ORIGINAL ARTICLE

EFFECTIVNESS OF PREEMPTIVE REGIMEN IN REDUCING NOCICEPTIVE *c-Fos* EXPRESSION IN THE SOMATOSENSORY CORTEX OF SURGICAL PAIN PARADIGM

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Background: Preemptive analgesia effectively diminishes postsurgical pain with fewer side effects, although its effectiveness and mechanism of action to reduced pain is still debatable. This study assessed the effectiveness of the preemptive regime in terms of neuronal *c-fos* expression on the somatosensory cerebral cortex (pain perception area of the spinothalamic tract) in both somatic and visceral pain models. **Methods:** Lab-base experimental investigation was carried out for two years (January 2022 to December 2023) at Khyber Medical University Peshawar. Eight to ten weeks old, eighteen Sprague-Dawley rats weighing between 150 and 250g were divided into: a) Somatic (skin incision only) (SG; n=9); b) Visceral pain groups (uterine incision was given and then closed) (DG; n=9). Each group was further categorised into Buprenorphine, Tramadol, and Saline subgroups; in addition, 2% lidocaine (7 mg/kg) was given preoperatively under general anaesthesia (isoflurane). *c-fos* mean cell count (MCC) and optical density (OD) were calculated on immunohistochemical slides two hours postoperatively. Data was analysed using MS Excel, SSPS version 25, Graph Pad Prism, and the Fiji Image J analyser. **Results**: In comparison to the saline group, buprenorphine and tramadol were significantly more effective in reducing the mean cell count and optical density of *c-fos*-expressing neuronal cells in the cortex ($p \leq 0.05$) in both the somatic and visceral pain groups [superficial (somatic) and deep (visceral/uterine) surgical pain models]. **Conclusion**: The study concluded that a sufficient preemptive regimen is a practical way to avoid post-operative discomfort and pain following a surgical procedure.

Keywords: Postoperative pain, Preemptive regimen, *c-fos* expression; Buprenorphine; Tramadol; Lidocaine; Immunohistochemistry

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INTRODUCTION

Surgical procedures result in postoperative pain, tension, and discomfort. It ranks among the primary reasons for morbidity sometimes leading to mortality.[1] After surgery, almost 50% of patients report having excruciating pain and suffering. Analgesics such as NSAIDs/COX-2 inhibitors, paracetamol, local anaesthetics, opioids (morphine, buprenorphine, and tramadol), and localised analgesia are frequently used to alleviate pain and problems following surgery.[2] Furthermore, no particular pharmacological class has been proven to be successful in lowering problems and discomfort following surgery while having manageable side effects; also, some of these side effects are severe or even lethal, which limits their usage in clinical settings.[3] In order to manage pain after surgery, a pre-emptive regime, which lessens sensory input preoperatively, has been occasionally employed as a palliative method to prevent or decrease central nervous system mechanisms of neuronal sensitization.[4] Although their effectiveness is still unknown.[5] Preemptive single doses of intraperitoneal tramadol or buprenorphine (S/C) along with lidocaine have been proven to lessen post-surgical analgesia in the recovery phase and spinal cord dorsal horn in previous studies.[6–8]

The most common side effect following surgery is inflammation, which is partly brought on by tissue damage and the release of inflammatory mediators that trigger *c-fos* expression in the neuronal cell bodies of the spinal cord, cortical, and subcortical brain regions, such as the primary and secondary somatosensory cortices (S1 and S2), the insular cortex (IC), and the anterior cingulate 2–4 hours post-surgery.[9,10] The S1 and S2 cortices may be the most significant in pain perception in response to visceral and somatic stimulation of pain.[11] The conduction of pain signals from the peripheral system to the cerebral cortex depends on the spinothalamic tract. It consists of three types of neurones: first-order, which sense pain at the site of injury; second-order, which sends these signals to the thalamus; and third- order, which sends signals from the thalamus to the cerebral cortex, where pain is perceived.[1,12] *c-fos* is a transcription factor, proto-oncogene, and the protein derivative of gene *c-fos*.[13] It monitors the nuclear

expression of *c-fos* in the central nervous system after painful stimulation. This method can be useful in examining how neurones respond to changes in gene expression in response to external painful stimulation in both functional and pathological circumstances and to a preemptive regime.[14]

