Haryana Vet. (March, 2022) **61(SI)**, 72-75

Research Article

ATRACURIUM AS A NEUROMUSCULAR BLOCKING AGENT IN BUFFALOES

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Received: 17.08.2021; Accepted: 07.09.2021

ABSTRACT

In present study, atracurium was injected intravenously at a dose rate of 0.4 mg/kg body weight in five healthy male buffaloes of 8-24 months age and changes in behavioural, physiological, haematological and biochemical parameters were evaluated. All the buffaloes showed ataxia at 1.02±0.17 min. and became laterally recumbent at 1.77±0.29 min. Endotracheal intubation was done at 8.91± 0.59 min. The neuromuscular block persisted for 33.65± 1.18 min. There was a significant ($p<0.05$) increase in heart rate and decrease in respiration rate at five minutes of atracurium administration. A significant increase in the haemoglobin, total erythrocyte count, platelet count, plasma glucose, triglycerides, calcium and potassium was noted at five minutes of administration of atracurium. It was concluded that atracurim produces an effective and safe muscle relaxation of medium duration in buffaloes.

Keywords: Atracurium, Buffaloes, Muscle relaxant

How to cite: Sangwan, A., Chaudhary, R.N., Kumar, A., Tiwari, D.K., Kumar, S., Sharma, M. and Bangar, Y.C. (2022). Atracurium as a neuromuscular blocking agent in buffaloes. *Haryana Vet.* **61(SI)**: 72-75.

Muscle relaxation of variable degrees can be achieved using local anaesthesia, centrally acting anaesthetic agents or using neuromuscular blocking agents. Complete muscle relaxation can not be achieved with simple techniques of local anaesthesia which also depends upon the temperament of the animals. Furious/ vicious/nervous and sometimes docile animals too are unsuitable subjects for local anaesthesia. Higher doses of general or local anaesthetics required for proper muscle relaxation have risks associated with cardiovascular and respiratory systems. Deep depression and immobility in the recovery period can predispose to complications of aspiration of regurgitated contents in ruminants and pneumonia in horses (Hall *et al.*, 2001). The neuromuscular blocking agents like atracurium directly affect the neuromuscular junction and can produce muscle relaxation without influencing central nervous and cardiovascular systems (Hall *et al.*, 2001). Neuromuscular blocking agents are mostly used as adjunct to anaesthesia for reducing the dose of anaesthetics, to facilitate tracheal intubation, skeletal muscle tone relaxation at light planes of inhalant or injectable anaesthesia, orthopaedic manipulations and prevention of patient movement during delicate ocular, neurological, or cardiac surgery (Taylor, 2006 and Martinez and Keegan, 2007).

Keeping in view the aforementioned points, the present study was conducted to evaluate the efficacy and safety of atracurium in buffaloes.

MATERIALS AND METHODS

Five experimental trials were conducted in apparently healthy male buffaloes of eight to twenty four months of age. The experiments were conducted with prior permission of the institutional animal ethical committee. All the buffaloes were maintained on standard ration and under similar managemental conditions. Based on the results of pilot trials, atracurium was given at 0.4 mg/kg body weight intravenously. Animals were kept off feed for 24 hours and off water for 12 hours prior to experiment. Following parameters were recorded to evaluate effects of atracurium:

Behavioural changes (weak time, down time, chin on ground, turning of neck, lateral recumbency, paddling of limbs, salivation, mouth gag application, intubation, extubation time, return to sternal recumbency, standing with ataxia, walking with ataxia, walking without ataxia), body reflexes (palpebral reflex, corneal reflex, tongue reflex, swallowing reflex, jaw tone, tail tone, fore limb tone, hind limb tone, prepuce tone, and scrotal tone), rectal temperature, heart rate, respiratory rate, haemoglobin, packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), total platelet count, lymphocyte count, monocyte count, granulocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), plasma glucose, urea nitrogen, creatinine, bilirubin, cortisol, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total plasma proteins, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), calcium, phosphorus, sodium, potassium and chloride.

