

The Therapeutic Effects of Quercetin in a Canine Model of Low-dose Lipopolysaccharide-Induced Sepsis Compared with Hydrocortisone

Alale Soltanian^{1*}, Bahman Mosallanejad², Mohammad Razi Jalali², Hossein Najafzadeh Varzi³, Masoud Ghorbanpour Najafabadi⁴

¹Graduated from the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

²Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

³Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

⁴Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Abstract

BACKGROUND: Canine low-dose sepsis model provides a reliable setting to study innovative drugs. Lipopolysaccharides (LPS), a major constituent of bacterial outer membrane, have been demonstrated to play a critical role in the initiation of pathogenesis. Lipopolysaccharide-induced sepsis has been extensively studied in laboratory animals; but its importance has mainly remained unknown in dogs.

OBJECTIVES: The aim of the present survey was to examine the effectiveness of quercetin, along with hydrocortisone on clinical and hematological alterations, and organ failure (liver and heart) in low-dose lipopolysaccharide-induced canine sepsis model.

METHODS: For this purpose, fifteen clinically healthy mixed dogs were randomly divided into three equal groups. Lipopolysaccharide (0.1 µg/kg, IV) was injected to dogs in group A (control). Group B was similar to group A, but quercetin bolus (2 mg/kg, IV, once) was injected 40 minutes after LPS injection. Group C was similar to group B; however, hydrocortisone bolus (2 mg/kg, IV, once) was administered instead of quercetin. Serum levels of glucose, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase isoenzyme muscle/brain (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin I (cTn-I) concentration were measured by commercial kits.

RESULTS: In control group, red blood cells (RBCs), hemoglobin (Hb), and hematocrit (HCT) significantly decreased and serum activities of AST, ALP, LDH, CK-MB, and plasma cTn-I significantly increased ($P < 0.05$). RBCs, Hb, and HCT significantly increased in quercetin group, compared with hydrocortisone and control groups ($P < 0.05$). Quercetin group significantly decreased LDH, CK-MB, and cTn-I compared with hydrocortisone and control groups ($P < 0.05$). Quercetin significantly decreased AST in comparison to control group and ALP in comparison to hydrocortisone group, also ($P < 0.05$).

CONCLUSIONS: These results suggest that quercetin protects RBCs in the early stages of sepsis and decreases organs dysfunction (heart and liver), therefore it has a positive influence on sepsis and may be more effective than routine corticosteroid (hydrocortisone) therapy.

KEYWORDS: Dog, hydrocortisone, lipopolysaccharide, quercetin, sepsis

Correspondence

Alale Soltanian, Graduated from the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
Tel: +98(51) 36010812, Fax: +98(51)36010812, Email: alale.soltani@gmail.com

Received: 2019-05-11

Accepted: 2019-08-14

Copyright © 2019. This is an open-access article distributed under the terms of the Creative Commons Attribution- 4.0 International License which permits Share, copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, even commercially.

How to Cite This Article

Soltanian, A., Mosallanejad, B., Razi Jalali, M., Najafzade Varzi, H., & Ghorbanpour Najafabadi, M. (2019). The Therapeutic Effects of Quercetin in a Canine Model of Low-dose Lipopolysaccharide-induced Sepsis Compared with Hydrocortisone. Iranian Journal of Veterinary Medicine, 13(4), 377-387.

Introduction

Sepsis is the clinical manifestation of systemic inflammatory response syndrome (SIRS) secondary to an underlying pathogenic organism. The clinical criteria that provide the best sensitivity and specificity in diagnosis of SIRS in septic dogs include temperature lower than 38.1 °C or higher than 39.2 °C, heart beat higher than 120 beats/min, respiratory rate higher than 20 breaths/min, white blood cells lower than $6 \times 10^3 /\mu\text{l}$ or higher than $16 \times 10^3 /\mu\text{l}$, and band cell higher than 3 percent (Balk, 2014). In the early stages of sepsis, red blood cell rigidity will occur because of the damage to the membrane proteins, due to reactive oxygen species (ROS), generated by inflammatory cells and ischemic tissues (Goyette et al., 2004). Decreased RBC deformability reduces blood flow and increases the required time for cells to transit the microcirculation. It also has a negative effect on oxygen delivery and contributes to organ dysfunction outside of hematologic system (Baskurt et al., 1998).

The dog is a commonly used animal model for pharmacokinetic and metabolic studies. Canine low-dose sepsis model provides a reliable setting to test new drugs before application in human or setting to study innovative drugs for use in dogs (De Vries et al., 2013). Considering that sepsis is an uncontrolled inflammatory response, it should be expected that the most appropriate treatment would be anti-inflammatory therapies such as pharmacologic doses of corticosteroids (Volbeda et al., 2015). Activation of multiple stress signaling processes such as oxidative stress and mitogen-activated protein kinases play pivotal roles in the pathogenesis of septic dysfunction. Nowadays, novel antisepsis strategies are being clinically assessed for the more effective treatment of sepsis (Pe-

nalva et al., 2017).

