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An Exploratory Evaluation of Dexamethasone and Chlorpheniramine Maleate Effects on Serum Biomarkers in Male Dromedary Camels Undergoing Electroejaculation

Derar Derar [1], Ahmed Ali [1], Mohamed Tharwat [2], Fahad A. Alshanbari [3,*], Yousef M. Alharbi [3] and Mohammed H. Elzagafi [4]

[1] Department of Clinical Sciences, College of Veterinary Medicine, Qassim University, Buraidah 51452, Saudi Arabia
[2] Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt
[3] Department of Medical Biosciences, College of Veterinary Medicine, Qassim University, Buraidah 51452, Saudi Arabia
[4] Department of Theriogenology, University Veterinary Hospital, Qassim University, Buraidah 51452, Saudi Arabia
* Correspondence: shnbry@qu.edu.sa

Simple Summary

Electroejaculation is used to collect semen from male camels, but it may be associated with physiological stress. This study examined whether dexamethasone, the antihistamine chlorpheniramine maleate, or their combination was associated with changes in 11 blood markers related to inflammation, cardiovascular status, and oxidative balance in 44 infertile male dromedary camels. Some markers differed among the four experimental groups, but none showed a statistically significant difference in the change from before to after electroejaculation. Therefore, the results do not demonstrate that either drug or their combination reduced the biomarker response to the procedure. Further studies incorporating serial sampling, analytically characterized assays, and direct assessments of animal welfare are needed.

Abstract

This study evaluated the effects of dexamethasone, chlorpheniramine maleate, and their combination on serum biomarkers in male dromedary camels subjected to electroejaculation (EEJ). Forty-four infertile males were allocated to four groups (n = 11 each): untreated control, dexamethasone (0.1 mg/kg), chlorpheniramine maleate (0.5 mg/kg), or a combination of both drugs. Treatments were administered intramuscularly approximately 30 min before EEJ. Blood samples were collected immediately before drug administration (approximately 30 min before EEJ) and 60 min after completion of EEJ. Eleven biomarkers were measured: serum amyloid A (SAA), atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), catalase (CAT), C-reactive protein (CRP), glutathione peroxidase (GPx), glutathione (GSH), haptoglobin (Hp), malondialdehyde (MDA), superoxide dismutase (SOD), and cardiac troponin I (cTnI). No significant treatment × time interactions or main effects of sampling time were detected for any of the biomarkers, indicating that statistically significant differences in pre-to-post EEJ changes among the treatment groups were not demonstrated. Significant overall treatment effects were observed for SAA, BNP, CRP, GSH, Hp, and MDA ($p < 0.05$), whereas ANP, CAT, GPx, SOD and cTnI showed no significant treatment effects. These treatment

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effects represent differences among groups averaged across sampling occasions and should not be interpreted as evidence of pharmacological attenuation of the EEJ-associated biomarker response. The absence of a measurable cTnI change within the sampling window should not be interpreted as proof that myocardial injury did not occur. Further studies incorporating larger sample sizes and additional sampling time points are warranted to characterize the temporal biomarker responses to EEJ and investigate potential treatment effects.

Keywords: dromedary camel; fertility; electroejaculation; anti-inflammatory; cardiac biomarkers; acute-phase proteins

1. Introduction

The dromedary camel (*Camelus dromedarius*) is an economically important livestock species in arid and semi-arid regions and contributes to milk and meat production, racing, breeding, and the preservation of valuable genetic resources. Assisted reproductive technologies are increasingly relevant to the genetic improvement and conservation of superior camel germplasm [1–3]. However, semen collection from male dromedary camels remains challenging because of species-specific reproductive behavior and semen characteristics. Electroejaculation (EEJ) is therefore used when collection with an artificial vagina is not feasible or unsuccessful [4,5].

Although EEJ is an established technique for semen collection in dromedary camels, its application may be associated with physiological responses involving autonomic, cardiovascular, endocrine, and inflammatory pathways [6]. These responses should be distinguished from pain, discomfort, and tissue injury, which represent separate outcomes requiring specific behavioral, clinical, or pathological assessments. Accordingly, changes in circulating biomarkers may provide information about selected physiological and inflammatory responses to the procedure but cannot, in isolation, establish the presence or severity of pain, discomfort, or tissue damage.

Although EEJ is useful in clinical and research settings, it raises animal-welfare concerns because repeated electrical stimulation, restraint, and associated muscular contractions may produce substantial physiological responses. Refining the procedure and reducing avoidable distress are important considerations in camel reproductive management [6,7].

Previous work in male dromedary camels has documented cardiovascular and metabolic changes following EEJ, including changes in heart and respiratory rates, acid–base status, blood gases, electrolytes, and biochemical variables [8]. Cardiac troponin I (cTnI) is used as a marker of myocardial cellular injury and has been discussed in camel medicine and veterinary biomarker research [9–11]. These observations support the evaluation of cardiovascular responses to EEJ, while recognizing that biomarker changes alone do not establish the presence or severity of clinical cardiac disease.

Cardiovascular responses may reflect both myocardial injury and changes in cardiac loading conditions. Atrial natriuretic peptide (ANP) and B-type/brain natriuretic peptide (BNP) are associated with myocardial stretch and hemodynamic loading, whereas cTnI provides complementary information about myocardial injury [12,13]. A population-based human cohort has also reported an association between male infertility and cardiovascular disease [14]; however, this epidemiological finding cannot be directly extrapolated to dromedary camels. Measuring these markers together may provide a broader assessment of cardiovascular responses than any single marker, although the

responses of ANP and BNP to EEJ in male dromedary camels remain insufficiently characterized.

The temporal characteristics of acute-phase proteins are important when interpreting their circulating concentrations following an acute procedure. SAA, CRP, and Hp may differ in the magnitude and timing of their responses, and measurable increases may require several hours or longer depending on the nature and intensity of the inflammatory stimulus. Nevertheless, evaluating these proteins at an early post-procedural interval may provide preliminary information about their early circulating profiles following EEJ. However, a single early sampling point cannot characterize the complete acute-phase response or exclude delayed changes in these biomarkers.

EEJ may also be associated with systemic inflammatory responses. Acute-phase proteins contribute to innate immune responses and can be useful indicators of inflammation and tissue stress [15]. In male dromedary camels, serum amyloid A (SAA) was reported to increase after EEJ, whereas haptoglobin (Hp) did not change significantly, indicating that acute-phase proteins can differ in their response patterns and kinetics [16]. C-reactive protein (CRP), SAA, and Hp may therefore provide complementary information about inflammatory status following the procedure.

Physiological stress can disturb redox homeostasis when reactive oxygen species production exceeds antioxidant defenses [17,18]. Malondialdehyde (MDA) is commonly used as an indicator of lipid peroxidation, while superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) are components of antioxidant defense [18,19]. Measuring MDA alongside antioxidant markers can help distinguish evidence of lipid peroxidation from changes in antioxidant status.

