Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Agomelatine alleviates pain-related behaviour in ovariectomized rats

Engin Korkmaz[a], Asiye Beytur[a], Suat Tekin[a,*]

[a]*Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Türkiye*

ARTICLE INFO

Keywords:
Agomelatine
Pain
Ovariectomy

Received: Dec 25, 2023
Accepted: Feb 02, 2024
Available Online: 27.02.2024

DOI:
10.5455/annalsmedres.2023.12.339

Abstract

Aim: Menopause is a physiological process that results in the cessation of ovarian follicle activity. Research suggests that postmenopausal women may experience changes in pain sensitivity. Ovariectomized (Ovx) rats are commonly used to mimic postmenopausal pain symptoms in research. Agomelatine is a unique antidepressant that acts as both a melatonergic receptor agonist and a 5-HT_{2C} receptor antagonist. Preclinical studies have suggested potential analgesic effects associated with agomelatine. This study aims to investigate the influence of agomelatine on pain behavior in Ovx rats.

Materials and Methods: Forty female *Sprague Dawley* rats were divided into four groups: Sham, Ovx, Ago20 and Ago40. The Ovx, Ago20 and Ago40 groups underwent bilateral ovariectomy, while the Sham group underwent all surgical procedures except ovarian ligation. After four months, the Sham and Ovx groups received vehicle, while the Ago20 (20mg/kg) and Ago40 (40mg/kg) groups received agomelatine by oral gavage for two months. Pain sensitivity assessments were conducted using electronic von Frey, hot plate, tail flick, and tail immersion methods after the final drug administration.

Results: In the Ovx group, there was an increase in pain sensitivity observed in the hot plate, electronic von Frey, tail flick, and tail immersion tests ($p < 0.05$). Agomelatine treatment significantly reduced the heightened nociceptive response ($p < 0.05$).

Conclusion: Agomelatine effectively attenuates the increased sensitivity to pain observed in Ovx rats.

Introduction

Menopause is a biological process characterised by the cessation of follicular activity, resulting in a significant decrease in serum oestrogen levels [1]. It usually occurs in women around their mid-forties. As women's average life expectancy has increased, a significant portion of their lives will be spent in the hypoestrogenic post-menopausal state [2]. Menopausal oestrogen depletion may contribute to the central regulation underlying chronic pain [3]. Estrogens are thought to affect pain through various mechanisms, such as modulating estrogen receptors in spinal neurons and the endogenous opioid system [3, 4]. Epidemiological studies have shown that women have a higher prevalence of painful conditions than men [5]. Experimental studies have demonstrated sex differences in functional and structural characteristics of pain pathways [6]. Ovx rats are frequently used to investigate changes in pain sensitivity in these conditions [3].

However, multiple studies have reported contradictory changes in pain sensitivity in castrated and Ovx animals. Many investigations into the effects of ovariectomy on pain sensitivity have been conducted in rodents, which have a short survival time post-surgery, contributing to the variability of observations [7]. Despite the variability observed in short-term studies, there are limited yet significant long-term studies that report increased pain sensitivity in Ovx rats [7-9].

Melatonin regulates circadian rhythms through melatonergic MT_1 and MT_2 receptors and has antioxidant, anti-neoplastic, anti-inflammatory and immunomodulatory effects [10]. Melatonin has the potential to be an analgesic through the action of MT_1 and MT_2 receptors [11]. Agomelatine is an antidepressant that acts as an MT_1 and MT_2 receptor agonist, similar to melatonin, while also acting as a 5-HT_{2C} receptor antagonist [12]. Several studies have demonstrated that agomelatine can reduce pain sensitivity in various disease models by acting on MT_1 and MT_2 receptors [13, 14]. Therefore, we investigated the effects of agomelatine on pain behaviour in long-term Ovx rats.