Flavonoids, a group of naturally available small molecules, have been known as a treatment of sepsis or acute systemic inflammation (Liao and Lin, 2015). One of these flavonoids is quercetin (Q) [3,3',4',5,7-pentahydroxyflavone], which is found in green and black tea, red wine, citrus fruit (i.e. apple), onions and some vegetables (Penalva et al., 2017). Quercetin possesses a broad range of pharmacological properties, that can act as a potent antimicrobial, anti-inflammatory, anti-oxidant, anti-allergic, anti-atherosclerotic, anti-diabetic, anti-proliferative, anti-mutagenic and anti-carcinogenic agent (Valentova et al., 2016; Afshari et al., 2018; Yao et al., 2018).

Among numerous members of the flavonoid family, quercetin has been recognized as the most powerful antioxidant. Quercetin protects RBCs against oxidative stress and displays an important structural feature responsible for free radical scavenging activity. It also possesses immunomodulatory activities against LPS-treated mouse peritoneal macrophages in sepsis model (Liao and Lin, 2015). Quercetin is an effective therapeutic strategy in the treatment of patients with liver damage and fibrosis (Wan et al., 2014). It has also been experimentally used in the treatment of ischemia and myocarditis (Javadi and Sahebkar, 2017). The discovery of new natural products extracted from medicinal plants or compounds derived from them, such as quercetin, hesperidin, vitamin C, horse chestnut extract and selenium can represent a valuable source of new medicinal agents for treatment of infectious diseases in dogs (Virginia et al., 2017).

To date, only a few studies have investigated bioavailability of flavonoids in dogs.

To the best of our knowledge, there is no research about the intravenous injection of quercetin to a large animal model of sepsis, and this research provides useful information regarding the therapeutic potential in sepsis. Moreover, studies comparing the efficacy of quercetin and hydrocortisone are lacking in canine sepsis, and clinical experience is limited. Therefore, the objective of the present survey was to evaluate the effects of quercetin in a canine model of low-dose lipopolysaccharide-induced sepsis. In this paper, we compared the therapeutics (anti-inflammatory) effects of quercetin with hydrocortisone in dog. Hematological changes, liver and heart enzymes abnormalities, and clinical signs were studied also.

Materials and Methods

Animals

Fifteen adult Mixed breed dogs were used with the age of 12-24 months, both sexes (eight female and seven male), and weighing 17-22 kg. All dogs appeared healthy, as determined by clinical examination, normal hemogram and biochemical profiles. The dogs were kept separately in a controlled environment at 22-25 °C. They were fed with a home-made diet. All procedures which might be associated with discomfort including venipuncture and administration of LPS were performed by an experienced veterinarian. This study was approved by the Animal Care and Research Committee of Shahid Chamran University of Ahvaz, Iran. The dogs were vaccinated against DHPPiL and Rabies. Antiparasite tablets (Caniverm) were given and Ivermectin was also administered to all animals. They were hospitalized and fasted for 12 h before the study. Water was available ad libitum. Determination of the age was accomplished based on the dental formulary.

Experimental protocol

To induce sepsis, all dogs received an IV bolus of a low-dose LPS (0.1 µg/kg, from *Escherichia coli* serotype O26: B6; Sigma, St. Louis, MO, USA), dissolved in normal saline solution (De Vries et al., 2013). Dogs were categorized into three equal groups, by simple random allocation as follows: Group A (control group) included five dogs that only LPS was injected, and no treatment was performed. Group B was similar to group A; but quercetin bolus (2 mg/kg, single dose, IV; Sigma-Aldrich Co., St. Louis, MO, USA), was injected 40 minutes (min) after LPS injection. Quercetin dosage was selected based on Reinboth et al.'s (2010) study. Group C was similar to group B; however, hydrocortisone bolus (2 mg/kg single dose, IV) was administrated instead of quercetin. Hydrocortisone dosage was in the pharmacologic range (Rochweg et al., 2018). The time of 40 minutes was selected based on a pilot study. When vomiting, metoclopramide (0.5 mg/kg single dose and IM) was administered to the affected dogs of three groups. Rectal temperature (T), heart rate (HR), and respiratory rate (RR) were measured in all dogs. The conjunctiva of the eye and mouth were also examined during challenge.

