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Effectiveness of preemptive regime; buprenorphine or tramadol with lidocaine and isoflurane on smooth post-operative recovery in novel uterine surgical model

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***Abstract*---**Introduction: Preemptive regime is proven to be effective component of balance anesthesia referring to smooth postoperative recovery in animal surgical models. Nature and method of induction as well as maintenance of general anesthesia along with suitable preemptive regime with minimal side effects, are the essential elements of research these days in animal surgical model. Objective: To determine the efficacy of preemptive regime (buprenorphine or tramadol with lidocaine and isoflurane) on a rat uterine surgical model pertaining to smooth postoperative recovery. Method: In this experimental study, uterine surgery was performed on 21 female Sprague dawley rats after dividing them into three study groups, each group comprising of 7 rats. In group A (BG) 7 rats were administered

buprenorphine +lidocaine along with general anesthesia. In group B (TG) tramadol+lidocaine was given, whereas in group C (SG), saline injection was given along with general anesthesia. Results: On comparison between three study groups, statistically significant variation was noted in heart rate at 1min and 10 mins (p value 0.02). Preemptive regime either tramadol or buprenorphine significantly reduced recovery timing ($p< 0.001$) along with pedal withdrawal reflex and righting reflex as compared to saline group ($p < 0.001$ and $p< 0.001$) respectively. Conclusion: Preemptive regime significantly reduced the recovery time hence smoothening the recovery from general anesthesia according to this uterine surgical model interpretation.

***Keywords*---**Preemptive regime, postoperative pain, Incision, animal surgical model, balance anesthesia, Righting reflex, pedal withdrawal reflex.

Introduction

Preemptive regime is proven to be effective component of balance anesthesia referring to smooth postoperative recovery in animal surgical models[1]. The choice of preemptive regime during general anesthesia in the animal model depends upon many factors like species, strain, gender and health condition of animal, nature, type and duration of surgical procedure, recovery time and research objective and goals[2]. Nature and method of induction as well as maintenance of general anesthesia along with suitable preemptive regime with minimal side effects, are the essential elements of research these days in animal surgical model[3]. However after extensive research work, the smooth, pain free post-operative recovery is still a nightmare, not only for the patients, but also for their attendants and as well as for doctors[4]. Researchers are now trying to develop 'animal pain models' to imitate distinct clinical diseases as well as surgical procedures, and understand underlying mechanisms of sympathetic stimulation in the CNS and its possible improvement with preemptive regime[5].

Various effective preemptive regime along with general anesthesia has been shown to diminish the neuro-humoral stress reaction to surgery in animal surgical model, thus reducing postoperative cardio-respiratory functions and subsiding complications during recovery phase as well as after recovery from anesthesia[6,7]. In animal model the post-operative recovery is customarily evaluated using anesthetic reflex especially righting reflex, Pedal withdrawal reflex, recovery from anesthesia and cardiovascular monitoring[8].

Balanced anesthesia constitute a combination of preemptive regime (analgesics), sedatives and anesthetics to induce anesthesia by giving lesser doses in comparison to if each of the components were to be used solitary[9]. This exercise holds the benefit of synergistic effect and also elude the unsolicited effects experienced when high doses of single component is used to induce anesthesia[10]. Balanced anesthesia holds the prospective for smooth induction, augmented

safety margins and when preemptive regimes are utilized, reduces pain for the duration of recovery and postoperative period.[5]

Buprenorphine and carprofen are frequently used analgesics in research and preemptive administration of these drugs has been recommended[11]. However there is no consensus on the most suitable analgesic agent for mice or on the optimal time of administration [12, 13]. Our goal in the current study is to develop a surgical model with administration of a novel preemptive regimen using a combination of analgesia, local anesthesia along with general anesthesia.

Buprenorphine or tramadol + lidocaine along with isoflurane is selected. These anesthetic regimen was compared with saline +isoflurane (control) to assess its ability of smooth recovery from anesthesia during recovery phase. Cardiovascular responses and behavioral changes were noted as parameters to assess smooth recovery.

