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CARNOSOL ALLEVIATES INFLAMMATION AND BACTERIAL TRANSLOCATION IN A RAT MODEL OF INTESTINAL ISCHEMIA-REPERFUSION INJURY

Özgür Cem Müsri[1], Serhat Oğuz[2], Şaban Gürcan[3], Taner Özgürtaş[4], Tülin Yalta[5], Gokhan Tazegul[6], Fatma Yalçın Müsri[7]

1 Department of General Surgery, Erzurum Region Training and Research Hospital, Erzurum, Turkey.
2 Department of General Surgery, Trakya University Medical Faculty, Edirne, Turkey
3 Department of Medical Microbiology, Trakya University Medical Faculty, Edirne, Turkey
4 Department of Biochemistry, University of Health Sciences, Gulhane School of Medicine, Ankara, Turkey.
5 Department of Pathology, Trakya University Medical Faculty, Edirne, Turkey
6 Department of Internal Medicine, Akdeniz University School of Medicine, Antalya, Turkey
7 Department of Medical Oncology, Erzurum Region Training and Research Hospital, Erzurum, Turkey.

The contact information of corresponding author:
Dr. Özgür Cem Müsri
Department of General Surgery, Erzurum Region Training and Research Hospital, Erzurum, Turkey
Telephone: + 90 506 598 28 88
E- mail: ozgurcemmusri@hotmail.com

Abstract

Intestinal ischemia-reperfusion injury (II/R) is a condition with significant morbidity and mortality. In this study, we aimed to demonstrate the protective effect of carnosol, an anti-oxidant, anti-inflammatory, anti-carcinogenic and anti-microbial agent, on pro-inflammatory cytokines, tissue angiopoietins, histopathological damage and bacterial translocation in rats subjected to II/R injury. Thirty male Sprague-Dawley rats were divided into 3 groups: control group, intestinal ischemia/reperfusion group (II/R) and carnosol group (II/R+C). Ischemia-reperfusion injury was conducted by laparotomy. All rats received 10^{10} colony forming units/milliliters *E.coli* 12 hours prior to surgery. II/R+C rats received 3 mg/kg/day carnosol per oral starting from 3 days prior to surgery. After 72 hours of post-surgical follow-up, rats were

re-laparotomized for specimen collection. II/R+C group had lower serum TNF-α, IL-1β and IL-6 levels when compared with II/R group (p=0.026, 0.006 and 0.045, respectively). II/R group had significantly higher tissue injury when compared with II/R+C group (p<0.001). II/R+C group rats had significantly reduced liver, spleen and mesenteric lymph node culture growths when compared with II/R group. Angiopoietin I and II levels were similar between groups. In conclusion, carnosol is a powerful anti-inflammatory and anti-microbial agent in II/R injury model. We showed effective reduction of pro-inflammatory cytokine profile, improvement of histopathological damage, and prevention of bacterial translocation.