Collection of blood and serum samples

Blood samples were collected from the cephalic or jugular vein. One ml of blood was poured into potassium EDTA-treated tubes (Becton Dickinson, Franklin Lakes, NJ, USA), at baseline, 40, 60, 120, 180, 240, 300, and 360 minutes to evaluate complete blood count (CBC) using an automatic impedance cell counter (Mindray 2800-vet, Montpellier, France). Moreover, five ml of blood were collected at baseline, 1, 3, and 6 h and poured into the non-heparinized tubes. Serum was obtained using centrifugation at

2500 rpm for 10 min after keeping blood for 20 min at room temperature. Serum levels of glucose, total protein, and albumin; ALT, AST, ALP, CK-MB, and LDH were measured by commercial kits using an auto-analyzer (BT-TARGA-3000 model). Moreover, the cTn-I concentration was measured by commercial kits according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was conducted using SPSS for Windows (release 24, IBM Inc., 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, one-way ANOVA analysis of variance and least significance difference (LSD) test. All information was expressed as Mean±SEM.

Results

Overall: All dogs completed the study without mortality. Typical clinical signs of sepsis including vomiting and lethargy were mainly observed after 40 min of LPS (0.1 µg/kg, IV) injection. Therefore, the time of forty min after injection, was selected as the starting time of treatment. Rectal temperature, heart rate, and respiratory rate were increased following LPS injection in control group ($P<0.01$ vs. baseline). Hydrocortisone increased the temperature (360 min, $P<0.05$ vs. control); but quercetin reduced temperature (300 min, $P<0.05$ vs. control). Quercetin also increased the heart rate (180 min, $P<0.05$ vs. control). Hydrocortisone had no significant effect on heart rate ($P>0.05$ vs. control). Both treatment groups had no significant effect on respiratory rate ($P>0.05$ vs. control) (Table 1).

Hematologic parameters: Severe leukopenia ($P<0.01$ vs. baseline) was observed

after 60 min of LPS injection, followed by a rapid decrease until the experiment ended at 360 min. The major leukocyte component that decreased in the blood was granulocytes (particularly neutrophils); while the proportion of monocytes remained relatively constant. Lymphocytes increased at 60 min ($P<0.01$ vs. baseline) and then decreased rapidly. A significant decrease in WBC and granulocyte was observed in hydrocortisone group ($P<0.01$ vs. control). Quercetin had no significant effect on WBC and granulocytes ($P>0.05$ vs. control).

We noticed a significant increase of RBC ($P<0.001$), Hb ($P<0.001$), and HCT ($P<0.05$) in quercetin group, compared with hydrocortisone group at 360 min. Hydrocortisone had no significant effect on these parameters, compared with control group ($P>0.05$) (Table 1).

Biochemical parameters: Changes for total protein, albumin, glucose, and ALT levels were not significant in any of the groups. Serum activity of AST, ALP, CK-MB, LDH, and cTn-I increased in control group following LPS injection ($P<0.05$ vs. baseline). In comparison between treatment groups and their effects on hepatic enzymes, quercetin decreased ALP ($P<0.05$), in comparison to hydrocortisone group at 360 min. Hydrocortisone had no significant effect on AST and ALP, versus control group ($P>0.05$).

Regarding cardiac enzymes, we observed decreased levels of LDH (at 360 min, $P<0.01$), CK-MB (at 180 min, $P<0.001$), and cTn-I (at 360 min, $P<0.001$) in quercetin group, compared with hydrocortisone group (Table 1). Hydrocortisone had no significant effect on LDH, CK-MB, and cTn-I versus control group ($P>0.05$).

Table 1. Different parameters (Mean±SEM) in three groups of control (0.1 µg/kg, IV), quercetin (2 mg/kg, IV) and hydrocortisone (2 mg/kg, IV) in the studied dogs.