Comprehending the processes underlying the preemptive regime antinociception functions in the aftermath of surgery will open the door to potentially novel, side-effect-free pain treatments.[8] Thus, the effectiveness of preemptive regime on the post-operative pain-induced *c-fos* expression in the somatosensory cortex in a well-validated model of surgical pain in rats was investigated in order to identify the functional neuro-anatomical target sites of the preemptive regime in post-surgery in the cerebral cortex as well as its effect on the postsurgical process.

MATERIAL AND METHODS

The Khyber Medical University Peshawar, Animal Care Committee gave approval for the investigation (No. DIR/KMU-EB/MB/000755). Every experimental method was structured in compliance with the National Institute of Health's Guide for the Handling and Caring of Laboratory Animals as well as our institution's policies.

The trial was conducted with eighteen female Sprague-Dawley rats (150–250 g); National Institute of Health Sciences, Islamabad). Animals had gratis access to water and food with a twelve-hour cycle of light-dark set-in stone. In an attempt to lessen stress, all animals were transferred to the lab and then acclimatised for a day for at least five days by being exposed to the general handling and anaesthetic operations. The rats were administered injections of either; Saline (1 ml/IP) or Buprenorphine (0.05 mg/kg/SC) + Lidocaine (7mg/kg/SC) or Tramadol (12.5 mg/kg/IP) + Lidocaine (7mg/kg/SC) just prior to the procedure. In a plexiglass chamber, general anaesthesia induction commenced with 1.5 L/min of oxygen and 5% isoflurane. The rats were positioned on the operating table once their righting reflex stopped working, and a face mask containing 2% isoflurane was placed on them to maintain general anaesthesia. For the surgical incision, the animal was positioned dorsal. An electrical clipper was employed to shave the site of surgery and remove the fur. For an aseptic approach, rat skin was prepared using a 10% povidine solution.

A rat's lower abdomen was cut with a two-centimetre transverse incision, and then the rectus sheet was separated by either 1.2 or 2 centimetres. In order to carefully avoid damaging the abdominal muscles during blunt dissection, a two- centimetre incision into the muscle layer was made using a stab incision. The bladder and peritoneum were carefully retracted, and a lower transverse uterine incision of approximately one centimetre was made. The uterus and abdominal muscles were sutured using absorbable 1/0 Vicryl sutures (Trucryl PGA, rounded bodies). 2/0 Vicryl (Trucryl PGA 75 cm, rounded bodies, ½ c) interruptive sutures were used to sew the skin.[6]

Isoflurane was stopped after the skin closure, and the rat was allowed to recover on 1.5 L/min of oxygen alone. To prevent hypothermia, each rat was covered in paper over the hot pad. Rats were then sacrificed for *c-fos* expression histological examination after two hours.[8] For the somatic pain model, only skin incision was given and closed with 2/0 Vicryl under general anaesthesia.[7]

