

The analgesic effects of L-arginine and its antagonist L-NAME in mice

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Summary

L-arginine-Nitric oxide pathway plays an important role in a series of neurobiological functions underlying behaviors including analgesic effect and has shown a role in pain feeling which is a mediator with modulation effect in dorsal root of ganglionic neurons of spinal cord. The goal of the present study is to clarify the influence of L-arginine-mediated nitric oxide (NO) on pain arbitration in both sexes of mice. The reactive time to thermal stimulus, latency period, tail withdrawal and the number of foot licking and flinching in hot plate test, tail flick and formalin tests were recorded. The results showed that L-NAME (nitric oxide synthase inhibitor) has had an antinociceptive activity demonstrated as prolonged reactive time to thermal stimulus, latency period for tail withdrawal and decreasing the number of foot licking and flinching in hot plate, tail flick and formalin tests. These findings might be attributed to that intensity of pain feeling is intercede due to interference of sex hormones in both sexes of mice. In addition, from the results of L-NAME on pain sensation, it may be concluded that L-arginine-nitric oxide pathway is extravital in male in comparison with female in pain sensation.

Key word: - L-arginine, analgesic, L- NAME, mice

التأثيرات التمسكينية للأرجنين ومضاده L-NAME في الفئران

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الخلاصة

يلعب مسلك أل-أرجنين-أوكسيد النتريت دور مهم في الوظائف العصبية الحيوية ومنها السلوكيات التي تتضمن التأثيرات التمسكينية فيلعب دوراً في الشعور بالألم حيث أظهر تأثيراً بسيطاً وتوليفياً في الجذر الظهري لأعصاب الحبل الشوكي العقيدية. تهدف الدراسة إلى توضيح تأثير أل-أرجنين وسيط أوكسيد النتريت في توليف الألم في كلا جنسي الفئران. سجل كل من الوقت الفعال للاستجابة الحرارية والفترة الفعالة لسحب الذيل وتقليل عدد مرات الإجمال ولعق القدم في اختبارات الصفيحة الساخنة كذلك تحريك الذيل والفورمالين لإدراك الألم. لوحظ من نتائج التجربة: أن L-NAME يمتلك الفعالية المضادة للألم حيث لوحظت من خلال إطالة الوقت الفعال للاستجابة الحرارية والفترة الفعالة لسحب الذيل وتقليل عدد مرات الإجمال ولعق القدم في اختبارات الصفيحة الساخنة وتحريك الذيل والفورمالين. عزيت هذه النتائج إلى اختلاف درجة الشعور بالألم بين ذكور وإناث الفئران بسبب تداخل الهرمونات الجنسية في هذا التأثير. إضافة إلى ذلك ونتيجة لتأثير L-NAME قد تختلف درجة الشعور بالألم نتيجة اختلاف فرط حيوية الإحساس بالألم في الذكور أكثر منه في الإناث. كلمات مفتاحية:- التأثيرات التمسكينية, أرجنين, الفئران.

Introduction

Pain is a complex phenomenon that is difficult to describe and measure. It can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (1). Nitric oxide is produced within the central nervous system (CNS) from L-arginine by a constitutive neuronal form of NO synthase (nNOS), an enzyme which is localized in neurons of the central nervous system. A role of nitric oxide (NO) in nociceptive signaling was initially based on the localization of neuronal nitric oxide saynthese (nNOS) in the superficial dorsal horn and intermediolateral cell column (2and3), which led to the notion that nitric oxide

(NO) regulates both autonomic tone and sensory transduction at the spinal cord level. Some reports have shown that reduction of nitric oxide (NO) induces antinociception (4and5). Female mice could tolerate pain for a longer time than male. Inhibition of nitric oxide synthesis by administration of nitric oxide synthase (NOS) inhibitor (L-NAME), resulted in diminished perception of pain in male but not in female mice (6). This effect of L-NAME could be reversed by the administration of L-arginine (7). It has been shown that the inflammatory pain induced by epinephrine intradermal injection in the hind paw of the rat is depended on sex hormones, and nitric oxide synthase inhibitors can antagonize pain only in male but not in female rats (8). It has been reported that sex is a factor that influences a variety of neurotransmitter systems and different mediators are important in the response to painful stimuli such as hot plate and tail flick in mice. The levels of stable metabolites of nitric oxide, nitrite and nitrate in the rat brain show sex differences i.e. female rats in comparison with males have lower levels in the cortex and hippocampus brain areas (9). In male mice, inhibition of nitric oxide synthase at the level of the brain but not at the spinal cord result in supraspinal analgesia. These results may suggest that sex steroid hormones such as estrogen have a role in the feeling of pain. The aim of this study is to clarify the influence of L-arginine-mediated nitric oxide (NO) on pain arbitration in both sexes of mice.