Groups Parameters	Control	Quercetin	Hydrocortisone	P-value
Temperature (°C)	39.5 ± 0.2 ^a	39.1 ± 0.1 ^b	39 ± 0.1 ^b	p=0.031
Heart rate (beats/min)	113.4 ± 3.1 ^a	101.3 ± 2.2 ^b	110.5 ± 0.5 ^{ac}	p=0.005
Respiratory rate (breath/min)	20.9 ± 1 ^a	20.1 ± 1.9 ^a	21.4 ± 0.1 ^a	p=0.776
WBC (x 10 ³ /µl)	11.2 ± 0.4 ^a	10.1 ± 1.5 ^a	8.5 ± 0.2 ^b	p=0.001
Granulocyte (x 10 ³ /µl)	75.6 ± 1.3 ^a	72.1 ± 1.7 ^a	65.8 ± 0.5 ^b	p=0.001
Lymphocyte (x 10 ³ /µl)	25.1 ± 0.2 ^a	23.4 ± 2.5 ^a	21.9 ± 1.4 ^a	p=0.414
Monocyte (x 10 ³ /ul)	4.4 ± 0.1 ^a	4.3 ± 0.1 ^a	4.4 ± 0.1 ^a	p=0.788
PLT (x 10 ³ /µl)	232.4 ± 12.9 ^a	252.7 ± 19 ^a	276.3 ± 26.1 ^a	p=0.336
RBC (x 10 ⁶ /µl)	6.5 ± 0.1 ^a	7.4 ± 0.2 ^b	6.5 ± 0.1 ^a	p=0.004
Hb (g/dl)	14.8 ± 0.1 ^a	17.7 ± 0.6 ^b	15 ± 0.2 ^a	p<0.001
HCT (%)	42.4 ± 1 ^a	54.9 ± 1.5 ^b	46.9 ± 0.5 ^a	p<0.001
MCV (fl)	65.5 ± 0.9 ^a	74.6 ± 1 ^b	68.7 ± 0.3 ^a	p<0.001
MCH (pg)	23.2 ± 0.7 ^a	23.9 ± 0.28 ^a	23.1 ± 0.1 ^a	p=0.903
MCHC (g/dl)	35.7 ± 1.2 ^a	32.2 ± 0.3 ^b	34.3 ± 0.01 ^a	p=0.013
Glucose (mg/dl)	71.7 ± 5.3 ^a	77.5 ± 4.5 ^a	77.2 ± 3.4 ^a	p=0.628
Protein (g/dl)	7 ± 0.2 ^a	6.6 ± 0.3 ^a	6.9 ± 0.1 ^a	p=0.521
Albumin (g/dl)	2.9 ± 0.1 ^a	2.6 ± 0.1 ^a	3.1 ± 0.2 ^a	p=0.058
ALT (iu/l)	33.6 ± 0.4 ^a	33.3 ± 1.6 ^a	33.5 ± 0.5 ^a	p=0.983
AST (iu/l)	32.7 ± 0.8 ^a	28.7 ± 1.5 ^b	31.3 ± 0.8 ^a	p=0.048
ALP (iu/l)	175.3 ± 3.3 ^a	169.1 ± 3.2 ^a	184.6 ± 3.6 ^b	p=0.023
LDH (iu/l)	655.7 ± 47.6 ^a	488.45 ± 10 ^b	627.5 ± 23.4 ^a	p=0.006
CK-MB (iu/l)	320 ± 7.7 ^a	171.2 ± 2.5 ^b	293.6 ± 15.2 ^a	p<0.001
cTn-I (ng/ml)	5.5 ± 0.3 ^a	2.23 ± 0.1 ^b	5.3 ± 0.2 ^a	p<0.001

Lowercase letters show significant differences in each row.

Discussion

In the present study, we evaluated the anti-inflammatory role of quercetin compared with hydrocortisone in a low-dose canine LPS-induced sepsis model. The first aim of the research was to identify the effect of quercetin treatment on erythrocytes. Levels of RBC, Hb, and HCT were significantly higher in quercetin group compared with hydrocortisone and control groups. Erythrocytes are more prone to oxidative damage due to the presence of high levels of polyunsaturated fatty acids in their membranes and high cellular concentrations of oxygen, especially during sepsis. Alterations in the properties of individual red blood cells have the potential to diminish oxygen carrying capacity, change the rheology of the microcirculation, and reduce blood flow in sepsis (Goyette et al., 2004).

In numerous studies, protective properties of quercetin have been discussed for RBC. This flavonoid was able to protect RBC against oxidative damage (membrane lipid peroxidation and hemolysis) caused by a variety of oxidants (Kitagawa et al., 2004; Asgary et al., 2005; Jamshidzadeh and Mehrabadi, 2010; Das et al., 2013). Quercetin was also able to prevent membrane lipid peroxidation and hemolysis of mouse RBC induced by phenyl hydrazine, acrolein, divicine and isouramil. Several studies have shown that quercetin inhibits LPS induced tumor necrosis factor α production in macrophages and LPS-induced IL-8 production in lung A549 cells. Quercetin inhibits production of inflammation-producing enzymes (cyclooxygenase and lipoxygenase) (Geraets et al., 2007). Antioxidant activity of quercetin is based on its ability to directly scavenge ROSs (such as hydroxyl radical, superoxide anion, singlet oxygen, peroxy

radicals and others). It also prevents the formation of ROS through chelating of transition metal ions, and promoting the antioxidant defense system (Vasquez-Garzon et al., 2009). Our results along with these studies which have shown quercetin effectiveness in protecting RBCs, suggests that quercetin supplementation may improve oxygen-delivery to organs.

Lipopolysaccharide of gram-negative bacteria has been recognized as a causative agent in myocardial depression during sepsis. It has been shown that the inflammatory responses induced by LPS in cardiomyocytes are characterized by an increased production of free radicals which leads to the activation of transcription factors and intracellular signaling pathways and to the induction of inflammatory mediators. All mediators may be involved in the depression of cardiac, liver and kidney function (Ceylan-Isik et al., 2010). According to the present study, quercetin significantly decreased ALP, LDH, CK-MB, and cTn-I in comparison to hydrocortisone group, while hydrocortisone had no significant effect on these parameters as compared to control group. Consistent with these results, studies in animals and human suggest that quercetin reduces the risk of cardiovascular diseases and liver damage (Wan et al., 2014).

