Effects of xylazine, lignocaine and their combination for lumbar epidural analgesia in water buffalo calves (Bubalus bubalis)

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ABSTRACT
The study was conducted to evaluate the effects of xylazine alone (0.05 mg/kg), lignocaine alone (2.0 mg/kg) and a combination of xylazine and lignocaine (0.05 mg/kg and 2.0 mg/kg, respectively) after lumbar epidural administration in water buffalo calves. Fifteen nonden- script, male water buffalo calves of 6–8 months of age and weighing between 55 and 75 kg were randomly placed in 3 groups (A, B and C). The agents were administered at the 1st lumbar epidural space. Clinico-physiological parameters such as analgesia, ataxia, sedation, salivation, heart rate, respiratory rate and rectal temperature were studied. Other haematological and biochemical parameters monitored were haemoglobin, packed cell volume, total leukocyte count, plasma glucose, cortisol, protein albumin, globulin, blood urea nitrogen, creatinine, ALT, sodium, potassium and chloride. The onset of analgesia was faster in group C (3.0 ± 0.44 min) compared with that of group B (4.4 ± 0.40 min) and group A (34.0 ± 1.86 min). Analgesia of the thorax, flank, inguinal region, hind limbs, perineum and tail was complete in group C, but mild to moderate in groups A and B. Ataxia was severe in groups B and C and mild in group A. Mild to deep sedation were produced by groups A and C animals. Longer duration and greater depth of analgesia was produced in animals in group C. Heart rate, respiratory rate and rectal temperature decreased in groups A and C. The haematological parameters decreased in all the groups. The biochemical parameters like glucose, cortisol, blood urea nitrogen, creatinine, ALT increased in all the animals. However, total proteins and albumin decreased in the 3 groups. Plasma electrolytes sodium, potassium and chloride did not show any significant change. The results of this study indicated a possible additive analgesic interaction between epidurally administered xylazine and lignocaine, without causing any marked systemic effects in water buffalo calves.

Key words: analgesia, epidural, lignocaine, lumbar, water buffalo calves, xylazine.

INTRODUCTION
Xylazine, an alpha-2-agonist, has been used to induce sacrococcygeal/lumbar sacral epidural/spinal analgesia in different species of domestic animals12,19,30. Epidural analgesia induced by alpha-2-agonists is superior to that induced by anaesthetic agents commonly used to provide local anaesthesia for surgery in standing cattle, because it has a prolonged duration of action and decreased disruption of the motor function of the hind limbs16. In these studies it was reported that the onset of action of epidural xylazine was delayed. Xylazine produces a longer duration of regional analgesia in many animal species. Lignocaine has been reported to enhance the onset of xylazine-induced epidural analgesia12. A literature search revealed that there are a few reports on the use of xylazine in combination with lignocaine or other drugs for caudal epidural analgesia in domestic animals12,20. However, to the authors’ knowledge there are no reports on the use of xylazine or its combination with either lignocaine or other drugs after their lumbar epidural administration in water buffalo (Bubalus bubalis). The study was therefore designed to assess the effects of xylazine alone or in combination with lignocaine on clinical, physiological, haematological and biochemical parameters after their lumbar epidural administration in water buffalo calves.

MATERIALS AND METHODS
Experimental animals
The experimental study was carried out using 15 nondescript, healthy, male water buffalo calves ranging from 6–8 months in age and weighing 55–75 kg.

Experimental design
The animals were divided into 3 groups (A, B and C) of 5 animals each. In group A, xylazine at 0.05 mg/kg, in group B lignocaine at 2.00 mg/kg and in group C a combination of xylazine and lignocaine (same doses as in groups A and B) were administered under aseptic conditions in the 1st lumbar epidural space using 18-gauge spinal needle. The study was approved by the Research Coordination and Monitoring Section of the Joint Director of Research of the Indian Veterinary Research Institute.