- Weak time: Time elapsed from administration of drug to onset of ataxia
- Down time: Time elapsed from administration of drug to onset of sternal recumbency
- Lateral recumbency: Time elapsed from administration of drug to lateral recumbency

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Recording of rectal temperature, ambient temperature, heart rate and respiratory rate was done before drug administration and at 5, 10, 15, 30, 45 minutes of administration of drug, at recovery and at 24 hours of recovery. Blood samples were collected from jugular vein before drug administration, at five minute of injection, at recovery and at 24 hours of recovery for haematological and biochemical studies. Haematological parameters were estimated in automatic analyser MS4 after collecting blood samples in vials containing EDTA. Blood samples for analysis of biochemical parameters were collected in two sets of test tubes. One set of test tubes containing 3.8% sodium fluoride solution (10 mg/ml of blood) for estimation of glucose and other set containing heparin (10 units/ml) for estimation of remaining parameters as mentioned earlier. The plasma was harvested by centrifugation at 3000 rpm for 20 minutes and then stored at -20 °C. The biochemical parameters were analysed with automatic analyzer using commercially available standard system pack kits. Sodium, potassium and chloride were analyzed with automatic electrolyte analyzer. The statistical analysis of data was done by one-way-analysis of variance and Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

All the buffaloes showed sluggish activities with ataxia at 1.02±0.17 min. and became laterally recumbent at 1.77±0.29 min. as shown in Table 1 and 2. After mouth gag application at 7.92±1.03 min., intubation was done at 8.91 ±0.59 min. This may be due to anticholinergic action of neuromuscular blocking agents at nicotinic acetylcholine receptors of the skeletal muscle cells. It inhibit the receptor coupled ion movement across the cell membrane and thereby inhibiting muscle contractions (Unwin, 2005). The tail tone loss in the buffaloes occurred at 3.23±0.39 min.

The fore limbs (6.93±1.45 min.) were relaxed earlier than the hind limbs (7.92±1.41 min.) while during regaining of reflexes, hind limb tone (21.56± 0.75 min.) appeared earlier followed by forelimb tone (23.48±2.17 min.). Corneal and palpebral reflexes were intact in all the buffaloes throughout the trial. Pin pricks stimulation in the region of abdomen and thorax showed the presence of cutaneous reflex as the muscle relaxants do not have analgesic property (Melegar, 2006). Jaw muscles were relaxed at 7.12±0.54 min. and animal intubated at 8.91± 0.59 min.

Sternal recumbency was regained at 29.56±1.23 min. Animals stood up without support showing slight ataxia at 31.01±1.21 min. and walked with ataxia at 32.03±1.23 min. while ataxia vanished at 33.65±1.18 min. of administration of atracurium. A wide variety of onset of action and duration of muscle relaxation at a number of different doses had been studied for atracurium (Gergis *et al.*, 1983).

There was no significant change in the ambient and rectal temperature as shown in Table 3. There was a significant

Table 1

Different behavioural characteristics induced by administration of atracurium in male buffaloes

Parameters	Mean ±SE (Min.)
Weak time	1.02± 0.17
Down time	1.10± 0.15
Chin on ground	1.21 ± 0.16
Turning of neck	1.50± 0.27
Lateral recumbency	1.77± 0.29
Paddling of limbs	3.07± 0.14
Salivation	6.18± 0.91
Mouth gag application	7.92± 1.03
Intubation	8.91± 0.59
Extubation time	24.66± 1.24
Return to sternal recumbency	29.56± 1.23
Standing with ataxia	31.01± 1.21
Walking with ataxia	32.03± 1.23
Walking without ataxia	33.65± 1.18

Table 2

Different body reflexes (Loss and Gain) induced by administration of atracurium in male buffaloes

Reflex	Loss Mean ± SE (Min.)	Gain Mean ± SE (Min.)
Tongue reflex	6.67± 0.21	14.95 ± 1.00
Swallowing reflex	8.42± 0.77	22.30± 1.16
Jaw tone	7.12± 0.54	25.91± 1.20
Tail tone	3.23± 0.39	21.50± 1.20
Forelimb tone	6.93± 1.45	23.48 ± 2.17
Hind limb tone	7.92 ± 1.41	21.56± 0.75
Prepuce tone	10.42±0.92	21.75± 0.45
Scrotal tone	11.97±1.57	24.39± 0.70

increase in heart rate (97.4±6.68 beats/min.) at 5 min. as compared to the base value (56.2±5.5 beats/min.). Atracurium belong to the group of benzyl isoquinoloine neuromuscular blocking agents and induce histamine release (Savarese *et al.*, 2000). Histamine has a positive ionotropic and chronotropic effect on H2 receptors of myocardium and its chronotropic effect in part may be due to catecholamines (Moss and Rosow, 1983).

