JoCASS
Çukurova Anestezi ve Cerrahi Bilimler Dergisi
2018 Adana

PROTECTIVE EFFICACY OF ROSMARINIC ACID ON ACUTE PANCREATITIS IN RATS

RATLARDA ROSMARİNİK ASİT'İN AKUT PANKREATİT ÜZERİNDEKİ KORUYUCU ETKİNLİĞİNİN İNCELENMESİ

Mehmet Rencber[1], Abdullah Oguz[1], Eda Yıldızhan[1]

1 *Department of General Surgery, Faculty of Medicine, Dicle University, Diyarbakır, Türkiye*
2 *Department of Histology and Embryology, Faculty of Medicine, Dicle University, Diyarbakır, Türkiye*

Sorumlu Yazar/Corresponding Author: Eda Yıldızhan E-mail: blgc_eda@hotmail.com
Geliş Tarihi/Received: 28.07.2022 Kabul Tarihi-Accepted: 18.08.2022 Available Online Date/Çevrimiçi Yayın Tarihi: 31.08.2022
Cite this article as: Rencber M, Oguz A, Yıldızhan E. Protective Efficacy of Rosmarinic Acid on Acute Pancreatitis in Rats. J Cukurova Anesth Surg. 2022;5(2):250-258.
Doi: 10.36516/jocass.1150397

Abstract

Aim: Acute pancreatitis is a serious disease, with an incidence of 5 - 35 in 100,000 individuals. New studies are constantly planned for the treatment of pancreatitis. Many studies have shown that Rosmarinic acid has antioxidant properties. In this study, we examined the protective effect of Rosmarinic acid on acute pancreatitis.

Methods: A total of 28 animals were used during the experiment, and 4 groups were formed with 7 animals in each group. Group 1 is the control group and no drugs were used during the experiment. The rats in Group 2 were administered 75 µg/kg Cerulein every hour intraperitoneally at one-hour intervals, a total of four times. Group 3 experimental animals were given 50 mg/kg Rosmarinic acid by per oral gavage. The rats in group 4 were given 50 mg/kg Rosmarinic acid per oral gavage after 75 µg/kg Cerulein was injected intraperitoneally every hour for a total of four times. Afterwards, all animals were sacrificed by exsanguination, blood samples and pancreatic tissue were taken for examination.

Results: Examination of pancreatic tissues revealed necrosis, edema and inflammation in the acute pancreatitis group. Both histopathological and serum values of the rosmarinic acid group were close to the control group. The use of Rosmarinic acid after acute pancreatitis had a positive effect on the pacreatic tissues and blood values, but still did not cause complete recovery.

Conclusions: In the case of acute pancreatitis, it was concluded that rosmarinic acid has a partial curative effect, but still does not provide a full recovery.

Keywords: Acute pancreatitis, Cerulein, Rosmarinic Acid.

Öz

Amaç: Akut pankreatit her yüz bin kişide 5-35 kişi arasında görülen ciddi bir hastane yatış sebebidir. Pankreatit durumunun tedavisi konusunda sürekli yeni çalışmalar planlanmaktır. Rozmarinik asit'in yapılan birçok çalışmada antioksidan özelliğe sahip olduğu gösterilmiştir. Bizde yaptığımız bu çalışmada Rosmarinik asit'in akut pankreatit üzerinde koruyucu etkinliğini incelemeyi amaçladık.

Yöntemler: Deney süresince toplam 28 hayvan kullanılmış olup, her grupta 7 hayvan olacak şekilde 4 grup oluşturuldu. Grup 1 kontrol grubu olup deney süresince hiçbir ilaç kullanılmadı. Grup 2'deki ratlara saatte bir 75 µg/kg Cerulein birer saat arayla intraperitoneal olarak, toplam dört defa enjekte edildi. Grup 3 deney hayvanlarına peroral gavaj yoluyla 50 mg/kg dozda Rosmarinik asit verildi. Grup 4'teki ratlara ise saatte bir 75 µg/kg Cerulein intraperitoneal olarak toplam dört defa olmak üzere enjekte edildikten sonra, Rosmarinik asit peroral gavaj yoluyla 50 mg/kg verildi. Sonrasında çalışma gruplarındaki tüm hayvanlar ekzanguinasyon ile sakrifiye edildi, kalpten alınan kan örnekleri ve pankreas dokusu inceleme amacıyla alındı.

