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Original Article *Surgery*

Sedative, analgesic and behavioral changes of caudal epidural injection of xylazine, magnesium sulphate in Egyptian water buffalo (*Bubalus bubalis*)

Ahmed Khalil[a*], Mohamed Zeineldinb [b,c], Atef Abd Al-Galil[d], Seham Abo-Kora[e] and Ahmed Sabek[f]

[a]Department of Veterinary Surgery, Anesthesiology and Radiology, College of Veterinary Medicine, Benha University, Benha, Egypt.
[b] Department of Animal Medicine, College of Veterinary Medicine, Benha University, Benha, Egypt.
[c]Carl R. Woese Institute of Genomic Biology, Infectious Genomic of One Health (IGOH), University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.
[d] Department of Veterinary Surgery, Anesthesiology and Radiology, College of Veterinary Medicine, Monofia University, ShebinEl-Kom, Egypt
[e] Department of Pharmacology, College of Veterinary Medicine, Benha University, Benha, Egypt.
[f] Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Benha University, Benha, Egypt.

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Corresponding author: Ahmed Khalil
Email: ahmed.khalil@fvtm.bu.edu.eg

ABSTRACT

Objective: To assess and compare the effects of epidural xylazine (XY), magnesium sulphate (MG) and their combination (XY-MG) in buffalo.
Design: Crossover study
Animals: Seven healthy, non-pregnant buffalo
Procedures: Buffaloes were allocated into one of three groups in a prospective randomized crossover design with two weeks washout period as the following: XY group, MG group and XY-MG group. The sedative, analgesic, and behavioral effects of XY, MG and their combination were evaluated prior to administration and at then after injection at 5, 10, 15, 30, 45, 60, 90 120, 150, 180, and 210 minutes.
Results: Clinical and hematological variables were evaluated at baseline (0 minute) and then at 15, 30, 45, 60, 90, 120, 180, and 210-minutes post-administration. Buffaloes received XY-MG combination showed the fastest onset and resulted in the longer duration of epidural analgesia. The longest duration of complete sedation was recorded in group XY-MG. All treatment protocols caused mild ataxia and affected the heart rate, respiratory rate and rectal temperature of buffalo. There was a significant decrease in the blood glucose at 30 minutes after injection in all groups, returning to the baseline level by 180 minutes in XY-MG group and 210 minutes in XY and MG groups.
Conclusion and clinical relevance: XY-MG combination may be used as epidural sedative and analgesic to control pain in buffalo.
Keywords: Buffalo, Epidural, Locomotion, Magnesium sulphate, Xylazine

1. Introduction

Pain control in veterinary practice can be achieved through the use of opioids, α-2-adrenoceptor agonists, local anesthetic, non-steroidal anti-inflammatory and multimodal medicine [1-6]. The clinical selection of analgesic drugs and route of administration are dependent upon on animal species and surgical procedures [7]. In ruminants, as an alternative to general anesthesia, caudal epidural analgesia is the most commonly used methods for pain control in many clinical conditions including dystocia, uterine prolapse, vaginal, rectal, perineal and bladder surgery [8; 9]. Because of the increased risk of problems such as regurgitation, bloating, and muscular injury, ruminants are poor candidates for general anesthesia [3]. As a result, surgical operations in standing animals under local anesthetic are favored [10].

Paravertebral nerve blocks, local anesthetic infiltration, intravenous regional limb perfusion, and epidural anesthesia are all common procedures. Several obstetrical surgeries, as well as surgical procedures of the anus, vulva, perineum, caudal udder, and scrotum, are performed under epidural anesthesia. As an additional control for tenesmus, epidural analgesia is used [11].

The first coccygeal intervertebral space (Co1-Co2) and the sacrococcygeal intervertebral space (S5-Co1) are the most popular locations for epidural administration of anesthetic drugs in large ruminant [12]. Constant-rate infusion of an analgesic into the epidural space and obtaining a sufficient duration of analgesia during potentially long surgeries could be performed only after the insertion of an epidural catheter and not through a single injection [13]. Placing an epidural catheter is a time-consuming procedure that requires a certain degree of expertise and carries a high risk of severe complications [14; 15].

Ultrasonographic guidance was thus often used in perineural and spinal analgesia to alleviate the complications of epidural procedure, recently, the practicability of ultrasound-guided epidural injection technique has been studied in human and small animals. As a result, there is a high likelihood of practicality and effectiveness and subsequent fewer complications [13]. Xylazine (XY), an alpha-2 agonist, is one of

the most commonly used analgesic solutions in cows and buffaloes [16]. The low molecular weight and lipophilic characteristics of XY improve its meningeal penetration when administered epidurally [17]. While XY is a potent and effective sedative and analgesic drug, it induces mild-to-moderate cardiopulmonary depression and ataxia [8; 18-20].

When compared to other local anesthetic drugs like lidocaine, epidural alpha-2 agonists like xylazine produce a longer duration of analgesia and less loss of motor function in large ruminants [8]. Magnesium is the fourth cation in the body and has a fundamental role in many cellular functions [21] Magnesium sulphate's analgesic effects are due to its antagonistic action on the N-methyl-D-aspartate -receptor that contributes to the development of central sensitization [22; 23]. Other feasible mechanisms of MG intervention include inhibition of catecholamine release that causes a subsequent reduction in neuronal activity [24]. Analgesic and sedative effects of using magnesium with epidural local anesthetics have been reported to increase the duration, hasten onset and increase the intensity of analgesia in goats [25], horses [26], and cattle [27]. To the authors' knowledge, the use of MG or its combination with XY in buffalo are not reported previously. This study was conducted to compare the effect of XY, MG and XY-MG combination on the sedative, analgesic, locomotors behavior, clinical and hematobiochemical parameters in buffalo.

