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Evaluation of intraperitoneal splash block by lignocaine and ropivacaine on stress parameters in dogs undergoing keyhole ovariohysterectomy

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The study evaluated the effects of splash block using local anaesthetic agents - lignocaine, ropivacaine, and their combination - on surgical stress and the associated alterations in oxidative and antioxidant parameters following keyhole ovariohysterectomy (OHE) in 24 intact bitches. The dogs were randomly allocated into four groups, receiving intraperitoneal administration of either normal saline (0.5 mL/kg), 2% lignocaine hydrochloride (2 mg/kg), 0.5% ropivacaine hydrochloride (3 mg/kg), or a combination of both drugs at the same concentrations and dosages. The splash block was applied before closure of the muscle layer to assess its influence on oxidant markers such as lipid peroxidation (LPO) and antioxidant parameters including catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH). The parameters were recorded at 0, 24, and 48 hours. Lipid peroxidation (LPO) increased at 24 hr and declined by 48 hr, although the values did not return to baseline (0-hr) levels. Group D exhibited significantly lower LPO values at all-time intervals compared to the other groups. Catalase (CAT), an enzymatic antioxidant, showed a reduction at both 24 and 48 hr, with no significant difference between these two time points, and no notable variation among the treatment groups. Superoxide dismutase (SOD) levels decreased significantly at 48 hr, again with no significant differences among groups. Glutathione (GSH) values gradually declined from 0 to 48 hr, with no significant intergroup variation.

Overall, the findings indicate that intraperitoneal administration of 2% lignocaine HCl and 0.5% ropivacaine HCl as a splash block may be beneficial as part of a multimodal approach to pain management and reduction of surgical stress following OHE in dogs.

Keywords: *Anti-oxidant, Canine ovariohysterectomy, Lignocaine, Ropivacaine, Splash block.*

Intraoperative topical administration of 2% lidocaine as a splash block on both ovaries (2 mg/kg each) helped to attenuate increases in blood pressure, heart rate, and respiratory rate during surgery, and thus enhance intraoperative haemodynamic stability, reduces surgical pain, and decreases the requirement for rescue analgesia (Cicirelli *et al*., 2022).

Although extensive human clinical and experimental studies have evaluated the efficacy of intraperitoneal local anaesthetic instillation for managing postoperative pain (Benito *et al*., 2016), similar investigations in veterinary medicine remain limited. Therefore, the present study documents the effects of splash block using lignocaine and ropivacaine on the oxidant–antioxidant balance in dogs undergoing keyhole OHE.

Materials and Methods

Stray female dogs presented for ovariohysterectomy (OHE) at the Animal Birth Control–Anti Rabies Facility, Shuhama, served as the subjects for this study. A total of 24 healthy female dogs weighing 4–18 kg were included. Upon arrival, the animals were housed in preoperative kennels, where baseline (0-hr) parameters were recorded. All dogs were maintained under similar management conditions throughout their stay. Food was withheld for 24 hr and water for 8–10 hr prior to surgery. Each dog was housed individually during both the preoperative and postoperative periods. Surgery was performed 24 hr after arrival, and parameters corresponding to the 24-hr interval were recorded immediately after completion of the procedure. Post-surgery, the animals were shifted to postoperative kennels. The 48-hr parameters were recorded the day after surgery, following which the dogs were released after a thorough clinical evaluation.

The dogs were randomly assigned into four groups (A, B, C, and D), each consisting of six animals. Keyhole OHE was performed in all groups. Prior to closure of the laparotomy incision, the following treatments were administered intraperitoneally as a splash block: group A received normal saline; group B, lignocaine HCl 2% (LOXR, 30 mL, Neon Laboratories Ltd., Maharashtra); group C, ropivacaine HCl 0.5% (Ropin-R, 5 mg/mL, 200 mL, Neon Laboratories Ltd., Maharashtra); and group D received a combination of lignocaine HCl (2%) and ropivacaine HCl (0.5%), as detailed in Table 1. Drug dosages were calculated based on body weight.

