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UNDERSTANDING THE SYNERGISTIC IMPACT OF ATROPINE WITH XYLAZINE AND KETAMINE ON RECOVERY TIME, HEART RATE AND RESPIRATORY RATE IN MALE RABBITS

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ABSTRACT

The present study aimed to investigate the synergistic or antagonistic impacts of atropine alone or in combination with xylazine and ketamine on recovery time, heart rate, and respiratory rate during surgical interventions in rabbits. Fifteen rabbits were randomly assigned into three groups; the first group (GA) was injected with atropine for 10 minutes followed by a full dose of ketamine-xylazine (XK) injection, the second group (GB) was injected with atropine for 10 minutes followed by a half-dose injection of xylazine-ketamine mixture (XK), and the third group (GC) was injected only with a complete dose of xylazine-ketamine mixture. Based on the observation of recovery time, we demonstrated that half doses of ketamine and xylazine significantly (P=0.0009 and 0.0051) increased the time of recovery compared to any other treatment groups. Furthermore, the GB and GC groups experienced a substantial decrease in recovery time, which was directly proportional to the used dose. The effects of complete and half doses of ketamine and xylazine on heart and respiration rates in male rabbits were observed. No significant difference in heart rates amongst all treated groups before the injection of doses; however, a significant (P=0.00012) decrease in recovery time was observed in the GC group, compared to other treated groups. Using atropine with xylazine and ketamine increased anaesthesia longevity when compared with ketamine and xylazine alone. Importantly, the anaesthetic effect of atropine with full or half doses of xylazine and ketamine was similar. Interestingly, we revealed that heart and respiratory rates are improved when atropine is applied with half doses of xylazine and ketamine.

keywords: Rabbits, Atropine, Xylazine, Ketamine, Vital Signs

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INTRODUCTION

Rabbits are sensitive to many chemicals, including anaesthesia, due to their sensitive nature, and it has been recorded that as high as 5.8% of fatalities are observed during administration of xylazine-ketamine anaesthesia (Henke *et al.*, 2005). During anaesthesia administration in rabbits, plasma catecholamine concentrations may rise due to their susceptibility to stress, which may culminate in fatalities (Clarke & Trim, 2013). The anaesthetic combinations, especially xylazine and ketamine, have historically been administered intramuscularly (IM). However, this pain relief combination is not strong enough for major surgeries and often causes a dangerous drop in blood pressure, which could explain some of the deaths seen (Avsaroglu et al., 2003). It is commonly known that the majority of anaesthetic procedures applied in rabbits result in a noticeable and protracted hypotension, particularly when the anaesthesia lasts longer than an hour (Marini *et al.*, 1992). While the loss of sensations allows medical and surgical treatments, anxiety, discomfort, and the amount of anaesthetic agent required are all reduced by premedication before anaesthesia. One of the most widely used preventive medications in veterinary medicine is xylazine. Moreover, it is a muscle relaxant, sedative, and analgesic that has been safely used with other medications to induce anaesthesia (Purohit *et al.*, 2008).

Ketamine produces a dissociative anaesthetic state and is categorized as an antagonist of the N-methyl-D-aspartate (NMDA) receptor. An arylcycloalkylamine with a structure similar to cyclidine, such as phencyclidine, eticyclidine, rolicyclidine, and tenocyclidine, is ketamine 2-(2-chlorophenyl)-2-(methylamino). In healthy animals, ketamine often enhances cardiovascular function, raising mean arterial pressure and heart rates. Due to its link to tachycardia, catalepsy, inadequate muscle relaxation, and muscle rigidity, it is rarely used alone. Instead, it is frequently used in combination with xylazine, diazepam, and acepromazine to reduce side effects (Tranquilli *et al.*, 2007). One of the cyclohexylamine classes of drugs, ketamine, is frequently used to induce and maintain anaesthesia. Because it acts quickly and has little effect on the heart and lungs, it is utilized in rabbits. As ketamine alone is insufficient to provide the required level of anaesthesia, it is frequently used in conjunction with preanesthetic medications, such as xylazine. Wide safety margins have been found when using xylazine and ketamine together, as it has been shown to be more effective in rabbits (Dupras *et al.*, 2001). Ketamine tends to produce hypertonus, inadequate muscular relaxation, persistent pain reflex reactions, and a violent anaesthesia recovery when taken as a stand-alone anaesthetic. Therefore, ketamine has been combined with other medications, such as xylazine, to alleviate these unwanted side effects. It has been noted that xylazine and ketamine together can provide effective anaesthesia in rabbits, albeit certain mortality is occasionally observed (Kiliç, 2004).

