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Original article

The clinical efficacy of pentoxifylline and l-glutamine on ischemia and reperfusion injury in cattle with displaced abomasum: a longitudinal study

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Abstract

This study aimed to assess the clinical efficacy of pentoxifylline (PTX) and L-glutamine (L-Gln) treatment on ischemia and reperfusion (I/R) injury in the abomasal tissue, acute phase response (APR), oxidative stress (OS), cytokine response, hemostatic, and coagulation disorders in the 96-h period before and after surgery in displaced abomasum (DA) cases. The study sample consisted of 48 dairy cows with DA that were categorized into four groups as group S (Sham group) (9 Left displaced abomasum (LDA)+3 Right displaced abomasum (RDA), group P (PTX) (10 LDA+2 RDA), group G (L-Gln) (10 LDA+2 RDA), and group P+G (PTX+L-Gln) (10 LDA+2 RDA). Acute-phase protein (Haptoglobin), oxidative stress indicators (malondialdehyde, nitric oxide, and glutathione), cytokines (tumor necrosis factor (TNF)-α and interleukin-1β (IL-1β), coagulation factors (D-Dimer, Antithrombin (ATIII), Thrombin-antithrombin complex, Plasminogen activator inhibitor-1), and enzyme activities (lactate dehydrogenase, gamma--glutamyl transferase, sorbitol dehydrogenase, glutamate dehydrogenase, adenosine deaminase, myeloperoxidase, and creatine phosphokinase) in blood serum samples and coagulometric analyses of blood plasma were performed in samples taken before the operation and at 30 and 60 min and 2, 5, 10, 24, 48, 72, and 96 h after the operation. In DA cases, while post-operative treatment procedures with PTX and L-Gln were effective in decreasing APR and OS, these were ineffective in prohibiting the inflammatory response coordinated by cytokines. For the treatment and prevention of I/R injury in the DA cases, PTX and L-Gln procedures hold promise with their effects on APR, OS, and hemostatic dysfunction. Additional treatment procedures are required for the suppression of inflammatory response, and the effectiveness of preconditioning treatment may be evaluated.

Key words: abomasal displacement, acute phase response, oxidative stress, cytokine response, hemostatic dysfunction, dairy cow

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Introduction

Displaced abomasum (DA) is a multifactorial disease frequently seen in high-yielding dairy cows. A majority (80–90%) of the cases occur in the first 3-4 weeks post-partum (Geishauser 1995, Sexton et al. 2007, Doll 2015). In DA cases, pathological changes such as ischemia, inflammation, and necrosis due to obstruction and/or strangulation occur and continue after restoration (reperfusion) of blood circulation causing functional disorders in organs (Sies 1997, Cevrioglu et al. 2004, Grosche et al. 2012). Ischemia/Reperfusion (I/R) injury is an event coordinated by cytokines, adhesion molecules, and neutrophil activation. During I/R, inflammatory damage and cell death occur due to the release of types of reactive oxygen by destructive pro-inflammatory cytokines and inflammatory cells in tissues. Anti-oxidative, anti-inflammatory, and anti-apoptotic treatment approaches may be considered for the prevention of I/R injury (Zhang et al. 2008). Various administrations are beneficial, for example, in experimental studies in rats to prevent I/R injury, inhibitors such as tumor necrosis factor (TNF)-α converting enzyme, matrix metalloproteinases (Souza et al. 2007), pentoxifylline (PTX) (El-Ghoneimi et al. 2007), and glutamine, an amino acid that decreases cytokine release in endotoxemic rats and demonstrates an organ-protective effect (Guiqi 2011) in experimental myocardial I/R cases. PTX is a methylxanthine derivative and provides protection against I/R injury in organs such as the intestines (Hammerman et al. 1999), liver (El-Ghoneimi et al. 2007), and lungs (Tazuke et al. 2003). It has antioxidant properties and regulates microcirculation by affecting capillary blood flow and tissue oxygenation (Hammerman et al. 1999, Mallick et al. 2004). L-Glutamine (L-Gln) possesses immunomodulatory, anti-catabolic/anabolic, gastrointestinal mucosal protective, and antioxidant activities (Mallick et al. 2004, Abilés et al. 2008), and its effect in reducing I/R injury has been identified (Ikeda et al. 2002, Wasa et al. 2005). Treatment of DA cases is done surgically. Information on reposition of the abomasum and the effects of reperfusion is limited (Pyörala et al. 1993, Hirvonen and Pyörala 1998). The post-surgical recovery period and the re-acquisition of production traits are considered to be closely linked to inflammation and the extent and duration of tissue damage (Grosche et al. 2012). In this context, ischemia and inflammatory changes in DA cases must be evaluated and considered in the treatment period.

The aim of this study was to evaluate the acute phase response (APR), oxidative stress (OS), inflammatory response, and coagulation system in the 96-hour period before and after surgery in dairy cows with DA and to investigate the effects of glutamine and pentoxifylline treatment on I/R injury.

Materials and Methods

Animals

The study sample consisted of 48 dairy cows diagnosed with DA (39 Left Displaced Abomasum/LDA and 9 Right Displaced Abomasum/RDA without volvulus) in the 2-4-week period (14–30 DIM) after giving birth and with no other post-partum disease. This study was approved by the Selcuk University Veterinary Faculty Local Ethics Committee (26.08.2011-2011/15). All animals diagnosed with displaced abomasum were examined for post-partum diseases (mastitis, metritis, retentio secundinarium, pneumonia, hoof problems, ketosis, etc.). Animals suffering from a disease other than DA were not included in this study.

Diagnosis of abomasal displacement and operation method protocols were as described earlier (Maden et al. 2018).

Experimental Design

Group S (n:12, 9 LDA + 3 RDA) – The surgery/sham group: In this group, only DA operation and serum infusion were made.

Group P (n:12, 10 LDA + 2 RDA) – The pentoxifylline/PTX group: In this group, pentoxifylline (CAS: 6493-05-6, Sigma-Aldrich Co. St Louis, USA) 10 mg/kg, BID, IV (Corum et al. 2019, Uney et al. 2019) were infused after 30 min of DA operation. PTX treatment was continued for three days.

Group G (n:12, 10 LDA + 2 RDA) – The L-glutamine/L-Gln group: In this group, L-Glutamine (CAS: 56-85-9, Merck KGaA, Darmstadt, Germany) 300 mg/kg, SID, IV were infused after 30 min of DA operation. L-Gln treatment was continued for three days.

Group P+G (n:12, 10 LDA + 2 RDA) – PTX + L-Gln group: In this group, PTX (10 mg/kg, BID, IV) and L-Gln (300 mg/kg, SID, IV) were infused after 30 min of DA operation. PTX and L-Gln treatment was continued for three days.

Long-acting tetracycline (Primamycin LA, Zoetis Animal Health Co Ltd, Istanbul, Turkey; 20 mg/kg body weight), which is an inhibitor of myeloperoxidase inhibitor (MPO), was administered IM every other day in all treatment groups, with low side effects on forestomach microflora and faunas. In all groups, fluid therapy was given during and after the surgery.