Bulgular: Pankreas dokularının ışık mikroskobik incelemelerinde akut pankreatit grubunda dokularda nekroz, ödem ve inflamasyon görüldü. Rosmarinik asit grubunun hem histopatolojik hem de serum değerlerinin kontrol grubuna yakın olduğu görüldü. Akut pankreatit sonrası Rosmarinik asit kullanımının pankreas dokularda ve kan değerlerinde pozitif etkili olduğu, fakat yine de tamamen iyileşmeye neden olmadığı tespit edildi.

Sonuç: Akut pankreatit durumunda Rosmarinik asit'in kısmen iyileştirici özelliğinin olduğu fakat yine de tam bir iyileşme sağlamadığı kanaatine varıldı.

Anahtar Kelimeler: Akut pankreatit, Cerulein, Rosmarinik Asit

Introduction

Acute pancreatitis is an inflammatory disease with mild to severe symptoms. In general, it is thought that in the beginning of acute pancreatitis, digestive zymogens lead to early intraacinar cell activation and once these enzymes are activated, they cause acinar cell damage[1]. Early acinar cell damage in acute pancreatitis leads to local inflammatory reactions. Then, a systemic inflammatory response syndrome (SIRS) occurs[2]. It is known that acute pancreatitis occurs in two stages as early and late stages. The severity of the disease is classified as mild, moderate or severe. The most common form is mild acute pancreatitis and it can cause organ failure, local or systemic complications, which usually resolves within the first week. Moderate acute pancreatitis results in transient organ failure and local complications. If severe acute pancreatitis lasts longer than 48 hours, permanent organ failure develops[3].
Rosmarinic acid (RA) is a polyphenolic antioxidant that is widely found in many plants[4-6].
In many studies, antioxidant, anticarcinogenic, anti-inflammatory, antidepressant and antimicrobial effects of RA has been revealed[7,8]. RA increases the expression of cytoprotective genes and affects several enzymes of the antioxidant system, and as a result it acts as an antioxidant [9].
In this study we examined whether RA can be an effective treatment for acute pancreatitis by utilizing its antioxidant, anti-inflammatory and other therapeutic properties and to add new findings to science on the treatment of such a serious and fatal disease.

Materials and Methods

- *Formation of Experimental Groups*

In this study, 28 Wistar Albino male rats were used, which were 8-10 weeks old and weighing 250-300 g. Rats were divided into 4 groups in groups of 7 in cages in an environment with a ventilation system.

Group 1 (n=7):
(Control Group)
Intraperitoneal (i.p.) saline was administered to the rats during the experiment.

Group 2 (n=7):
(Acute Pancreatitis Group)
Seventy-five µg/kg Cerulein was administered to the rats every hour at one-hour intervals, a total of four times.

Group 3 (n=7):
(Rosmarinic acid group)
Peroral (p.o.) Rosmarinic acid was administered at a dose of 50 mg/kg via gavage.

Group 4 (n=7):
(Acute pancreatitis + Rosmarinic acid group)
50 mg/kg Rosmarinic acid per oral gavage after 75 µg/kg Cerulein was injected intraperitoneally every hour for a total of four times.

We obtained Cerulein from Sigma-Aldrich, (St. Louis, MO, USA) to initiate acute pancreatitis.
At the end of the experiment Ketamine HCl (90 mg/kg, Pfizer Inc, USA) + Xylazine HCl (10 mg/kg, Bayer HealthCare AG, Germany) i.p. was administered to the rats and the rats were sacrificed by exsanguination under general anesthesia and the study was terminated. The collected pancreatic tissues were fixed in containers containing 10% buffered formol (Sigma #SZBE2450V).

