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Research Paper

Optimizing xylazine-ketamine combination dose to reach surgical anesthesia in medium duration surgeries of Wistar Rats

Shahzada Mudasir Rashid [1*], Manzoor.R.Mir[1], Sheikh Bilal Ahmad[1], Majid Shafi[2], Nuzhat Showkat[1], Rahil Razak[1], Insha Amin[1], Tavsief Ahmad[3], Ishraq Hussain[1], Gowhar Gul[4], Hina Bhat[5]

[1]Division of Veterinary Biochemistry; [2]Division of Veterinary Pathology, [3]Division of Animal Genetics & Breeding, [4]Division of Animal Nutrition, [5]Division of Animal Biotechnology, Faculty of Veterinary Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alesteng Jammu and Kashmir (India)
*e-mail: *mudasir@skuastkashmir.ac.in*

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ABSTRACT

Background: Our study was designed in optimizing appropriate dose of ketamine-Xylazine (KX) cocktail in Wistar rats for conduct of medium duration surgeries like orchiectomy. Four different doses were analyzed for better safety and action. Method: Male Wistar rats in the age group of 7-8 weeks weighing 170-200g, divided into four groups (n=6), received freshly prepared Ketamine-Xylazine at dose rate of K- 87mg/kg b wt, X- 13mg/kg b. wt to group 1; K- 43.5 mg/kg b wt, X- 13mg/kg b. wt to group 2; K- 87mg/kg b wt , X- 6.5mg/kg b.wt to group 3 and K- 43.5 mg/kg b wt, X- 6.5mg/kg b.wt to group 4 animals, through intraperitoneal route as single injection. The anesthetic effect was interpreted and correlated with the physiological parameters. Result: Full dose of KX in group 1 showed adequate muscle relaxation, profound analgesia, and fast induction with longer recovery time and highest post operational complication. Half dose of KX did not achieve induction, adequate muscle relaxation, and analgesia in group 4 rats. A decrease in the duration of anesthesia, recovery time and post-operative complications as compared to group 1 were observed in group 2 rats. In group 3 rats, adequate muscle relaxation, profound analgesia with added attributes of reduced recovery time and post op complications were observed as compared to group 1. Conclusion: KX administered to group 3 animals through intraperitoneal route were found most suitable, with better margin of safety in wistar rats for performing medium duration surgeries

Key words: Analgesia, ketamine, Orchiectomy, Rat, Xylazine.

Rat and mice have proved their potential to be the most relevant candidates for biomedical research attributed to their small size, short life span, easy handling, learnable skills with genetic, physiological and anatomical similarity to man. Rats offer many advantages over mouse including their applicability as disease models, cognition and memory, proportional size of important organs, excellent models of breast cancer, mechanistic studies of human reproduction, more relevant diabetic models and availability of a volume of data generated from previous studies (Abouelfetouh *et. al., 2021*), (Alexander, *e.t al.,* 2022). Notably, several surgical/invasive procedures are desired that requires surgical anesthesia with a precision to save the animal life for completion of experimental trial. Hence, many safety measures with accuracy are to be taken into account.

Mostly these procedures are expected to conclude successfully in half hour duration, provided critical care measures have been accomplished. One of the surgical procedures in male rats is neutering/castration or orchiectomy notably performed in prostate research and in pet rats by the removal of testicles from scrotal sac. The mostly used anesthetic combination is xylazine and ketamine. Ketamine is a dissociative anesthetic with prompt onset and a fair therapeutic index. It increases the sympathetic tone and thus it is combined with other drugs like xylazine, potentiating and balancing its anesthetic affect (Arnbjerg, 1979) A synergetic effect of Ketamine-Xylazine (KX) combination in terms of extended analgesia and immobility has

been established. These effects as reported have been achieved in most rats by the administration of KX combination at the dose of 87mg/Kg of b wt. and 13mg/Kg of b wt. respectively in rats (Bryda, 2013). Sufficient and dose centric information is still scarce in the available literature, nevertheless KX combination varying from 65/4 to 100/13mg/Kg has been reported for executing mice surgeries (Hsu, 1986). More research and reproduction of a specific KX-dose needs further validations and in this context we validate a specific combination dose of KX-combination as safe and reliable surgical anesthetic agent.