For immunohistochemical examination, coronal slices at the level of the brain's vertex (bregma -3.30/-4.00) were taken. Standard procedures were followed to create paraffin blocks, and three randomly selected 5µm coronal brain slices were used for examination. PBS (phosphate buffer saline) was used to wash the fixed tissue segments three times after they had been cleaned of xylene in descending order of alcohol concentration and de-waxed in an oven for fifteen minutes. Following optimisation, 0.01 M PBS containing 0.3% Triton X-100 and 10% normal goat serum was used to dilute a mouse monoclonal *c-fos* antibody (1:100; ab 208942). To hide the antibody site, the segments were heated in the microwave for 40 minutes after being treated with 2% retrieval solution (PH = 6.5), treated with distilled water thrice. After 15 minutes of permeabilization at room temperature (RT) using 0.4% Triton X in 1% PBS, the materials were then washed with distilled water. 5% peroxidase (DM 821, Dako) was applied to sections as a blocking agent for 10 minutes at RT, then areas were cleaned with distilled water and sections were treated with *c-fos* (the primary antibody; ab 208942) for an hour at a concentration of 1:100. The second antibody, avidin (biotinylated horseradish peroxidase complex; HRP), was administered for 45 minutes following two washes with distilled water. The sections were twice rinsed before being treated for 15 minutes with DAB-Chromogen (Deko) and 30 seconds of haematoxylin staining. The portions were prepared for examination.

The somatosensory cortex of the cerebrum was investigated using nuclei labelled with *c-fos* (bregma -3.30/-4.00A). A computer-aided camera attached to a Nikon E600 light microscope was used to take pictures of the sections. Two parameters were calculated (three sections per rat): (i) total immuno-positive *c-fos* neuronal cells per region; and (ii) *c-fos* optical density per area, using image J software, also referred to as Fiji. ANOVA (one-way analysis of variance) was utilised to tally and evaluate the total quantity of *c-fos* positive neurones in the cerebral cortex.[15] In addition to the student T-test, a post hoc "least significant difference (LSD)" was employed to assess the significant values between the

groups. The information was provided as mean ± standard deviation (SD) with a 95% confidence interval around the mean difference with alpha value of 0.05.

The number of immune-positive cells in the sub-regions (23400 um^2) was estimated using the OD of *c-fos*-expressing- cells in the somatosensory cerebral cortex. By a Nikon E600 light microscope, images were captured at 20X x 100µm magnification, from three sections per slide. The total immunoreactive areas of each brain were divided by the total area of the pertinent sub-regions on the three sections to determine the overall optical density value for each brain.[8,16]

For each 8-bit image, maximum intensity was equal to 255. Therefore,

$$Optical\ Density = \log\left(\frac{maximum\ intensity}{mean\ intensity}\right)$$

RESULTS

At 2 hours postsurgical uterine incision or skin incision, a vigorous increase in *c-fos* expression was found in somatosensory cerebral cortex. We further investigated the effect of tramadol and buprenorphine (preemptive regime) along with lidocaine on these increased *c-fos* positive neurones in the cerebral cortex.

As shown in Figure-1, more densely labelled *c-fos*-positive neuronal cells were seen in the cerebral cortex's post-sensory region after skin incision as compared to the tramadol and buprenorphine groups. At two hours after minor surgery (somatic pain group), the number as well as optical density of *c-fos* immunoreactive cells were statistically significant when three subgroups were compared by ANOVA (p<0.001; Figure-2). These results suggested the effectiveness of a preemptive regimen in the suppression of postoperative pain. Post hoc with Bonferroni correction showed that, as compared to saline group, tramadol and buprenorphine group effectively suppressed the *c-fos* expression in the somatosensory area (p<0.001). Although the efficacy of tramadol in suppressing *c-fos* is greater than buprenorphine but statistically insignificant when both are compared with a t-test. (p>0.05; Figure-2)

Expression of *c-fos* immune-positive cells caused by surgical incision in the visceral pain group (Figure-3 & 4) in the cerebral cortex was extensively suppressed when rats were pre-treated with a preemptive regimen (buprenorphine and tramadol); (p<0.001; Figure-4 A, B). These results suggested the neutralising effect of the preemptive regimen on the uterine-surgery-induced activation of the sensory area of cerebral cortex. In the visceral pain group, tramadol suppresses the *c-fos* more effectively than buprenorphine, but we found no statistically significant association. (p>0.05).

