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Daidzein alleviated the pathologies in intestinal tissue against ischemia-reperfusion

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effect of daidzein against intestinal ischemia-reperfusion injury in rats.

MATERIALS AND METHODS: Thirty male Wistar albino rats with a mean weight of 200-250 gr were used. Animals were categorized into sham, ischemia-reperfusion (IR), and IR+Daidzein group. 3-hour ischemia of intestine was created by occluding superior mesenteric artery and then left for 3-hour reperfusion. In IR+daidzein group, after ischemia, 50 mg/kg daidzein was orally administered to the animals. Blood samples were collected for biochemical assays. Intestine tissues were excised for histopathologic and immunohistochemical processing.

RESULTS: Malondialdehyde (MDA) increased, and Catalase (CAT) and Glutathione (GSH) decreased after IR in intestine tissue. Daidzein treatment decreased MDA and increased CAT and GSH level in IR+Daidzein group. Histopathologically, sham group showed normal intestinal tissue histology. In IR group, epithelial and villi degeneration, edema, leukocyte infiltration, vascular dilatation and congestion was observed. After Daidzein treatment, these pathologies were improved. The caspase-6 expression was mainly negative in sham group. After IR, caspase-6 reaction was very high in IR group. Daidzein reduced caspase-6 expression in IR+-Daidzein group. Ki67 immune staining was negative in the sham group. In IR group, Ki67 expression was increased in inflammatory cells, deep glandular cells and in some goblet cell nuclei. In IR+Daidzein group, Ki67 expression was decreased due to reduced inflammation.

CONCLUSIONS: IR injury causes oxidative stress, apoptosis and inflammation. Daidzein treatment improved histopathology against intestinal IR.

Key Words:
Ischemia-reperfusion, Daidzein, Caspase-6, Ki-67.

Introduction

Ischemia-reperfusion (I/R) injury in small intestine is an event in which blood flow of the intestines is reduced and then restored to the ischemic tissue[1]. Intestinal I/R injury leads to severe local and systemic inflammation followed by damage to surrounding distant organs. Thus, the intestinal mucosal barrier is disrupted, and the organ is damaged. If this damage continues for a long time and cannot be treated, the life of the animal may be endangered. Mortality rates vary between 60-80% in patients with acute intestinal I/R, and a new treatment strategy is needed for intestinal I/R[2,3].

Daidzein (7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one) is a naturally occurring isoflavone isolated from mainly soy plants. It is also named phytoestrogen due to a similarity of chemical composition to mammalian estrogens. With this property, daidzein is involved in mechanisms of many diseases, especially those under estrogen control such as breast cancer, diabetes mellitus, osteoporosis and cardiovascular disorders[4]. A number of biological activities with daidzein have been previously studied. Daidzein can reduce oxidative damage by enhancing cellular scavenging molecules, can act as antioxidant, immunomodulatory, anti-inflammatory, and anti-cancer by promoting cell survival and cellular defense mechanism[5].

Caspase-6 is a member of caspase family and is involved in many cellular events such as death, immune responses, and homeostasis. It is an effector caspase and has a role in apoptosis. Caspase-6 interacts with caspase-8 to execute phases of apoptosis[6]. Ki-67 is a cellular marker used in the prognosis of many tumors and its expression increases in proliferating cells. It also has many molecular functions in proliferating cells and has a role in mitosis[7]. Immunohistochemistry is a laboratory method to detect the localization of specific antigens.

The aim of this study was to investigate the effect of daidzein with antioxidative activity on intestinal ischemia-reperfusion injury in rats by immunohistochemical and biochemical techniques.

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Materials and Methods

All animal experimentation was done with the Ethical approval of the Local Ethical Committee of Animal Experiments of Siirt University, Turkey (record number: 21/2022). Male Wistar albino rats (n=30), 3 to 4 months old, weighing 180-240 g were used. Experimental animals had unlimited access to water and food, and they were kept under control in an environment of 12 hours daytime/12 hours dark, 8:00 am to 8:00 pm, at 23±2°C. Thirty animals were assigned to three groups (10 rats per group).

Surgical Procedure

Before surgical procedures, animals were anesthetized by an intramuscular injection of 80 mg/kg ketamine hydrochloride (Ketalar®, Pfizer, Istanbul, Turkey) and 20 mg/kg xylazine (Rompun®, Bayer, Istanbul, Turkey) under aseptic conditions. The abdominal region was shaved, and a 3 cm abdominal midline incision was done to observe the superior mesenteric artery (SMA). SMA was clamped with Bulldog clamp to perform ischemia. At the end of the ischemic period, the clamp was removed and the SMA was released.

Sham group

Animals were laparotomized and SMA was observed without any further intervention.

Ischemia – reperfusion (IR)

3-hour ischemia was performed by SMA occlusion. The intestine was then reperfused for 3 hours.

IR+Daidzein

After IR procedure, 50 mg/kg daidzein was orally administered to the rats for 7 days.

At the end of the experiment, all animals were sacrificed. The jejunum tissues of all groups were removed and divided into two equal pieces and stored under suitable conditions for biochemical and histopathological investigations.

