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Research Article

ISOFLURANE SPARING EFFECT OF FLUNIXIN MEGLUMINE AND TRAMADOL IN BUFFALOES UNDERGOING DIAPHRAGMATIC HERNIORRHAPHY

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ABSTRACT

The effect of flunixin meglumine and tramadol on isoflurane sparing in buffaloes undergoing diaphragmatic herniorrhaphy was investigated in the present study. Fifteen buffaloes undergoing diaphragmatic hernia surgery under general anaesthesia were randomly assigned to three groups having five buffaloes each. Intramuscular injection of atropine (0.04 mg kg) and xylazine (0.05 mg kg) were given to all buffaloes. In group-I (AXPI/Control group), no analgesic was administered. In group-II (AXFPI), injection of flunixin meglumine @ 2.0 mg/kg and in group-III (AXTPI) injection of tramadol @ 2.0 mg/kg were given intravenously 15 minutes after premedication. In all groups, anaesthesia was induced with IV propofol (till effect) and maintained with isoflurane with a variable vaporizer setting for a uniform surgical plane of anaesthesia. The amount of isoflurane used (ml) was computed and equilibrated for 400 kg body weight of animals and 40 minutes of anaesthesia. Administration of flunixin meglumine and tramadol to buffaloes undergoing diaphragmatic herniorrhaphy reduced the amount of isoflurane required by 13.14% and 16.89%, respectively, as compared to the control group.

Keywords: Buffaloes, Diaphragmatic herniorrhaphy, Isoflurane, Flunixin meglumine, Tramadol

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Ruminants are poor subject for general anaesthesia especially in dorsal recumbency during the trans-abdominal approach of diaphragmatic herniorrhaphy as the ventilation-perfusion is markedly mismatched. An ideal anaesthetic agent produces sleep, amnesia, analgesia and muscle relaxation. All these characteristics cannot be provided by a sole agent and therefore, combination of drugs is used which is referred to as "balanced anaesthesia" (Thurmon and Short, 2007). For long duration surgeries the use of less isoflurane for maintenance of anaesthesia is significantly importantas it will lessen the side effects of isoflurane such as respiratory depression, hypotension, and lower cardiac output (Hikasa *et al.*, 2002). Many drugs especially analgesics have been used for this purpose.

The effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the minimum alveolar concentration (MAC) of volatile anaesthetics have been investigated in different animals. Flunixin is a commonly used NSAID in bovines and has analgesic properties in post-surgical pain (Reid and Nolan, 1991), which exhibits its effect by inhibiting the COX (Cyclooxygenase). It's use in different ruminant species, for the treatment of various inflammatory conditions like endotoxemia, mastitis and musculoskeletal disorders, has been reported (Rantala *et al.*, 2002).

Tramadol is an opioid μ-receptor agonist that provides analgesia by increasing release and decreasing reuptake of serotonin and norepinephrine in the spinal cord (Desmeules *et al.*,1996). Coetzee *et al.* (1996) reported that addition of tramadol in the anaesthetic protocol of dog reduced the quantity of isoflurane required for maintenance of anaesthesia. Also it significantly reduces the MAC of sevoflurane in dogs (Seddighi *et al.*, 2009). But literature is scarce related to use of flunixin and tramadol for sparing effect on isoflurane in buffaloes. Therefore, a study was planned to study the isoflurane sparing effect of flunixin meglumine and tramadol to lessen the cardiopulmonary depression in buffaloes undergoing diaphragmatic herniorrhaphy (DH) premedicated with atropine and xylazine followed by propofol induction.

MATERIALS AND METHODS

Animals: A total of 15 buffaloes with diaphragmatic hernia were reported to the Veterinary Clinical Complex, LUVAS, Hisar between May 2020 and June 2021. After clinical examination and radiographic diagnosis, they were subjected to rumenotomy followed by diaphragmatic herniorrhaphy on the next day. After rumenotomy, the animals were kept off feed and off water, and weighed to calculate the doses of drugs for general anaesthesia. The physiological parameters were recorded and blood samples were collected aseptically from jugular vein of buffaloes before rumenotomy, before drug administration on the day of herniorrhaphy, at different interval during and after herniorrhaphy, and at 24 hours of recovery to analyse haematological and biochemical alterations. The different parameters recorded were behavioural changes,

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rectal temperature, heart rate, respiratory rate, non-invasive blood pressure, SpO_2, haemoglobin, total leucocyte count, differential leucocyte count, packed cell volume, blood urea nitrogen, plasma creatinine, alanine amino transferase, aspartate amino transferase, total plasma proteins, albumin, globulin, A: G ratio. The study used an unequal randomization procedure.