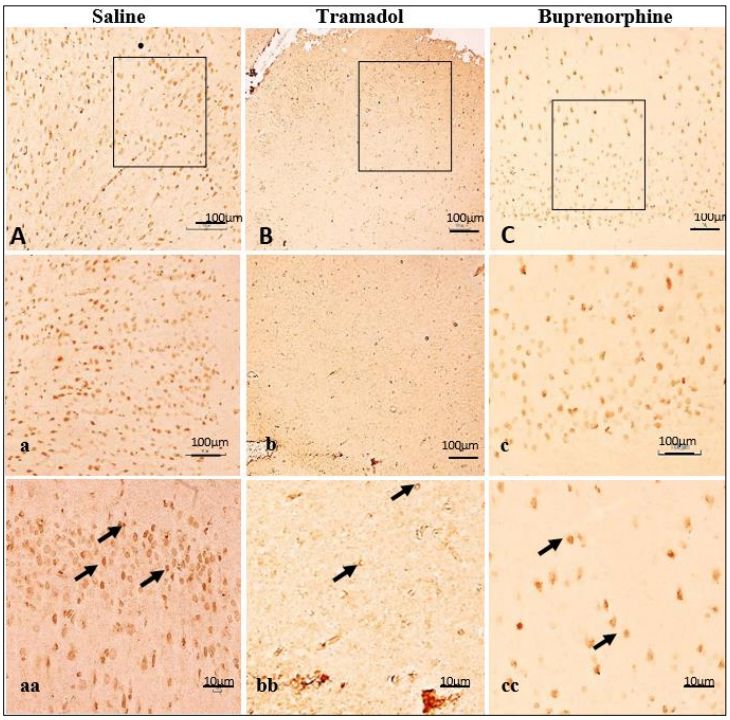

Figure 1: Cerebral cortex displaying *c-fos* expression in superficial pain group

Photomicrographic depiction (A-C) demonstrates expression of *c-fos* in cerebral cortex (somatosensory area) of superficial pain group. Cerebral cortex in Figure A-C shows markedly reduced quantity of *c-fos* positive neurons two hours post-surgery in rats treated with preemptive regimen (buprenorphine and tramadol) versus saline subgroup (internal control subgroup). Immunostaining nuclear *c-fos* positive cells are black in colour. [Scale bar: A-C= 100X. × 100µm, a-c=200X× 100µm, and aa-cc=400X × 10µm].

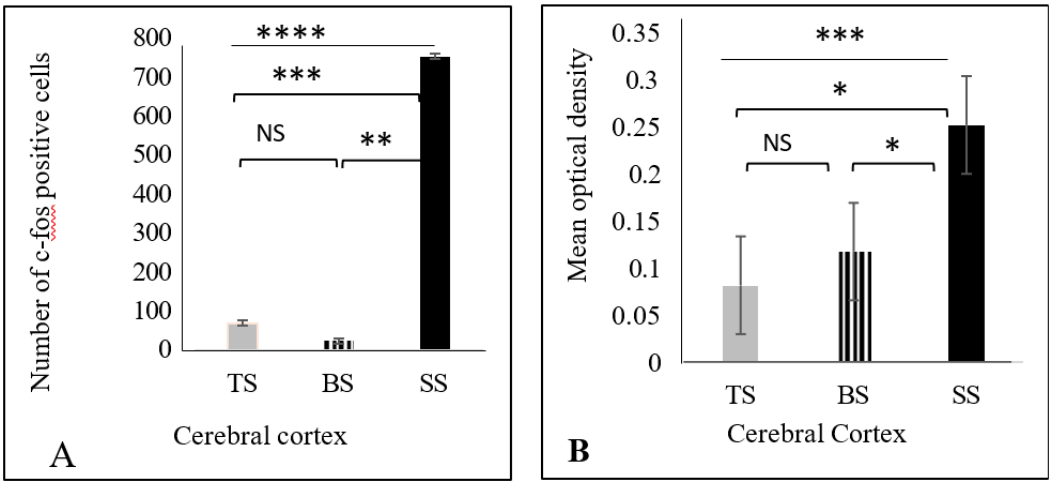

Figure-2: Cerebral cortex displaying *c-fos* expression and mean ± SD of *c-fos* positive cells (number and optical density) in superficial pain group.

