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Research Article

INFLUENCE OF GENDER AND OBESITY ON ANALGESIC MODULATION OF TRAMADOL IN RATS

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ABSTRACT

Objective: The objective of this study was to investigate the influence of gender and obesity on analgesic modulation of tramadol in Wistar rats.

Methods: This study was carried out in two sets of experiments. In Set I experiment - 48 rats (body weight ≤150 g), 24 each male and female rats were randomly divided into two groups (n=6/group) (Group I - Control; 0.9% NaCl; 1 ml/kg/day i.p. and Group II - Tramadol 10 mg/kg/day i.p.) for each nociception model - plantar test and acetic acid-induced writhing test. The treatment duration was of 5 days. On the last day of treatment (i.e., on the 5th day), paw withdrawal latency (PWL) was assessed using plantar test, and writhing movements were observed following administration of 0.8% acetic acid; 10 ml/kg i.p. Set II experiment was repeated like Set I experiment among rest 48 high-fat diet-fed rats (body weight ≥300 g).

Results: For both males and females, PWL was significantly decreased ($p<0.05$) in obese control groups compared to lean control groups. A number of writhing movements were significantly increased ($p<0.01$ for males and $p<0.001$ for females) in obese control groups compared to lean control groups. In tramadol-treated obese rats, PWL was significantly decreased ($p<0.01$ for males and $p<0.05$ for females), and number of writhing movements were significantly increased ($p<0.01$ for both males and females) in comparison with the tramadol-treated lean rats.

Conclusion: The present study revealed that obese female rats experience more pain sensation to noxious stimuli compared to lean male rats and also the analgesic effect of tramadol is more pronounced in lean male rats compared to obese female rats.

Keywords: Pain threshold, Sex hormone, Opioids, Body weight, Writhing, Plantar, Acetic acid.

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INTRODUCTION

According to the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience arising from actual or potential tissue damage or described in terms of such damage [1]. Sex differences in pain and its control have long been a debated issue for scientists and health-care providers hoping to optimize pain treatment for the individual. The current scenario toward evidence-based medicine has further highlighted this issue. In the year 2007, the sex, gender, and pain special interest group of the IASP issued a consensus paper highlighting the need for inclusion of both males and females in pre-clinical and clinical studies on pain and its management [2]. In one of the randomized clinical trials, females have been shown to experience greater intensity of post-operative pain and less tolerance to pain compared to men [3].

Rosseland and Stubhaug stated gender difference in response to opioids, but their findings are inconsistent [4]. Some studies suggest that female patients experience greater analgesic efficacy compared to males following administration of mixed opioid agonist-antagonists, whereas other studies with morphine demonstrate that women require a higher drug dose compared to males to achieve analgesia of the same degree [3-5].

Several studies have positively correlated the experience of pain with an increase in body mass index (BMI) [6,7]. The causal relationship between the two remains unclear: It is not known whether obesity causes chronic pain, chronic pain causes obesity, or some other factors cause both concurrently. Obesity is hypothesized to lead to pain because of excess mechanical stresses and its proinflammatory state.

Obesity can affect pain threshold, emotional mood, and quality of life. Differences in pain thresholds and emotional mood may have implications for pain and depression management. Differences in pain thresholds may have implications for pain management, as they may account in part for the variability in analgesic requirements between individuals. In obese patients, pain thresholds would make it possible to predict the need for prescriptions of drugs with a narrow therapeutic margin, such as morphine [8]. McKendall and Haier conducted a study based on the hypothesis claiming that analgesic opiates are increased in obesity [9]. They applied a constant pressure of approximately three pounds to the tip of the thumb on 56 obese and non-obese patients to measure the time interval until the first sensation of pain. However, contrary to the expectations, the obese patients were found to be more sensitive to pain. Roane and Porter used tail pinch or tail flick pain stimulation tests and found that Zucker rats were more sensitive to pain [10].