Most animals, post-xylazine administration, experience respiratory depression depending on the species and dosage, bradycardia, bradyarrhythmias, and hypotension (Amarpal *et al.*, 2010). The α_2 adrenergic agonist xylazine is a dose-dependent drug that induces bradycardia, hypertension, and hypotension. Its effects include sedation, analgesia, and muscular relaxation. The kidney excretes ketamine and xylazine after they are mostly processed by liver cytochrome P450 enzymes (Baniadam *et al.,* 2004).

Parasympathetic impulses to the cardiopulmonary system, glands, and

smooth muscle are blocked by anticholinergics (e.g., atropine sulphate). As a result, they suppress bronchial and salivary gland secretions, slow the heart (bradycardia), and inhibit vaso-vagal reflexes. Anticholinergic medications that lessen ketamine-induced tachycardia and hypersalivation include atropine sulphate (A) and are frequently used as pre-anaesthetics (Wellington *et al.*, 2013).

Since there is no single drug that is considered perfect for anaesthesia, anaesthesia is typically produced by combining two or more drugs. Gaseous and injectable anaesthetics are used to induce and maintain anaesthesia (Tonner, 2005). Although gaseous anaesthetics provide good control over the level of anaesthesia, their use is limited by equipment complexity and a lack of skilled operators. (Brattwall *et al.*, 2012) The choice of anaesthetic is based on the availability, safety, and development of surgical tolerance for the drug (Fu *et al*., 2025). The level of anaesthesia known as surgical tolerance occurs when an animal is totally unaware of its surroundings and is not capable of feeling pain. This is the most important factor to consider when choosing an anaesthetic protocol (Orrell *et al.*,2025). Surgical interventions are carried out, and the length of the interventions depends on the duration. The α2-agonists, such as xylazine (X), and mostly dissociative medications, like ketamine (K), are frequently used together (Alves *et al.*, 2009). However, it has been shown that not all animals develop surgical tolerance when receiving the KX combination at prescribed doses. Therefore, to induce surgical tolerance, additional drugs are administered. Due to their safety and surgical tolerability, acepromazine and KX are considered an ideal combination. Since acepromazine is not sold in the region, a replacement must be found, and different KX dosages adjusted in conjunction with the new drug are to be explored. Anticholinergic medications, including atropine sulphate (A), are frequently used as pre-anaesthetics because they lessen the bradycardia and hypersalivation exacerbated by ketamine. Furthermore, veterinary clinics commonly combine atropine with KX (Wellington *et al.*, 2013). Therefore, the objective of the current study was to determine the optimal dosage of KX alone or in combination with atropine to elicit minimal cardio-pulmonary effects while maximizing recovery time without exacerbating animal distress during anaesthesia.

MATERIALS AND METHODS

Ethical approval:

This research was designed and done in accordance with all applicable national and international regulations as well as ethical principles followed by the Animal Care and Use Committee/College of Veterinary Medicine, University of Basrah/Iraq. (Approval number: 67/37/2025).

Animals:

The study was conducted on 15 adult male rabbits with body weights ranging from 1.5 to 2.0 kg. The animals were placed in the cages at the Veterinary Medicine College, University of Basrah. Physical examinations were conducted on all animals to ensure their health before starting the experiment (Alrafas *et al.*, 2023). The rabbits were randomly divided into three groups (as outlined earlier by (Albozachri *et al.*, 2017)

- Group GA (*n*=5): After 10 minutes, Group A received intramuscular injections of the following pre-medication: atropine 1% (Norvel, India), 0.5 mg/kg B.W.; xylazine 2% (Alfasan, Holland); and ketamine hydrochloride 10%, 10 mg/kg and 50 mg/kg B.W., respectively.
- Group GB (*n*=5): After 10 minutes, Group B received intramuscular injections of the following pre-

medication: atropine 1%, 0.5 mg/kg B.W.; xylazine 2%; and ketamine hydrochloride 10%, 5 mg/kg and 25 mg/kg B.W., respectively.