Methodology

The study protocol was approved by Ethical Committee of Khyber Medical University Peshawar. Experiments were done on adult female Sprague-Dawley rats. Their weight was between (150- 250mg) and were housed two animals per cage in the central animal facility with free access to food and tap water. 12:12-h light–dark cycle was used at 22±2 ℃ temperature. All surgical experiments were performed between 0900 and 1400 hrs. All the rats were treated in accordance with ethical guideline for investigations and keeping in view of 3R principles of animal research[14].Random sampling technique was used in this study. The sample size was calculated from "resource equation approach"[15]. The study subjects were assigned to the groups as follows:

1. Study groups: Skin and uterine incision+preemptive regime (n=21)
2. (BG): Buprenorphine +Lidocaine + Isoflurane (n=7)
3. (TG): Tramadol+ Lidocaine+ Isoflurane (n=7)
4. (SG): Saline +isoflurane (n=7)

Rats were anesthetized with inhaled Isoflurane (5% for induction and 2% for maintenance along with oxygen) and put on spontaneous ventilation by general anesthetic machine. Intramuscular Penicillin injection was given to each rat. Pre-emptive regimen was given before start of surgery, comprising intra-peritoneal tramadol (12.5mg/kg/IP) or buprenorphine subcutaneously (0.05-0.1mg/kg/SC) along with lidocaine (7mg/SC) at incision site to group A and B. The control group (C) received only saline injection S/C before the procedure. The site of incision was shaved and scrubbed with 10% Povidine-iodine solution. Then a 2cm transverse lower abdominal skin incision was given, carefully to avoid tearing abdominal muscles during blunt dissection. After retracting peritoneum and bladder, about 1cm long lower transverse uterine segment incision was given. Incision was then closed with a continuous suture of 2-0 Vicryl. Abdominal

muscles and skin were closed in the reverse order. After recovery, rats were housed in individual cages.

A number of anesthetic parameters were assessed during the recovery. The time for return of righting reflex was assessed as the time interval between the end of surgical procedure and the time the rat takes to right itself .The recovery time was assessed as time interval between end of surgical procedure to return of normal activity whereas the pedal withdrawal reflex was determined after the end of surgical procedure to the return of this reflex. The normal activity of rats was determined when the animal was able to stand. Heart rate and breathing rate was noted as heart rate /min and breaths/min using abdominal excursion at 1 and 10 minutes. Oxygen saturation was noted after surgery at 1 and 10 minutes by using pulse oximetry.

Mean and standard deviation for numerical variables like pedal withdrawal reflex, righting reflex, recovery from anesthesia, oxygen saturation, heart and breathing rate were analyzed by applying ANOVA followed by post hoc analysis and Bonferroni and Holm correction to avoid both type I and type II error[16]. The standard deviation set at 1.96 which corresponds to 95% confidence interval. The statistical analysis was performed using SPPS version 22 and MS Excel.

Results

As mentioned earlier, this study experiments comprised of three groups viz; Tramadol +lidocaine group (TG), Buprenorphine + lidocaine group (BG) and Saline group (SG). Parameters used to evaluate smooth recovery from anesthesia along with their findings are:

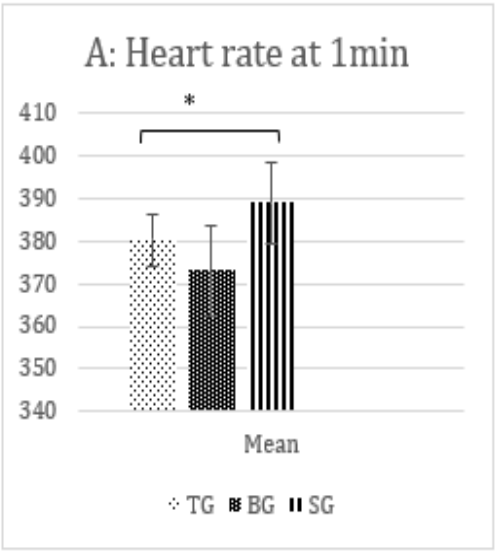
Breathing rate, heart rate and SpO2 were recorded as vital signs. According to our findings , mean heart rate at 1 minute and 10 minutes was faster in saline group compared to tramadol, buprenorphine (p = 0.02). On the contrary, the mean breathing rate at 1 minute and 10 minutes varied but it was not statistically significant ($p \geq 0.05$). (Fig II).

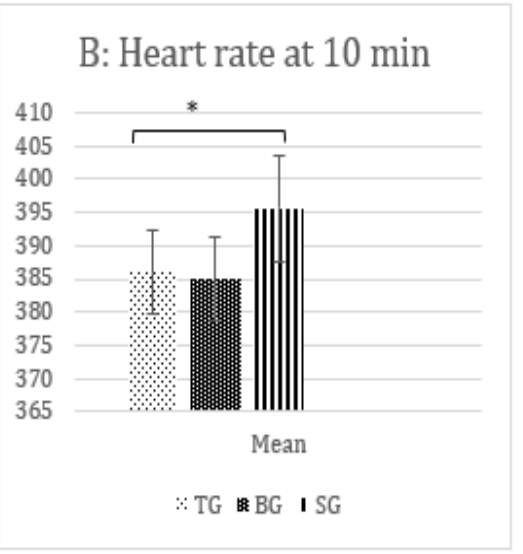
However no variation was noted in the oxygen saturation at 1 min and 10 mins within three groups ($P \geq 0.05$) (Fig II).