*Corresponding author:
Email address: suat.tekin@inonu.edu.tr (Suat Tekin)

Materials and Methods

Animals

The study was conducted after receiving approval (2022/14-2) from the local ethics committee of Inonu University Faculty of Medicine Animal Experiments. Forty female *Sprague Dawley* rats, aged 6 months and weighing 200-250 g, were used in this study. The number of animals used in the experiments was determined by power analysis. At least 10 animals were required in each group, with an average weight of 200-250g and a standard deviation of 25g. A 4% deviation, type 1 error (α) of 0.05 and type 2 error (β) of 0.80 were also considered. The study was carried out at the Inonu University Laboratory Animal Facility. The rats were kept in a regulated environment with a consistent temperature of 22±2°C and humidity of 50±10% under a 12:12 h light-dark cycle. During the experiment, the rats were given standard rodent chow and had unlimited access to water.

Experiment plan

The animals were administered a combination of 100 mg/kg ketamine (Keta-Kontrol, Doğa İlaç, Türkiye) and 10 mg/kg xylazine (Sanalazin20, Santavet, Türkiye) for anaesthesia before undergoing bilateral ovariectomy. The dorsolateral region was shaved and disinfected with 70% alcohol. An incision was made approximately 1.5 cm posterior to the right and left ribs to expose the abdominal muscles. The muscles were dissected to access the peritoneum. The adipose tissue was then carefully retracted until the ovaries were visible. Periovarian fat was also carefully retracted around the incision site to avoid detaching a small portion of ovarian tissue. The ovaries were then ligated and removed from the distal horn of the uterus. Finally, the uterine horn was reintegrated into the abdominal cavity and the muscles and skin were sutured. The sham groups, in which the ovaries were not ligated, underwent the same procedure [15].

Before the surgical procedure, the animals were randomly assigned to either the sham (n=10) or Ovx (n=30) groups based on their average body weight. Four months post-surgery, the Ovx rats were further divided into three groups with similar body weight averages: Ovx, Ago20, and Ago40 (n=10). Agomelatine (Valdoxan, Servier, Türkiye) was dissolved in normal saline. Sham and Ovx groups were administered a vehicle, while the Ago20 and Ago40 groups received two doses of agomelatine (20 and 40 mg/kg) via oral gavage for a period of two months. The dose was selected based on prior experiments that have demonstrated the efficacy of agomelatine [16]. Figure 1 shows the experiment's flow diagram.

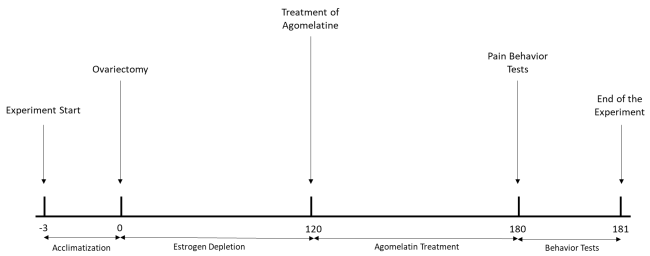

Figure 1. Flow diagram of this experiment.

Nociceptive behavior tests

Nociceptive behavior tests were conducted as previously described in Ovx rats [17, 18]. The animals were acclimatized to the apparatus 24 hours prior to the tests. The tests were conducted by a single researcher 24 hours after the last oral gavage administration. To reduce potential bias in the results, the researchers were blinded to the tests.

Hot plate

Hot plate test was performed by placing the animals on a warm surface inside a transparent Plexiglas cylinder measuring 15 cm in diameter and 22.5 cm in height to prevent escape. The hot plate analgesimeter was set at a temperature of 52°C [19, 20]. Response latency was recorded using an electronic timer, either by jump or paw lick. To prevent tissue damage, a cut-off time of 20 seconds was determined. Each rat was measured three times, with at least 10 minute intervals between each measurement, and the average value was calculated [21].

Electronic von Frey

The animals were housed in 20x20 cm enclosures with a raised metal mesh floor 40 cm above the surface. To evaluate withdrawal thresholds, an electronic von Frey hair apparatus (Ugo Basile, Italy) was used, which applied forces ranging from 0 to 100 g in increments of 0.2 g. Pulsed stimuli were directed to the mid-plantar region of the right hind paw through the mesh floor, and withdrawal thresholds were promptly displayed on the screen. Paw sensitivity was assessed by measuring the minimum pressure required to elicit an immediate and strong withdrawal reflex, excluding any voluntary movements attributable to the stimulus. The stimuli were applied to each hind paw at 5-minute intervals, and the final dataset was obtained by averaging five measurements [22].