Restraint and epidural injection technique
The animals were restrained in standing position with cotton rope halters in a cattle crush. The 1st lumbar space was palpated (between 1st and 2nd lumbar vertebrae). The area of injection was clipped, shaved and painted with povidone iodine for aseptic epidural injection. The needle was inserted just to the right of the lumbar spinous process on a line 1.5 cm behind the cranial edge of the 2nd lumbar transverse process. An initial skin weal was produced with 2% lignocaine hydrochloride using a fine needle. The spinal needle (14 gauge, 12 cm long) was directed ventrally and medially at an angle of 10–13° from the vertical for a distance of 7.5 cm at which point the needle entered the vertebral canal. The needle was passed further to penetrate the interarcuate ligament where a little resistance was felt. As it entered the epidural space the syringe was mounted on the hub and air was injected for loss of resistance. The air bubbles entered the syringe, which confirmed the correct placement of the needle in the epidural space. The injection was then made. The correctness of epidural injection was further confirmed as the injectate freely and easily entered the space and minimal resistance on injection and minimal pressure on the syringe plunger was needed11. The volume was kept constant at 3.5 ml in all the animals irrespective of the drug used. Wherever the volume remained below 3.5 ml it was made up to 3.5 ml by adding normal physiological saline. The animals were
allowed to move out of the crush to record different clinical parameters.

**Measured parameters**

The animals were observed for clinical, physiological, haematological and biochemical parameters. The onset of analgesia (the time from injection of the drug(s) to loss of sensation) was recorded by response to pin pricks every 5 min at the flank region. Depth of analgesia and area of desensitisation was recorded in the thorax, flank, inguinal region, on the hind limbs, perineum and tail at 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min by observing response to pin pricks. The reactions were graded on a 0 to 3 score scale where 0: no analgesia, strong reaction to pin pricks; 1: mild analgesia, weak response to pin pricks; 2: moderate analgesia, occasional response to pin pricks, and 3: strong/complete analgesia, no response to pin pricks. Ataxia was recorded at the same time intervals as analgesia and was graded on a 0–4 score scale where 0: walking without staggering; 1: able to stand but walks with little ataxia; 2: able to stand but walks with extreme ataxia; 3: sternal recumbency but animal is able to flex and extend the limbs, and 4: sternal recumbency and animal is unable to flex or extend its limbs. While grading sedation the animals were observed for their ability to sit with or without support along with the position of head and eyelids. Sedation was recorded and graded on a scale of 0–3 where 0: standing alert, keeping the head high or normal, position of eyelids normal; 1: standing but appears tired, dropping of head and eyelids; 2: able to sit without support, dropping of head and eyelids, and 3: unable to sit without support, dropping of head and eyelids. Duration of analgesia (in min) was considered as time from loss of sensation from any region to return of sensation at all the sites. The time interval to stand without support was also recorded.

During blood sampling the animals were tied in the crush using cotton rope halters. After collection of samples they were allowed to move out of the crush for further evaluation. A total of 7–8 mtl of blood was collected for haematological and biochemical parameters in test tubes containing EDTA at 30 min, 120 min and 24 h after administration of drugs. For haematological parameters 1 mtl of blood was used for the determination of haemoglobin (Hb), packed cell volume (PCV) and total leucocyte count (TLC). Two mtl of blood in heparin was used for cortisol estimation and 2 mtl in NaF for glucose estimation before and 30, 60, 120 min and 24 h after injection of the drugs. The plasma samples were submitted for the estimation of glucose (GOD/POD method), cortisol (RIA method), total protein, albumin, globulin (modified Biuret and Dumas method), urea nitrogen (DAM method), creatinine (Alkaline Picrate method), ALT (Span diagnostic kits using standard procedure), Na, K (Flame photometry) and chloride (Colorimetric method using Span diagnostic kits).

**Statistical analysis**

The data were analysed using ANOVA for repeated measures for comparison of mean ± SE values between different groups at different time intervals. Paired t-test with Bonferroni correction was used for comparison of mean ± SE at different time intervals with the base values of the respective treatments as per the standard procedures. The values were considered as significant at \( P < 0.05 \) and \( P < 0.01 \).