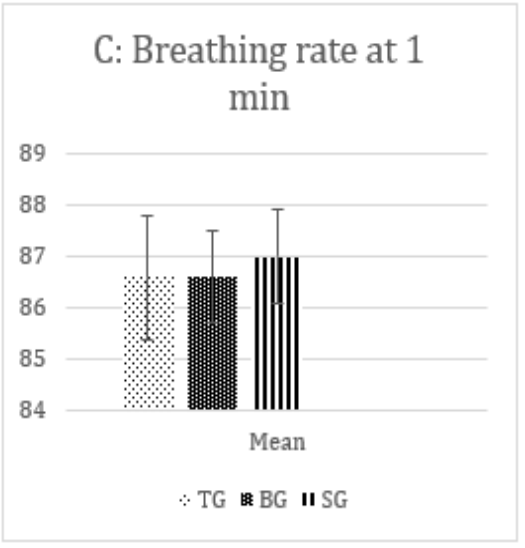
Significant variation was noted in the timing of righting reflex (p= 0.0004), pedal withdrawal reflex (p=0.0004) and recovery from anesthesia (p=0.0002), when the three groups of uterine surgical model were compared by applying one way ANOVA test. (Fig III). Post hoc analysis with Bonferroni and Holm correction was applied and significant variation was found between the groups. When the highly significant value of righting reflex was explored through Bonferroni correction, Saline (SG) against tramadol group (TG) was significant (p = 0.0007), Saline group with buprenorphine group was also significant (p= 0.009), but no statistical significance was found when the TG and BG were compared (p =0.09) (Table I).

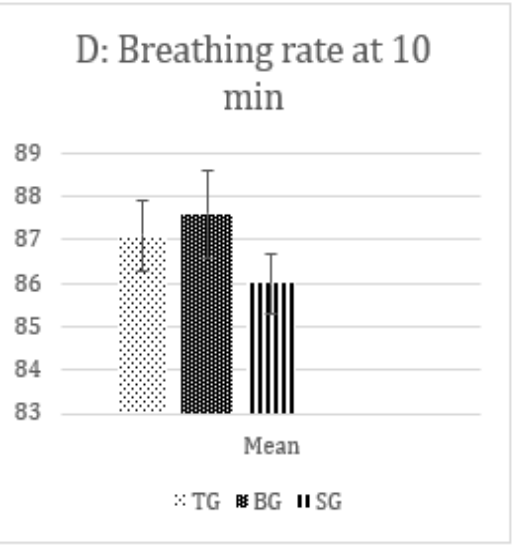
When Bonferroni correction was applied to Pedal withdrawal reflex, values were also significantly increased in saline group compared with preemptive regime of TG ($p < 0.001$) and BG (p =0.005), (Table I).

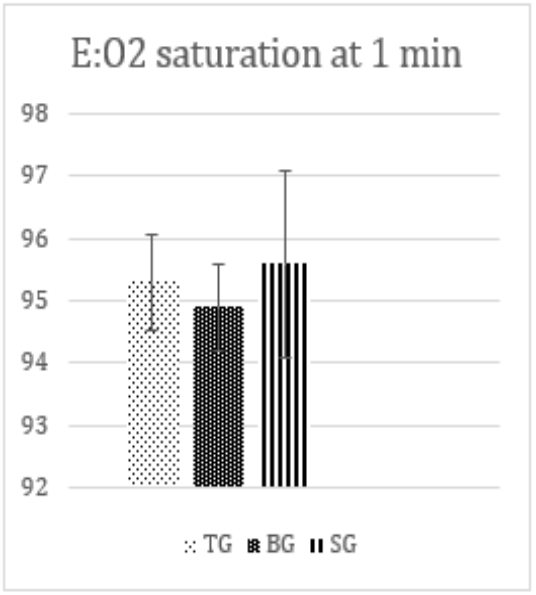
Similarly the mean recovery time from anesthesia was statistically significant when SG was compared with BG (p = 0.003) and TG (p = 0.0004), (table I). No significant variation was noted when both the preemptive regime groups were compared i-e TG versus BG (p = 0.1). (Table I)











