Int J Basic Sci Med. 2022;7(1):28-33 Original Article

http://ijbsm.zbmu.ac.ir/ doi 10.34172/ijbsm.2022.06

Comparison of Analgesic Effect of Rosmarinic Acid With Piroxicam in Mice

Mohsen Moradi[1], Mahdi Mashhadi Akbar Boojar[2*], Mehdi Saberi[2], Hamidreza Rahimi Salkuyeh[1], Maryam Ghorbani[2], Hasan Amanpour[1], Fateme Salem[2]

[1]Student Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran

***Correspondence to**
Mahdi Mashhadi Akbar Boojar,
Email: mahdimashhadi@yahoo.com

Received January 22, 2022
Accepted March 25, 2022
Published online March 31, 2022

Abstract

Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used drugs in the treatment of pain and inflammation. Although, their side effects i.e.; gastrointestinal and renal adverse effects, limit the use of these drugs. Therefore, investigating new compounds with higher efficacy and fewer side effects is considerable issue. In this study, the analgesic activity of rosmarinic acid at different doses was compared with piroxicam (known standard NSAID).

Methods: To evaluate the analgesic effects of rosmarinic acid, two well-known methods, Hot plate, and Tail-flick, were used. The experiments were performed in 10 groups (6 male mice per group) including the experimental groups (rosmarinic acid), the positive control group (piroxicam), and the negative control group (normal saline). All administrations were through intraperitoneal injection. The obtained data were analyzed separately by SPSS software and an independent student *t* test.

Results: Rosmarinic acid at doses of 10 mg/kg and higher had significant analgesic effects compared with the control group, 30 minutes after injection in both hot plate and tail flick tests ($P<0.05$). Concomitant injection of 50 mg/kg of rosmarinic acid with 15 mg/kg of piroxicam produced significant painkiller effects when compared with the dose of 15 mg/kg piroxicam injection alone.

Conclusion: Rosmarinic acid was able to exert analgesic effects in pharmacological tests in a dose-dependent manner. Concomitant use of rosmarinic acid and piroxicam may lead to synergic pain relief, especially in acute and severe pain in patients, reducing the therapeutic dose of piroxicam and subsequently, its side effects.

Keywords: Analgesic, Hot plate, Rosmarinic acid, Rosmarinus officinalis, Tail flick

Please cite this article as follows: Moradi M, Mashhadi Akbar Boojar M, Saberi M, Rahimi Salkuyeh H, Ghorbani M, Amanpour H, et al. Comparison of analgesic effect of rosmarinic acid with piroxicam in mice. Int J Basic Sci Med. 2022;7(1):28-33. doi:10.34172/ijbms.2022.06.

Introduction

Pain is an unpleasant sensation that is caused by damage to various tissues and introduced as a warning factor that indicates the presence or possibility of injury in an organ.[1] Both acute and chronic pain cause problems in humans, limiting or disabling daily activities.[2] For this reason, continuously, humans have been looking for a way to relieve pain.[3] According to a study by the American Pain Association, 50 million people of all ages in the United States suffer from pain, which costs more than $ 100 million to control.[4]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs in this field, which unfortunately in many cases their gastrointestinal, hepatic, and renal complications are important factors in limiting their use.[5] Opioids, another class of analgesic drugs, has serious side effects, besides dependence and tolerance, which limit their administration.[6] Regard to adverse effects of chemical mentioned drugs, herbal medicines could be considered as suitable alternative.[7]

