The Effect of Cerebral Ventricular Injection Extract of Myristica Fragrans on Fear Behavior in Male Adult Rats of Wistar

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Abstract: Myristica fragrans plant is one of the herbal plants which has medical and cure effects on nerves. By pay attention to the disadvantages of the psychological drugs for body, the researchers want to have a research on the relations between these drugs and fear. In this study, the researchers put extract of Myristica fragrans (50%) with the 24.53% humidity is the values of (600,300,150 μg per rat) with six sample that each with the value of 1 μL. In the control group, they inject 1 micro L saline to the injection of cerebral ventricular. Also in other positive control group, 20 mg kg⁻¹ Pentylenetetrazol (PTZ) inject to the Intra Peritoneum (IP) after these injections, they evaluate the fear behaviors with the Elevated plus-maze test. The results of this test shows that PTZ is as the positive control group (p<0.05) can increase the residence time in the close arm more than the saline control group. In comparison, the extract of Myristica fragrans is all doses could increase the specified criteria and decrease the residence time in the open arm. But to the statistical way, two doses (600, 300 μg per rat) have the various levels (p<0.05), the saline control group. But when the extract of Myristica fragrans with PTZ was injected to the animal, it reduced the operation of PTZ. In fact, the maximum dose of extract (600 μg per rat) could show variation to the positive control group PTZ.

Key words: Fear, injection of cerebral ventricular, elevated plus-maze, Myristica fragrans, coparison, Iran

INTRODUCTION

The human always was concerned by the illness and pain. So, they find the plants, the best ones. The great men like Zakaria Razi, Abo Ali Sina were the most famous men that knew the plants. By the improvement in sciences such as chemistry, the synthesis of the plants even they could find ways to product artificial plants. It becomes very normal to use the plants for the medical care even, it has been taught at universities. Now-a-days, the researchers use the extract of the plants that changes to the juice or tablets and drugs. Fear is the important reaction to the danger, it includes the autonomic and neuroendocrine reactions and behaviors and of course fear is a physiological defensive response to the damage (Kaplan and Sadock, 1998).

In considerable to the proof of the harmful effect of the hallucinogenic drugs so, we decide to search the negative effect of these drugs. In terms of fear, previous researches showed that Myristica may cause bad headaches, drowsiness, fainting and shaking (Pellow and File, 1986). Myristica can weak Monamine Oxidase (MAO) inhibitor and it acts as an antioxidant (Sonavane et al., 2002). When someone is influenced by this plant at first of poisonous part. The patient arouse by this drug because of the hallucinogenic, he/she feels hallucination and it causes the arousal of the central nerves system and anticholinergic (Weil, 2006).

MATERIALS AND METHODS

To do this research, we use the male adult Wister rats with the weight of 200±20 g. These rats (weight 120 g) are provided from the Razi Institute in Hesarak (Fig. 1). Then, they transfer to the animal protection of Rasoul Akram’s University. They protected in special cages for rats on the group of 3-4 numbers. They provide their food (pellet) from Javane laboratory animal company in Mashhad. The rats have enough food, except the time of the test. The physical situation for production of these rats is the lightening, darkness period, 12 h (7 am to 7 pm) temperature: 22±2°C. Laboratory equipment and instruments required for testing are as follows:

- Ohaus scales for weighing rat with a 10 g accuracy, blade modified to revise the animal hair, a much number of 1 mL insulin syringes to inject drugs (the number of animals and a number) and accessories described as scissors, scalpel, clamps, forceps, described within etc.
- Gauge 21 syringes, lot of stainless steel for making injection cannula, kulis difficulty 0.5 dirty miter, cutter, papar, lighting study.
Extracting modeling: *Myristica fragrans* due to being native in India is produced dried. In biostatistics point of view, it has been confirmed by Biostatistics Department of Mashhad, Ferdosi University in terms of genus and species. For extracting *Myristica fragrans*, soaking it in alcohol 50% was done. In this model, first measure a certain amount of *Myristica fragrans* (500 g), mill it then add 400 CC ethanol 50% and put it in Ban mar in temperature of 30-40°C to extract it completely. Once in a while stir the mixture to make extracting easier. Alkaloid found in *Myristica fragrans* seeds solve in ethanol 50% well (Nichols and Sanders-Bush, 2001). After complete extraction of alkaloid that is a mixture of ethanol and the seed of the mentioned plant, we smooth the mixture by Buchner funnel, filter study and vacuum pump to eliminate the gross out of the mixture (Demetriades et al., 2005). In this step, we need to eliminate the solvent and make a thick liquid to do this, the mixture is put in Rotary machine in the temperature of 40°C and rotation of 60. Finally, the extract is ready to use.

