

## Oxidative stress studies in buffaloes undergoing inhalant and total intravenous anaesthesia

Ram Niwas<sup>1</sup>, R.N. Chaudhary<sup>2\*</sup>, Dinesh<sup>1</sup> and Rajesh Kumar<sup>3</sup>

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004 (Haryana)

<sup>1</sup>Assistant Professor, <sup>2</sup>Professor and Head, Department of Veterinary Surgery and Radiology; <sup>3</sup>Scientist, Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences, Hisar

DOI No.: 10.5958/0973-9726.2026.00004.X

Accepted: March 2026

The study was conducted on 18 female buffaloes diagnosed with diaphragmatic hernia, which were randomly allocated into three groups (n=6 each). All animals were premedicated with atropine sulphate, xylazine hydrochloride, and butorphanol, followed by anaesthetic induction with thiopentone sodium. Anaesthesia was maintained using a thiopentone continuous rate infusion in the TT group, isoflurane in the TI group, and sevoflurane in the TS group, all delivered via a partial rebreathing system. Enzymatic and non-enzymatic oxidative stress markers, along with antioxidant parameters, were evaluated at six different time points. The results indicated that total intravenous anaesthesia was associated with a lower degree of physiological stress compared to inhalant anaesthesia using either isoflurane or sevoflurane in buffaloes undergoing diaphragmatic herniorrhaphy.

**Keywords:** Anaesthesia, Buffalo, Isoflurane, Sevoflurane, Thiopentone, Total Intravenous Anaesthesia

Diaphragmatic hernia (DH) is a severe digestive disorder requiring a two-step surgical approach, typically rumenotomy followed by herniorrhaphy. Buffaloes affected with DH are often presented late and in poor body condition; therefore, the anaesthetic protocol should be minimally stressful to ensure successful outcomes with fewer complications.

Most anaesthetic agents are known to induce physiological stress in animals (Desborough, 2000). Assessment of reactive oxygen species (ROS) is an important indicator of stress and a useful parameter for evaluating the safety of anaesthetic protocols (Lee *et al.*, 2015). Although several comparative studies on total intravenous anaesthesia (TIVA) and inhalation anaesthesia exist across species, limited literature is available in buffaloes.

The body possesses enzymatic and non-enzymatic antioxidant defence mechanisms against ROS (Balaban *et al.*, 2005). However, as individual antioxidant components are difficult to measure separately, total antioxidant capacity (TAC) is commonly used as an integrated indicator of overall antioxidant status (Ghiselli *et al.*, 2000). A reduction in TAC reflects increased oxidative stress (Cao *et al.*, 1993; Benzie and Strain, 1996). Malondialdehyde (MDA), a product of lipid peroxidation, is widely regarded as a reliable biomarker of oxidative stress (Janero, 1990; Georgieva, 2005; Giera *et al.*, 2012).

Heat shock proteins (HSPs), initially identified in response to heat stress, are now known to be expressed under various stressful conditions (Fernandez *et al.*, 2010). These highly conserved proteins are essential for cellular function and protein homeostasis (Ellis and Vandervies, 1991) and exhibit cytoprotective roles through upregulated expression during stress (Santoro, 2000; Christopher *et al.*, 2010). Although thiopentone has been suggested to possess some antioxidant properties, it is less potent than propofol in this regard. However, findings regarding its effects on oxidative stress remain inconsistent.

Total intravenous anaesthesia is widely used under field conditions and in institutions lacking anaesthesia machine facilities. Considering the importance of buffaloes in the Indian subcontinent and the limited comparative data on TIVA versus inhalation anaesthesia based on stress-related parameters, the present study was conducted.

### Materials and Methods

The study was conducted on 18 clinical cases of female buffaloes diagnosed with diaphragmatic hernia. The animals were randomly allocated into three groups (TT, TI, and TS), irrespective of age and physiological status, with six animals in each group. For surgical management, a left flank laparorumenotomy was performed on the first day to retrieve any reticular foreign bodies and to completely evacuate ruminal contents, thereby reducing the risk of regurgitation and aspiration during general anaesthesia. On the following day, diaphragmatic herniorrhaphy was performed.