Tail flick latency

Thermal antinociceptive activity was assessed using a tail flick device (Ugo Basile, Varese, Italy). The distal portion of the tail was positioned over a radiant heat source set at 52°C, and the duration of tail movement was recorded. To prevent potential tissue damage from the radiant heat, a maximum cut-off time of 15 seconds was established [23].

Tail immersion

During the study, the rats' lower tails were submerged in a beaker filled with cold water (0-2 °C), and the duration of tail flicking was recorded in seconds. To prevent potential tissue damage, a maximum cut-off time of 15 seconds was set [24].

Termination of experiment

Forty-eight hours after the last dose of agomelatine, the animals were decapitated.

Statistical analysis

The statistical analysis was executed using the IBM SPSS Statistics 22.0 software package for Windows. Quantitative data were expressed in terms of mean ± standard deviation (SD), with an evaluation of normal distribution adherence conducted through the Shapiro-Wilk test. Group-wise comparisons of quantitative variables were performed utilizing the Kruskal-Wallis H test. In the presence of statistically significant differences, post hoc analyses were executed employing the Mann-Whitney U test, with Bonferroni correction implemented for multiple comparisons. A predetermined alpha level of $p < 0.05$ was established as the threshold for statistical significance.

Results

After 8 weeks of treatment with agomelatine (starting 4 months after surgery), pain behaviour tests were performed. The OVX group (37.70 ± 3.48, n = 10) showed lower pain thresholds in the electronic von Frey test compared to the sham group (54.19 ± 5.46, n = 10). Pain-like behaviour in the von Frey test was significantly improved in rats in the Ago20 (48.95 ± 4.36, n = 10) and Ago40 (50.29 ± 3.48, n = 10) groups compared to untreated Ovx rats (37.70 ± 3.48, n = 10) ($p < 0.05$; Figure 2).

Thermal hyperalgesia was observed in Ovx rats (5.11 ± 1.21, n = 10) subjected to the tail flick test, i.e. there was a significant ($p < 0.05$) decrease in tail flick latency to radiant heat in Ovx rats (5.11 ± 1.21, n = 10) compared to sham rats (8.12 ± 1.32, n = 10) in the tail flick test.

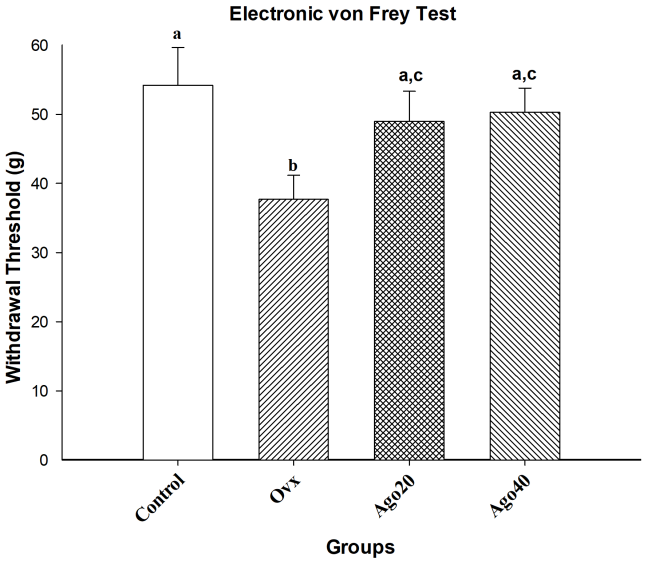

Figure 2. Electronic von Frey test. The data were subjected to statistical analysis using the Kruskal-Wallis H test for overall comparisons. Post hoc multiple comparisons were conducted employing the Bonferroni-corrected Mann-Whitney U test. The results are expressed as mean ± SD (standard deviation). Different superscript letters (a, b, c) denote statistically significant differences between groups at a significance level of $p < 0.05$. The sample size for each group was n=10.

