

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Analgesic Modulation of Tramadol, Amitriptyline and Gabapentin in Male and Female Wistar Rats

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### ABSTRACT

The central and peripheral analgesic response of tramadol, amitriptyline and gabapentin in male and female Wistar rats was measured using hot plate, tail-flick and acetic acid-induced writhing tests. In the first two methods withdrawal latency was measured at baseline and after administration of either tramadol (10 mg/kg, i.p.), amitriptyline (10 mg/kg, i.p.) or gabapentin (50 mg/kg, i.p.) at 15, 30, 60 and 120 minutes. In acetic acid induced writhing method, same drugs were given to both sex groups and then after 30 minutes, 2% acetic acid (2ml/kg, i.p.) was given and after 10 minutes number of writhes were counted. Tramadol showed significantly higher pain threshold in male rats than the female rats in response to hot plate test, tail-flick test and acetic acid induced writhing test. Amitriptyline treated male rats showed significantly higher pain threshold than the female rats in response to tail-flick method only. There was less number of writhing movements in gabapentin treated male rats as compared to female rats. The three drugs show different response in male and female rats. However, in some models there was no gender difference

**Keywords:** male and female rats, nociception, tramadol, amitriptyline, gabapentin, analgesic, acetic acid

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## INTRODUCTION

Studies suggest that sex is one of the factors influencing severity of pain sensation. Women report more pain than men and are at greater risk for developing many forms of chronic pain [1]. Numerous studies report sex differences in the analgesic effects of opioids. In humans, the mu agonist morphine and the mixed-action opioids pentazocine, butorphanol and nalbuphine produced greater or more prolonged analgesia in women than in men [2]. Sex differences in opioid antinociception also are widely reported in rodents, although they tend to be in the opposite direction to those observed in humans. Variables that may influence the magnitude of sex differences in opioid antinociception in rodents include opioid efficacy/selectivity, intensity of the noxious stimulus used in the pain test, and subject genotype (i.e. rodent strain). It is not yet known whether the opposite sex differences observed in human vs. animal studies reflect a true genotypic difference or are due to the different conditions under which opioid analgesia has been examined in human versus animal subjects.

Evidence from both human and animal studies also supports the use of tricyclic antidepressants like amitriptyline and newer anticonvulsants like gabapentin in neuropathic pain and in a number of specific chronic pain syndromes [3-8]. Earlier studies show that the analgesic response of selective serotonin reuptake inhibitor paroxetine does not vary in two sexes. Drugs like morphine (3mg/kg, i.p) and gabapentin (25mg/kg, i.p) administered to Sprague-Dawley rats, decreased postoperative mechanical hyperalgesia, but did not show any gender difference [9]. The aim of this study was to study whether the sex differences modulates the central as well as peripheral analgesic activity of tramadol, amitriptyline and gabapentin in the adult Wistar rats using different nociception animal models.

## MATERIALS AND METHODS

### Animals

Adult male and female Wistar albino rats weighing 150–300 g were housed in separate polypropylene cages, maintained under standard conditions with temperature (22–24<sup>o</sup> C), 12- h light/ dark cycle and relative air humidity 40–60%. Rats had continuous access to normocaloric standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and to tap water. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines on the use and care of experimental animals.

### Drugs and Reagents

Tramadol, Amitriptyline and Gabapentin were procured from Cadila Healthcare Ltd., Wockhardt Ltd., Intas Pharmaceuticals respectively. The other chemical reagents used in the

study were obtained from Merck Chemicals, Bangalore, India. Acetic acid (2%v/v) was used as writhing agent.

## **Experimental Procedure**

A total of 48 adult Wistar albino rats (24 male and 24 female) were divided into three groups based on the administered test drug. Each group contained 8 male and 8 female rats. Tramadol (10mg/kg), amitriptyline (10mg/kg) and gabapentin (50 mg/kg) was administered intraperitoneally to both male and female rats of their respective group 30 minutes prior to evaluation of analgesic activity with each nociception animal model [4].