Materials and Methods:

Three hundred and twenty male and female mice (160 of each sex), weighing (25-30) gm. with an average of (27.5 ± 0.02) gm. were used in the experiments of the present study. They were kept under suitable environmental conditions of $(20-25) ^\circ\text{C}$ in an air-conditioned room, (12) hours light and nourished ad libitum. Pretreatment values were taken for all segments of the study for both sexes separately. Fifty male and fifty female mice were used to perform tail flick and hot plate tests. Five animals of each sex were given 160, 200 or/and 240 mg/kg, B.W. of L-arginine orally daily for 15 or 30 days. Other groups of five mice of both sexes were likewise treated with 100 mg/kg, B.W. of L-NAME intraperitoneally. Similar groups were given D.W. and served as control (figure 1). The tail flick test was described by (10). The noxious motivation was produced by immersing approximately (1) cm of the tip of the tail into a $(56.0 \pm 0.5) ^\circ\text{C}$ water bath and the latency period for tail withdrawal (second) was recorded, while the hot plate reactive time (second) to thermal stimulus was checked for certain different groups in hot plate apparatus which heated to $(55 \pm 1) ^\circ\text{C}$, as described by (11,12and13). Formalin test was applied by placing each mouse in a transport cage, (10) microliters of (2%) formalin were injected subcutaneously into the planter region of the right hind paw, which produced pain that can be measured in terms of nociceptive response namely flinching and licking of injected paw, the number of licking and flinching were recorded which measured immediately after formalin injection and continued for (60) min (14,15and16). Six groups of mice for each sex (5 mice) were treated in the same manner as in the tail flick and hot plate tests except one group was given normal saline intraperitoneally (figure 2).

Data were analyzed by using completely Randomized Design in factorial experimental (Two-way) ANOVA. For calculation the effect (SPSS package 2008) probability of $(P < 0.05)$ were considered as significant differences.