There was a significant decrease in respiratory rate at 5 min. (11.2±0.97 breaths/min.), with a minimum value of 8.2±0.58 breaths/min. at 15 min. as compared to the base value (18.8±0.86 breaths/min.). This may be due to the relaxation of the respiratory muscles. A non-significant reduction in respiration rate in dogs for 40 minutes have been reported by Suresh (1996).

There was a significant increase in haemoglobin, total erythrocyte count and total leucocytes count at five minutes as shown in Table 4. Dhankhar *et al.* (2016) and Dhankhar *et al.* (2020) reported similar findings using pancuronium and vecuronium in buffalo calves. These changes may be due to hypoxia induced by lowered respiration rate in response to which erythropoietin

Table 3

Effects of atracurium administration on rectal temperature, heart rate and respiratory rate in male buffaloes

Parameters (Units)	Before drug admn.	At 5 min.	At 10 min.	At 15 min.	At 30 min.	At 45 min.	At recovery	At 24 hrs. of Recovery
Ambient temperature (°C)	15.64^{a}±0.25	15.64^{a} ±0.25	15.64^{a} ±0.25	15.64^{a} ±0.25	15.72^{a} ±0.28	15.70^{a} ±0.25	15.76^{a} ±0.25	15.62^{a} ±0.14
Rectal temperature (°C)	37.50^{a} ±0.23	37.38^{a} ±0.23	37.06^{a} ±0.24	37.14^{a} ±0.29	37.18^{a} ±0.15	37.26^{a} ±0.19	37.66^{a} ±0.26	37.44^{a}±0.28
Heart rate (beats /min.)	56.2^{ac}±5.50	97.4^{b}±6.68	65.6^{c} ±4.02	52.2^{ac}±1.28	55.2^{ac}±2.87	57.4^{ac}±4.12	56.0^{ac}± 2.47	56.4^{a} ±3.19
Respiratory rate (breaths/min.)	18.8^{c} ±0.86	11.2^{b} ±0.97	10.4^{ab} ±0.68	8.2^{a} ±0.58	9.6^{ab} ±0.81	10.6^{b} ±0.51	14.2^{b}±0.66	19.8^{c} ±0.66

Means with different superscripts vary significantly ($p<0.05$) within rows

Table 4

Effect of atracurium administration on haematological parameters in male buffaloes

Parameters (Units)	Before Drug admn.	At 5 Min	At recovery	At 24 hrs. of recovery
Haemoglobin (g/dl)	8.80^{a} ± 0.55	11.38^{b} ± 0.52	10.06^{ab} ± 1.50	9.38^{a} ± 0.57
Packed cell volume (%)	30.90^{a} ± 1.56	33.86^{a} ± 1.31	33.06^{a} ± 3.81	31.40^{a} ± 1.21
TEC (x10^{6} /mm³)	6.12^{a} ± 0.45	7.69^{b} ± 0.37	6.57^{ab} ± 0.93	5.99^{a} ± 0.30
TLC (x10^{3}/mm³)	8.58^{aa} ± 1.43	10.44^{b} ± 1.33	9.38^{ab} ± 1.04	8.18^{a} ± 1.62
Total platelets count (x10^{3}/mm³)	217.20^{a} ± 22.68	236.00^{b} ± 21.50	210.40^{a} ± 23.80	225.60^{b} ± 24.03
Lymphocytes (%)	53.72^{a} ± 3.39	56.64^{a} ± 2.44	52.88^{a} ± 2.07	61.26^{a} ± 6.54
Monocytes (%)	2.04^{a} ± 0.38	1.84^{a} ± 0.20	2.22^{a} ± 0.23	1.58^{a} ± 0.12
Granulocytes (%)	44.24^{a} ± 3.04	41.52^{a} ± 2.36	44.90^{a} ± 1.89	37.16^{a} ± 6.48
MCV (fl)	48.90^{a} ± 1.58	49.30^{a} ± 1.66	49.20^{a} ± 1.51	48.60^{a} ± 1.48
MCH (pg)	14.42^{a} ± 0.57	14.94^{a} ± 0.53	15.06^{a} ± 0.38	14.98^{a} ± 0.29
MCHC (%)	31.60^{a} ± 1.12	32.42^{a} ± 1.13	32.24^{a} ± 1.56	34.16^{a} ± 1.33