Research in dromedary camels has considered inflammatory and oxidative biomarkers in health, disease, and stress-related contexts [15,16,20–22]. Independent studies in bulls and pampas deer have also examined physiological, hormonal, biochemical, and behavioral responses associated with electroejaculation, highlighting the importance of species-specific protocols and direct welfare assessment [7,23]. However, the short-term oxidative response specifically associated with EEJ and the potential influence of pharmacological pretreatment remain insufficiently defined.

Dexamethasone and chlorpheniramine maleate were selected because they act through different pharmacological pathways that may be relevant to the physiological responses associated with EEJ. Dexamethasone is a glucocorticoid with established anti-inflammatory properties, whereas chlorpheniramine maleate is an H1-receptor antagonist that can interfere with histamine-mediated vascular and inflammatory responses. Their combined administration was therefore intended to investigate whether targeting these distinct pathways could modify selected biochemical responses to EEJ. The selected doses and intramuscular route were based on the intended pharmacological use and the available product information. Both treatments were administered approximately 30 min before EEJ according to the administration interval selected for the study; however, the onset and magnitude of their effects on individual biomarkers in dromedary camels were not directly established.

Dexamethasone is a potent glucocorticoid with anti-inflammatory actions and can alter inflammatory signaling and acute-phase responses [24,25]. However, the effects of dexamethasone pretreatment on cardiac, inflammatory, and oxidative biomarkers following EEJ have not been established in male dromedary camels.

Histamine participates in inflammatory and vascular responses, including vasodilation and increased vascular permeability, and antihistamines are used to modify histamine-mediated effects [26,27]. The potential effects of antihistamine treatment alone or in combination with dexamethasone therefore warrant investigation in this species.

The available evidence indicates that EEJ can be associated with cardiovascular and acute-phase biomarker changes in male dromedary camels [8,16]. Glucocorticoids can also affect acute-phase protein gene expression, although such effects depend on biological context [28]. However, the simultaneous responses of cardiac biomarkers, acute-phase proteins, and oxidative stress markers, and the extent to which pharmacological pretreatment alters these responses, remain poorly understood.

Accordingly, this study evaluated the effects of dexamethasone, chlorpheniramine maleate, and their combination on 11 serum biomarkers in male dromedary camels undergoing EEJ. The selected biomarkers represented cardiovascular status (cTnI, ANP, and BNP), systemic inflammation (SAA, CRP, and Hp), and oxidative damage or antioxidant status (MDA, SOD, CAT, GSH, and GPx). We hypothesized that EEJ would be accompanied by measurable biomarker changes and that pretreatment, particularly combined treatment, would modify the pre-to-post response. The treatment-by-time interaction was therefore central to evaluating whether biomarker changes following EEJ differed among the treatment groups.

2. Materials and Methods

2.1. Animals, History, and Breeding Soundness Examination

A total of 44 male dromedary camels (*Camelus dromedarius*), aged 6–12 years, were enrolled at the University Veterinary Hospital, Qassim University, Saudi Arabia. All animals had documented histories of infertility, classified as primary (failure to impregnate females despite regular breeding exposure, with no previous successful conception) or secondary (inability to impregnate following one or more previous successful conceptions). Body condition scores ranged from 2.5 to 4 on a 1–5 scale [29]. Infertility duration varied, and many males had served between 30 and 150 females without a confirmed pregnancy. Before inclusion in the study, all animals underwent general clinical examination to assess their overall health status and identify apparent systemic diseases. Routine hematological and biochemical investigations were not performed in all animals but were conducted when clinically indicated. Animals with apparent clinical signs of systemic disease were not considered eligible for inclusion.

Breeding-soundness assessment included a detailed history of libido, mating ability, previous reproductive performance, medical history, trauma, and previous pharmacological treatment. The enrolled males displayed normal sexual drive and copulatory behavior, including necking, olfactory investigation, mounting in response to a receptive female, and maintenance of the mating posture, penile erection, and ejaculation. Genital examination and reproductive assessment were conducted with reference to previously reported camel genital parameters [30].

Each camel underwent a general clinical examination and detailed evaluation of the external and internal genital organs. Ultrasonography was performed using a SonoScape X3V unit (SonoScape Medical Corp., Shenzhen, China) equipped with an interchangeable 5–10 MHz linear-array transducer. Reproductive examinations followed previously described clinical protocols [31].

2.2. Experimental Design and Treatment Groups

The animals were assigned sequentially to four experimental groups according to their order of admission, with 11 animals allocated consecutively to each group: the control group, dexamethasone group, antihistamine group, and combined dexamethasone plus antihistamine group. Dexamethasone sodium phosphate (Vétoquinol, 2 mg/mL) and chlorpheniramine maleate (Riemser/Illium, 10 mg/mL) were administered

at doses of 0.1 mg/kg and 0.5 mg/kg body weight, respectively. Allocation was sequential rather than based on a randomization procedure:

Group 1 (control): No pharmacological treatment before EEJ.

Group 2 (dexamethasone; D): Dexamethasone sodium phosphate (0.1 mg/kg body weight, intramuscularly) approximately 30 min before EEJ.

Group 3 (antihistamine; AH): Chlorpheniramine maleate (0.5 mg/kg body weight, intramuscularly) approximately 30 min before EEJ.

Group 4 (D + AH): Dexamethasone sodium phosphate (0.1 mg/kg body weight) and chlorpheniramine maleate (0.5 mg/kg body weight), both administered intramuscularly approximately 30 min before EEJ. The selected doses were based on available veterinary prescribing information and clinical dosing practices for large domestic animals, particularly cattle, because specific pharmacokinetic and dose-response data for these drugs in dromedary camels were limited.

2.3. Blood Sampling Protocol

Blood was collected at two time points: (i) immediately before drug administration (approximately 30 min before EEJ) and (ii) 60 min after completion of EEJ, while the animal was resting in sternal recumbency. The mean duration of EEJ was 18 ± 2.31 min. The post-procedure sample was intended to characterize the early response to EEJ. Blood was collected into plain vacutainer tubes without anticoagulant. Samples clotted at room temperature for 30 min and were centrifuged at 1700× *g* for 15 min. Serum was separated and stored at −20 °C until analysis.

2.4. Electroejaculation Procedure

Prior to electroejaculation (EEJ), each camel was placed in lateral recumbency following the procedure reported earlier [31]. The animal was physically restrained with ropes and lightly sedated with an intravenous injection of xylazine HCl (0.2 mg/kg body weight; Bomazine 10%, BOMAC Laboratories Ltd., Auckland, New Zealand). The rectum was emptied of feces before the procedure began.

Semen collection was performed with an ElectroJac 6 electroejaculator (Neogen, Lexington, KY, USA) set to automatic mode. In this mode, the device delivers a sequence of 40 stimulation cycles, each at a slightly higher intensity than the one before. Every cycle lasted 2 s and was followed by a 2 s rest. The unit ran on a 220-volt, 40-cycle alternating current supply. Voltage reaching the probe was controlled with two knobs. One knob, the power step control, set the maximum probe voltage across seven increments (power steps) from 10 to 40 volts. At each power step, the stimulator knob was turned gradually from zero up to the maximum voltage and back to zero over 2 to 3 s. The voltage was then raised to the next power step, and the sequence was repeated. After ejaculation started, the voltage was not raised any further, unless a higher setting was needed to obtain a complete ejaculate.