From a long time ago Rosemary, a native herbal plant in different areas of Iran, has been used as a tonic, relieving fatigue and weakness agent, anti-depressant, anti-flatulence, appetite stimulant, antiseptic, diuretic, perspiration stimulant, bile flow stimulant, and antispasmodic. Rosmarinic acid, (2R)-2-[[(2E)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]] oxy]-3-(3,4-dihydroxyphenyl), propanoic

acid) is one of the most abundant caffeic acid esters that was first isolated from Rosemary in 1958 and studied as a major biologically active component of this plant.[8] It is generally found in several plants from the Lamiaceae (the Mint) family, such as *Rosmarinus officinalis* L. (rosemary), *Thymus vulgaris* L. (thyme), *Mentha spicata* L. (spearmint), *Origanum vulgare* L. (oregano), *Ocimum basilicum* L. (sweet basil), and some other medicinal herbs.[9] Many therapeutic effects of rosemary such as anticonvulsant and anti-anxiety, anti-inflammatory, antioxidant, and anti-apoptotic effects have been attributed to rosmarinic acid.[10] It has been shown that rosmarinic acid reduces the expression of cyclooxygenase (COX)-2 and prostaglandin E2 (PGE2).[11]

In traditional medicine, rosemary has been introduced as a remedy to relieve muscle and joint pain (especially in topical use) and some studies have evaluated the analgesic effects of its alcoholic extract and considered further study on the main components of this extract.[12] Therefore, the present study seeks to investigate the analgesic effects of pure rosmarinic acid in a mice model in comparison with piroxicam and its possible additive interaction in co-administration with piroxicam as one of the oldest and most common analgesic drugs of NSAIDs family.

Materials and Methods

Tools and Chemicals

The equipment and materials used in this study included the following: standard rosmarinic acid material, purity > 90%, purchased from Sigma Company (USA), piroxicam ampoules purchased from Chemi Darou Industrial Co. (Tehran, Iran), insulin syringe, animal cage, digital scale, Hot plate, and Tail flick device made by Tajhiz Gostar Omid Iranian Company (Tehran, Iran).

Study Population (Animals)

In this study, 66 male NMRI mice weighing 20 to 30 g were used and the experiments were performed in 11 groups with 6 mice in each group (according to similar studies) for testing.[12] The interval between the hot plate and tail-flick tests was performed on mice, observing the wash-out period (5 half-lives after drug injection). Mice were injected intra-peritoneally, as described in Table 1.

According to previous studies, the LD50 of rosmarinic acid was determined by intraperitoneal injection in mice, 561 mg/kg.[13] In the present study, the highest dose for injection was about one-fifth of this value (100 mg/kg). Then, to assess other doses, the rosmarinic acid dose was halved in each group for better comparison. In previous studies, the effective dose of piroxicam with analgesic effects in mice was determined to be 30 mg/kg.[14] Therefore, in the present study, two doses, 15 and 30 mg/kg were used.

Pain Assessment Tests

In this study, hot plate and tail flick pharmacological tests were used to evaluate the acute analgesia. Quick responses, reliability of results, and presenting quantitative results are some of the advantages of these two methods. Also, these two tests show the sensory and reactive function of central nervous systems and related spinal reflexes, typically involved in controlling and improving pain.[15]

Table 1. Groups and Dose of Drug Administered in Pharmacological Tests

Number of Groups	Dose	Drug Received
Control	1 mL	Normal saline
1	0.05 mL (5 mg/kg)	Rosmarinic acid
2	0.1 mL (10 mg/kg)	Rosmarinic acid
3	0.2 mL (20 mg/kg)	Rosmarinic acid
4	0.5 mL (50 mg/kg)	Rosmarinic acid
5	1 mL (100 mg/kg)	Rosmarinic acid
6	0.1 mL (30 mg/kg)	Piroxicam
7	0.05 mL (15 mg/kg)	Piroxicam
8	0.1 mL piroxicam (30 mg/kg) + 0.5 mL rosmarinic acid (50 mg/kg)	Rosmarinic acid + piroxicam
9	0.05 mL piroxicam(15 mg/kg) + 0.5 mL rosmarinic acid (50 mg/kg)	Rosmarinic acid + piroxicam