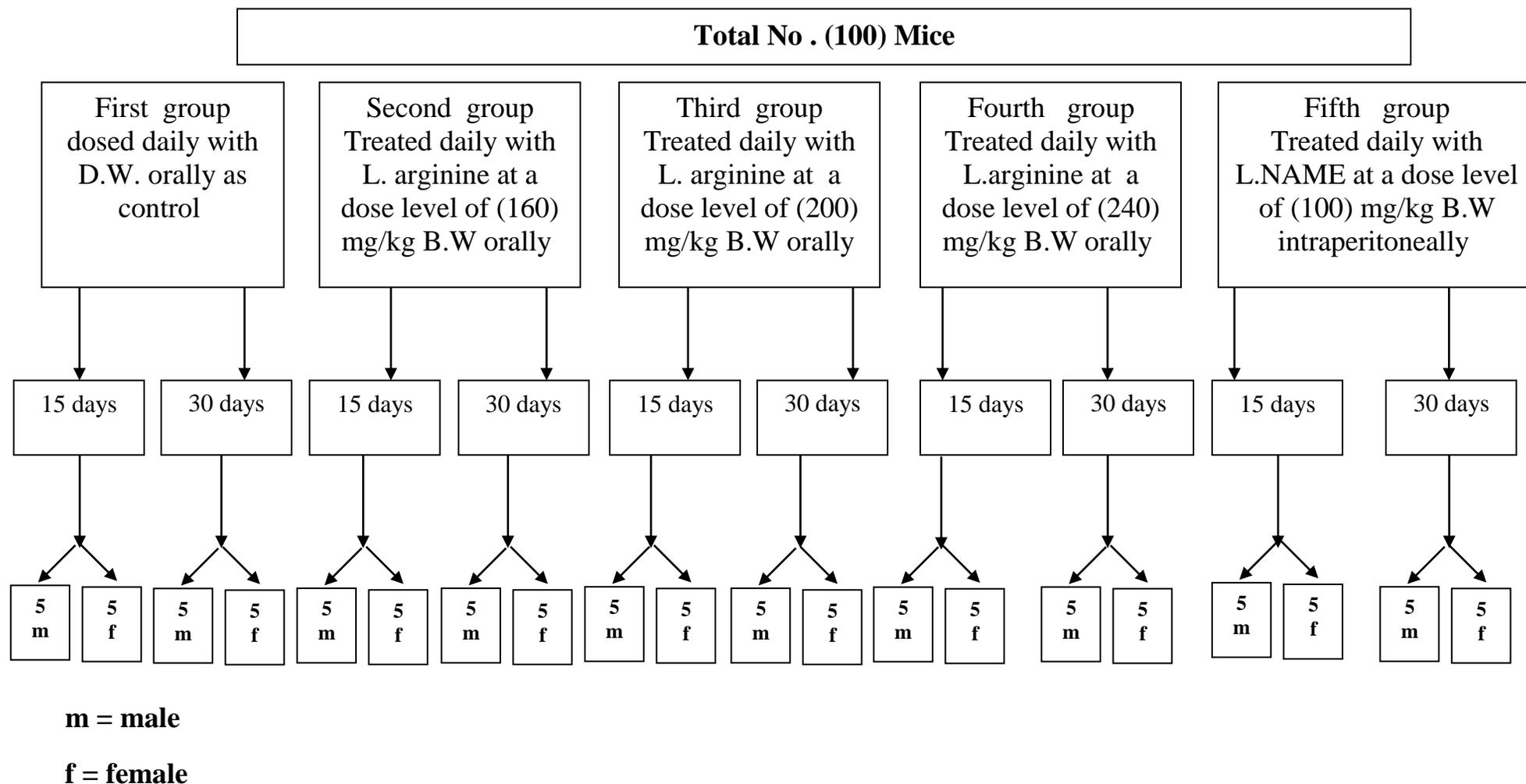
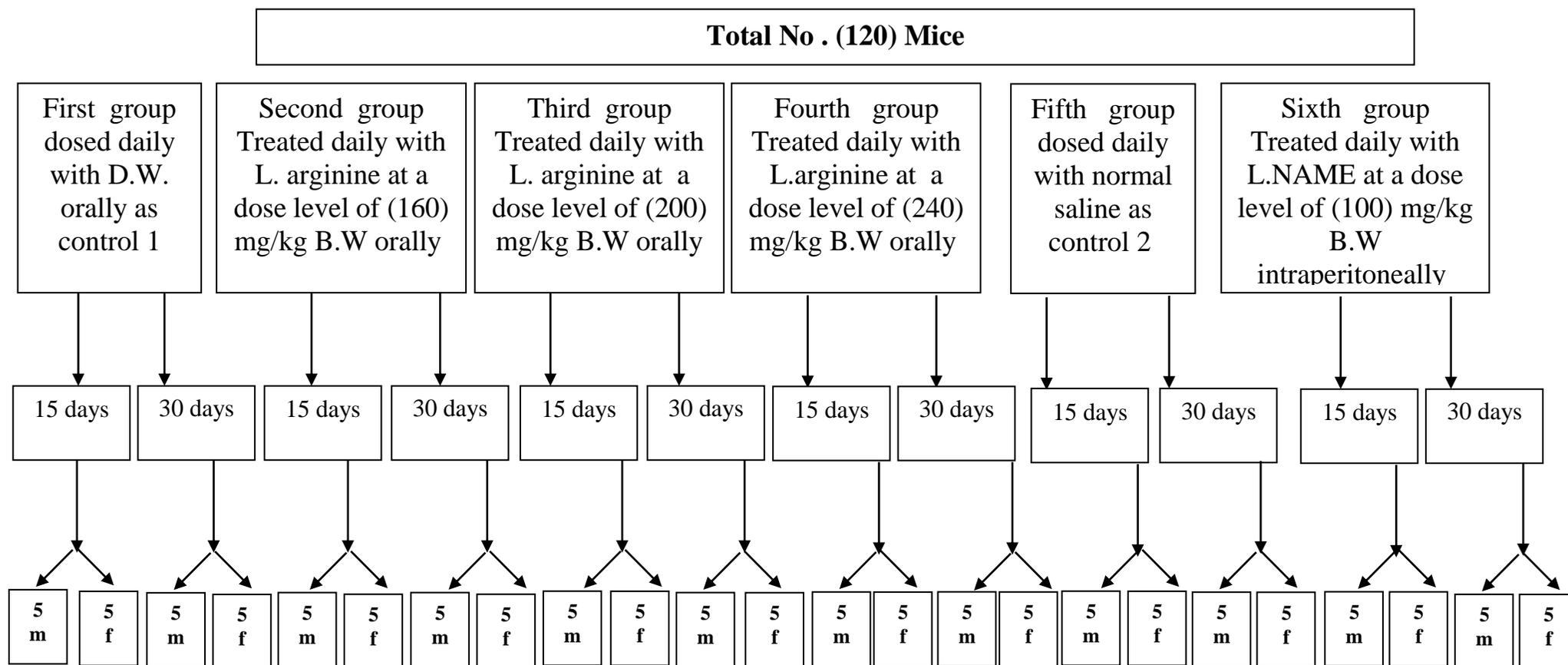