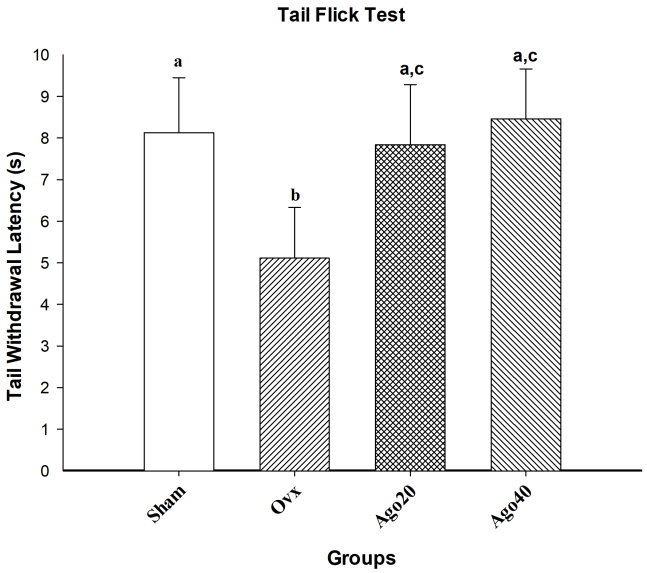

Figure 3. Tail-flick test. The data were subjected to statistical analysis using the Kruskal-Wallis H test for overall comparisons. Post hoc multiple comparisons were conducted employing the Bonferroni-corrected Mann-Whitney U test. The results are expressed as mean ± SD (standard deviation). Different superscript letters (a, b, c) denote statistically significant differences between groups at a significance level of $p < 0.05$. The sample size for each group was n=10.

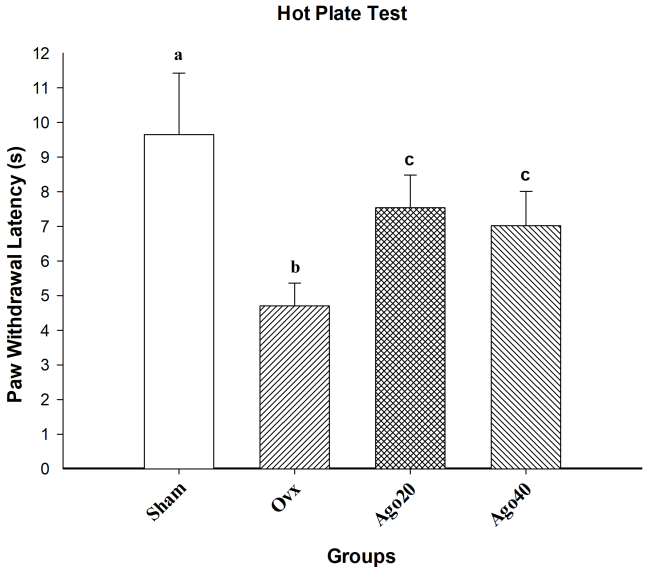

Figure 4. Hot-plate test. The data were subjected to statistical analysis using the Kruskal-Wallis H test for overall comparisons. Post hoc multiple comparisons were conducted employing the Bonferroni-corrected Mann-Whitney U test. The results are expressed as mean ± SD (standard deviation). Different superscript letters (a, b, c) denote statistically significant differences between groups at a significance level of $p < 0.05$. The sample size for each group was n=10.

Treatment of the Ago20 (7.84 ± 1.44, n=10) and Ago40 (8.46 ± 1.19, n=10) groups with agomelatine prevented