Keywords: Carnosol, intestinal ischemia-reperfusion, antioxidants

KARNOSOL SIÇANLARDA BAĞIRSAK İSKEMİ-REPERFÜZYON MODELİNDE İNFLAMASYON VE BAKTERİYEL TRANSLOKASYONU HAFİFLETİR

Özet

İntestinal iskemi-reperfüzyon hasarı (II/R), önemli morbidite ve mortaliteye sahip bir durumdur. Bu çalışmada, anti-oksidan, anti-enflamatuvar, anti-karsinojenik ve anti-mikrobiyal bir ajan olan karnosolün II/R modeli uygulanan sıçanlarda pro-inflamatuar sitokinler, doku anjiopoietinleri, histopatolojik hasar ve bakteriyel translokasyon üzerindeki koruyucu etkisini göstermek amaçlanmıştır. Otuz erkek Sprague-Dawley sıçanı 3 gruba ayrıldı: kontrol grubu, intestinal iskemi/reperfüzyon grubu (II/R) ve karnosol grubu (II/ R+C). İskemi-reperfüzyon hasarı laparotomi ile sağlandı. Tüm sıçanlara ameliyattan 12 saat önce 10^{10} koloni oluşturan birim/mililitre *E.coli* uygulandı. II/R+C grubuna, ameliyattan 3 gün önce başlayarak oral 3 mg/kg/gün karnosol uygulandı. Ameliyat sonrası takipten 72 saat sonra, sıçanlar örnek toplama için yeniden laparotomize edildi. II/R+C grubunun II/R grubuna göre serum TNF-α, IL-1β ve IL-6 düzeyleri anlamlı derecede azalmıştı (sırasıyla p=0.026, 0.006 ve 0.045). II/R grubunun, II/R+C grubuna göre daha şiddetli doku hasarına maruz kaldığı görüldü (p<0.001). II/R+C grubu, II/R grubu ile karşılaştırıldığında, karaciğer, dalak ve mezenterik lenf nodu kültür pozitifliğinin anlamlı düzeyde azalmış olduğu gösterilmiştir. Anjiyopoietin I ve II düzeyleri

gruplar arasında benzerdi. Sonuç olarak, karnosol güçlü bir anti-inflamatuar ve anti-mikrobiyal ajandır. Bu çalışmada karnosolun II/R modelinde pro-inflamatuar sitokin profilinin etkili bir şekilde azaltan, histopatolojik hasarı düzelten ve bakteriyel translokasyonu önleyen bir ajan olduğu gösterilmiştir.

Anahtar kelimeler: Karnosol, intestinal iskemi-reperfüzyon, antioksidan

Introduction

Ischemia-reperfusion injury is characterized by increased free oxygen radicals and excessive consumption of membrane phospholipids due to resumption of blood flow (1-3). Intestinal ischemia-reperfusion injury (II/R) is a condition with significant morbidity and mortality (4). Disruption of intestinal mucosal integrity, bacterial translocation, and as a result, a pro-inflammatory cytokine profile by endotoxin-induced monocyte and macrophage activation has major roles in II/R pathogenesis (5-9).

Angiopoietins, major angiogenic substances in the intestinal tissue, may also have a role in regulating vascular endothelial response to II/R as well. Best described angiopoietins are Ang-1 and Ang-2. Ang-1 exhibits anti-apoptotic and anti-inflammatory activity by inhibiting leukocyte adhesion and reducing vascular permeability, whereas Ang-2 induces a pro-inflammatory pattern and potentiates endothelial response to TNF-α and IL-1β. Literature regarding angiopoietins in response to II/R injury are scarce (10-14).

Carnosol, along with carnosic and rosmarinic acid, is one of the major phenolic diterpenes found in rosemary (Rosmarinus officinalis). Previous studies show that carnosol exert its' anti-inflammatory effects by inhibiting several pro-inflammatory cytokines. Anti-inflammatory effects of carnosol are also associated with anti-oxidant and anti-microbial effects (15-18).

Carnosol has been largely investigated for anti-tumoral, anti-inflammatory and anti-oxidant activities in vitro and in vivo (19-20). It has been shown experimentally that carnosol can eliminate lung and liver damage secondary to II/R via its' anti-inflammatory and anti-

oxidant effects. In addition, it has been experimentally shown that carnosol reduces ischemia-reperfusion injury associated with oxidative stress of lung transplantation (21-24). Although anti-tumorogenic effects of carnosol have been studied in many cancer types, it has been reported that carnosol may show anti-carcinogenic effects in various cancers by inducing apoptosis and inhibiting the cell cycle division (25). Carnosol has been shown to suppress several pro-inflammatory pathways in chronic inflammatory processes (26-27). In another study, it has been shown to improve endothelial dysfunction in diabetic microangiopathy by means of anti-oxidative mechanisms (28). In addition, the protective effect of a single dose of carnosol has been shown in patients with ischemia-reperfusion injury due to acute kidney injury (29).

Carnosol is reported to be a powerful anti-inflammatory and anti-microbial substance. We hypothesized that carnosol may also be protective against II/R injury by its' anti-inflammatory, anti-oxidant and anti-bacterial activity. Therefore, we aimed to study the potential protective effects and effective mechanisms of carnosol in a murine model of II/R injury.