Surgery and prescription drugs: After the fixation of the animal in stereotaxic device using PACKSYSUS atlas, the coordination of ventricular AP = -0.8 and ML = 1.6 from the Braga area was determined and needle 21 was used as a guide cannula in ventricular. Total 5 days after the surgery in each prescription, the animal receives 1 µL of the extract injection in ventricular area by injection cannula no. 27 and Hamilton syringe.

Experimental groups:
- Control Group: in this group, male rats receive 1 µL of saline via i.c.v.
- Positive control group: in this group, male rats receive 20 mg kg⁻¹ of PTZ via i.p.
- The groups injecting via i.c.v receive 600, 300, 150 µg per rat of the extract
- The groups of having 600, 300, 150 µg per rat of the extract via i.c.v also receive PTZ via i.p. It is important to mention that these groups were 6 (N = 6)

Behavioral experiment: Behavioral assessment system for fear is done by elevated plus-maze. This device contains two open arms and two blind closed arms, crossing each other in a neutral zone called maze. The closed arms are surrounded by 40 cm walls (Hosseinzadeh and Sadeghnia, 2007). The size of the open and close corridors is 5x100 cm. The height of the device
from the floor is 50 cm and during the experiment there is a light of 100 V in the height of 120 cm from the maze in a way that the light is the same in all parts. During the experiment, all the parameters were measured carefully by observation and stopwatch. Entry number and the time spend when the animal is kept in the closed and open corridors is captured as can be shown in Fig. 2.

**Histological work:** After all the experiments, all brain samples were examined to confirm the correct location of cannula by methylene blue and after 22 days were kept in formalin 10% and in terms of histological work was examined (Paximus, 1986). In case of the wrong location of cannula, the sample was eliminated from the experiment (Fig. 3).

**Locomotion test:** According to this test, displacement of the animal is assessed by statistic analysis using software called SPSS, one way Anova variance and Tukey test in order to assure the animal health during this displacement in each arms. According to paksynius atlas, the coordination is chosen in a way that causes less harm to the brain tissue (Paximus, 1986). If the location of the cannula is accurate, animal according to locomotion must not be different from other groups. The accuracy of the cannula location was confirmed, too.

**Indicators for assessment:** After the injection of the mentioned mixture, the animal was kept in a very quiet place for 5 min and then was put in the middle of the maze facing a closed arm, the light was provided by the 100 V located above the maze in the height of 120 cm during the 5 min that the animal was moving around the maze, these parameters were measured:

- OAE (%) is the entry number of rats to each open arm
- OAT (%) is the duration of time that the animal stays in open arm
- CAE (%) is the entry number of rats to each open arm
- CAT (%) is the duration of time that the animal stays in closed arm

Staying in the close or open arm means when all four legs of the animal is located in the mentioned arm (Hallstrom and Thuvander, 1997). For each animal the percentage of the entrance of the arms and staying duration is calculated in this way. The effect of i.e.v. in Oleander in all experiences using statistic analysis, the SPSS software, one-way anova variance and Tukey test were done and the results were drawn by Excel software. In all cases (p<0.05) showed a valuable result.

**RESULTS**

The process of these statistic achievements via Tukey test and one way anova variance have been shown in Table 1. Comparison of mean and standard deviation percent open arm time in the presence of animals after injection of positive control group (PTZ, 20 mg per rat) compared with the control group (saline) has been shown in Fig. 4. In Fig. 4, N = 6, p<0.05 and unsimilar letters are significantly and the data analyzed by anova one way is shown.