There is need for further studies to investigate the influence of gender and obesity on pain threshold and analgesic modulation of opioids. This might help to modify the practice of prescribing analgesic medication according to gender and BMI. Tramadol is a synthetic, centrally acting analgesic agent with two distinct, synergistic mechanisms of action, and acting as both a weak opioid agonist and an inhibitor of monoamine neurotransmitter reuptake [11]. Earlier, we had reported the analgesic modulation of some analgesics in male and female Wistar rats [12]. Consequently, the aim of the present study was to investigate the effect of tramadol on pain threshold among male and female Wistar rats using plantar test (Hargreaves' method) and acetic acid-induced writhing test.

MATERIALS AND METHODS

Drugs and chemicals

Active pharmaceutical ingredient of tramadol was obtained from Sigma-Aldrich, Bangalore (India). Acetic acid and other chemicals were purchased from Merck Life Sciences Pvt. Ltd., Mumbai (India).

Normal saline (0.9% Sodium chloride) was purchased from pharmacy of Kasturba Hospital, Manipal, Karnataka (India).

Animals

A total of 96 Wistar rats (48 each male and female) weighing 100–150 g were housed in separate polypropylene cages, maintained under standard conditions with temperature (22–24°C), 12-h light/12-h dark cycle, and relative air humidity 40–60%. The animals were acclimatized to the laboratory conditions for 1 week before the start of the experiment. The animals were provided with a normal pellet diet (Amrit Feeds Ltd., Pune, India) and water *ad libitum*. The experimental protocol was approved by the institutional animal ethics committee (IAEC/KMC/41/2014), and experiments were conducted according to the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines.

Experimental design

A total of 96 Wistar rats were included in this study. The study was carried out in two sets of experiments. In Set I experiment - 48 lean rats (body weight 100–150 g), 24 each male and female rats were randomly divided into two groups (n=6/group) (Group I - control; 0.9% NaCl; 1 ml/kg/day i.p. and Group II - tramadol 10 mg/kg/day i.p.) for each nociception model - plantar test and acetic acid-induced writhing test (Fig. 1). Set II experiment was repeated like Set I experiment among rest 48 high-fat diet-fed (Vanaspati dalda + Coconut oil; 3:1; 10 ml/kg/day for 90 days) obese rats (body weight ≥300 g) (Fig. 2). The treatment duration of this study was 5 days. On the last day of treatment (i.e., on the 5th day), 15 min after administration of normal saline/tramadol, paw withdrawal latency (PWL) was assessed using plantar test, and writhing movements were observed following administration of 0.8% acetic acid; 10 ml/kg i.p [12-14].

High-fat diet-induced obesity model

For Set II experiment, 48 Wistar rats (100–150 g, 24 males and 24 females) were housed as two rats/cage. In addition to the normal pellet diet, these animals were orally fed mixture of Vanaspati dalda + Coconut oil (3:1) - 10 ml/kg/day for 90 days. After 90 days, these rats attained body weight approximately ≥300 g. Thereafter, Set II experiment was repeated like Set I experiment on these 48 obese (body weight ≥300 g).

Nociception models

Plantar test (Hargreaves' method)

Thermal pain threshold to radiant heat (IR-90) was quantified using the paw withdrawal test (Fig. 3). Rats were placed in a Perspex enclosure, without restraint and a movable infrared radiant heat source placed directly under the plantar surface of the hind paw (Ugo Basile, Como, Italy). The PWL to radiant heat was defined as the time from onset of the radiant heat to the withdrawal of the rat hind paw. The cutoff time for PWL was 30 s. Testing was alternated between hind paws and carried out at 3 min intervals. The average of three estimations was taken to yield mean PWL. Before any testing was carried out, rats could adjust to their environments for at least 10 min.