Procedure for anaesthesia: The buffaloes were randomly divided into three groups (I, II and III) of five animals each. All buffaloes were premedicated with atropine (0.04 mg/kg) and subsequently xylazine (0.05 mg/kg at an interval of 15 minutes. After fifteen minutes of xylazine treatment, buffaloes were made in lateral recumbency for induction of anaesthesia. Then, in group I (AXPI) no separate analgesic was given, however, in groups II (AXFPI) and III (AXTPI), intravenous flunixin (2.0 mg/kg) and intravenous tramadol (2.0mg/kg) were given, respectively. Induction was performed with propofol (till effect approximately @ 1.3 mg/kg b. wt.) as per Table 1. Intubation with a cuffed endotracheal tube (inner diameter 20 mm, Surgivet®, Smith medical firm, UK) was performed after induction and attached to a large animal anaesthetic machine (Vetland® Veterinary anaesthesia system, Louisville, KY, USA). Isoflurane was administered via an agent-specific vaporizer (Dräger®, USA) in combination with oxygen via a semi-closed rebreathing system for maintenance. For premedication quality, induction quality, maintenance quality, and recovery quality, number ratings ranging from 1 to 4 (1-poor, 2-fair, 3-good, 4-excellent) were assigned (Bodh *et al.*, 2015). Vaporizer settings was adjusted and recorded to maintain the surgical plane consistently by monitoring body reflexes. The physical reaction of the treated animal to surgical stimulation during diaphragmatic herniorrhaphy was used to assess the drug's qualitative and subjective effects (sedation, analgesia, muscular relaxation). During the maintenance of anaesthesia, numerical values ranging from 0 to 3 (0-no effect, 1-mild effect, 2-moderate effect, 3-deep effect) were utilised for sedation, analgesia, and muscular relaxation as per the criteria laid by Bodh *et al.* (2015). For diaphragmatic herniorrhaphy, the animal was placed in dorsal recumbency and operated on using a post-xiphoid trans-abdominal route. Throughout the procedure, all of the animals were given normal saline. With the last skin suture was applied, isoflurane was stopped, but the animals were permitted to breathe oxygen until their swallowing and coughing reflexes recovered when the endotracheal tube was removed. Post-operatively, after recovery Streptopenicillin (5 g) and meloxicam (0.5 mg/kg) were administered intramuscularly for five days, respectively along with antiseptic dressing of incision sites.

Calculation of liquid isoflurane usage: The fresh gas flow rate (FGF) and vaporizer settings were recorded the different time interval given. The quantity of isoflurane consumed in each group was determined using formula (Senthilkumar *et al.*, 2013).

Isoflurane vapour delivered (mL) =Vapourizer setting (%) x FGF (Litre/minute) x Duration (minute) x 10

By aggregating the isoflurane vapour delivered for each of the FGF and vaporizer settings used, the total isoflurane vapours delivered (mL) during the complete duration of anaesthesia was computed. For statistical comparison, the total isoflurane vapour value obtained was equilibrated to 400 kg body weight and a 40-minute operation duration for each animal as:

Isoflurane vapour delivered for 400 kg and 40-minute basis (mL) = (Total isoflurane vapour delivered in mL x 400 x 40)/ (Body weight x Duration of maintenance).

Using Avagadro's principle, the volume of liquid isoflurane consumed was determined, and correction factors were used on account of the effect of ambient temperature and atmospheric pressure in Operation Theatre. = Isoflurane vapour delivered for 400 kg and 40-minute basis (ml) x181.4 ÷ (ambient temp/273) x (760/ barometric pressure mm/Hg)

Haemato-biochemical Parameters: Blood specimens were processed for hematological parameters using an automatic haematoanalyzer (MS4®; MeletSchloesing Laboratories, Evalic, France), and plasma from heparinized blood was collected by centrifugation at 2500 rpm for 15 minutes for biochemical parameters using an automated random access blood chemistry analyzer (EM 200®; Erba Mannheim, Germany) with commercially available kits.