2.5. Biomarker Analysis

Serum was analyzed for 11 biomarkers using commercial enzyme-linked immunosorbent assay (ELISA) kits or an enzyme-activity assay, according to the manufacturers' instructions. Serum amyloid A (SAA), haptoglobin (Hp), cardiac troponin I (cTnI), atrial natriuretic peptide (ANP), and B-type/brain natriuretic peptide (BNP) were measured by sandwich ELISA. Catalase (CAT) and C-reactive protein (CRP) were measured by sandwich ELISA. Glutathione peroxidase (GPx) was measured using a sandwich ELISA, whereas glutathione (GSH) was measured using a competitive ELISA. Malondialdehyde (MDA) was measured by competitive ELISA. Superoxide dismutase (SOD) activity was measured using the xanthine oxidase method. Assays were performed in duplicate.

ELISA absorbance was read at 450 nm and SOD absorbance at 550 nm using a microplate reader. Concentrations or activities were calculated from standard curves. The assays used in this study were kits specifically designated by the manufacturers for camel analytes. Therefore, the measurements were performed using assays appropriate for the study species, rather than relying solely on extrapolation from assays developed for other species. Manufacturer-recommended procedures were followed for sample preparation and analysis, in accordance with the respective kit protocols. Catalase (CAT) and glutathione peroxidase (GPx) were quantified using ELISA-based assays; therefore, their results represent immunoreactive protein concentrations rather than functional enzyme activities. In contrast, superoxide dismutase (SOD) was measured using an enzyme-activity assay. Glutathione (GSH) was assessed as a non-enzymatic antioxidant, whereas malondialdehyde (MDA) was measured as an indicator of lipid peroxidation. Intra- and inter-assay coefficients of variation were within manufacturer specifications (<10–15%). Manufacturer-reported kit specifications are summarized in Table 1.

Table 1. Manufacturer-reported specifications of the commercial kits and assay used to measure serum biomarkers.

Biomarker	Manufacturer	Kit/Catalog No.	Method	Sensitivity	Detection Range
SAA	SunLong Biotech Co., Ltd., Room 1-315, Kongle Changqing Building, No. 160 Guangye Road, Gongshu District, Hangzhou, China.	SL0059Cm	Sandwich ELISA	6 pg/mL	30–2000 pg/mL
Hp		SL0058Cm	Sandwich ELISA	12 ng/mL	40–1800 ng/mL
cTn I		SL0020Cm	Sandwich ELISA	2.6 pg/mL	15–1000 pg/mL
ANP	MyBioSource, Inc. P.O. Box 153308, San Diego, CA 92195-3308, USA.	MBS063175	Sandwich ELISA	5.0 pg/mL	31.2–1000 pg/mL
BNP		MBS9378293	Sandwich ELISA	2.0 pg/mL	15.6–500 pg/mL
Catalase		MBS740466	Sandwich ELISA	0.1 ng/mL	0–25 ng/mL
CRP		MBS055862	Sandwich ELISA	0.1 µg/mL	0.625–20 µg/mL
GPx	Antibodies.com Limited, 6 Cambridge Technopark, Newmarket Road, Cambridge, UK	A247241	Sandwich ELISA	0.094 ng/mL	0.156–10 ng/mL
Glutathione	Elabscience, Biomedical Park, No. 858 Gaoxin Road, Wuhan, China	E-EL-0026	Competitive ELISA	0.94 µg/mL	1.56–100 µg/mL
MDA	Thermo Fisher Scientific (Bender MedSystems), Campus Vienna Biocenter 2, 1030 Vienna, Austria.	EEL160	Competitive ELISA	18.75 ng/mL	0–2000 ng/mL
SOD	ELK Biotechnology, Wuhan, China.	(not specified) ELK Biotechnology	Xanthine oxidase	Not applicable	U/mL

2.6. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 17.0). The data were analyzed using a two-way mixed-design repeated-measures analysis of variance (ANOVA), performed using the General Linear Model–Repeated Measures procedure. Treatment group (control, dexamethasone, chlorpheniramine maleate, and dexamethasone plus chlorpheniramine maleate) was included as the between-subjects factor, whereas sampling time (before medication administration and 60 min after completion

of electroejaculation) was included as the within-subjects factor. The main effects of treatment and sampling time, as well as the treatment × time interaction, were evaluated separately for each biomarker. Because only two sampling occasions were included, the assumption of sphericity was inherently satisfied. Where significant effects were detected, pairwise comparisons were performed using Fisher's least significant difference (LSD) test. Statistical significance was set at $p < 0.05$.

The principal experimental question was whether pharmacological treatment modified the pre-to-post electroejaculation (EEJ) biomarker response, as assessed by the treatment × time interaction. This interaction evaluated whether changes between the two sampling occasions differed among the experimental groups. Treatment main effects, representing differences among groups averaged across the two sampling occasions, were considered exploratory. Accordingly, significant treatment main effects were not interpreted independently as evidence of treatment-mediated attenuation of the EEJ-associated biomarker response. As no single primary biomarker outcome was prospectively designated, the analyses of the 11 investigated biomarkers were considered exploratory.

3. Results

Treatment, sampling-time, and treatment-by-time effects for the 11 biomarkers are summarized in Table 2. Significant treatment main effects were detected for SAA, BNP, CRP, GSH, Hp and MDA. No significant main effect of sampling time or treatment × time interaction was detected for any biomarker, indicating that the pre-to-post EEJ changes did not differ significantly among the experimental groups.

Table 2. *p*-values for treatment, sampling time, and treatment × time effects from the mixed-design ANOVA.

Biomarker	Treatment *p*	Time *p*	Treatment × Time *p*
SAA (pg/mL)	0.001	0.88	0.91
ANP (pg/mL)	0.489	0.71	0.91
BNP (pg/mL)	0.002	0.87	0.89
Catalase (ng/mL)	0.32	0.42	0.51
CRP (µg/mL)	0.01	0.64	0.89
Glutathione Peroxidase (ng/mL)	0.09	0.15	0.18
Glutathione (µg/mL)	0.01	0.97	0.18
Haptoglobin (ng/mL)	0.040	0.81	0.990
MDA (ng/mL)	0.002	0.76	0.94
SOD activity (U/mL)	0.90	0.63	0.53
cTnI (pg/mL)	0.53	0.99	0.8

Baseline serum concentrations of the 11 investigated biomarkers in the four experimental groups before drug administration and electroejaculation are presented in Table 3. Baseline concentrations did not differ significantly among groups for any biomarker (all $p > 0.05$).

Table 3. Baseline serum concentrations of the investigated biomarkers in infertile male dromedary camels (n = 11 per group).