Graphs (A, B) displaying mean count of *c-fos* expression cells, and optical density respectively in cerebral cortex of rats pretreated with saline (SS) versus tramadol (TS) and buprenorphine (BS). Statistically significant result obtained after comparing three subgroups by ANOVA followed by Bonferroni correction (p<0.01). Straight line shows one way ANOVA, while bracket line shows post-hoc test with Bonferroni correction. Error bar shows SD and n=3.

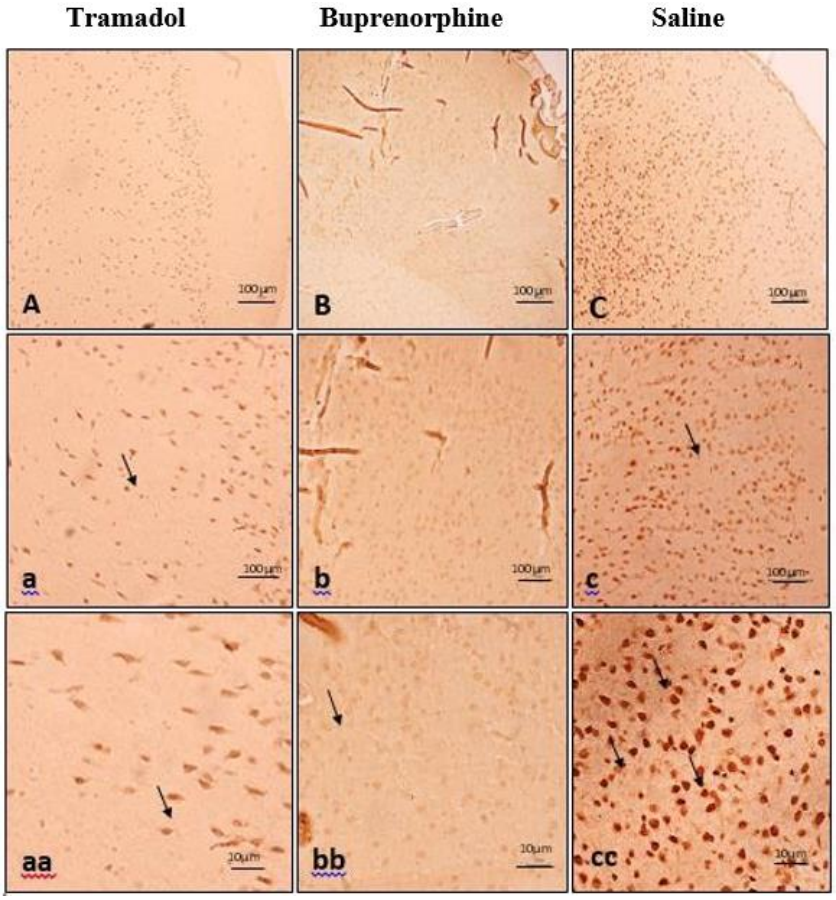

Figure 3: Cerebral cortex displaying *c-fos* expression in deep pain group

The expression of *c-fos* in cerebral cortex (premotor region) in the deep pain group is illustrated photomicrographically. Two hours after surgery, the cerebral cortex in rats given a prophylactic regimen (buprenorphine and tramadol) exhibited a significantly lower count of *c-fos* positive neurones compared to the saline subgroup (internal control subgroup) (p<0.01). Black is the colour of immunostaining nuclear *c-fos* positive cells shown by arrows. The scale bars (A–C), (a–c), and (aa–ii) correspond to 100X × 100 µm, 200X × 100 µm, and 400 X ×10 µm, in that order.

