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Comparative evaluation of therapeutic effects of silymarin and hydrocortisone on clinical and hematological alterations, and organ injury (liver and heart) in a low-dose canine lipopolysaccharide-induced sepsis model

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Abstract

The present study aimed to examine the effectiveness of silymarin compared to hydrocortisone on clinical and hematological alterations and organ injury (liver and heart) in a low-dose canine lipopolysaccharide (LPS)-induced sepsis model. Fifteen clinically healthy dogs were randomly categorized into three equal groups: Two dogs in group A, LPS (0.10 μg kg^{-1}, IV) was injected (control, n = 5); Group B was similar to group A, with the difference that silymarin bolus (10.00 mg kg^{-1}, IV, once) was injected 40 min after LPS injection. Group C was similar to group B with the difference that hydrocortisone bolus (2.00 mg kg^{-1}, IV, once) was administrated instead of silymarin. Five mL of blood was collected at baseline, 1, 3, and 6 hr of the study. Septic control dogs experienced a significant reduction in red blood cells count (RBC), hemoglobin (Hb), and hematocrit (HCT) and a significant elevation in serum activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase isoenzyme MB (CK-MB), and plasma cardiac troponin I (cTnI) concentration. We noticed a significant increase in RBCs, Hb, and HCT, and a significant decrease in AST, ALP, LDH, CK-MB, and cTnI in the silymarin group in comparison with hydrocortisone and control group. Our results suggested that silymarin had a positive influence on sepsis due to protecting RBCs, and decreasing organ (heart and liver) injury. These findings supported the hypothesis that silymarin could be more effective than routine corticosteroid therapy in sepsis.

Introduction

Sepsis is a common cause of morbidity and mortality in veterinary medicine.[1,2] It is the systemic inflammatory response to identifiable infection, most often of bacterial origin. Systemic inflammation is manifested clinically as two or more of the following signs: hyper- or hypothermia, tachycardia, tachypnea, and an abnormal leukogram (ie, leukocytosis, leukopenia, or high-band neutrophil count).[2-4]

In the early stages of sepsis, reactive oxygen species (ROS) are generated by inflammatory cells and ischemic tissues. The ROS will cause damage to the membrane protein of red blood cells and will induce RBC deformability. This deformability has the potential to reduce blood flow and increase the time required cells to transit the microcirculation. Moreover, it has a negative effect on oxygen-delivery and contributes to organ dysfunction outside the hematologic system.[5,6] The ROS are also known to depress cardiac function and contractility and produce cellular injury.[7]

Any microbial organism (e.g., fungus, parasite, and virus) can cause sepsis.[8] However, bacterial infections are the most common cause of sepsis in dogs and Escherichia coli is the most common isolate.[9] Intravenous administration of lipopolysaccharide of gram-negative bacteria is being used to induce sepsis in dogs.[10,11] Compared to rodents, where LPS doses were in the milligram range, only microgram doses elicit sufficient clinical, a hematological and biochemical response in dogs

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which are comparable to human sepsis. Canine low dose sepsis model provides a reliable setting to test new drugs before application in humans or to study innovative drugs for canine use.[12]

To decrease the high rate of morbidity and mortality of sepsis, several interventions have been suggested including early antibiotics, corticosteroids, and supportive care. Exogenous administration of corticosteroids at a moderate dose, i.e., < 400 mg of hydrocortisone may help reverse sepsis-associated organ dysfunction. [13] Despite the useful applications assigned to hydrocortisone, it has been associated with many serious adverse effects.[14-16]

Flavonoids were found to protect the host from severe inflammation in different septic situations and might provide a modern hope for the treatment of sepsis or acute systemic inflammation.[17] Silymarin is a flavonoid, isolated from the fruits and seeds of the milk thistle, *Silybum marianum*.[18] Silymarin standardized dry extract is a bioflavonoid complex consisting of at least seven flavonolignans. Silibinin is the most active constituent of silymarin, followed by silicristin, silidianin, and isosilybin. Silibinin is a mixture of two stereoisomers: silibinin A and silibinin B.[19] Clinical and experimental studies have shown silymarin to act by antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, and immunomodulatory mechanisms.[20] Silymarin has been used in alcoholic liver diseases,[21] liver cirrhosis,[22] Amanita mushroom poisoning,[23] viral hepatitis,[24] toxic and drug-induced liver diseases[25] and in diabetic patients.[26] Silymarin have also been shown to possess an important cardioprotective activity and promise a relatively inexpensive way available for the treatment of ischemia-reperfusion injury.[27]

Studies have reported the beneficial effects of silymarin for the treatment of sepsis in Wistar rats and mice LPS-induced sepsis models.[28,29] To the best of our knowledge, there is no study on the intravenous injection of silymarin to a large animal model of sepsis, and this study provides meaningful data regarding the therapeutic potential of this healthful dietary constituent in sepsis. Moreover, studies comparing the efficacy of silymarin and hydrocortisone in canine sepsis are lacking and clinical experience is limited. Therefore, the present survey aimed to evaluate and compare the effects of silymarin and hydrocortisone on clinical and hematological alterations, and organ injury (heart and liver), in a low-dose canine LPS-induced sepsis model.