Statistical evaluations: Two-way ANOVA with repeated measures was used to determine the significant difference between treatments and time points. The pair wise comparisons were done using Duncan's multiple range test (Duncan, 1955). The significance difference was considered if $P < 0.05$.

RESULTS AND DISCUSSION

Isoflurane was selected as maintenance agent of interest because at present it is the most widely used inhalation anaesthetic in medical as well as veterinary patients. It is also being widely used in buffaloes (Singh *et al.*, 2013; Bodh *et al.*, 2015; Chaudhary and Tayal, 2020; Yadav *et al.*, 2021). The reduction in isoflurane for maintaining surgical plane of general anaesthesia will

Table 1. Time and route of drug administration along with dose rate in buffaloes undergoing diaphragmatic herniorrhaphy

Drug	Dose Rate	Time and Route of administration of different drugs
Atropine	0.04 mg	Atropine I/M ↓10 min
Xylazine	0.05 mg	Xylazine I/M ↓15 min
Flunixin meglumine	2 mg	No analgesic (Group-I)/ Flunixin meglumine I/V (Group -II)/ Tramadol I/V (Group-III)
Tramadol	2 mg	↓5 min
Propofol	till Effect (@1.3 mg/kg)	Propofol (I/V)
Isoflurane	0-5%	Inhalation

Table 2. Amount of isoflurane utilized (on 400 kg and 40 min basis in ml) by individual buffaloes of group-I, II and III during diaphragmatic herniorrhaphy Isoflurane liquid utilized in (ml)

Animals	Group-I	Group-II	Group-III
Animal A	62.00	54.45	57.34
Animal B	65.09	54.16	42.35
Animal C	54.66	56.99	52.58
Animal D	73.92	48.66	44.95
Animal E	51.04	52.14	57.66
Mean ±S.E.	61.34±4.02	53.28±1.39	50.58±3.15

Table 3. Depicting amount of isoflurane utilized (on 400 kg and 40 min basis in ml) during diaphragmatic herniorrhaphy by different groups of buffaloes.

Groups	Isoflurane Liquid Utilized in (ml)	Percentage Isoflurane utilized of Control group	Percentage reduction from control group
Group-I	61.34±4.02[a]	100%	0%
Group-II	53.28±1.39[b]	86.86%	13.14%
Group-III	50.58±3.15[b]	83.11%	16.89%

($P<0.05$)

obtund isoflurane-related undesirable effects (Hikasa *et al.*, 2002). So, any reduction in its dose without compromising the quality of anaesthesia will be advantageous.

Tramadol has been used to control moderate pain in humans for decades in many countries but veterinarians have recently become interested in tramadol to control postoperative pain. Tramadol is an atypical centrally acting opioid analgesic with one-tenth the potency of morphine (Coetzee *et al.*, 1996). However, the reports about the inhalant sparing effect of tramadol for various species (rats and dogs) verified that tramadol turned out to reduce minimum alveolar concentration of inhalants (de Wolff *et al.*, 1999 and Seddighi *et al.*, 2009). Not only requirement of inhalants but doses of injectable anaesthetics for the induction can be decreased with drug combinations.

Flunixin meglumine a nonsteroidal anti-inflammatory drug (NSAID), is a commonly used as analgesic in domestic ruminants. In sheep, flunixin produced a small but significant increase in the pain threshold to mechanical stimulation (Chambers *et al.*, 1995). This change in threshold was blocked by naloxone or atipamezole, suggesting that flunixin's effect was mediated by opioids and adrenergic mechanisms. The effects of NSAIDs on the MAC of volatile anaesthetics have been investigated in other species but not in buffaloes (Alibhai and Clarke., 1996; Ko *et al.*, 2000).

