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ORIGINAL ARTICLE

# EFFECT OF PREEMPTIVE REGIMEN ON *C-FOS* EXPRESSION IN DORSAL ROOT GANGLION OF RAT UTERINE SURGICAL PAIN MODEL

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## ABSTRACT

**Background**: In animal surgical models, the preemptive regime has been shown to be an effective part of balanced anaesthesia with regard to pain-free postoperative recovery. The study investigated the impact of pre-emptive analgesic regimens on pain and *c-fos* expression in the spinal dorsal root ganglion (1st order neuron) following uterine surgery in rats.

**Materials & Methods**: The Khyber Medical University in Peshawar's Ethical Committee of gave its approval to the study protocol (ASRB/IBMS/IRBE/4TH meeting/2023/9821-18, dated 03/04/23). The study was conducted in KMU Peshawar (April 2023 to January 2024). Eighteen Sprague-Dawley adult female rats were used in the experiments (calculated by resource equation) and divided into two study groups: Superficial and Deep pain groups, each group further divide into three study groups: Buprenorphine +Lidocaine, Tramadol+ Lidocaine, Saline under general anesthesia. By using ANOVA, post hoc analysis and Bonferroni and Holm correction, the mean and standard deviation for numerical variables were calculated. The 95% confidence interval corresponded to a standard deviation of 1.96. SPPS version 22 and MS Excel were used to conduct the statistical analysis.

**Results**: The results showed that the groups treated with buprenorphine or tramadol had significantly reduced *c-fos* expression, indicating lower pain levels compared to the saline group $p>0.05$ in both study groups.

**Conclusion**: Preemptive regime appeared to suppress *c-fos* expression in DRG 1st order neurons of Spinothalamic tract in uterine pain surgical model. Among the two analgesics compared, buprenorphine appeared to be more potent in suppressing *c-fos* than tramadol.

**Keywords**: Buprenorphine; *c-fos* expression; dorsal root ganglion; image J; immunohistochemistry; Preemptive regimen; postoperative pain; tramadol.

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## INTRODUCTION

The effectiveness of preventive analgesic treatments (given before the onset of pain), including epidural analgesia, local anaesthetics, wound infiltration, and the use of nonsteroidal anti-inflammatory drugs (NSAID), has been established in a number of situations.[1] However, researchers continue to look for strategies to lessen postoperative discomfort. Postoperative pain is defined as troublesome sensory or emotional event which is related to actual or possible tissue after surgery.[2] It is influenced by a number of variables, including the type of surgery done as well as patient cultures, beliefs, genetics, past experiences with pain and ability to withstand it. Despite these improvements in analgesic approaches, inappropriate postoperative pain management still occurs.[3] Due to the possible unfavourable effects of postoperative pain, both the patient and the surgeon are most concerned with its effective control and therapy.[4] Acute postoperative pain effects 80% of these patients, and pain treatment can have positive clinical, psychological and intuitive results for 25% of them.[5]

The dorsal root ganglia (DRG), is an important diagnostic target for pain treatment. For controlling peripheral and central sensory processes, such as somatic pain, neuropathic pain, and inflammation, the DRG is crucial which is 1st order neuron in pain pathway (spinothalamic tract).[6] Numerous studies have shown that a variety

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of pain stimuli, including thermal, mechanical, and chemical stimulations, cause the production of *c-fos* in the brain and spinal cord (DRG).[7]one of the challenges faced involves minimizing the brain-tissue response, which would otherwise create an environment that is detrimental for the accurate functioning of such probes. Following the implantation process, the brain reacts with a sterile inflammation response and resulting astrocytic glial scar formation, potentially resulting in neuronal cell loss around the implantation site. Such alterations in the naïve brain tissue can hinder both the quality of neuronal recordings, and the efficacy of deep-brain stimulation. In this study, we chronically implanted a glass-supported polyimide microelectrode in the dorsolateral striatum of Sprague–Dawley rats. The effect of high-frequency stimulation (HFS) and *c-fos* proto-oncogene is referred to as an early response gene[9], and expressed in 30 to 60 minutes after the painful stimuli in neuronal nuclei specially DRG.[8,9] The detection of *c-fos* expression in the central nervous system (CNS) is recognised as a trustworthy method for assessing the efficacy of pain-producing and preventive interventions like anaesthesia or analgesia. This is due to the topographic association of the pattern of *c-fos* activation with nociceptive primary afferent spinal projections.[10,11]