2. MATERIALS AND METHODS

2.1. Ethics Statement

The study procedures have been performed in accordance with recommendations of the guidelines for care and use of animals at the College of Veterinary Medicine, Benha University, Egypt. The research protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the College of Veterinary Medicine, Benha University (BUFVTM-01-07-2018).

2.2. Animals and Experimental Design

The experiment was carried out in the educational ruminant farm at the College of Veterinary Medicine, Benha University. A total of seven non-pregnant buffaloes (4-6 years old and weighting between 454 and 572 kg) were selected and used in this study in a prospective simple randomized crossover design, with the main investigator unaware of the drug used. Prior to enrollment, all buffalos were subjected to a complete physical and clinical examination to ensure that they were clinically healthy including, assessment of heart rate (HR), respiratory rate (fR), rectal temperature, lung sound, mucous membranes, and ruminal movement. The buffaloes were maintained during the whole experiment under the same management and feeding conditions. Prior to the experiment, food and water were withheld for 24 and 6 hours, respectively. Buffaloes were allocated randomly to receive each of epidural treatment with a wash-out period of 2 weeks between treatments. According to the treatment, the group were: XY group receiving xylazine HCL at 0.05 mg/kg body weight (Xylaject 2%, Adwia Co., Country), MG group receiving 1 mL of magnesium sulphate 10% solution (Magnisol, magnesium sulphate 10% ampoules, Memphis Co., Egypt), and XY-MG group receiving the same dosing levels of each drug. To avoid the bias due to the different volume between groups; 1:1 dilution with saline solution was applied to keep the volumes equal between groups (complete all doses till 5 ml with normal saline solution). To avoid drug compounding safety concerns, 1 mL of 2% XY was mixed with 1 mL of 10% MG and the XY-MG combination was centrifuged and microscopically examined for precipitate.

2.3. Ultrasonography guidance epidural injection

All treatments were performed using ultrasonographic guided technique through caudal sacrococcygeal epidural injection. All buffaloes were restrained in a standing position in metal stanchion and sacrococcygeal area was shaved, aseptically scrubbed, and prepared for epidural catheterization. Ultrasonographic guided epidural injection was performed using ultrasonographic machine (CHISON ECO 3 Expert, Medical Imaging Equipment, Zhejiang, China) with an adjusted 6.0 MHz Micro-Convex transducer probe. The probe was placed on a sagittal plane over the sacrum (appeared as double echogenic layered structure with distal shadowing) and moved caudally till discriminate the first anechoic space that represent the sacrococcygeal joint (Fig.1A).

Local anesthetic agent (2mL, lidocaine HCL 2%, AstraZeneca co., *Egypt*) was injected over the injection site to desensitize the skin and minimize reaction during insertion of the needle (Fig. 1B). The probe was then placed in a transverse plane to distinguish the sacrococcygeal epidural space (Fig. 1C). The anechogenic epidural space appeared approximately at 2.5-3 cm from skin surface. The hypoechoic vertebral canal (red pinpointed) is ventrally bordered by the cranial epiphysis of the first coccygeal vertebra (v) and laterally bordered by erector spinae muscle (Fig.1D). Using ultrasonographic guidance, the epidural catheter was successfully placed into the epidural space and directed cranially using a 17-gauge, 5 cm long spinal needle (Tuohy needle, reusable technique needle, Tuohy; thin wall, Becton Dickinson, and Company; NJ, USA).

The successful needle insertion was confirmed by observing an echogenic needle between the double laminae of the dura mater. Once the needle in epidural space, the catheter (Flextip Plus, Arrow Epidural Anesthesia Catheter Arrow International Reading, PA, USA) was inserted and then moved 4 cm beyond the needle tip. Finally, the needle was removed, and the catheter was secured in correct position till completion of injection using adhesive tape and sutured to skin. The free catheter end was protected by capped and wrapped using sterile gauze to ensure that injections into the catheter were sterile (Fig. 1E, F and G). Finally, the calculated dose of each treatment was injected within a time of 10 seconds.

2.4. Epidural analgesia and sedation evaluation

The person allocated to assess analgesia was blinded to all treatments. Scores of analgesia and sedation were recorded at baseline (0 minute before drug administration) and then after injection at 5, 10, 15, 30, 45, 60, 90 120, 150, 180, and 210 minutes. Time to onset of analgesia was recorded each minute after epidural injection till loss of sensation by evaluating the animal's response to pin pricks implemented in the perineal region (base of the tail, anus, perineum and upper hind limb). Pinpricks were made by using a 21-gauge, 2.5-cm long hypodermic needle inserted through the skin into the underlying tissues [28]. If there was no response to superficial pinpricks, an additional two to three pricks were applied at 2 cm depth (only on area we can reach this depth) to confirm the complete desensitization. Time from injection to appearance of analgesia and sedation has been recorded and considered as time to onset of analgesia and sedation, respectively. The time between appearance and disappearance of analgesia and sedation had also been documented and considered as duration of analgesia and sedation, respectively. Analgesia and sedation were evaluated while the buffalo standing in a stanchion. All values were scored according to modified simple descriptive numerical scale (0 to 3) by the observer, modified from [1] (Table 1).

Table 1. Scoring system for analgesia, sedation and ataxia after epidural injection of xylazine (XY), magnesium sulphate (MG) and xylazine-magnesium sulphate (XY-MG) combination in buffalo.