Material and Methods

Experimental animals

Thirty male Sprague-Dawley rats (200-250 g) supplied by **(censored)** University Animal Center were housed in an air-conditioned room with 12-h light and dark cycles, with stable temperature (22±2 centigrade Celsius). All experimental protocols were approved and ethics approval was gained from **(censored)** University Animal Care and Use Committee.

Animals were divided into three groups: (1) control rats (C) (n=10) received a sham operation with intraperitoneal vehicle administration, (2) study group rats (II/R) (n=10) were laparatomized, intraperitoneal vehicle was administered and II/R surgery was conducted, (3) carnosol group rats (II/R+C) (n=10) received pre-operative and intraperitoneal carnosol administration along with II/R surgery.

Experimental procedure

All rats were followed up for 7 days prior to surgery and received the same nutrients**.** II/R+C rats received 3 mg/kg/day carnosol per oral (100% crystalline carnosol, Cayman Chemical Company, Ann Arbor, USA) diluted in 10% dimethyl sulfoxide (DMSO) daily, starting from 3 days prior to surgery. Rats were kept fasted 1 day prior to surgery. All rats received 1 milliliter of 10^{10} colony forming units/milliliters *E.coli* 12 hours prior to surgery. Ketamine (30 mg/kg, ketamine hydrochloride, Ketalar; Pfizer, İstanbul, Turkey) and xylazine hydrochloride (3 mg/kg, Rompun; Bayer, İstanbul, Turkey) were used as anesthetics during surgery.

During laparotomy, superior mesenteric artery was occluded with an atraumatic microvascular clamp. Ischemia was confirmed with a loss of intestinal arterial pulse and a fading of intestinal color. Control and II/R groups received 0.2 cubic centimeters of DMSO during laparotomy, whereas II/R+C group received 3mg/kg carnosol intraperitoneally. After 60 minutes of ischemia, reperfusion was initiated by removing the clamp. Reperfusion was confirmed with visualization of intestinal arterial pulses and reddening of intestinal tissue. Operations were concluded after 2 hours of reperfusion.

Sample collection, cytokine assays

After 72 hours of post-surgical follow-up, rats were re-laparotomized under sterile conditions to collect microbiologic, biochemical and histopathological samples from the blood, liver, spleen, mesenteric lymph nodes (MLN) and ileum. Blood was collected by cardiac puncture. Tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β) and interleukin-6 (IL-6) were measured using radioimmunoassay kits. Half of the ileal tissue samples were fixed in 10% formaldehyde, whereas the other half were kept in a phosphate buffer solution under -80°C to measure intestinal angiopoietins (Ang-1 and Ang-2) via enzyme-linked immunosorbent assay.

Microbiological and histopathological assays

Blood samples were cultured in aerobic and anaerobic culture flasks (Bact/Alert, Biomerieux, France) and were incubated for 7 days. For positive cultures, conventional cultures were conducted under appropriate aerobic and anaerobic conditions and positive cultures were automatically defined using an automatic system (VITEK2, Biomerieux, France).

Tissue samples (MLN, liver and spleen) were removed under sterile conditions, minced and homogenized after adding 1 milliliter of thiogluconate. Samples were planted into appropriate agar cultures and were incubated for 72 hours. Bacterial densities in positive cultures were calculated as colony forming units/gram and positive cultures were automatically defined using an automatic system (VITEK2, Biomerieux, France).

Tissue samples from ileum kept in 10% formaldehyde underwent routine histologic preparation, embedded in paraffin. Random tissue sections were cut, mounted on slides and stained with hematoxylin and eosin. Tissue damage was characterized and quantified using Chiu classification (18).

Statistical analysis

SPSS version 22.0 was used for statistical analysis (IBM, Chicago, Illinois). Quantitative data were expressed as means ± standard deviation. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Frequencies were expressed where appropriate. Values of $p<0.05$ were considered significant.