In the tail flick test, the thermal stimulator of the light rays of the lamp was 8.5 V. The intensity of radiation in this device was variable and controllable and included degrees 1 to 11 that in all experiments, the intensity of light emitted was adjusted to 5. Piroxicam and rosmarinic acid in different doses were injected intraperitoneally and according to the schedule. Immediately and 30, 60, and 120 minutes after injection, the mice were placed in a special holding chamber, the beam of radiation began to shine, and at the same time and the latency time for mice to remove their tail was recorded. To prevent heat damage, the cut-off time of the experiment was 15 seconds.[16]

The hot plate test shows a complex response to an acutely painful and generally non-inflammatory stimulus. This device is a plate that is heated by electricity, at a temperature of 55 ± 0.5°C. After adjusting the temperature, the mice were placed on the hot plate of the device, and the time elapsed to observe the animal's reaction to the thermal stimulus was recorded. In order to adapt the mice to the conditions, the animal was placed in the device half an hour before the experiment. The cut-off time was adjusted to 50 seconds. [17]

Statistical Analysis

All data were expressed as mean ± SD. For statistical analysis, an independent student t-test and SPSS software (version 19.0) were used. In each test, the P value of < 0.05 was considered significant.

Time and Place of the Study

The present study was conducted in two months, in Baqiyatallah University of Medical Sciences, in standard animal care settings in terms of light, temperature (22 to

25°C), and free access to water and food. Also, the animals were kept in a cycle of 12 hours of light and 12 hours of darkness. The cages with a size of 24×13.5×13 cm were provided by Tajhiz Gostar Omid Iranian Company and the number of animals in each cage was 6. All the principles of safety with laboratory materials and animals (use of gloves while working, avoiding direct contact with materials, use of masks and hoods when preparing materials, etc) were implemented before starting work.

Results

Comparison of Rosmarinic Acid and Piroxicam Results With Tail Flick Test

The most analgesic effects of rosmarinic acid were obtained at a dose of 100 mg/kg. At 30 minutes after injection, the groups receiving rosmarinic acid at doses of 10-100 mg/kg had significant analgesic effects ($P<0.001$) compared with the control group. At 60 minutes after injection, the groups receiving doses 100 and 50 of rosmarinic acid exhibited significant analgesic effects ($P<0.05$) compared to the control group. At 120 minutes after injection, the maximum dose of rosmarinic acid (100 mg/kg) had significant analgesic effects ($P<0.05$) compared to the control group. Piroxicam in both doses of 15 and 30 mg/kg, 30, 60, and 120 minutes after injection, was able to produce significant analgesic effects ($P<0.01$) compared to the control group. A delayed tail retraction was also more pronounced in the first 30 minutes after injection.

In the tail-flick test, simultaneous administration of rosmarinic acid (50 mg/kg) and piroxicam (30 mg/kg) at 30 minutes after injection had significant analgesic effects after 30 minutes when compared to the injection of piroxicam alone with the dose of 30 mg/kg ($P<0.05$) (Figure 1).

Comparison of Rosmarinic Acid and Piroxicam Results With Hot Plate Test

Thirty minutes after injection, the groups receiving rosmarinic acid at doses of 10 mg/kg and higher had significant analgesic effects compared to the control group. 60 minutes after injection, only doses of 50 and 100 mg/kg of rosmarinic acid produced significant analgesic effects compared to the control group. 120 minutes after injection, none of the injectable doses of rosmarinic acid produced significant analgesic effects. Piroxicam at both doses of 15 and 30 mg/kg at all time intervals after injection (30, 60, and 120 minutes) had significant analgesic effects ($P<0.001$) compared with the control group. This comparison was performed 30 minutes after injection of these compounds. Simultaneous injection of rosmarinic acid, 50 mg/kg and piroxicam, 15 mg/kg, had significant analgesic effects compared with injection of piroxicam, 15 mg/kg, alone. However, this difference was not significant at 60 and 120 minutes later in the hot plate test (Figure 2).

Concomitant injection of rosmarinic acid at a dose of 50 mg/kg and piroxicam at a dose of 30 mg/kg at 30 minutes after injection had no significant painkiller effects compared with injection of piroxicam at a dose of 30 mg/kg alone.