Score	Analgesia	Sedation	Ataxia
0	No analgesia (strong response to avoid pinpricks such as try to kick)	No sedation (Animal standing alert and conscious)	Normal (Animal walking without staggering)
1	Mild analgesia (weak response to pinpricks such as sudden lateral movement of the hind quarter)	Mild (Animal appeared tired with slight lowering of head carriage, and reduced awareness)	Mild (animal standing with little spacing between hind limbs, slight stumbling while walking, and easily able to continue walking)
2	Moderate analgesia (occasional response to pinpricks such as turning the head towards the site of stimulation)	Moderate (clear ptosis of eye lids, prolapsed third eyelid, lethargy and ptyalism)	Moderate (animal standing with wide spacing between hind limbs, marked stumbling while walking, knuckling of the fetlocks and walking but very ataxic)
3	Complete analgesia (no avoidance response to pinpricks)	Deep (clear lethargy, extreme lowering of head, standing with wide stance and need to lean on stanchions for support)	Sever (animal unable to stand and became recumbent)

2.5. Locomotor behavior observation

Buffalos were observed before the treatment and the normal locomotion was observed and recorded then locomotion was evaluated after treatment at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, and 210 minutes to follow up the degree of ataxia (abnormal locomotion). The examiner who was unaware of the injected drugs stood a suitable distance from the animals to make a good observation. By focal observation, the examiner observed the locomotion of the animals by allowing the animal to get out from the stanchion for few steps and return again. All values were scored according to modified simple descriptive numerical scale (0 to 3) by the observer, as described by [1] (Table 1).

2.6. Clinical parameters evaluation

Clinical variables including HR, f_R and rectal temperature were evaluated at baseline (0 minute) and then at 15, 30, 45, 60, 90-, 120-, 180-, and 210-minutes post-administration.

2.7. Hematological and biochemical analyses

Blood samples were collected at each time by jugular vein puncture for both hematological and biochemical analyzes. Blood samples were collected at baseline (0 minutes) and then at 15, 30, 60, 120, 180, and 210 minutes after treatment. The hematological blood sample was taken with anticoagulant Ethylene diamine-tetraacetic acid for determination of total erythrocytes count (RBCs), hemoglobin (HB), packed cell volume (PCV), white blood cells (WBCs), neutrophils and lymphocytes using haematology analyzer (Model XF9080, Perlong Medical Machine Co., Ltd, Nanjing, China). The biochemical blood sample was collected using plain tube without anticoagulant, centrifuged at 1008 g or 10 minutes, and clear, nonhaemolyzed serum samples were stored at −20°C until further testing. The serum samples were then used for assessing blood glucose, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine using commercial kits provided by Spectrum Diagnostics and spectrophotometric analysis (Clinical Chemistry Analyzer ERBA CHEM 7, ERBA, Germany).

2.8. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 20 (IBM Armonk, NY, USA) and JMP Pro 13 (SAS Institute Inc., NC, USA). Normality of the data distribution was evaluated by the Shapiro-Wilk test. Comparisons between treatments and within each treatment were performed using a mixed-model Analysis of variance (ANOVA) with the animal as a random effect and time-point as a repeated effect. For multiple pairwise comparisons, the Bonferroni procedure was used to correct the p value. Furthermore, the Dunnett's multiple comparison test has been used to compare each time point within the treatments with their respective baseline values. Significance was set at (p<0.05) and all values were presented as the mean ± standard deviation (SD).

3. RESULTS

Table 2. Degree of analgesia in xylazine (XY), magnesium sulphate (MG) and xylazine-magnesium sulphate (XY-MG) combination groups at baseline (prior to treatment) and at different time points post treatment.

Treatment	Baseline	post administration										
	T0	T5	T10	T15	T30	T45	T60	T90	T120	T150	T180	T210
XY	0 (0-0) ^{1a}	1 (0-1) ^{1a}	3 (2-3) ^{2b}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	2 (1-2) ^{2a}	1 (0-2) ^{1a}	1 (0-1) ^{1a}
MG	0 (0-0) ^{1a}	0 (0-1) ^{1a}	1 (0-2) ^{1a}	2 (2-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (1-2) ^{2a}	1 (1-2) ^{1a}	1 (0-1) ^{1a}	0 (0-0) ^{1a}
XY-MG	0 (0-0) ^{1a}	1 (0-2) ^{2a}	3 (3-3) ^{2b}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2a}	3 (3-3) ^{2b}	3 (3-3) ^{2b}	3 (3-3) ^{2b}

Variables with different superscript letters at the same column are significantly different at $p \leq 0.05$
Variable with different superscript numbers in the same row are significantly different at $p \leq 0.05$.

Table 4. Degree of sedation in xylazine (XY), magnesium sulphate (MG) and xylazine-magnesium sulphate (XY-MG) combination groups at baseline and at different time points post treatment.

Treat-ment	Baseline	post administration										
	T0	T5	T10	T15	T30	T45	T60	T90	T120	T150	T180	T210
XY	0 (0-0) ^{1a}	0 (0-0) ^{1a}	1 (0-1) ^{1a}	2 (1-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (1-2) ^{2a}	1 (1-2) ^{2b}	0 (0-1) ^{1a}	0 (0-0) ^{1a}
MG	0 (0-0) ^{1a}	0 (0-1) ^{1a}	1 (0-1) ^{1a}	1 (1-2) ^{1a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (1-2) ^{2a}	1 (0-2) ^{2a}	1 (0-2) ^{1a}	0 (0-1) ^{1a}	0 (0-0) ^{1a}
XY-MG	0 (0-0) ^{1a}	0 (0-1) ^{1a}	1 (0-2) ^{2a}	2 (2-2) ^{2b}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2a}	2 (2-2) ^{2b}	2 (2-2) ^{2b}	1 (1-2) ^{1a}

Variables with different superscript letters at the same column are significantly different at $p \leq 0.05$
Variable with different superscript numbers in the same row are significantly different at $p \leq 0.05$.