Comparison of mean and standard deviation percentage of time spend of animal experimental group treated with the values 600, 300 and 150 µg per rat extract nutmeg snooker in the open arm the device Elevated plus-maze compared to control group (saline) and positive control PTZ with Article PTZ (20 mg Kg⁻¹) are shown in Fig. 5. In Fig. 5, n = 6, p<0.05 and unsimilar characters with
Table 1: Results of statistic achievements via Tukey test and one way anova variance

<table>
<thead>
<tr>
<th>Groups</th>
<th>Simple size</th>
<th>1st injection</th>
<th>2nd injection</th>
<th>OAT (%) compared with the control</th>
<th>OAE (%) compared with the control</th>
<th>CAT (%) compared with the control</th>
<th>CAE (%) compared with the control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N = 6</td>
<td>N/C saline (1 µL)</td>
<td>-</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Positive control (PTZ)</td>
<td>N = 6</td>
<td>I.C.V saline (1 µL)</td>
<td>1P PTZ 20 mg kg⁻¹</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Myristica, f.</td>
<td>N = 6</td>
<td>I.C.V 600 µg µL⁻¹</td>
<td>1P PTZ 20 mg kg⁻¹</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Extract of Mf</td>
<td>N = 6</td>
<td>I.C.V 600 µg µL⁻¹</td>
<td>-</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Extract of Mf</td>
<td>N = 6</td>
<td>I.C.V 300 µg µL⁻¹</td>
<td>1P PTZ 20 mg kg⁻¹</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Extract of Mf</td>
<td>N = 6</td>
<td>I.C.V 150 µg µL⁻¹</td>
<td>1P PTZ 20 mg kg⁻¹</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Extract of Mf</td>
<td>N = 6</td>
<td>I.C.V 150 µg µL⁻¹</td>
<td>-</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Increase</td>
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<table>
<thead>
<tr>
<th>Groups</th>
<th>OAT (%) compared with PTZ</th>
<th>OAE (%) compared with PTZ</th>
<th>CAT (%) compared with PTZ</th>
<th>CAE (%) compared with PTZ</th>
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<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Positive control (PTZ)</td>
<td>-</td>
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<tr>
<td>Myristica, f.</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease</td>
<td>Decrease</td>
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<tr>
<td>Extract of Mf</td>
<td>Decrease</td>
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<td>Extract of Mf</td>
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<td>Extract of Mf</td>
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Fig. 4: OAT (%) vs. negative and positive control

Fig. 5: OAT (%) vs. extract Myristica f.+PTZ

Fig. 6: OAE (%) vs. extract Myristica f.+PTZ

eperimental group treated with the values 600, 300 and 150 µg per rat extract nutmeg snooker in the open arm, the device elevated plus-maze compared to control group (saline) and positive control PTZ with Article PTZ (20 mg kg⁻¹) are shown in Fig. 6.

In Fig. 6, n = 6, p<0.05 and unsimilar characters with significant differences by statistical analysis and data anova one-way is shown. Comparison of mean and SD percent of time spend of animal experimental group treated with the values 600, 300 and 150 µg per rat extract nutmeg snooker in the close arm elevated plus-maze compared to control group (saline) and positive control PTZ with Article PTZ (20 mg kg⁻¹) are shown in Fig. 7.

In Fig. 7, n = 6, p<0.05 and unsimilar characters with significant differences by statistical analysis and data anova one-way is shown. Comparison of mean and standard deviation percent of entry number of animal
experimental group treated with the values 600, 300 and 150 μg per rat extract nutmeg snooker in the close arm Elevated plus-maze compared to control group (saline) and positive control PTZ with Articulate PTZ (20 mg kg⁻¹) have been shown in Fig. 8.

In Fig. 8, n = 6, p<0.05 and non-homologous characters with significant differences by statistical analysis and data one way anova is shown.

Comparison of mean and SD percente of time spend of animal experimental group treated with the values 600, 300 and 150 μg per rat extract nutmeg snooker in the open arm, the device elevated plus-maze compared to control group (saline) and positive control PTZ without PTZ (20 mg kg⁻¹) have been shown in Fig. 9. In Fig. 9, n = 6, p<0.05 and non-homologous characters with significant differences by statistical analysis and data one way anova is shown.