## **Evaluation of analgesic activity**

### **Hot plate method**

The hot plate test was performed according to Woolfe and Mac Donald (1944). The surface of the hot plate measured 25.3 cm<sup>2</sup>, and was surrounded by 30 cm high Plexiglas walls with standard cage bedding covering the floor. Each rat was placed on the Eddy's hot plate which was kept at 55°C ± 1°C, and the withdrawal latency was noted by observing either the licking of the hind paws, jumping or the rotation movements at baseline (before drug administration), 15<sup>th</sup>, 30<sup>th</sup>, 60<sup>th</sup>, and 120<sup>th</sup> minute after drug administration. Withdrawal latency in seconds was measured and an increase in time interval was indicative of analgesia. A cut off time of twenty seconds was used to avoid tissue injury.

### **Tail flick method**

Antinociceptive response was determined with the tail-flick test by measuring the latency of the tail-flick response. Rats were gently held while the tail put on analgesimeter. The tail-flick response at baseline (before administering test drug), 15<sup>th</sup>, 30<sup>th</sup>, 60<sup>th</sup>, and 120<sup>th</sup> minute after drug administration was elicited by applying the radiation of heat from a heated nichrome wire to the dorsal surface at 1-1.5cm from the tip of the tail. Time between placing the tail of the rat on the radiant heat source and sharp withdrawal of the tail was recorded as "withdrawal latency". Cut off time of ten seconds was imposed in all sets of experiments taken as maximum latency so as to rule out thermal injury while noting down the reaction time of tail-flick.

### **Acetic acid induced writhing method**

Writhing was induced by an intra-peritoneal injection of 2% acetic acid (2ml/kg) thirty minutes after the test drug administration. 10 minutes after administering acetic acid, the number of writhing movements such as abdominal constriction/elongation of body/arching of back/hind limb extension/forelimb extension/trunk twisting were cumulatively counted over 20 minutes further for nociceptive evaluation [10,11].

## STATISTICAL ANALYSIS

Data were analyzed using SPSS software package version 16.0 (Statistical Program for Social Science). Comparison of antinociception effect between male and female rats of each group at different time intervals was done by general linear model repeated measures test for hot plate, tail-flick method and by nonparametric two independent samples test (Mann-Whitney test) for acetic acid induced writhing method. P value less than 0.05 was considered as statistically significant.

## RESULTS

### Analgesic activity using Hot plate method (Table-1)

**Tramadol-** Pain threshold was higher in males as compared to females. There was a significant difference ( $p= 0.04$ ) between male and female rats at 15 minute. Difference between the two groups was maximum at 15 minute and reduced thereafter.

**Amitriptyline-** There was no significant difference ( $p= 0.06$ ) between the analgesic response of males and females to amitriptyline. The reaction time in males was lower than females at 30 and 60 minute but by 120 minute the withdrawal reaction time was more in males.

**Gabapentin-** The difference between the reaction time in males and females was not significant ( $p= 0.83$ ). At 120 minute the withdrawal time was more in females.

**Table-1: Effect of drugs on withdrawal latency during Hot plate method**

Groups	Withdrawal latency (in seconds) Mean $\pm$ Std. Error of Mean				
	0 <sup>th</sup> min.	15 <sup>th</sup> min.	30 <sup>th</sup> min.	60 <sup>th</sup> min.	120 <sup>th</sup> min.
Tramadol M	5.54 $\pm$ 0.40	8.43 $\pm$ 1.08	5.82 $\pm$ 0.57	5.30 $\pm$ 0.57	4.18 $\pm$ 0.25
Tramadol F	5.14 $\pm$ 0.45	5.43 $\pm$ 0.27	4.66 $\pm$ 0.26	4.47 $\pm$ 0.29	4.20 $\pm$ 0.31
Amitriptyline M	5.10 $\pm$ 0.21	6.44 $\pm$ 0.38	6.44 $\pm$ 0.17	5.82 $\pm$ 0.20	6.05 $\pm$ 0.35
Amitriptyline F	4.60 $\pm$ 0.47	5.56 $\pm$ 0.65	6.87 $\pm$ 0.96	6.12 $\pm$ 0.77	4.98 $\pm$ 0.42
Gabapentin M	5.16 $\pm$ 0.37	6.90 $\pm$ 0.68	6.43 $\pm$ 0.37	5.66 $\pm$ 0.33	4.68 $\pm$ 0.29
Gabapentin F	5.56 $\pm$ 0.25	5.84 $\pm$ 0.31	6.31 $\pm$ 0.24	5.52 $\pm$ 0.17	5.23 $\pm$ 0.23