Table 5. Degree of abnormal locomotion (ataxia) in xylazine (XY), magnesium sulphate (MG) and xylazine-magnesium sulphate (XY-MG) combination groups at baseline and at different time points post treatment.

Treatment	Baseline	Time post administration										
	T0	T5	T10	T15	T30	T45	T60	T90	T120	T150	T180	T210
XY	0 (0-0) ^{1a}	0 (0-0) ^{1a}	1 (0-1) ^{1a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (0-1) ^{2a}	1 (1-1) ^{1b}	1 (1-1) ^{1b}	0 (0-0) ^{1a}
MG	0 (0-0) ^{1a}	0 (0-0) ^{1a}	0 (0-1) ^{1a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (0-1) ^{2a}	0 (0-1) ^{1a}	0 (0-1) ^{1a}	0 (0-0) ^{1a}	0 (0-0) ^{1a}
XY-MG	0 (0-0) ^{1a}	0 (0-1) ^{1a}	1 (0-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2a}	1 (1-1) ^{2b}	1 (0-1) ^{1a}	0 (0-1) ^{1a}

Variables with different superscript letters at the same column are significantly different at $p \leq 0.05$
Variable with different superscript numbers in the same row are significantly different at $p \leq 0.05$.
(XY) xylazine, (MG) magnesium sulphate and (XY-MG) xylazine-magnesium sulphate combination

Table 3. Onset and duration of sedation and analgesia after IV injection of different treatments.

Treatment	Analgesia		Sedation	
	Onset	Duration	Onset	Duration
XY	10.71 ± 1.4^{a}	132.9 ± 5.5^{b}	15.2 ± 0.81^{b}	97.71 ± 2.05^{b}
MG	13.8 ± 1.1^{a}	100.4± 7.7^{c}	19.4 ± 2.12^{a}	69.71 ± 6.52^{c}
XY-MG	7.4 ± 1.0^{b}	200.4± 17.5^{a}	12.42 ± 1.72^{b}	170.85 ± 6.8^{a}

Variables with different superscript letters at the same column are significantly different at $p \leq 0.05$
(XY) xylazine, (MG) magnesium sulphate and (XY-MG) xylazine-magnesium sulphate combination

Physical examination revealed that all buffaloes were clinically healthy with no apparent abnormal signs at baseline. Drug compatibility test revealed non precipitate in the centrifuged XY-MG combination microscopically. The insertion of the epidural catheter and the epidural injection using ultrasonographic guidance were easily performed and were well tolerated by all buffaloes. None of a difficulties or inflammation were encountered during the second injection after the 2 weeks wash out period. Epidural administration of XY-MG mixture or each single drug resulted in complete analgesia of the base of the tail, perineum, and upper hind limbs at different time point compared to baseline. All buffaloes received XY-MG, XY and MG showed a maximum degree of analgesia (score 3) but at a different time of onset and for varying length (Table 2). Buffaloes received XY-MG combination showed the fastest onset of analgesia (7.4 ± 1) min followed by those received XY (10.71 ± 1.4) min and MG (13.8 ± 1.1) min. The combination of XY-MG resulted in longer duration of epidural analgesia when compared to XY or MG alone. The maximum analgesic effect in XY-MG group, followed by XY group and finally the MG group (Table 3).

The maximum sedation observed in the studied buffaloes was scoring 2 which were recorded at a different time of onset and last for a different duration according to the received treatment. The longest duration of complete sedation (score 2) was recorded in group XY-MG begin at T15 till T180 (Table 4).

All injected agents affected locomotor behavior of buffaloes in the form of mild ataxia (Table 5). All groups achieved a score 1 regarding locomotion behavior within 15 minutes and that the only difference was in duration (Table 5). No animals achieved score 3 (sternal recumbency).

Table 6. Heart rate, respiratory rate and rectal temperature values as affected by different treatments.