Materials and Methods

Animals. Fifteen clinically healthy adult mixed breed dogs were used with the age of 12-24 months, both sex (eight female and seven male), and weighing 17.00 - 22.00 kg. The dogs were hospitalized one month before the study. Vaccination (DHPPiL and Rabies) and anti-parasitic therapy were performed during hospitalization. Dogs have fasted for 12 hr before the study and water was available *ad libitum*. The determination of the age was accomplished based on dental formulary. This research proposal received ethical approval by Shahid Chamran University of Ahvaz Research Committee, Ahvaz, Iran (Act No. 9338402; 2017.01.30).

Experimental protocol. A pilot study was conducted to find the approximate time of sepsis symptoms occurrence in one dog. The dog showed the incidence of sepsis, 40 min after LPS (0.10 μg kg^{-1}, IV) injection. Therefore, 40 min after LPS injection, was selected as a time to start the treatments. To induce sepsis, all dogs received an IV bolus of a low-dose LPS (0.10 μg kg^{-1}, from *Escherichia coli* serotype O26: B6; Sigma, St. Louis, USA), dissolved in normal saline solution.[12] Dogs were divided into three equal groups (n = 5), by simple random allocation, as follow. In control group LPS was injected, and no treatment was performed. In silymarin group, a single dose of silymarin bolus (10 mg kg^{-1}, IV; Sigma) was injected 40 min after LPS injection. Silymarin dosage was selected based on Varzi *et al*. study.[30] In hydrocortisone group, a single dose of hydrocortisone (Caspian Tamin, Rasht, Iran) bolus (2.00 mg kg^{-1}, IV) was administrated. Hydrocortisone dosage was in the physiologic range recommended by Annane and Papich.[31,32] In the case of emesis, a single dose of metoclopramide (0.50 mg kg^{-1}, IM; Tehran Chemie Pharmaceutical Co., Tehran, Iran) was used for all dogs in three groups.

Collection of blood and serum samples. Blood samples were collected from the cephalic or jugular vein. One mL of blood was collected in potassium EDTA-treated tubes (Becton Dickinson, Franklin Lakes, USA), at baseline, 40, 60, 120, 180, 240, 300, and 360 min to evaluate complete blood count (CBC) using an automatic impedance cell counter (Mindray, BC- 2800 Vet; Nanjing Poweam Medical Co., Ltd., Jiangsu, China). Moreover, 5.00 mL of blood was collected at baseline, 1, 3, and 6 hr and poured into the plane tube. Serum was obtained using centrifugation at 700 *g* for 10 min after keeping blood for 20 min at room temperature. Serum levels of glucose, total protein and albumin, and also serum activity of alanine aminotransferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP), creatine kinase isoenzyme MB (CK-MB), and lactate dehydrogenase (LDH) were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an auto-analyzer (BT1500; Biotecnica, Rome, Italy). Also, the plasma cardiac troponin I (cTnI) concentration was measured by canine cardiac troponin I ELISA commercial kit (CTNI-4-HS; Life Diagnostics Inc., West Chester, USA) according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was conducted using SPSS for Windows (version 24.0; IBM, Chicago, USA). A *p*-value of 0.05 or less was taken as a criterion for a statistically significant difference. Statistical analysis was

performed using repeated-measures ANOVA (treatment= independent factor, time= independent factor, outcome variable= dependent factor), one-way ANOVA analysis of variance (treatment= independent factor, time= dependent factor) and least significance difference (LSD) test. Mauchly's test of sphericity was used to evaluate whether the sphericity assumption was violated. When the probability of Mauchly's test statistic was greater than or equal to ($p > 0.05$), we failed to reject the null hypothesis that the variances were equal. Therefore, we concluded that the assumption was not violated. In instances where Mauchly's test was significant, modifications were needed to be made to the degrees of freedom so that a valid F-ratio was obtained. We used Greenhouse-Geisser correction in these cases. All parameters were expressed as mean ± SEM.

Results

Overall observations. All dogs completed the study without mortality. After 40 min of LPS injection, typical clinical signs of sepsis including vomiting and lethargy were observed. Rectal temperature (T), heart rate (HR) and respiratory rate (RR) were increased following LPS injection in the control group ($p < 0.01$ vs. baseline). Hydrocortisone attenuated the T increase by 82.00% (360 min, $p < 0.05$ vs. control) and silymarin had no significant effect on T ($p > 0.05$ vs. control), (Fig. 1). Both two treatment groups had no significant effect on HR and RR ($p > 0.05$ vs. control), (Table 1).