Biomarker	Control	D	AH	D + AH	Baseline ANOVA *p*
SAA (pg/mL)	1633 ± 1240	2562.38 ± 1690	1367 ± 1258	850 ± 984	0.247
ANP (pg/mL)	219 ± 321	304 ± 313	385 ± 316	241 ± 312	0.671
BNP (pg/mL)	273 ± 140	173 ± 180	251 ± 161	92 ± 134	0.063
CAT (ng/mL)	2178 ± 2019	2789 ± 1801	1538 ± 1240	1638 ± 1409	0.393

CRP (µg/mL)	32.75 ± 3.47	36.10 ± 5.79	29.14 ± 14.75	25.98 ± 16.01	0.446
GPx (ng/mL)	1.66 ± 0.071	2.1 ± 0.070	1.87 ± 0.034	2.11 ± 0.018	0.632
GSH (µg/mL)	5.62 ± 7.07	7.99 ± 7.19	4.74 ± 7.01	4.98 ± 6.10	0.767
Hp (ng/mL)	1129 ± 675	406 ± 73	910 ± 732	996 ± 773	0.269
MDA (ng/mL)	738 ± 637	941 ± 446	587 ± 405	412 ± 281	0.111
SOD (U/mL)	66.64 ± 40.93	46.67 ± 34.21	54.22 ± 35.72	60.84 ± 34.86	0.776
cTnI (pg/mL)	707 ± 82	765 ± 45	313 ± 327	223.48 ± 254	0.474

A significant treatment main effect was detected for SAA. Post hoc comparisons indicated lower estimated SAA concentrations in the combined-treatment group than in the control, D, and AH groups. Neither the main effect of sampling time nor the treatment-by-time interaction was significant (Figure 1).

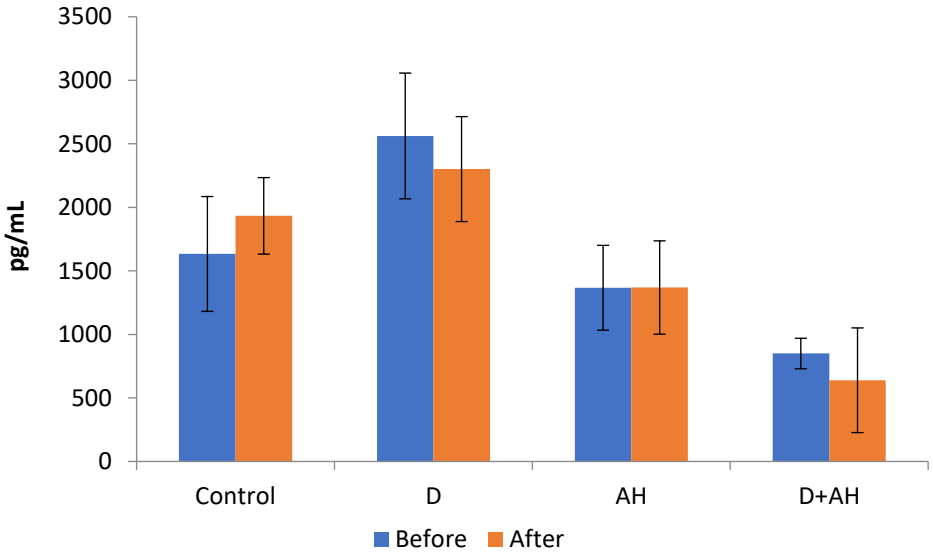


Figure 1. Serum amyloid A (SAA) concentrations in male dromedary camels in the control, dexamethasone (D), chlorpheniramine maleate (AH), and combined-treatment (D + AH) groups before and 60 min after electroejaculation (EEJ). D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

No significant treatment main effect was detected for ANP, and neither the sampling-time effect nor the treatment-by-time interaction was significant (Figure 2).

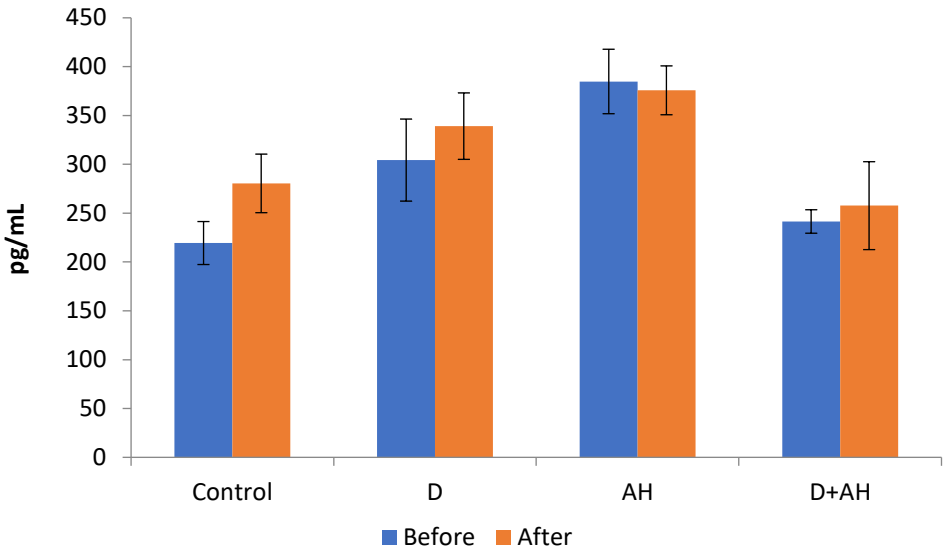


Figure 2. Atrial natriuretic peptide (ANP) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

A significant treatment main effect was observed for BNP. Pairwise comparisons indicated differences between control and D, control and D + AH, D and AH, and AH and D + AH; estimated BNP concentrations were higher in the control and AH groups than in the D and D + AH groups, respectively. The sampling-time effect and treatment-by-time interaction were not significant (Figure 3).

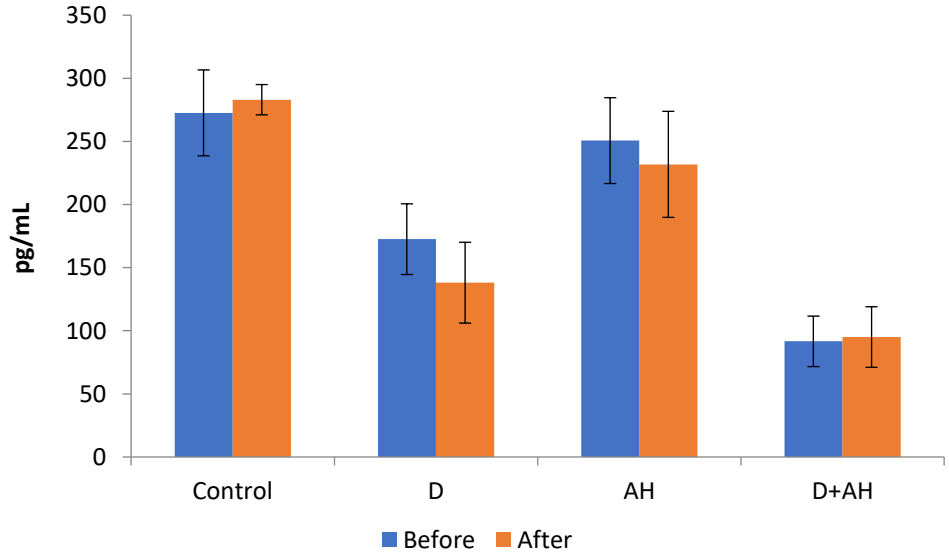


Figure 3. B-type/brain natriuretic peptide (BNP) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

No significant treatment main effect was detected for CAT. Neither sampling time nor the treatment-by-time interaction was significant (Figure 4).