On the day of herniorrhaphy, all animals were premedicated with i.m. administration of atropine sulphate (0.045 mg/kg), xylazine (0.05 mg/kg), and butorphanol (0.02 mg/kg). Anaesthesia was induced with thiopentone sodium (6 mg/kg, i.v.). Following induction and placement of a mouth gag, endotracheal intubation was performed using an appropriately sized cuffed tube, which was connected to a large animal anaesthesia machine to deliver oxygen.

Anaesthesia was maintained with a thiopentone continuous rate infusion (0.40 µg/kg/min) in the TT group (TIVA), while isoflurane and sevoflurane were used in the TI and TS groups, respectively, via a partial rebreathing circuit. All animals were positioned in

\*Corresponding author; E-mail: mncvet@luvas.edu.in

dorsal recumbency for herniorrhaphy. Blood samples were collected at six time points: before rumenotomy, 15 min before premedication, 5 and 30 min after induction, and 2 and 24 hr post-herniorrhaphy. Samples were analyzed for enzymatic and non-enzymatic stress markers using standard laboratory protocols. The data were analysed statistically and the differences were noted at  $P < 0.05$ .

## Results and Discussion

Within groups, all parameters reflecting reactive oxygen species (ROS) levels increased at 15 min before premedication compared to baseline (before rumenotomy), while reduced glutathione levels decreased, likely due to its role in neutralizing excess oxygen free radicals (Table 1). The overall increase in HSP 60 expression after rumenotomy across all groups may reflect an elevated stress response. HSP 60 primarily functions as a molecular chaperone, ensuring proper protein folding and regulating apoptosis during physiological stress (Hartl and Hayer, 2002; Kregel, 2002). However, basal expression of HSP family transcripts in the absence of stress has also been reported (Meistertzheim *et al.*, 2009; Tucker *et al.*, 2009). The relatively low, non-significant expression of HSP 60 in the sevoflurane group at 30 min post-induction may be attributed to reduced mitochondrial ROS production, helping to preserve mitochondrial integrity via the PI3K/Akt signaling pathway, as reported in a rat model by Wang *et al.* (2016).

The observed increase in HSP 90 expression across all groups may be due to the cumulative stress associated with both surgical procedures, as HSP 90 is known to be upregulated in response to oxidative stress through transcriptional and translational mechanisms (Morimoto and Santoro, 1998). However, buffaloes maintained under isoflurane exhibited significantly higher HSP 90 expression at 30 min post-induction compared to those maintained under thiopentone (IV) and sevoflurane anaesthesia. Additionally, HSP 90 levels in the sevoflurane group were non-significantly higher than those in the thiopentone (IV) group.

Lipid peroxidation levels were elevated in all groups at 24 hr post-herniorrhaphy, which may be attributed to increased ROS generation associated with a prolonged stress response, resulting in enhanced cellular membrane damage. On intergroup comparison, buffaloes maintained on isoflurane showed significantly higher lipid peroxidation levels than those maintained on thiopentone (TIVA) at 30 min post-induction. Similarly, buffaloes maintained on sevoflurane also exhibited higher lipid peroxidation levels compared to the TIVA group.

Significantly lower levels of reduced glutathione were observed within all groups at 24 hr post-herniorrhaphy, which may be attributed to the depletion of glutathione reserves due to increased

exposure to reactive oxygen species (ROS) during oxidative stress (Eunkeyoung *et al.*, 2021). Although no statistically significant differences were observed between the groups, the reduction in glutathione levels during the intraoperative period (at 5 and 30 min after induction) was comparatively less in buffaloes maintained on TIVA than in those receiving inhalant anaesthesia. This suggests a lower degree of ROS generation in the TT group during anaesthesia.

Values of both total antioxidant capacity (TAC) and superoxide dismutase (SOD) were significantly elevated at 2 hr post-herniorrhaphy in all groups, irrespective of the anaesthetic maintenance agent. SOD, an important enzymatic antioxidant, and TAC, which reflects the overall antioxidant status of the body, exhibited a similar pattern of variation across different time intervals. The significant increase in TAC and SOD at 2 hr post-herniorrhaphy may be attributed to an acute oxidative stress response, wherein antioxidant production is upregulated to counteract increased oxidant levels (Eunkeyoung *et al.*, 2021). Additionally, volatile anaesthetics used in the inhalant groups may have contributed to greater ROS generation (Bedirli *et al.*, 2012), further amplified by inflammatory mediators released during surgery (Eunkeyoung *et al.*, 2021). The findings of the present study are in agreement with Lee (2012), who reported an initial increase in TAC following propofol and thiopental anaesthesia in dogs, followed by a gradual decline over time.