Hematological findings. A significant increase of RBC (1.05 fold, $p < 0.001$; 1.37 fold, $p < 0.01$), Hb (88.00%, $p < 0.001$; 1.01 fold, $p = 0.05$), and HCT (1.06 fold, $p < 0.05$; 1.88 fold, $p < 0.01$) was noted in silymarin group, compared with hydrocortisone and control group at 360 min, respectively. Hydrocortisone had no significant effect on these parameters, compared with control group (Fig. 1). Following LPS injection in control group, MCV was decreased and MCHC was increased ($p < 0.05$ vs. baseline, for both). Silymarin increased MCV and decreased MCHC by maximum 18.00% and 30.00%, at 360 min, respectively ($p < 0.05$ for both vs. control). Hydrocortisone had not significant effects on these parameters compared to the control group (Table 1). Severe leukopenia ($p < 0.01$ vs. baseline) was observed after 60 min of LPS injection followed by a rapid increase until the experiment ended at 360 min. The major leukocyte component that was decreased in the blood was granulocytes (particularly neutrophils), while, the proportion of monocytes has stayed relatively constant. A significant decrease in WBC and granulocyte was observed in silymarin ($p < 0.001$ for both vs. control), and hydrocortisone group ($p < 0.001$ for both vs. control). The effectiveness of silymarin in reducing WBC and granulocyte was more than that of hydrocortisone ($p < 0.01$ for both), (Fig. 2).

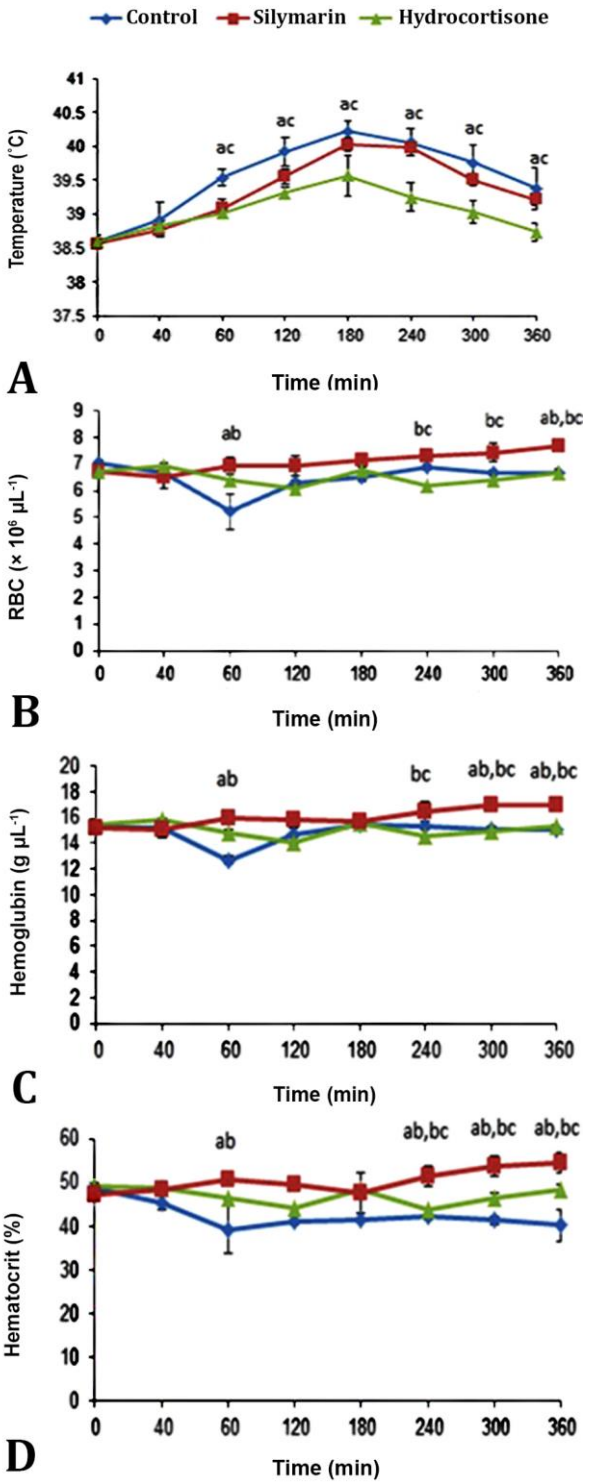

Fig. 1. Changes in **A)** Total white blood cells (WBC), **B)** Granulocytes, **C)** Lymphocytes, and **D)** Monocytes in control, silymarin, and hydrocortisone groups in a low-dose canine LPS-induced sepsis model. Letters shows a significant difference between groups including control and silymarin (ab); control and hydrocortisone (ac); silymarin and hydrocortisone (bc). Bars indicate SEM.