Figure (1): Experimental Design of Tail Flick and Hot Plate Test



m = male

f = female

Figure (2): Experimental Design of Formalin Test

Results and Discussion

The results of the effects of L-arginine and L-NAME on tail flick latency period table (1) and hot plate reactive time to thermal stimulus table (2) showed significant ($p < 0.05$) differences between post-treatments (15 and 30) days as compared with control and pretreatment groups, which displayed decrease in L-arginine treated groups and increase in L-NAME treated groups. Results showed significant increase ($p < 0.05$) in latency period displayed by females. Number of licking and flinching increase significantly ($p < 0.05$) in mice treated with L-arginine, on the other hand L-NAME cause significant reduction ($p < 0.05$) in number of licking and flinching in comparison with control groups table (3). There were significant ($p < 0.05$) differences between early and late phase after formalin injection and significant gender differences in all treated groups, which was significantly lower in female than in male mice in the number of licking and flinching.

The results of this experiment point to the participation of L-arginine-nitric oxide (NO) pathway in mediation of acute pain in female mice is not significant as in male mice. It has been shown that the inflammatory pain induced by epinephrine intradermally injection in the hind paw of the rat was dependent on sex hormones, because there were gender and sex hormone related differences in pain and nociception (17 and 18), which proposed that the modulatory effect of sex steroids on both nociceptive mechanisms of central and peripheral nervous system (CNS) that both oestrogen and androgen receptors are present on small-diameter dorsal root ganglion (DRG) neurons (19 and 20). Furthermore, sex hormones affect expression of protein kinase C (PKC), protein kinase A (PKA) and nitric oxide synthase (NOS) activity which were implicated in peripheral nociceptive mechanisms, and these messengers signaling pathways contribute to epinephrine induced hyperalgesia in males but not in females, due to suppression by oestrogen, which decreases epinephrine-induced hyperalgesia in females by suppressing contributions of protein kinase C (PKC) and protein kinase A (PKA) to pain signaling, (8). Furthermore, the gender differences in pain might be due to differences in nitrite and nitrate levels (nitric oxide metabolites) and activities founded in brain areas of cortex, hippocampus, corpus striatum, midbrain and cerebellum which is higher in adult male than female rats (9). Several reports had suggested a role of L-arginine-NO-cGMP pathway in central and peripheral nociceptive processing (21, 22 and 23), while nitric oxide (NO) mediated the N-methyl-D-aspartate (NMDA) produced facilitation of the nociceptive tail-flick reflexes which depends on the activity of spinal cord neurons (24 and 25). Nitric oxide (NO), which derived from L-arginine plays a role in nociceptive signaling due to localization of neuronal nitric oxide synthase (nNOS) in the superficial dorsal horn and intermediolateral cell column of spinal cord (2 and 3). Hot plate test was a marker test of supraspinal analgesia whereas, tail-flick was considered to be a measure of spinally mediated antinociception since the hot plate test was widely considered to be sensitive to drugs acting supraspinally (26 and 27). L-NAME, nitric oxide synthase inhibitor produce opioid-independent antinociceptive effects in the mouse, suggesting the role of NO-cGMP system in supraspinal transmission of nociceptive information and the antinociceptive in the mouse due to L-NAME is not antagonized by naloxone and is thus, independent of endogenous opioid release (21 and 22). In conclusion, the present data show that in male mice the inhibition of (NOS) in the brain but not in the spinal result in supraspinal analgesia. The response to the pain inhibition which was assessed by hot plate and tail-flick tests, may be sex steroid hormones related, like oestrogen interact with L-arginine-nitric oxide system and had involved a mechanism of pain feeling.