Oxidant and antioxidant parameters were recorded at three intervals: 0 hr (preoperative), 24 hr (immediately post-surgery), and 48 hr (postoperative).

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Oxidant status was assessed through lipid peroxidation (LPO; nmol MDA/mL) estimated using the TBARS method described by Nichans and Samuelson (1968). Antioxidant parameters included catalase (CAT; µmol H, O, decomposed/min/g) estimated using the method of Clairborne (1985), superoxide dismutase (SOD; U/g), and glutathione (GSH; nmol/mL). Data collected were subjected to statistical analysis using SPSS (Version 20, SPSS Inc., Chicago, IL) following the procedures of Snedecor and Cochran (1989). Results were expressed as Mean±SE.

Results and Discussion

Table 1: Therapeutic modalities in different groups.

Group	No. of animals	Drug/Local anaesthetic	Dose rate
A	6	Normal Saline Solution (NSS)	0.5 mL/kg
B	6	Lignocaine Hydrochloride (2%)	2 mg/kg
C	6	Ropivacaine Hydrochloride (0.5%)	3 mg/kg
D	6	Lignocaine Hydrochloride (2%) and Ropivacaine Hydrochloride (0.5%)	2 mg/kg and 3 mg/kg

The Mean±SE values of lipid peroxidation (nmol MDA/mL) at 0, 24, and 48 hr for the different groups are presented in Table 2. All recorded values remained within the normal physiological range. A significant increase (P<0.05) in LPO levels was observed at the 24-hour interval across all groups. No significant differences were noted among the intervals within groups A, B, and C. However, in group D, the LPO values at both 0 and 24 hr were significantly lower compared to the corresponding values in the other groups.

Surgical incision, introduction of foreign material, the inflammatory response, and accumulation of cellular debris lead to increased oxygen consumption and, consequently, elevated production of reactive oxygen species (ROS). In addition, anaesthesia-induced hypothermia during surgery is often compensated by mild shivering, further increasing metabolic demand and oxygen consumption. These factors predispose patients to enhanced free radical generation, resulting in lipid peroxidation (LPO) and oxidative stress (Serin *et al*., 2008). Assessment of LPO levels, along with antioxidant parameters, provides a reliable indication of oxidative stress status. Plasma malondialdehyde (MDA) concentrations reflect the extent of lipid peroxidation within cells and are widely used as a biomarker for LPO (Yazar *et al*., 2003). In the present study, plasma MDA levels increased following surgery; however, in the test group, the changes observed at 48 hr were not statistically significant. These findings are consistent with the observations reported by Sakundech *et al.* (2020).

Table 2: Mean±SE values of lipid peroxidation (nmol MDA/mL) in all the groups at various time intervals.

Groups	Observation Intervals (hr)		
	0	24	48
A	9.80 ± 0.22^{aB}	12.24 ± 0.22^{cB}	11.31 ± 0.22^{bB}
B	9.51 ± 0.22^{aB}	11.95 ± 0.22^{cB}	11.02 ± 0.22^{bB}
C	8.96 ± 0.22^{aB}	11.40 ± 0.22^{cB}	10.48 ± 0.22^{bB}
D	8.58 ± 0.23^{aA}	11.02 ± 0.23^{cA}	10.09 ± 0.23^{bB}

Means bearing different superscripts in upper case differ significantly in columns (P < 0.05)
Means bearing different superscripts in lower case differ significantly in rows (P < 0.05)

The Mean±SE values of catalase activity (µmol H, O, decomposed/min/g) at 0, 24, and 48 hr for the different groups are presented in Table 3. All recorded values fell within the normal physiological range. Catalase levels at the 0-hour interval were significantly higher (P<0.05) in all groups compared to the values at 24 and 48 hr. However, no significant differences were observed among the groups at any of the recorded time intervals.