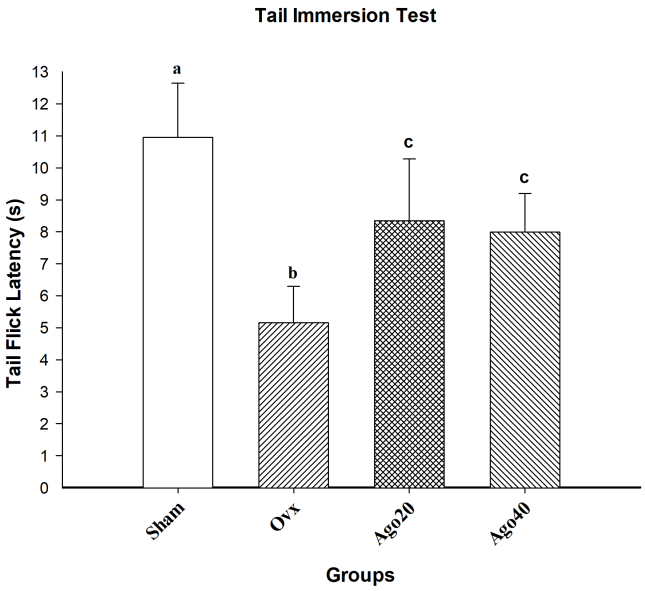

Figure 5. Tail immersion test. The data were subjected to statistical analysis using the Kruskal-Wallis H test for overall comparisons. Post hoc multiple comparisons were conducted employing the Bonferroni-corrected Mann-Whitney U test. The results are expressed as mean ± SD (standard deviation). Different superscript letters (a, b, c) denote statistically significant differences between groups at a significance level of $p < 0.05$. The sample size for each group was n=10.

the development of thermal hyperalgesia, i.e. there was no significant difference between the agomelatine-treated groups and the sham group ($p > 0.05$; Figure 3).

In the hot-plate test, the paw withdrawal threshold was statistically lower in the Ovx group (4.7 ± 0.66) compared to the sham group (9.65 ± 1.77); administration of agomelatine to Ago20 (7.54 ± 0.94) and Ago40 (7.02 ± 0.99) rats partially reversed this behaviour ($p < 0.05$; Figure 4).

The tail immersion test showed a significant decrease in tail flick latency in the Ovx group (5.16 ± 1.11) compared to the sham group (10.95 ± 1.69). This behaviour was partially reversed in the Ago20 (8.35 ± 1.92) and Ago40 (7.99 ± 1.20) groups ($p < 0.05$; Figure 5).

Discussion

Research suggests that menopausal women may experience higher incidence of musculoskeletal pain, headache, backache and fibromyalgia due to the decline in estrogen [25]. Managing pain in menopausal women is a clinical challenge due to the complex underlying mechanisms [26]. The role of sex steroids in acute nociception is still unclear. Studies have reported that rats exposed to estrogen deficiency through Ovx are more sensitive to painful stimuli than intact rats [8, 18, 27]. Conversely, an another study disclosed that rats subjected to ovariectomy exhibited diminished nociceptive responses during the inflammatory pain period [28]. The reason for these differences may depend on the duration of ovariectomy, as adipose tissue remains an important source of estrogen even after ovary removal. Adipose tissue-derived estrogen can regulate several gene expressions [29]. After ovariectomy, gene expression may take a long time to change due to estrogen depletion [30]. The onset of hyperalgesia and allodynia in Ovx rodents has been reported to take up to 4 months [31].

In this study, agomelatine was administered in the fourth month of the experiment, after hormonal depletion and the development of hyperalgesia. At the termination of the study, an escalation in pain sensitivity was discerned among Ovx rats through behavioral assessments, encompassing the tail flick, hot plate, tail immersion, and von Frey tests. Long-term studies (4-6 months) monitoring the effects of hormonal depletion after ovariectomy reported increased pain sensitivity in rodents, which is similar to our findings [8, 9].

In this study, agomelatine treatment effectively reversed the increased sensitivity observed in behavioral tests (tail flick, hot plate, tail immersion, and von Frey tests) in both groups. Similarly to our study, agomelatine treatment improved and effectively reversed increased pain sensitivity in the hot plate test in rats with visceral nociception [32]. Also, in rats with diabetic neuropathy, agomelatine significantly decreased allodynic responses in behavioral tests of thermal and mechanical hyperalgesia, consistent with the findings in our study [14]. In parallel with our study, agomelatine demonstrated antinociceptive potential in behavioral tests of pain. The authors hypothesized that this effect was mediated by MT_1 and MT_2 receptors. The study found that the antinociceptive effect of agomelatine was abolished by the administration of melatonergic receptor antagonists, indicating the important role of melatonergic agonism in reducing pain sensitivity [13].