Table 1. Some biochemistry and coagulation parameters as controls in healthy dairy cows (n:12) in the postpartum period (2-4 week period, 14-30 DIM).

Hp (µmol/L)	MDA (nmol/mL)	NO (µmol/L)	GSH (mmol/L)	TNF-α (ng/L)	IL-1β (pg/mL)	
196±21.0	53.6±3.27	4736±548	624±6.49	5346±692	380±28.7	
LDH (ukat/L)	GGT (ukat/L)	SDH (ng/mL)	GDH (ng/mL)	ADA (ng/mL)	MPO (ukat/L)	CK (mg/dL)
1674±140	16.2±1.77	71.6±8.77	33±3.99	10.6±1.87	132±17.0	69.5±14.3
D-Dimer (µg FEU/L)	TAT (ng/mL)	ATIII (mg/dL)	PAI-1 (ng/mL)	PT (s)	APTT (s)	FIB (g/L)
731±63.1	19.3±2.47	155±21.1	21.2±1.84	30.7±0.95	40.4±1.36	336±21.0

Hp (haptoglobin); MDA (malondialdehyde); NO (nitric oxide); GSH (glutathione); TNF-α (tumor necrosis factor-alpha); IL-1β (interleukin-1beta); LDH (lactate dehydrogenase); GGT (gamma-glutamyl transferase); SDH (sorbitol dehydrogenase); GDH (glutamate dehydrogenase); ADA (adenosine deaminase); MPO (myeloperoxidase); CK (creatine kinase); D-dimer; TAT (thrombin-antithrombin complex); ATIII (antithrombin III); PAI-1 (plasminogen activator inhibitor-1); PT (prothrombin time); APTT (activated partial thromboplastin time); FIB (fibrinogen).

Blood Sampling and Laboratory Analysis

Blood samples were collected from the jugular vein of the dairy cows diagnosed with DA before the operation, as well as 30 and 60 min, and 2, 5, 10, 24, 48, 72, and 96 h after the operation. Blood samples with an anticoagulant (Ethylenediamine Tetraacetic acid) were used for hematological analysis (complete blood count), and citrated blood samples were used for coagulometric analysis (prothrombin time/PT, activated partial thromboplastin time/APTT, and fibrinogen/FIB). Blood samples without any anticoagulant were used for the analysis of coagulation factors (D-Dimer, Thrombin-antithrombin complex/TAT, Plasminogen activator inhibitor-1/PAI-1), the indicators of oxidative stress status (malondialdehyde/MDA, nitric oxide/NO, glutathione/GSH), acute-phase protein (Haptoglobin/Hp), cytokines (TNF-α and IL-1), and enzyme activities (lactate dehydrogenase/LDH, gamma-glutamyl transferase/GGT, sorbitol dehydrogenase/SDH, glutamate dehydrogenase/GDH, adenosine deaminase/ADA, myeloperoxidase/MPO, creatin phosphokinase/CPK). Blood serum was analyzed by ELISA tests (ELX800 universal microplate reader, ELX50 universal washer, BIOTEK, USA) for coagulation factors, the indicators of oxidative stress status, acute phase proteins, and some enzyme activities (ADA, MPO, SDH, GDH) used bovine specific commercial test kits (Shanghai Sunred Biological Technology Co Ltd, Shanghai, China). Enzyme activities (LDH, GGT, CK) were measured by an autoanalyzer (Architect G 8000, Abbott, USA), and plasma PT and APTT times, and FIB concentrations were analyzed by a coagulometer (Sysmex CA 560, Siemens, Germany). In this study, control values in healthy dairy cows (postpartum 2-4-week period, 14-30 DIM, n:12) were determined for comparison (Table 1).

Statistical Analysis

Data were analyzed by statistical software (SPSS 22.0 for Windows® package, Chicago, IL), and the level of significance was set at $p<0.05$. Statistical procedures were first performed with the "Kolmogorov Smirnov" test to determine the homogeneity of the obtained data. For variance analysis, the "Wilcoxon Signed Rank Test" method was used for recurrent comparisons in non-homogeneous groups. For the parametric data, one-way ANOVA with Tukey's test was used for multiple group comparisons. The significance level of the study was $p<0.05$

Results

To assess the data, differences between the treatment groups (P, G, and G+P) and group S were examined. All data are presented in Tables 2-5. In this study, we found that Hp concentration at 48 h was significantly low ($p < 0.05$) in all treatment groups, serum MDA concentration at 48 h was significantly low ($p<0.05$) in group G, and that while serum GSH concentration after the operation was significantly high ($p<0.05$) in group G+P, it was significantly low ($p<0.05$) at 48 h in all treatment groups. On the other hand, except for serum IL-1β concentration being significantly low post-operation and at 5 h in group G+P, it was found that serum TNF-α concentrations at 72 h were significantly high ($p<0.05$) in groups P and G (Table 2). The following concentrations were found consistently at a significantly high ($p<0.05$) level: SDH concentration in group P, GDH concentration in groups P and G, ADA concentration in groups G and G+P, and CK concentration in groups P and G (Table 3).

In this study, we observed an insignificant ($p>0.05$)

Table 2. Acute phase response. oxidative stress and cytokine values before and after operation in sham and treatment groups.