Discussion

In this study, the analgesic effect of rosmarinic acid was tested by hot plate and tail-flick tests in mice. Time latency in animals' response to a thermal stimulus, evaluated. Whereas the tail flick test reaction represents the spinal response, hot plate test principally produces a supraspinal response.[15] Rosmarinic acid is one of Polyphenol compounds which are a major family of naturally occurring organic compounds characterized

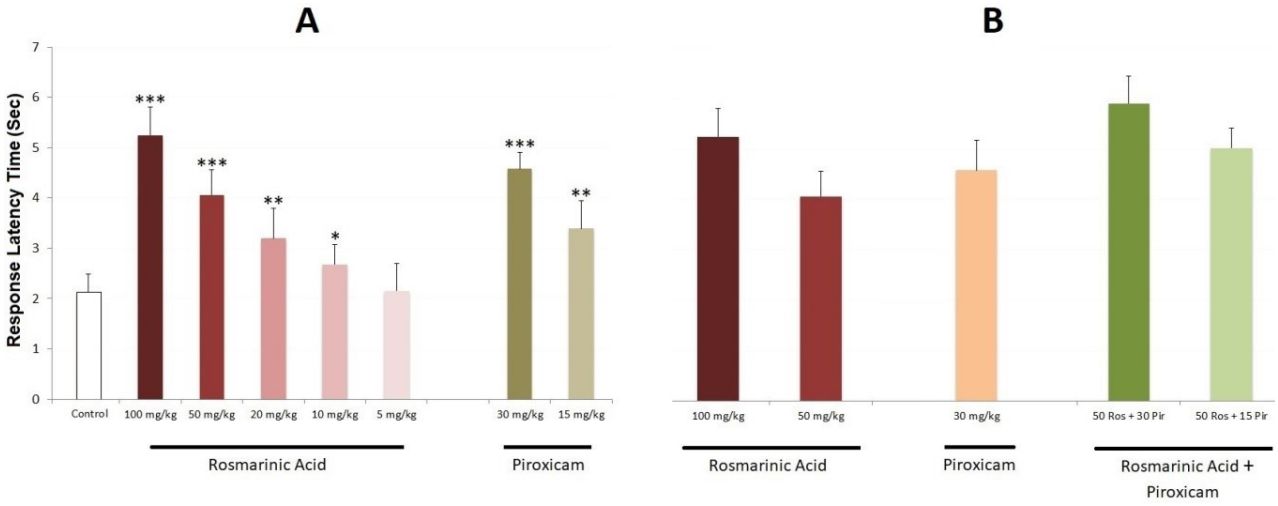

Figure 1. (A) Comparison of analgesic effects of Piroxicam and Rosmarinic acid in tail-flick test in 30 minutes after Rosmarinic acid and Piroxicam injection in mice. The data represent the tail retraction time presented as Mean ± SD (n = 6). The results obtained from each group were analyzed separately by independent student t-test and SPSS software. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ show significant analgesic effects compared to control. (B) Comparison of co-injection of rosmarinic acid and piroxicam with the injection of piroxicam alone.