MATERIALS AND METHODS

Procurement of Animals: Seven to eight weeks old, 24 male wistar rats were purchased from animal house facility of IIIM Jammu-India. The experimental trial was conducted in animal house facility of FVSc & AH Shuhama, where necessary arrangements for animal housing, feedings are intact. The rats utilized in this study were selected from the reservoir rats dedicated to a research designed for studying the effect of natural compounds on benign prostatic hyperplasia. Animals were housed in polycarbonate cages with corncob bedding in a temperature controlled room. The animal house was maintained at temperature of 22^0C and 50-60% humidity with adaptation to a light-dark cycle (12 hours light:12 hours dark), The rats were acclimatized to human touch by handling them 4 min daily for 5 days in a week for a period of first 2 weeks.

Ethical approval: The ethical approval was sought from institutional animal ethical committee of CPCSEA vide IAEC Reference Number AU/FVS/PS-57/3379, Dated: 05-07-2021.

Animal Grouping: All rats were prepared for general anesthesia for providing analgesia in performing the surgical procedure of bilateral orchiectomy. The xylazine-ketamine cocktail in different doses were testified to ascertain the most suitable dose with greater margin of safety. The animals were randomly divided in 4 groups with 6 animals in each group (n=6). At the time of surgery, group 1 received freshly prepared Ketamine-Xylazine at dose rate of K- 87mg/kg b wt, X- 13mg/kg b.wt (1K/1X); Group 2: K- 43.5 mg/kg b wt, X- 13mg/kg b.wt (1/2K/1X) ; Group 3: K- 87mg/kg b wt , X- 6.5mg/kg b.wt (1K/1/2X) and group 4: K- 43.5 mg/kg b wt, X- 6.5mg/kg b.wt (1/2K/1/2X) (Table 1).

Table 1: Ketamine Xylazine cocktail administered in different groups

Anesthetic Drug	Group 1	Group 2	Group 3	Group 4
Ketamine	87mg/kg b wt	43.5 mg/kg b wt	87mg/kg b. wt	43.5 mg/kg b wt
Xylazine	13mg/kg b.wt	13 mg/kg b.wt	6.5 mg/kg b.wt	6.5mg/kg b.wt