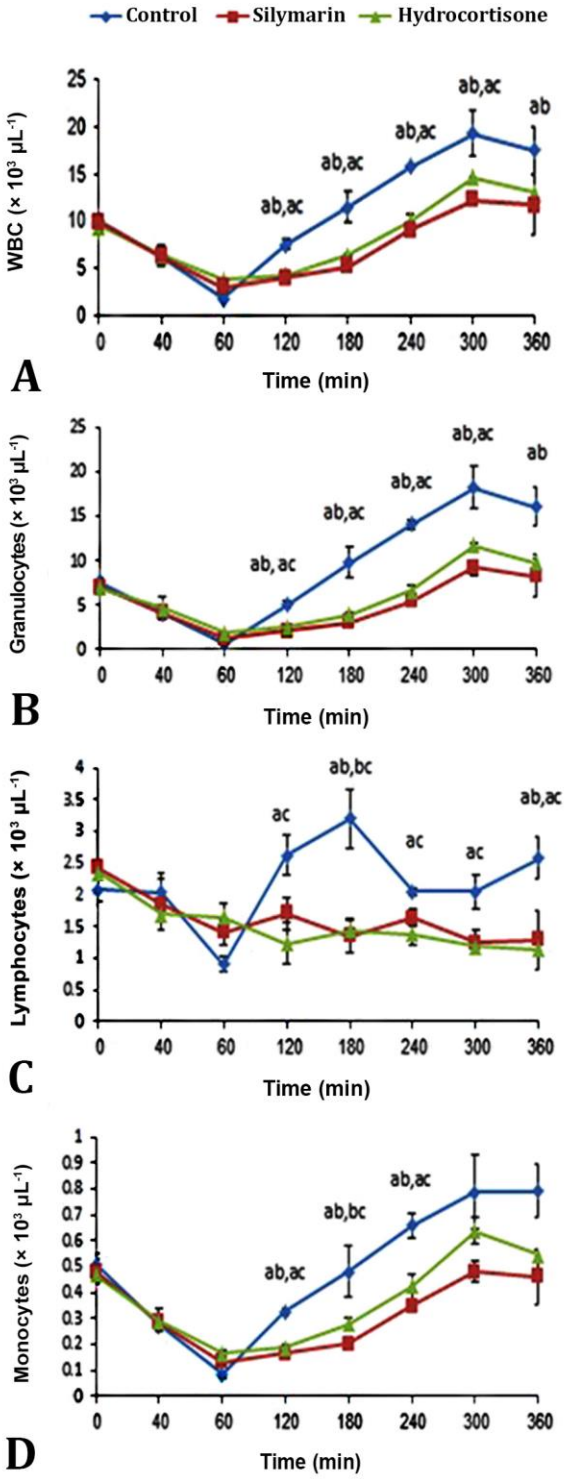

Fig. 2. Changes in **A)** Total white blood cells (WBC), **B)** Granulocytes, **C)** Lymphocytes, and **D)** Monocytes in control, silymarin, and hydrocortisone groups in a low-dose canine LPS-induced sepsis model. Letters shows a significant difference between groups including control and silymarin (ab); control and hydrocortisone (ac); silymarin and hydrocortisone (bc). Bars indicate SEM.

Biochemical parameters. Treatment and time-treatment interactions were not significant for total protein, albumin, glucose, and ALT levels in any of the groups (Table 1). Serum activity of AST, ALP, CK-MB, LDH, and cTnI were increased in the control group following low-dose canine LPS-induced sepsis ($p < 0.05$ vs. baseline).

In comparison between treatment groups and their effects on hepatic enzymes, silymarin decreased AST by (16.00%, $p < 0.05$; 29.00%, $p < 0.01$), and ALP by (1.05 fold, $p < 0.001$; 84.00%, $p = 0.001$), when compared to hydrocortisone and control group, respectively, at 180 min. In contrast, the hydrocortisone had no significant effect on AST and ALP versus the control group ($p > 0.05$), (Fig. 3).

In comparison between treatment groups and their effect on cardiac enzymes, we observed decreased levels of LDH (33.00%, $p < 0.05$; 39.00%, $p < 0.01$; 360 min), CK-MB (27.00%, $p < 0.001$; 35.00%, $p < 0.001$; 180 min), and Troponin-I (69.00%, $p < 0.001$; 75.00%, $p < 0.001$; 360 min) in silymarin group compared to hydrocortisone and control group, respectively (Fig. 4).

