A comparison of acute pain thresholds between the male and female and the effect of alcoholic *Datura Stramonium* seed extract

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A B S T R A C T

Background and Objective: Opposite observations about the discrepancy in the pain perception between male and female has been reported. In this regard, pain thresholds among male and female rats in different phases of oestrous cycle were evaluated via tail immersion test. In addition, efficiency of alcoholic extract of *Datura stramonium* as an analgesic plant in the modulation of acute pain threshold has been studied.

Materials and Methods: The control groups of male and female rats were exposed to the tail immersion test using 52˚C water. In the experimental group, the extract of *Datura stramonium* was injected intraperitoneally 25-30 minutes before the tail immersion test. Afterwards, in both control and experimental female rats the vaginal smear samples were taken.

Results: In the female rats in proestrous and dioestrous phases, the pain thresholds are significantly lower than that of rats in oestrous phase ($p<0.01$). Also, male rats in addition to having lower pain threshold relative to whole females ($p<0.05$) (the average of 4 phases) treated with *Datura stramonium*, show a pain threshold that is significantly lower than that of females of oestrous and metoestrous phases ($p<0.001$). Furthermore, proestrous and dioestrous groups with lower pain thresholds relative to rats in oestrous and metoestrous phases show significant differences ($p<0.001$).

Conclusion: The female rats in the different phases of oestrous cycle have different pain thresholds and the extract of *Datura stramonium* is more efficient in the pain relief of female (especially oestrous stage) than that of male rats.

Key Words:
Datura Stramoniu
Acute pain
Oestrous cycle

1. Introduction

There is a vast body of evidence indicating significant differences between the response of male and female subjects to a variety of experimental and clinical situations. Comparisons of responses to noxious heat stimuli among females and males have yielded inconsistent results. For example, some investigators have reported lower pain threshold among females (1), whereas others have reported no sex difference in thermal pain threshold (2). These differences are likely to result from the influence exerted by gonadal hormones, as suggested by studies here significant fluctuations in the responsiveness to noxious stimulation or opiate drug therapy were found in ovariectomized female rats in different

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stages of the estrous cycle (3). The rapid effect of estrogen on the nociception via non- genomic mechanism is reported (4). In frequent researches the gender-dependent effect of oestrogen on the second messenger pathways is observed. In most of studies the excitatory and inhibitory effects of oestrogen on the second messengers' activation in females (5, 6) and males (7, 8) are reported respectively. There are variations of pain threshold at each of the four different phases of oestrous cycle in rats. It is reported that female rats in the proestrous and oestrous phases are more sensitive to pressure pain rather than other phases of oestrous cycle (9). However, more sensitivity to the heat pain in the oestrous and metoestrous phases is reported (10). On the other hand, ovariectomy accompanied by oestrogen treatment induces different responses (10). In spite of development of pharmacological knowledge and also the presence of chemical substances for pain relief, using medicinal plant is recommended because of having fewer side effects, easier accessibility and being more economic (11). There are some reports pointing out the use of *Datura stramonium* as an analgesic plant (12). In this regard, some species of datura are introduced as antinociceptives (13). In this research, according to the existence of inconsistency about nociception between male and female, primarily the acute pain thresholds of male and female (different stages of oestrous cycle) rats were compared using tail immersion test. Furthermore, the effect of *Datura stramonium* seed extract as an Iranian folk and antinociceptive plant on the pain threshold in male and different phases of female rats was aimed.

2. Materials and Methods

2.1. Animals

Adult male and female NMRI rats weighting 195-220 g (Razi Institue, Iran) were used. Four rats were housed in each cage at a temperature of 21±2 ºC and light-dark cycling 12 h with food and water provided ad libitum. In each of control and experimental groups, five series of rats including one group of 8 males and 4 groups of females (6-8 rats at each phases of oestrous cycle) were used.