Clinical parameters	Treatment XY	MG	XY-MG
Heart rate	beats per minute		
0 (base line)	51.5±7.6^{a1}	50.85±9.5^{a1}	49.7±8.2^{a1}
15 min	40.3±5.2^{a2}	35.4±6.2^{b2}	39.2±5.8^{a2}
30 min	40.6±6.1^{a2}	37.6±5.1^{b2}	39.3±4.1^{ab2}
45 min	38.7±5.3^{a2}	36.6±4.3^{b2}	37.2±5.4^{ab2}
60 min	37.2±7.1^{a2}	37.4±6.1^{a2}	37.6±3.4^{a2}
90 min	38.9±6.9^{a2}	37.9±6.2^{a2}	37.1±5.9^{a2}
120 min	37.9±4.6^{a2}	37.9±4.4^{a2}	37.8±6.5^{a2}
180 min	39.6±5.8^{c2}	44.8±4.9^{b1}	46.8±4.4^{a1}
210 min	42.4±8.4^{b2}	49.5±7^{a1}	50.2±4.7^{a1}
Respiratory rate breaths per minutes			
0 (base line)	33.7±3.4^{a1}	33.14± 4.1^{a1}	35.28±5.1^{a1}
15 min	18.7± 3.3^{a2}	16.5± 4.5^{b2}	19.6± 2.6^{a2}
30 min	22.4±3.6^{a2}	17.2±1.6^{c2}	19.4±3.4^{b2}
45 min	16.4±2.9^{c2}	19.5±2.3^{b2}	22.6±4.3^{a2}
60 min	16.3±3.5^{a2}	17.6±2.5^{a2}	18.2±2.9^{a2}
90 min	17.1±3.8^{c2}	19.2±1.9^{b2}	26.4±3.9^{a1}
120 min	24.5±1.7^{b2}	18.9±2.6^{c2}	31.5±3.5^{a1}
180 min	21.9±3.4^{c2}	26.7±2.5^{b2}	29.4±4.1^{a1}
210 min	29.8±2.6^{b1}	26.1±1.8^{c1}	33.6±4.4^{a1}
Rectal temperature C°			
0 (base line)	38.8±0.5^{a1}	38.3±0.4^{a1}	38.6±0.5^{a1}
15 min	37.6±0.6^{a2}	37.2±0.2^{a2}	37.4±0.5^{a2}
30 min	37.6±0.3^{a2}	37.3±0.4^{a2}	37.4±0.4^{a2}
45 min	37.1±0.5^{a2}	37.2±0.6^{a2}	37.3±0.6^{a2}
60 min	37.5±0.4^{a2}	37.1±0.5^{a2}	37.2±0.3^{a2}
90 min	37.3±0.6^{a2}	37.3±0.4^{a2}	37.6±0.5^{a2}
120 min	37.8±0.5^{a2}	37.6±0.4^{a1}	37.9±0.4^{a1}
180 min	38.4±0.6^{a1}	38.1±0.5^{a1}	38.6±0.5^{a1}
210 min	38.3±0.3^{a1}	38.1±0.4^{a1}	38.8±0.4^{a1}

Variables with different superscript letters at the same column are significantly different at $p \leq 0.05$
Variable with different superscript numbers in the same row are significantly different at $p \leq 0.05$.

XY- MG group showed higher heart rate than MG group (at 15th, 30th and 45th minutes) and XY group (at 180th and 210th). XY MG group showed higher respiratory rate than XY group and MG group from 45th minutes 210th minutes post drug administration. Treatments had no significant effect on temperature throughout the different periods (Table. 6). Epidural injection of different treatment did not have a significant effect on haemato-biochemical variables (Table 7), except a significant decrease in blood glucose compared to baseline value at 30 minutes after injection in all treatment groups, returning to the baseline level by 180 minutes in XY-MG group.

4. Discussion

The current study revealed that epidural injection of XY-MG combination in buffalo resulted in fast onset, prolonged duration of action, profound analgesia and sedation as comparable to epidural injection of XY or MG alone. In large ruminant, epidural injection of xylazine produce prolonged duration of analgesia and decrease disruption of motor function when compared to local anesthetic agents as lidocaine [8]. Similarly, epidural MG has been studied previously with no apparent adverse effects in human such as hypotension and digestive disturbance [29], equine [26], cattle [27] and canine [23]. Magnesium inhibits calcium influx and antagonizes NMDA receptor channels in a noncompetitive manner [22; 24; 30]. These benefits have motivated researchers to look into using MG as a postoperative analgesic adjuvant [31-33]. To best of our knowledge, the use of epidural MG or XY-MG combination has not been reported in buffalo.

Selection of XY and MG dose for the present study was based primarily on previous published literature [1; 27]. In our study, insertion of epidural catheter and epidural injection using ultrasonographic guidance were easily performed and well tolerated by all buffaloes. This method was used to guarantee that the catheter was in the correct position during injection and that the complete calculated dose was delivered into the epidural space. Epidural administration of XY-MG mixture or each single drug resulted in complete analgesia of tail, perineum, and hind limbs compared to baseline plausibly due to successful placing of the epidural catheters in sacrococcygeal region using ultrasonographic guidance and injection of sufficient concentrations of analgesic drug in the sacrococcygeal space. In the present study all buffaloes, regardless of the treatment received, showed a maximum degree of analgesia (score 3), but at a different time of onset and for different duration. The XY-MG group revealed the significant fastest onset and the significant longest duration (7.4 ± 1.0 and 200.4± 17.5; respectively) followed by XY group (10.71 ± 1.4 and 132.9 ± 5.5; respectively) and finally the MG group (13.8 ± 1.1 and 100.4± 7.7; respectively). The time of onset after injection of epidural XY in buffaloes was previously reported to be approximately 10-15 minutes [1]. In rats, the time to onset after intrathecal injection of MG was 8.4 ± 1.5 minutes [34]. Xylazine-induced analgesia has been reported to be mediated through the alpha2-adrenoceptors in substantia gelatinosa of dorsal horn in the spinal cord. In the present study, combination of XY-MG resulted in a longer-lasting maintenance of maximum analgesic intensity. The synergistic effect of MG when injected with epidural opioids and/or local anesthetics has been reported previously in many studies as with lidocaine in equine [26] and cattle [27] bupivacaine in human [35] and ketamine in sheep [33]. The extended duration of epidural analgesia of magnesium sulphate - lidocaine combination has been found to be owing to the pH-lowering effect [26]. More crucially, magnesium sulphate is a noncompetitive NMDA receptor antagonist with analgesic qualities allowing for significantly longer epidural anesthetic

duration [22; 23; 30]. Other feasible mechanisms of MG intervention include inhibition of catecholamine release that causes a subsequent reduction in neuronal activity [24].