- *Histopathological Analysis*

Pancreatic tissue samples washed in tap water for 12 hours after fixation were passed through increasing alcohol series for dehydration. Sections taken after routine histological tissue follow-up were stained with Hematoxylin & Eosin (H&E) for histological evaluation.
Paraffin depolymerization was provided in an oven at 58°C for 1 hour before staining

on tissue samples taken on positively charged slides. Hematoxylin-Eosin (H&E) staining protocol was applied to the sections taken from the oven for histological evaluation. Edema, acinar cell necrosis, and inflammation in the pancreatic tissue were evaluated using Schoenberg's Pancreatic Injury Scoring System, which ranges from 0 to 4[10].

- *Biochemical Analysis*

The blood samples taken were centrifuged at 3000/min for 10 minutes and the serum samples were separated and sent to the Biochemistry Laboratory. Serum amylase level, serum OSI, pancreatic OSI, TNF-α, IL-1β, IL-6 values were measured.

- *Measurement of total antioxidant status (TAS)*

It is a method that measures the body's total antioxidant capacity against strong free radicals. In this study, venous blood samples taken from rats in all groups were taken into EDTA tubes and centrifuged at 900 g at 4°C for 10 minutes. For evaluating the total antioxidant status (TAS) level from blood samples, we purchased the kits from Rel Assay Diagnostics (Gaziantep, Turkey). An automated measurement method which was improved by Erel[11] was used to analyze the TAS of the supernatantphase. “mmol/L” was used as the measurement unit of TAS.

- *Measurement of total oxidant status (TOS)*

In this study, venous blood samples taken from rats in all groups were taken into EDTA tubes and centrifuged at 900 g at 4°C for 10 minutes. For evaluating the total oxidant status (TOS) level from blood samples, we purchased the kits from Rel Assay Diagnostics (Gaziantep, Turkey). An automated measurement method which was improved by Erel[12] was used to analyze the TOS levels of the supernatant fragments. “μmol/L” was used as the measurement unit of TOS.

- *Measurement oxidative stress index (OSI)*

OSI indicates the degree of oxidative stress and is calculated using following formula[13]: OSI: (TOS/TAS) × 100.

- *Statistical Analysis*

Statistical analyzes of the data were done with SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA). The compatibility of the data with the assumption of normal distribution was examined by applying the Shapiro Wilk test. The Kruskal Wallis test, which is one of the non-parametric tests, was applied to the values that did not show normal distribution, and the Mann Whitney U test was performed between the two groups for the differences between the variables that were found to be significant. Significance level was accepted as significant in case of $p<0.05$ value.

Results

- *Histopathological Findings*

In the histopathological analysis of pancreatic tissue, when all study groups were evaluated in terms of edema, hemorrhage, inflammation and necrosis, no pathological findings were found in the pancreatic tissues of the control group (Figure 1). Similarly, no pathological findings were found in the pancreatic tissue of the Rosmarinic acid group (Figure 1). Numerous cell necrosis and cytoplasmic vacuoles were found in the pancreatic tissues of the group receiving cerulein (Figure 1). When the pancreatic tissues of the Cerulein+ Rosmarinic acid group were examined, it was observed that the severity of the findings decreased compared to the group that received Cerulein, but it was not as normal as the control or Rosmarinic acid group (Figure 1).