	Groups	Sampling time (Mean±SE)										
		Before operation	After operation	30th minutes	60th minutes	2nd hours	5th hours	10th hours	24th hours	48th hours	72nd hours	96th hours
Hp µmol/L	S	228±29.1	276±53.1	286±28.1	278±41.4	552±76.0	455±59.3	375±77.5	405±63.7	865±104^{A}	435±53.0	378±50.6
	P	299±61.8	330±47.4	326±40.8	414±56.5	626±54.8	494±126	419±77.4	447±108	427±99.9^{B}	483±106	492±90.1
	G	290±42.5	313±45.5	290±30.8	463±51.2	653±80.8	358±72.0	388±58.2	466±86.6	368±53.7^{B}	385±40.6	539±53.8
	G+P	325±47.3	324±34.2	282±32.7	470±70.6	488±58.5	429±44.4	402±57.2	397±52.3	334±39.7^{B}	397±51.1	469±68.1
MDA nmol/mL	S	53.8±4.68^{AB}	44.2±1.71^{B}	56.2±8.47	59.2±6.35	92.1±12.1	76.2±15.5	70.8±13.1	64.0±11.5	120±8.83^{A}	66.2±11.0	71.9±14.1
	P	38.1±5.87^{B}	44.9±5.05^{B}	57.4±6.79	84.7±12.9	99.3±9.05	94.2±22.3	86.0±13.9	60.7±12.6	83.7±15.4^{AB}	104±13.7	78.5±9.72
	G	46.8±3.55^{AB}	60.8±5.69^{AB}	64.1±8.29	91.2±14.9	86.2±13.3	80.0±16.9	73.0±14.1	76.6±14.7	69.0±16.5^{B}	75.2±13.3	54.7±8.76
	G+P	59.6±3.26^{A}	70.5±4.57^{A}	55.2±6.75	85.5±11.4	112±14.1	77.9±16.0	98.2±13.0	91.3±117.3	85.7±10.9^{AB}	62.1±6.00	65.4±5.26
NO µmol/L	S	995±222	1316±286	884±81.3	744±41. ^{B}	1463±41.1	738±192	771±157	500±48.0	1057±78.8	549±55.7	1030±97.4^{AB}
	P	1259±310	998±128	795±84.3	2268±666^{A}	2105±250	660±203	1430±410	473±82.2	1255±266	1056±189	1276±159^{A}
	G	1013±332	1578±253	764±71.1	1718±247^{AB}	1918±345	957±336	1226±374	490±48.1	819±183	738±222	1284±129^{A}
	G+P	846±171	1432±180	715±48.2	1204±141^{AB}	1718±470	716±332	733±212	466±51.3	778±213	784±92.5	656±56.4^{B}
GSH mmol/L	S	766±105	657±20.6^{B}	877±89.4	748±98.7	831±46.6^{AB}	609±15.8	627±5.01	563±38.1	814±42.3^{A}	625±17.7	678±43.0
	P	797±87.5	753±43.7^{B}	737±106	864±68.9	901±47.5^{A}	638±6.81	628±7.59	619±4.31	661±18.3^{B}	677±29.7	650±25.8
	G	698±44.9	979±93.8^{AB}	710±75.3	814±47.6	905±37.4^{A}	574±28.1	599±11.9	612±9.69	638±4.66^{B}	627±4.51	647±6.05
	G+P	738±44.9	1280±134^{A}	764±47.3	757±30.4	743±20.1^{B}	605±12.3	655±34.1	608±23.4	625±5.28^{B}	618±26.4	614±4.94
TNF-α ng/L	S	9799±1127^{A}	6104±1680	816±159	868±148^{B}	1113±290	2870±868	603±131	2431±582	1195±179^{AB}	876±157^{B}	1042±194^{AB}
	P	3728±673^{B}	6282±1090	933±214	1710±301^{AB}	1464±194	4821±1181	694±141	1791±830	1891±382^{A}	1701±187 ^{A}	1467±231^{A}
	G	5985±1572^{AB}	4125±817	1012±138	2262±253 ^{A}	1785±280	3785±1112	479±150	1203±315	818±234 ^{B}	1315±203^{AB}	1019±174^{AB}
	G+P	5149±1841^{AB}	2587±746	823±138	1766±234^{AB}	1716±208	3628±792	491±109	2366±489	810±272^{B}	870±166^{B}	664±86.5^{B}
IL-1β pg/mL	S	494±73.1	583±63.1^{A}	289±69.4	219±66.4	229±32.7	116±25.5^{A}	126±26.7	41.2±12.5	268±31.6^{AB}	150±26.5	351±226
	P	370±33.5	436±41.7^{AB}	209±35.2	190±17.5	222±30.9	113±22.4^{A}	180±51.9	47.4±18.1	380±58.6^{A}	222±56.3	105±23.1
	G	512±103	437±46.3^{AB}	312±84.3	238±24.8	236±33.6	85.0±14.4^{AB}	162±44.8	26.2±2.94	257±39.3^{AB}	156±43.7	113±18.4
	G+P	296±51.4	285±43.0^{B}	171±27.4	169±19.1	206±29.6	31.7±5.58^{B}	159±30.9	26.7±3.65	208±33.7^{B}	215±98.1	109±25.4

$^{A, B, C}$; Different letters in the same line are statistically significant (Tukey test, p<0.05); S (Sham group); P (Pentoxifylline-PTX group); G (L-Glutamine group); P + G (Pentoxifylline/PTX + L-Glutamine group); Hp (haptoglobin); MDA (malondialdehyde); NO (nitric oxide); GSH (glutathione); TNF-α (tumor necrosis factor-alpha); IL-1β (interleukin-1 beta).

decrease in plasma PT (sec.) duration at 96 h in group P and at 2 h in group G, compared to the positive control group (group S) in DA cases; a significant decrease ($p<0.05$) was observed in all other groups, including that in group G+P following surgery and at 2 and 10 h post-surgery. Except an insignificant decrease ($p>0.05$) before surgery in group P, a significant decrease ($p<0.05$) at all measurement times, a significant decrease ($p<0.05$) post-surgery and at 2 h in group G+P, and an insignificant decrease ($p>0.05$) at all other measurement times were recorded for APTT duration (Table 4). Fibrinogen level was significantly higher ($p<0.05$) in group P (except at 2 and 5 h) and group G (except at 2 h) compared to that in the positive control group, while an insignificant difference ($p>0.05$) was identified in group G+P. A significant increase ($p<0.05$) in PAI-1 level was observed only in group G+P at 10 h, and a significant increase ($p<0.05$) in plasma D-dimer concentration was determined only in group G+P at 48 h. In all groups, PAI-1 and D-dimer concentrations were observed to be higher at all times than that of the healthy control group.

Discussion

In this longitudinal study, APR, OS, inflammatory response, hemostatic dysfunction, and coagulation activation in the 96-h period, including before and after the surgery in DA cases, were evaluated, and the clinical efficacy of PTX and L-Gln treatment on I/R damage developing in the abomasal tissue were evaluated.

In this study, we observed enhanced APR, OS, inflammatory response, hemostatic dysfunction, and coagulation system activation in DA cases and confirmed abomasal tissue damage due to these effects. PTX and L-Gln treatment were found to be effective in decreasing APR and OS but ineffective in suppressing the cytokine-mediated inflammatory response. Therefore, PTX and L-Gln treatment can be used following DA surgery along with an additional drug to suppress the inflammatory response.

In this study, PTX and L-Gln treatment were used for the first time in cattle with DA cases. Intravenous infusion of the drug alone (PTX, 10 mg/kg, BID) and in combination with L-Gln (10 mg/kg, BID and 300 mg/kg, SID) was well tolerated. No side effects

Table 3. Blood serum biochemical values before and after operation in sham and treatment groups.