### Analgesic activity using tail-flick method-(Table-2)

**Tramadol-** Pain threshold was higher in males as compared to females. There was a significant difference ( $p= 0.012$ ) between male and female rats. Female rats had the maximum tail-flick latency at 60minute and reduced thereafter.

**Amitriptyline-** There was a significant difference ( $p < 0.001$ ) between males and females. The maximum effect was seen in males at 15 minute whereas in female rats it was at 30minute time interval after drug administration.

**Gabapentin-** The difference between tail flick latency was not significant ( $p= 0.291$ ) in two groups. But, the tail-flick latencies at different time intervals were comparable between male and female rats. The tail flick latency was more in males as compared to female rats throughout 15, 30, 60 and 120 minute. But at 120 minute, the reaction time increased in both sex groups.

**Table-2:Effect of drugs on tail withdrawal latency during Tail flick test**

Groups	Tail withdrawal latency (in seconds)				
	0 <sup>th</sup> min.	15 <sup>th</sup> min.	30 <sup>th</sup> min.	60 <sup>th</sup> min.	120 <sup>th</sup> min.
Tramadol M	0.97± 0.06	1.24± 0.06	1.18± 0.06	1.18± 0.12	1.24± 0.21
Tramadol F	0.79± 0.03	0.94± 0.07	1.00± 0.09	1.02± 0.13	0.88± 0.03
Amitriptyline M	1.76± 0.16	4.17± 0.27	1.91± 0.18	2.12± 0.21	1.78± 0.11
Amitriptyline F	1.26± 0.11	1.62± 0.16	1.74± 0.24	1.31± 0.09	1.33± 0.27
Gabapentin M	0.91± 0.06	1.315±0.15	1.16± 0.08	1.09± 0.07	1.20± 0.13
Gabapentin F	0.97± 0.05	1.13± 0.06	1.00± 0.08	1.02± 0.06	1.09± 0.06

**Analgesic activity: Acetic acid induced writhing method-(Table-3)**

**Tramadol-** The writhing counts decreased significantly in male rats after administering Tramadol. The difference between number of writhing was statistically significant ( $p= 0.041$ ) in male and female rats.

**Amitriptyline-** In number of writhing, there was no statistically significant difference between male and female rats ( $p= 0.132$ ). But, total number of writhes was comparable between both sexes with high variation in male rats.

**Gabapentin -** There was a statistically significant difference in number of writhing between male and female rats ( $p= 0.002$ ). But, comparing the number of writhing among these three drugs, it was observed that highest number of writhing was seen in Gabapentin treated male and female rats.

**Table-3: Effect of drug treatment on number of writhes during Acetic acid induced writhing test**

Groups	Number of writhes Mean (SEM)
Tramadol M	15.00 ± 3.96
Tramadol F	28.00 ± 3.29
Amitriptyline M	9.00 ± 3.51
Amitriptyline F	17.00 ± 1.57
Gabapentin M	37.00 ± 2.24
Gabapentin F	80.00 ± 6.38

## DISCUSSION

Sex-related differences in the perception and modulation of pain have been reported [2,12-14]. Controversial results have been reported as to whether there is a sex difference in the analgesic effect of morphine.