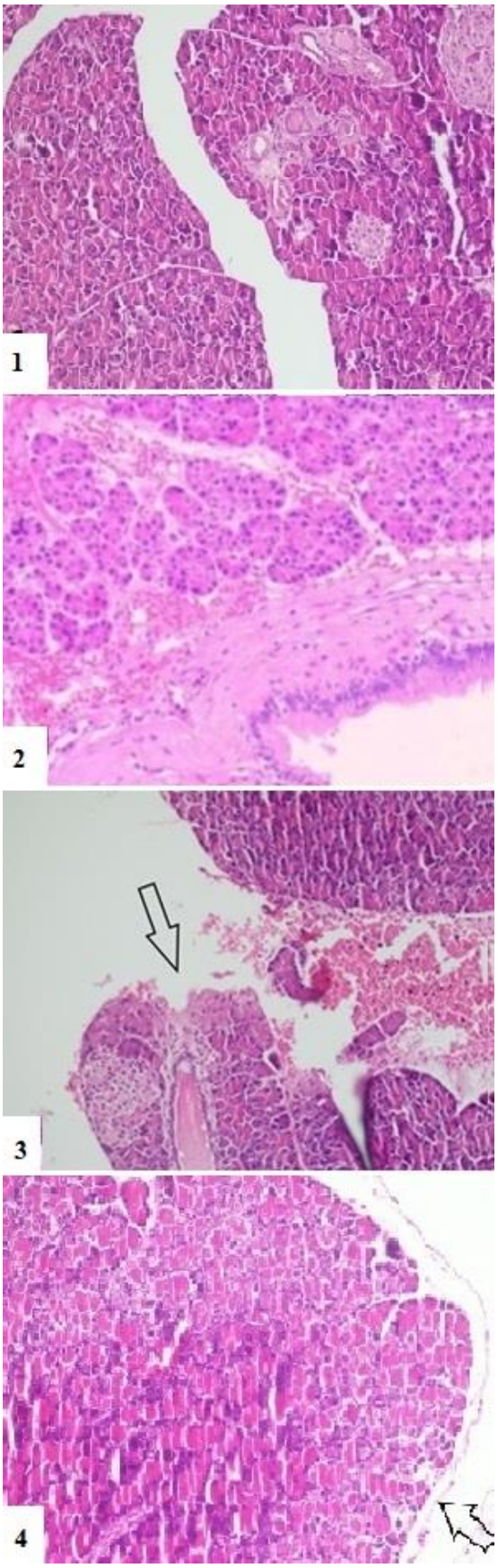

Figure 1.H&E Staining of the pancreas of the groups (H&E:Hematoxylin and Eosin)

According to the statistical analyzes made between the groups.

When examined in terms of edema, it was seen that the Acute pancreatitis + Rosmarinic acid group had the highest score compared to the other groups, while no difference was observed between the other groups ($p<0.05$).

In the evaluation of necrosis, Acute pancreatitis group had the highest scoring, while no necrosis was found in the Control and Rosmarinic acid groups. Acute pancreatitis Rosmarinic acid group had a lower score compared to the acute pancreatitis group ($p<0.05$).

In the hemorrhage evaluation, no hemorrhagic changes were found in the study groups except the acute pancreatitis group ($p<0.05$).

In inflammatory changes, it was observed that the acute pancreatitis+ Rosmarinic acid group had lower inflammation compared to the acute pancreatitis group ($p<0.05$).

The mean±standard deviation and p values of the histopathological (edema, inflammation, hemorrhage and necrosis) changes that were statistically analyzed are summarized in Table 1.

- *TAS and TOS Analysis*

As a result of the evaluation of TAS levels in both tissue and serum, it was found that there was no statistically significant difference between the groups ($p>0.05$).

As a result of the evaluation of TOS levels in tissue and serum, it was found that it was significantly higher in the acute pancreatitis group compared to all other groups ($p<0.05$). It was found that it was lower in the Acute pancreatitis+ Rosmarinic acid group compared to the Acute pancreatitis group, but it was significantly higher compared to the control and Rosmarinic acid groups ($p<0.05$).

In the OSI calculations made in line with these data.

The OSI level in pancreatic tissue was found to be the highest in the Acute pancreatitis group compared to the other groups

Table 1: The mean±standard deviation and p values of the histopathological analyzes (edema, necrosis, hemorrhage and inflammation), biochemical analyzes (serum Amylase, TNF-α, IL-1β, IL-6), TAS (in serum and tissue), TOS (in serum and tissue), Serum OSI, Pancreatic OSI.