- Group GC (*n*=5): Xylazine 2% and ketamine hydrochloride 10% were given intramuscularly to Group (C) at doses of 10 mg/kg and 50 mg/kg BW, respectively.

Experimental design:

Several parameters were measured as outlined by Henke *et al.* (2005). Two minutes prior to injection, baseline measurements of heart rate (HR, beats/minute) and respiratory rate (RR, breaths/minute) were taken with a stethoscope before any anaesthetic agents were administered. At this baseline moment, thoracic movement was also noted and documented.

Induction and Maintenance of Anaesthesia:

An intramuscular injection was used to produce anesthesia. The lack of pedal response was considered the first sign of anesthesia. The time between losing the pedal reflex and regaining it was used to calculate the length of surgical anesthesia (Abduljaleel, 2024).

Cardiopulmonary Parameter Monitoring:

Cardiopulmonary parameters, including RR, HR, and thoracic movement, were recorded at the following time points:

- Baseline (2 minutes pre-injection).
- Every 5 minutes during surgical anaesthesia.
- Every 5 minutes during the initial recovery phase.
- Every 10 minutes during the later stages of recovery.

Recovery Period Definition:

The time from the beginning of surgical anaesthetic (loss of pedal reflex) until the animal recovered the righting reflex and was able to stand steadily was referred to as the recovery period. The following reflexes were monitored to assess recovery:

- Palpebral reflex: Return of blinking.
- Pedal reflex: Return of response to toe pinch.
- Righting reflex: The ability to return to a normal standing position.
- Muscle tone: Gradual return of normal muscle tone.

Total recovery time was calculated from the start of surgical anaesthesia to the point where the animal exhibited a stable standing position (Abduljaleel, 2024).

Statistical analysis:

Statistical analysis was performed using GraphPadPrism software (San Diego, CA, USA). Experiments were repeated at least three times to confirm reproducibility. To describe statistical differences, a one-way ANOVA, followed by Tukey's post-hoc comparison test, was used unless otherwise noted. Significance was determined to have a p-value of 0.05 (*), 0.01 (**), 0.001 (***), or 0.0001 (****) (Jasim *et al*., 2025).

RESULTS

Effect of different doses of combination (K+X) on the time of recovery in male rabbits:

The data presented in Table 1 showed the effect of complete and half doses of ketamine and xylazine on male rabbits. The result indicated a significant (p= 0.009 and 0.0051) increment in time of recovery in animals belonging to GA compared to any other treatment groups. Additionally, time of recovery decreased significantly in GB and GC groups with decreasing doses.

Cardiopulmonary effects of different doses of combination (K+X) in rabbits: -

We next analyzed the effect of complete and half doses of ketamine and xylazine on heart rate and rate of respiration in male

rabbits. Analysis of the data showed no significant difference in heart rate among all treated groups before drug injection. However, during recovery time, the heart rate in GC dropped considerably (P=0.00012) in comparison to other treated groups (Table 2). No changes in respiratory rate between groups before treatment were observed. The results showed a significant change in respiratory rate in animals belonging to group C (P=0.0013) after recovery, and it was not applicable in animals belonging to the GA and GB groups (Table 3).

Table 1: Effect of different doses of combination (K+X with or without A) on time recovery.

Groups	Time of recovery (minute)
GA (A(K+X) complete dose)	182.50± 9.57^{A***}
GB (A(K+X) half dose)	125.00± 12.90^{B**}
GC (K+X)	102.50± 17.07^{C*}
LSD	22.50

^{A,B,C} Values in different superscript letters within column are significantly different at P < 0.05 (*), 0.01 (**), 0.001 (***), or 0.0001 (****), respectively.

Table 2: Effect of different doses of combination (K+X with or without A) on heart rate.