Results

Serum cytokines and intestinal angiopoietins

Pro-inflammatory cytokines were measured using radioimmunoassay. II/R group had markedly higher levels of TNF- α, IL-1β, and IL-6 compared to control group (p=0.026, 0.006 and 0.045, respectively). Interestingly, II/R+C group had similar pro-inflammatory cytokine levels with control group; carnosol administration reduced pro-inflammatory cytokines profoundly.

Intestinal angiopoietins (Ang-1 and Ang-2) were measured using frozen tissue samples via enzyme-linked immunosorbent assay. Although II/R group had lower mean Ang-1 and higher mean Ang-2 levels, all three groups were statistically similar (Table 1).

Table 1;Serum cytokine and tissue angiopoietin levels of control and study groups

	Control (n=10)	**II/R (n=10)**	**II/R + C (n=10)**
Serum cytokines			
TNF-α (pg/mL)	0.88±0.27	1.18±0.19	0.88±0.24
IL-1β (pg/mL)	23.82±7.01	34.47±10.49	21.61±7.53
IL-6 (ng/L)	131.82±8.48	143.43±11.64	132.22±10.44
Tissue Ang levels			
Ang-1 (pg/g protein)	4.81+2.70	3.18+1.73	5.58+4.41
Ang-2 (pg/g protein)	126.51+79.18	217.37+163.94	164.18+115.77

Notes: All values are expressed as mean±standard deviation (SD). II/R: Intestinal ischemia/reperfusion. II/R+C: Intestinal ischemia/reperfusion plus carnosol. TNF-α: Tumor necrosis factor-α, IL-1β: Interleukin-1β, IL-6: Interleukin-6, Ang-1: Intestinal tissue angiopoietin-1, Ang-2: Intestinal tissue angiopoietin-2.

Bacterial translocation

Blood and tissue samples (MLN, liver and spleen) were cultured in aerobic and anaerobic culture flasks and conventional cultures were conducted under appropriate aerobic and anaerobic conditions for positive samples.

Blood cultures and all anaerobic tissue cultures were negative. Control group had no aerobic culture growth in MLN, liver and spleen cultures. However, 4 out of 10 II/R rats had hepatic and MLN, 5 out of 10 rats had splenic aerobic culture growth. All growths were identified as *E.coli*. Interestingly, II/R+C rats had no aerobic tissue culture growth.

Histopathological damage

Control group rats had no terminal ileum damage (Grade 0). II/R group had profound terminal ileum damage, associated with structural villi damage and inflammatory infiltration (Grade 4). Carnosol administration had significantly down-graded inflammation in terminal ileum histology, mostly associated with presence of Grunhagen's sub-epithelial space (70% Grade 1 and %30 Grade 2).

Discussion

In this study, we aimed to investigate the effect of carnosol, an anti-oxidant agent, on pro-inflammatory cytokines, tissue angiopoietins, histopathological damage and bacterial translocation in rats subjected to II/R injury. Herein, we demonstrate carnosol as a powerful anti-inflammatory agent in II/R injury model. Our main findings suggest carnosol alleviates inflammation by reducing the markedly increased serum pro-inflammatory cytokines and inhibiting bacterial translocation in response to II/R injury.

Carnosol is the major phenolic anti-oxidant found in rosemary (*Rosmarinus officinalis*). As an anti-oxidant, carnosol has been previously demonstrated to have protective effects against ischemia-reperfusion induced lung, hepatic and renal damage as an anti-oxidant, anti-inflammatory and anti-cancer agent (21-23). Experimental studies previously showed

protective effect of carnosol was associated with a strong inhibition of TNF-α expression (30-32). Tsai *et al* (33) demonstrated carnosol inhibits inflammation associated TNF-α and IL-1β increase *in vitro*. Mengoni *et al* (34) previously demonstrated carnosol effectively reduced TNF-α and inhibited IL-1β increase even in low titrations. Additionally, several authors demonstrated in II/R injury, administration of carnosol reduces IL-6 levels, which is a powerful chemo attractant and a pro-inflammatory cytokine (21-22). These results are further validated in our study; we demonstrated carnosol significantly reduced serum TNF-α, IL-1β and IL-6 levels in II/R injury model. Carnosol is a powerful anti-inflammatory molecule which inhibits pro-inflammatory cytokine profile in response to II/R injury.