Male rats in control and experimental groups were chosen randomly. In female cases, selection and division has been scheduled under oestrous cycle situation. Since vaginal epithelium of female rats show histological variation during each stage of oestrous cycle, after pain test performing, vaginal smear samples were taken from rats and stained. Based on the histological identification of vaginal smears that were determined microscopically, females were categorized in phases of oestrous cycle (proestrous, oestrous, metoestrous and dioestrous).

2.2. Preparation of plant extract

The medicinal plant of datura was provided from the local market and was scientifically identified by the department of botany of Shaheed Beheshti University. To prepare the alcoholic extract, one hundred grams of cleaned datura seeds was crushed and mixed at ratio of 1 to 4 with methanol 70% for 24 hours at room temperature. The alcohol of filtered solution was evaporated in a 50 ºC organ bath and was prepared with a final concentration of 25% (14).

2.3. Tail immersion test

Acute pain assessment was carried out via a tail immersion test using 52˚C water (15). In control groups of male and female, after holding the rats in restrainer for 20 minutes, animals' tail were put in the 52 ˚C water for 5 times with seven minutes intervals and the time course that rats pull out the tails from hot water was measured and considered as pain threshold. The threshold average of these times for each rat was calculated.

In the experimental group, the extract of datura stramonium 50 mg/kg was injected intraperitoneally 25-30 minutes prior to the tail immersion test. Afterwards, in the both control and experimental groups the vaginal smear samples were taken from the females.

2.4. Vaginal smear staining

The papanicolaou method was performed to stain the smear (16). The samples were developed on the slide and the total area of slide was covered by absolute alcohol to be stabilized. After evaporation in the air, slides were dipped in 50%, 70% and 80% alcohol for 5 seconds respectively. Then they were rinsed in water for 5 seconds and were incubated in hematoxylin

solution for 4 minutes. Later, the extra staining was removed from the slides by dipping in water. Slides were washed in acid alcohol for 5 seconds and were put in water. In the next step, they were dipped in 50%, 70% and 80% alcohol for 5 seconds each one, respectively and entered in OG6 solution for one minute. Then they were washed in 2 dishes of absolute alcohol, each one for 5 seconds and were rinsed in EA50 for 4 minutes. Slides were entered in 3 absolute alcohol solutions. Finally, to transparent the slides, they were dipped in xylol solution.

2.5. Statistical analysis

Data from pain assessment were expressed as means ± S.E.M. Comparisons were carried out using one way analysis of variance (ANOVA) followed by post-hoc Tukey test and p values less than 0.05 were considered as a significant difference.

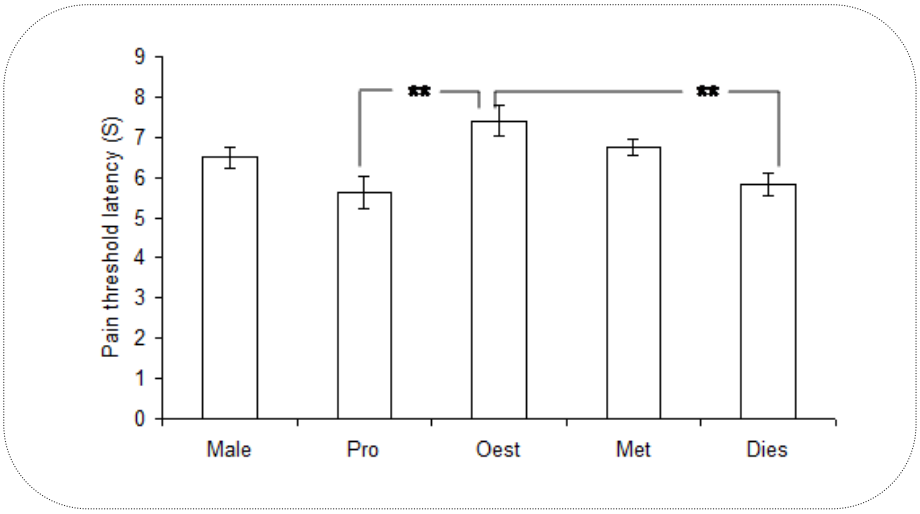

Figure 1: A comparison of acute pain threshold of male rats with female rats in different phases of oestrous cycle. Bars indicate pain average considering standard deviations. One group of 8 males and 4 groups of females (6-8 rats at each phases of oestrous cycle) were used $p > 0/01$