	Groups	Sampling time (Mean±SE)										
		Before operation	After operation	30th minutes	60th minutes	2nd hours	5th hours	10th hours	24th hours	48th hours	72nd hours	96th hours
LDH ukat/L	S	1911±177	1882±181	2142±234	2395±142	2329±212	2180±181	2122±149	1976±157	2169±160	2081±137	2030±106
	P	1765±183	1729±206	2050±121	2237±158	2136±161	2259±174	2152±132	2204±187	2025±220	2315±86.4	2174±203
	G	2206±196	1833±315	2175±254	1723±244	1998±218	2050±234	2012±220	2111±209	1970±262	2251±165	2198±184
	G+P	2020±168	2344±178	2460±112	2047±184	2186±96.5	1801±318	1916±255	1911±306	2093±195	2175±135	2241±119
GGT ukat/L	S	32.3±4.32	40.5±9.88	45.7±8.52	48.5±9.27	51.4±10.8	68.3±12.9	53.8±8.71	40.1±8.74^{B}	43.1±8.14	56.9±11.2	60.0±16.0
	P	34.5±7.78	40±8.19	55.6±13.6	62.9±12.4	52.0±10.0	81.6±14.5	55±9.90	49.9±9.02^{AB}	55.8±8.81	50.8±6.69	50.1±6.32
	G	49.2±8.42	63.5±14.3	61.4±12.0	66.8±15.6	62.0±10.6	69.8±11.5	70.5±11.9	62±10.5^{AB}	77.2±12.1	60.8±9.91	64.4±9.52
	G+P	33.4±6.83	66±13.4	69.8±15.7	59±13.6	53.4±7.94	75.4±11.8	68±11.2	79.5±11.2^{A}	65.8±15.3	73.7±17.6	73.9±14.4
SDH ng/mL	S	43.2±5.42	38.0±6.24^{B}	33.4±3.79	32.8±8.12	70.0±32.2	18.6±1.78^{B}	35.0±8.96 ^{B}	13.9±2.29	28.3±3.40^{AB}	21.9±3.05^{B}	19.0±3.95^{AB}
	P	42.8±5.61	66.6±9.88^{A}	45.0±3.37	47.2±5.04	42.5±4.97	34.2±3.73^{A}	67.1±8.58^{A}	16.3±1.81	38.9±6.24^{A}	38.7±4.49^{A}	35.3±4.54^{A}
	G	45.7±5.66	59.3±7.38^{AB}	38.2±6.41	40.6±5.06	41.8±4.78	22.2±3.57^{B}	36.7±3.06^{B}	22.0±3.01	23.9±4.62^{AB}	26.1±3.06^{AB}	25.0±6.29^{AB}
	G+P	84.2±23.2	35.1±4.41^{B}	36.6±6.52	32.2±5.70	27.5±3.12	15.1±2.88^{B}	24.7±5.55^{B}	13.2±2.78	20.6±4.01^{B}	28.0±4.75^{AB}	15.6±2.37^{B}
GDH ng/mL	S	19.4±1.99^{B}	24.1±4.12^{B}	33.3±10.4	24.5±2.95^{AB}	14.9±1.69	41.6±5.65	36.6±3.27	27.4±3.91	13.7±0.85^{B}	35.2±4.09	28.4±4.33
	P	30.7±2.90^{AB}	31.0±5.58^{B}	36.9±11.9	27.7±3.74^{A}	12.8±2.58	36.8±5.20	31.49±4.04	31.8±5.31	33.0±3.50^{A}	38.5±6.36	37.4±6.00
	G	30.6±4.56^{AB}	58.8±11.5^{A}	41.6±10.8	16.8±1.94^{B}	15.8±1.87	33.9±4.69	36.2±2.91	25.3±3.65	28.2±4.07^{A}	34.7±6.49	31.1±4.80
	G+P	32.3±3.17^{A}	22.7±4.78^{B}	34.1±9.49	15.2±1.00^{B}	13.2±1.38	32.2±5.01	38.8±2.36	28.5±3.03	32.9±3.93^{A}	48.7±5.92	36.9±2.86
ADA ng/mL	S	11.8±2.85	9.95±1.91^{AB}	3.49±0.56	1.95±0.36^{B}	12.7±2.36	4.22±0.70	4.96±1.53	2.97±0.29^{AB}	15.3±6.44	4.63±0.60	5.19±0.66
	P	9.19±3.22	14.3±3.40^{A}	3.37±0.70	12.0±4.17^{AB}	14.6±6.14	4.35±1.04	5.55±0.60	2.81±0.40^{B}	12.3±2.97	9.8±3.88	11.6±3.03
	G	8.43±2.73	9.79±2.29^{AB}	4.25±0.91	17.7±2.96^{A}	8.78±1.16	6.47±1.63	5.4±2.54	3.28±0.40^{AB}	8.35±2.76	5.87±0.73	7.49±1.20
	G+P	9.82±2.23	3.75±0.55^{B}	2.88±0.34	13.4±3.00^{A}	10.1±2.17	8.05±2.05	8.76±2.37	4.40±0.53^{A}	10.4±2.54	4.42±0.41	6.43±1.27
MPO ukat/L	S	231±31.5	254±37.2	287±50.9	156±23.2	146.6±37.2	288±173	174±21.8	70.4±11.0	143±32.9	367±216	171±36.0
	P	222±31.2	170±36.0	194±38.1	176±36.7	140±31.8	66.0±10.8	192±36.6	88.3±14.2	223±40.0	146±56.5	622±235
	G	207±41.5	259±45.2	189±36.3	111±23.5	167±36.0	40.9±11.8	204±37.4	91.6±14.9	200±35.1	283±119	195±83.3
	G+P	248±46.1	208±42.7	208±36.3	93.4±23.5	142±30.0	61.4±6.75	247±37.0	62.7±12.0	209±46.9	197±47.3	215±74.0
CK mg/dL	S	94.8±37.3	91.0±22.5	147±41.5	168±39.8	189±44.0^{B}	267±76.8^{B}	319±50.1^{B}	525±101	295±70.8	221±41.4	107±15.3^{B}
	P	208±51.0	407±162	492±133	462±113	528±132^{A}	711±125^{A}	877±207^{A}	1224±448	688±176	492±74.2	244±49.0^{AB}
	G	174±48.1	388±78.8	289±50.3	423±117	358±67.7^{AB}	513±66.6^{AB}	819±134^{AB}	762±179	695±109	521±59.2	318±50.9^{A}
	G+P	228±83.1	352±107	376±122	316±82	366±85.2^{AB}	610±136^{AB}	635±124^{AB}	803±151	697±171	428±126	230±57.5^{AB}

$^{A, B, C}$; Different letters in the same line are statistically significant (Tukey test, $p<0.05$); S (Sham group); P (Pentoxifylline-PTX group); G (L-Glutamine group); P + G (Pentoxifylline/PTX + L-Glutamine group); LDH (lactate dehydrogenase); GGT (gamma-glutamyl transferase); SDH (sorbitol dehydrogenase); GDH (glutamate dehydrogenase); ADA (adenosine deaminase); MPO (myeloperoxidase); CK (creatine kinase).

other than increased salivation and agitation were observed during PTX infusion.

The limitations of this study were the varying levels of abomasal inflammation and severity of damage in DA cases, and the precise time of displacement development was not known, and the treatment was initiated after the operation. In this context, the preconditioning treatment, shown earlier to be effective in experimental studies (Ikeda et al. 2002, Wasa et al. 2005, El-Ghoneimi et al. 2007, Guiqi 2011), may be investigated.

In DA cases, as a result of the obstruction and increase in luminal pressure after displacement, blood circulation in the abomasal mucosa is affected; besides, ischemic mucosal damage occurs, and APR, OS, hemostatic function disorders, and DIC (Disseminated Intravascular Coagulation) occur in response to this injury (Constable et al. 1992a, b, Irmak and Turgut 2005, Doll et al. 2009, Maden et al. 2012). There is limited information on abomasal repositioning and reperfusion following DA surgery, and surgery is considered to be the most radical treatment (Pyörala et al. 1993, Hirvonen and Pyörala 1998).