MT_1 and MT_2 receptors are located in the thalamus, anterior cingulate cortex, and dorsal horns of the spinal cord, which are important targets for pain relief [33]. Preclinical studies have demonstrated that the MT_2 receptor mediates antinociceptive effects [11, 34]. In an experimental pain model in mice, pyromelatine, which is an agonist for both MT_1 and MT_2 receptors, was found to have an antinociceptive effect in the von Frey test, similar to our study [35]. Additionally, a selective partial agonist for MT_2 receptors demonstrated analgesic effects by modulating antinociceptive pathways in two different pain models in rats. In the same study, the compound reduced allodynia in the tail flick test, which is consistent with our findings [36].

Furthermore, the serotonergic system is significant in pain modulation via 5-HT receptors. While there are conflicting reports on the impact of 5-HT_{2C} receptors on pain, they are predominantly associated with a pronociceptive effect [37]. In various rodent pain models, it was observed that 5-HT_{2C} receptors were increased [38]. Chenaf et al. investigated the mechanism of the antinociceptive effect of agomelatine. The study found that the antinociceptive effect of agomelatine was eliminated when administered with a specific 5-HT_{2C} receptor antagonist that strongly binds to the 5-HT_{2C} receptor. These results suggest that blocking the 5-HT_{2C} receptor is crucial for the antinociceptive effect of agomelatine [13].

Therefore, the pain behaviour modulation observed in our study may be attributed to the interactive effects of agomelatine as both a melatonergic receptor agonist and

a serotonergic receptor antagonist.

This study examines the effects of agomelatine on the nociceptive response in Ovx rats. The findings suggest that the nociceptive hypersensitivity response in long-term Ovx rats can be used as a translational model of menopause. The results indicate that agomelatine has a partial analgesic effect on ovariectomy-induced pain. These observations suggest that agomelatine may be considered as an additional therapeutic agent for this condition.

However, it is important to note that our study has two limitations. Firstly, there was no group that received estrogen together with agomelatine. Secondly, all behavioural tests were performed on the same day.

Conclusion

In summary, this study suggests that the administration of agomelatine may alleviate post-ovariectomy pain. However, further research is necessary to elucidate the underlying molecular mechanisms. The results indicate that agomelatine holds promise as a prospective therapeutic intervention for alleviating postmenopausal pain symptoms.

Acknowledgements

Not applicable.

Availability of data and materials

The data generated in the present study are available upon request from the corresponding author.

Author's contributions

Conceptualization, E.K.; methodology, E.K.and A.B. software, S.T.; formal analysis, S.T.; data curation S.T.; writing, original draft preparation, E.K.; All authors have read and agreed to the published version of the manuscript.

Ethical approval

This study received approval from the Ethical Committee of Experimental Animals at the Faculty of Medicine, Inonu University, with the assigned approval number 2022/14-2. The authors affirm that there are no ethical conflicts to disclose.

Declaration of competing interests/Conflict of interest

The authors declare that they have no competing interests.