**RESULTS**

The fastest onset of analgesia was recorded with group C (3.0 ± 0.4 min) followed by group B (4.4 ± 0.40 min), and group A (34.0 ± 1.86 min). The time of onset of analgesia was significantly \( (P < 0.05) \) higher in group A than in groups B and C. The difference was not significant between group B and group C. Animals in group C showed complete analgesia in the area of the thorax, flank, inguinal region and hind limbs from 45 to 90 min; thereafter it declined and moderate to mild analgesia were persisted till the end of observation (Figs 2–5). Analgesia in the perineum and tail area lasted for 10–15 min. All animals in group A were able to stand during the entire post-injection period. However, the animals showed various degrees of ataxia starting from 15 min up to the end of the observation (Fig. 6). The animals in group B showed extreme ataxia of hind limbs immediately after injection and were unable to stand. However, only little ataxia was observed later on. Ataxia further increased in group C where animals assumed sternal recumbency. Thereafter the animals were able to stand but showed extreme ataxia during walking. All the animals in groups B and C rose to their feet when encouraged. Mild to deep sedation and salivation was observed after 20 min in group A and animals remained standing but appeared tired with drooping heads (Fig. 7). Group B did not produce any sign of sedation. Group C produced moderate to deep sedation and salivation up to 60 min, but
the animals were able to sit without support in sternal recumbency (Fig. 7). Mild sedation persisted thereafter, till the end of observation and the animals were able to stand but appeared tired. Ruminal movements decreased significantly in groups A and C. However, maximum decrease in ruminal movements was observed in group C. Frequent urination was noticed in most of the animals in groups A and C, and the animals in both these groups urinated at different intervals during the post-injection period.

A significant decrease in heart rate was observed in groups A and C (Fig. 8). Respiratory rate decreased in groups A and C (Fig. 9). Rectal temperature decreased in groups A and C (Fig. 10). Longer duration and more depth of analgesia (Fig. 11) were observed in group C. Haemoglobin (Hb), packed cell volume (PCV) and total leukocyte count (TLC) decreased in all the groups after epidural injection of different drugs. The values for PCV and Hb returned to baseline at 24 hours in all the groups. However, the values for TLC showed no significant increase at 24 h in all the groups (Table 1).

Values for plasma glucose increased for variable duration in different groups. In animals in groups A and B a significant increase in glucose level at 30 min \( (P < 0.05) \) and 60 min \( (P < 0.01) \) was observed. In animals in group C a significant \( (P < 0.01) \) rise of glucose up to 120 min was recorded (Table 2). Thereafter the values for plasma glucose returned to pre-administration levels within 24 h.

In animals in group A the plasma cortisol levels significantly \( (P < 0.01) \) increased at the 30 min interval and remained elevated up to 120 min. Animals in group B also showed significant \( (P < 0.01) \) increase up to 120 min of observation. Similarly, animals in group C showed significant \( (P < 0.01) \) increase from 30 min to 120 min interval. The increase in group C was more and significantly \( (P < 0.05) \) different from groups A and B.

In animals in group A the increase in plasma creatinine was significant \( (P < 0.05) \) from 30 min to 120 min post-injection. In group B a non-significant increase persisted for up to 60 min and values returned to baseline at 120 min. The animals in group C showed a significant \( (P < 0.01) \) increase up to 30 min.

In animals in group A a significant \( (P < 0.05) \) increase in blood urea nitrogen (BUN) was observed at 30 min. It increased significantly \( (P < 0.01) \) at 60 min. In group B animals a significant \( (P < 0.05) \) increase was observed at 30 min and 60 min and at the end it returned to baseline in both groups (A and B). In group C a significant \( (P < 0.01) \) increase in BUN at 60 min and 120 min but did not return to base value even after 24 h.

ALT showed a significant \( (P < 0.01) \) increase from 30 min to 120 min in the animals in group A, a slight variation in group B and increase in group C. The values in all groups returned to baseline after 24 h.