In current study, several forms of analgesia will be paired with local and general anesthesia in a rat uterine surgery model to investigate morphological, molecular and behavioural changes in rat's central nervous system. Combining general anaesthesia with local anaesthetic and analgesia may help to reduce pain transmission because general anaesthesia by itself cannot relieve pain. Additionally, this regimen may help to reduce adverse effects related to GA (general anesthesia) dose, which is crucial in the elderly, hypertensive, and people who are not suitable for prolonged general anaesthetic induction.[12,13] The study investigated the impact of pre-emptive analgesic regimens on pain and *c-fos* expression in the spinal dorsal root ganglion (1st order neuron) following uterine surgery in rats.

## MATERIALS AND METHODS

The study protocol, approved by the ethical committee (ASRB/IBMS/IRBE/4TH meeting/2023/9821-18, dated 03/04/23). The lab based experimental study (non-probability sampling technique) was carried out at Institute of Basic Medical sciences (IBMS) Khyber Medical University of Peshawar and National Institute of Health Islamabad in the year April 2023- January 2024.

Adult female Sprague-Dawley rats weighing 150-250 grams. The rats were housed in a controlled environment and subjected to a 12:12-hour light-dark cycle at 22°C.[14] A total of eighteen rats were randomly divided into two groups (each group n=9): [15] Superficial pain group (SG) and Deep pain group (DG). The Superficial pain group was further divided into three subgroups: Saline, Buprenorphine and tramadol (n=3). The sample size was calculated by resource equation.[16]

**Surgery:** The surgical procedure involved making a 3 cm Pfannenstiel incision in the lower abdomen, followed by peritoneal and uterine incisions. The wounds were closed with absorbable sutures.[15,17] Two hours after surgery, the rats were euthanized, and their spinal cords and dorsal root ganglia (DRG) were extracted for analysis.

For **immunohistochemistry**, we used *c-fos* antibody on dorsal horn sections. Paraffin blocks of the dorsal horn were prepared and sectioned at 5 microns. The sections were deparaffinized, rehydrated, and subjected to antigen retrieval. A blocking agent was applied to prevent nonspecific binding, followed by formalin fixation. The c-fos primary antibody (ab 208942) was then added, followed by a secondary antibody, and the detection was carried out using DAB staining. Finally, the slides were counterstained, mounted, and prepared for analysis, following standard IHC protocols.[15]

**Image J**: Images were analyzed using a compound microscope at 10x, 20x, and 40x magnifications. ImageJ (Fiji) was used to analyze the *c-fos* expression by calculating mean cell count of c-fos positive cells. The process involved recording a macro, using "H-DAB" staining via color deconvolution, and cell count from selected images.[15]

## RESULTS

This was lab-based experimantal study in which 18 rat were equally divided in two groups. The study aimed to develop a model of uterine surgical pain and evaluate the impact of a pre-emptive regimen on *c-fos* expression in the dorsal root ganglion (DRG) of rats. DRGs were processed for immunohistochemistry, and *c-fos* expression was quantified by counting *c-fos*-positive neurons in superficial (figure 1) and deep pain group. (Fig 3)

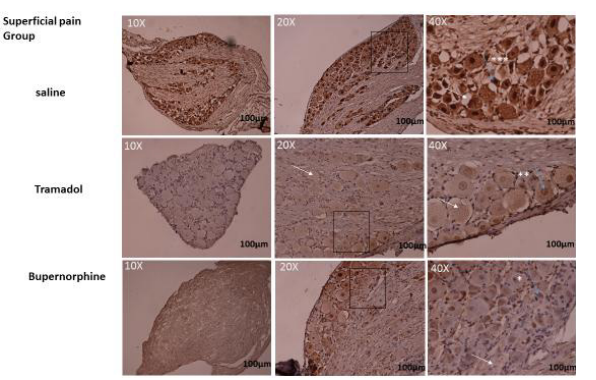


**Figure 1:** Shows that mean cell count of c-fos expressing cells were significantly higher in the saline group (no pre-emptive regimen) as compared to the tramadol and buprenorphine subgroups (preemptive regimen in superficial pain group) in

superficial pain group. $p < 0.05$. Each group (n=3 rats). The photomicrographs shows c-fos expression in the saline (A), tramadol (B) and Buprenorphine (C) at 2 hours post-surgery. Scale: 100um and photographs were taken at 10x, 20x and 40x power of microscope.