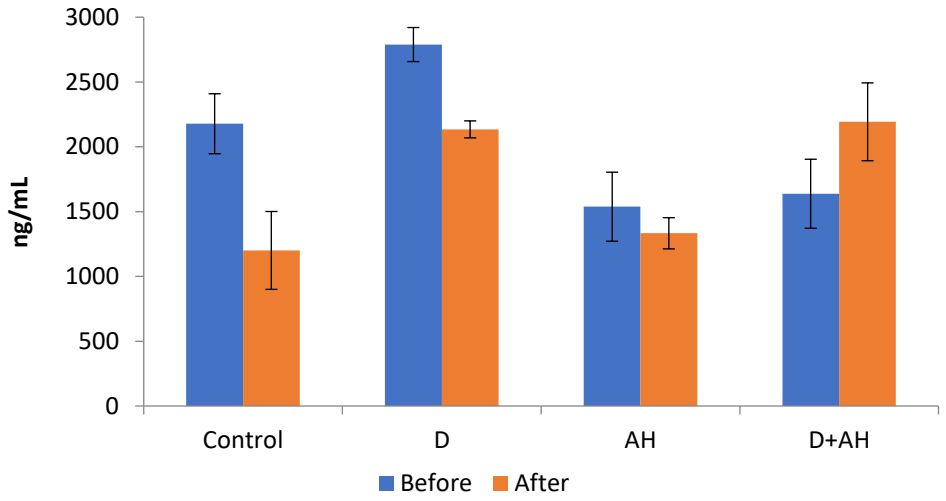


Figure 4. Catalase (CAT) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

A significant treatment main effect was detected for CRP. Estimated CRP concentrations were lower in the D + AH group than in the control and D groups. The sampling-time effect and treatment-by-time interaction were not significant (Figure 5).

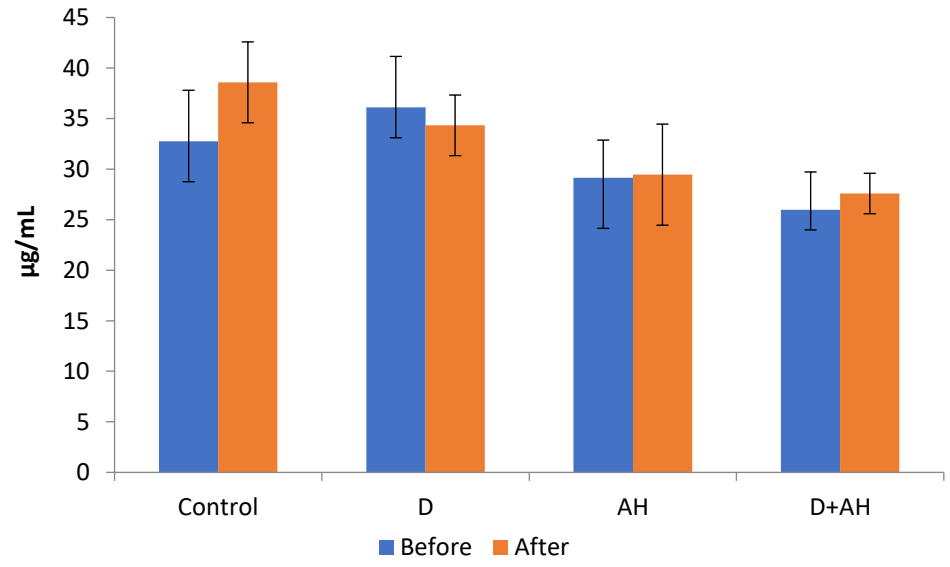


Figure 5. C-reactive protein (CRP) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

No significant treatment main effect was detected for GPx, and neither sampling time nor the treatment-by-time interaction was significant (Figure 6).

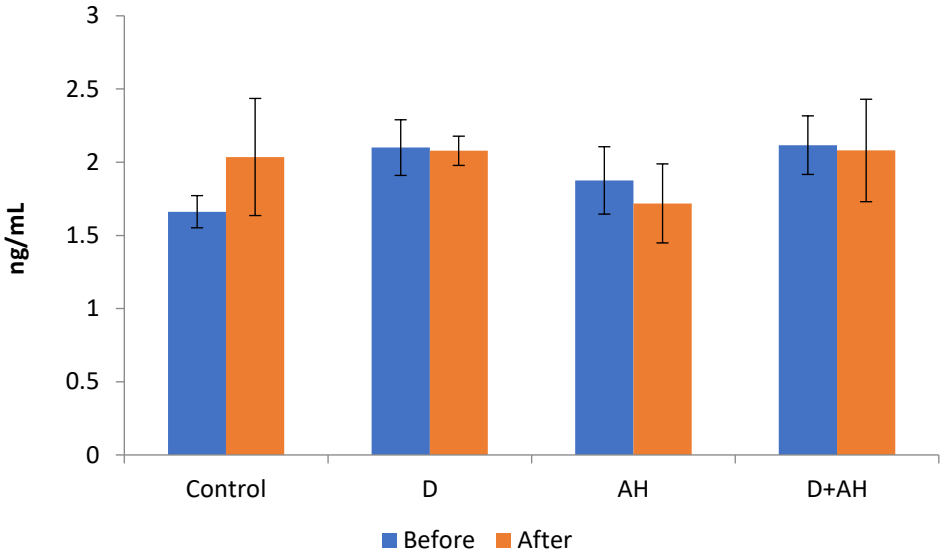


Figure 6. Glutathione peroxidase (GPx) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

A significant treatment main effect was detected for GSH. Estimated GSH concentrations were higher in the control and D groups than in the AH and D + AH groups. Neither the sampling-time effect nor the treatment-by-time interaction was significant (Figure 7).