A slight but non-significant decrease in protein, albumin, A:G ratio and an increase in globulin was observed between 30 min to 120 min in all the treated groups.

Changes in plasma electrolytes (Na, K and Cl) were only transient and after 120 min values returned to near-normal in all 3 groups.

**DISCUSSION**

**Analgesia**

In large ruminants, including water buffalo calves, caudal epidural block is a commonly used block. However, under this block, in many instances, the motor fibres of the hind limb are partially or completely blocked by the drugs used. In

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**Fig. 3:** Analgesia at flank after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \( (P < 0.05) \) among groups.

**Fig. 4:** Analgesia at inguinal region after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \( (P < 0.05) \) among groups.

**Fig. 5:** Analgesia at hind limbs after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \( (P < 0.05) \) among groups.
the present investigation lumbar epidural route was used to achieve analgesia of the entire flank, lateral and ventral abdominal region. The lumbar vertebrae are difficult to palpate in comparison with the caudal vertebrae. However, after practice the site may be easily approached. The analgesia produced by the block gives ample opportunity to perform surgical interventions in these regions in standing animals since motor fibres of the hind limbs are not affected.

The early onset in group C has been attributed to the predominant conduction blockade of spinal nerve roots by lignocaine. Xylazine alone, on the other hand, is known to produce a delayed onset of action in comparison with lignocaine. The combination produced complete analgesia of thorax, flank, inguinal region and hind limbs after the injection of drugs and thereafter it declined and moderate to mild analgesia was maintained till the end of observation. It also produced complete analgesia at the perineum and tail but only for a short duration. Xylazine-induced analgesia has been reported to be mediated through the alpha₂-adrenoceptors in substantia gelatinosa of dorsal horn in the spinal cord. It has also been reported that the alpha₂-adrenoceptor agonists provide a local depot of the drug at the spinal cord level which are released slowly over a longer period of time. It has been observed that the distribution of anaesthetic solution in the cerebrospinal fluid determines the uptake of anaesthetics. Furthermore, uptake was found to be the greatest where the concentration of solution was greater (i.e. at the site of injection) and decreased above and below the site of highest concentration.

The marked analgesia at the thorax, flank and inguinal region in the present study may probably be due to higher concentration of anaesthetic solution deposited at the site of injection, i.e. lumbar epidural space from where the spinal nerves innervate these regions. The caudal migration of anaesthetic solution resulted in a lesser degree of analgesia at hind limbs, perineum and tail. Longer duration and greater depth of analgesia in group C suggested an additive interaction between xylazine and lignocaine, which confirms the findings in goats after spinal administration of xylazine and lignocaine.

Ataxia
All animals in group A were able to stand during the entire post-injection period. However, a few animals showed little to extreme ataxia while walking. Alpha₂-agonists result in a selective blockade of sensory fibres so that analgesia is

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**Fig. 6:** Motor incoordination after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \((P < 0.05)\) among groups.

**Fig. 7:** Sedation after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \((P < 0.05)\) among groups.

**Fig. 8:** Heart rate (beats/min) after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \((P < 0.05)\) among groups.

**Fig. 9:** Respiratory rate (breaths/min) after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves. Values with different letters differ significantly \((P < 0.05)\) among groups.
achieved without hind limb dysfunction\textsuperscript{12}. The ataxia seen with epidural administration of xylazine in this study may be related to its postulated local anaesthetic properties at the spinal cord level\textsuperscript{23} probably due to structural similarity with lignocaine\textsuperscript{4} or as a result of its systemic uptake through venous sinuses. The animals in group B showed extreme ataxia of hind limbs immediately after injection and were unable to stand. However, only little ataxia was observed later on. Lignocaine indiscriminately blocks the sensory, sympathetic and motor fibres\textsuperscript{4,12}. Blockade of motor fibres might be the reason for extreme ataxia initially; however, as the effect of lignocaine wore off the animals regained normal gait. Ataxia further increased in group C and the animals assumed sternal recumbency. Thereafter the animals were able to stand but showed extreme ataxia while walking. The extreme ataxia and sternal recumbency in group C animals may be due to the additive effect of xylazine with lignocaine at the spinal level. Sternal recumbency after sacrococcygeal injection of lignocaine and xylazine has also been described in sheep\textsuperscript{20}.