In the present study, MDA levels (an indicator of lipid peroxidation) were significantly higher in the TI group than in the TT group at 30 min post-induction. This increase may be attributed to isoflurane-induced oxidative stress (Senoner *et al.*, 2019), further potentiated by the release of inflammatory mediators during surgery (Su *et al.*, 2019). Elevated intraoperative stress has also been reported in conditions such as aortic clamping and unclamping during human cardiac surgery (Senoner *et al.*, 2019). Additionally, MDA levels at 30 min post-induction were higher in the TS group compared to the TT group.

No statistically significant ( $P < 0.05$ ) variation in cortisol levels was observed either between or within groups. A similar finding was reported by Church *et al.* (1994), who concluded that anaesthesia alone did not significantly alter plasma cortisol levels in dogs undergoing major abdominal, orthopaedic, and thoracic surgeries. However, in the present study, a trend toward higher cortisol levels was observed at 24 hr post-herniorrhaphy compared to baseline values. Additionally, cortisol concentrations were non-significantly higher at 2 hr post-herniorrhaphy compared to values recorded at 30 min post-induction. The increase in cortisol was more pronounced in buffaloes maintained under inhalant anaesthesia (isoflurane) than in those maintained on thiopentone (TIVA). Saidu *et al.* (2016) similarly reported elevated serum cortisol levels following surgery in goats, with