I/R injury is a condition coordinated by cytokines, adhesion molecules, and neutrophil activation. In DA cases, the ischemic effects and tissue necrosis in the abomasal tissue are assessed by evaluating acute-phase proteins (Hp, SAA), oxidative stress indicators (MDA, NO), and various enzymes (LDH, ALP, CK) (Reeves et al. 1990, Souza et al. 2007, Grosche et al. 2012). MDA and NO have been described as oxidative stress indicators in clinical and experimental studies (Sies 1997, Cevrioglu et al. 2004, Jurczuk et al. 2007, Bian et al. 2008). I/R stimulates free oxygen radical production and the release of products such as NO and MDA. In this case, MDA may be used as a sensor for tissue damage and reperfusion. NO is released in cases of brain ischemia as a result of the early movement of leucocytes to the brain parenchyma. Under these circumstances, NO may be a sensor indicating the

Table 4. Coagulation factors and clotting times before and after operation in sham and treatment groups.

	Groups	Sampling time, Median (minimum-maximum values)										
		Before operation	After operation	30th minutes	60th minutes	2nd hours	5th hours	10th hours	24th hours	48th hours	72nd hours	96th hours
D-Dimer µg FEU/L	S	821 (388-1814)	792.5 (359-223)	636.5 (431-2900)	678 (367-2295)	617.5 (277-3371)	652 (437-2028)	950.5 (447-2195)	710 (412-1547)	1001.5^{B} (548-1834)	1429 (512-2962)	1856.5 (648-2819)
	P	1523.5 (436-2262)	1450 (418-2872)	1316.5 (378-2306)	523 (234-2269)	644.5 (315-3242)	726.5 (293-2195)	1044 (405-2195)	993.5 (580-2020)	700^{B} (415-2020)	1522 (554-2672)	2269 (714-2672)
	G	828 (411-2274)	1154 (383-2872)	849 (365-2381)	477 (240-2810)	543 (320-2986)	945 (422-2195)	850.5 (336-1943)	1110 (477-2020)	1092^{AB} (404-2519)	1763.5 (518-2521)	1115 (327-2872)
	G+P	971 (467-2177)	1458.5 (537-2761)	1077 (584-2095)	699 (506-1820)	1120.5 (465-2509)	1357 (456-2195)	953 (430-1784)	1083 (512-1467)	1719.5^{A} (914-2641)	2186 (792-2672)	1712 (538-2833)
TAT ng/mL	S	31.1 (10.7-105.2)	20.2 (12.3-56.3)	23.8 (8.50-95.5)	25.65 (12.2-64.7)	26.1 (15.0-73.2)	15.45 (10.4-50.2)	15.9 (11.7-47.8)	16.2 (13.6-45.7)	20.45 (11.3-39.1)	18.7 (6.20-37.6)	22.55 (10.9-43.1)
	P	50.0 (11.5-118.5)	30.35 (8.90-49.2)	29.0 (9.40-90.6)	19.25 (6.20-75.6)	38.75 (9.30-69.3)	27.3 (10.3-80.9)	18.15 (6.20-80.1)	25.65 (11.4-53.2)	20.3 (10.1-56.3)	24.20 (8.20-60.8)	32.0 (9.90-62.6)
	G	22.0 (10.3-144.4)	22.45 (9.30-55.1)	12.85 (5.20-74.3)	22.4 (7.70-79.30)	20.35 (10.3-73.3)	22.8 (7.80-69.1)	18.25 (7.90-60.7)	21.35 (11.8-50.5)	17.55 (5.90-46.8)	18.50 (9.20-46.1)	27.35 (10.6-68.3)
	G+P	48.5 (13.6-110.8)	28.15 (11.6-58.4)	23.85 (8.50-96.7)	30.4 (15.7-56.2)	29.15 (13.6-61.4)	24.9 (13.8-45.1)	28.8 (16.2-45.3)	25.25 (13.9-32.7)	19.15 (4.50-41.1)	26.4 (12.0-47.2)	33.3 (5.30-46.4)
ATIII mg/dL	S	387.4 (150.3-1067.4)	254.95 (75.5-617.9)	427.2 (166.5-1020.1)	264.75 (194.7-719.6)	298.75^{B} (225.3-581.2)	256.95^{B} (188.8-1634.6)	276.4^{AB} (210-6158.5)	192.25 (86.9-515.6)	229.65 (95.1-506.3)	547.75 (132.1-719.6)	489.55 (135.3-610.3)
	P	447.15 (129.3-1339.6)	360.1 (92.6-833.3)	313.3 (128.8-884.8)	267.9 (132.8-761.5)	376.9^{B} (128.9-940.2)	728.85^{AB} (217.1-2488.9)	1410.05^{A} (182.3-4697.2)	220.75 (114.5-721.6)	304.4 (109.2-678.6)	525.9 (121.9-689.6)	534.75 (93.1-713.8)
	G	334.3 (132.1-1233.8)	446.55 (66.9-809.1)	276.65 (121.7-918.7)	379 (133.9-867.9)	275.15^{B} (157.5-710.2)	499.05^{B} (152.8-2906.1)	200.5^{AB} (146.4-3047.3)	286.2 (53.9-737.8)	461.05 (84.6-721.6)	517.15 (138.1-561.7)	323.85 (68.7-924.2)
	G+P	510.45 (176.8-1173.4)	422.3 (205.5-629.2)	636.9 (204.4-1154.9)	443.75 (239.9-593.1)	713.7^{A} (223.3-5943.9)	2069.95^{A} (223.5-3964.3)	326.05^{B} (178.1-621.2)	285.2 (164.3-433.3)	438.6 (135.5-712.8)	549.5 (120-722.8)	344.8 (130.1-625.3)