Comparison of mean and standard deviation percent of entry number of animal experimental group treated with the values 600, 300 and 150 μg per rat extract nutmeg snooker in the open arm the device elevated plus-maze compared to control group (saline) and positive control PTZ without PTZ (20 mg kg⁻¹) have been shown in Fig. 10. In Fig. 10, n = 6, p<0.05 and non-homologous characters with significant differences by statistical analysis and data one way anova is shown. Comparison of mean and standard deviation percent of
entry number of animal experimental group treated with the values 600, 300 and 150 µg per rat extract nutmeg snooker in the close arm Elevated plus-maze compared to control group (saline) and positive control PTZ without PTZ (20 mg kg⁻¹) have been shown in Fig. 12. In Fig. 12, n = 6, p<0.05 and non-homologous characters with significant differences by statistical analysis and data anova one-way is shown.

**DISCUSSION**

The cause of this affections is influenced by the compounds and mechanisms some of the most effective compounds in this branch are some of the alkaloid that can pass from the black of cerebrospinal blood. Two of the most important alkaloids that exist in Myristica fragrans are Myristicine and Trymiristine (Hallstrom and Thuander, 1997). The researches on this plant (Myristica fragrans) show that Myrisicine can cause provoking effects on central nerve system. It is a hallucination drug and can weak Monoamine Oxidase (MAO) inhibitor and it acts as an antioxidant and its’ structure is similar to serotonin agonists. Based on the researches, the reaction of Myristica fragrans and diazepam drugs or Buspiron had seen on rats, also eating Myristica fragrans and flunitrazepam simultaneously could cause death. These alkaloids may metabolize one structure like Amphetamine by the effect of hallucinogen like LSD.

To cure the patient, the hallucino can use to converse the effect of amphetamine like thing (Demetriades et al., 2005). In this research, the extract of Myristica fragrans includes compounds of alkaloid Myristicine and Trymiristine. To study the fear behavior, this drug with the doses of (600, 300 and 150 µg per rat) by the method of i.e.v with the PTZ drug in the feature of I.P and without this drug (PTZ) inject to the animal (n = 6). The research has been done in two steps. Step one the various values from the extract of the Myristica fragrans with PTZ in the form of I.P simultaneously and the next step to complete the research the same value of the extract from the 1st step without PTZ is studied because they want to study the effect of the extract alone and compare it with the previous results. To obtained results, the noticed extract without PTZ can instigation anxiety and decreases the percent of time of the animal spend in the open arm to the comparison to saline and statistically, the doses of the extract (600 and 300 µg per rat) have the meaningful variation (p<0.05) with the control group.

As it has shown in Fig. 12, two noticed doses reduced the percentage of the time of the presence in the open arm in comparison with the positive control group (PTZ) but it was not meaningful and the presence of the numbers of the entrance to the open arm in all doses is reduced in comparison to the control group (saline) too but it could not act like the power of PTZ in fact it is in the middle of the positive and negative control group.

These results statistically were not meaningful but these results complete the others. In the percentage of time of the presence of the extract in close arm in all doses could increase the time in comparison to the control group (saline) therefore statistically, the middle dose 300 µg per rat could prove this. In comparison to the positive control group (PTZ), the doses (600, 300 and 150 µg per rat) act like PTZ but the least dose (150 µg per rat) in this criteria in comparison to the positive control group shows the reduction and becomes between two positive and negative control groups. Based on the results, all doses of extract could increase the percentage of numbers entrance to close arm in comparison to the control group (saline) but statistically this increase is not meaningful and also these doses can show reduction in comparison to the positive control group and become between two positive and negative control groups.

The results of the other step of the research that include the extract of Myristica fragrans with the PTZ show that the percentage of the time of the presence in the open arm in all doses was decreased in comparison to the control group (saline) in fact the doses of (300 and 150 µg per rat) the expressed statistically are proved these results too and show the meaningful variation to the control group but in comparison to the positive control group (PTZ), the doses 600 and 300 µg per rat could increase this time but the less dose (150 µg per rat) could not do this and act like this drug and statistically the greatest dose (600 µg per rat) could show the increase of time in presence in open arm and have the meaningful level (p<0.05) in comparison to the positive control group.

By considering the statistical results, there is a meaningful variation between the most and the less doses 600-150 µg per rat that shows the dose dependend to cause the effects. Of course, about the number of
entrance criteria to the open arm (CAE%), the greater dose, 600 µg per rat and dose, 300 µg per rat shows the increase in the number of entrance to the open arm in comparison to the both control groups and could cause the reduction in the PTZ effects but 150 µg per rat dose could not confront with this material and the results show the value of the expressed in the plant and it is completely dose dependent.