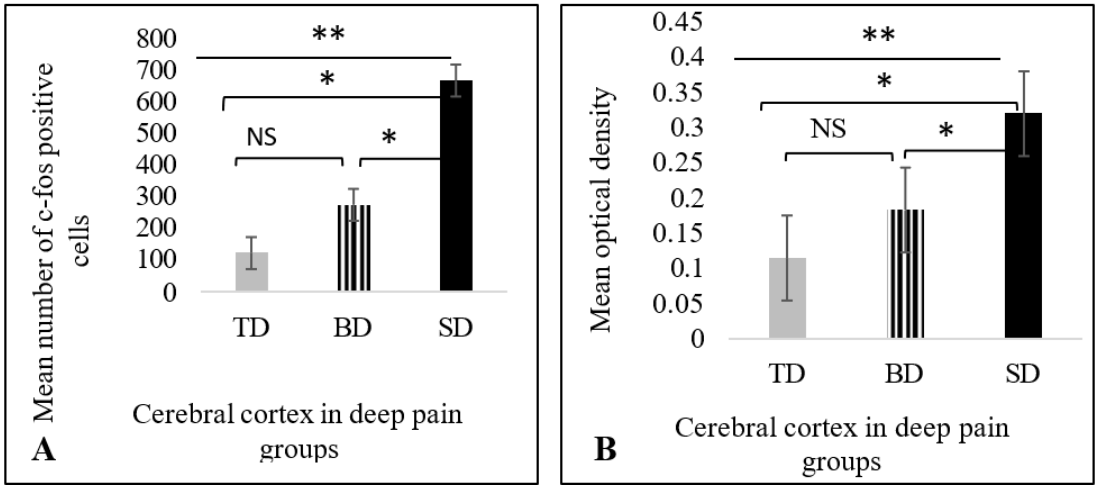

Figure 4: Cerebral cortex showing *c-fos* positive cells and mean cells count and optical density in the deep pain group

The mean number of c-fos positive cells and optical density in the cerebral cortex of rats treated with saline (SD) as opposed to tramadol (TD) and buprenorphine (BD) subgroups are shown in the above graphs (A, B). After comparing the three subgroups using ANOVA, a statistically significant result is displayed ($p \leq 0.05$). Straight line shows ANOVA, bracket display post hoc test, and error bar shows standard deviation.

DISCUSSION

A preemptive regime for postoperative pain management entails giving analgesic (pain-relief) therapy before the procedure to lessen pain following surgery, which is different from conventional postoperative pain management (offers pain relief following the procedure).[17] Post-surgical pain is an apprehension for patients, their attendants, and medical professionals, despite advancements in pain management therapy.[18] Therefore, preemptive pain management has drawn a lot of attention in recent years. Keeping in view the strategic importance of the preemptive regimen, postoperative pain was evaluated in the cerebral cortex after creating the somatic and visceral/uterine surgical pain models.

The *c-fos* expression at the neuronal level in the central nervous system was evaluated for further verification of this research project. The *c-fos* expression was appraised numerically as well as in terms of optical density by using Image J software. In the deep/visceral pain group (uterine surgical pain model), full suppression of *c-fos* expression was attained with tramadol and buprenorphine as part of preemptive regimen in comparison to the saline subgroup. Notably, the outcomes of this research are consistent with earlier study that found a similar decrease in overall *c- fos* expression in the dorsal horn of the spinal cord in the preemptive regimen groups as compared to saline groups.[8] The determination of *c-fos* expression at the cortex level was found to be statistically significant in both main study groups. These findings corresponded with research by Xiao Cuicui *et al.,* (2021) that established the notable upsurge in the *c-fos* protein expression in the anterior cingulate cerebral cortex as early as one hour following nerve injury, indicating amplification of *c-fos* expression due to early neuropathic pain.[19] *c-fos*, a proto-oncogene and a transcription factor, is useful for identifying neuronal activity in the brain and understanding reaction of brain to postoperative pain stimulation.[20] Insights into the neuronal mechanisms and pathways involved in pain perception, pain regulation, and the effects of analgesic medicines can be gained from the *c-fos* expressing cells in the cortex of the cerebrum after analgesia. Opioid analgesics, NSAIDs, and local anesthetics may inhibit the expression of *c-fos* in particular cortical areas linked to pain perception.[21] This decrease in *c-fos* expression implies that medications effectively lowered the neural activity linked to pain signals, corroborating prior findings.[8,22] Freitas ATA *et al.,* in 2016 shows a similar reduction in expression of *c-fos* positive cells in cerebral cortex as in the current study, where treatment with acupuncture with analgesia decreased the expression of *c-fos* in the brains of the rats in comparison with that of the animals in the control group, which is aligned with the current study. [23]