Groups	Time	Heart rate (bpm)
GA (A(K+X) complete dose)	before injection	156.25 ±4.78^{A*}
	Recovery time	140. 00±8.16^{b**}
GB (A(K+X) half dose)	before injection	157.50±12.58^{A*}
	Recovery time	152.50±9.57^{a***}
GC (K+X)	before injection	161.25±8.53^{A*}
	Recovery time	125.00±5.77^{c*}
LSD	before injection	N.S
	Recovery time	15.00

$^{A, B, C, a, b, c}$ Values in different superscript letters within column are significantly different at P < 0.05 (*), 0.01 (**), 0.001 (***), or 0.0001 (****), respectively.

Table 3: Effect of different doses of combination (K+X with or without A) on respiratory rate.

Groups	Time	Heart rate (bpm)
GA (A(K+X) complete dose)	before injection	45.50 ± 5.00 ^{A*}
	Recovery time	42.50±9.57 ^{c*}
GB (A(K+X) half dose)	before injection	45.00±10.00^{A*}
	Recovery time	45.00±10.00^{b**}
GC (K+X)	before injection	45.00±5.77^{A*}
	Recovery time	55.00±5.77^{a***}
LSD	before injection	N.S
	Recovery time	NS

$^{A, B, C, a, b, c}$ Values in different superscript letters within column are significantly different at P < 0.05 (*), 0.01 (**), 0.001 (***), or 0.0001 (****), respectively.

DISCUSSION

The current study aimed to determine the optimal dosage of KX alone or in combination with atropine to elicit minimal cardiopulmonary effects while maximising recovery time without exacerbating animal distress during

anaesthesia. The continuous anaesthesia protocol used for rabbits in veterinary clinics and the veterinary teaching hospital in Basrah City, Iraq, is the xylazine with ketamine protocol, but this protocol gives a short recovery period, so we added atropine in a full dose and in a half dose to find out which protocol gives a longer period so that we can recommend its use in clinical cases that require a longer period of surgery, such as fracture cases.

In GA, atropine was injected as a premedication dose and KX was injected 10 minutes later. In GB, atropine was injected as a premedication level followed by injection of half of the KX dose ten minutes later. In GC, KX was injected alone without atropine. The study showed a significant difference when using a full dose of atropine in increasing the recovery time compared with using half a dose or without using atropine. Our findings suggest that using atropine in combination with xylazine and ketamine is different from using xylazine and ketamine alone (Albozachri *et al.*, 2017).

Hedenqvist (2008) and Hedenqvist *et al.* (2002) have reported that ketamine and xylazine can be widely used to induce anaesthesia in rabbits to perform temporary surgical anaesthesia in these animals. Because of its association with tachycardia, catalepsy, inadequate muscle relaxation, and muscle rigidity, ketamine is rarely used alone. Instead, it is often used in combination with xylazine to reduce the negative effects. Since xylazine and ketamine are highly soluble in fat and can be redistributed to muscles and adipose tissues, this may be due to their widespread distribution in the body (Amarpal *et al.*, 2010). The effectiveness of anticholinergic medications varies significantly depending on the type. They have beneficial effects by preventing bradycardia and the accumulation of salivary secretions that can occur during anaesthesia. They are often recommended in research with laboratory animals, even though there is little evidence of their effectiveness in these animal species. Prevention of bradycardia is particularly important when these laboratory animals are being used for prolonged surgical and experimental procedures. In experimental animals (e.g., mice and rabbits), alpha-2-adrenergic drugs such as xylazine are often included in injectable anaesthetic combinations, which cause significant reductions in heart rate (Eisele, 1990).

The study showed a significant difference in heart rate and respiratory rate between different treatment groups. We have shown that the use of half of the dose with atropine was accompanied by equal respiratory rates and heart rate before the injection of anaesthesia, compared to the first and third groups, due to the use of a lower dose of anaesthesia. Similar observations were also noticed by Hedenqvist (2008). The use of the full dose of anaesthesia with atropine offered a significant difference in reducing heart and respiration rates compared with animals treated without atropine. Passive collapse of diseased airways can occur as a result of anaesthetic drugs and declining respiratory rate in rabbits has previously been demonstrated by several studies. In general, the xylazine component of the mixture is most likely to cause a drop in the heart rate and respiration (Borkowski *et al.*, 1990; Eesa, 2010).