Angiopoietins, major angiogenic substances in the intestinal tissue, were shown to have regulatory roles in endothelial response to inflammatory stimuli. Ang-1 may have anti-inflammatory effects whereas Ang-2 has pro-inflammatory effects that potentiates endothelial response to TNF-α and IL-1β (10-14). Although not statistically significant, our study showed a decrease in anti-inflammatory Ang-1 and an increase in pro-inflammatory Ang-2 in response to II/R, which were reversed with carnosol administration. Further studies are needed to prove a causative relationship between inflammatory response to II/R injury and angiopoietins, and the effect of carnosol on angiopoietin levels.

Carnosol also demonstrates anti-microbial effects. Several authors, such as Bernardes *et al* (35) previously demonstrated carnosol has a powerful anti-microbial effect on oral pathogens. In addition, Oluwatuyia *et al (*36) showed carnosol had better anti-bacterial effect on methicillin resistant *S.aureus* when compared with erythromycin and tetracycline. Bozin *et al* (37) previously demonstrated carnosol as a powerful anti-bacterial agent against *Esherichia coli, Salmonella typhi, Salmonella enteritidis* and *Shigella sonnei* as well. In our study, nearly half of the rats in II/R group had mesenteric, splenic and hepatic aerobic culture growths with *E.coli*, as a result of bacterial translocation. Interestingly, carnosol inhibited bacterial translocation, confirming an anti-microbial effect on II/R injury model as well.

II/R injury is characterized by extensive histopathological damage. Several studies showed ischemia-reperfusion related tissue damage was associated with edema, hemorrhage and neutrophilic infiltration, which was alleviated by carnosol administration (21-22-38). In our

study, consistent with the literature, we demonstrated II/R injury causes a significant damage to terminal ileum, and carnosol reduces inflammatory changes in terminal ileum.

Intestinal ischemia, which may develop due to occlusive or non-occlusive causes, is a syndrome which start in intestines with reduced intestinal blood flow and then turns into a highly mortal multi-organ failure. Most of the research in this area focused on the advancement of techniques for early detection of ischemia and the development of new therapeutic approaches targeting post-ischemic reperfusion. Therefore, the proposed therapeutic interventions for protection against reperfusion injury have been aimed at inhibiting oxidant damage and neutrophil activation.

In the present study, we demonstrate carnosol effectively reduces pro-inflammatory cytokine profile, prevents bacterial translocation and reduces histopathological damage; however, we failed to demonstrate any strong association with angiopoietins. We believe carnosol may be a potential therapeutic agent for II/R in daily surgical practice. However, further studies are needed to confirm the therapeutic effects of carnosol in humans.

References

1. Zimmerman BJ, Granger DN. Reperfusion injury. Surg Clin North Am 1992; 72:65-83.
2. Sorkine P, Szold O, Halpern P, Gutman M, Greemland M, Rudick V, et al. Gut decontamination reduces bowel ischemia-induced lung injury in rats. Chest 1997; 112:491-5.
3. Schoenberg MH, Beger HG. Reperfusion injury after intestinal ischemia. Crit Care Med 1993; 21:1376-86.
4. Tullis MJ, Brown S, Gewertz BL. Hepatic influence on pulmonary neutrophil sequestration following intestinal ischemia-reperfusion. J Surg Res. 1996; 66: 143-6.

5. Börjesson A, Norlin A, Wang X, Andersson R, Folkesson HG. TNF-alpha stimulates alveolar liquid clearance during intestinal ischemia-reperfusion in rats. Am J Physiol Lung Cell Mol Physiol 2000; 278: L3-12.