Parameters	Control group	Rosmarinic Acid (RA) group	Acute Pancreatitis (AP) group	AP + RA group	P
TAS (nmoltroloxequiv/mg protein) –pancreas	0.32 ±0.29	0.39±0.33	0.35±0.14	0.55±0.29	N.S.
TAS- Serum	0.78±0.06	0.84±0.05	0.87±0.26	0.83±0.16	N.S.
TOS (nmol H2O2 equiv/mg protein)– pancreas	326.29±109.9	320.95±103.1	1561.10±647.89[a,b]	1202.03±344.50[a,b]	<0.001
TOS - Serum	565.58±223.95	553.29±87.64	1638.01±910.11 [a,b]	908.01±247.22 [a,b]	0,003
OSI-Pancreas	186823.29±196128.44	257782.26±322786.03	507711.84±293901.92[a]	297670.46±202272.78	N.S.
OSI-serum	66172.92±22672.39	70628.2±8369.91	183723.88±60828.14[a,b]	115854.27±52963.21[a,b]	0.005
Serum- TNF-α(pg/mL)	44.45±2.13	47.26±3.74	133.71±25.75[a,b]	46.19±2.42[c]	0,001
Serum- IL 1β(pg/mL)	421.3±24.21	442.45±44.54	793.92±307.89[a,b]	452.16±32.70[c]	0,012
Serum IL-6(pg/mL)	25.93±3.64	26.55±1.75	54.17±30.78[a,b]	28.59±3.87[c]	0,004
Serum Amilase (IU/L)	760.85±96.88	674.85±46.84	2042.42±593.21[a,b]	1473.71±339.31[a,b]	<0.001
Pancreatic Tissue Edema Formation	0.0±0.0	0.0±0.0	0.0±0.0	1.00±0.57	<0.001
Development of Pancreatic Tissue Necrosis	0.0±0.0	0.0±0.0	1.14±0.69	0.14±0.37	0.001
Pancreatic Tissue Hemorrhagic Changes	0.0±0.0	0.0±0.0	0.42±0.53	0.0±0.0	0.018
Pancreatic Tissue Inflammatory Changes	0.0±0.0	0.0±0.0	1.28±1.11	0.42±0.53	0.01

Acute Pancreatitis group (AP), Acute Pancreatitis+ Rosmarinic Acid group (AP+RA), Not significant (N.S.).

a: Different from the control group, b: Different from the RA group, c: Different from the AP group, d: Different from the AP+RA group

($p<0.05$), but there was no significant difference between the other groups ($p>0.05$).
OSI level in the serum was found to be higher in the Acute pancreatitis group compared to all other groups ($p<0.05$). It was found that it was lower in the acute pancreatitis and rosmarinic acid group compared to the acute pancreatitis group, but higher than the control and rosmarinic acid groups ($p<0.05$). It was found that it was lower in the acute pancreatitis + rosmarinic acid group compared to the acute pancreatitis group, but higher than the control and rosmarinic acid groups ($p<0.05$).
TAS and TOS values, mean±standard deviation and p values of pancreatic OSI and serum OSI levels are summarized in Table-1.

- *Biochemical Analysis*

According to the results of serum amylase, serum Oxidative Stress Index (OSI), pancreatic OSI, IL-1β, IL-6 and TNF-α levels that we have checked as a result of the blood samples we have taken.
Serum amylase levels were found to be significantly higher in the acute pancreatitis group compared to all other groups ($p<0.05$). It was found to be lower in the acute pancreatitis + Rosmarinic acid groups compared to the acute pancreatitis group ($p<0.05$).
TNF-α levels were found to be significantly higher in the Acute pancreatitis group compared to all other groups ($p<0.05$), while it was lower in the Acute pancreatitis+ Rosmarinic acid group ($p<0.05$).
While IL-1β levels were found to be similarly low in the control group and Rosmarinic acid groups ($p<0.05$), they were higher in the acute pancreatitis+ Rosmarinic acid group compared to these two groups ($p<0.05$).
IL-6 levels were found to be lower in the Acute pancreatitis+ Rosmarinic acid group compared to the Acute pancreatitis group ($p<0.05$).
The mean±standard deviation and p values of serum Amylase, TNF-α, IL-1β, IL-6 levels are summarized in Table-1.