Different behavioural parameters related to the development of CNS depression and recovery from CNS depression in buffaloes undergoing diaphragmatic herniorrhaphy by administration of different anaesthetic combinations are shown in table 4. No significant difference was seen in comparing muzzle dryness, weak time, down time, loss of palpebral reflex, relaxation of jaw muscle, loss of tongue reflex, loss of swallowing reflex and intubation of different groups. Time for regain of muscle tone was significantly higher in buffaloes administered tramadol followed by flunixin because tramadol has properties similar to opioids so causes some sedation, however, flunixin is a NSAID.

Effects of different anaesthetic combinations on physiological and hemodynamic parameters in buffaloes are shown in table 5. There were no significant differences within groups observed during the entire period except in group-1 where decreases in rectal temperature at 30 minutes of isoflurane inhalation and at recovery were reported. It may be due to individual differences. The respiratory rate of group-III (AXTPI) was significantly lower in comparison to group-I (AXPI) at 30 minutes of isoflurane inhalation. Within group-II (AXFPI) and III (AXTPI) respiration rate was significantly lower at recovery and at 30 minutes of isoflurane inhalation than before rumenotomy as tramadol has depressant effects on CNS also, like other opioids. No significant change was observed in heart rate before rumenotomy and before drug administration (premedication) on day of herniorrhaphy. But on the day of herniorrhaphy heart rate of groups-III was significantly lower than group-I at 5 minutes of

Table 4. Different behavioural aspects (Mean S.E. in minutes) of the development of CNS depression and recovery from CNS depression in buffaloes undergoing diaphragmatic herniorrhaphy produced by administration of different anaesthetic combinations in groups

Parameters	Mean±SE (Minute)		
	Group IAXPI	Group IIAXFPI	Group IIIAXTPI
Muzzle dryness°	13.2 ± 0.58^{A}	13.00 ± 0.55^{A}	13.20 ± 0.58^{A}
Weak time°°	9.40 ± 0.51^{A}	9.80 ± 0.49^{A}	9.60 ± 0.51^{A}
Down time°°	13.4 ± 0.60^{A}	14.20 ± 0.58^{A}	13.80 ± 0.49^{A}
Loss of palpebral reflex*	2.20 ± 0.37^{A}	2.40 ± 0.24^{A}	2.60 ± 0.24^{A}
Relaxation of jaw muscle*	2.60 ± 0.24^{A}	2.40 ± 0.24^{A}	2.60 ± 0.24^{A}
Loss of tongue reflex*	3.20 ± 0.37^{A}	2.80 ± 0.37^{A}	3.20 ± 0.20^{A}
Loss of swallowing reflex*	3.80 ± 0.37^{A}	3.60 ± 0.40^{A}	3.60 ± 0.24^{A}
Intubation*	4.40 ± 0.24^{A}	4.40 ± 0.24^{A}	4.20 ± 0.20^{A}
Regain of alar reflex†	10.80 ± 1.46^{AB}	9.60 ± 0.81^{A}	9.00 ± 0.55^{A}
Extubation†	17.20 ± 1.39^{BC}	13.60 ± 1.29^{A}	14.20 ± 0.37^{AB}
Regaining of muscle tone†	21.20 ± 1.59^{B}	14.60 ± 1.33^{A}	15.60 ± 0.51^{A}
Regaining of head righting reflex†	23.00 ± 0.89^{BC}	17.80 ± 1.88^{A}	19.20 ± 0.86^{AB}
Return to sternal recumbency†	25.80 ± 0.86^{A}	21.60 ± 1.94^{A}	23.40 ± 1.57^{A}
Standing with ataxia†	31.60 ± 2.42^{AB}	29.60 ± 2.71^{AB}	27.00 ± 1.64^{A}
Complete recovery†	41.60 ± 2.04^{A}	42.40 ± 1.72^{A}	36.80 ± 1.69^{A}

°after administration of atropine; °°after administration of xylazine
*after administration of propofol; †after discontinuation of isoflurane
Means with different superscripts (A/B/C/D) in a column show significant difference between groups (P<0.05)

Table 5. Effects of anaesthetic combinations of goup I, group II and group III on Physiological parameters in buffaloes undergoing diaphragmatic herniorrhaphy (Mean±S.E.)

