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PAIN BEHAVIOR AFTER PLANTAR INJECTION OF FORMALIN IN RATS

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ABSTRACT

This study aims to review rats' pain behavior after plantar injection with 1, 2.5 and 5 percent formalin. There were 6 rats in the first group, i.e. the control group. The respond of these rats to pain after the plantar injection with normal saline was reviewed. There were also three other groups each of which had 6 rats. In these groups, the somatic pain was created through a plantar injection with 1, 2.5 and 5 percent formalin with a 50µL volume and then it was reviewed. In order to review the data, the analysis of variance method was used as well as the GLM procedure in the SAS software. In addition, Tukey test was used to compare the means. According to the results, the plantar inject of normal saline made no significant change in licking or hitting the injected food after 5 minutes or in the interval of 15 to 40 minutes. The only significant difference was seen in the first five minutes (0-5) (P<0.05). On the other hand, the plantar injection of formalin at 1, 2.5 and 5-percent concentrations led to licking and hitting the injected foot in the time intervals of 0 -5, 15 -20, 20 – 25, 25 – 30, 30 – 35, and 35 – 40. It can be concluded that the behavioral response to pain in this study can be narrowed down to 2 phases of hitting and licking the spot of injection on the foot.

KEY WORDS
Pain Behavior, Saline, Formalin, Rat

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INTRODUCTION

Sensing pain and perceiving it are some of the most important functions of the nervous system which provides the necessary information associated with a damaging or a potentially damaging stimuli. It designs the suitable reaction given the type of the stimulus. Pain is a complex phenomenon and includes both sensory and emotional components. This means that pain is a sensory experience which is accompanies by incentive responses and also by somatic and motor adaptations. From this perspective, nociception is an essential process is a necessary process and it is a prerequisite for a living creature to survive [1]. To put it simply, pain is a protective sensory experience to make the person aware of harms and damages to the body tissues. One of body's defense mechanisms that protects the tissues and organs against harm is the sense of pain which makes the central nerve system aware of the part of body that is being damaged. That is how the patient would think of a solution. Adjustment of pain is a complex process which depends on many physiological, neural and hormonal factors. Sensitivity to pain and nociception might be increased or decreased because of some environmental occurrences along with changes in the chemical mediators that are released in the body. It is significantly important to know these chemical mediators in order to soothe the pain. Numerous parts of the central nervous system play roles in transferring and processing different types of pain. Some of the most important ones are Hypothalamus, thalamus, somatosensory cortex, cingulate cortex, hippocampal formation, amygdala, Sylvius periaqueductal gray matter, Habeluna, insular cortex, striatum and cerebellum [2]. Pain is a sensory experience which is together with sensory and emotional components. There is a difference in the sensory component since it depends on the spinothalamic tracts to the ventral posteriomedial and ventral posteriolateral nucleus of thalamus. The nociceptors send the information to the somatosensory parts of cortex 1 and 2. It is because of the sensory processing in the aforementioned upper levels of the cortex that the quality of pain, the painful stimulus, the intensity and duration of pain are perceived. The emotional responses to the painful stimulus include attentions, awareness, somatic and autonomic reflexes, endocrine responses and emotional changes. These mentioned factors are responsible for the undesirable essence of the painful stimulus. Emotional responses to the transmission in multiple ascending pathways depend not on trigeminal and spinothalamic tracts but on the spinoreticular tract and the spinocerebellar tract as well. The parts of the cortex that play a role in these responses are insula and cingulate gyrus. Moreover, a few tracts were recently discovered which directly connect the spinal cord to the limbic system including amygdala, without thalamic relays [1]. There are considerable evidences that suggest that sensory stimuli are even perceived without the cortex and this is true for pain in particular. These areas in the cortex are apparently responsible for an accurate and significant discriminating interpretation of pain and some of its emotional components. However, the cortex is not needed for the nociception alone [3].