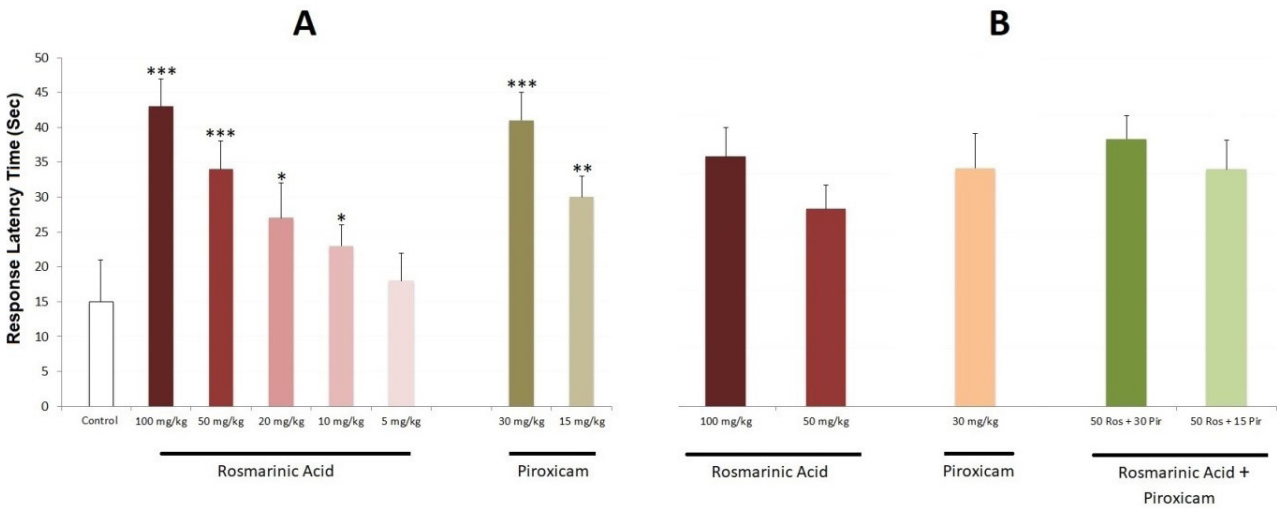

Figure 2. (A) Comparison of analgesic effects of Piroxicam and Rosmarinic acid in Hot plate test in 30 minutes after Rosmarinic acid and Piroxicam injection in mice. The data represent the time taken to start the reaction to the thermal stimulus as Mean ± SD (n = 6). The results obtained from each group were analyzed separately by independent student *t* test and SPSS software. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ show significant analgesic effects compared to control. (B) Comparison of co-injection of rosmarinic acid and piroxicam with the injection of piroxicam alone.

by several phenol units.[18] They have been investigated in various studies for their antioxidant, antitumor, antibacterial, hepatoprotective, and beneficial effects on the cardiovascular system and anti-inflammatory activities.[19,20]

The present data revealed that rosmarinic acid at doses of higher than 10 mg/kg had analgesic effects, which is consistent with the available information in ethnopharmacology of medicinal plants that introduces Rosemary as an analgesic and anti-inflammatory herb.[21-23] The most analgesic effects of rosmarinic acid were observed at doses of 50 and 100 mg/kg and data indicates the dose-dependent effects of rosmarinic acid. Also, the effects of rosmarinic acid were reduced during the experiment over time, which indicates a short half-life (about 45 minutes in humans) of this compound.[24]

The rapid onset of action of an analgesic agent is a remarkable criterion for developing a new compound in this field.[25] At 30 minutes post-injection, rosmarinic acid at doses of 50 and 100 mg/kg had comparable analgesic effects with piroxicam at 15 mg/kg. Due to its rapid onset of action, rosmarinic acid can be a suitable alternative for piroxicam in acute pain due to its rapid onset of action.[26]

The combined use of different analgesic agents to achieve greater efficiency has a long history in pain control.[27] The evaluation of analgesic effects in two groups who received the same dose of piroxicam along with rosmarinic acid showed that adding rosmarinic acid could increase the analgesic effects of piroxicam. Therefore, due to the observed synergistic and/or potentiating effects, combination of rosmarinic acid and piroxicam can be used in more severe pains and better and higher response can be obtained.