6. Simpson R, Alon R, Kobzik L, Valeri CR, Shepro D, Hechtman HB. Neutrophil and nonneutrophil-mediated injury in intestinal ischemia-reperfusion. Ann Surg 1993; 218: 444-53.

7. Towfigh S, Heisler T, Rigberg DA, Hines OJ, Chu J, McFadden DW, et al. Intestinal ischemia and the gut-liver axis: an in vitro model. J Surg Res 2000; 88:160-4.

8. Turnage RH, Guice KS, Oldham KT. Endotoxemia and remote organ injury following intestinal reperfusion. J Surg Res 1994; 56: 571-8.

9. Köksoy C, Kuzu MA, Kuzu I, Ergün H, Gürhan I. Role of tumour necrosis factor in lung injury caused by intestinal ischaemia-reperfusion. Br J Surg 2001; 88:464-8.

10. Thurston G, Rudge JS, Ioffe E, Zhou H, Ross L, Croll SD, et al. Angiopoietin-1 protects the adult vasculature against plasma leakage. Nat Med 2000; 6: 460-3.

11. Kim I, Moon SO, Park SK, Chae SW, Koh GY. Angiopoietin-1 reduces VEGF-stimulated leukocyte adhesion to endothelial cells by reducing ICAM-1, VCAM-1, and E-selectin expression. Circ Res 2001; 89:477-9.

12. Pizurki L, Zhou Z, Glynos K, Roussos C, Papapetropoulos A. Angiopoietin-1 inhibits endothelial permeability, neutrophil adherence and IL-8 production. Br J Pharmacol 2003; 139:329-36.

13. Mandriota SJ, Pepper MS. Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. Circ Res 1998; 83:852-9.

14. Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med 2006; 12:235-9.

15. Yao H, Chen Y, Zhang L, He X, He X, Lian L, et al. Carnosol inhibits cell adhesion molecules and chemokine expression by tumor necrosis factor-α in human umbilical vein endothelial cells through the nuclear factor-κB and mitogen-activated protein kinase pathways. Mol Med Rep 2014; 9:476-480.

16. Schwager J, Richard N, Fowler A, Seifert N, Raederstorff D. Carnosol and Related Substances Modulate Chemokine and Cytokine Production in Macrophages and Chondrocytes. Molecules 2016; Apr 8;21(4):465.

17. Rocha J, Eduardo-Figueira M, Barateiro A, Fernandes A, Brites D, et al. Anti-inflammatory effect of rosmarinic acid and an extract of Rosmarinus officinalis in rat models of local and systemic inflammation. Basic Clin Pharmacol Toxicol 2015; 116: 398-413.

18. Oliveira Gde A, de Oliveira A E, da Conceicao E C, Leles M I. Multiresponse optimization of an extraction procedure of carnosol and rosmarinic and carnosic acids from rosemary. Food Chem 2016; 211; 465-473.

19. Ortuno J, Serrano R. Banon S. Antioxidant and antimicrobial effects of dietary supplementation with rosemary diterpenes (carnosic acid and carnosol) vs vitamin E on lamb meat packed under protective atmosphere. Meat Sci 2015; 110: 62-69

20. Sanchez C, Horcajada M N, Membrez Scalfo F, Ameye L, Offord E, Henrotin Y. Carnosol inhibits pro-inflammatory and catabolic mediators of cartilage breakdown in human osteoarthritic chondrocytes and mediates cross-talk between subchondral bone osteoblasts and chondrocytes. PLoS ONE 2015; 10:8:e0136118.

21. Tian XF, Yao JH, Zhang XS, Zheng SS, Guo XH, Wang LM, et al. Protective effect of carnosol on lung injury induced by intestinal ischemia/reperfusion. Surg Today 2010; 40:858-65.

22. Yao JH, Zhang XS, Zheng SS, Li YH, Wang LM, Wang ZZ, et al. Prophylaxis with carnosol attenuates liver injury induced by intestinal ischemia/reperfusion. World J Gastroenterol 2009; 15:3240-5.