**Table 1:** Mean±SE values of stress parameters in buffaloes undergoing herniorrhaphy at different time intervals.

Parameters	Groups	Before rumenotomy	Diaphragmatic herniorrhaphy			Recovery phase	
			15 min before premedication	5 min after induction	30 min after induction	2 hr after herniorrhaphy	24 hr after herniorrhaphy
HSP 60 (ng/mL)	TT	12.35 ±1.10	13.56 ±2.02	13.80 ±1.92	12.88 ±1.93	13.67 ±2.23	14.81 ±2.51
	TI	8.41 ±1.31	9.98 ±1.23	9.23 ±1.14	9.60 ±1.16	9.90 ±1.03	10.98 ±0.90
	TS	9.14 ±0.62	10.53 ±1.11	11.19 ±1.49	9.58 ±1.07	10.90 ±1.24	12.56 ±1.35
HSP 90 (ng/mL)	TT	57.02 <sup>a</sup> ±7.63	74.60 <sup>abA</sup> ±6.00	80.98 <sup>ba</sup> ±5.96	84.14 <sup>ba</sup> ±3.91	82.92 <sup>b</sup> ±11.36	111.03 <sup>c</sup> ±9.82
	TI	73.26 ±11.58	105.36 <sup>B</sup> ±12.11	103.74 <sup>B</sup> ±14.46	104.19 <sup>B</sup> ±13.38	107.49 ±6.84	124.88 ±16.97
	TS	66.88 ±6.08	86.65 <sup>A</sup> ±10.84	89.90 <sup>A</sup> ±8.03	92.96 <sup>A</sup> ±12.65	100.29 ±13.53	119.03 ±17.82
Lipid peroxidation (mg/dL)	TT	5.86 <sup>a</sup> ±0.70	7.91 <sup>ab</sup> ±0.74	7.58 <sup>ab</sup> ±0.74	5.96 <sup>aA</sup> ±0.44	6.70 <sup>a</sup> ±0.69	9.46 <sup>b</sup> ±0.65
	TI	6.78 ±0.78	8.20 ±0.59	7.54 ±0.89	8.36 <sup>B</sup> ±0.57	8.13 ±0.40	9.37 ±0.94
	TS	7.12 ±1.42	7.74 ±1.05	7.87 ±0.57	6.96 <sup>AB</sup> ±0.84	8.08 ±0.91	9.10 ±1.00
Reduced-glutathione (mg/dLequi. to Vit-C)	TT	68.38 <sup>d</sup> ±3.64	51.40 <sup>c</sup> ±2.76	45.25 <sup>c</sup> ±2.34	44.55 <sup>c</sup> ±3.49	34.46 <sup>b</sup> ±2.83	23.71 <sup>a</sup> ±2.30
	TI	59.29 <sup>c</sup> ±4.20	52.84 <sup>bc</sup> ±5.69	48.91 <sup>bc</sup> ±4.60	40.25 <sup>ab</sup> ±3.91	41.94 <sup>ab</sup> ±3.09	30.09 <sup>a</sup> ±3.98
	TS	55.94 <sup>b</sup> ±6.52	43.89 <sup>ab</sup> ±6.57	41.29 <sup>a</sup> ±3.52	42.92 <sup>ab</sup> ±4.20	39.94 <sup>a</sup> ±3.47	30.97 <sup>a</sup> ±1.62
Cortisol (ng/mL)	TT	56.22 ±15.34	73.29 ±12.75	60.81 ±5.28	54.47 ±8.47	64.81 ±10.29	65.84 ±3.63
	TI	55.60 ±12.87	72.95 ±12.57	70.82 ±13.6	88.19 ±10.09	99.14 ±8.25	53.70 ±11.17
	TS	68.58 ±10.55	72.03 ±13.58	65.25 ±8.50	71.11 ±10.19	72.23 ±5.77	76.67 ±12.98
TAC (mg/dL)	TT	1.26 <sup>a</sup> ±0.17	1.78 <sup>ab</sup> ±0.18	1.95 <sup>bc</sup> ±0.18	2.17 <sup>bc</sup> ±0.24	2.40 <sup>c</sup> ±0.18	1.72 <sup>ab</sup> ±0.13
	TI	1.39 <sup>a</sup> ±0.11	2.24 <sup>bc</sup> ±0.14	2.15 <sup>bc</sup> ±0.16	1.93 <sup>b</sup> ±0.13	2.44 <sup>c</sup> ±0.10	1.85 <sup>b</sup> ±0.13
	TS	1.12 <sup>a</sup> ±0.09	1.84 <sup>bc</sup> ±0.21	1.75 <sup>b</sup> ±0.10	1.90 <sup>bc</sup> ±0.09	2.15 <sup>c</sup> ±0.10	1.61 <sup>b</sup> ±0.12
SOD (per mg protein)	TT	0.69 <sup>a</sup> ±0.05	1.12 <sup>bc</sup> ±0.07	1.08 <sup>bc</sup> ±0.08	0.96 <sup>b</sup> ±0.06	1.22 <sup>c</sup> ±0.05	0.93 <sup>b</sup> ±0.06
	TI	0.63 <sup>a</sup> ±0.08	0.89 <sup>ab</sup> ±0.09	0.97 <sup>bc</sup> ±0.09	1.08 <sup>bc</sup> ±0.12	1.20 <sup>c</sup> ±0.09	0.86 <sup>ab</sup> ±0.06
	TS	0.51 <sup>a</sup> ±0.04	0.77 <sup>ab</sup> ±0.07	0.82 <sup>bc</sup> ±0.07	0.88 <sup>bc</sup> ±0.13	1.06 <sup>c</sup> ±0.12	0.73 <sup>ab</sup> ±0.07

Means with different superscripts (a/b/c) in a row and (A/B/C) in column shows significant difference within group and between groups, respectively ( $P < 0.05$ ).

increased values persisting up to 48 hr before declining markedly after 72 hr. In contrast, Church *et al.* (1994) observed a return to baseline cortisol levels within 24 hr post-surgery in dogs.

The present study concludes that parameters indicative of oxidative stress were generally higher,

while reduced glutathione levels, reflecting antioxidant capacity, were lower in buffaloes maintained under inhalation anaesthesia (isoflurane/sevoflurane) compared to those maintained on thiopentone (TIVA) during anaesthesia. Therefore, total intravenous anaesthesia (TIVA) using thiopentone was associated

with a lower stress response than inhalation anaesthesia with either isoflurane or sevoflurane in buffaloes undergoing diaphragmatic herniorrhaphy.