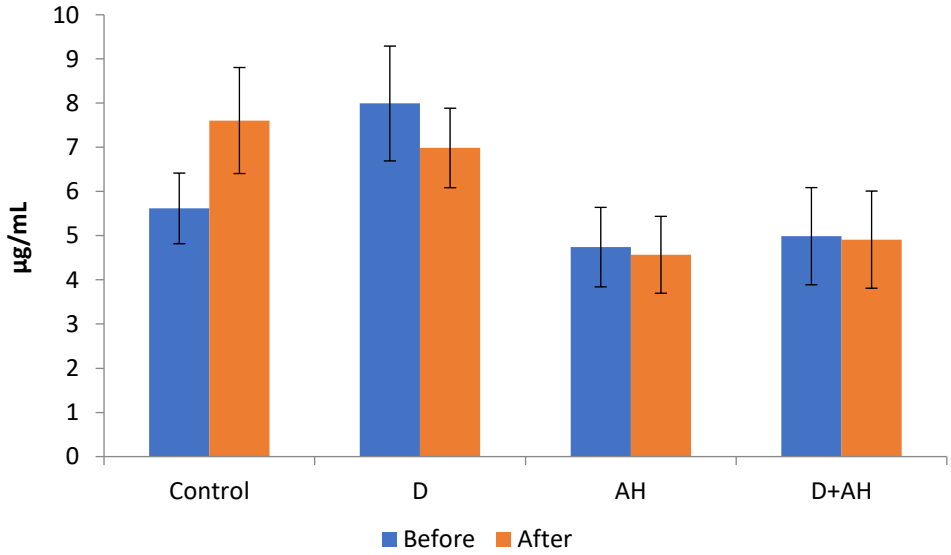


Figure 7. Glutathione (GSH) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

A significant treatment main effect was detected for Hp. Estimated Hp concentrations were lower in the D group than in the control, AH, and D + AH groups. The sampling-time effect and treatment-by-time interaction were not significant (Figure 8).

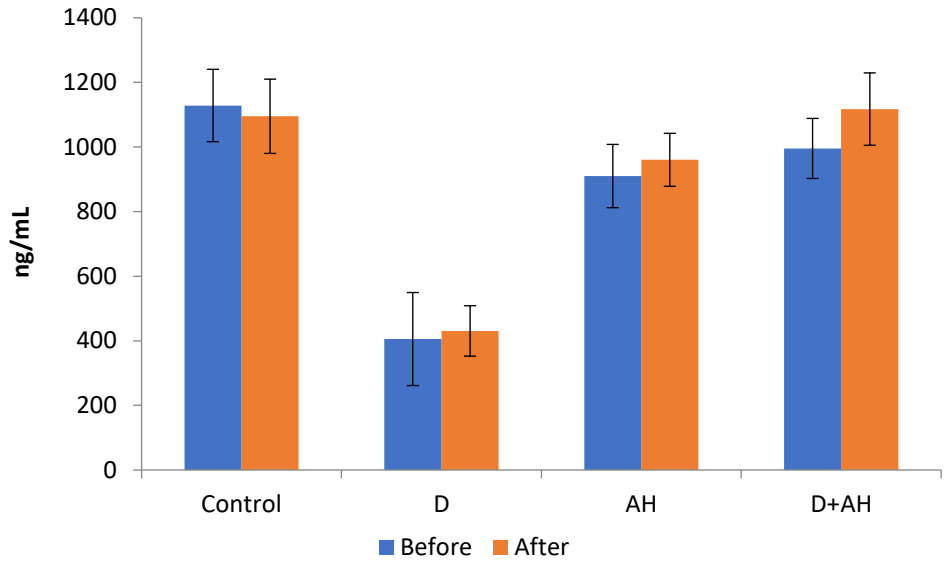


Figure 8. Haptoglobin (Hp) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

A significant treatment main effect was detected for MDA. Estimated MDA concentrations were lower in the D + AH group than in the D and AH groups. Neither the sampling-time effect nor the treatment-by-time interaction was significant (Figure 9).

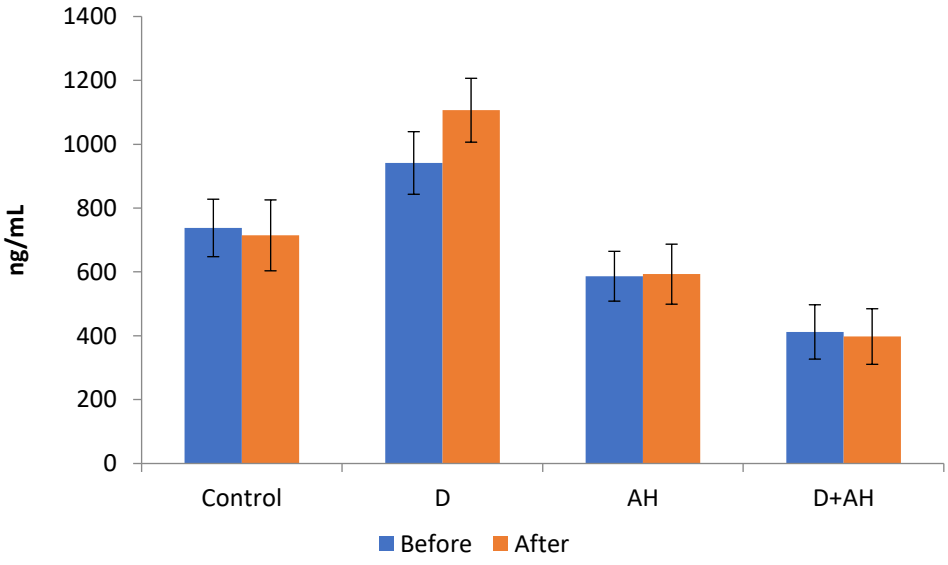


Figure 9. Malondialdehyde (MDA) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

No significant treatment main effect was detected for SOD. Neither sampling time nor the treatment-by-time interaction was significant (Figure 10).

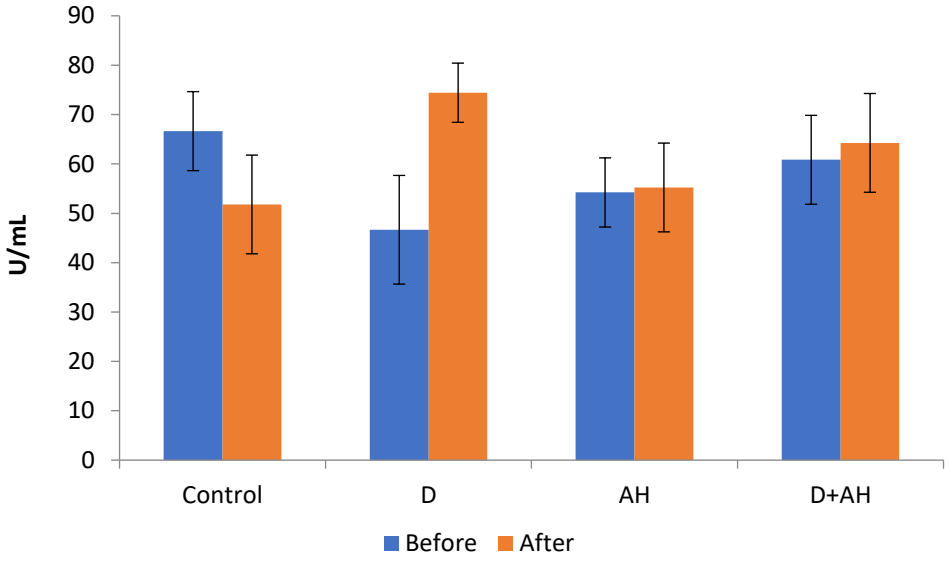


Figure 10. Superoxide dismutase (SOD) activity in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

No significant treatment main effect was detected for cTnI. Neither sampling time nor the treatment-by-time interaction was significant (Figure 11).