Parameters	Groups	Diaphragmatic Herniorrhaphy						
		Before Rumenotomy	Before drug administration	At 5min of propofol	At 15min of isoflurane	At 30min of isoflurane	At Recovery	At 24 hrs Recovery
Rectal Temperature (°C)	G-I	37.98 ± 0.31^{ab}	38.56 ± 0.69^{bB}	37.66 ± 0.2^{ab}	37.70 ± 0.39^{ab}	37.34 ± 0.32^{a}	37.44 ± 0.1^{a}	37.58 ± 0.21^{ab}
	G-II	37.9 ± 0.41	37.64 ± 0.24^{AB}	36.6 ± 0.92	36.9 ± 0.82	37.02 ± 0.93	36.76 ± 0.5	36.82 ± 0.67
	G-III	37.24 ± 0.16	37.3 ± 0.31^{A}	37.58 ± 0.3	37.62 ± 0.37	37.48 ± 0.4	37.02 ± 0.46	36.86 ± 0.44
Heart rate (beat/min)	G-I	46.8 ± 1.83^{a}	48 ± 2.12^{ab}	51.6 ± 2.94^{abC}	56.2 ± 2.44^{bB}	53.4 ± 3.06^{abB}	51.6 ± 2.56^{abB}	51.6 ± 2.48^{abB}
	G-II	46.4 ± 1.63	47 ± 2.02	48.4 ± 2.2^{BC}	48.6 ± 3.12^{A}	48.6 ± 2.04^{AB}	45.0 ± 1.18^{A}	45.8 ± 1.43^{A}
	G-III	44.4 ± 1.57	45.2 ± 1.16	44.2 ± 1.28^{AB}	45.2 ± 1.66^{A}	46.8 ± 0.73^{A}	45.6 ± 1.69^{A}	44.4 ± 1.03^{A}
Respiratory Rate (breath/min)	G-I	12 ± 0.84	12.4 ± 1.17	12 ± 1.9	12.6 ± 1.44	13.2 ± 0.37^{B}	10.8 ± 0.66	10.4 ± 0.6
	G-II	12.6 ± 0.51^{c}	12.4 ± 0.51^{bc}	11.2 ± 0.97^{abc}	11.4 ± 0.51^{abc}	12.6 ± 1.17^{cAB}	9.8 ± 0.49^{a}	10.2 ± 0.37^{ab}
	G-III	12.6 ± 0.4^{b}	11.8 ± 0.37^{ab}	12.4 ± 0.93^{ab}	11 ± 0.71^{ab}	10.2 ± 0.8^{aA}	11.6 ± 0.81^{ab}	11.2 ± 0.8^{ab}
Spo_2(%)	G-I	99.2 ± 0.37	98.6 ± 0.51	99.6 ± 0.24	99.4 ± 0.24	97.6 ± 0.51	94 ± 4	96.6 ± 1.94
	G-II	99 ± 0.32	99.2 ± 0.2	99 ± 0.32	98.4 ± 0.68	97.8 ± 1.16	97.8 ± 0.58	98 ± 0.71
	G-III	99 ± 0.32^{b}	98.8 ± 0.37^{b}	98.6 ± 0.24^{b}	98.2 ± 0.58^{ab}	94 ± 2.28^{a}	95.8 ± 1.98^{ab}	96 ± 1.76^{ab}
Systolic Blood Pressure (mmHg)	G-I	99 ± 3.79	95.8 ± 2.63	96.8 ± 7.31	106.8 ± 15.19	108.2 ± 10.07	100 ± 3.24	101.6 ± 3.26
	G-II	91.8 ± 1.28	92.6 ± 1.12	85.2 ± 4.75	95.4 ± 8.64	102.4 ± 9.91	92.2 ± 2.15	95.2 ± 3.14
	G-III	97.6 ± 1.47	93.6 ± 2.77	103.4 ± 3.06	125 ± 3.86	136.8 ± 7.36	106.4 ± 6.61	94.6 ± 3.7
Diastolic Blood Pressure (mmHg)	G-I	60.6 ± 3.54	54 ± 4.46	60.4 ± 6.68	74.6 ± 16.29	62.4 ± 7.01^{A}	61.2 ± 4.72	60.4 ± 4.53
	G-II	58.2 ± 1.69	57.2 ± 1.11	55.4 ± 5.02	55.6 ± 4.61	64 ± 5.43^{AB}	59 ± 1.1	60 ± 3.18
	G-III	62.8 ± 3.37^{a}	60 ± 2.59^{a}	72.8 ± 2.46^{ab}	81.8 ± 6.72^{bc}	95.4 ± 6.27^{cC}	66 ± 5.69^{a}	65.4 ± 3.94^{a}
Mean Arterial Blood Pressure (mmHg)	G-I	72.6 ± 3.67	66.4 ± 3.68	72.6 ± 7.1^{AB}	84.8 ± 16.95	77.8 ± 7.75^{A}	72.6 ± 3.12	77.4 ± 5.16^{AB}
	G-II	69 ± 1.3^{ab}	66.2 ± 1.16^{ab}	$64.6 \pm 4.66a^{A}$	65 ± 4.05^{a}	76.8 ± 6.38^{bA}	68.4 ± 1.21^{ab}	$68.6 \pm 2.11ab^{A}$
	G-III	74.2 ± 2.4^{a}	70.6 ± 2.4^{a}	84.8 ± 2.82^{abB}	96.4 ± 5.84^{bc}	107.4 ± 6.52^{cB}	79.2 ± 6.26^{a}	74.4 ± 3.87^{aAB}