Table 7. Hematobiochemical parameters as affected by different treatments.

Parameters	Groups	0 minute (baseline)	15 minutes	30 minutes	60 minutes	120 minutes	180 minutes	210 minutes
RBCs (x 10^{12} L^{-1})	XY	8.41± 1.11^{1a}	8.13±0.9^{1a}	9.32±1.3^{1a}	9.2±1.01^{1a}	8.83±1.7^{1a}	9.52±1.2^{1a}	9.76±0.7^{1a}
	MG	9.21±1.08^{1a}	8.82±1.2^{1a}	9.7±1.4^{1a}	10.21±1.2^{1a}	10.01±2.1^{1a}	9.89±1.4^{1a}	9.01±1.2^{1a}
	XY-MG	8.91±1.3^{1a}	9.15 ±1.6^{1a}	8.74±1.1^{1a}	9.19±1.3^{1a}	10.32±1.6^{1a}	9.59±1.6^{1a}	9.84±1.4^{1a}
Hb (g dL^{-1})	XY	12.2±1.3^{1a}	11.3±1.2^{1a}	11.1±1.4^{1a}	12.2±1.1^{1a}	11.7±0.9^{1a}	12.3±1.01^{1a}	12.1±0.8^{1a}
	MG	12.1±1.7^{1a}	11.8±1.7^{1a}	12.3±1.2^{1a}	12.3±1.1^{1a}	12.3±0.7^{1a}	12.4±0.3^{1a}	12.9±0.7^{1a}
	XY-MG	12.7±1.5^{1a}	12.1±1.2^{1a}	11.6±1.1^{1a}	12.1±0.9^{1a}	12.2±1.2^{1a}	11.5±0.5^{1a}	12.3±1.1^{1a}
PCV (%)	XY	34.8 ± 0.6^{1a}	36.8± 1.01^{1a}	35.08±1.3^{1a}	33.1 ± 1.18^{1a}	32.9 ± 1.11^{1a}	31.6 ± 1.25^{1a}	32.4 ± 2.2^{1a}
	MG	33.93±0.4^{1a}	35.17± 1.4^{1a}	36.01±1.8^{1a}	31.8±1.21^{1a}	33.72 ± 1.6^{1a}	32.8 ± 1.4^{1a}	34.5 ± 1.2^{1a}
	XY-MG	36.17±0.9^{1a}	34.7± 1.1^{1a}	36.33±1.6^{1a}	36.4 ± 1.3^{1a}	35.2 ± 1.9^{1a}	34.9 ±1.5^{1a}	35.3 ± 1.04^{1a}
WBCs (x 10^{9} L^{-1})	XY	8.65±0.42^{1a}	9.89±0.02^{1a}	10.01±1.13^{1a}	11.31±0.61^{1a}	9.42±0.44^{1a}	8.79±0.52^{1a}	9.15±1.01^{1a}
	MG	8.94±0.31^{1a}	9.59±0.23^{1a}	9.68±0.63^{1a}	8.99±1.1^{1a}	8.91±1.02^{1a}	9.24±0.82^{1a}	10.11±0.91^{1a}
	XY-MG	8.19±0.62^{1a}	8.89±0.44^{1a}	9.68±1.3^{1a}	9.54±2.1^{1a}	8.72±0.2^{1a}	9.18±1.5^{1a}	9.82±1.04^{1a}
Neutrophil (%)	XY	15.01 ± 3.01^{1a}	24.02±4.11^{1a}	22.17±2.5^{1a}	20.17 ±4.22^{1a}	24.72±1.81^{1a}	22.59±2.31^{1a}	20.67 ±2.52^{1a}
	MG	19.67±2.11^{1a}	22.33 ±1.31^{1a}	24.21±4.21^{1a}	22.19 ±4.44^{1a}	23.61±2.23^{1a}	24.55±3.52^{1a}	22.17 ±3.6^{1a}
	XY-MG	18.2 ± 2.91a	16.2± 5.011a	19.17± 3.111a	17.17 ±2.911a	21.00±3.871a	21.50±4.561a	19.67 ±4.411a
Lymphocyte (%)	XY	47.00 ± 6.111a	55.24±4.131a	52.33±4.221a	57.17± 3.91a	54.02±6.211a	52.67±5.51a	55.09 ±3.171a
	MG	54.67 ±2.141a	55.03 ± 5.11a	51.67 ± 6.71a	49.67 ±8.211a	53.67 ±4.81a	56.67 ±5.321a	51.67 ±3.111a
	XY-MG	52.83±1.911a	48.2±4.311a	53.67 ± 2.91a	48.67 ±5.231a	49.67 ±1.91a	51.67 ±4.221a	52.67 ±4.141a
Glucose (mmol L^{-1})	XY	4.88±0.631a	3.15±0.231a	1.62±0.361b	2.91±1.011b	2.6±0.441b	2.88±0.511b	4.25±0.221a
	MG	4.32±1.221a	3.64±1.921a	1.95±0.441b	2.13±1.661b	2.08±2.111b	2.75±1.631b	3.76±1.511a
	XY-MG	4.68±0.311a	3.01±0.861a	2.68±0.421b	2.42±1.011b	3.03±1.121b	3.99±1.21a	4.25±0.811a
ALT (U L^{-1})	XY	6.19±0.21	7.2±0.221a	8.91±0.321a	8.95±0.461a	9.01±0.211a	6.21±0.291a	8.41±0.371a
	MG	7.3±0.311a	7.5±1.111a	8.9±0.521a	8.95±0.231a	9.01±0.311a	6.2±0.351a	7.2±0.521a
	XY-MG	6.4±0.731a	6.5±1.61a	8.1±0.491a	8.5±0.341a	8.9±0.361a	5.2±2.541a	6.3±0.411a
AST (U L^{-1})	XY	68.7 ± 10.41a	62.3 ± 7.31a	68.8 ± 4.61a	62.3 ± 5.51a	63.5 ± 3.21a	60.9 ± 1.71a	59.9 ± 4.81a
	MG	64.5 ± 4.41a	59.2 ± 2.11a	64.3 ± 5.21a	62.1 ± 5.11a	62.9 ± 4.61a	61.4 ± 3.81a	63.5 ± 2.51a
	XY-MG	66.3 ± 8.11a	64.1 ± 5.21a	61.9 ± 4.31a	64.2 ± 4.51a	62.4 ± 2.91a	62.5 ± 4.71a	61.8 ± 6.21a
ALP (U L^{-1})	XY	112.3±6.21a	100.4±8.91a	109.6±4.61a	104.9±7.31a	116.6±3.81a	108.52±5.51a	108.35±5.11a
	MG	116.4±3.11a	107.2±6.361a	101.2±8.11a	103.2±9.11a	109.2±1.91a	107.12±4.61a	109.15±6.21a
	XY-MG	109.1±3.31a	103.4±7.221a	106.6±7.31a	101.9±7.51a	100.8±8.91a	108.18±8.51a	105.15±6.61a
BUN (mmol L^{-1})	XY	4.78 ± 0.791a	3.81±0.49 1a	3.12±0.821a	3.56±1.011a	3.39±1.211a	3.01±1.421a	3.62±0.851a
	MG	4.13 ± 1.21a	4.01±0.491 1a	3.88±1.011a	3.09±2.211a	3.71±0.851a	3.92±0.551a	3.73±1.041a
	XY-MG	3.82+1.491a	3.64 ± 0.71a	4.01±1.4 1a	3.9±1.111a	3.7±1.411a	3.9±1.251a	4.11±1.221a
Creatinine (µmol L^{-1})	XY	117.57±16.71a	114.2±26 .11a	122.3±22.31a	118.5±13.51a	114.5±18.11a	121.7±14.21a	123.9±13.21a
	MG	101.2±11.31a	103.1±18 .31a	111.1±12.51a	106.3±16.61a	110.3±13.41a	118.5±11.21a	109.7±16.61a
	XY-MG	122.5±17.61a	113.2±14.11a	111.6±16.51a	109.4±21.21a	118.2±20.31a	126.1±19.41a	120.5±20.11a