In this regard, formalin has been widely used in order to review pain in this study. Usage of formalin and its behavioral responses have come to be known as formalin test. The formalin test is one of the standard tests for measuring the responses to the chemical painful stimuli. This test was presented to the world by Dubisson and Dennis (1977)[4]. Just like any other aldehydes, formaldehyde combined with amine groups has a toxic effect on the cell depending on the ceoncentration. Although formalin has been rather well-known as a painful matter for a couple of years now, but it was because of the invention of Dubisson and Dennis (1977) that two separate phases of the sensing pain. The formalin test is a test that measures the pain of rats. The first phase starts immediately after the injection in about 5 minutes and the second phase starts around 20 minutes after the injection. In this method, a way has been presented to quantify pain. It included raising, licking, biting the injected leg and reduction of the weight that leg could bear based on the entire time that was spent in various behavioral conditions. Some changes were made in this test and since then, it has been used as one of the valid tests for measuring pain. It seems that two separate stimuli cause the two-phased pain sensing behaviors seen in this test. The role of bradykinins, cytokines and arachidonic acid metabolites has been strongly emphasized in one or both of the formalin pain. The first phase is neurogenic, the direct stimulation of nociceptors. According to the results of the experiments, the matter P and bradykinins are involved in the first phase; whereas, histamine, serotonin, prostaglandins and bradykinin play their roles in the second phase. In a study, the B_2 antagonists of bradykinin inhibited pain responses in the second phase but were not effective in the first one. The role they played showed the environmental inflammatory processes in the second phase of the formalin pain. On the other hand, the nonsteroidal anti-inflammatory drugs such as indomethacin decreased the pain in the second phase.; while it couldn't do so in the first phase [5, 6]]. Numerous methods have been presented in order to assess the formalin pain. For instance, after injecting back of the foot or plantar surface of the rat, some researchers only measured a single parameter including throwing, shaking, jerking/flinching or licking the foot. On the contrary, some have used some fixed characteristics for scoring.

In this regard, formalin has been widely used and its behavioral responses have been standardized and this test has come to be known as the formalin test. 0.1 to 5 percent formalin was used in this test. The volume of formalin varied from 5 to 10 microliters. This substance was injected to various parts of the body including back of the hand and foot, upper lip, intraperitoneal and in the colon, in order to create and then review the behavioral and hormonal responses, visceral and somatic pains.

After formalin was injected, the behavioral responses such as licking, biting, chewing and holding that certain body part still have been recorded in two phases. The first phase is the 5 to 10 minutes immediately after the injection (called neurogenic pain) and the second phase starts 15 to 20 minutes after the injection for about 30 to 40 minutes when an increase is seen in the behavior (called inflammatory pain). There is a 5 to 15-minute time interval between the two phases when the responses to pain decrease.

Responses to pain were recorded through measuring the duration of licking and biting the injected foot. According to the cited experiences, it is way better to record the behaviors of the rats than to use the scoring method.

A research studied duration of licking and biting the toe of the injected foot with 5-percent formalin in the time intervals of 0 – 5 minutes and 20 – 40 minutes after the injection. The first phase is neurogenic and it is created by the direct stimulation of nociceptors and there is no chemical mediator in this phase.

In the second phase, the formalin pain is an inflammatory pain and it is created through the involvement of inflammatory mediators such as prostaglandins, bradykinins, histamines and enzymes.

In brief, this study showed that varapamyl, nifedipine and dilitiazem inhibit formalin-induced pain behavior and it has analgesic and anti-inflammatory effects. These effects can be associated with the role of calcium channels in nerve cells on one hand and the release of inflammatory mediators from the cells on the other. However, further reviews are needed in order to investigate the exact role of the calcium channels inhibiting inflammatory pain in human beings and other living creatures [4].

The formalin test was also used for reviewing the analgesic effects of Eugenol, because this test has been known as a valid research model among various aspects of chronic pain. It determines the spinal central sensitivity after the environmental inflammation. There are also numerous reports that indicate that neurotransmitter systems such as the substance P, glutamate, serotonin and histamine play role in the formalin responses [7].

In order to review the pain mechanisms, formalin has been used as a painful substance at various concentrations when injected in different parts of the body. According to these studies, it has been specified that a two-phased pain is created after the plantar injection of formalin . Histamine is frequently used for reviewing the inflammation mechanisms. Histamine stimulates the neural fibers transmitting pain and releases pain-related neuropeptides. A sense of itch and pain is created in the skin when histamine is injected [8].

MATERIALS AND METHODS

In this study, adult male Wistar rats were used. These rats weighed 200 to 250 grams and they were bought from Faculty of Veterinary Medicine of Tehran University. The rats were divided into groups of six and they were put in plastic cages in a room with desirable temperature of about 23±2°C, desirable environmental conditions and 12 hours of light. They were fed with commercial pellet food. They had 24/7 access to food and water. All of the experiments were performed in the time interval of 8 to 15 hours. Commercial 37-percent formalin was used and normal saline was added to it in order to prepare the 5, 1 and 2.5% formalin solution.