Discussion

Acute pancreatitis is an inflammatory disease that can result in acute inflammation or necrosis of the pancreatic gland parenchyma[14]. The prognosis of acute pancreatitis generally depends on the presence of accompanying complications such as organ failure and infected pancreatic necrosis. Although the incidence of this disease is increasing, unfortunately we have no specific treatment methods to diminish the symptoms and course of the disease yet[15]. In our study, we examined the protective effects of Rosmarinic acid, which is an alternative treatment for pancreatitis.
Fan et al found in their study that acute pancreatitis is characterized by the onset of necrosis and peripancreatic tissue inflammation. These findings are similar to severe acute pancreatitis in humans[16]. Luo et al showed in their study that rosmarinic acid pretreatment significantly improved the pathological change in the pancreas. They also found that it caused a decrease in serum amylase and lipase activity[17]. Similarly, Mccue et al showed in their study that Rosmarinic acid reduces serum amylase level[18,19]. In our study, we observed that necrotic changes and inflammation had a very high score in the acute pancreatitis group, in terms of histopathological evaluation. In addition, we found that administration of Rosmarinic acid after acute pancreatitis caused decreases in serum amylase values.
IL-1β and TNF-α are major proinflammatory mediators and are responsible for all other systemic complications[20]. Seyed Abbas Metal showed that it was highest in the acute pancreatitis group in their studies[21].In our study, we observed that serum TNF-α values were lower in the group treated with Rosmarinic acid after acute pancreatitis. In other studies, it has been confirmed that Rosmarinic acid has positive results thanks to its anti-inflammatory and antioxidant effects[22-25]. In our study, we observed that rosmarinic acid decreased serum amylase, serum TNF-α, IL-1β and IL-6 values positively.

Ilhan et al showed in their study that there was a significant increase in TAS levels of the group receiving rosmarinic acid[26-27]. However, in this study, we found that there was no statistically significant difference as a result of the evaluation of TAS levels in both tissue and serum. Our study was not compatible with this study in this context.

Conclusion

As a result of the evaluation of biochemical parameters and histopathological findings; It was observed that rosmarinic acid had a partial healing effect on pancreatic tissue and blood values in acute pancreatitis, but still did not cause complete recovery. In line with this information, we came to the conclusion that more comprehensive studies with different doses and durations are required in order to fully understand the curative effect of rosmarinic acid in acute pancreatitis, by which mechanisms it occurs at the cellular level.

Author contributions

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

Authors declared no financial support.

Ethical approval

The experiment was approved by Experiments of the Dicle University Local Ethics Committee2021/23 with protocol number approved.

References

1. Saluja AK, Steer ML. Pathophysiology of pancreatitis. Digestion. 1999;60:27–33. doi: 10.1159/000051450.
2. Bhatia M, Wong FL, Cao Y, et al. Pathophysiology of Acute Pancreatitis. Pancreatology. 2005;5:132–44. doi: 10.1159/000085265
3. Banks PA, Bollen TL, Dervenis C, et al. Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013 ;62(1):102-111. doi: 10.1136/gutjnl-2012-302779.
4. Han S, Yang S, Cai Z, et al. Anti-Warburg effect of rosmarinic acid via miR-155 in gastric cancer cells.Drug Des Devel Ther. 2015; 9: 2695–703. doi: 10.2147/DDDT.S82342
5. Miranda LE, Capellini VK, Reis GS, et al. Effects of partial liver ischemia followed by global liver reperfusion on the remote tissue expression of nitric oxide synthase: lungs and kidneys. Transplant Proc. 2010; 42(5): 1557–62. doi: 10.1016/j.transproceed.2010.02.097.
6. Xu Y, Jiang Z, Ji G, et al. Inhibition of bone metastasis from breast carcinoma by rosmarinic acid. Planta Med. 2010; 76(10): 956–62. doi: 10.1055/s-0029-1240893
7. Chu X, Ci X, He J, et al. Effects of a natural prolyl oligopeptidase inhibitor, rosmarinic acid, on lipopolysaccharide-induced acute lung injury in mice. Molecules. 2012; 17(3): 3586–98. doi: 10.3390/molecules17033586
8. Xu Y, Xu G, Liu Li, et al. Anti-invasion effect of rosmarinic acid via the extracellular signal regulated kinase and oxidation- reduction pathway in Ls174-T cells. J Cell Biochem. 2010; 111(2): 370–379. doi: 10.1002/jcb.22708
9. Moon DO, Kim MO, Lee JD, et al. Rosmarinic acid sensitizes cell death through suppression of TNF-alpha-induced NF-kappaB activation and ROS generation in human leukemia U937 cells. Cancer Lett. 2010; 288(2): 183–191. doi: 10.1016/j.canlet.2009.06.033
10. Schoenberg M H, Büchler M, Gaspar M, et al. Oxygen free radicals in acute pancreatits of the rat. Gut 1990; 31: 1138-43. doi: 10.1136/gut.31.10.1138
11. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem, 2004; 37: 112–19. doi: 10.1016/j.clinbiochem.2003.10.014
12. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 2005; 38: 1103–11. doi: 10.1016/j.clinbiochem.2005.08.008
13. Tüfek A, Tokgöz O, Aliosmanoglu I, et al. The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats. Int J Surg. 2013;11(1):96–100.