Co-administration of rosmarinic acid with a low dose of piroxicam produced equivalent analgesic effects compared with high-dose of piroxicam alone. Therefore, using rosmarinic acid with a lower dose of piroxicam or other NSAIDs almost produces the same analgesic effects. Thus the dose of NSAIDs will be reduced and as a result, drop many side effects. Unfortunately, a high rate of dose-dependent gastrointestinal bleeding has been reported in patients taking piroxicam and other related drugs.[28]

Despite the many biological properties of rosmarinic acid, the mechanism of action of these effects has not been fully understood. Many of these effects may be due to the neuroprotective or anti-antioxidant effects of rosmarinic acid.[29,30] Pain is centrally regulated by various neuronal complex systems such as opioid, dopaminergic, serotonergic, and noradrenergic pathways.[31] Rosmarinic acid inhibits the monoamine transporters and monoamine oxidase as well as modulation of the opioid receptors activity.[32,33]

It is possible that at least a part of the analgesic effects of rosmarinic acid is due to its anti-inflammatory properties, which is largely attributed to the inhibition of enzymes involved in prostaglandin biosynthesis.[34] Sastry et al. demonstrated that rosmarinic acid is a potent inhibitor of the key enzyme phospholipase A2 in response to stress conditions.[35] Scheckel et al also showed that rosmarinic acid is the selective inhibitor of COX-2 in human cancer and nonmalignant cell lines.[36] Inhibition of inflammatory cytokines such as interleukins 1 and 6, interferon-gamma, and matrix-9 metalloproteinases has been reported for rosmarinic acid in many cell culture studies.[11,23,37] NF-κB transcription factor is also an important pro-inflammatory factor that has played an important role in initiating the inflammatory process in various studies and it can be supposed that antioxidant factors such as rosmarinic acid have a significant effect in inhibiting this factor.[38]

Conclusion

Conclusively, in the present study, rosmarinic acid was able to prolong the latency time of response to the analgesic stimulus in pharmacological tests, indicating the potent analgesic effects of rosmarinic acid (even comparable to piroxicam). Considering the result of this study and other related investigations, it can be concluded that rosmarinic acid has central analgesic effects which interfere with CNS pathways and peripheral analgesic effects with possible anti-inflammatory mechanisms.

Acknowledgments

We would like to thank the deputy of research of Baqiyatallah University of Medical Sciences for financial support for this study in the form of a dissertation in the general doctorate of pharmacy.

Authors' Contribution

MM, MMAB, and MG were involved in planning the work and designed the project. MS, HRS and MMAB performed the experiments. HA, FS and MMAB wrote the article. All authors provided critical feedback and helped shape the research, analyze data, and prepare the manuscript.

Competing Interests

No conflict or competing financial interests exist.

Ethical approval

All protocols of the ethics committee of Baqiyatallah University of Medical Sciences were observed in keeping and transporting animals. This study has been approved with the code of ethics number IR.BMSU.REC.1398.310.

Financial Support

No funding.