There were 6 rats in the first group, i.e. the control group. The respond of these rats to pain after the plantar injection with normal saline was reviewed. There were also three other groups each of which had 6 rats. In these groups, the somatic pain was created through a plantar injection with 1, 2.5 and 5 percent formalin with a 50µL volume and then it was reviewed.

In order to review the sense of pain in all groups, the formalin test was used. This text was first presented by Dubisson (1977) and it is now a valid method used for reviewing chronic pain. In the present study, the somatic pain was created through a plantar injection with 1, 2.5 and 5 percent formalin with a 50µL volume and then it was reviewed. As it was already mentioned, using various concentrations of formalin creates pain in the rats' plantar. On the other hand, Responses to pain were recorded through measuring the duration of licking and biting the injected foot. According to the cited experiences, it is way better to record the behaviors of the rats than to use the scoring method (4). In this method, we have a plantar subcutaneous injection of formalin.

The rats were lightly kept with a towel and they are injected with 50 µL formalin solution with the concentration of 1 percent on the foot plantar using the needle number 28. When the foot plantar is injected with diluted formalin, the animal immediately reacts by pulling back the foot and whines and tries to run. The rats are instantly put inside the pain mirror so that their behavioral response to pain would be reviewed. The response to pain caused by plantar injection of formalin has two phases. In this present, the animal's behavior in the first five minutes and in the time interval of 15 to 40 minutes were considered as the first and second phase of pain. Figure 3-4 shows the animal licking the injected body part after it was injected with formalin in the pain mirror device.

Fig. 1 : Licking the injected part of the body after plantar injection of formalin in the pain mirror device.

Review of pain behavior

The pain mirror device was used to create the behavior caused by plantar injection of formalin which was then reviewed. This device has one basis and one box. The box is made of shatterproof glass with the dimensions of 25×30×30 on a framework and it has a mirror with a 45-degree angle [Fig. 2]. It is because of the 45-degree angle that every move the animals make can be monitored. There are various factors that might make the animals stressed such as putting them in a cage, keeping them awake, separating one from the group, moving them from one room to another with different lighting and smell. Since there are numerous stressful factors, thus the researchers must try to minimize these factors [9]. In order create a compliance between the animals and the environment, they are transferred to the laboratory four hours before the experiments begin and they are placed inside the glass box in the pain mirror device half an hour before the beginning of the test. The animals are taken out of the box to be injected with formalin and are put back in it after the injection. [Fig. 2] illustrates the pain mirror box used in this study.

Fig.2: The pain mirror device used in this study.

The statistical analysis method

The data obtained from plantar injection with normal saline (control group) and with formalin (experimental group) was analyzed using the statistical method factor repeated measures (factorial) and then the Duncan. On the other hand, the data obtained from the experiment where the solution was injected was analyzed by one-way analysis of variance method (ANOVA) and then the Duncan test was used. The significance level has been $P<0.05$. In the experiments associated with determining the response, the proper dose of the substance has been specified by processing different nonlinear models such as second-pseudo, broken line, broken line with two breaks and exponential function, etc. The rate of determination coefficient has been selected as the best model and the desirable response is obtained from it. The GLM procedure of the SAS software was used for the analysis of variance and the Tukey method was used to compare the means.

RESULTS

[Fig. 3 and 4] illustrate the duration of licking and hitting the injected foot after the plantar injection with normal saline and formalin at the concentrations of 1, 2.5 and 5%. Figure 5 and 6 show them in 5-minute intervals (0 – 5 and 15 – 40).

The plantar injection with normal saline did not cause a considerable behavior in 5-minute intervals and between 15 and 40 minutes after the beginning of the experiment. The only significant difference it made was in the first 5 minutes (0 – 5) ($P<0.05$). Plantar injection of formalin at concentrations of 1, 2.5 and 5% made the animals lick or hit their foot in the following time intervals: 0 – 5, 15 -20, 20 – 25, 25 – 30, 30 – 35, and 35 – 40.

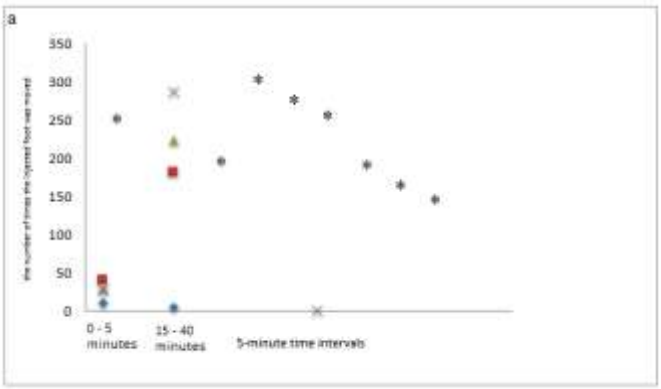

Fig. 3: The number of times the injected foot was moved (plantar injection of formalin).