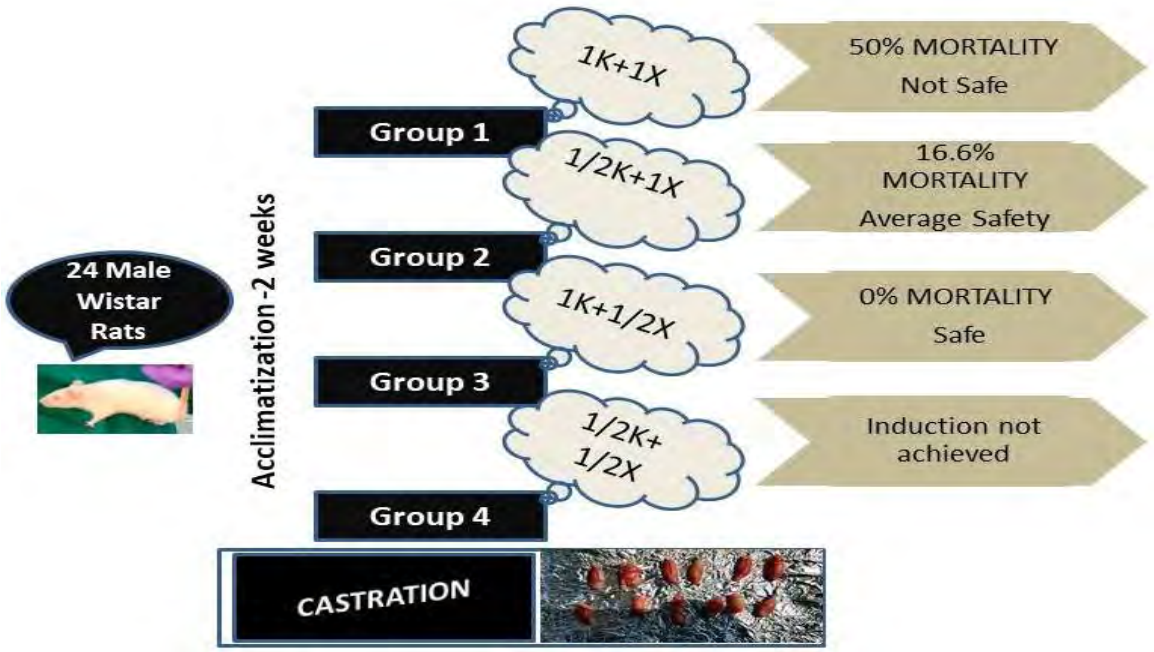

Fig. 1: Experimental Design involving 24 Male Wistar Rats, acclimatized for 2 weeks received different doses of anesthesia specific to each group. 1K+1/2 X administered to group 3 proved safe and effective

The anesthetic drugs were administered through a single injection through intraperitoneal route. Assessments of anesthetic action of drugs in different doses were made as per recording various physiological/clinical parameters as given in table 2.

Table 2: Clinical/Physiological criteria to evaluate anesthetic dose effects

S. No	Clinical/ Physiological Criteria	Description	Assessment
1.	Unconsciousness	Loss of awareness and feeling of an animal surroundings	Early ≤ 1min. Mid: More than 1min but less than 3 min. Late ≥ 3 minutes
2.	Induction time	Measured in minutes from the onset of KX injection to its effect	Ultra short ≤ 1min. Short: More than 1min but less than 5 min. Long ≥ 5 minutes.
3.	Immobility	Inability to move	Early ≤ 1min. Mid: More than 1min but less than 3 min. Late ≥ 3 minutes
4.	Muscle Relaxation	Loss of muscle tone except diaphragmatic.	Low Medium Adequate
5.	Analgesia	Absence of pain in response to painful stimulus Measured on visual analog scale of pain response to pin break	Mild (rats respond to any stimuli on whole body) Moderate (rats respond to stimuli on highly sensitive areas) Profound (no response to stimuli)
6.	Plane of Anesthesia achieved based on GUDEL'S Classification	The various planes are categorized based on the monitoring of vitals, respond of pupil to light, muscle tone etc.	Plane 1/Light Plane II/Medium Plane III/Deep Plane IV/ Very deep
7.	Duration of anesthesia	Measured in minutes from unconsciousness to recovery	Ultra-short (that lasts less than five minute) Short (that lasts less than thirty minutes) Long (lasts more than thirty minutes)
8.	Recovery time	Measured in minutes from regaining consciousness to full strength movements	Short (less than ten minutes) Moderate (from ten to twenty minutes) Long (more than twenty minutes)

Statistical Analysis: Student t test (Unpaired) for continuous variables and Chi square test for categorical variables were performed and a probability value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The group 1 wistar rats administered with Ketamine-Xylazine at dose rate of K- 87mg/kg b wt, X- 13mg/kg b.wt showed mid-level unconsciousness, short induction time, adequate muscle relaxation, profound analgesia, fast induction, achieved surgical anesthesia suitable for long duration surgeries. The recovery time was longer with highest death rate as post-operative complication as compared other groups (Table 3) (Figure 2).

Group 2 rats administered with KX combination @ K- 43.5 mg/kg b wt, X- 13mg/kg b.wt also showed mid-level unconsciousness, adequate muscle relaxation, profound analgesia, and achieved surgical anesthesia suitable for long duration surgeries. However, the induction time increased and a significant decrease in the duration of anesthesia, recovery time and post-operative complications as compared to group 1 were observed (Table 3) (Fig. 2).