Chronic restraint stress following surgery was found by Meng Y *et al*., 2021, to increase postoperative hyperalgesia in rats, as evidenced by a considerable elevation of *c-fos* in the brain. This discovery is consistent with the observation that the saline group exhibits higher levels of *c-fos* expression in the cerebral cortex.[24] This research showed that, following either buprenorphine or tramadol treatment, there was no statistically significant difference ($p>0.05$) in the number or optical density of c-*fos* cells. This suggests that the rat's pain signal transmitted from the peripheral nociceptors to the spinal nociceptive neurones and hence forth to the cerebral cortex is expressed in terms of *c-fos*-positive neurones and reduces effectively in both the preemptive groups as compared to the saline group.

CONCLUSION

This study validated the potential role of preemptive regime in post-operative pain. The research project highlighted the advantages of combinations of various analgesic drugs to maximise pain relief and reduce the risk of adverse effects linked to high doses of certain medications. This study emphasised the significance of preemptive analgesia in the comprehensive treatment of postoperative pain, with the potential for application to benefit human subjects.

Conflict of interest

The author(s) have no potential conflicts of interest.

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AUTHORS' CONTRIBUTION

SMK: Investigation, Study Design, Writing, Data Collection, Data Analysis. NB: Supervision, Proof writing -Review & Editing, formal Analysis. SHH: Study Design, Methodology, Formal Analysis. SS, SM: Graphical Abstract, Lecture Search, Proof Writing. The final draft of the work has been critically examined and approved by all authors, who also bear responsibility for its content and similarity index.