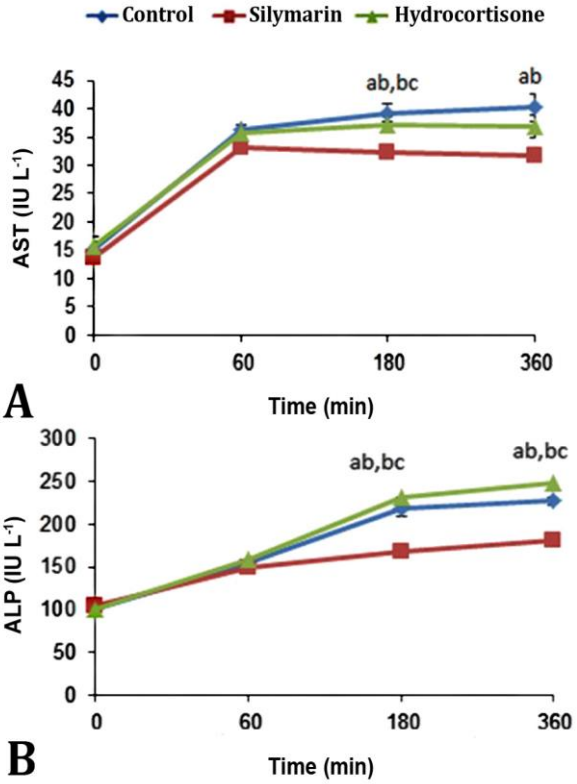

Fig. 3. Changes in **A)** aspartate aminotransferase (AST) and **B)** alkaline phosphatase (ALP) in control, silymarin, and hydrocortisone groups in a low-dose canine LPS-induced sepsis model. Lowercase letters show a significant difference between groups including control and silymarin (ab), control and hydrocortisone (ac), and silymarin and hydrocortisone (bc). Bars indicate SEM.

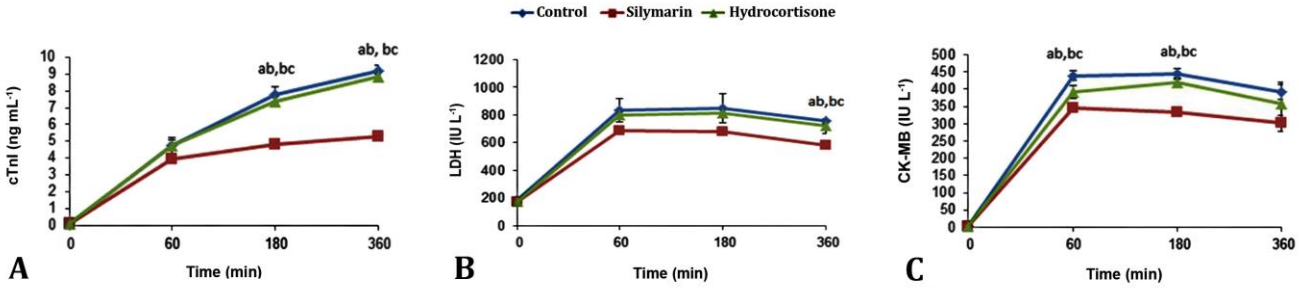

Fig. 4. Changes in **A)** cardiac troponin I (cTnI), **B)** lactate dehydrogenase (LDH), and **C)** creatine kinase-MB (CK-MB) in control, silymarin and hydrocortisone groups in a low-dose canine LPS-induced sepsis model. Lowercase letters show a significant difference between groups including control and silymarin (ab), control and hydrocortisone (ac), and silymarin and hydrocortisone (bc). Bars indicate SEM.

Table 1. Effects of different interventions on a low-dose canine LPS-induced sepsis model. The *p*-values correspond to comparisons between the groups. Values are expressed as mean ± SEM.