A recent study demonstrated that low to moderate oral dose of quercetin for two weeks increased plasma quercetin concentrations dose-dependently in healthy individuals, confirming its bioavailability (Egert et al., 2008). A mixture of grapefruit flavones was orally administered to adult beagle dogs. Interestingly, the measured plasma concentrations in that study were in the nanomolar range. The median maximum plasma concentrations of the most abun-

dant metabolite, naringenin, was only 238 nmol/l. Absolute bioavailability of quercetin is only 4% in dogs. As in other species like human subjects, rats and pigs, the oral bioavailability of quercetin, after the application of the quercetin monoglucoside isoquercitrin is significantly higher compared with the application of the aglycone (Reinboth et al., 2010).

Quercetin has been found to have beneficial effects on myocardial injury (Boots et al., 2008). Cardioprotective effects of quercetin were evaluated in Isoproterenol-treated rats. Their result showed that pretreatment with quercetin normalized increased levels of serum troponins and increased intensities of serum lactate dehydrogenase-1 and 2 isoenzyme bands in the studied rats due to scavenging free radicals, improving antioxidants and maintaining Ca^{2+} levels (Punithavathi and Stanely Mainzen Prince, 2011). Quercetin has the capacity to protect the myocardial tissue against global ischemia and reperfusion injury, due to its antioxidant and cyto-protective effects (Ikizler et al., 2007). In a rat model of adenine-induced chronic kidney disease, treatment with quercetin improved renal function, reduced oxidative stress factors, and kidney inflammation (Yang et al., 2018).

Quercetin exerts inflammation and immune modulating activity in several murine models of autoimmunity. Animal experiments also support an anti-inflammatory effect in vivo. Quercetin can also ameliorate various toxin-induced liver injuries, such as ethanol and acetaminophen (De David et al., 2011). Zhang et al., (2014) studied the therapeutic detoxification of quercetin against carbon tetrachloride (CCl₄) induced acute liver injury. Quercetin decreased CCl₄-increased serum activities of alanine

and aspartate aminotransferases when orally taken 30 min after CCl₄ intoxication. The results of a histological evaluation further evidenced the ability of quercetin to protect against CCl₄-induced liver injury. Wan et al., (2014) investigated the effects of quercetin on hepatitis and hepatic fibrosis induced by immunological mechanism. Treatment with quercetin significantly decreased the ALT and AST levels, and markedly attenuated the pathologic changes in the liver. Their results suggest that quercetin may be an effective therapeutic strategy in the treatment of patients with liver damage and fibrosis.

A range of clinical conditions are associated with a dysregulation of inflammatory responses. Although the most common of these is sepsis, high concentrations of cytokines are also generated by ischemia-reperfusion, trauma, acute rejection, antigen-specific immune responses, and different acute inflammatory states (Landry and Oliver, 2001). An ideal therapeutic agent for sepsis should be able to preserve tissues from multiple insults; ultimately preserving organ and system function and increasing survival (Shapiro et al., 2009). In the present study, though hydrocortisone (2 mg/kg) attenuated the temperature and WBC significantly increased compared with control group, it does not fulfill the above criteria. On the other hand, quercetin showed significant RBC protection properties, which leads to oxygen-delivery improvement and reperfusion. It also protected liver and heart from ischemic damage caused by sepsis. Here in this research, the effects of hydrocortisone have been investigated in low-dose canine sepsis model for the first time and we also compared its effects with modern flavonoid therapy, quercetin. Our results are along

with studies in animals and human which have shown relations between usage of quercetin (as a flavonoid that can be consumed in significant amounts in the daily diet) and improvement in overall health; suggesting the potential effectiveness of quercetin for sepsis. In conclusion, the obtained data confirms that quercetin as a modern flavonoid therapy may be more effective than routine corticosteroid therapy in sepsis. It protects RBCs in the early stages of sepsis and decreases organs dysfunction (heart and liver), therefore quercetin has a positive influence on sepsis. These results suggest that quercetin might serve as a valuable protective agent in inflammatory diseases. Further studies are needed to better characterize the mechanisms of action underlying the beneficial effects of quercetin on immunity in dogs.