**Number of cells count of c-fos-expressing cells in superficial pain group:**

*c-fos* – positive cells were detected in all the experimental groups in superficial pain group (Fig. 2). Statistically significant result was obtained when we compared saline group with tramadol group ($p< 0.01$) and saline Vs. buprenorphine ($p< 0.01$) but it was not statistically significant when tramadol group was compared to buprenorphine group ($p < 0.2$), although mean *c-fos* cell count buprenorphine is less than that of tramadol group

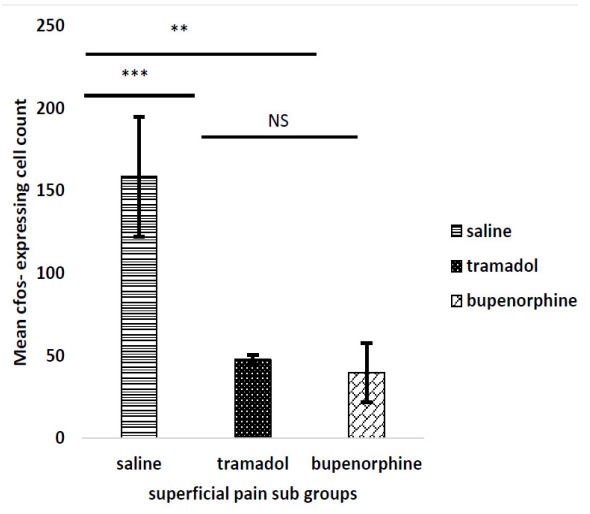


***Figure 2*:** shows marked c-fos expressing cell count in the saline group because there was no pre-emptive regimen given in the saline group as compared to the tramadol and buprenorphine groups, where a pre-emptive regimen was given c-fos expression was suppressed. ($p \leq 0.05$). Error bar shows the stander deviation.

**Number of cells count of *c-fos*-expressing cells in deep pain group:**

In present experimental study showed that the pain effect after uterine surgery in saline group were of great magnitude than preemptive regimen as shown in figure 3.

The number of *c-fos* positive cells is shown as mean ± SD. Data were analysed with a one-way ANOVA followed by a post hoc test. Statistically significant results were obtained when we compared saline Vs buprenorphine ($p < 0.003$), saline Vs tramadol ($p< 0.002$), but it was not statistically significant when two pre-emptive regime was compared that was tramadol Vs buprenorphine ($p =0.03$) were compared, after applying post hoc test followed by Bonferroni correction. (Fig. 4). This may be due to similar analgesic efficacy between the two pre-emptive regimens.

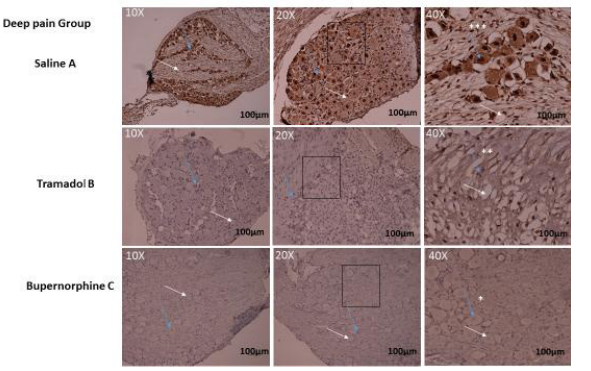


**Figure 4:** Representative photomicrograph of C-fos stained DRG slides of Deep pain Group at 10x (A), 20x (B) and 40x (C) each scale bar @ scale bar=100um. Blue Arrow showing positive cells and white Arrow showing negative cells.