References

1. Steeds CE. The anatomy and physiology of pain. Surgery (Oxford). 2009;27(12):507-511. doi:10.1016/j.mpsur.2009.10.013
2. Patel NB. Physiology of pain. In: Guide to Pain Management in Low Resource Settings. Washington, DC: International Association for the Study of Pain (IASP); 2010;13-17.
3. Zahradnik HP, Hanjalic-Beck A, Groth K. Nonsteroidal anti-inflammatory drugs and hormonal contraceptives for pain relief from dysmenorrhea: a review. Contraception. 2010;81(3):185-196. doi:10.1016/j.contraception.2009.09.014
4. Gaskin DJ, Richard P. The economic costs of pain in the United States. J Pain. 2012;13(8):715-724. doi:10.1016/j.jpain.2012.03.009
5. Patrono C, Baigent C. Nonsteroidal anti-inflammatory drugs and the heart. Circulation. 2014;129(8):907-916. doi:10.1161/circulationaha.113.004480
6. Busse JW, Wang L, Kamaleldin M, et al. Opioids for chronic noncancer pain: a systematic review and meta-analysis. JAMA. 2018;320(23):2448-2460. doi:10.1001/jama.2018.18472
7. Qi F, Li A, Inagaki Y, et al. Chinese herbal medicines as adjuvant treatment during chemo- or radio-therapy for cancer. Biosci Trends. 2010;4(6):297-307.
8. Petersen M. Rosmarinic acid: new aspects. Phytochem Rev. 2013;12(1):207-227. doi:10.1007/s11101-013-9282-8
9. Sik B, Hanczné EL, Kapcsándi V, Ajtony Z. Conventional and nonconventional extraction techniques for optimal extraction processes of rosmarinic acid from six Lamiaceae plants as determined by HPLC-DAD measurement. J Pharm Biomed Anal. 2020;184:113173. doi:10.1016/j.jpba.2020.113173
10. Amoah SK, Sandjo LP, Kratz JM, Biavatti MW. Rosmarinic acid--pharmaceutical and clinical aspects. Planta Med. 2016;82(5):388-406. doi:10.1055/s-0035-1568274
11. Ghasemzadeh Rahbardar M, Amin B, Mehri S, Mirnajafi-Zadeh SJ, Hosseinzadeh H. Anti-inflammatory effects of ethanolic extract of *Rosmarinus officinalis* L. and rosmarinic acid in a rat model of neuropathic pain. Biomed Pharmacother. 2017;86:441-449. doi:10.1016/j.biopha.2016.12.049
12. Fonteles AA, de Souza CM, de Sousa Neves JC, et al. Rosmarinic acid prevents against memory deficits in ischemic mice. Behav Brain Res. 2016;297:91-103. doi:10.1016/j.bbr.2015.09.029
13. Petersen M, Simmonds MS. Rosmarinic acid. Phytochemistry. 2003;62(2):121-125. doi:10.1016/s0031-9422(02)00513-7
14. Saganuwan SA, Orinya OA. Toxico-neurological effects of piroxicam in monogastric animals. J Exp Neurosci. 2016;10:121-128. doi:10.4137/jen.s40144
15. Malmberg AB, Bannon AW. Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Curr Protoc Neurosci. 1999;6(1):8-9. doi:10.1002/0471142301.ns0809s06
16. Silva JR, Silva ML, Prado WA. Analgesia induced by 2- or 100-Hz electroacupuncture in the rat tail-flick test depends on the activation of different descending pain inhibitory mechanisms. J Pain. 2011;12(1):51-60. doi:10.1016/j.jpain.2010.04.008
17. Menéndez L, Lastra A, Hidalgo A, Baamonde A. Unilateral hot plate test: a simple and sensitive method for detecting central and peripheral hyperalgesia in mice. J Neurosci Methods. 2002;113(1):91-97. doi:10.1016/s0165-0270(01)00483-6
18. Nunes S, Madureira AR, Campos D, et al. Therapeutic and nutraceutical potential of rosmarinic acid-cytoprotective properties and pharmacokinetic profile. Crit Rev Food Sci Nutr. 2017;57(9):1799-1806. doi:10.1080/10408398.2015.1006768
19. Nadeem M, Imran M, Aslam Gondal T, Imran A, Shahbaz M, Muhammad Amir R, Wasim Sajid M, Batool Qaisrani T, Atif M, Hussain G, Salehi B. Therapeutic potential of rosmarinic acid: A comprehensive review. Applied Sciences. 2019;9(15):3139.
20. Elufioye TO, Habtemariam S. Hepatoprotective effects of rosmarinic acid: insight into its mechanisms of action. Biomed Pharmacother. 2019;112:108600. doi:10.1016/j.biopha.2019.108600
21. Emami F, Ali-Beig H, Farahbakhsh S, et al. Hydroalcoholic extract of Rosemary (*Rosmarinus officinalis* L.) and its constituent carnosol inhibit formalin-induced pain and inflammation in mice. Pak J Biol Sci. 2013;16(7):309-316. doi:10.3923/pjbs.2013.309.316
22. Keshavarzian S, Shahgholian N. Comparison of the effect of topical application of rosemary and menthol for musculoskeletal pain in hemodialysis patients. Iran J Nurs Midwifery Res. 2017;22(6):436-441. doi:10.4103/ijnmr.IJNMR_163_16
23. Luo C, Zou L, Sun H, et al. A review of the anti-inflammatory effects of rosmarinic acid on inflammatory diseases. Front Pharmacol. 2020;11:153. doi:10.3389/fphar.2020.00153
24. Bhatt R, Mishra N, Bansal PK. Phytochemical, pharmacological and pharmacokinetics effects of rosmarinic acid. J Pharm Sci Innov. 2013 Apr;2(2):28-34.
25. Moore ND. In search of an ideal analgesic for common acute pain. Acute Pain. 2009;11(3-4):129-137. doi:10.1016/j.acpain.2009.09.003
26. Hobbs DC. Piroxicam pharmacokinetics: recent clinical results relating kinetics and plasma levels to age, sex, and adverse effects. Am J Med. 1986;81(5b):22-28.
27. Schröder W, Tzschentke TM, Terlinden R, et al. Synergistic interaction between the two mechanisms of action