Table (1): The effect of L-arginine treated orally and L-NAME intraperitoneally daily on tail flick analgesic test latency period (second) in male and female mice.

Periods of treatment and sex Groups	Pre-treatment		Post-treatment (15 days)		Pre-treatment		Post-treatment (30 days)	
	Male	Female	Male	Female	Male	Female	Male	Female
D.W. as control.	6.00±0.30 ^{Bc}	9.00±0.22 ^{Ac}	-	-	6.80±0.33 ^{Bc}	9.60±0.20 ^{Ac}	-	-
L-arginine (160) mg / kg B. W.	4.00 ±0.01 ^{Ba}	6.06±0.11 ^{Aa}	2.50±0.20 ^{Cb}	4.66±0.08 ^{Db}	4.00±0.23 ^{Ba}	5.60±0.11 ^{Aa}	2.60±0.19 ^{Cb}	4.00±0.09 ^{Db}
L-arginine (200) mg / kg B. W.	4.00 ±0.01 ^{Ba}	6.20±0.10 ^{Aa}	2.40±0.03 ^{Cb}	4.70±0.12 ^{Db}	4.00±0.23 ^{Ba}	6.62±0.10 ^{Aa}	2.30±0.01 ^{Cb}	4.62±0.04 ^{Db}
L-arginine (240) mg / kg B. W.	4.16 ±0.08 ^{Ba}	6.00±0.11 ^{Aa}	2.40±0.04 ^{Cb}	4.74±0.14 ^{Db}	4.20±0.01 ^{Ba}	6.30±0.10 ^{Aa}	2.34±0.06 ^{Cb}	4.80±0.08 ^{Db}
L-NAME (100) mg /kg B. W.	9.00 ±0.11 ^{Bb}	7.72 ±0.10 ^{Ab}	12.60±0.24 ^{Ca}	10.40±0.16 ^{Da}	10.16±0.14 ^{Bb}	7.70±0.70 ^{Ab}	13.00±0.20 ^{Ca}	11.00±0.11 ^{Da}

Values are presented as Mean ±SE

Small letters denoted to (P<0.05) different between treated groups of certain sex.

Capital letters denoted to (P<0.05) gender differences.

Number = 5mice/group.

Table (2): The effect of L-arginine treated orally and L-NAME intraperitoneally daily on hot plate analgesic test the reactive time to thermal stimuli (second) in male and female mice.