Based on the results of this study, it is plausible to conclude that the use of atropine with xylazine and ketamine can increase the recovery period from anaesthesia compared with ketamine and xylazine alone. The effect on recovery time remained similar when atropine was used with half doses of ketamine and xylazine. We also observed improved heart rate and respiratory rates when atropine was used along with half doses of xylazine and ketamine.

CONCLUSIONS

To improve recovery time, it is imperative to use atropine before injecting xylazine and ketamine. Pre-medicating with atropine is proposed to reduce the amount of anaesthetic agent needed for anaesthesia in rabbits. Moreover, when utilized as a pre-medication, the combination of xylazine and ketamine as an anaesthetic has a minimal impact on heart and breathing rate.

NOVELTY STATEMENT

Despite many cases of side effects of anaesthesia, the field of anaesthesia remains a challenge in veterinary medicine, and few researchers have shed light on the use of anaesthesia in rabbits. Our finding offers reduced use of anaesthesia with prolonged recovery timing.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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AUTHOR'S CONTRIBUTION

MRA: Contributed to the preparation of the animals. AWA: Contributed to calculating the doses and monitoring the animals. MFA and AAA: Analysis and interpretation of data, as well as writing and revision of the manuscript. AAI, and MMJ: Contributed to monitoring the animal during the anaesthetic pre- and post-injection period. WMMS: Contributed to the statistical analysis and contacted the journal for publishing

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فهم التأثير التآزري للأتروبين مع الزيلازين والكيتامين على وقت التعافي، ومعدل ضربات القلب، ومعدل التنفس في ذكور الأرانب

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ركزت هذه الدراسة على استكشاف التأثير المشترك أو المعاكس لدواء الأتروبين سواء بمفرده أو حين يُدمج مع الزيلازين والكيتامين لتقييم انعكاس ذلك على مدة التعافي ومعدل ضربات القلب والتنفس خلال العمليات الجراحية التي أُجريت على الأرانب وتم تخصيص خمسة عشر أرنباً عشوائياً لثلاث مجموعات حيث حُقنت المجموعة الأولى بالأتروبين لعشر دقائق تبعتها جرعة كاملة من خليط الكيتامين والزيلازين أما المجموعة الثانية فحُقنت بالأتروبين للمدة ذاتها لكن تبعتها نصف الجرعة الكاملة من الخليط فيما حُقنت المجموعة الثالثة بجرعة كاملة فقط من خليط الزيلازين والكيتامين وكشفت الملاحظات بخصوص وقت التعافي أن استخدام نصف الجرعة من الكيتامين والزيلازين أدى إلى زيادة كبيرة في وقت التعافي مقارنة بأي من المجموعات الأخرى بالإضافة إلى ذلك شهدت المجموعتان الثانية والثالثة انخفاضاً ملحوظاً في وقت التعافي وكان هذا الانخفاض مرتبطاً بشكل مباشر بحجم الجرعة المستخدمة ولوحظت تأثيرات الجرعات الكاملة والنصف كاملة على معدلي القلب والتنفس في ذكور الأرانب ولم يُسجل أي فرق يُذكر في معدلات ضربات القلب بين المجموعات قبل حقن الأدوية إلا أن المجموعة الثالثة سجلت انخفاضاً كبيراً في وقت التعافي مقارنة بالمجموعات المعالجة الأخرى ومن المهم الإشارة إلى أن إدراج الأتروبين مع الزيلازين والكيتامين زاد من استمرارية التخدير مقارنة باستخدام الكيتامين والزيلازين وحدهما وكان التأثير التخديري للأتروبين متشابهاً سواء استُخدم مع جرعات كاملة أو نصف كاملة من الزيلازين والكيتامين والأمر اللافت أن الدراسة كشفت عن تحسن في معدلات ضربات القلب والتنفس عند تطبيق الأتروبين مع نصف جرعة من الزيلازين والكيتامين.