The percentage of the time of the presence in the closed arm (CAT%) in all doses cause increase in comparison to the saline control group and statistically, the doses (300-150 µg per rat) have proved this result and have a meaningful variation to the control group but show reduction in comparison to the positive control group and reduce the PTZ effect. This reduction is related to the extract of the plant. Because the greatest dose (600 µg per rat) has the meaningful variation (p<0.05). It must be noticed that statistically, there is variation between the less and the most doses (600-150 µg per rat) that shows the dose dependence to cause the effects.

By considering the percentage of the numbers of entrance to the closed arm (CAE%), the greatest dose (600 µg per rat) shows the reduction for the number of entrance to the closed arm in comparison to the both control groups and cause reduction about the PTZ effects. The dose (300 µg per rat) causes reduction for this criteria in comparison to the positive control group and reduces the PTZ effect but the dose (150 µg per rat) could not confront with this material and the results show the value of the extract and it is completely dose dependent. In fact, statistically the greatest dose of the extract could have a meaningful variation (p<0.05) with the PTZ and reduces the PTZ effects. It is important to consider that in this step, all doses had received the PTZ simultaneously, results show that in comparison to the 1st step that showed the increase of anxiety this step shows the reaction between these two materials.

Anxiety showed property creation but in the 2nd stage with an anxiety-causing substance was used to simultaneously, consider the effects of reduced material and show the interaction between the brain systems involved in visitors. Based on studies, Pentyleneetetrazole (PTZ) drug at a specific dose (20 mg per rat) is a property anxiogenic that this trend in the study and also statistically significant as was proven (Hosseinzadeh and Sadeghnia, 2007). As we know GABA is a neurotransmitter inhibitory through by activating chloride channels decreases neuronal activity. PTZ increased permeability and flow of potassium ion in the membrane including Na and Ca membrane excitability of neurons. Also, this chemical increases extracellular glutamate and aspartate levels in the amygdala and the frontal cortex and be synaptic glutamate removal ability is reduced by PTZ. PTZ as a (GABA A) receptor antagonist through connection for connecting positions picrotin caused blocking of GABA receptors and through involve GABAergic system creates fear in animals. Also, important material impact PTZ-hormone corticosterone mediate effects of anxiety, it is creation.

As we know, electrical stimulation of amygdala increases plasma corticosterone levels probably a result of HPA axis is the amygdala. Serotonergic system in the brain stimulates fear and anxiety is important (Nichols and Sanders-Bush, 2001).

As noted, one of safron alkaloid compounds in the extract is available, acted like serotonin agonist and serotonin-like effects have increased concentrations of serotonin through serotonin reuptake inhibition and probably through the serotonergic system involved in creating fear behavior is involved.

The other hand, studies have shown that the similarity of building molecular alkaloid Myristicine and Trymristine with combining serotonin can many of these compounds to bind to receptors serotonergic justify that probably as a composition competition for occupied positions of operation with serotonin to compete.

The characteristics of plants like Myristica, being the mono-amino oxides as research has shown that serotonin and noradrenalin increases act MAO. In this study, Myristica extract without PTZ on fear behavior in animals was shown. Probably also be dose dependent. p<0.05 and the creation of anxiety effect was proven. However, this extract administered with PTZ administration caused an interaction (interaction) between the two were combined. And showed reduced function PTZ. Probably due to the interaction of two serotonergic system and is GABAergic. Research conducted has shown that several compounds such as those compounds due to receptor 5HT (1A) and 5HT3 and NMDA and GLY can act stimulatory effects of PTZ to reduce substance.

As mentioned compounds in the extract of Myristica is like agonist serotonin acts and according to the results of this study, probably receptor agonist 5HT (1A) and 5HT3 has been through the involvement of these receptors, the effects of this chemical compound reduce effect of anxiety creation caused by PTZ.

**CONCLUSION**

Finally, it was concluded that probably, the noticed extract could cause the fear action and the fear depends on the doses of the extract but when recommended with
the PTZ simultaneously reduced the PTZ operation. It may be was interaction between of the serotonergic and GABAergic systems in brain and they reduced the effect of each other.

ACKNOWLEDGEMENT

The researchers wish to thanks Prof. Hadi Arabshahi from Ferdowsi University of Mashhad for his support of writing up the study.

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