Acknowledgments

This study was supported by research fund (Grant) of Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

- Afshari, M., Rahimmalek, M., Miroliaei, M. (2018) Variation in polyphenolic profiles, antioxidant and antimicrobial activity of different achillea species as natural sources of antiglycative compounds. *Chem Biodivers*, 15(8), e1800075. <https://doi.org/10.1002/cbdv.201800075>. PMID: 29779268.
- Asgary, S., Naderi, G.H., Askari, M.S. (2005) Protective effect of flavonoids against red blood cell hemolysis by free radicals. *Exp Clin Cardiol*, 10(2), 88-90. PMID: 19641665.
- Balk, R.A. (2014) Systemic inflammatory response syndrome (SIRS). *Virulence*, 5(1), 20-26. <https://doi.org/10.4161/viru.27135>. PMID: 24280933.
- Baskurt, O.K., Gelmont, D., Meiselman, H.J. (1998) Red blood cell deformability in sepsis. *Am J Respir Crit Care Med*, 157(2), 421-427. <https://doi.org/10.1164/ajrccm.157.2.9611103>. PMID: 9476853.
- Ceylan-Isik, A.F., Zhao, P., Zhang, B., Xiao, X., Su, G. and Ren, J. (2010) Cardiac overexpression of metallothionein rescues cardiac contractile dysfunction and endoplasmic reticulum stress but not autophagy in sepsis. *J Mol Cell Cardiol*, 48(2), 367-378. <https://doi.org/10.1016/j.yjmcc.2009.11.003>. PMID: 19914257.
- Das, D.K., Chakraborty, A., Sinha, M., Manna, K., Mukherjee, D., Chakraborty, A., Bhattacharjee, S., Dey, S. (2013) Modulatory role of quercetin against gamma radiation-mediated biochemical and morphological alterations of red blood cells. *Int J Radiat Biol*, 89(6), 471-481. <https://doi.org/10.3109/09553002.2013.767989>. PMID: 23363054.
- De David, C., Rodrigues, G., Bona, S., Meurer, L., Gonzalez-Gallego, J., Tunon, M.J. (2011) Role of quercetin in preventing thioacetamide-induced liver injury in rats. *Toxicol Pathol*, 39(6), 949-957. <https://doi.org/10.1177/0192623311418680>. PMID: 21885874.
- De Vries, F., Leuschner, J., Jilma, B., Derhaschnig, U. (2013) Establishment of a low dose canine endotoxemia model to test anti-inflammatory drugs: effects of prednisolone. *Int J Immunopathol Pharmacol*, 26(4), 861-869. <https://doi.org/10.1177/039463201302600404>. PMID: 24355221.
- Egert, S., Wolfram, S., Bosy-Westphal, A., Boesch-Saadatmandi, C., Wagner, A.E., Frank, J., Rimbach, G., Mueller, M.J. (2008) Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. *J Nutr*, 138(9), 1615-1621. <https://doi.org/10.1093/jn/138.9.1615>. PMID: 18716159.
- Geraets, L., Moonen, H., Brauers, K., Wouters, E.F., Bast, A., Hageman, G.J. (2007). Di-