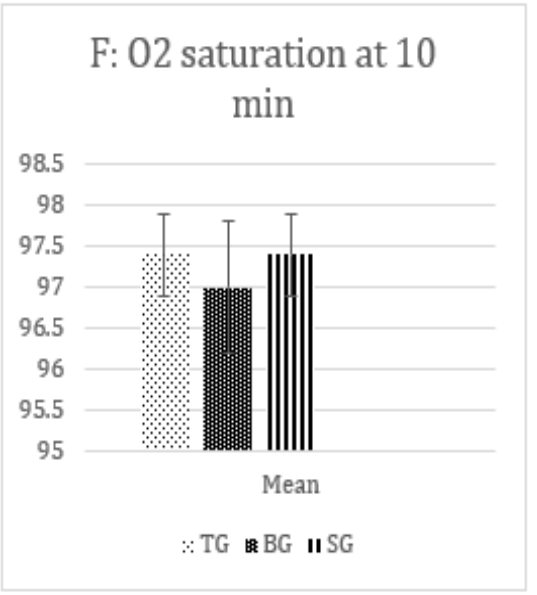
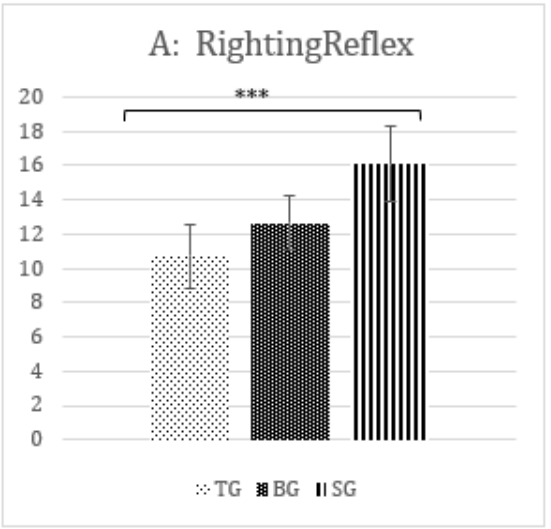
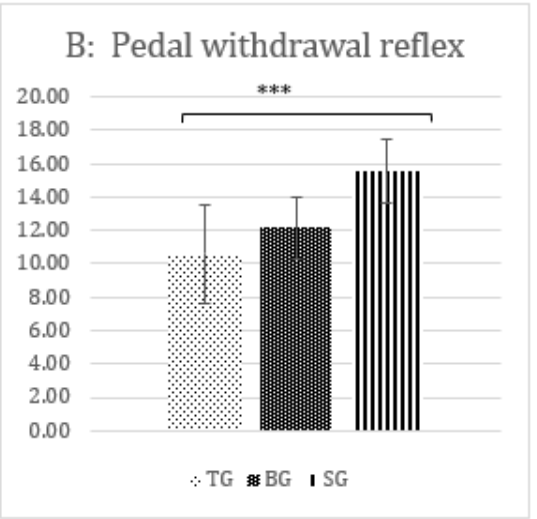


Figure II: Comparison of Oxygen saturation, breathing and heart rate between groups. Data is shown as mean ± standard error of the mean. Saline is used as internal control group (SG). In figure A and B vital sign monitoring during the recovery shows increased in heart rate per min in SG group compared to other groups at 1min and 10 min (p =0.02). Mean breathing rate in figure C & D shows that the breathing rate of rats in SG is more than that of TG, BG group at 1 min and decreased at 10mins but not statistically significant ($p \geq 0.05$). There was no statistical variation noted in SpO2 concentration in groups of figure E and F at 1 min and at 10 min with $p>0.05$. Error bar shows standard deviation.





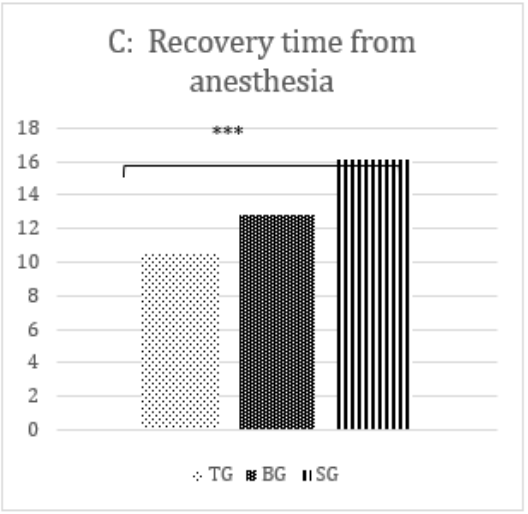


Figure above bars shows time (in minutes) of righting reflex, pedal withdrawal reflex and recovery from anesthesia in three study groups. The data is shown as mean ± standard error of the mean. In figure A, B & C the righting reflex timing, pedal withdrawal reflex and recovery from anesthesia is higher in saline SG (internal control group) as compared to BG and TG groups (preemptive regimen) with (***p < 0.001). Error bars show standard deviations

Figure III: Comparison of mean righting reflex, Pedal withdrawal reflex and recovery timing (in minutes) between the study groups.