PAI-1 ng/mL	S	57.1 (10.7-159.5)	29.9 (5.10-81.6)	31.4 (18.9-118.7)	44.75 (22.1-87.1)	44.95 (18.2-105.6)	29.2 (17.4-79.4)	29.4^{B} (15.9-36.3)	39.15 (12.7-77.2)	44.15 (12.2-87.7)	29.65 (13.8-90.3)	32.05 (16.6-87.2)
	P	24.5 (14.3-107.9)	27.2 (10.3-69.1)	40.45 (21.6-137.1)	39.0 (15.4-134.6)	49.5 (15.5-173.1)	51.4 (17.6-97.4)	28.45^{B} (17.4-57.8)	43.55 (9.70-96.6)	52.85 (14.0-96.6)	31.15 (4.10-163.1)	34.6 (12.7-155.1)
	G	34.95 (18.2-149.6)	23.75 (10.6-40.5)	24.7 (13.9-149.7)	36.05 (16.1-115.1)	34.95 (4.70-121.8)	24.65 (18.0-77.9)	25.65^{B} (18.6-54.9)	52.25 (13.1-96.6)	42.35 (14.1-124)	33.05 (12.6-105.9)	28.5 (10.5-56.6)
	G+P	36.0 (23.2-99.2)	26.5 (9.80-53.7)	45.45 (26.3-138.8)	38.85 (29.8-79.7)	33.4 (20.9-108.3)	30.35 (18.2-71.7)	49.1^{A} (24.8-77.0)	56.0 (23.4-75.1)	41.45 (18.1-79.2)	26.8 (17.9-109.8)	25.2 (9.70-59.6)
PT s	S	85.2^{A} (28.9-116.5)	87.05^{A} (62.1-96.5)	71.8^{A} (29.3-87.2)	79.85^{A} (24.4-95.5)	39.9^{A} (22.3-82.4)	78.3^{A} (32.1-92.0)	88.9^{A} (31.0-112.2)	66.8^{A} (28.7-93.5)	69.35^{A} (33.6-77.8)	64.45^{A} (35.8-86.5)	71.35^{A} (38.3-89.3)
	P	35.65^{B} (27.0-44.7)	24.85^{C} (19.7-39.5)	32.5^{B} (25.0-34.7)	32.8^{B} (24.2-43.2)	27.35^{B} (20.7-36.0)	41.75^{B} (30.8-69.8)	41.1^{C} (30.3-67.5)	39.0^{B} (27.9-61.6)	36.95^{B} (19.7-42.9)	44.0^{B} (31.2-78.8)	51.45^{AB} (31.2-77.6)
	G	37.45^{B} (27.9-60.1)	30.9^{C} (19.5-38.4)	32.8^{B} (27.3-80.7)	35.1^{B} (25.1-42.3)	30.15^{AB} (25.7-37.4)	40.15^{B} (31.6-52.0)	38.3^{C} (29.6-49.2)	31.8^{B} (24.0-36.8)	30.65^{B} (26.9-38.0)	32.3^{B} (24.7-47.4)	38.0^{B} (32.1-86.7)
	G+P	76.8^{A} (31.3-96.2)	39.65^{B} (23.7-81.9)	66.05^{A} (26.6-92.1)	64.5^{A} (26.2-92.6)	21.4^{B} (17.2-77.4)	70.4^{A} (34.1-90.6)	74.6^{B} (32.8-85.7)	62.0^{A} (25.9-84.6)	72.15^{A} (32.4-89.6)	70.3^{A} (29.2-84.7)	67.5^{AB} (25.1-92.8)
APTT s	S	61.9^{A} (42.9-84.6)	71.85^{A} (53.6-84.7)	41.35^{A} (30.2-48.1)	52.1^{A} (36.1-78.1)	46.1^{A} (33.5-63.3)	45.1^{A} (27.0-84.3)	58.3^{A} (46.0-84.2)	60.9^{A} (23.4-86.7)	45.05^{A} (34.0-103.8)	54.75^{A} (34.1-78.1)	50.8 (41.2-77.7)
	P	47.8^{AB} (37.9-95.9)	40.95^{BC} (25.7-82.7)	32.25^{B} (25.8-37.4)	31.25^{B} (25.5-37.7)	33.8^{B} (24.1-56.3)	34.5^{B} (26.3-41.3)	47.8^{BC} (37.7-65.8)	35.95^{C} (24.6-56.2)	35.5^{BC} (24.6-44.4)	32.3^{C} (24.2-50.5)	45.45 (27.0-54.2)
	G	42.55^{B} (26.9-60.0)	35.45^{C} (25.1-51.3)	30.35^{B} (20.5-40.2)	30.35^{B} (20.5-47.6)	34.85^{B} (28.4-38.6)	31.65^{B} (23.3-43.5)	39.25^{C} (31.4-58.9)	52.85^{BC} (27.1-62.6)	30.1^{C} (24.1-53.7)	33.6^{BC} (23.1-53.4)	39.15 (27.6-77.8)
	G+P	55.8^{A} (36.7-86.7)	48.35^{B} (30.4-77.2)	41.25^{A} (24.7-57.9)	48.0^{A} (29.5-69.5)	29.65^{B} (25.2-62.6)	45.05^{A} (32.7-69.1)	59.8^{AB} (43.1-87.8)	55.5^{AB} (43.1-75.5)	44.15^{AB} (31.0-82.6)	48.7^{AB} (27.9-62.4)	46.9 (25.8-58.4)
FIB g/L	S	180.05^{C} (100-521.7)	156.4^{B} (122-216.4)	158.4^{B} (105.1-449.7)	165.55^{C} (76.9-411.6)	295.45 (102.3-766)	165.55^{B} (103.8-511.4)	201^{B} (92.3-467.7)	181^{B} (123.4-488.3)	263.3^{B} (76.9-478)	169.45^{B} (94.9-454.9)	157.6^{C} (110.3-269.7)
	P	525.55^{A} (262-699.1)	568.55^{A} (290.3-807.1)	510.1^{A} (323.7-930.3)	418.85^{AB} (195.1-745.4)	425.5 (310.9-807.1)	313.45^{AB} (174.6-580.9)	462.55^{A} (280-881.7)	656.7^{A} (310.9-825.1)	492.3^{A} (244-668.3)	560.3^{A} (318.6-907.4)	537.15^{A} (321.1-724.9)
	G	449.85^{AB} (210.6-791.7)	493.95^{A} (231.1-732.6)	489.25^{A} (305.7-827.7)	535.9^{A} (303.1-897.1)	352 (128.3-822.6)	397^{A} (290.3-781.4)	466.4^{A} (282.6-840.6)	457.45^{A} (274.9-732.6)	485.7^{A} (303.1-686.3)	461.3^{A} (282.6-604)	420.65^{AB} (174.6-686.3)
	G+P	279.75^{BC} (164.3-735.1)	233.9^{B} (138.4-624.6)	277.45^{B} (115.4-735.1)	190.05^{BC} (130.9-735.1)	448.35 (128.6-686.3)	316.7^{AB} (118-686.3)	401.8^{AB} (102.6-470.3)	304.25^{B} (195.1-493.4)	260.7^{B} (110.2-555.1)	182.3^{B} (89.7-532)	236.3^{BC} (74.3-717.1)