Means with different superscripts vary significantly ($p<0.05$) within rows.

hormone is secreted resulting in compensatory increase in total erythrocyte count (Weiss and Wardrop, 2010a). The increase in the level of leukocytes had been reported in cattle, dog and cats due to excitement, fear, restraining and venipuncture (Weiss and Wardrop, 2010a). Total Platelet count increased significantly at five minutes and at 24 hours of recovery. Epinephrine mediated spleenic contraction in excited animal may cause physiologic thrombocytosis (Weiss and Wardrop, 2010b). In dogs, the proportion of reticulated platelets was increased after administration of erythropoietin (Wolf *et al.*, 1997) which in turn may be raised endogenously under hypoxic conditions (Weiss and Wardrop, 2010c).

There was a significant increase in glucose (121.26 ±12.4 g/dl) at 5 min. as shown in Table 5. High value of glucose at 5 min. may be due to increase in plasma cortisol level which causes gluconeogenesis (Toshihiko *et al.*, 1996). Cortisol level remained non-significantly high till recovery which may be due to the release of ACTH from the anterior pituitary gland under hypoxia (Castognoli *et al.*, 1961).

There was a significant increase in triglycerides (18.60±0.65 mg/dl) at 5 min. The precursor of acetyl co-A used for synthesis of long chain fatty acids (LCFA) is acetate or glucose with former being more important in ruminants. The LCFA-Co-A forms triglycerides after esterification (Kaneko *et al.*, 2008).

Potassium level increases significantly (5.91±0.19 mmol/L) at 5 min. which may be due to hypoxia which reduces intracellular ATP in skeletal muscles leading to opening of ATP-sensitive K+ channel (Woll *et al.*, 1989). In acidemia resulting from hypoventilation, hydrogen ion enter the intracellular compartment in exchange with potassium ions leading to increased potassium ion level (Reece *et al.*, 2015). Similar findings had been reported by Dhankhar *et al.* (2016) and Dhankhar *et al.* (2020).

CONCLUSION

On the basis of the findings of present study, it can be concluded that atracurium at a dose rate of 0.4 mg/kg body weight given intravenously induces a rapid, safe and effective medium duration of neuromuscular blockade without much effects on haemato-biochemical parameters in buffaloes.