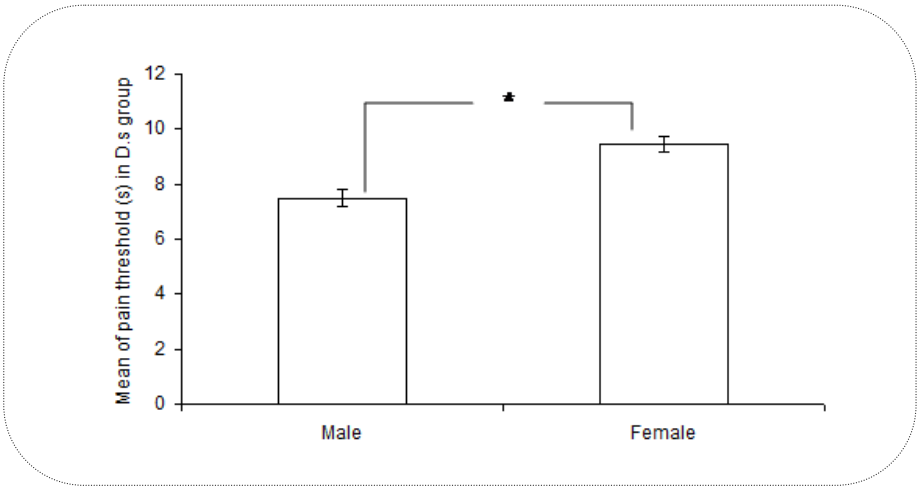

Figure 2: A comparison between pain threshold between male and female groups treated with datura seed extract .One group of 8 males and 4 groups of females (6-8 rats at each phases of oestrous cycle) were used $* p > 0/05$

3. Results

3.1. Effect of heat stimulation in control group

Our results and statistical analysis show no significant differences in pain perception between male and female rats. As it is shown in figure 1, there is a significant difference in acute pain threshold between rats in proestrous (5.36± 0.4) and oestrous (7.41±0.38) phases and also between rats in oestrous and dioestrous (5.83± 0.99) phases ($p<0.01$).

3.2. Effect of alcoholic *Datura stramonium* seed extract on the tail immersion test-induced pain threshold

Figure 2 illustrates that there was a significant difference in pain threshold between male (7.5± 0.3) and female (9.45±0.3) rats ($P<0.05$). A comparison between the results of experiment (table 1) shows that pain threshold of male and female rats (average of 4 phases) treated with datura have a significant difference ($P<0.05$).

Table 1 indicates that the most antinociceptive effect of datura was observed in rats in oestrous phase and the least effect was observed at dioestrous stage. Our results show that there is a significant difference between pain threshold of male rats (7.5± 0.3) and female rats of oestrous stage (11.28± 0.31), male and female rats of metoestrous stage (10.29±0.240), female rats of proestrous (7.26±0.25) and oestrous(11.28± 0.31), female rats of proestrous (7.26±0.25) and metoestrous (10.29±0.240), female rats of oestrous (11.28± 0.31) group and dioestrous (6.94± 0.28), and finally between female rats of metoestrous (10.29±0.240) and dioestrous ($p<0.001$).

3.3. Comparison of the effect of alcoholic *Datura stramonium* seed extract on pain threshold between male and female rats

As can be seen in figure 3, the average of pain threshold in the rats treated with datura, in male rats was 7.5±0.3 and in females was 9.45± 0.3. In the control group this average was 6.49±0.25 and 6.45±0.17 in male and female rats, respectively. Therefore, the pain threshold in both sexes in the treated group was significantly higher than control rats ($p<0.001$).