$^{A, B, C}$; Different letters in the same line are statistically significant (Tukey test, $p<0.05$); S (Sham group); P (Pentoxifylline-PTX group); G (L-Glutamine group); P + G (Pentoxifylline/PTX + L-Glutamine group); D-dimer; TAT (thrombin-antithrombin complex); ATIII (antithrombin III); PAI-1 (plasminogen activator inhibitor-1); PT (prothrombin time); APTT (activated partial thromboplastin time); FIB (fibrinogen).

Table 5. Hematological findings before and after operation in sham and treatment groups.

Parameters	Groups	Sampling time (Mean±SE)					
		Before operation	After operation	24th hours	48th hours	72nd hours	96th hours
WBC (10^9 cells/L)	S	8.59±0.76^{B}	8.32±0.78^{B}	8.95±1.08^{B}	8.07±0.87	8.97±1.11^{B}	8.60±1.05
	P	19.8±3.59^{A}	21.2±4.30^{A}	22.8±3.50^{A}	17.2±2.73	19.3±3.55^{A}	14.2±3.21
	G	9.02±1.51^{B}	10.9±2.28^{AB}	10.0±1.95^{B}	8.67±1.08	9.98±1.93^{B}	11.6±2.90
	G+P	8.45±2.55^{B}	10.3±3.97^{AB}	8.86±2.68^{B}	15.5±6.22	10.7±1.75^{AB}	7.15±0.99
LYM (10^9 cells/L)	S	4.62±0.75^{B}	3.62±0.36	4.6±0.79^{B}	3.82±0.45	4.48±0.83^{B}	4.06±0.66
	P	12.2±3.55^{A}	13.6±4.22	15.5±3.90^{A}	10.2±2.73	13.6±3.64^{A}	8.45±3.13
	G	4.82±1.02^{B}	6.95±2.02	5.93±1.58^{B}	3.75±0.64	5.42±1.54^{AB}	7.16±2.89
	G+P	3.27±0.76^{B}	5.08±2.59	3.12±0.80^{B}	10.0±4.86	6.37±1.91^{AB}	3.27±0.72
MON (10^9 cells/L)	S	0.21±0.02^{AB}	0.20±0.02	0.22±0.02	0.19±0.02	0.28±0.07	0.27±0.07
	P	0.29±0.03^{A}	2.23±1.97	1.74±1.47	1.32±1.06	0.95±0.65	0.23±0.02
	G	0.20±0.03^{AB}	0.22±0.03	0.21±0.03	0.23±0.02	0.24±0.03	0.22±0.02
	G+P	0.16±0.02^{B}	0.18±0.04	0.15±0.02	0.25±0.05	0.20±0.02	0.17±0.02
GRAN (10^9 cells/L)	S	3.75±0.71	4.47±0.67	4.13±0.69	93.3±88.9	4.5±0.74	4.26±0.59
	P	7.30±0.88	7.52±0.96	6.99±0.89	6.73±0.46	12.3±5.26	5.53±0.53
	G	3.99±0.81	3.81±0.62	3.85±0.74	4.69±0.79	4.31±0.63	4.26±0.60
	G+P	5.02±1.87	5.08±1.49	5.58±1.87	5.27±1.40	4.44±0.64	3.70±0.58
RBC (10^{12} cells/L)	S	7.38±0.48	7.3±0.36	6.66±0.27	6.62±0.26	6.63±0.31	6.36±0.24
	P	7.28±0.34	7.15±0.32	6.84±0.36	6.94±0.39	7.03±0.39	6.64±0.33
	G	7.16±0.21	7.29±0.16	6.56±0.20	6.53±0.24	6.45±0.23	6.35±0.23
	G+P	6.72±0.24	7.01±0.24	7.16±0.31	6.86±0.19	6.71±0.16	6.35±0.14
PCV (L/L)	S	32.7±2.39	32.4±1.36	29.0±0.85	28.6±0.93	28.8±1.17	27.6±0.88
	P	31.4±1.54	30.4±1.49	29.4±1.34	29.7±1.61	29.8±1.54	28.2±1.22
	G	31.8±1.32	32.3±1.19	28.5±0.88	28.5±0.99	28.4±1.05	27.9±0.82
	G+P	30.6±1.48	32.0±1.44	31.3±1.08	30.9±1.22	30.5±1.44	28.8±1.24
Hb (mmol/L)	S	10.7±0.39	11.2±0.55 ^{AB}	10.1±0.29	10.1±0.25	10.1±0.26	9.71±0.23
	P	10.3±0.68	11.0±0.49 ^{AB}	10.6±0.52	10.5±0.44	10.4±0.62	10.1±0.46
	G	11.0±0.37	11.3±0.27 ^{A}	10.3±0.25	10.1±0.30	9.98±0.29	10.1±0.26
	G+P	10.7±0.42	11.0±0.43 ^{AB}	11.0±0.44	10.8±0.39	10.7±0.40	10.1±0.40
MCV (um^3)	S	44.3±1.06	44.8±0.94	43.8±0.83	43.5±1.00	43.8±1.07	43.8±1.09
	P	43.2±0.86	42.6±1.03	43.3±0.78	43.1±0.79	43.7±1.22	42.8±0.84
	G	44.5±1.24	44.3±1.26	43.7±1.24	43.9±1.16	44.2±1.22	44.2±1.18
	G+P	45.6±1.39	45.8±1.36	45.0±1.32	45.0±1.55	43.5±2.45	45.2±1.34
MCHC (mmol/L)	S	33.5±1.37	34.5±1.04	35±0.87	35.6±1.11	35.3±0.99	35.2±0.88
	P	32.7±1.06	36.3±0.61	36.0±0.84	35.7±0.83	34.9±0.79	36.0±0.87
	G	34.7±0.78	35.4±0.70	36.3±0.72	35.3±0.83	35.3±0.58	34.7±0.02
	G+P	35.3±0.83	34.8±0.87	35.1±0.71	35.4±0.70	35.3±0.68	35.4±0.56
RDW	S	12.4±0.22	12.7±0.21	12.8±0.21	12.4±0.19	12.6±0.17	12.6±12.6
	P	12.3±0.31	12.7±0.33	12.5±0.34	12.8±0.31	13.0±0.39	12.8±12.8
	G	12.6±0.26	12.5±0.30	12.7±0.25	12.5±0.28	12.7±0.32	12.7±12.7
	G+P	12.4±0.18	12.5±0.25	12.5±0.18	12.5±0.17	12.4±0.21	12.4±12.4
PLT (10^9 cells/L)	S	572±120	647±87.6^{A}	507±102^{A}	382±67.5	582±79.7^{A}	569±101^{A}
	P	285±29.7	267±27.3^{B}	275±40.9^{AB}	269±23.4	304±52.2^{B}	246±22.3^{B}
	G	294±55.6	307±54.1^{B}	202±17.6^{B}	217±16.1	210±18.3^{B}	224±33.0^{B}
	G+P	381±89.2	362±64.9^{B}	562±102^{A}	424±94.5	358±94.2^{AB}	292±49.2^{B}

$^{A, B, C}$; Different letters in the same line are statistically significant (Tukey test, $p<0.05$); S (Sham group); P (Pentoxifylline-PTX group); G (L-Glutamine group); P + G (Pentoxifylline/PTX + L-Glutamine group); WBC (white blood cell); LYM (lymphocyte); MON (monocyte); GRAN (granulocyte); RBC (red blood cell); PCV (packed cell volume); HGB (hemoglobin); MCV (mean corpuscular volume); MCHC (mean corpuscular hemoglobin concentration); RDW (red blood cell distribution width); PLT (platelet).