Our study, using the hot-plate, tail-flick as the model of central analgesia and writhing tests as peripheral analgesia, shows a clear sex difference in the potency of systemically administered tramadol. It is a synthetic codeine analog that is a weak  $\mu$ -receptor agonist with opioid and non-opioid like properties. In central as well as peripheral nociception models, with tramadol the pain threshold was higher in males as compared to females. Due to the faster onset of action, the maximum analgesic response of tramadol in males has been seen at 15 minute after drug administration and decreased thereafter (Figure 1) in hot plate method but in tail-flick method may be due to development of some adaptive mechanism, the tail-flick reaction time again increased at 120 min in male rats. In peripheral analgesic action, number of writhes were less in males as compared to female rats (Figure 2). Sex-specific differences in gonadal hormones, genetic factors, central nervous system pain and pain modulation circuitry, pharmacokinetic/pharmacodynamic factors and psychosocial factors have been advanced as potential mechanisms for sex differences in opioid analgesia. There are many possible molecular mechanisms via which estrogen might diminish Orphanin FQ (OFQ)-induced antinociception, including decreasing the expression of the (opioid receptor like-1) ORL1 receptor and/or its coupling to G-proteins which would secondarily modify the affinity of OFQ to the ORL1 receptor. Estrogen receptors ( $ER\alpha/ER\beta$ ) are present in spinal dorsal horn neurons and estrogen; in addition to altering the expression of opioid peptides alter the expression of the ORL1 receptor gene and protein in the trigeminal region and the hypothalamus. Reciprocally, the requirement for testosterone in mediating the antinociceptive effects of OFQ in the male could be attributable to up regulating expression of the ORL1 gene or enhancing coupling of OFQ receptors to ( $G_i/G_o$ ) proteins and thus to downstream effectors [2].

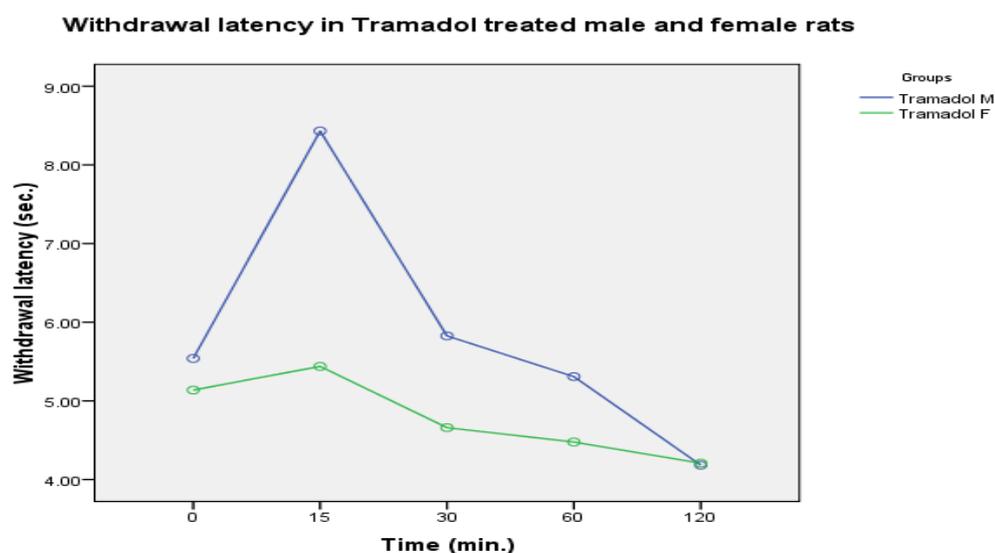


Figure 1 shows withdrawal latency of male and female rats treated with tramadol in hot plate method