doi: 10.1016/j.ijsu.2012.12.003.
14. Irrera N, Bitto A, Interdonato M, et al. Evidence for a role of mitogen-activated protein kinases in the treatment of experimental acute pancreatitis. World J. Gastroenterol. 2014; 20 (44): 16535–43.
doi: 10.3748/wjg.v20.i44.16535
15. Kambhampati S, Park W, Habtezion A. Pharmacologic therapy for acute pancreatitis. World J. Gastroenterol. 2014;20 (45): 16868–80.
doi: 10.3748/wjg.v20.i45.16868
16. Fan YT, Yin GJ, Xiao WQ, et al. Rosmarinic acid attenuates sodium taurocholate-induced acute pancreatitis in rats by inhibiting nuclear factor-kappa B activation. Am. J. Chin. Med. 2015; 43 (6): 1117–35.
doi: 10.1142/S0192415X15500640
17. Luo C, Zou L, Sun H, et al. A Review of the Anti-Inflammatory Effects of Rosmarinic Acid on Inflammatory Diseases. Front Pharmacol. 2020; 28 (11):153.
doi: 10.3389/fphar.2020.00153
18. McCue PP, Shetty K. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. Asia Pacific Journal of Clinical Nutrition. 2004;13: 101-6.
19. Alagawany M, Abd El-Hack ME, Farag MR, et al. Rosmarinic acid: modes of action, medicinal values and health benefits. Animal Health Research Reviews, 2017;18(2): 167-76.
doi: 10.1017/S1466252317000081.
20. J Norman, "The role of cytokines in the pathogenesis of acute pancreatitis," The American Journal of Surgery. 1998; 175 (1):76–83.
doi: 10.1016/S0002-9610(97)00240-7
21. Mirmalek SA, Boushehrinejad AG, Hassan Yet al. Antioxidant and Anti-Inflammatory Effects of Coenzyme Q10 on L-Arginine-Induced Acute Pancreatitis in Rat. Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity. 2016; 2016: 8.
doi: 10.1155/2016/5818479
22. Ramalho LNZ, Pasta ÂAC, Terra VA, et al. Rosmarinic acid attenuates hepatic ischemia and reperfusion injury in rats. Food and chemical toxicology. 2014; 74: 270-8.
doi: 10.1016/j.fct.2014.10.004.
23. Ahmed MM. Rosmarinic acid attenuates the hepatotoxicity induced by ethanol in rats. American Journal of Biochemistry. 2016; 6(3): 82-90.
doi: 10.5923/j.ajb.20160603.03
24. Oğuz A, Böyük A, Ekinci A, et al. Investigation of antioxidant effects of rosmarinic acid on liver, lung and kidney in rats: a biochemical and histopathological study. Folia morphologica. 2020; 79 (2): 288-95.
doi: 10.5603/FM.a2019.0087
25. Sadeghi A, Bastin AR, Ghahremani H, et al. The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Molecular biology reports. 2020; 47(5): 3557-66.
doi: 10.1007/s11033-020-05447-x.
26. Ilhan N, Bektas I, Susam S, et al. Protective effects of rosmarinic acid against azoxymethane-induced colorectal cancer in rats. J Biochem Mol Toxicol. 2021;12:e22961.
doi: 10.1002/jbt.22961.
27. Mohammed FS, Karakas M, Akgul H, et al. Medicinal Properties of Allium Calocephalum Collected from Gara Mountain (Iraq). Fresenius Environmental Bulletin. 2019; 28 (10): 7419-26.