Periods of treatment and sex Groups	Pre-treatment		Post-treatment (15 days)		Pre-treatment		Post-treatment (30 days)	
	Male	Female	Male	Female	Male	Female	Male	Female
D.W. as control	6.80±0.20 ^{Ac}	8.20±0.20 ^{Bc}	-	-	6.82±0.28 ^{Ac}	8.60±0.23 ^{Bc}	-	-
L-arginine (160) mg / kg B. W.	4.00 ±0.22 ^{Ba}	6.22 ±0.20 ^{Ab}	2.34 ±0.24 ^{Db}	4.80 ±0.09 ^{Cb}	4.80 ±0.28 ^{Ba}	6.00 ±0.23 ^{Ab}	2.30 ±0.30 ^{Db}	4.70 ±0.09 ^{Cb}
L-arginine (200) mg / kg B. W.	4.60 ±0.18 ^{Ba}	6.00 ±0.13 ^{Ab}	2.30 ±0.25 ^{Db}	4.76 ±0.10 ^{Cb}	4.40 ±0.28 ^{Ba}	7.00±0.44 ^{Ab}	2.20 ±0.28 ^{Db}	4.66 ±0.10 ^{Cb}
L-arginine (240) mg / kg B. W.	4.22 ±0.22 ^{Ba}	6.00 ±0.13 ^{Ab}	2.30 ±0.24 ^{Db}	4.72 ±0.09 ^{Cb}	4.16 ±0.27 ^{Ba}	6.20 ±0.40 ^{Ab}	2.24 ±0.25 ^{Db}	4.60 ±0.14 ^{Cb}
L-NAME (100) mg /kg B. W.	12.87 ±0.08 ^{Db}	10.30±0.34 ^{Ca}	19.80±0.73 ^{Aa}	12.00 ±0.20 ^{Ba}	12.60 ±0.25 ^{Db}	10.00 ±0.16 ^{Ca}	20.00 ±0.72 ^{Aa}	12.22 ±0.60 ^{Ba}

Values are presented as Mean±SE

Small letters denoted to (P<0.05) different between treated groups of certain sex.

Capital letters denoted to (P<0.05) gender differences.

Number = 5mice/group.

Table (3): The effect of L-arginine treated orally and L-NAME intraperitoneally daily on formaline analgesic test (the nociceptive response) in male and female mice.

Periods of treatment and sex Groups	Nociceptive response (Number of licking and flinching)							
	Early phase (0-5) min. after injection				Late phase (15-45) min. after injection			
	Male		Female		Male		Female	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
D.W. as control 1 group before injection of formaline	34.20 ±2.20 ^{Aa}	35.00 ±1.60 ^{Aa}	29.20 ±2.30 ^{Ba}	30.00±1.62 ^{Ba}	21.00 ±1.74 ^{Ca}	21.00±1.40 ^{Ca}	12.00 ±0.94 ^{Da}	18.60±0.74 ^{Da}
L-arginine (160) mg / kg B. W.	40.00 ±0.71 ^{Ab}	39.60 ±0.71 ^{Ab}	33.20 ±1.04 ^{Bb}	34.20 ±1.30 ^{Bb}	23.40 ±3.04 ^{Cb}	23.60 ±0.63 ^{Cb}	21.20 ±1.81 ^{Db}	21.00 ±0.67 ^{Db}
L-arginine (200) mg / kg B. W.	40.00 ±0.81 ^{Ab}	41.00 ±0.93 ^{Ab}	33.00 ±1.25 ^{Bb}	34.00 ±1.14 ^{Bb}	24.20 ±3.06 ^{Cb}	24.60 ±3.01 ^{Cb}	21.00 ±3.05 ^{Db}	20.60 ±0.53 ^{Db}
L-arginine (240) mg / kg B. W.	40.20 ±0.72 ^{Ab}	41.00±0.85 ^{Ab}	34.30±0.54 ^{Bb}	35.00 ±1.12 ^{Bb}	23.60±2.70 ^{Cb}	24.40±2.29 ^{Cb}	21.20±2.60 ^{Db}	20.80±0.28 ^{Db}
normal saline as a control 2 group before injection of formalin.	34.00±2.10 ^{Aa}	34.00±2.60 ^{Aa}	28.80±2.30 ^{Ba}	28.60±1.60 ^{Ba}	22.00±3.90 ^{Ca}	21.40±1.70 ^{Ca}	18.80±2.26 ^{Da}	19.00±1.32 ^{Da}
L-NAME (100) mg /kg B.W.	19.20±2.23 ^{Bc}	18.40±2.30 ^{Bc}	24.40±1.60 ^{Ac}	24.00±1.6 ^{Ac}	10.20±2.23 ^{Cc}	10.00±2.10 ^{Cc}	16.00±1.60 ^{Dc}	16.80±0.24 ^{Dc}

Values are presented as Mean ±SE

Small letters denoted to (P<0.05) different between treated groups of certain sex.

Capital letters denoted to (P<0.05) gender differences in early and late phase and between early and late phase .

Number = 5mice/group.

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