## References

- Balaban, R.S., Nemoto, S. and Finkel, T. 2005. Mitochondria, oxidants, and aging. *Cell* **120**: 483-495.
- Bedirli, N., Demirtas, C.Y., Akkaya, T. Salman, B., Alper, M., Bedirli, A. and Pasaoglu, H. 2012. Volatile anaesthetic preconditioning attenuated sepsis induced lung inflammation. *J. Surg. Res.* **178**: E17-E23.
- Benzie, I.F.F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **239**: 70-76.
- Cao, G., Alessio, H.M. and Cutler, R.G. 1993. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radic. Biol. Med.* **14**: 303-311.
- Christopher, G., Evans, L.C. and Jason, E.G. 2010. Heat shock protein 70 (Hsp70) as an emerging drug target. *J. Med. Chem.* **53**: 4585-4602.
- Church, D.B., Nicholson, A.I., Ilkiw, J.E. and Emslie, D.R. 1994. Effect of non-adrenal illness, anaesthesia and surgery on plasma cortisol concentrations in dogs. *Res. Vet. Sci.* **56**: 129-131.
- Desborough, J.P. 2000. The stress response to trauma and surgery. *Br. J. Anaesth.* **85**: 109-117.
- Ellis, R.J. and Vandervies, S.M. 1991. Molecular chaperones. *Annu. Rev. Biochem.* **60**: 321-347.
- Eunyoung, K., Zhao, Z., Rzasa, J.R., Glassman, M., Bentley W.E., Chen, S., Kelly, D.L. and Payne, G.F. 2021. Association of acute psychosocial stress with oxidative stress: Evidence from serum analysis. *Redox Biol.* **47**: 1-34.
- Fernandez, R.R., Concepcion, Y.M. and Zulueta, G. 2010. 70 kDa heat-shock proteins in surgical stress: thoracotomy vs. herniorrhaphy. *Cirurgia Espanola* **88**: 23-29.
- Ghiselli, A., Serafini, M., Natella, F. and Scaccini, C. 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic. Biol. Med.* **29**: 1106-1114.
- Hartl, F.U. and Hayer, H.M. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **29**: 1852-1858.
- Janero, D.R. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* **9**: 515-540.
- Kregel, K.C. 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J. Appl. Physiol.* **92**: 2177-2186.
- Lee, J.Y. 2012. Oxidative stress due to anesthesia and surgical trauma and comparison of the effects of propofol and thiopental in dogs. *J. Vet. Med. Sci.* **74**: 663-655.
- Lee, Y.M., Song, B.C. and Yeum, K.J. 2015. Impact of volatile anaesthetics on oxidative stress and inflammation. *Biomed Res. Int.* 242709. doi: 10.1155/2015/242709.
- Meistertzheim, A.L., Lejart, M., LeGoic, N. and Thébault, M.T. 2009. Sex-, gametogenesis, and tidal height-related differences in levels of HSP70 and metallothioneins in the Pacific oyster *Crassostrea gigas*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **152**: 234-239.
- Morimoto, R.I. and Santoro, M.G. 1998. Stress-inducible responses and heat shock proteins: New pharmacologic targets for cytoprotection. *Nat. Biotechnol.* **16**: 833-838.
- Saidu, A.M., Bokko, Paul, B., Mohammed, A., Bukbuk, D.N. and Igwenagu, E. 2016. Serum cortisol of Sahel goats following rumenotomy with assorted anaesthetics and sutures. *Int. J. Vet. Sci. Med.* **4**: 23-26.
- Santoro, M.G. 2000. Heat shock factors and the control of the stress response. *Biochem. Pharmacol.* **59**: 55-63.
- Senoner, T., Schindler, S., Stattner, S., Ofner, D., Troppmair, J. and Primavesi, F. 2019. Associations of oxidative stress and postoperative outcome in liver surgery with an outlook to future potential therapeutic options. *Oxid. Med. Cell. Longev.* 1-18.
- Su, L.J., Zhang, J.H., Gomez, H., Raghavan, R., Hong, X., Dongxue, X., Jiang, F. and Peng, Z.Y. 2019. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxid. Med. Cell. Longev.* 1-13.
- Tucker, N.R., Ustyugov, A., Bryantsev, A.L., Konkel, M.E. and Sheldon, E.A. 2009. Hsp27 is persistently expressed in zebrafish skeletal and cardiac muscle tissues but dispensable for their morphogenesis. *Cell Stress Chaperones.* **14**: 521-533.
- Wang, Z., Ye, Z., Huang, G., Wang, N., Wang, E. and Guo, Q. 2016. Sevoflurane post-conditioning enhanced hippocampal neuron resistance to global cerebral ischemia induced by cardiac arrest in rats through PI3K/Akt survival pathway. *Front. Cell. Neurosci.* **10**: 271. doi: 10.3389/fncel.2016.00271.