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Table 5

Effects of atracurium administration on biochemical parameters in male buffaloes

Parameters (Units)	Before Drug admn.	At 5 min.	At recovery	At 24 hrs. of recovery
Glucose (mg/dL)	$70.96^{a} \pm 5.13$	$121.26^{b} \pm 12.4$	$106.86^{ab} \pm 13.91$	$75.36^{a} \pm 1.86$
Triglycerides	$15.58^{a} \pm 0.73$	$18.60^{b} \pm 0.65$	$16.56^{ab} \pm 0.67$	$14.70^{a} \pm 0.82$
Total cholesterol (mg/dL)	$62.40^{a} \pm 2.46$	$65.80^{a} \pm 2.69$	$66.00^{a} \pm 2.85$	$61.20^{a} \pm 1.77$
HDLC (mg/dL)	$38.98^{a} \pm 2.81$	$39.44^{a} \pm 3.32$	$39.36^{a} \pm 4.05$	$40.14^{a} \pm 3.85$
LDLC (mg/dL)	$12.81^{a} \pm 2.23$	$12.08^{a} \pm 1.71$	$11.89^{a} \pm 1.64$	$12.15^{a} \pm 1.85$
Cortisol (ng/L)	$28.98^{a} \pm 2.05$	$33.34^{a} \pm 3.37$	$30.33^{a} \pm 1.83$	$27.83^{a} \pm 2.61$
Total Bilirubin (mg/dl)	$0.17^{a} \pm 0.03$	$0.17^{a} \pm 0.03$	$0.23^{a} \pm 0.06$	$0.21^{a} \pm 0.07$
Bilirubin Direct (mg/dL)	$0.10^{a} \pm 0.02$	$0.09^{a} \pm 0.01$	$0.12^{a} \pm 0.02$	$0.11^{a} \pm 0.01$
Indirect Bilirubin (mg/dl)	$0.07^{a} \pm 0.03$	$0.07^{a} \pm 0.02$	$0.11^{a} \pm 0.05$	$0.09^{a} \pm 0.06$
Total protein (g/dL)	$8.60^{b} \pm 0.18$	$7.77^{ab} \pm 0.47$	$7.32^{a} \pm 0.19$	$8.53^{b} \pm 0.15$
Albumin (g/dL)	$3.03^{a} \pm 0.15$	$2.96^{a} \pm 0.08$	$3.16^{a} \pm 0.11$	$3.05^{a} \pm 0.06$
Globulin (g/dL)	$3.57^{a} \pm 0.20$	$3.81^{a} \pm 0.47$	$3.36^{a} \pm 0.17$	$3.48^{a} \pm 0.19$
A:G ratio	$0.86^{a} \pm 0.07$	$0.82^{a} \pm 0.08$	$0.89^{a} \pm 0.06$	$0.89^{a} \pm 0.06$
ALT (IU/L)	$29.82^{ab} \pm 3.53$	$37.02^{b} \pm 1.64$	$36.02^{b} \pm 1.13$	$29.76^{a} \pm 0.32$
AST (IU/L)	$45.50^{a} \pm 1.55$	$47.04^{a} \pm 2.60$	$43.62^{a} \pm 3.13$	$42.70^{a} \pm 1.57$
GGT (IU/L)	$20.65^{a} \pm 1.07$	$22.57^{a} \pm 1.06$	$21.49^{a} \pm 1.07$	$21.28^{a} \pm 1.28$
ALP (IU/L)	$85.20^{a} \pm 14.13$	$92.60^{a} \pm 15.27$	$91.80^{a} \pm 13.52$	$86.60^{a} \pm 11.10$
LDH (IU/L)	$520.20^{a} \pm 42.86$	$527.40^{a} \pm 45.69$	$514.80^{a} \pm 47.48$	$525.20^{a} \pm 48.78$
BUN (mg/dL)	$25.18^{a} \pm 6.61$	$23.02^{a} \pm 6.77$	$23.96^{a} \pm 7.25$	$25.52^{a} \pm 4.18$
Creatinine (mg/dL)	$1.17^{a} \pm 0.04$	$1.14^{a} \pm 0.03$	$1.15^{a} \pm 0.04$	$1.11^{a} \pm 0.03$
Calcium (mg/dL)	$10.54^{a} \pm 0.27$	$11.24^{b} \pm 0.17$	$11.82^{c} \pm 0.21$	$10.24^{a} \pm 0.36$
Phosphorus (mg/dL)	$6.30^{a} \pm 0.66$	$6.27^{a} \pm 0.49$	$6.20^{a} \pm 0.63$	$7.09^{a} \pm 0.47$
Sodium (mmol/L)	$138.46^{a} \pm 0.92$	$141.20^{a} \pm 0.93$	$140.42^{a} \pm 1.97$	$139.14^{a} \pm 1.60$
Potassium (mmol/L)	$4.39^{a} \pm 0.33$	$5.91^{b} \pm 0.19$	$4.10^{a} \pm 0.15$	$4.48^{a} \pm 0.19$
Chloride (mmol/L)	$104.36^{a} \pm 1.67$	$102.76^{a} \pm 1.09$	$101.92^{a} \pm 2.81$	$105.44^{a} \pm 1.28$

Means with different superscripts vary significantly ($p<0.05$) within row.

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