Acetic acid-induced writhing test

Writhing movement was induced by administering an intraperitoneal injection of 0.8% acetic acid (10 ml/kg), 15 min after the tramadol/normal saline administration. After 10 min of acetic acid administration, the number of writhing movements such as abdominal constriction/elongation of body/arching of back/hind limb extension/forelimb extension/trunk twisting (Fig. 4) was cumulatively counted over 20 min further for nociceptive evaluation [12-14].

Statistical analysis

Using Statistical Package for the Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, USA), data were expressed as mean ± standard deviation and analyzed by one-way analysis of variance followed by *post hoc* Tukey test. A level for p≤0.05 was considered as statistically significant.

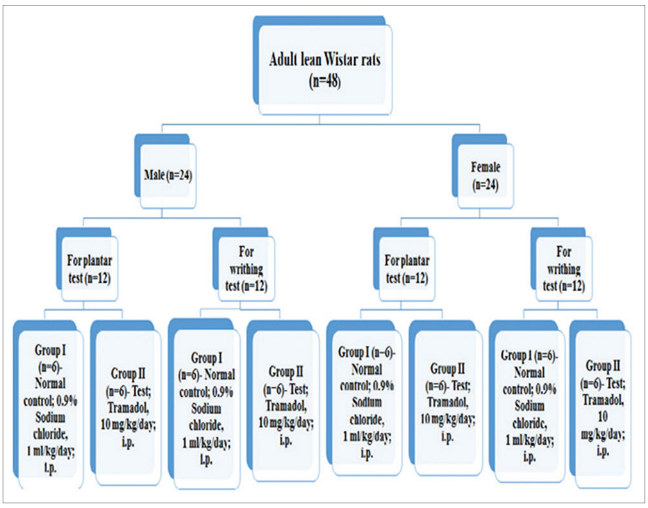

Fig. 1: Experimental design for lean Wistar rats (Set-I experiment)

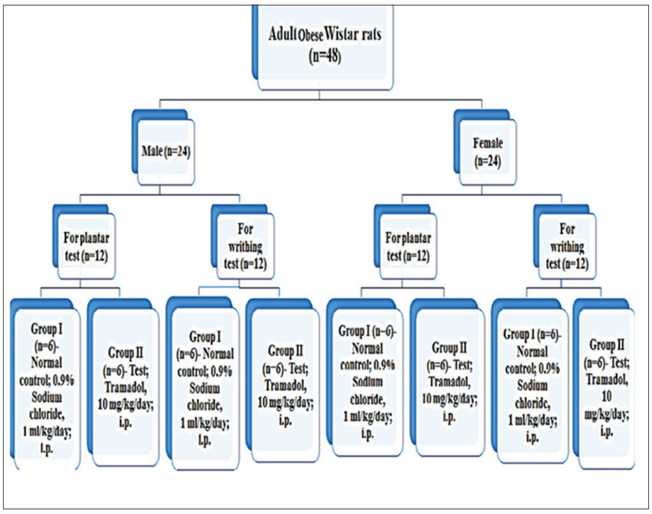

Fig. 2: Experimental design for obese Wistar rats (Set-II experiment)

