes before running tests. As mentioned, administration of the drugs was performed via intra peritoneal injection.

Plant extraction procedure

Melissa officinalis(varangboo) was obtained from zardband medicinal herbs company, Gilan, and identified and confirmed by the systematic researcher of the university. The plant was gathered in its blooming season, the month of July. 100 grams of ground M.Officinalis was added to 2 litres of 75% ethanol and placed on a shaker for a duration of 5 hours. This mixture was placed in a dark location with a temperature of 42± 3 degrees Celsius for 72 hours. Subsequent to this, the mixture was passed thrice through a filter paper, poured into plates in small quantities and placed in an oven with a temperature of 38 degrees Celsius in order to vaporize the solvent. Eventually, the resulting powder obtained from M.Officinalis was transferred to a dark container and stored in a refrigerator for conservation. Doses of 25 mg/ kg of M.Officinalis were prepared by mixing the aforementioned powder with saline.

Training

In training days, subjects were placed with considerable care on the platform of the memory-testing machine. The duration which they remained on this platform was recorded. The machine would be activated for a duration of 15 minutes, as a result of the mouse leaving the platform and all four limbs coming to contact with the steel rods, sending electric pulses to their hands and feet. The mice would be removed and stored in their cage after every experiment.

Testing

In order to evaluate the effect of the drugs, the testing session was performed 24 hours subsequent to the training session. Training and testing sessions have similar execution methods, with the sole difference being the lack of electric shock in the testing session.

The difference between the duration in which the subjects would leave the platform, also known as the step down latency, was used as a parameter in memory evaluation. At the exact time which the subject's stepped down from the platform and all four limbs were placed on the steel rods covering the bottom of the cage, the chronometer would be stopped and the time recorded. The maximum allowance which the subjects could stay on the platform was determined as 180 seconds.

Drug treatment

Four groups received saline 30 minutes pre-training and saline, varangboo extract and morphine 30 minutes pre- testing. 5 groups received morphine 30 minutes pre-training and saline, varangboo and morphine 30 minutes pre- testing. 2 groups received varangboo extract 30 minutes pre- testing and saline and morphine 30 minutes pre-testing. The average duration which the subjects would remain on the wooden platform was recorded. It is necessary to mention that the control group received saline prior to both training and testing sessions.

Data analysis

In order to process the gathered data, SPSS software was used. Data processing was performed using ANOVA analysis, paired samples test with a P value lesser than determined as meaningful. The charts were drawn using Microsoft Excel and average charts were shown based on Mean±SEM.

Results

The effect of pre-training and pre-testing morphine administration and based on SDL duration:

Subjects who had received morphine (3 mg/kg) prior to both training and testing session, showed weaker memory compared to that of the control group (saline + saline) ($P<0.01$). The memory of those who had received saline pre- training and morphine pre-testing was significantly stronger than the control group ($P<0.05$). Those who had received morphine pre- training and pre- testing, had a weaker memory when compared to the control group ($P<0.05$) (chart 1).

The effect of varangboo hydro-alcoholic extract pre-training administration on SDL duration in subjects being treated with morphine:

Subjects who had received varangboo (25 mg/kg) pre- training and testing had increases memory when compared to the control group (saline + saline) ($P<0.05$). Pre-training administration of saline and pre-test morphine administration (3mg/kg) improved the memory of the subjects compared to saline administration prior to both sessions ($P<0.05$). subjects who had received varangboo extract (25 mg/kg) pre- training and morphine (3mg/kg) pre- testing, showed a significant weakening of memory when compared to the control group ($P<0.05$). Saline administration pre- training and pre-test morphine administration (3 mg/kg), improved the memory of the subjects compared to those who had received varangboo (25 mg/kg) extract pre-training and morphine (3 mg/kg) pre-test ($P<0.01$) (chart 2).

The effect of pre-test varangboo hydro-alcoholic extract administration on SDL duration in subjects being treated with morphine:

Subjects who had received morphine (3 mg/kg) pre- training and testing had weaker memory when compared to the control group (saline + saline)

The effect of pre-training and pre-test morphine administration on SDL duration

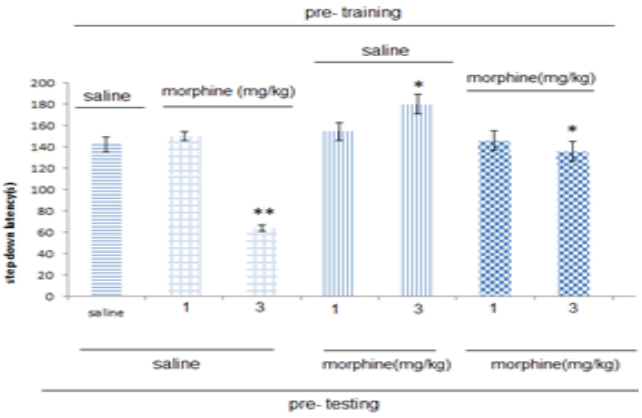

Chart 1; comparing the effect of pre-training and pre-testing morphine administration on SDL duration, compared to the control group.
**P<0.01 indicates a meaning full difference compared to the control (saline + saline) group.
*P<0.05 indicates a meaning full difference compared to the control (saline + saline) group.
The columns are representative of Mean±SEM (n=6).