failure of tissue perfusion (non-reflow phenomenon) (Cano et al. 2003). MDA and MPO, indicators of lipid peroxidation and ischemic damage in DA cases, display a significant increase in RDA, whereas an insignificant increase in LDA cases and NO levels were lower in both groups compared to that in the control group. While low levels of NO in DA cases in this study were assessed as indicators of the development of apoptosis, MPO, MDA, and NO measurements may be used in the assessment of tissue damage and apoptosis, whereas SAA and Hp concentrations may be used to evaluate inflammatory responses (Maden et al. 2012). In this study, Hp concentration at 48 h was significantly low ($p<0.05$) in all treatment groups, serum MDA concentration at 48 h was significantly low ($p<0.05$) in group G, and while serum GSH concentration post-surgery was significantly high ($p<0.05$) in group G+P, and significantly low ($p<0.05$) at 48 h in all treatment groups. On the other hand, with the exception of serum IL-1β concentration being significantly low post-surgery and at 5 h in group G+P, serum TNF-α concentrations at 72 h were significantly high ($p<0.05$) in groups P and G (Table 2). On assessing these parameters together, changes particularly in the APR, OS, and cytokine parameters 48 h post-surgery were noticeable. In this respect, the drop in serum Hp levels at 48 h indicating the development of APR and OS in DA cases reflects the effectiveness of treatment in decreasing acute phase response, and the drop in MDA and GSH concentrations in groups G and G+P indicates that the combination of L-Gln and PTX is useful in reducing the development of oxidative stress in DA cases (Reeves et al. 1990, Hammerman et al. 1999, Coster et al. 2004, Mallick et al. 2004, Souza et al. 2007, Zhang et al. 2008, Grosche et al. 2012, Maden et al. 2012, An et al. 2015). With the exception of a significantly high ($p<0.05$) level in group P at 60 min, serum NO concentration remained low at other measurement times in all groups, which may be due to tissue perfusion insufficiency and apoptosis development (Cano et al. 2003, Maden et al. 2012). With the exception of serum IL-1β concentration being at a significantly low ($p<0.05$) level post-surgery and at 5 h in group G+P, cytokine concentrations remained at a high level in all groups. This was interpreted as an insufficient inhibitory effect of treatment administrations on pro-inflammatory cytokines. PTX, acts as a TNF-α inhibitor in experimental intestinal I/R injury, is effective on the lipid end-product MDA following the reperfusion period, and on the key antioxidant GSH and the polymorph nuclear leucocytes index MPO activity. PTX treatment has a significant protective effect against I/R injury (Sener et al. 2001); it increases the levels of cytoprotective HO-1 (hemin) antioxidants (glutathione and superoxide dismutase) during intestinal I/R injury caused by intravenous infusion of L-Gln and also decreases TNF-α levels (Mallick et al. 2004). The current study is a clinical study, and treatment administrations were started 30 min after surgery. Considering the current data, the results demonstrate that PTX and L-Gln treatment is ineffective in the suppression of ongoing inflammatory response.

In RDA cases, a significant increase was observed in serum ADA, AST, CK, creatine kinase MB isoenzyme (CK-MB), GGT, and MPO enzyme activities; however, in LDA cases, there was a significant increase in ADA and the changes in SDH, GDH, ADA, and CK concentrations in treatment groups remained significantly high (Table 3). These changes in enzyme activities in DA cases may be related to hepatic lipidosis (Maden et al. 2012, Moreira et al. 2012, Ok et al. 2013) and abomasal tissue damage (Fürll et al. 2004, Stojević et al. 2005, Maden et al. 2012, Kayano and Kida 2015) included in DA etiology. High MDA and low NO concentrations in this study, together with high MPO enzyme activities (Cano et al. 2003, Cevrioglu et al. 2004, Karatepe et al. 2009) in all groups, and increase in ADA and CK enzyme activities (Maden et al. 2012) indicate mucosal tissue damage in the abomasum. High GGT enzyme levels in AD cases may be related to abomasal damage (Maden et al. 2012) and, together with liver-specific SDH and GDH enzyme activities, to hepatic dysfunction and damage (Moreira et al. 2012, Kayano and Kida 2015).

In DA cases, hemostatic dysfunction and DIC may develop as a result of defects in the abomasal mucosa (Maden et al. 2012, Maden et al. 2018). DIC is assessed through hemostatic test results such as moderate thrombocytopenia, prolonged PT, low fibrinogen, and an increase in fibrinolytic products (FDP, D-Dimer) (Stokol 2012). In cows with DA, a statistically significant increase in D-dimer concentration in group G+P at 48 h and high D-dimer levels in all groups relative to the control group was interpreted to be due to fibrinolytic system activation, and its consistently high level in the post-operative period was attributed to the presence of hypercoagulation, ischemia, and inflammation (Acosta et al. 2003, Altinyollar et al. 2006, Delgado et al. 2009, Wittek et al. 2010, Grosche et al. 2012). An increase in PAI-1 is said to indicate hypercoagulation and fibrinolysis inhibition (Collatos et al. 1994). In this study, high levels of PAI-1 before and after surgery in DA cases indicate hypercoagulation and fibrinolysis suppression (Gando and Hayakawa 2016).

Coagulation activation during DIC is higher and may cause a decrease in ATIII level (Sobiech et al. 2008, Radwińska 2010, Di Loria et al. 2012, Sobiech et al. 2013). ATIII level remains low due to surgery (particularly hemodilution) and may reach normal

levels on day 5 post-surgery (Sørensen 1996). Also, a low level of ATIII due to hemostatic system activation is expected to exhibit a drop partially consistent with the increase in TAT (Kloek et al. 2010, Levi and van der Poll 2010). In this study, no difference was observed in serum TAT levels in all groups (Table 4). In DA cases, high serum TAT concentration, development of DIC (Kloek et al. 2010, Levi and van der Poll 2010), and post-operative decrease are attributed to changes related to surgery (Sørensen 1996). The normal serum TAT levels and high ATIII levels are attributed to coagulation activation.

Early studies (Irmak and Turgut 2005, Karakurum et al. 2009) have reported prolonged APTT in DA cases in cattle. On the other hand, there was no significant difference in APTT in cattle with LDA (Ogurtan et al. 2003). Prolonged prothrombin time (PT) is a key indicator for the development of severe DIC (Bick 1994, Cöl and Durgun 2011), and while it was determined in RDA (Irmak and Turgut 2005), no change was observed in cattle with LDA (Ogurtan et al. 2003). This study demonstrated a significant decrease ($p<0.05$) in plasma PT (sec.) in all groups (Table 4). Fibrinogen level was found to be significantly high in groups P, and G. Low fibrinogen levels have been reported in DIC cases (Estrin et al. 2006, Jaillardon et al. 2012, Stokol 2012). Fibrinolysis is an important component of DIC, and, similar to an increase in D-dimer and FDPs levels, it is an indicator of fibrin breakdown. In this study, the hypofibrinogenemia and increase in D-dimer in group S may be deemed to indicate coagulation activation in DA cases. High fibrinogen concentrations in treatment groups have been considered to be of inflammatory origin. These results indicate the post-operative response of the hemostatic system to surgery and I/R injury (Sørensen 1996) and to the activation of the coagulation system in the reperfusion stage (Irmak and Turgut 2005, Sobiech et al. 2008, Karakurum et al. 2009, Kloek et al. 2010).

To conclude, APR, OS, inflammatory response, and coagulation system activation develops in relation to I/R effects and abomasal damage in DA cases. Post-operative treatment administrations using PTX and L-Gln were found to be effective in decreasing APR, OS, coagulation activation, and the risk of developing DIC and ineffective in suppressing the cytokine-coordinated inflammatory response. Therefore, PTX and L-Gln treatment could be helpful after DA surgery and suppress inflammatory response along with additional drugs, which may be needed to improve this treatment protocol. Although these results are promising, clinical and therapeutic uses of these results warrant further research; besides, preconditioning treatment may be evaluated.

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