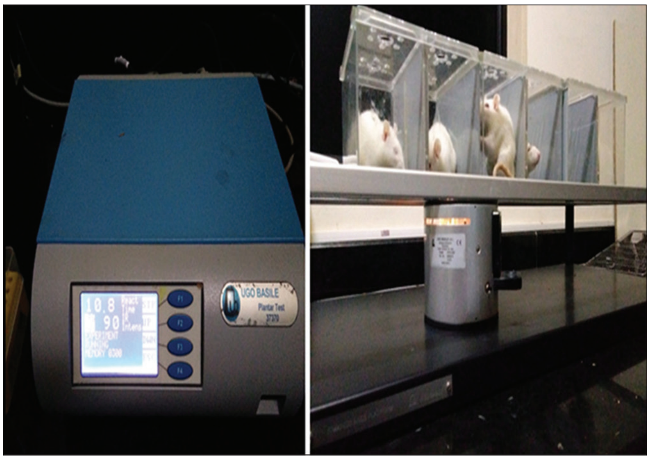

Fig. 3: Hargreaves apparatus for plantar test

RESULTS

For both lean and obese rats, PWL was significantly decreased ($p<0.001$) and number of writhing movements were significantly increased ($p<0.001$) in female control groups compared to male control groups. Furthermore, in tramadol-treated female rats, PWL was significantly decreased ($p<0.01$ for lean and $p<0.05$ for obese groups) and number of writhing movements were significantly increased ($p<0.001$ for both lean and obese groups) in comparison with the tramadol-treated male rats (Figs. 5 and 6).

For both males and females, PWL was significantly decreased ($p<0.05$) in obese control groups compared to lean control groups. Number of writhing movements were significantly increased ($p<0.01$ for males and $p<0.001$ for females) in obese control groups compared to lean control groups. In tramadol-treated obese rats, PWL was significantly decreased ($p<0.01$ for males and $p<0.05$ for females) and number of writhing movements were significantly increased ($p<0.01$ for both males and females) in comparison with the tramadol-treated lean rats (Figs. 7 and 8).

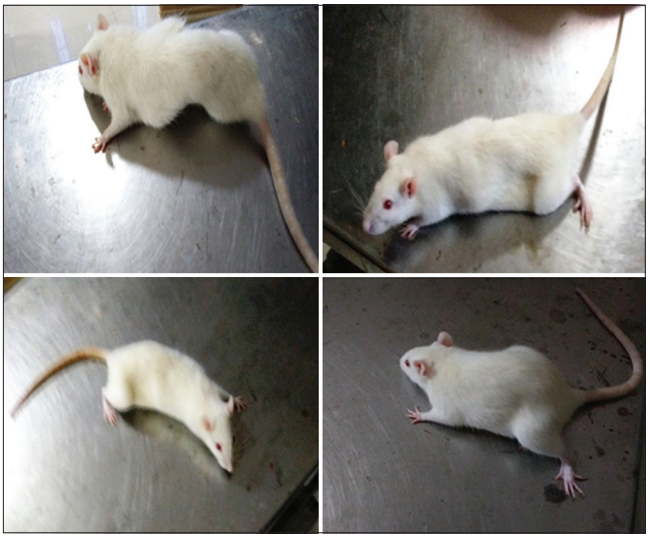

Fig. 4: Acetic acid-induced writhing movements

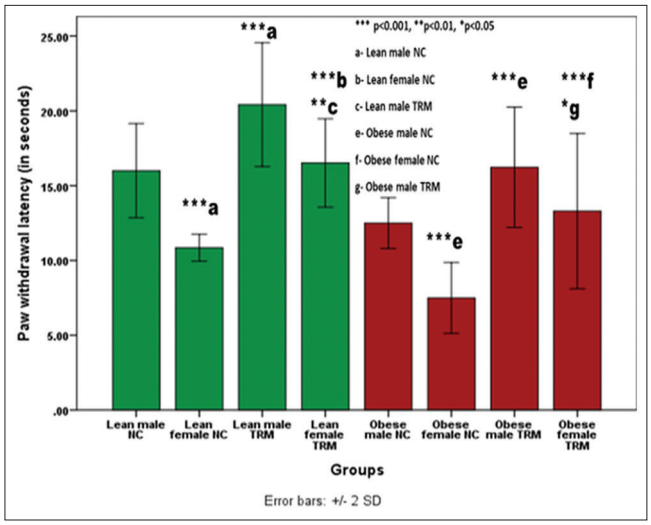

Fig. 5: Overall, comparison of paw withdrawal latency among both lean and obese male and female rats. n=6, number of rats in each group; NC: Normal control; TRM: Tramadol. Values are mentioned as mean. Error bars, ±2 standard deviation