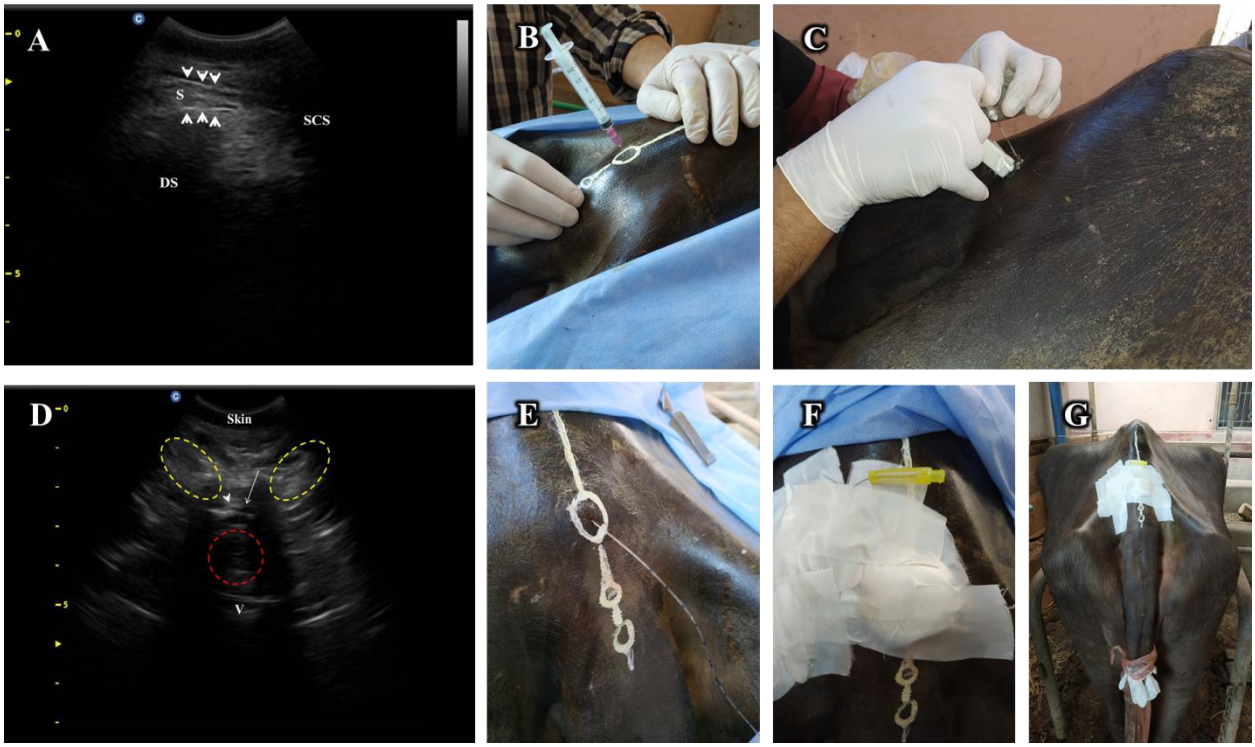

Figure 1. Ultrasonography guided Insertion of epidural catheter in the epidural space through the sacrococcygeal joint. **(A)** Sagittal ultrasonographic plane of sacrococcygeal area; sacrum (S), double echogenic layers (arrow heads), distal shadowing (DS) and sacrococcygeal space (SCS). **(B)** Injection of subcutaneous local anesthetic to desensitize the skin over the injection site. **(C)** Ultrasonographic guided introduction of the needle. **(D)** Transverse plane of sacrococcygeal space; the echogenic epidural catheter (arrowhead) inside the anechoic epidural space (arrow), the hypoechoic vertebral canal (red pinpointed), the cranial epiphysis of the first coccygeal vertebra (v) and laterally bordered by Erector Spinae Muscle (yellow pinpointed). **(E, F and G)** The inserted catheter secured and fixed catheter in position using adhesive tape.

Using XY, MG or their combination in this study resulted in mild-to-moderate sedation with a shorter onset of action and a longer period of sedation in XY-MG group. Similar to previous studies, XY treated-buffalo appeared tired with a slight lowering of head carriage, clear ptosis of eyelids, and prolapsed third eyelid [1; 16]. Due to mild to moderate sedation recorded when MG was injected alone, the sedative effects of magnesium could not be overlooked in the current research which may be attributed to its systemic absorption. As similar; [32] reported that magnesium analgesic effect occurred at the supra-spinal level might be related to its systemic absorption.

All injected agents affected locomotors activity of animals in form of mild ataxia. The ataxia score observed in the current study was 1 in all treatment groups. The ataxia observed after XY or XY-MG combination injection may be attributed to the local anesthetic properties or alpha-2 agonist mediated central sedative effects of XY [8]. Similarly, the extent and duration of ataxia after XY administration were proved in a variety of species [1; 36]. It was also confirmed that MG may be safely used for epidural analgesia without causing any motor deficits [23]. While sedation and motor deficient with subsequent ataxia were considered as an unfavorable effect of epidural anesthesia, our dosing strategy provided an acceptable degree of sedation and ataxia which means using of these protocols in buffalo anesthesia are safe with little effect on locomotion as animals can walk with mild ataxia without falling down or sternal recumbency and it is very important for welfare issue.

In agreement with previous studies, our results revealed a significant reduction in f_R in XY, MG and XY-MG injected buffalos after drug administration compared to baseline [8; 36]. The exact mechanism of oligopnea may be attributable to direct depression of respiratory center by alpha-2 agonists after systemic absorption [36]. Similarly, significant reduction in HR was observed in all buffaloes after injection with XY, MG and XY-MG combinations. A significant decrease in HR is considered as a classical response after the administration of alpha-2 agonists in buffaloes [16], cattle [8], sheep [37], goats [38], and equine [39]. Bradycardia following the administration of alpha-2 agonists might be attributed to augmentation of vagal tone from