23. Peng CH, Su JD, Chyau CC, Sung TY, Ho SS, Peng CC, et al. Supercritical fluid extracts of rosemary leaves exhibit potent anti-inflammation and anti-tumor effects. Biosci Biotechnol Biochem 2007; 71:2223-32.

24. Kawamura T, Momozane T, Sanosaka M, Sugimura K, Iida O, Fuchino H, et al. Carnosol Is a Potent Lung Protective Agent: Experimental Study on Mice. Transplant Proc 2015: 47(6):1657-61.

25. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Anti-Carcinogenic Effects of Carnosol-An Updated Review. Curr Drug Discov Technol 2018; 15(1):32-40.

26. Lee DY, Hwang CJ, Choi JY, Park MH, Song MJ, Oh KW, Son DJ, Lee SH, Han SB, Hong JT. Inhibitory Effect of Carnosol on Phthalic Anhydride-Induced Atopic Dermatitis via Inhibition of STAT3. Biomol Ther 2017; 1;25(5):535-544.

27. Oliviero F, Scanu A, Zamudio-Cuevas Y, Punzi L, Spinella P. Anti-inflammatory effects of polyphenols in arthritis. J Sci Food Agric 2018; 98:5:1653-1659.

28. Li X, Zhang Q, Hou N, Li J, Liu M, Peng S, Zhang Y, Luo Y, Zhao B, Wang S, Zhang Y, Qiao Y. Carnosol as a Nrf2 Activator Improves Endothelial Barrier Function Through Antioxidative Mechanisms. Int J Mol Sci 2019; 18:20(4).

29. Zheng Y, Zhang Y, Zheng Y, Zhang N. Carnosol protects against renal ischemia-reperfusion injury in rats. Exp Anim 2018; 1:67(4):545-553.

30. Chen CC, Chen HL, Hsieh CW, Yang YL, Wung BS. Upregulation of NF-E2-related factor-2-dependent glutathione by carnosol provokes a cytoprotective response and enhances cell survival. Acta Pharmacol Sin 2011; 32:62-9.

31. Chae IG, Yu MH, Im NK, Jung YT, Lee J, Chun KS, et al. Effect of Rosemarinus officinalis L. on MMP-9, MCP-1 levels, and cell migration in RAW 264.7 and smooth muscle cells. J Med Food 2012; 15:879-86.

32. Yao H, Chen Y, Zhang L, He X, He X, Lian L, et al. Carnosol inhibits cell adhesion molecules and chemokine expression by tumor necrosis factor-α in human umbilical vein

endothelial cells through the nuclear factor-κB and mitogen-activated protein kinase pathways. Mol Med Rep 2014; 9:476-80.

33. Tsai TH, Chuang LT, Lien TJ, Liing YR, Chen WY, Tsai PJ. Rosmarinus officinalis extract suppresses Propionibacterium acnes-induced inflammatory responses. J Med Food 2013; 16:324-33.

34. Mengoni ES, Vichera G, Rigano LA, Rodriguez-Puebla ML, Galliano SR, Cafferata EE, et al. Suppression of COX-2, IL-1β and TNF-α expression and leukocyte infiltration in inflamed skin by bioactive compounds from Rosmarinus officinalis L. Fitoterapia 2011; 82: 414-21.

35. Bernardes WA, Lucarini R, Tozatti MG, Souza MG, Silva ML, Filho AA, et al. Antimicrobial activity of Rosmarinus officinalis against oral pathogens: relevance of carnosic acid and carnosol. Chem Biodivers 2010; 7:1835-40.

36. Oluwatuyi M, Kaatz GW, Gibbons S. Antibacterial and resistance modifying activity of Rosmarinus officinalis. Phytochemistry 2004; 65: 3249-54.

37. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J Agric Food Chem 2007; 55:7879-85.

38. Mester A, Magyar Z, Sogor V, Tanczos B, Stark Y, Cherniavsky K, et al. ischemia-reperfusion leads to early systemic micro-rheological and multiorgan microcirculatory alterations in the rat. Clin Hemorheol Microcirc 2018; 68:(1):35-44.