Different superscripts (A/B/C/D) in a column show significant difference between groups (P<0.05)
Different superscripts (a/b/c/d) in a column show significant difference with in rows (P<0.05)

Table 6. Effects of anaesthetic combinations of goup I, group II and group III on haematological parameters in buffaloes undergoing diaphragmatic herniorrhaphy (Mean±S.E.)

Parameters (Units)	Groups	Before rumenotomy	Diaphragmatic Herniorrhaphy					
			Before drug admn. (preme -dication)	At 5 min. of Propofo-linduction	At 15 min. of Isofluran-einhalation	At 30 min. of Isofluran-einhalation	At recovery	At 24 hrs of recovery
TLC ($x10^3/mm^3$)	I	9.9 ± 0.92^a	10.3 ± 1.03^a	10.5 ± 1.16^a	9.78 ± 0.95^a	9.64 ± 0.84^a	9.32 ± 1.11^a	8.35 ± 1.14^a
	II	11.39 ± 0.95^a	11.28 ± 1.02^a	10.64 ± 0.9^a	9.69 ± 1.13^a	9.06 ± 1.01^a	10.39 ± 1.16^a	10.44 ± 1.24^a
	III	9.76 ± 0.75^a	10.14 ± 0.74^a	9.9 ± 1^a	9.26 ± 0.76^a	9.36 ± 1.09^a	9.53 ± 1.22^a	9.35 ± 0.77^a
Packed cell volume (%)	I	25.6 ± 3.31^a	27.8 ± 4.03^a	27.8 ± 4.67^a	27 ± 3.77^a	25.6 ± 4.5^a	26.4 ± 3.91^a	26.8 ± 2.84^a
	II	27.6 ± 3.22^a	28.2 ± 3.06^a	28.6 ± 3.11^a	29.8 ± 2.71^a	28 ± 4.06^a	28 ± 4.09^a	26.4 ± 2.32^a
	III	26.4 ± 1.83^a	27.8 ± 2.22^a	26.6 ± 2.23^a	29.4 ± 2.11^a	22.6 ± 2.29^a	28.2 ± 1.88^a	25.2 ± 2.82^a
Total Erythrocyte Count ($x10^6/mm^3$)	I	5.34 ± 0.2^a	5.95 ± 0.26^a	5.84 ± 0.3^a	5.28 ± 0.2^a	5.43 ± 0.24^a	5.64 ± 0.28^a	5.77 ± 0.18^a
	II	5.14 ± 0.36^a	5.22 ± 0.34^a	5.15 ± 0.27^a	5.34 ± 0.3^a	5 ± 0.24^a	5.01 ± 0.09^a	5.21 ± 0.14^a
	III	5.4 ± 0.51^a	5.71 ± 0.41^a	5.3 ± 0.41^a	5.22 ± 0.28^a	4.84 ± 0.37^a	5.08 ± 0.37^a	5.5 ± 0.45^a
Thrombocyte ($x10^3/mm^3$)	I	236 ± 18.7^a	229.4 ± 20.54^a	196.2 ± 14.45^a	208 ± 9.86^a	224.2 ± 27.18^a	226 ± 21.71^a	229.2 ± 12.89^a
	II	272 ± 14.31^a	274.2 ± 22.47^a	273 ± 16.52^a	235.2 ± 3.92^a	257.2 ± 21.31^a	234.4 ± 16^a	267.8 ± 20.31^a
	III	242.2 ± 11.7^a	253.2 ± 13.48^a	228.4 ± 14.84^a	242.4 ± 12.82^a	233.4 ± 32.57^a	253.8 ± 11.38^a	238.6 ± 8.76^a
MCV (fl)	I	62.44 ± 1.63^a	63.24 ± 1.37^a	63.38 ± 1.06^a	62.24 ± 1.18^a	62.48 ± 1.34^a	61.9 ± 1.3^a	62.78 ± 1.34^a
	II	67.2 ± 2.3^a	67 ± 2.25^a	67.12 ± 2.17^a	66.94 ± 2.47^a	66.9 ± 2.39^a	66.38 ± 2.14^a	66.56 ± 2.05^a
	III	66.48 ± 2.42^a	65.64 ± 2.09^a	65.26 ± 2.25^a	65.16 ± 2.55^a	64.4 ± 2.23^a	64.16 ± 2.29^a	64.74 ± 2.61^a