### Sedation

Mild to deep sedation and salivation was observed in the post-injection period in group A and the animals remained standing but appeared tired, with drooping of the head and eyelids. Animals in group B did not produce any sign of sedation. Group C produced moderate to deep sedation with salivation but the animals were able to sit without support in sternal recumbency. Mild sedation persisted until the end of observation and the animals were able to stand but appeared tired. The sedation produced in this group may be the manifestation of central effects of alpha\texttextdagger-agonist xylazine probably after its absorption from the epidural space.

### Ruminal movements

Ruminal movements decreased significantly in group A, and in group C where maximum decrease in ruminal movements was observed. The decrease in ruminal movements might be attributed to the fact that xylazine, after quick absorption from the epidural space, attached to alpha\texttextdagger-adrenergic receptors in the central nervous system (CNS) and fore stomach muscle thereby inhibiting reticulo-ruminal contractions as observed after administration of alpha\texttextdagger-agonists in cattle\textsuperscript{32}. Frequent urination at different intervals was noticed in most of the animals in groups A and C. This is probably due to inhibition of production and release of antidiuretic hormone\textsuperscript{11}. Hyperglycaemia produced by alpha\texttextdagger-agonists may be another factor responsible for increased urine output. The increased glucose level might have acted as an osmotic diuretic\textsuperscript{8}. Increased urinary output following xylazine administration has also been reported in different species of animals\textsuperscript{11}. Salivation was recorded in most of the animals.

### Heart rate

A significant decrease in heart rate was observed in groups A and C. This decrease could be explained by several

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**Table 1: Haematological parameters after lumbar epidural administration of xylazine, lignocaine and a combination of xylazine and lignocaine in buffalo calves.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>A</td>
<td>8.60 ± 0.40\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8.96 ± 0.33\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9.12 ± 0.36\textsuperscript{a}</td>
</tr>
<tr>
<td>PCV</td>
<td>A</td>
<td>24.04 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>26.40 ± 1.94</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>24.20 ± 0.97</td>
</tr>
<tr>
<td>TLC</td>
<td>A</td>
<td>4.98 ± 0.21\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4.68 ± 0.40\textsuperscript{ab}</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.30 ± 0.27\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

Values with different letters differ significantly (*P < 0.05; **P < 0.01) among groups.
mechanisms, which include decreased sympathetic outflow from CNS, inhibition of noradrenaline release from sympathetic nerve terminals, direct depression of cardiac pace maker and conduction tissue, increased vagal tone and a direct increase in the release of acetylcholine from parasympathetic nerves in the heart. However, the mechanism of decreased heart rate was not examined in this study. Similar findings were reported after epidural administration of xylazine in cattle, sheep and goats. However, no change in heart rate was observed in group B.

Respiratory rate
Respiratory rate (RR) decreased in groups A and C. A decrease in RR may be due to direct depression of the respiratory centres by xylazine. Respiratory depression was more pronounced in group C in comparison with that seen in group A and group B. This indicates an additive depressant effect of both classes of drugs on the respiratory system and confirmed the earlier findings. A slight respiratory depression by lignocaine alone in the present study probably may be due to the blockade of nerves innervating the muscles of respiration. Similar results have also been reported in cattle.

Rectal temperature
Rectal temperature decreased in groups A and C. Hypothermia was due to reduced basal metabolic rate and muscle activity and depression of thermoregulatory centres. The alpha-agonists also have been found to depress the hypothalamic nor-adrenergic alpha2-receptors to cause hypothermia. No significant change in rectal temperature was recorded in group B which confirms the earlier results of a non-significant change in rectal temperature in goats. Contrary to the findings of the present study, a decrease in rectal temperature has been observed following administration of lignocaine in cattle and buffalo and it was believed to be due to heat loss from relaxation of thoracic and abdominal skeletal muscles.