Table 3: Mean±SE values of catalase (µmol H_2O_2 decomposing/min/g) in all the groups at various time intervals

Groups	Observation Intervals (hr)		
	0	24	48
A	3.19 ± 0.08^{b}	2.21 ± 0.08^{a}	1.80 ± 0.08^{a}
B	3.42 ± 0.08^{b}	2.45 ± 0.08^{a}	2.04 ± 0.08^{a}
C	3.44 ± 0.08^{b}	2.47 ± 0.08^{a}	2.06 ± 0.08^{a}
D	3.71 ± 0.08^{b}	2.73 ± 0.08^{a}	2.32 ± 0.08^{a}

Means bearing different superscripts in lower case differ significantly in rows (P < 0.05)

Primarily located in peroxisomes (Nordberg and Arner, 2001), catalase (CAT) is a key component of the enzymatic antioxidant defence system and is considered one of the most responsive antioxidants against oxidative stress. It plays an essential role in detoxifying excess hydrogen peroxide by converting it into water and oxygen (Gogoi *et al*., 2018). A reduction in CAT activity is commonly associated with the healing process and the accompanying inflammatory cascade, which elevates oxidative stress. In the present study, CAT values did not differ significantly among the groups, a finding similar to the observations of Sakundech *et al*. (2020). However, closer examination of the data revealed that the CAT activity at 24 and 48 hr was significantly lower than at 0 hr across all groups, which aligns with the reports of Szczubial *et al*. (2015). Additionally, CAT values at 48 hr did not differ significantly from those recorded at 24 hr.

The Mean±SE values of superoxide dismutase (U/g) at 0, 24, and 48 hr in the different groups are

Table 4: Mean±SE values of superoxide dismutase (U/g) in all the groups at various time intervals

Groups	Observation Intervals (hr)		
	0	24	48
A	0.32±0.01[b]	0.29±0.01[b]	0.20±0.01[a]
B	0.34±0.01[b]	0.31±0.01[b]	0.22±0.01[a]
C	0.35±0.01[b]	0.32±0.01[b]	0.23±0.01[a]
D	0.36±0.01[b]	0.33±0.01[b]	0.23±0.01[a]

Means bearing different superscripts in upper case differ significantly in columns (P < 0.05)

presented in Table 4. All observed values fell within the normal physiological range. Superoxide dismutase activity at 0 hr was significantly higher than at 48 hr across all groups (P<0.05). However, the values at 0 hr did not differ significantly from those recorded at 24 hr. Furthermore, no significant differences were observed among the groups at any of the evaluated time intervals.

In the present study, SOD concentrations showed a gradual decline from 0 to 48 hr, likely reflecting increased ROS production following surgical stress. The rise in ROS also leads to elevated hydrogen peroxide levels, which may further contribute to enhanced lipid peroxidation.

The Mean±SE values of glutathione (nmol/mL) at 0, 24, and 48 hr for the different groups are presented in Table 5. All measured values remained within the

Table 5: Mean±SE values of glutathione (nmol/mL) in all the groups at various time intervals

Groups	Observation Intervals (hr)		
	0	24	48
A	1.24±0.04[c]	1.02±0.04[a]	1.16±0.04[b]
B	1.30±0.04[c]	1.09±0.04[a]	1.23±0.04[b]
C	1.33±0.04[c]	1.11±0.04[a]	1.26±0.04[b]
D	1.41±0.04[c]	1.20±0.04[a]	1.34±0.04[b]

Means bearing different superscripts in upper case differ significantly in columns (P < 0.05)

normal range. A significant decreasing trend in glutathione concentration was observed from 0 to 48 hr across all groups (P<0.05).

The decline observed in the present study from 0 to 48 hr suggests increased oxidative demand following surgical stress. No significant differences were noted among the groups at any time point. These findings align with those of Serin *et al*. (2008), who reported approximately a 20% reduction in glutathione concentration after abdominal surgery.

In conclusion, the findings of this study suggest that the intraperitoneal application of lignocaine HCl (2%) and ropivacaine HCl (0.5%) as a splash block is beneficial and can be effectively incorporated into a multimodal approach to pain management and stress reduction following ovariohysterectomy in dogs.

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