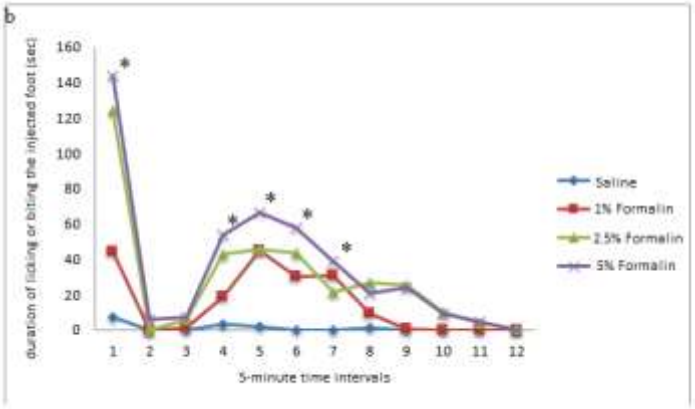

Fig.4: Duration of licking and biting the foot (plantar injection of formalin)

* Shows that there is a significant difference at the level of P<0.05 compared with plantar injection with normal saline and other time intervals take 5 minutes.

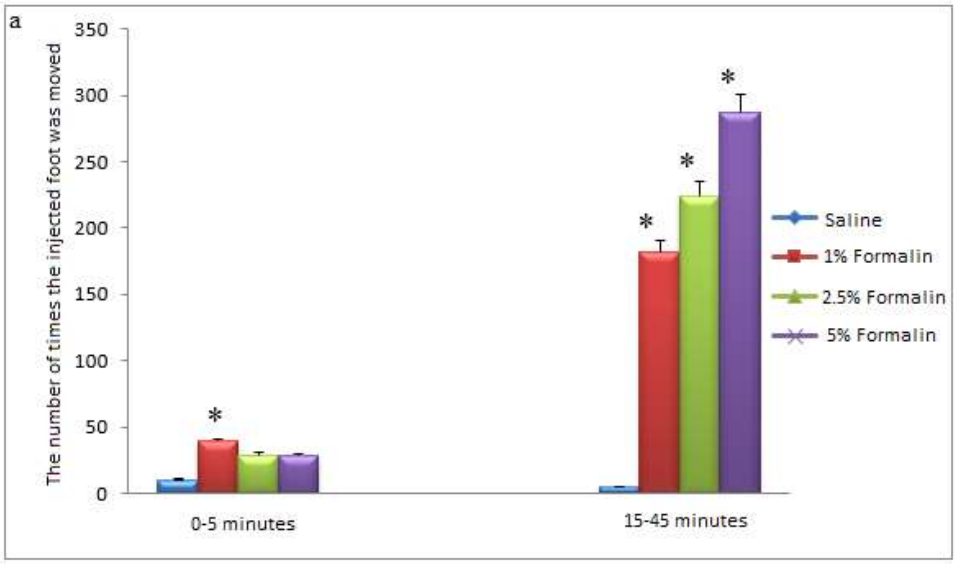

Fig.5 : The number of times the injected foot was moved (plantar injection of formalin).