Haematological parameters
Haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC) decreased in all the groups after epidural injection of different drugs. The values for PCV and Hb returned to baseline at 24 hours in all the groups, however, the values for TLC showed an increase at 24 h in all the groups. Pooling of the circulating blood cells in the spleen or other reservoirs secondary to decreased sympathetic activity could be the reason for the decrease in Hb, PCV and TLC recorded in this study as also reported with other tranquillisers in dogs. Decreases in these parameters was also reported after epidural administration of xylazine in cattle. The decrease in PCV and Hb during the period of anaesthesia or sedation may also be due to shifting of fluid from extravascular compartment to intravascular compartment in order to maintain the normal cardiac output in the animals. A rise in neutrophil count and a decrease in lymphocytes were recorded in all the groups in the present study. This could be attributed to the adrenocortical stimulation and subsequent effect of glucocorticoids on circulating neutrophils. These observations suggested that xylazine when used alone or in combination with lignocaine has more or less the same effects on haematological parameters.

Biochemical parameters
A significant increase in glucose levels was observed at different intervals in all the animals. Thereafter the values for plasma glucose returned to pre-administration level. There have been many investigations into the hyperglycaemic effect of xylazine. Hyperglycaemia observed in the present study may probably be due to an alpha-adrenergic inhibition of insulin release by stimulation of alpha2-receptors in the pancreatic beta cells and to an increased glucose production in the liver. The exact mechanism of xylazine-induced hyperglycaemia was not investigated in the present study. However, it has been suggested that hyperglycaemia may be due to a rise in adrenergic hormonal stress.

Increased levels of cortisol recorded after the epidural injection of drug(s) might be attributed to the stimulation of...
The increase in plasma creatinine in all groups may be attributed to temporary inhibitory effects of these drugs on the renal flow or as a result of prerenal azotaemia, which in turn might have caused a rise in plasma creatinine values. However, it is uncertain whether it might be due to renal damage because all values returned to baseline at 24 h. Administration of xylazine has also been reported to cause a rise in creatinine level in goats and cattle. The increase in BUN may be attributed to a temporary inhibitory effect of drugs on renal blood flow or as a result of prerenal azotaemia, which in turn might have caused a rise in BUN. Also, increased hepatic urea production from amino acid degradation could account for the observed increase in BUN. Changes in protein levels were only mild and transient and did not indicate severe changes in hormonal levels or protein metabolism. Changes in plasma electrolytes (Na, K and Cl) were only transient as the drugs could not affect these indices and after 120 min values returned to near-normal in all the 3 groups and are thus of little clinical significance.

**CONCLUSION**

Clinico pathological, haematological and biochemical effects of lumboperidural xylazine (0.05 mg/kg), lignocaine (2.0 mg/kg), and a xylazine and lignocaine (0.05 mg/kg and 2.0 mg/kg) combination were studied in water buffalo calves. The study revealed that the combination of xylazine and lignocaine produced greater depth of analgesia for a longer duration compared with groups A (xylazine) and B (lignocaine). Furthermore, it was found that the xylazine and lignocaine combination did not have any adverse effect on the physiological parameters like heart rate, respiratory rate and rectal temperature. The transient changes produced by these drugs on different body systems waned after standing recovery. The alteration seen in different haematological and biochemical parameters were the outcome of general stress probably due to unpleasant experience and handling of the animals during the study. As the changes were transient and more or less the same in all the 3 groups and returned to baseline at 24 h of observation. The results of this study indicated a possible additive analgesic interaction between epidurally administered xylazine and lignocaine, without causing any marked systemic effects in water buffalo calves. It is concluded that the combination used in the study did not have deleterious effects on normal physiological mechanisms of the body and is thus safe for use in water buffaloes.

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