In Group 3 the parameters like unconsciousness, induction time, muscle relaxation, anesthesia and plane of anesthesia were similar to group 2. The duration of anesthesia was comparatively reduced; however, there was reduction in recovery time and post-operative complications in this group (Table 3) (Fig. 2).

Table 3: The clinical effect of four different doses of Ketamine-Xylazine cocktail

Criteria	Group 1	Group 2	Group 3	Group 4
Dose of Anesthesia K/X in mg/kg b.wt	87/13	43.5/13	87/6.5	43.5/6.5
Unconsciousness	Mid	Mid	Mid	Not achieved
Induction time	3.5±1	5±1	5±1	Induction not achieved
Immobility	Mid	Mid	Mid	Late
Muscle Relaxation	Adequate	Adequate	Adequate	Low
Analgesia	Profound	Profound	Profound	Mild
Plane of Anesthesia achieved based on GUDEL'S Classification	Deep	Deep	Deep	Light
Duration of anesthesia	60±5	40±5	32±5	Nil
Recovery time	20±3	13±2	8±3	3±1
Post-operative complications-Death Rate	50%	16.6%	0	0

In Group 4 administered with KX combination at dose rate of K- 43.5 mg/kg b wt, X- 6.5mg/kg b.wt (the dose of KX was reduced to half as compared to Group 1), the anesthetic clinical effects could not be reached as unconsciousness and induction time was not achieved with low level of muscle relaxation, mild analgesia, light plan anesthesia and no post-operative complications.

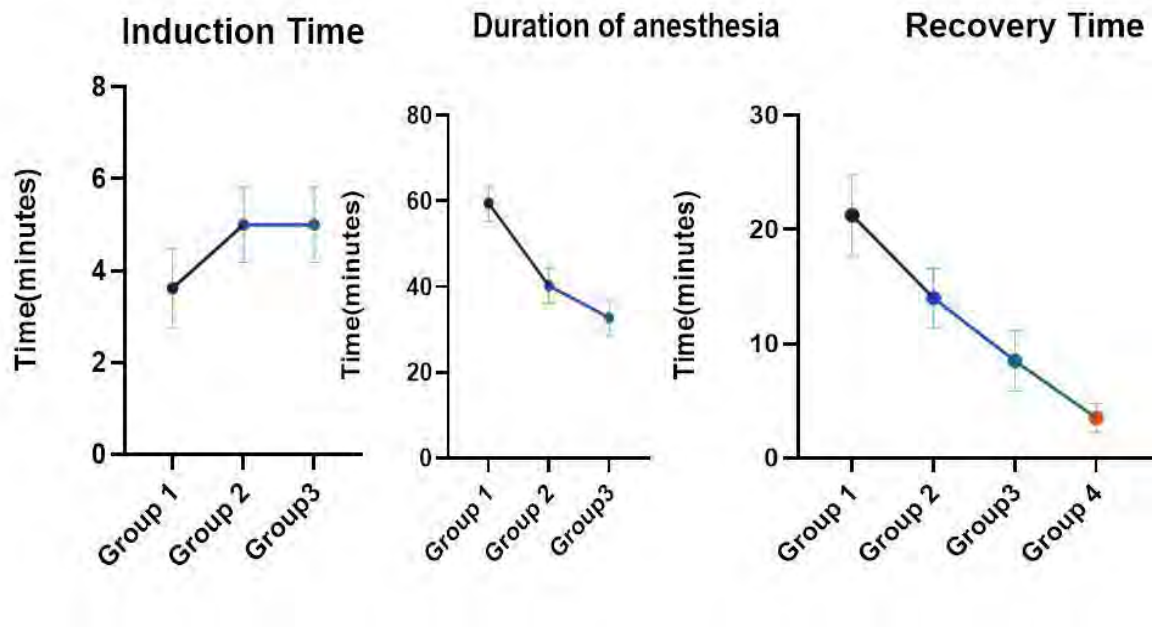

Fig. 2: Graphs showing induction time, duration of anesthesia and recovery time in different groups using different dose of KX cocktail

The other signs similar to all groups were decreased rectal temperature, respiratory rate and bradycardia. This experimental design involving 24 Male Wistar Rats aimed to testify the safe and effective dose of KX cocktail, showed 1K+1/2 X administered to group 3 as safe and effective dose (Fig. 1).