- etary flavones and flavonoles are inhibitors of poly(ADP-ribose)polymerase-1 in pulmonary epithelial cells. *J Nutr*, 137(10), 2190-2195. <https://doi.org/10.1093/jn/137.10.2190>. PMID: 17884996.
- Goyette, R.E., Key, N.S., Ely, E.W. (2004) Hematologic changes in sepsis and their therapeutic implications. *Semin Respir Crit Care Med*, 25(6), 645-659. <https://doi.org/10.1016/10.1055/s-2004-860979>. PMID: 16088507.
- Ikizler, M., Erkasap, N., Dernek, S., Kural, T., Kaygisiz, Z. (2007) Dietary polyphenol quercetin protects rat hearts during reperfusion: enhanced antioxidant capacity with chronic treatment. *Anadolu Kardiyol Derg*, 7(4), 404-410. PMID: 18065337.
- Javadi, B., Sahebkar, A. (2017) Natural products with anti-inflammatory and immunomodulatory activities against autoimmune myocarditis. *Pharmacol Res*, 124, 34-42. <https://doi.org/10.1016/j.phrs.2017.07.022>. PMID: 28757189.
- Kitagawa, S., Sakamoto, H., Tano, H. (2004) Inhibitory effects of flavonoids on free radical-induced hemolysis and their oxidative effects on hemoglobin. *Chem Pharm Bull (Tokyo)*, 52(8), 999-1001. <https://doi.org/10.1248/cpb.52.999>. PMID: 15305001.
- Landry, D.W., Oliver, J.A. (2001) The pathogenesis of vasodilatory shock. *N Engl J Med*, 45(8), 588-595. <https://doi.org/10.1056/NEJMra002709>. PMID: 11529214.
- Liao, Y., Lin, J. (2015) Quercetin intraperitoneal administration ameliorates lipopolysaccharide-induced systemic inflammation in mice. *Life Sci*, 137(1), 89-97. <https://doi.org/10.1016/j.lfs.2015.07.015>. PMID: 26209141.
- Penalva, R., Gonzalez-Navarro, C.J., Gamazo, C., Esparza, I., Irache, J.M. (2017) Zein nanoparticles for oral delivery of quercetin: Pharmacokinetic studies and preventive anti-inflammatory effects in a mouse model of endotoxemia. *Nanomedicine*, 13(1), 103-110. <https://doi.org/10.1016/j.nano.2016.08.033>. PMID: 27615118.
- Punithavathi, V.R., Stanely Mainzen Prince, P. (2011) The cardioprotective effects of a combination of quercetin and α -tocopherol on isoproterenol-induced myocardial infarcted rats. *J Biochem Mol Toxicol*, 25(1), 28-40. <https://doi.org/10.1002/jbt.20339>. PMID: 20979156.
- Reinboth, M., Wolfram, S., Abraham, G., Unge-mach, F.R., Cermak, R. (2010) Oral bioavailability of quercetin from different quercetin glycosides in dogs. *Br J Nutr*, 104, 198-203. <https://doi.org/10.1017/S000711451000053X>. PMID: 20230651.
- Rochwerg, B., Oczkowski, S.J., Siemieniuk, R.A.C., Agoritsas, T., Belley-Cote, E., D'Aragon, F., Duan, E., English, S., Gossack-Keenan, K., Alghuroba, M., Szczeklik, W., Menon, K., Alhazzani, W., Sevransky, J., Vandvik, P., Annane, D., Guyatt, G. (2018) Corticosteroids in Sepsis: An Updated Systematic Review and Meta-Analysis. *Crit Care Med*, 46(9), 1411-1420. <https://doi.org/10.1097/CCM.0000000000003262>. PMID: 29979221.
- Shapiro, H., Lev, S., Cohen, J., Singer P. (2009) Polyphenols in the prevention and treatment of sepsis syndromes: rationale and pre-clinical evidence. *Nutrition*, 25(10), 981-997. <https://doi.org/10.1016/j.nut.2009.02.010>. PMID: 19502006.
- Valentova, K., Sima, P., Rybkova, Z., Krizan, J., Malachova, K., Kren V. (2016) (Anti) mutagenic and immunomodulatory properties of quercetin glycosides. *J Sci Food Agric*, 96(5), 1492-1499. <https://doi.org/10.1002/jsfa.7251>. PMID: 25960089.
- Vasquez-Garzon, V.R., Arellanes-Robledo, J., Garcia-Roman, R., Aparicio-Rautista, D.I., Villa-Trevino, S. (2009) Inhibition of reactive oxygen species and pre-neoplastic lesions by quercetin through an antioxidant defense mechanism. *Free Radic Res*, 43(2), 128-137. <https://doi.org/10.1080/10715760802626535>. PMID: 19115120.
- Virginia, D.V., Owen, H., Poapolathep, A., Mario, G. (2017) Natural substances as new potential strategies for the treatment of leishmaniasis in dogs. *Am J Anim Vet Sci*, 12(3), 169-175. <https://doi.org/10.3844/ajavsp.2017.169.175>.
- Volbeda, M., Wetterslev, J., Gluud, C., Zijlstra, J.G., van der Horst, I.C.C., Keus, F. (2015) Glucocorticosteroids for sepsis: systematic review with meta-analysis and trial sequential anal-

ysis. *Intensive Care Med*, 41(7), 1220-1234. <https://doi.org/10.1007/s00134-015-3899-6>. PMID: 26100123.

Wan, Y., Tang, M.H., Chen, X.C., Chen, L.J., Wei, Y.Q., Wang, Y.S. (2014) Inhibitory effect of liposomal quercetin on acute hepatitis and hepatic fibrosis induced by concanavalin A. *Braz J Med Biol Res*, 47(8), 655-661. <https://doi.org/10.1590/1414-431x20143704>. PMID: 25098714.

Yang, H., Song, Y., Liang, Y.N., Li, R. (2018) Quercetin treatment improves renal function and protects the kidney in a rat model of adenine-induced chronic kidney disease. *Med Sci Monit*, 24, 4760-4766. <https://doi.org/10.12659/MSM.909259>. PMID: 29987270.

Yao, Z., Gu, Y., Zhang, Q., Liu, L., Meng, G., Wu, H., Xia, Y., Bao, X., Shi, H., Sun, S., Wang, X., Zhou, M., Jia, Q., Wu, Y., Song, K., Gao, W., Guo, C., Niu, K. (2018) Estimated daily quercetin intake and association with the prevalence of type 2 diabetes mellitus in Chinese adults. *Eur J Nutr*, 11(1), e0144179. <https://doi.org/10.1007/s00394-018-1713-2>. PMID: 29754250.

Zhang, J., Shi, L., Xu, X., Huang, S., Lu, B., Ji, L. (2014) Therapeutic detoxification of quercetin against carbon tetrachloride-induced acute liver injury in mice and its mechanism. *J Zhejiang Univ Sci B*, 15(12), 1039-1047. <https://doi.org/10.1631/jzus.B1400104>. PMID: 25471833.