DISCUSSION

The present study has demonstrated the influence of gender and obesity on analgesic modulation of tramadol in Wistar rats using plantar test and acetic acid-induced writhing test. This study has revealed the lower pain threshold in obese female rats than the lean male rats. In experimentally induced pain studies, the majority of results show that women are comparatively less tolerant and more sensitive to noxious stimulation than men [15-17]. Sex-related differences in pain may also reflect differences in the endogenous opioid system. The most studied of the endogenous pain modulatory systems is the endogenous opioid system, and sex differences in the functioning of this system could arise based on several different mechanisms. First, sex differences could result from differences in the distribution, expression, or sensitivity of opioid receptors in regions of the central nervous system involved in nociceptive processing. Sex-specific differences in gonadal hormones, genetic factors, central nervous system pain and pain

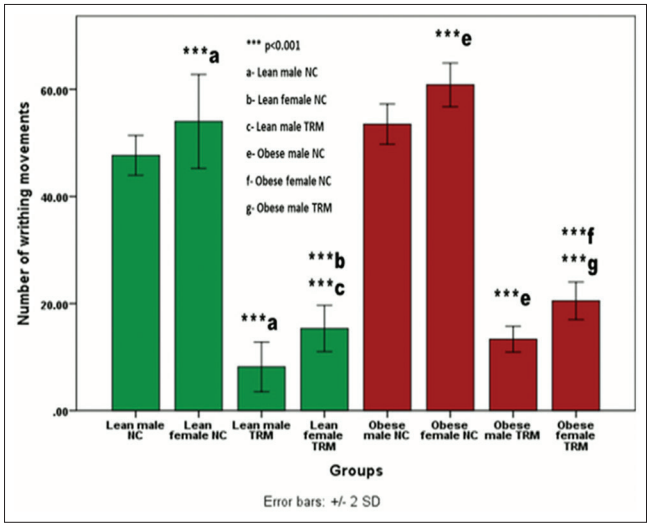

Fig. 6: Overall, comparison of number of writhing movements among both lean and obese male and female rats. n=6, number of rats in each group; NC: Normal control; TRM: Tramadol. Values are mentioned as mean. Error bars, ±2 standard deviation

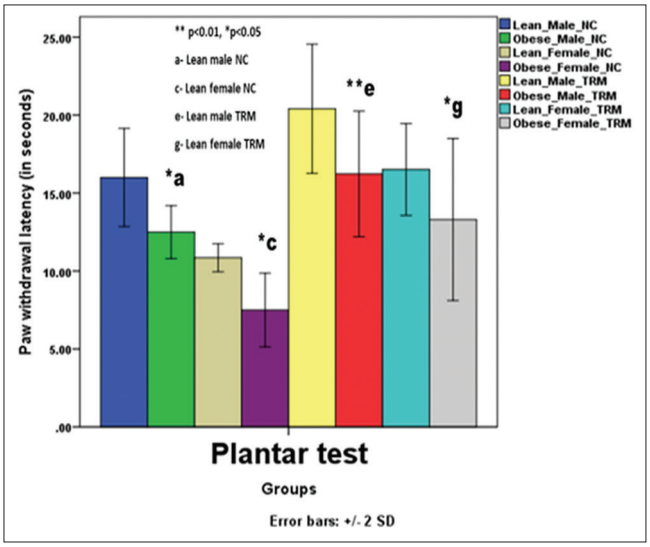

Fig. 7: Comparative analysis of paw withdrawal latency for lean versus obese rats. n=6, number of rats in each group; NC: Normal control; TRM: Tramadol. Values are mentioned as mean. Error bars, ±2 standard deviation