The current study was aimed at ascertaining the reliable, adjustable and safe anesthetic dose of Xylazine-Ketamine combination for providing better analgesia during medium duration procedure of orchiectomy.

Xylazine is categorized as α_2 agonist frequently used as sedative and a non-opioid anesthetic agent exclusively in animal practice and not in humans (Iannaccone *et al* 2009). The drug binds to α_2 adrenergic receptors located in the brain, spinal cord and blood vessels (Kacinko *et al* 2022). This drug can cause nervous and respiratory depression, decrease in cardiac output and even death (Muir et al 1977). Therefore, Xylazine is often used in combination with other drugs like ketamine, diazepam, butorphanol, medetomidine etc. for general anesthesia (Nishimur *et. al.,* 1992) (Sakaguchi *et. al.,* 1996).

The KX-combination as anesthetic agents is in vogue providing good sedation, analgesia with stable cardiopulmonary functions in animals (Tammam *et. al.,* 2019). The literature suggests the use of KX-combination in rat and mice with doses having a wide difference. KX combination varying from 65/4 to 100/13mg/Kg has been reported for mice [5]. KX combination in rats @ dose of 87mg/Kg of b wt. 13mg/Kg of b wt [4], 45 mg/Kg 21 mg/Kg of b wt. (Van 1977) and 43.5 mg/Kg 6.5 mg/Kg of b wt. [5] has been reported reliable in promoting anesthesia in rats. The data is immensely variable inviting further research for validation.

Our study showed better results with KX-combination in the dose of X- 6.5mg/kg b.wt and K- 87mg/kg b wt administered to Group 3, with good margin of safety, profound analgesia and adequate muscle relaxation in performing medium duration surgeries like orchiectomy. The study conducted by Van *et al* is in contrary with the present study as administration of KX in dose given to Group1 yielded post-operative complications with the death of 50% of animals due to respiratory arrest. This combination provided adequate level of analgesia and anesthetic duration but was little promising in margin of safety. Tammam *et al* arrived at optimal intraperitoneal dose of KX cocktail (K-43.5 mg/Kg b wt and X-6.5 mg/Kg B Wt) in Wistar rats with chronic liver injury as optimal dose. In our study the same dosage was used in group 4, the results are in contrary as this dose failed to achieve adequate level of unconsciousness, analgesia and muscle relaxation in Wistar rats.

For performing medium duration surgical procedures of Wistar rats with highest safety margin along with better recovery time is established in this study in Group 3 animals. Our study is in agreement with that of Van *et al* in choosing the dose of ketamine and Tammam *et al* in selecting the dose of Xylazine for optimal results.