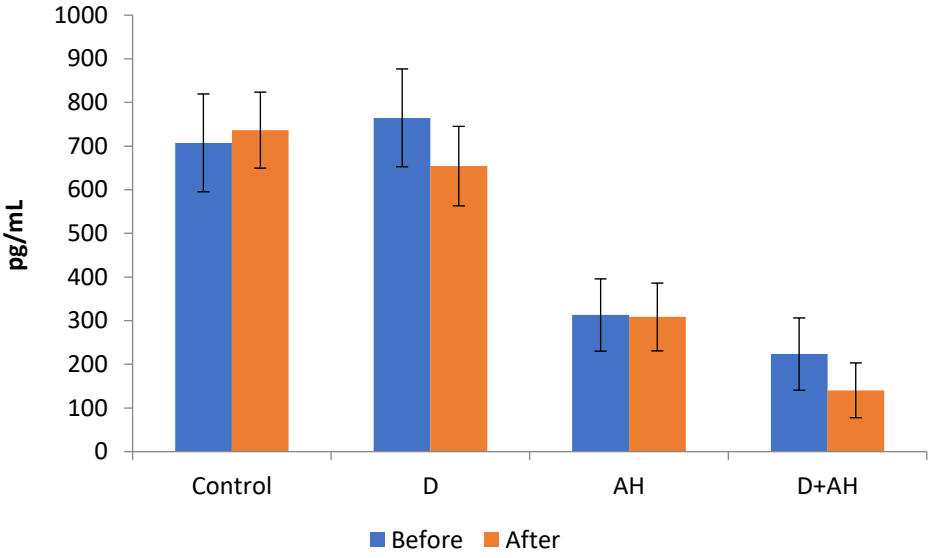


Figure 11. Cardiac troponin I (cTnI) concentrations in male dromedary camels in the control, D, AH, and D + AH groups before and 60 min after completion of EEJ. D, dexamethasone; AH, chlorpheniramine maleate; D + AH, combined treatment; EEJ, electroejaculation. Values are presented as means ± SE; error bars represent standard errors.

4. Discussion

This study examined whether pretreatment with dexamethasone, chlorpheniramine maleate, or their combination modified the early serum biomarker response to EEJ in male dromedary camels. The principal finding was that significant treatment main effects were detected for selected biomarkers, but no significant treatment × time interaction was detected for any of the 11 biomarkers. Because baseline samples were collected

before medication administration, treatment main effects, which represent differences among groups averaged across both sampling occasions, cannot be interpreted as evidence that the drugs reduced the response to EEJ. The treatment × time interaction is the relevant test for determining whether pre-to-post changes differed among groups, and this interaction was nonsignificant for all biomarkers. Therefore, the present findings do not demonstrate that either drug, alone or in combination, modified the early biomarker response to EEJ.

Among the inflammatory biomarkers, significant treatment main effects were detected for SAA, CRP, and Hp, with lower estimated concentrations in selected group comparisons. These patterns were not accompanied by significant sampling-time effects or treatment × time interactions and therefore do not establish an acute EEJ-associated change or treatment-specific attenuation. Previous work in male dromedary camels reported an immediate increase in SAA after EEJ, whereas Hp did not change significantly [16]. A review of acute-phase proteins in camels emphasizes that their circulating concentrations vary according to the nature of the condition and the sampling interval [15]. In another camel study, Hp remained elevated for several days following an inflammatory insult, illustrating that its response may extend beyond the early post-procedural period [15]. In the present study, the second sample was collected 60 min after completion of EEJ. Consequently, this sampling schedule may not have captured either immediate transient changes or delayed acute-phase responses. The absence of significant changes at the sampled time point should therefore not be interpreted as evidence that EEJ did not induce an inflammatory response. Serial sampling over a longer post-procedure period is needed to characterize the temporal profiles of SAA, CRP, and Hp following EEJ.

For the cardiovascular markers, a significant treatment main effect was detected for BNP, whereas ANP and cTnI showed no significant treatment, sampling-time, or treatment × time interaction effects. Natriuretic peptides reflect aspects of myocardial stretch and hemodynamic loading, whereas cTnI is used to assess myocardial cellular injury [9–13]. Earlier work in camels reported changes in cardiac variables following EEJ [8]. The BNP findings should be interpreted cautiously, particularly when considering the concentrations measured at baseline before medication administration. The overall treatment effect represents differences among groups averaged across the two sampling occasions and should not be regarded as evidence of a pharmacological effect. In addition, the absence of a significant treatment × time interaction indicates that differences in pre-to-post BNP changes among the experimental groups were not statistically demonstrated. Evidence from cattle supports the clinical use of cTnI in evaluating cardiac and noncardiac disorders [10], while high-sensitivity troponin literature emphasizes that troponin concentrations must be interpreted within their clinical and analytical context [11]. In the present study, no significant treatment, sampling-time, or treatment × time interaction effect was detected for cTnI. Nevertheless, the absence of a statistically detectable change within the investigated sampling interval does not necessarily exclude other physiological or cardiac responses to EEJ.

The oxidative-stress findings were mixed. MDA concentrations were lower in the combined-treatment group than in the D and AH groups, whereas GSH concentrations were higher in the control and D groups than in the AH and D + AH groups. MDA is an indicator of lipid peroxidation and oxidative damage, whereas GSH represents a component of non-enzymatic antioxidant defense. Thus, lower MDA concentrations may be consistent with reduced lipid peroxidation but cannot, by themselves, establish an improvement in overall oxidative balance, particularly when GSH showed a different pattern. Neither MDA nor GSH showed a significant treatment × time interaction; therefore, the observed treatment main effects represent group differences averaged

across sampling occasions rather than evidence of treatment-induced improvement or deterioration in oxidative status. These markers provide complementary, but not interchangeable, information about redox status [17–19]. Studies of camel lameness and transport-related stress also show that oxidative and stress-associated biomarkers vary with the condition examined [21,22]. Chlorpheniramine-related changes in oxidative and inflammatory signaling have been reported in a rodent model, but extrapolation to camel serum following EEJ should be done cautiously [32].

CAT, GPx, and SOD showed no significant treatment, sampling-time, or treatment × time interaction effects. CAT and GPx were quantified as immunoreactive protein concentrations using ELISA, whereas SOD was measured as enzyme activity. Consequently, the CAT and GPx findings do not establish that their respective functional enzyme activities were unchanged. The possibility that subtle differences were not detected under the assay conditions cannot be excluded. Biological variability, the relatively small sample size (11 animals per group), and the single post-EEJ sampling point may also have limited the detection of modest, transient, or delayed responses. The manufacturer-reported assay sensitivities and detection ranges are provided in Table 1 and should be considered when interpreting the measurements. The response of antioxidant defenses can also depend on the type and intensity of the stressor and the baseline physiological status of the animals [17–22]. Accordingly, the absence of statistically significant differences in CAT, GPx, and SOD does not establish that EEJ caused no oxidative challenge.