References

1. Yu Z, Jiao Y, Zhao Y, Gu W. Level of Estrogen in Females—The Different Impacts at Different Life Stages. Journal of Personalized Medicine. 2022;12(12):1995.
2. Mosconi L, Berti V, Dyke J, Schelbaum E, Jett S, Loughlin L, et al. Menopause impacts human brain structure, connectivity, energy metabolism, and amyloid-beta deposition. Scientific reports. 2021;11(1):10867.
3. Amandusson Å, Blomqvist A. Estrogenic influences in pain processing. Frontiers in neuroendocrinology. 2013;34(4):329-49.
4. Amandusson Å, Blomqvist A. Estrogen receptor-α expression in nociceptive-responsive neurons in the medullary dorsal horn of the female rat. European journal of pain. 2010;14(3):245-8.
5. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choinière M. A systematic literature review of 10 years of research on sex/gender and pain perception–part 2: do biopsychosocial factors alter pain sensitivity differently in women and men? Pain. 2012;153(3):619-35.
6. Vacca V, Marinelli S, Pieroni L, Urbani A, Luvisetto S, Pavone F. Higher pain perception and lack of recovery from neuropathic pain in females: a behavioural, immunohistochemical, and proteomic investigation on sex-related differences in mice. PAIN®. 2014;155(2):388-402.
7. Fillingim RB, Ness TJ. Sex-related hormonal influences on pain and analgesic responses. Neuroscience & Biobehavioral Reviews. 2000;24(4):485-501.
8. Ceccarelli I, Fiorenzani P, Massafra C, Aloisi AM. Long-term ovariectomy changes formalin-induced licking in female rats: the role of estrogens. Reproductive Biology and Endocrinology. 2003;1:1-9.
9. Pajot J, Ressot C, Ngom I, Woda A. Gonadectomy induces site-specific differences in nociception in rats. Pain. 2003;104(1-2):367-73.
10. Singh M, Jadhav HR. Melatonin: functions and ligands. Drug discovery today. 2014;19(9):1410-8.
11. Xie S, Fan W, He H, Huang F. Role of melatonin in the regulation of pain. Journal of pain research. 2020:331-43.
12. Millan MJ. Agomelatine for the treatment of generalized anxiety disorder: focus on its distinctive mechanism of action. Therapeutic Advances in Psychopharmacology. 2022;12:20451253221105128.
13. Chenaf C, Chapuy E, Libert F, Marchand F, Courteix C, Bertrand M, et al. Agomelatine: a new opportunity to reduce neuropathic pain—preclinical evidence. Pain. 2016;158(1):149-60.
14. Aydın TH, Can ÖD, Demir Özkay Ü, Turan N. Effect of subacute agomelatine treatment on painful diabetic neuropathy: involvement of catecholaminergic mechanisms. Fundamental & Clinical Pharmacology. 2016;30(6):549-67.
15. Moazen P, Taherianfard M, Ahmadi Soleimani M, Norozpor M. Synergistic effect of spexin and progesterone on pain sensitivity attenuation in ovariectomized rats. Clinical and Experimental Pharmacology and Physiology. 2018;45(4):349-54.
16. Promsan S, Thongnak L, Pengrattanachot N, Phengpol N, Sutthasupha P, Lungkaphin A. Agomelatine, a structural analog of melatonin, improves kidney dysfunction through regulating the AMPK/mTOR signaling pathway to promote autophagy in obese rats. Food and Chemical Toxicology. 2022;165:113190.
17. Dohke T, Iba K, Hanaka M, Kanaya K, Okazaki S, Yamashita T. Teriparatide rapidly improves pain-like behavior in ovariectomized mice in association with the downregulation of inflammatory cytokine expression. Journal of bone and mineral metabolism. 2018;36:499-507.
18. da Silva Moreira SF, Medeiros LF, de Souza A, de Oliveira C, Scarabelot VL, Fregni F, et al. Transcranial direct current stimulation (tDCS) neuromodulatory effects on mechanical hyperalgesia and cortical BDNF levels in ovariectomized rats. Life Sciences. 2016;145:233-9.
19. Ozcan S, Kelestemur MM, Hekim MG, Bulmus O, Bulut F, Bilgin B, et al. Asprosin, a novel therapeutic candidate for painful neuropathy: an experimental study in mice. Naunyn-Schmiedeberg's Archives of Pharmacology. 2022;395(3):325-35.
20. Deuis JR, Dvorakova LS, Vetter I. Methods used to evaluate pain behaviors in rodents. Frontiers in molecular neuroscience. 2017;10:284.
21. Delgado MA, Ferreira LA, dos Santos Gomes BJ, Leite IKO, Gomez MV, Castro-Junior C. Preclinical study in a postoperative pain model to investigate the action of ketamine, lidocaine, and ascorbic acid in reversing fentanyl-induced, non–glutamate-dependent hyperalgesia. PAIN Reports. 2023;8(2):e1062.
22. Di Cesare Mannelli L, Pacini A, Corti F, Boccella S, Luongo L, Esposito E, et al. Antineuropathic profile of N-palmitoylethanolamine in a rat model of oxaliplatin-induced neurotoxicity. PloS one. 2015;10(6):e0128080.
23. Campos AR, Santos FA, Rao VS. Ketamine-induced potentiation of morphine analgesia in rat tail-flick test: role of opioid-, α2-adrenoceptors and ATP-sensitive potassium channels. Biological and Pharmaceutical Bulletin. 2006;29(1):86-9.
24. Yemitan OK, Adeyemi OO. Mechanistic assessment of the analgesic, anti-inflammatory and antipyretic actions of Dalbergia saxatilis in animal models. Pharmaceutical biology. 2017;55(1):898-905.
25. Li L-H, Ling D-D, Lin H, Wang Z-C, Sun Z-R, Zhang Y-Q, et al. Ovariectomy induces hyperalgesia accompanied by upregulated estrogen receptor α and protein kinase B in the rat spinal cord. Physiology & behavior. 2023;271:114342.