The effect of varangboo extract administration on the memory of the subjects being treated with morphine

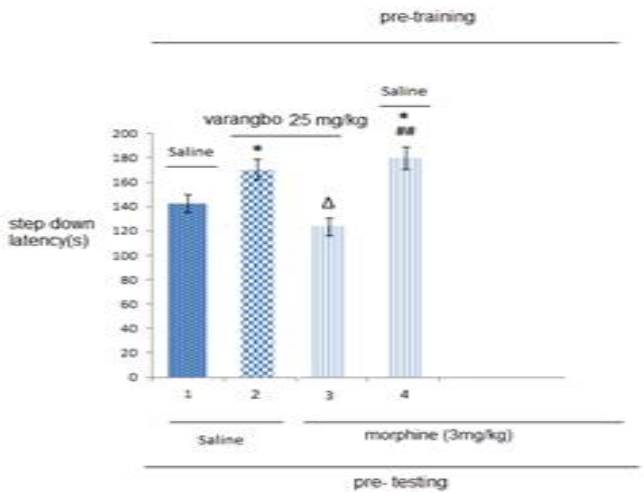

Chart 2; comparing the effect of pre-training Melissa officinalis extract (25 mg/kg) and saline administration on SDL duration in patients receiving morphine and saline, compared to the control group (Saline + saline).
##P<0.01 indicates a meaning full difference compared to the varangboo + morphine group.
*P<0.05 indicates a meaningful difference compared to the control (saline + saline) group.
$^{\Delta}$P<0.05 shows significant difference when compared to the varangboo + saline group.
The columns are representative of Mean±SEM (n=6).

(P<0.01). Pre-training administration of morphine (3 mg/kg) and pre-test administration of varangboo extract (25 mg/kg) weakened memory when compared to the pre-training administration of saline and pre-test varangboo extract (25 mg/kg) sessions (P<0.01) (chart 3).

The effect of pre-test varangboo extract administration on the memory of subjects treated with morphine:

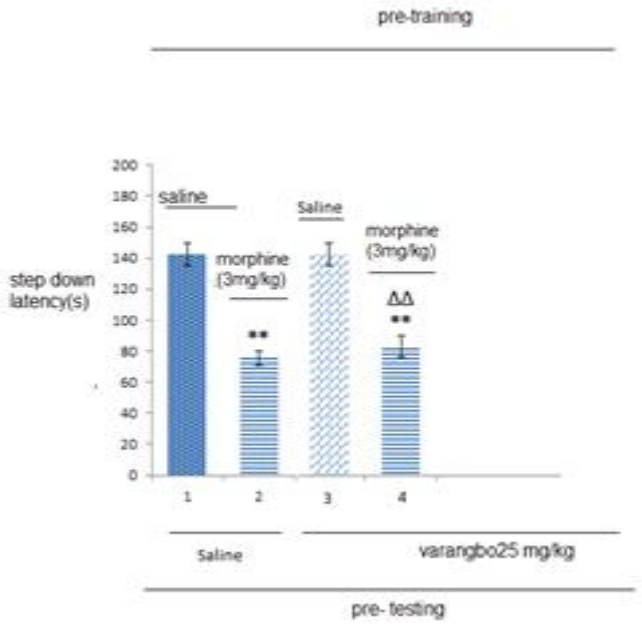

Figure 3. comparing the effect of pre-training morphine and saline administration on SDL duration in groups receiving varangboo (25 mg/kg) and saline, pre-test, with the control group.
**P<0.01 indicates significant variation when compared to the saline + saline group.
$^{\Delta\Delta}$ P<0.01 indicates significant differences when compared to the saline + varangboo .
The columns are representative of Mean±SEM (n=6).

Discussion

The hippocampus is responsible for the consecutive events which form our memories and forms connections between them through mutual factors (10). The results of this study reveal that pre-test morphine administration with a dosage of 3 mg/kg damages the memory. The formation of spatial memory in the hippocampus may be severely affected by hormonal factors, medications and various substances as well as the activity of cell receptors and molecular mediators (11). The effect of morphine on learning and memory is outstanding and many reports have been made discussing this adverse effect on morphine dependent learning in animals (12). Isquirdo et al (1979) reported that endogen opioids affect processes which are involved in the formation and retention of memories. The finds indicate that opioids positively affect the neuroplasticity of the hippocampus neural network. (13). some studies reveal that opioid agonists such as morphine and beta endorphin have a high affinity for μ opioid receptors which inhibit cholinergic activities of the hippocampus. It has also been reported that μ and δ receptors are located at the base of cholinergic terminals; terminals which are naturally inhibited by the opioid system (14). The finds of this study reveal that pre-test morphine administration (3 mg/kg), improves their memory. Also, administrating morphine to subjects who had received this drug pre-training also improves the damaged memory formed on that day. In This condition, which is known as state dependent learning, in order to retain and retrieve memories obtained recently, the condition and physiological conditions of the subjects must be similar to that of their learning sessions (15). The actual mechanism of state dependent learning has not been revealed yet (16). It noteworthy to mention that morphine has double sided effects, which are dependent on when this drug is administrated. If subjects are administered pre-test doses of morphine, memory retention is improved in subjects with experimentally induced amnesia, which is dependent on the dose and time of drug administration (17).

The overall results of this study show that interaction between Melissa officinalis and morphine damages memory retention. Opioids, such as morphine, which possess a high affinity for μ opioid receptors, are located in the cholinergic terminals of the hippocampus; attachment of morphine to these receptors induces the release of acetyl choline (18 & 19).

Since M.Officinalis shows affinity towards muscarinic and nicotinic acetyl choline receptor in the brain (5), it is also proposed that pre-training administration of morphine leads to the attachment of this drug to μ receptors located in cholinergic termini and inhibits the release of acetyl choline; thus when M.Officinalis is administrated beforehand, it is unable to attach to nicotinic receptors, improving memory retention as a result.

Conclusion

In general, the finds of the current study reveal that drug interactions between morphine and Melissa officinalis hydro-alcoholic extract damages the memory of the subjects.

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