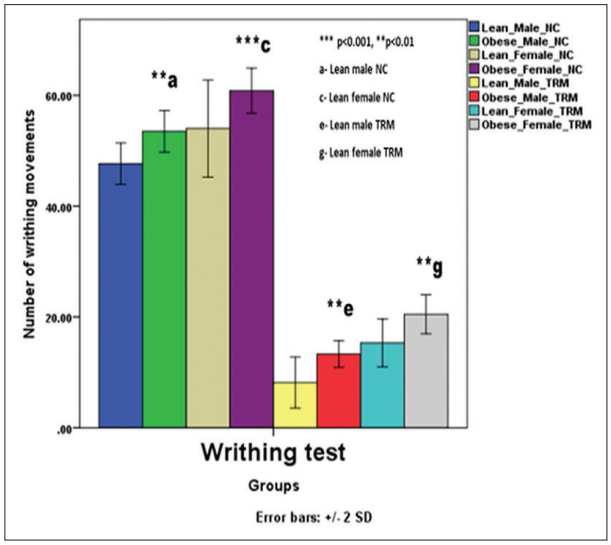

Fig. 8: Comparative analysis of number of writhing movements for lean versus obese rats. n=6, number of rats in each group; NC: Normal control; TRM: Tramadol. Values are mentioned as mean. Error bars, ±2 standard deviation

modulation circuitry, pharmacokinetic/pharmacodynamic factors, and psychosocial factors have been advanced as potential mechanisms for sex differences in opioid analgesia. Jomo *et al.* suggests that there are many possible molecular mechanisms through 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 [18]. Reciprocally, the requirement for testosterone in mediating the antinociceptive effects of OFQ in the male could be attributable to upregulating expression of the ORL1 gene or enhancing coupling of OFQ receptors to (Gi/Go) proteins and thus to downstream effectors [18]. Kepler *et al.* suggests that including gender as an independent variable, morphine does not produce the same degree of antinociception in male and female, especially following the induction of persistent pain [19]. Dahan *et al.* suggests that animal studies show a tendency for opioids to act more efficaciously in male, human studies are less clear in the presence and direction of any sex effect [20]. According to Palmeira *et al.*, the differences in pain perception related to sex may be associated with hyperalgesia in women but also to the hypoactivity of the inhibitory system of pain in females [21]. In a prospective observational study on 120 patients (60 males and 60 females), conducted by Hussain *et al.*, reported that female patients exhibited greater intensity of pain and required higher doses of analgesics compared to males in the immediate post-operative period to achieve a similar degree of analgesia [22].

Endocrine changes during the obesity may be responsible for the difference in the pain threshold. Leptin is a peptide (146 amino acid - long protein encoded by the obesity [ob] gene) secreted by adipose cells that act as a major regulator for food intake and energy homeostasis. Recent studies have shown that leptin, an adipocytokine, played a significant role in nociceptive behavior induced by nerve injury in rats [23,24]. Maeda *et al.* reported that the peripheral effect of leptin on neuropathic pain is mediated via macrophage stimulation [24]. Whereas, central effect of leptin on neuropathic pain is possibly due to the up-regulation of N-methyl-d-aspartate (NMDA) receptors following nerve injury [23]. This could be the reason for more pain in obese rats.

CONCLUSION

The present study revealed that obese female rats experience more pain sensation to noxious stimuli compared to lean male rats and also the analgesic effect of tramadol is more pronounced in lean male rats compared to obese female rats. Additional research to elucidate the mechanisms driving sex differences and obesity in pain responses is needed to foster future interventions to reduce these disparities in pain and analgesic modulation of tramadol. Henceforth, we are carrying out this study further on molecular level to find out the possible correlation among sex hormones, obesity, pain threshold, and tramadol. Gender and body mass index specific tailoring of pain treatments may become a conceivable outcome in the foreseeable future.

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AUTHORS' CONTRIBUTIONS

The first author has designed the study, carried out the experimental part of the work, data compilation, statistical analysis, interpretation of results, and manuscript writing. The second author has guided and monitored the experiment, statistical analysis, interpretation of result, and corrected the manuscript. The third author has coguided the first author.

CONFLICTS OF INTEREST

All the authors declare that they do not have any conflicts of interest.

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