26. Meriggiola MC, Nanni M, Bachiocco V, Vodo S, Aloisi AM. Menopause affects pain depending on pain type and characteristics. Menopause. 2012;19(5):517-23.
27. Sanoja R, Cervero F. Estrogen-dependent abdominal hyperalgesia induced by ovariectomy in adult mice: a model of functional abdominal pain. Pain. 2005;118(1-2):243-53.
28. Gaumond I, Arsenault P, Marchand S. Specificity of female and male sex hormones on excitatory and inhibitory phases of formalin-induced nociceptive responses. Brain research. 2005;1052(1):105-11.
29. Deslypere JP, Verdonck L, Vermeulen A. Fat tissue: a steroid reservoir and site of steroid metabolism. The Journal of Clinical Endocrinology & Metabolism. 1985;61(3):564-70.
30. Cyr M, Bosse R, Di Paolo T. Gonadal hormones modulate 5-hydroxytryptamine2A receptors: emphasis on the rat frontal cortex. Neuroscience. 1998;83(3):829-36.
31. Zhang W, Wu H, Xu Q, Chen S, Sun L, Jiao C, et al. Estrogen modulation of pain perception with a novel 17β-estradiol pretreatment regime in ovariectomized rats. Biology of sex Differences. 2020;11:1-13.
32. Kasap M, Can Ö D. Opioid system mediated anti-nociceptive effect of agomelatine in mice. Life Sci. 2016;163:55-63.
33. Uz T, Arslan AD, Kurtuncu M, Imbesi M, Akhisaroglu M, Dwivedi Y, et al. The regional and cellular expression profile of the melatonin receptor MT1 in the central dopaminergic system. Molecular brain research. 2005;136(1-2):45-53.
34. Ambriz-Tututi M, Granados-Soto V. Oral and spinal melatonin reduces tactile allodynia in rats via activation of MT2 and opioid receptors. Pain. 2007;132(3):273-80.
35. Liu Y-Y, Yin D, Chen L, Qu W-M, Chen C-R, Laudon M, et al. Piromelatine exerts antinociceptive effect via melatonin, opioid, and 5HT 1A receptors and hypnotic effect via melatonin receptors in a mouse model of neuropathic pain. Psychopharmacology. 2014;231:3973-85.
36. Lopez-Canul M, Palazzo E, Dominguez-Lopez S, Luongo L, Lacoste B, Comai S, et al. Selective melatonin MT2 receptor ligands relieve neuropathic pain through modulation of brainstem descending antinociceptive pathways. Pain. 2015;156(2):305-17.
37. Liu QQ, Yao XX, Gao SH, Li R, Li BJ, Yang W, et al. Role of 5-HT receptors in neuropathic pain: potential therapeutic implications. Pharmacological Research. 2020;159:104949.
38. Ren LQ, Wienecke J, Chen M, Møller M, Hultborn H, Zhang M. The time course of serotonin 2C receptor expression after spinal transection of rats: an immunohistochemical study. Neuroscience. 2013;236:31-46.