REFERENCE

1. Liu S, Kelliher L. Physiology of pain—a narrative review on the pain pathway and its application in pain management. Dig Med Res 2022;5(56):1–10.
2. Choi M, Wang L, Coroneos CJ, Voineskos SH, Paul J. Managing postoperative pain in adult outpatients: a systematic review and meta-analysis comparing codeine with NSAIDs. CMAJ 2021;193(24):E895–905.
3. Tan J, Dou N, Bai G, Qiu W, Zhao Y. Research status of preemptive analgesia and its application in clinical anesthesia. J Clin Nurs Res 2021;5(2):73–6.
4. Xuan C, Yan W, Wang D, Li C, Ma H, Mueller A, *et al*. Efficacy of preemptive analgesia treatments for the management of postoperative pain: a network meta-analysis. Br J Anaesth 2022;129(6):946–58.
5. Tan H, Wang C, Jiang Y, Shi Q, Liang W, Li D. Postoperative effect of sufentanil preemptive analgesia combined with psychological intervention on breast cancer patients. BMC Anesthesiol 2023;23(1):170.
6. Mumtaz S, Baseer N, Habib SH, Peshawar U, Zaman PA, Wazir NU, *et al*. Effectiveness of preemptive regime: buprenorphine or tramadol with lidocaine and isoflurane on smooth postoperative recovery in novel uterine surgical model. Int J Health Sci (Qassim) 2022;6(S7):49234–43.
7. Mumtaz S, Baseer N, Habib SH, Waheed N, Saleem S, Malik S. Impact of preemptive analgesic regime on the rats' anesthetic recovery period in somatic pain model. Gomal J Med Sci 2023;21(4):273.
8. Mumtaz S, Baseer N, Habib SH. Concomitant use of preemptive analgesia with local and general anesthesia in rat uterine surgical pain model. Mol Pain 2024;2:2–29.
9. Lee GJ, Kim YJ, Lee K, Oh SB. Patterns of brain c-Fos expression in response to feeding behavior in acute and chronic inflammatory pain condition. Neuroreport 2021;32(15):1269–77.
10. Cosi C, Millar M, Beltran M, Sherry L, Gatti-McArthur S. Quantitative analysis of RNAscope staining for c-Fos expression in mouse brain tissue as a measure of neuronal activation. MethodsX 2021;8:101348.
11. Bushnell MC, Villemure C, Strigo I, Duncan GH. Imaging pain in the brain: the role of the cerebral cortex in pain perception and modulation. J Musculoskelet Pain 2002;10(1–2):59–72.
12. De Ridder D, Adhia D, Vanneste S. The anatomy of pain and suffering in the brain and its clinical implications. Neurosci Biobehav Rev 2021;130:125–46.
13. Santos PL, Brito RG, Matos JPSCF, Quintans JSS, Quintans-Júnior LJ. Fos protein as a marker of neuronal activity: a useful tool in the study of the mechanism of action of natural products with analgesic activity. Mol Neurobiol 2018;55(6):4560–79.
14. Cruz FC, Rubio FJ, Hope BT. Using c-Fos to study neuronal ensembles in corticostriatal circuitry of addiction. Brain Res 2015;1628:157–73.
15. Beretta CA, Liu S, Stegemann A, Gan Z, Wang L, Tan LL, *et al*. Quanty-cFOS, a novel ImageJ/Fiji algorithm for automated counting of immunoreactive cells in tissue sections. Cells 2023;12(5):704.
16. Cizkova K, Foltynkova T, Gachechiladze M, Tauber Z. Comparative analysis of immunohistochemical staining intensity determined by light microscopy, ImageJ and QuPath in placental Hofbauer cells. Acta Histochem Cytochem 2021;54(1):21–29.
17. Byrne K, Smith C. Preemptive analgesia: an unobtainable goal? J Cardiothorac Vasc Anesth 2019;33(2):460–1.
18. Szedlák B, Mitre C, Fülesdi B. Preemptive and preventive analgesia—an important element in perioperative pain management. Orv Hetil 2018;159(17):655–60.
19. Xiao C, Liu D, Du J, Guo Y, Deng Y, Hei Z, *et al*. Early molecular alterations in anterior cingulate cortex and hippocampus in a rodent model of neuropathic pain. Brain Res Bull 2021;166:82–91.
20. Medinarales JC, Bautista-Carro MA, Serrano-Bello G, Sánchez-Teoyotl P, Vásquez-Ramírez AG, Iannitti T. Persistent peripheral inflammation and pain induces immediate early gene activation in supraspinal nuclei in rats. Behav Brain Res 2023;446:114395.
21. Wang Y, Liu Y, Liu J, Wang M, Wang Y. Coadministration of curcumin and hydromorphone hydrochloride alleviates postoperative pain in rats. Biol Pharm Bull 2022;45(1):27–33.
22. Kudo K, Takahashi T, Suzuki S. The changes of c-Fos expression by motor cortex stimulation in the deafferentation pain model. Neurol Med Chir (Tokyo) 2014;54(7):537–44.
23. de Freitas ATAG, Lemonica L, De Faveri J, Pereira S, Henao MDB. Preemptive analgesia with acupuncture monitored by c-Fos expression in rats. J Acupunct Meridian Stud 2016;9(1):16–21.
24. Meng Y, Zhuang L, Xue Q, Zhang J, Yu B. NLRP3-mediated neuroinflammation exacerbates incisional hyperalgesia and prolongs recovery after surgery in chronic stressed rats. Pain Physician 2021;24(7):E1099.

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