اثرات درمانی کوئرتستین در مدل سپسیس القایی با لیپوپلی ساکارید در دوز پایین در سگ و مقایسه آن با هیدروکورتیزون

آلاله سلطانیان^{۱*}، بهمن مصلی نژاد^۲، محمد راضی جلالی^۳، حسین نجف‌زاده ورزی^۴، مسعود قربانپور نجف‌آبادی^۴

^۱دانش‌آموخته دانشکده دامپزشکی شهید چمران اهواز، اهواز، ایران
^۲گروه علوم درمانگاهی، دانشکده دامپزشکی شهید چمران اهواز، اهواز، ایران
^۳گروه علوم پایه، دانشکده دامپزشکی شهید چمران اهواز، اهواز، ایران
^۴گروه پاتوبیولوژی، دانشکده دامپزشکی شهید چمران اهواز، اهواز، ایران

(دریافت مقاله: ۱۷ اردیبهشت ماه ۱۳۹۸، پذیرش نهایی: ۲۳ مرداد ماه ۱۳۹۸)

چکیده

زمینه مطالعه: مدل سپسیس با دوز پایین در سگ، شرایط مناسبی را برای مطالعه داروهای جدید در دسترس قرار می‌دهد. لیپوپلی ساکاریدها (LPS)، یکی از اجزاء اصلی غشای بیرونی باکتری‌ها، نقش مهمی در شروع پاتوژنز بیماری دارند. سپسیس ناشی از القاء لیپوپلی ساکارید، تا حد زیادی در حیوانات آزمایشگاهی مطالعه شده است، اما اهمیت آن در سگ‌ها، عمدتاً ناشناخته است.

هدف: هدف از انجام تحقیق حاضر، ارزیابی تأثیر گذاری کوئرتستین در مقایسه با هیدروکورتیزون، بر تغییرات بالینی، هماتولوژیکی و نارسایی اندامها (کبد و قلب)، در مدل سپسیس القاء شده توسط لیپوپلی ساکارید با دوز پایین، در سگ بود.

روش کار: بدین منظور، ۱۵ قلاده سگ سالم از نظر بالینی و از نژاد مخلوط، به‌صورت تصادفی، به سه گروه مساوی تقسیم شدند. سگ‌های گروه A (کنترل)، LPS (۰/۱ g/kg) (IV) را دریافت کردند. گروه B، مشابه گروه A بود، با این تفاوت که، ۴۰ دقیقه پس از تزریق LPS، کوئرتستین (۲ mg/kg، IV، یک‌بار) تزریق شد. گروه C، مشابه گروه B بود؛ با این تفاوت که به‌جای کوئرتستین، هیدروکورتیزون (۲ mg/kg، IV، یک‌بار)، تجویز شد. مقادیر سرمی گلوکز، پروتئین تام، آلبومین، ALP، AST، ALT، کراتینین کیناز ایزوآنزیم ماهیچه/مغز (LDH (CK-MB)، و تروپونین قلبی I (cTn-I)، با استفاده از کیت‌های تجاری اندازه‌گیری شدند.

نتایج: در گروه کنترل، پس از تزریق LPS، کاهش معنی‌داری در تعداد تام گلبول‌های قرمز (RBCs)، هموگلوبین (Hb) و هماتوکریت (HCT)؛ و همچنین، افزایش معنی‌داری در فعالیت سرمی آنزیم‌های LDH، ALP، AST، ایزوآنزیم CK-MB و cTn-I نشان دادند ($P < 0/05$). گروه کوئرتستین به‌صورت معنی‌داری، در مقایسه با گروه‌های هیدروکورتیزون و کنترل، مقادیر Hb، RBCs و HCT را افزایش داد ($P < 0/05$). همچنین، گروه کوئرتستین در مقایسه با هیدروکورتیزون و کنترل، کاهش معنی‌داری در فعالیت سرمی آنزیم‌های LDH، CK-MB، و cTn-I نشان داد ($P < 0/05$). علاوه بر این، کوئرتستین به‌صورت معنی‌داری، AST را در مقایسه با گروه کنترل و ALP را در مقایسه با گروه هیدروکورتیزون، کاهش داد ($P < 0/05$).

نتیجه‌گیری نهایی: این نتایج نشان می‌دهند که کوئرتستین از RBCها در مراحل اولیه‌ی سپسیس محافظت می‌کند و نارسایی اندامها (کبد و قلب) را کاهش می‌دهد؛ بنابراین، آن دارای تأثیر مثبت بر درمان سپسیس است و ممکن است مؤثرتر از درمان کورتیکواستروئیدی معمول (هیدروکورتیزون) باشد.

واژه‌های کلیدی:

سگ، هیدروکورتیزون، لیپوپلی ساکارید، کوئرتستین، سپسیس