Parameters	Control	Silymarin	Hydrocortisone	*p*-value
Temperature (˚C)	39.50 ± 0.20 [c]	39.30 ± 0.05	39.00 ± 0.10 [a]	$p = 0.040$
Heart rate (Beats per min)	113.40 ± 3.10	106.60 ± 2.80	110.5 ± 0.50	$p = 0.180$
Respiratory rate (Breath per min)	20.90 ± 1.00	19.30 ± 1.70	21.40 ± 0.10	$p = 0.448$
WBC (× 10^3 µL^{-1})	11.20 ± 0.40 [bc]	7.70 ± 0.50 [a]	8.50 ± 0.20 [a]	$p < 0.001$
Granulocyte (× 10^3 µL^{-1})	9.40 ± 0.40 [bc]	5.00 ± 0.40 [a]	6.00 ± 0.20 [a]	$p < 0.001$
Lymphocyte (× 10^3 µL^{-1})	2.20 ± 0.05	1.60 ± 0.20	1.50 ± 0.10	$p = 0.106$
Monocyte (× 10^3 µL^{-1})	0.50 ± 0.30 [bc]	0.32 ± 0.02 [a]	0.37 ± 0.01 [a]	$p = 0.017$
PLT (× 10^3 µL^{-1})	232.40 ± 12.90	253.20 ± 45.50	276.30 ± 26.10	$p = 0.621$
RBC (× 10^6 µL^{-1})	6.50 ± 0.10 [b]	7.10 ± 0.20 [ac]	6.50 ± 0.10 [b]	$p = 0.05$
Hb (g µL^{-1})	14.80 ± 0.10 [b]	16.00 ± 0.40 [ac]	15.00 ± 0.20 [b]	$p = 0.019$
HCT (%)	42.40 ± 1.00 [b]	50.40 ± 1.20 [ac]	46.90 ± 0.50 [b]	$p < 0.001$
MCV (fl)	65.50 ± 0.90	72.90 ± 2.60	68.70 ± 0.30	$p = 0.025$
MCH (pg)	23.20 ± 0.70	22.90 ± 0.60	23.10 ± 0.10	$p = 0.903$
MCHC (g µL^{-1})	35.70 ± 1.20	31.90 ± 0.50	34.30 ± 0.01	$p = 0.01$
Glucose (mg µL^{-1})	71.70 ± 5.30	80.70 ± 5.00	77.20 ± 3.40	$p = 0.416$
Protein (g µL^{-1})	7.00 ± 0.20	6.40 ± 0.20	6.90 ± 0.10	$p = 0.101$
Albumin (g µL^{-1})	2.90 ± 0.10	2.70 ± 0.10	3.10 ± 0.20	$p = 0.107$
ALT (IU L^{-1})	33.60 ± 0.40	32.50 ± 0.30	33.50 ± 0.50	$p = 0.172$
AST (IU L^{-1})	32.70 ± 0.80 [b]	27.80 ± 0.40 [ac]	31.30 ± 0.80 [b]	$p = 0.001$
ALP (IU L^{-1})	175.30 ± 3.30 [b]	150.80 ± 2.80 [ac]	184.60 ± 3.60 [b]	$p < 0.001$
LDH (IU L^{-1})	655.70 ± 47.60 [b]	530.50 ± 9.50 [ac]	627.50 ± 23.40 [b]	$p = 0.036$
CK-MB (IU L^{-1})	320.00 ± 7.70 [b]	246.00 ± 7.20 [ac]	293.60 ± 15.20 [b]	$p = 0.001$
Troponin I (ng mL^{-1})	5.50 ± 0.30 [b]	3.50 ± 0.10 [ac]	5.30 ± 0.20 [b]	$p < 0.001$

[abc] Superscript letters show a significant difference in each column.

Discussion

In this study, levels of RBC, Hb, HCT, and MCV were significantly higher in the silymarin group in comparison with hydrocortisone and control group ($p < 0.05$).

Roozbeh *et al.* evaluated the effects of silymarin and/or vitamin E on oxidative stress markers and Hb levels. Their results demonstrated that oral supplementation with silymarin alone or in combination with vitamin E led to a decrease in plasma malondialdehyde (MDA) levels, an increase in the level of RBC glutathione peroxidase (GPx), and an increase in Hb levels.[33] Adhikari and Arora indicated that silymarin augmented the proliferation of blood cells and modulated the immune responses. They measured the erythrocyte count, Hb, HCT, and total leukocyte count. Silymarin oral administration pretreatment increased the hematological constituents of blood.[34] Our results in agreement with these studies showed the silymarin effectiveness in protecting RBCs and suggested that silymarin supplementation improved oxygen-delivery to organs.

According to the present study, silymarin significantly decreased AST, ALP, LDH, CK-MB, and cTnI in comparison with the hydrocortisone group ($p < 0.05$), while, hydrocortisone had no significant effect on these parameters compared to control group ($p < 0.05$). These findings supported that silymarin decreased organ (heart and liver) injury in the present study.

Silymarin is known to protect some organs including the brain,[35,36] liver,[21-26] kidney[37,38], and gastrointestinal tract.[39] Silymarin has afforded cardiac protection against hyperglycemia-induced apoptosis in cardiomyocytes and

also reduced AST, ALT, and the AST/ALT ratio in rats with alloxan-induced diabetes.[40] In agreement with our results, serum cTnI and CK-MB, indicators of cardiac lesions, were reduced by silymarin in an Acrolein (a highly reactive aldehyde)-induced cardiac disease in mice.[41] Silymarin also counteracted the increase in the cardiac enzymes and cTnI concentration induced by cisplatin, toward near-normal levels.[42] Rao and Viswanath reported that administration of silymarin before ischemia–reperfusion-induced myocardial infarction maintained the levels of marker enzymes (LDH, CK, and CK-MB) compared to isoproterenol-injected rats.[43] Silymarin has demonstrated combined effectiveness in improving liver and myocardial injury.[44] Silymarin prevented a large part of the damage that occurred to ischemic organs upon its reperfusion.[27]