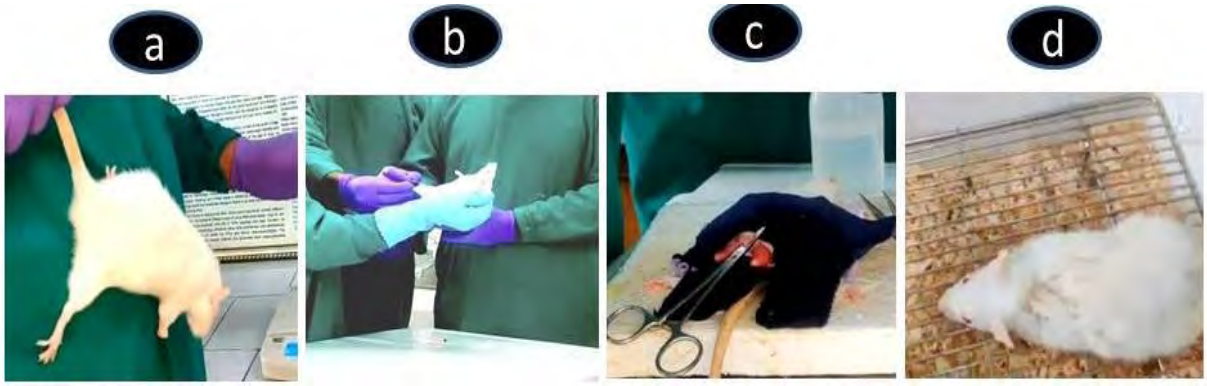

Fig. 3: Steps from anesthesia to recovery (a-Weighing and examination of rat, b-intraperitoneal injection of KX, c-Surgical Procedure, d- Post anesthetic recovery)

CONCLUSION

The appropriate dose of KX cocktail to be given as single injection through intraperitoneal route in Wistar rats for conduct of medium duration surgeries like orchiectomy were arrived to be K- 87mg/Kg and X-6.5 mg/Kg of b wt, for better safety.

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REFERENCES

Abouelfetouh, M.M., Liu, L., Salah, E., Sun, R., Nan, S. and Ding M., *et al.* 2021. The effect of xylazine premedication on the dose and quality of anesthesia induction with alfaxalone in goats. *Animals*, **11**:723.

Alexander, R.S., Canver, B.R., Sue, K.L. and Morford, K.L. 2022. Xylazine and overdoses: trends, concerns, and recommendations. *Am J Public Health,* **112:**1212–6.

Arnbjerg, J. 1979. Klinisk anvendelse af Ketamine-Xylazine til bedovelse af katte. *Nordisk veterinaermedicin*.

Bryda, E.C. 2013. The Mighty Mouse: the impact of rodents on advances in biomedical research. *Mo Med,* **110:**207.

Hsu, W.H., Bellin, S.I., Dellmann, H.D. and Hanson, C.E. 1986. Xylazine-ketamine-induced anesthesia in rats and its antagonism by yohimbine. *J Am Vet Med Assoc*, **189:**1040–3.

Iannaccone, P.M. and Jacob, H.J. 2009. Rats! Vol. 2, Disease models & mechanisms. *The Company of Biologists Limited*, 206–10.

Kacinko, S.L., Mohr. A.L.A., Logan, B.K. and Barbieri, E.J. 2022. Xylazine: Pharmacology Review and Prevalence and Drug Combinations in Forensic Toxicology Casework. *J Anal Toxicol*. **46:**911–7.

Muir, W.W., Skarda, R.T. and Milne, D.W. 1977. Evaluation of xylazine and ketamine hydrochloride for anesthesia in horses. *Am J Vet Res*, **38:**195–201.

Nishimura, R., Sakaguchi, M., Mochizuki, M., Sasaki, N., Takahashi, H., Tamura, H., *et al*. 1992. A balanced anesthesia with a combination of xylazine, ketamine and butorphanol and its antagonism by yohimbine in pigs. *J Vet Med Sci*, **54:**615–20.

Sakaguchi, M., Nishimura, R., Sasaki, N., Ishiguro, T., Tamura, H. and Takeuchi, A. 1996. Anesthesia induced in pigs by use of a combination of medetomidine, butorphanol, and ketamine and its reversal by administration of atipamezole. *Am J Vet Res,* **57:**529–34.

Tammam, O.Y., Taha, A.A. and El-Sherif, M.W. 2019. Optimization of Xylazine-Ketamine anesthetic dose in mice suffering chronic liver injury. *J Anes Cri*, **11**:6–8.

Van, P.L. 1977. Ketamine and xylazine for surgical anesthesia in rats. *J Am Vet Med Assoc*, **171**:842–4.