4. Discussion

Our results indicate that the acute pain threshold does not show any significant difference between male and female rats. This result does not have coordination with some previous reports about the effect of gender on pain threshold (17). This discrepancy could be raised from different reasons. For instance, in the mentioned report and much of similar researches,

Table1: A comparison of antinociceptive effect of datura on acute pain threshold in male and different phases of female rats

Gender	Female Male	Proestrous	Oestrous	Metoestrous	Dioestrous
Average of pain threshold	7.5 ± 0.30	7.36 ± 0.25	$11.28 \pm 0/31$	10.92 ± 0.24	6.94 ± 0.28
Male Female		***		***	***
Proestrous			***	***	
Oestrous	***	***			***
Metoestrous	***	***			***
Dioestrous	***		***	***	

One group of 8 males and 4 groups of females (6-8 rats at each phases of oestrous cycle) were used. Significant difference between two horizontal and vertical groups is shown with *** ($p > 0/01$)

female rats are used independent from their in oestrous cycle phases which affects the conclusion greatly. On the other hand, we have used rats from NMRI strain which are different from rats used in the other experiment. Since male and female rats from various strains in neuropathic pain threshold have different responses (18), the inconsistency in our research might be related to the strain of rats used. However, it is also reported that at the first phase of formalin-induced (formalin 1%) pain, nociception in the male and female rats was similar (19). This report is consistent with our result.

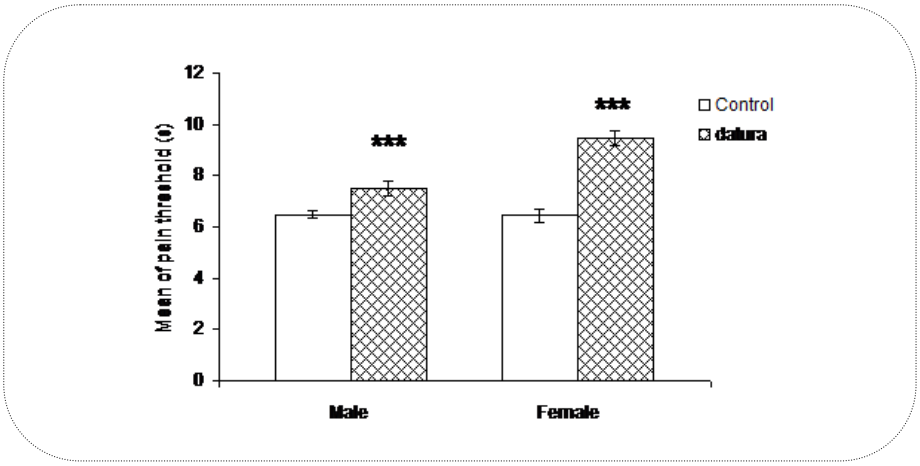

Figure 3: Acute pain thresholds induced by tail immersion test in control and treated groups of male and female rats. Number of female rats at each phase was 6-8 and in male group were 8. *** P<0.001