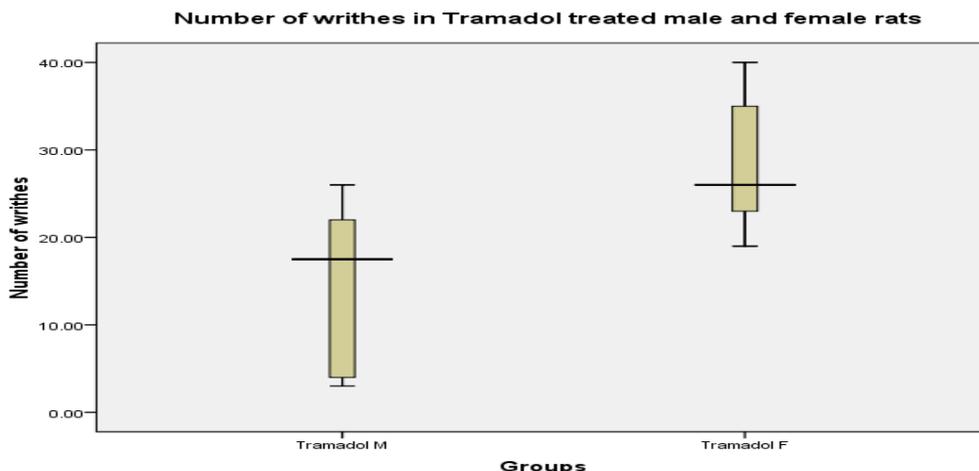


Figure 2 shows number of writhes in male and female rats treated with Tramadol

No gender difference has been seen in antinociceptive effect of selective serotonin reuptake inhibitor paroxetine when used as an adjuvant agent in some painful conditions [15]. In the present study, we have examined the analgesic effects of a tricyclic antidepressant amitriptyline, a blocker of both nor-epinephrine and 5-hydroxytryptamine that is currently used for clinical pain control, using hot plate, tail-flick and acetic acid-induced writhing test in male and female rats. In both sexes, amitriptyline showed the significant difference in reaction time in tail-flick method but not in hot plate and acetic acid induced writhing method. The abilities of antidepressants to express antinociceptive activity may vary dependent on the type of noxious stimuli used to evoke behavioral reactions [16]. In hot plate method, as compared to female rats the reaction time in males was more at 15 minute might be due to faster metabolism in males and again more at 120 minute. In tail-flick method, the male rats have the higher pain threshold than the female rats (Figure 3) and even the onset of action of amitriptyline in male rats is faster as compared to female rats. In acetic acid induced writhing method, amitriptyline comparatively reduced the number of writhes more prominently in males than female rats. But, in male rats there was more variation in total number of writhes. So, the significant difference did not come in both sex groups. The inhibition of writhing behavior observed in the antidepressants is based on changes in the pain threshold rather than on some impairment of motor activity [16]. The detailed neurochemical mechanisms involved in the analgesic properties of amitriptyline in both sexes is still unclear.

The present study also demonstrated that, gabapentin, a newer antiepileptic drug, did not produce a significant analgesic modulation in male and female rats using hot plate and tail-flick model for pain testing. The difference between the reaction time in males and females was not significant ( $p= 0.83$ ). At 120 minute the withdrawal time was more in females. But, the tail-flick latencies at different time intervals were comparable between male and female rats. There was increased tail flick latency in both males as well as female rats at 120 minute. Even though, the number of writhes produced after the administration of gabapentin was more as compared to tramadol and amitriptyline but in male rats it was lower than the female rats (figure 4). One interesting possibility is that gender differences in drug metabolism could result in a sex difference in analgesic effect [9]. It has been reported

that GBP decreases glutamate and glutamergic synaptic transmission presynaptically[17].The mechanism by which gabapentin exerts its analgesic action is unknown, but in animal models of analgesia, gabapentin prevents allodynia and hyperalgesia. In one recent study with gabapentin it has been put forth that various systems like arachidonate, nitroergic and serotonergic play an important role in the antinociceptive activity of gabapentin and that this effect occurred both centrally and peripherally [18]. Also gabapentin showed antiallodynic effect but different effects on mechanical and thermal hyperalgesia in another recent study.[19]