Different superscripts (A/B/C/D) in a column show significant difference between groups ($P<0.05$)
Different superscripts (a/b/c/d) in a column show significant difference with in rows ($P<0.05$)

propofol induction. Within group-I, heart rate increased at 15 minutes of isoflurane inhalation during anaesthesia than at before rumenotomy.

SpO_2 did not show significant variation between groups during the entire protocol. Within the group-III significant decrease in SpO_2 percent was observed at 30 minutes of isoflurane inhalation than at base value as well as at recovery and at 24 hours of recovery.

There were no significant changes in systolic BP (mm Hg) between groups during whole anaesthetic period, however, significant changes were observed in diastolic BP (mm Hg) in group-III than group I at 30 minute of isoflurane inhalation. No significant differences were observed between groups in mean arterial blood pressure before rumenotomy, before drug administration (premedication), at 15 minute of isoflurane inhalation and at recovery. At 5 minutes of propofol induction the MABP of group-II was significantly lower than MABP of group-III. Mean arterial blood pressure was significantly higher at 30 minute of isoflurane inhalation of group III than other groups.

Decreasing trend in haemoglobin levels were observed during anaesthesia in all groups at 5 minutes of propofol induction, at 15 minute and 30 minute of isoflurane inhalation and at recovery period. Significant change observed in group-II at 24 hours of recovery. Significant difference in TLC was observed between groups-I, II and group-II from lower to higher. No significant variation was observed in different groups before rumenotomy, before drug administration, at 15 minute and 30 minute of isoflurane inhalation, at recovery and at 24 hours of recovery. At 5 minutes of propofol induction TLC of group-I was significantly higher than group-II and group-III. There were no significance differences between different groups in other haematological parameters.

The amount of isoflurane utilized (on 400 kg and 40 min basis in ml) by individual buffaloes and in group is shown in table 2 and table 3. The volumes of isoflurane utilized during anaesthesia for groups Group-III (AXTPI) and Group-II (AXFPI) were significantly lower in comparison to control Group-I (AXPI). Similar reports regarding tramadol has been reported in rats and dogs (de Wolff *et al.*, 1999 and Seddighi *et al.*, 2009), however, Doherty *et al.* (2004) hypothesized that in goats flunixin could potentiate the reduction of the MAC with opioids like morphine but in this study, the isoflurane sparing effect might be due to the potentiation of analgesic effect of xylazine which have been used as sedative.

CONCLUSION

Tramadol and flunixin meglumine have significant dose sparing effect (16.89% and 13.14%, respectively) in

buffaloes undergoing diaphragmatic herniorrhaphy anaesthetized by atropine-xylazine-propofol combination without any added anaesthetic complication.

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