of tapentadol in analgesia. J Pharmacol Exp Ther. 2011;337(1):312-320. doi:10.1124/jpet.110.175042
28. Giercksky KE. Piroxicam and gastrointestinal bleeding. Am J Med. 1986;81(5B):2-5.
29. Boonyarikpunchai W, Sukrong S, Towiwat P. Antinociceptive and anti-inflammatory effects of rosmarinic acid isolated from *Thunbergia laurifolia* Lindl. Pharmacol Biochem Behav. 2014;124:67-73. doi:10.1016/j.pbb.2014.05.004
30. Erkan N, Ayranci G, Ayranci E. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. 2008;110(1):76-82. doi:10.1016/j.foodchem.2008.01.058
31. Pace MC, Mazzariello L, Passavanti MB, Sansone P, Barbarisi M, Aurilio C. Neurobiology of pain. J Cell Physiol. 2006;209(1):8-12. doi:10.1002/jcp.20693
32. Takeda H, Tsuji M, Inazu M, Egashira T, Matsumiya T. Rosmarinic acid and caffeic acid produce antidepressive-like effect in the forced swimming test in mice. Eur J Pharmacol. 2002;449(3):261-267. doi:10.1016/s0014-2999(02)02037-x
33. Gamaro GD, Suyenaga E, Borsoi M, Lermen J, Pereira P, Ardenghi P. Effect of rosmarinic and caffeic acids on inflammatory and nociception process in rats. ISRN Pharmacol. 2011;2011:451682. doi:10.5402/2011/451682
34. Rocha J, Eduardo-Figueira M, Barateiro A, et al. Anti-inflammatory effect of rosmarinic acid and an extract of *Rosmarinus officinalis* in rat models of local and systemic inflammation. Basic Clin Pharmacol Toxicol. 2015;116(5):398-413. doi:10.1111/bcpt.12335
35. Sastry Yarla N, Bishayee A, Vadlakonda L, et al. Phospholipase A2 isoforms as novel targets for prevention and treatment of inflammatory and oncologic diseases. Curr Drug Targets. 2016;17(16):1940-1962. doi:10.2174/1389450116666150727122501
36. Scheckel KA, Degner SC, Romagnolo DF. Rosmarinic acid antagonizes activator protein-1-dependent activation of cyclooxygenase-2 expression in human cancer and nonmalignant cell lines. J Nutr. 2008;138(11):2098-2105. doi:10.3945/jn.108.090431
37. Sadeghi A, Bastin AR, Ghahremani H, Doustimotlagh AH. The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Mol Biol Rep. 2020;47(5):3557-3566. doi:10.1007/s11033-020-05447-x
38. Cao W, Hu C, Wu L, Xu L, Jiang W. Rosmarinic acid inhibits inflammation and angiogenesis of hepatocellular carcinoma by suppression of NF-κB signaling in H22 tumor-bearing mice. J Pharmacol Sci. 2016;132(2):131-137. doi:10.1016/j.jphs.2016.09.003