the CNS, inhibition of norepinephrine release from sympathetic nerve terminals, direct depression of cardiac pacemaker, vagal stimulation or direct increase in the release of acetylcholine from parasympathetic nerves in the heart [40]. Magnesium causing cardiorespiratory depression by sympathetic blockade and inhibition of catecholamine release [41; 42]. A significant decrease in HR is considered as a classical response after the administration of alpha-2 agonists in buffaloes [16], cattle [8], sheep [37], goats [38] and equine [39]. Cardiopulmonary depression of xylazine was attributable to increasing the parasympathetic activity and by decreasing the sympathetic

outflow from CNS and depression of respiratory center by alpha-2 agonists after systemic absorption [36].

Epidural injection of different treatment revealed a significant decrease in blood glucose compared to baseline value at 30 minutes after injection in all treatment groups, returning to the baseline level by 180 minutes in XY-MG group and 24 hours in XY and MG groups. While the exact mechanism of hypoglycemia caused by epidural XY, XY-MG combination was not investigated in this study, a previous report suggested that hypoglycemia could be caused by increased hepatic glucose utilization mediated by increased insulin signaling in the cerebral cortex and hypothalamus [43]. While the results of this study were compelling and could open new avenues of investigation ultrasonographic guided epidural injection of a novel epidural combination of XY-Mg in buffalo, the experimental limitations of our study somewhat hinder the applicability of these findings to the broader buffalo population. The analyses were performed on a relatively small number of animals. Control group was not inserted in the study. While this sample size was similar to that used in equivalent, published studies in ruminant [44; 45], data from a larger cohort of animals is required before definitive conclusions can be made.

It is concluded that epidural injection of XY-MG combination in buffalo resulted in a prolonged duration of action with no adverse effects as sternal recumbency when compared to XY or MG alone and could be used efficiently and safely in clinical practice as an intraoperative and postoperative analgesia to provide long-lasting local anesthesia and sedation without severe alteration of locomotor behavior in buffaloes. However, further future studies are warranted to determine the utility of different doses of XY-MG combination for surgical procedures before final recommendations can be made.

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Declarations

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Conflict of interest

The authors declare that there is no conflict of interests.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

Ahmed Khalil, Ahmed Sabek, Mohamed Zeineldin, Atef Abd Al-Galil: designed the experiment, conducted the experiment, data analysis, Seham Abo-Kora: performed the laboratory analyses, Ahmed Khalil, Mohamed Zeineldin: writing the manuscript; Ahmed Sabek: editing and reviewing the manuscript.

Consent to participate

Not applicable

Consent for publication

All authors agree to publish the findings of the current research.

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