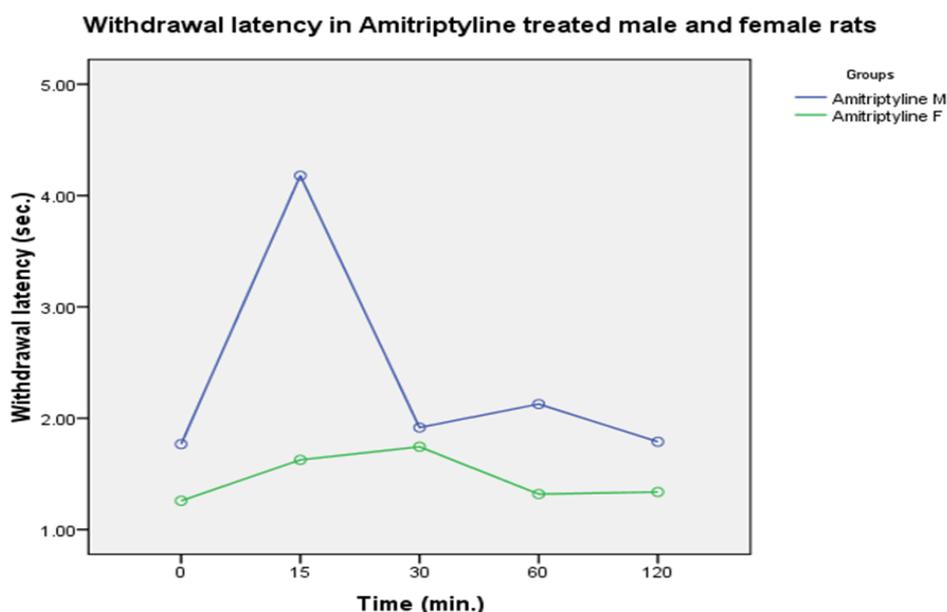


Figure 3 shows withdrawal latency in male and female rats treated with amitriptyline in tail flick method

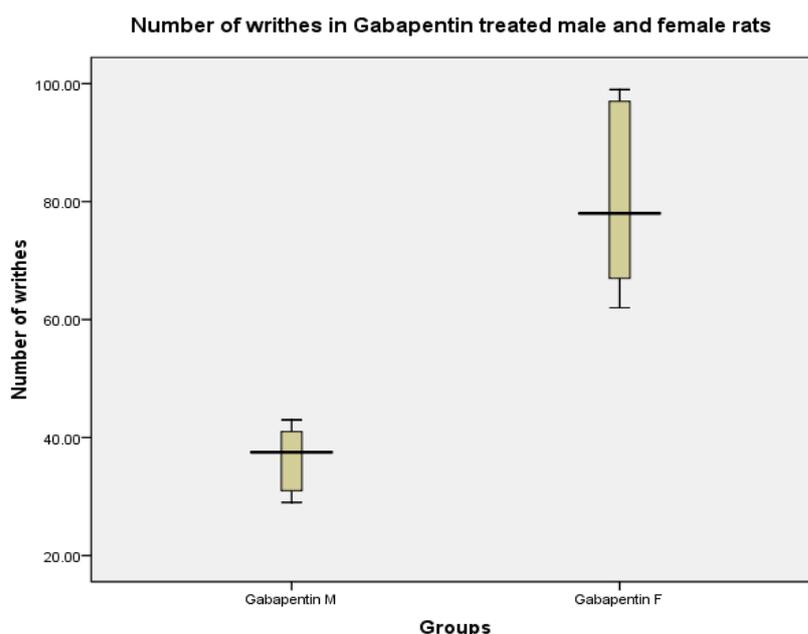


Figure 4 shows number of writhes in male and female rats treated with gabapentin

To conclude, sex related differences were observed in in all models in Tramadol treated group, in tail flick in amitriptyline group. Gabapentin showed differential effect in peripheral analgesic model i.e writhing test. Further studies would be necessary to clarify the detailed mechanisms on the present behavioral findings and whether sex specific management of clinical pain is required.

#### ACKNOWLEDGEMENT

The authors are thankful to Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, for support in this study.

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