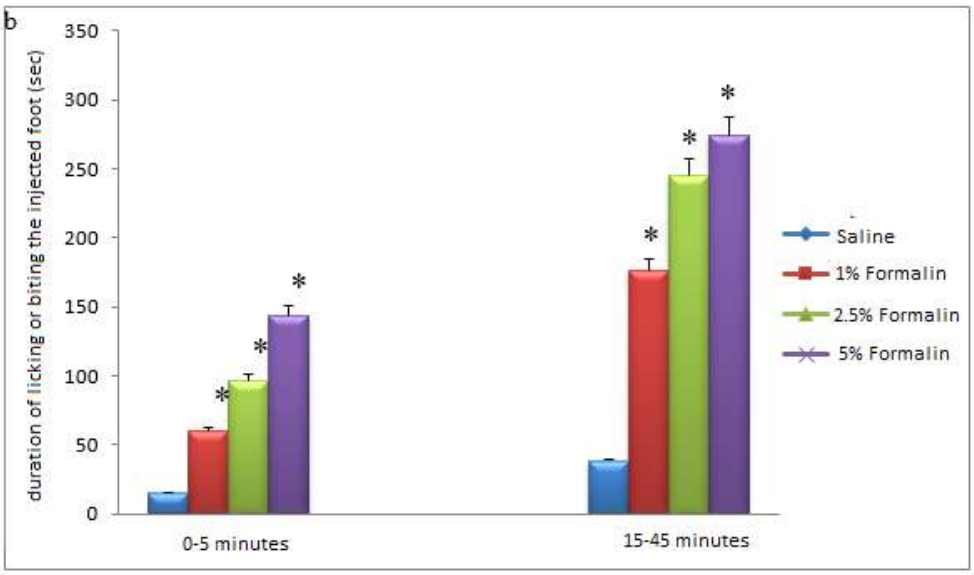

Fig. 6: Duration of licking and biting the foot (plantar injection of formalin).

* Shows that there is a significant difference at the level of P<0.05 compared with plantar injection with normal saline and other time intervals take 5 minutes.

CONCLUSION

This study aims to review rats' pain behavior after plantar injection with 1, 2.5 and 5 percent formalin. The results of the study show that injecting the inner level of the paw of rats with normal saline with a volume of 50µL creates an insignificant behavioral reaction just in the first 5 minutes after the injection. Depending on the research method, in the control group, various volumes of normal saline is used in most researches and perhaps it is mostly because of the fact that this solution is isotonic which means that it does not create the reactions associated with tonicity and pressure at the place of injection. The likelihood of creation of mild reaction to pain in the first five minutes after the injection with normal saline can be because of the fact that the needle is used for a subcutaneous injection. Despite the fact that the needle number 28 has been used for the subcutaneous injection in the present study, even if any other needle was used, only putting the needle in the body tissues would create pain reactions because the nociceptors are stimulated. In previous studies, in the first five minutes after injecting the foot plantar of rats with normal saline with a volume of 50µL, a weak pain reaction has been reported.

Figure 3 shows the duration of licking the foot plantar or hitting it after the injection with formalin at the concentrations of 1, 2.5 and 5%. A significant difference (P<0.05) was seen in the first, third and eighth time interval after the plantar injection of formalin at the concentrations of 1, 2.5 and 5%. In other words, in terms of time, formalin creates a two-phased pain (first phase: minutes 0 to 5 and second phase: minutes 15 to 40). Therefore, there is no contrast between this study and previous reports regarding the two phases of the pain caused by injection with formalin, which becomes totally clear. The first phase is neurogenic and it is created by the direct stimulation of nociceptors and there is no chemical mediator in this phase. In the second phase, the formalin pain is an inflammatory pain and it is created through the involvement of inflammatory mediators such as prostaglandins, bradykinins, histamines and enzymes.
The results of the present study showed that a two-phased pain behavior is felt when the foot plantar of rats is injected with formalin. These two phases are: hitting or licking the injected body part.
The formalin solutions (0.2 to 10%), as substances that cause pain, are used in order to review the tonic pain mechanisms through frequently injection various parts of body, especially foot plantar and face and in order to report the two-phased pain behaviors with an interphase between these two (5, 8, 10). Although subcutaneously injecting the ear of a rabbit or sheep with formalin has always created a one-phased pain for 10 minutes after the injection [11].

In this test, various concentrations of formalin have been used (from 0.1 to 5%). The volume of formalin varied from 5 to 10 microliters. This substance was injected to various parts of the body including back of the hand and foot, upper lip, intraperitoneal and in the colon, in order to create and then review the behavioral and hormonal responses, visceral and somatic pains.

After formalin was injected, the behavioral responses such as licking, biting, chewing and holding that certain body part still have been recorded in two phases. The first phase is the 5 to 10 minutes immediately after the injection (called neurogenic pain) and the second phase starts 15 to 20 minutes after the injection for about 30 to 40 minutes when an increase is seen in the behavior (called inflammatory pain). There is a 5 to 15-minute time interval between the two phases when the responses to pain decrease. Subcutaneously injecting the upper lip of rats with 5% formalin creates a two-phased pain

(12). In 1995, Clowly et al reviewed the effect of injecting rats with various concentrations of formalin on their pain behavior. They concluded that concentrations of 0.5 and higher create a two-phased pain; thus, the suggested formalin at concentrations of 0.5 to 5 for creating pain in rats' foot plantar [12]. Nonetheless, the pain behavior obtained from this study was a two-phased pain behavior shown as licking and hitting the injected body part. This means that this study complies with others.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

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