Our results about acute pain threshold in female rats in different phases of oestrous cycle show that there is a significant difference between oestrous rats with rats in dioestrous and proestrous phases. In conclusion, female rats at dioestrous and proestrous phases have lower pain thresholds relative to oestrous phase. This result confirms previous investigations expressing higher pain threshold in oestrous and raised from metoestrous phases of female rats (10). These discrepancies can be different hormonal levels and their receptors under experimental conditions. Different phases of oestrous cycle with different levels of sex hormones can affect the pain-related responses. The plasma oestrogen level has the highest amount and then dioestrous, metoestrous and oestrous respectively. (20). Regarding to the fact that the estrogen level in male rats is similar to its amount in female rats from oestrous phase (20), and the result of present study in which the pain thresholds in male rats and oestrous phase of female rats are similar, one can conclude that pain threshold is probably influenced by plasma estrogen level (21,22). This result confirms the algesiogenic effect of oestrogen that is in consistency with previous reports (23, 24) and probably the various degrees of nociception in different phases of oestrous cycle are based on plasma oestrogen levels. The main neurochemical reason for gender dependency of nociception is not scientifically clear, but there is a possibility for interfering of endogen opioid system in the regulation of nociception (25, 26). The lowest amount of β-endorphin has been seen in the afternoon of proestrous phase and at the evening of oestrous (27). The highest amount of μ receptor in the brain has been seen at noon of proestrous and in the evening of oestrous (28). The high level of oestrogen, after an initial increment of receptors in preoptic area results in the reduction of those receptors whose time course is similar to oestrous cycle (25). The effect of sex steroids on density of some opioid receptors and probably to affinity of receptors with their ligands is also reported (25, 28 and 29). Although the quantity differences of μ receptors in the afternoon of dioestrous possibly is related to involvement of other factors

such as progesterone level and presence or lack of LH surge (30), oestrogen treatment inhibits the antinociceptive effect of morphine in ovariectomized mice (23).

Since oestrogen treatment increases the opioid receptors, the inhibition of morphine-induced antinociceptive effect of oestradiol, might be resulted from the reduction of central and hypothalamic β-endorphin (31, 32). However, the inhibitory effect of oestradiol on the opioid receptors is also reported (32). It has been seen that in female rats there is an enhancement of nociceptive response threshold induced by oestrogen treatment, which is resulted from simultaneous activation of spinal receptors and (33). In the male rats receptors are probably involved instead of receptors (34). Since it is believed that attenuating effect of oestradiol on the antinociceptive effect of morphine in female is related to a sufficient and high dose of oestradiol which could induce a positive feedback on LH surge (30, 32), it is possible that this opposite result is an effect of the our dosage that is close to attenuating dose of oestradiol on the opiod function that decreased the pain threshold.

In the second part of our research, the effect of datura on the acute pain threshold of male and female rats was studied. Our result is in consistency with our previous research on the reduction of chronic pain by datura seed extract in male rats (14). It is shown that pain threshold of male rats treated with datura is significantly lower than that female group treated with datura. Datura seed extract increases pain threshold in the male and female but it has more efficiency in the female. This difference could be resulted from difference in the mechanism of datura action in two sexes. One possibility is the involvement of different opioid receptors in the pain control of datura seed extract in male and female rats. Based on previous report about the inhibitory effect of naloxone as an opioid antagonist on antinociceptive effect of datura (13), alkaloidal compounds of datura probably work in interaction with opioid system to decrease the pain. The existence of a cholinergic synapse in pain control opioidergic pathway (35) and the presence of alkaloid compounds in datura with anticholinergic effect induce the possibility of interaction of alkaloid compounds and opioid system effect in antinociceptive effect of datura. Datura increases the pain threshold of female rats in oestrous and metoestrous phases more than that of proestrous and dioestrous phases. A comparison of this result with the result of first part about pain threshold in intact female rats shows that we can not rule out the possibility that the effect of datura is somehow related to oestrogen level of plasma i.e. in the proestrous and dioestrous with more oestrogen in plasma the datura effect is less. According to the mediation of opioid receptors in oestrogen effect (33, 34) and interaction of alkaloid and anticholinergic effects of datura with opiod system (35), the effect of datura at different stages of oestrous cycle with different amounts of oestrogen could be justified. Since the difference in nociception in different phases of oestrous cycle might be related to oestrogen amount of blood, oestrogen measurement and pain assessment in different stages of oestrous cycle simultaneously and also considering oestrogen effect on nociception in ovariectomized rats could be useful. In addition, studying both morphine and datura effects on pain perception together is another way to assess interaction between datura and opioidergic system.

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