Comparisons with other electroejaculation studies should take account of differences in species, anesthesia, stimulation protocols, and sampling schedules. In bulls, electroejaculation has been investigated using electroencephalographic, behavioral, hormonal, and metabolite measurements, demonstrating the value of assessing several domains rather than relying on circulating biomarkers alone [7]. In anesthetized pampas deer, electroejaculation was accompanied by changes in heart rate, respiratory rate, cortisol, and other biochemical measures [23]. These studies provide comparative evidence that physiological responses to EEJ can be multidimensional, but their findings cannot be directly transferred to dromedary camels because of differences in species and experimental protocols. The present study's single post-procedure sampling point and use of xylazine sedation in all animals should also be considered when comparing results across studies. In particular, the absence of a group undergoing the same handling and sedation without EEJ limits the ability to distinguish the contribution of the procedure itself from other experimental factors.

All 11 biomarkers were re-assayed using newly purchased camel-specific commercial kits and stored serum samples, and the revised measurements replaced the previously reported values. The manufacturer-reported assay sensitivities and detection ranges are presented in Table 1. The revised Hp measurements, for example, were within the manufacturer's stated detection range. Nevertheless, the use of camel-specific commercial kits does not, by itself, establish complete independent analytical validation in dromedary serum. Manufacturer-reported sensitivity and detection range describe the specifications provided for the commercial assays but do not replace independent assessments of analytical performance. Recovery, dilution-linearity, and other independent analytical validation procedures were not conducted in the present study. These considerations should be borne in mind when interpreting relatively small differences among the experimental groups. The reported concentrations should be regarded as measurements obtained using the specified commercial assays and should not be considered established reference values for dromedary camels.

The potential clinical application of dexamethasone and chlorpheniramine maleate in camels subjected to EEJ should be considered in relation to their pharmacological ac-

tions, potential benefits, and possible adverse effects. Dexamethasone is a glucocorticoid with potent anti-inflammatory effects, whereas chlorpheniramine maleate is a first-generation H1-antihistamine that antagonizes histamine-mediated responses [24–28]. Their combined administration was intended to explore the potential effects of targeting these distinct pathways. The selected doses were based on available veterinary prescribing information and clinical dosing practices in large domestic animals, as specific pharmacokinetic and dose–response information for these drugs in dromedary camels is limited. The approximately 30 min interval between drug administration and EEJ was selected as a pretreatment period; however, the onset and duration of the relevant pharmacological effects under this schedule have not been specifically established in camels. Dexamethasone may produce systemic, dose- and context-dependent adverse effects [24,25,28], while sedation and anticholinergic effects are relevant considerations for first-generation H1-antihistamines [26,27]. The clinical significance of these potential effects following the doses and administration schedule used in the present study remains uncertain because treatment-related adverse events were not systematically evaluated. Importantly, the present findings do not demonstrate that either drug, alone or in combination, attenuated the biomarker response to EEJ or improved animal welfare. Their use should not be recommended solely on the basis of differences in selected serum biomarkers; any clinical application would require demonstrable welfare benefits and an acceptable safety profile in dromedary camels.

Several limitations should be considered when interpreting the present findings. Each group included 11 animals, and no a priori sample-size calculation was performed, which may have limited the detection of modest effects. Allocation was sequential according to order of admission rather than randomized, so temporal or selection-related confounding cannot be excluded. Numerical variation in baseline concentrations among groups should be considered when interpreting overall treatment effects, although no statistically significant baseline differences were detected for any of the 11 biomarkers. Only one post-EEJ sample was obtained, 60 min after completion of the procedure; transient or delayed responses may therefore have been missed. All animals received xylazine sedation, and no xylazine-only group without EEJ was included. The absence of a handling-only control and a vehicle-injected control means that effects associated with restraint, blood collection, intramuscular injection, and additional handling cannot be fully separated from the effects of EEJ or the study drugs. The sampling schedule was designed primarily to assess the acute physiological response to EEJ and the potential prompt prophylactic effects of the administered treatments during the immediate procedural period. This objective was based on the importance of preventing or minimizing severe acute physiological disturbances that could potentially compromise animal safety. Therefore, the findings should be interpreted within the specific sampling interval investigated and should not be extrapolated to immediate post-medication effects or delayed biomarker responses.

All enrolled animals had a history of infertility, which may limit generalizability to fertile males; routine hematological and biochemical screening was not performed in every animal. In addition, although the biomarkers were re-assayed using newly purchased camel-specific commercial kits, independent analytical validation and detailed assay performance assessments were not conducted. Finally, animal welfare was not assessed directly through behavioral scoring, validated pain assessment, or complementary physiological stress measurements such as cortisol and cytokines. The biomarkers measured here provide information about selected physiological responses but cannot independently establish the degree of pain, discomfort, or distress experienced by the animals.

Future studies should include adequately powered, randomized designs with appropriate handling and vehicle controls, serial post-procedure sampling, analytically characterized assays, and a broader panel of physiological stress markers. Pharmacokinetic and dose-response information in dromedary camels would also help refine the selected doses and determine the appropriateness of the pretreatment interval for the drugs investigated. Direct behavioral and physiological welfare assessments, together with systematic monitoring of adverse events, are necessary to determine whether any biomarker modulation is accompanied by measurable welfare benefits. Overall, the present findings do not support the use of dexamethasone, chlorpheniramine maleate, or their combination as a strategy to reduce EEJ-associated physiological stress or improve animal welfare in male dromedary camels.

5. Conclusions

The present study identified differences among experimental groups in several serum biomarkers in male dromedary camels subjected to EEJ. However, because some differences may have been present before drug administration and no significant treatment × time interaction was detected for any of the 11 biomarkers, these findings do not establish a causal pharmacological effect of dexamethasone, chlorpheniramine maleate, or their combination on the biomarker responses to EEJ. The results should therefore be interpreted cautiously and considered exploratory. Further studies incorporating randomized allocation, appropriate control groups, serial sampling, and direct animal welfare assessments are needed to clarify the physiological effects of EEJ and evaluate the potential benefits and safety of these pharmacological interventions. At present, the findings do not provide sufficient evidence to support the use of either drug, alone or in combination, as a strategy to reduce EEJ-associated physiological stress in male dromedary camels.

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Institutional Review Board Statement: The animal study protocol was approved by the Animal Care and Welfare Committee of the Deanship of Scientific Research at Qassim University, Saudi Arabia (No. 58/26-27; 12 March 2026), and the study was conducted in accordance with the relevant institutional regulations for animal care.

Informed Consent Statement: Written informed consent was obtained from the owners before the semen collection of the studied animals.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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