Table I: Post hoc analysis with Bonferroni and Holms correction for anesthetic reflexes in three study groups. Bonferroni correction is taken as 0.005.

Intergroup comparison	**Righting reflex**		**Pedal withdrawal reflex**		**Recovery from anesthesia**	
	p-value	**Holm**	**P-value**	**Holm**	**p-value**	**Holm**
SG vs TG	**<0.001**	0.01	**<0.001**	0.0083	**0.003**	0.008
SG vs BG	**0.009**	0.012	**0.005**	0.01	**<0.001**	0.006
TG vs BG	0.086	0.025	0.102	0.025	0.1	0.025

Discussion

In this study we observed smooth recovery of Sprague drawley rats from anesthesia by using balanced anesthesia comprising of isoflurane along with preemptive regimen tramadol+ lidocaine and buprenorphine + lidocaine. We meticulously determined the presence or absence of reflexes and vital sign responses such as, heart rate, as well as respiratory rate during recovery period from anesthesia after developing uterine surgical model. Salam A (2022) and Adetola R A, (2013) et al., reported use of buprenorphine and tramadol as commonly used analgesics in post-operative period to relieve patients from pain and are easily available and they are reported to have advantages over other analgesia [17,18]. However in our study tramadol and buprenorphine were given before the start of surgery along with local anesthesia and general anesthesia and their effectiveness was assessed on the recovery phase of anesthesia. Tranquilli et al.,(2015) also reports that despite anesthesia animal does not consciously perceive pain, surgical manipulations also act as stimulus which yields vital sign changes, such as raised blood pressure, heart rate [19] and brain activity

changes[20]. Moreover, anesthetics effectively induce unconsciousness, but research does not report analgesic effect [21] in the recovery period. The capacity of analgesics in unification with anesthetics to maximize peri-anesthetic stability is endorsed in our research.

Metry et al., (2019) stated that cardiopulmonary functions monitoring in animal models during and after surgery in postoperative period is crucial for assessing the effectiveness of balanced anesthesia [22]. Sumitra *et al.*, (2004) reported that during postoperative period significant respiratory depression was associated with combine used of Ketamin-xylazine [23]. In present study we noted tachycardia in saline group which indicates coarse recovery in the absence of preemptive analgesia. The mean breathing rate is slightly faster in saline group as compared to preemptive groups, but still it's not statistically significant when compared within the groups.

In current study we noted that pedal withdrawal reflex, righting reflex and recovery from anesthesia is significantly high in saline groups as compared to preemptive groups. This denotes that preemptive regimen reduces the duration of recovery period. Vincent *et al.*, (2021) also stressed that pedal withdrawal test are parameters used to assess smooth recovery from anesthesia after surgery along with righting reflex [24, 25]. Adetola R A in (2013) indicated that the pedal withdrawal reflex disappears showing the depth of anesthesia, furthermore combined used of analgesia with local and general anesthesia is more effective than without analgesia and local anesthesia [18]. This finding is in agreement with previous studies, but it needs further validation.

Maintaining optimum temperature is necessary during and after the surgery because it affects the quality and speed of recovery from anesthesia [26]. Keeping this in view we kept the temperature at 22±2 ℃. Furthermore, proper anesthetic machine was used for delivery of 2% Isoflurane to avoid buildup of carbon dioxide, which may also effect the recovery period.

Conclusion

Preemptive regime either tramadol or buprenorphine significantly reduced recovery timing ($p< 0.001$) along with pedal withdrawal reflex and righting reflex as compared to saline group ($p < 0.001$ and $p< 0.001$) respectively. We conclude that the administration of preemptive regimen significantly shortens the recovery time and causes smooth recovery from general anesthesia in a uterine surgical model. We suggest that the surgical model may be replicated in other settings to validate our findings.

Limitation of our study:

Manual monitoring of cardiopulmonary events was done due to limited resources.

Conflict of interest: No reported conflicts.

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