An ideal therapeutic agent for sepsis should be able to preserve tissues from multiple insults, ultimately preserving organ and system function and increasing survival.[17] Although hydrocortisone (2.00 mg kg^{-1}) attenuated the temperature and WBC was increased significantly compared to the control group in the present study, it did not fulfill the above criteria. On the other hand, silymarin showed significant RBC protection properties, which led to oxygen-delivery improvement and reperfusion and protected liver and heart from ischemic damage caused by sepsis. Here in this study, the effects of hydrocortisone in low-dose canine sepsis models were investigated for the first time and we also compared its effects with modern flavonoid therapy, silymarin. Our results were in agreement with studies in animals and humans that showed relations between usage of silymarin in sepsis and improvement in overall health, suggesting the potential effectiveness of silymarin for sepsis. In conclusion, these findings supported the hypothesis that silymarin is a modern flavonoid therapy and could be more effective than routine corticosteroid therapy in sepsis.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Rau S, Kohn B, Richter C, et al. Plasma interleukin-6 response is predictive for severity and mortality in canine systemic inflammatory response syndrome and sepsis. Vet Clin Pathol 2007; 36(3): 253-260.
2. Kenney EM, Rozanski EA, Rush JE, et al. Association between outcome and organ system dysfunction in dogs with sepsis: 114 cases (2003-2007). J Am Vet Med Assoc 2010; 236(1): 83-87.
3. Hauptman JG, Walshaw R, Olivier NB. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. Vet Surg 1997; 26(5): 393-397.
4. Silverstein D. Systemic inflammatory response syndrome and sepsis, Part 1: Recognition and Diagnosis. Today's Vet Prac 2015; 5: 38-44.
5. Baskurt OK, Gelmont D, Meiselman HJ. Red blood cell deformability in sepsis. Am J Respir Crit Care Med 1998; 157(2): 421-427.
6. Goyette RE, Key NS, Ely EW. Hematologic changes in sepsis and their therapeutic implications. Semin Respir Crit Care Med 2004; 25(6): 645-659.
7. Pattanaik U, Prasad K. Reactive oxygen species and endotoxic shock: effect of dimethylthiourea. J Cardiovasc Pharmacol Ther 2001; 6(3): 273-285.
8. Bone RC. Gram-negative sepsis. Background, clinical features, and intervention. Chest 1991; 100(3): 802-808.
9. Greiner M, Wolf G, Hartmann K. A retrospective study of the clinical presentation of 140 dogs and 39 cats with bacteremia. J Small Anim Pract 2008; 49(8): 378-383.
10. Cai B, Deitch EA, Ulloa L. Novel insights for systemic inflammation in sepsis and hemorrhage. Mediators Inflamm 2010; 642462, doi: 10.1155/2010/642462.
11. Yu DH, Kim B, Park J. Pathophysiologic and immunologic changes in a canine endotoxemia over a period of 24 hours. J Vet Med Sci 2012; 74(5): 537-544.
12. De Vries F, Leuschner J, Jilma B, et al. Establishment of a low-dose canine endotoxemia model to test anti-inflammatory drugs: Effects of prednisolone. Int J Immunopathol Pharmacol 2013; 26(4): 861-869.
13. Annane D. The role of ACTH and corticosteroids for sepsis and septic shock: An update. Front Endocrinol (Lausanne) 2016; 20(7): 70. doi: 10.3389/fendo. 2016.00070.
14. Schimmer BP, Parker KL. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones. In Goodman & Gilman's the pharmacological basis of therapeutics. 11th ed. Brunton L, Lazo J, Parker K (Eds). New York, USA: McGraw-Hill 2006; 1649-1677.
15. Anagnostis P, Athyros VG, Tziomalos K, et al. Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: A hypothesis. J Clin Endocrinol Metab 2009; 94(8): 2692-2701.
16. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments, and mechanistic insights. Mol Cell Endocrinol 2011; 335(1): 2-13.
17. Shapiro H, Lev S, Cohen J, et al. Polyphenols in the prevention and treatment of sepsis syndromes:

Rationale and pre-clinical evidence. Nutrition 2009; 25(10): 981-997.
18. Rainone F. Milk thistle. Am Fam Physician 2005; 72(7): 1285-1288.
19. Kroll DJ, Shaw HS, Oberlies NH. Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. Integr Cancer Ther 2007; 6(2): 110-119.
20. Sherif IO, Al-Gayyar MMH. Antioxidant, anti-inflammatory and hepatoprotective effects of silymarin on hepatic dysfunction induced by sodium nitrite. Eur Cytokine Netw 2013; 24(3): 114-121.
21. Nanda V, Gupta V, Sharma SN, et al. Effect of Liverubin™ on hepatic biochemical profile in patients of alcoholic liver disease: A retrospective study. Minerva Med 2014; 105(6 Suppl 2):1-8.
22. Lucena MI, Andrade RJ, de la Cruz JP, et al. Effects of silymarin MZ-80 on oxidative stress in patients with alcoholic cirrhosis. Results of a randomized, double-blind, placebo-controlled clinical study. Int J Clin Pharmacol Ther 2002; 40(1): 2-8.
23. Mengs U, Pohl RT, Mitchell T. Legalon® SIL: The antidote of choice in patients with acute hepatotoxicity from amatoxin poisoning. Curr Pharm Biotechnol 2012; 13(10): 1964-1970.
24. Fried MW, Navarro VJ, Afdhal N, et al. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: A randomized controlled trial. J Am Med Assoc 2012; 308(3): 274-282.
25. Lo D, Wang YT, Wu MC. Hepatoprotective effect of silymarin on di(2-ethylhexyl) phthalate (DEHP) induced injury in liver FL83B cells. Environ Toxicol Pharmacol 2014; 38(1): 112-118.
26. Srivastava RK, Sharma S, Verma S, et al. Influence of diabetes on liver injury induced by antitubercular drugs and on silymarin hepatoprotection in rats. Methods Find Exp Clin Pharmacol 2008; 30(10): 731-737.
27. Zholobenko A, Modriansky M. Silymarin and its constituents in cardiac preconditioning. Fitoterapia 2014; 97: 122-132.
28. Kang JS, Jeon YJ, Park SK, et al. Protection against lipopolysaccharide-induced sepsis and inhibition of interleukin-1beta and prostaglandin E_2 synthesis by silymarin. Biochem Pharmacol 2004; 67(1): 175-181.
29. Toklu HZ, Akbay TT, Velioglu-Ogunc A, et al. Silymarin, the antioxidant component of *Silybum marianum*, prevents sepsis -induced acute lung and brain injury. J Surg Res 2008; 145(2): 214-222.
30. Varzi HN, Esmailzadeh S, Morovvati H, et al. Effect of silymarin and vitamin E on gentamicin-induced nephrotoxicity in dogs. J Vet Pharmacol Ther 2007; 30(5): 477-481.
31. Annane D. Corticosteroids for septic shock. Crit Care Med 2001; 29(7 Suppl): S117-S120.
32. Papich MG. Saunders handbook of veterinary drugs: Small and large animal. 4th ed. Philadelphia, USA: Saunders 2016; 382.
33. Roozbeh J, Shahriyari B, Akmali M, et al. Comparative effects of silymarin and vitamin E supplementation on oxidative stress markers, and hemoglobin levels among patients on hemodialysis. Ren Fail 2011; 33(2): 118-123.
34. Adhikari M, Arora R. The flavonolignan-silymarin protects enzymatic, hematological, and immune system against γ-radiation-induced toxicity. Environ Toxicol 2016; 31(6): 641-654.
35. Wang PY, Kaneko T, Tsukada H, et al. Time courses of hepatic injuries induced by chloroform and by carbon tetrachloride: Comparison of biochemical and histo-pathological changes. Arch Toxicol 1997; 71(10): 638-645.
36. Hou YC, Liou KT, Chern CM, et al. Preventive effect of silymarin in cerebral ischemia–reperfusion-induced brain injury in rats possibly through impairing NF-κB and STAT-1 activation. Phytomedicine 2010; 17(12): 963-973.
37. Turgut F, Bayrak O, Catal F, et al. Antioxidant and protective effects of silymarin on ischemia and reperfusion injury in the kidney tissues of rats. Int Urol Nephrol 2008; 40(2): 453-460.
38. Senturk H, Kabay S, Bayramoglu G, et al. Silymarin attenuates the renal ischemia/reperfusion injury-induced morphological changes in the rat kidney. World J Urol 2008; 26(4): 401-407.
39. Alarcón de la Lastra AC, Martín MJ, Motilva V, et al. Gastroprotection induced by silymarin, the hepatoprotective principle of *Silybum marianum* in ischemia-reperfusion mucosal injury: Role of neutrophils. Planta Med 1995; 61(2): 116-119.
40. Tuorkey MJ, El-Desouki NI, Kamel RA. Cytoprotective effect of silymarin against diabetes-induced cardio-myocyte apoptosis in diabetic rats. Biomed Environ Sci 2015; 28(1): 36-43.
41. Taghiabadi E, Imenshahidi M, Abnous K, et al. Protective effect of silymarin against acrolein-induced cardiotoxicity in mice. Evid Based Complement Alternat Med 2012; 2012: 352091. doi: 10.1155/2012/352091.
42. El-Awady El-SE, Moustafa YM, Abo-Elmatty DM, et al. Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies. Eur J Pharmacol 2011; 650(1): 335-341.
43. Rao PR, Viswanath RK. Cardioprotective activity of silymarin in ischemia– reperfusion-induced myocardial infarction in albino rats. Exp Clin Cardiol 2007; 12(4), 179-187.
44. Salamone F, Galvano F, Gammazza AM, et al. Silibinin improves hepatic and myocardial injury in mice with nonalcoholic steatohepatitis. Dig Liver Dis 2012; 44(4): 334-342.