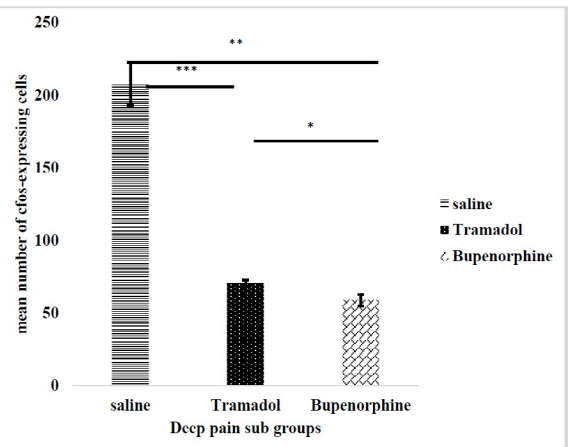


**Figure 3:** shows in the deep pain group c fos expressing cells were significantly higher in the saline group (no preemptive regimen) as compared to the In tramadol and buprenorphine (preemptive regimen). $p < 0.05$.

## DISCUSSION

This study explored the impact of pre-emptive analgesics (tramadol, buprenorphine, and saline) on *c-fos* expression in the dorsal root ganglion (DRG) of rats after uterine surgery. Findings revealed that the saline-treated control group had higher *c-fos* expression, indicating more neuron activation due to pain, compared to the drug-treated groups.

The nucleus of dorsal horn ganglions in the present study experienced postoperative pain that generated noxious painful stimuli that affected the level of *c-fos*.[18] The identical outcome was reported in 2015 by Tranquilli et al. In the study, we discovered that the saline-treated control group had greater levels

of *c-fos* expression in the DRG than the drug-treated groups. This is congruent with what may be anticipated in the absence of analgesic treatment.[19] The comparison of the results to an untreated group and the significance of anticipatory analgesia in lowering pain-related neuronal activity are both supported the effectiveness of pre-emptive regimen in the study. Furthermore, monitoring the nuclear expression of *c-Fos*, a protein that results from the *c-fos* gene, is thus a well-established and reliable anatomical technique for the functional mapping of neuronal activity and can be useful to examine neurons' capacity to respond with changes in gene expression to external stimulation under physiological and pathological conditions.[20]

In current study, tramadol-treated animals had considerably lower c-fos expression in the DRG than the control group. The opioid-like analgesic tramadol works as a moderate agonist at the -opioid receptor and inhibits serotonin and norepinephrine reuptake.[21] According to Toshifumi et al.'s result, which is consistent with other research, the lower expression of c-fos indicates that tramadol effectively inhibited neuronal activity in the DRG, which is accordance to our study.[22]

Additionally, Pre-fos expression was markedly decreased in the DRG, similar to what was observed in rats given buprenorphine. The -opioid receptors are partially agonists and antagonists of buprenorphine.[23] This study confirms Salam A and Adetola RA et al. and co-workers' findings that buprenorphine may be effective as a preventive analgesic for pain related to uterine surgery.[24,25]

Overall, the results of this study suggest that prophylactic administration of tramadol, buprenorphine, lidocaine, and isoflurane reduce pain-related neuronal activity in dorsal root ganglia of the spinal cord. It is essential to keep in mind that this study was conducted on animal models and that more research is necessary to confirm these findings in clinical settings before using pre-emptive analgesic regimens in human patients. Investigating the long-term effects and safety profiles of these analgesics in preventative use is also necessary to determine their therapeutic usefulness.

## CONCLUSION

The results of this hypothetical investigation showed a substantial reduction in c-fos expression in the Dorsal Root Ganglion following preemptive tramadol, buprenorphine in presence of isoflurane administration in a rat uterine surgical pain model. These findings suggest that, in this specific scenario, the prophylactic use of these drugs may successfully reduce pain-related neuronal activity in the central nervous system, particularly in the dorsal root ganglion.

**Recommendation:** It is advised behavior-related elements should be investigated in more advanced study. Apart from the *c-fos* and DRG it is advised to investigate the DRG cells' inputs and outputs. It is recommended to use alternative preventive measures.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.


## AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

| | |
|---|---|
| Conception or Design: | IK, NB |
| Acquisition, Analysis or Interpretation of Data: | IK, NB, SM, MLA, AK |
| Manuscript Writing & Approval: | IK, NB, SM, MLA, SW |

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.