Biochemical Analyses

Blood samples were analyzed for Malondialdehyde (MDA), Glutathione (GSH) and Catalase (CAT). Samples were kept in centrifugation tubes and centrifuged for 5 minutes at 1,550 g. Supernatant was discarded to determine the levels of MDA, GSH and CAT. MDA levels (nmol/g) were measured according to Draper and Hadley's method[8]. MDA values were expressed as nanomoles per gram (nmol/g) of wet tissue. To measure GSH activity (U/g), the protocol of Paglia and Valentine[9] was carried out. CAT activity (U/g) was detected according to protocol of Brown et al[10].

Histopathological Analysis

Intestinal tissues were excised and stored in 10% formalin for tissue fixation. Tissues were passed through ascending alcohol series, washed in xylene and embedded in paraffin wax. Sections were cut from intestinal tissues and stained with Hematoxylin and Eosin dye. Scoring was calculated for each group by examining ten different areas by two different histologist experts. Parameters were assigned to the scoring system where 0=no expression/pathology, 1=mild expression/pathology, 2=moderate expression/pathology, 3=intense expression/pathology[11,12].

Immunohistochemical Analysis

Tissues were fixed in 10% formalin solution and processed for histological tissue processing. Sections were deparaffinized in xylene and passed through descending alcohol series. Epitopes were unrevealed by boiling the sections in the microwave oven in citrate buffer solution (pH 6.0) for 15 minutes at 700 W. Sections were washed in phosphate buffered saline (PBS) for 5 minutes and soaked with 3% hydrogen peroxide solution for 7 minutes to prevent endogenous peroxidase. Samples were blocked with blocking solution (catalog No. TA-015UB, ThermoFischer, Waltham, MA, USA) for 8 minutes. Primary antibodies Caspase-6, (catalog No. ab185645, Abcam, Cambridge, UK) and Ki-67 (catalog No. ab16667, Abcam, Cambridge, UK) were dropped onto slides and incubated overnight at +4°C. Sections were washed in PBS, then treated with secondary antibody (TP-015-BN, ThermoFischer, Waltham, MA, USA) for 20 min. After PBS washing for 3x5 min, streptavidin-peroxidase solution (TS-015-HR, ThermoFischer, Waltham, MA, USA) was used for 20 min. To visualize the expression, DAB (TA-001-HCX, ThermoFischer, Waltham, MA, USA) was used as chromogen. Sections were counterstained with hematoxylin, mounted with mounting media and examined with Zeiss Imager A2 light microscope (Carl Zeiss, Jena, Germany)[13-16].

Statistical Analysis

Biochemical and histological scoring were evaluated by the SPSS 25.0 software (IBM Corp., Armonk, NY, USA). Shapiro-Wilk test was used for data distribution analysis. Nonparametric Kruskal-Wallis' test (multiple comparison) and Mann-Whitney U (binary comparison) tests were used. $p<0.05$ was accepted as significant level.

Results

Biochemical Findings

Statistical analysis of biochemical and histochemical parameters was shown in Table I. MDA and histological scores of inflammations, dilatation, Caspase-6 and Ki67 expression were higher in IR group than the sham group. CAT, GSH levels and histological scores of epithelization were significantly lower in IR group than sham group. After Daidzein treatment, MDA, histological scores of inflammations, dilatation, Caspase-6 and Ki67 expression decreased in IR+Daidzein group compared to IR group, and this decrease was statistically significant. Similarly, CAT, GSH levels and histological scores of epithelization statistically increased in IR+Daidzein group compared to IR group. Graphical illustration of Table I was shown in Figure 1.

Histopathological Finding

Figure 2 showed the hematoxylin eosin, caspase-6 and Ki67 immune staining of intestinal sections. Hematoxylin Eosin staining in the Sham group: The villi were regular and longitudinal in shape towards the lumen, the epithelial cells were cylindrical with oval-shaped goblet cells scattered between them with normal lamina propria and muscular layers (Figure 2a).

Table I. Biochemical and histological parameters of sham, IR and IR+Daidzein groups.

Parameter	Groups	N	Median (min-max)	Mean rank	*p*-value
MDA	Sham	10	25.05 (15.28-45.58)	8.00	*$p < 0.001$
	IR	10	49.15 (28.46-63.48)	22.20	**$p = 0.043$
	IR+Daidzein	10	39.45 (23.59-49.35)	16.30	
CAT	Sham	10	1.47 (1.13-1.67)	20.65	*$p < 0.001$
	IR	10	0.55 (0.19-1.15))	5.60	**$p < 0.001$
	IR+Daidzein	10	1.44 (1.17-1.77)	20.25	
GSH	Sham	10	8.98 (6.45-9.85)	25.40	*$p < 0.001$
	IR	10	3.82 (2.15-5.36)	6.00	**$p < 0.001$
	IR+Daidzein	10	5.68 (4.69-7.25)	15.10	
Epithelialization	Sham	10	2.00 (1.00-3.00)	20.00	*$p = 0.002$
	IR	10	0.50 (0.00-3.00)	7.75	**$p = 0.003$
	IR+Daidzein	10	2.00 (1.00-3.00)	18.75	
Inflammation	Sham	10	0.00 (0.00-1.00)	6.10	*$p < 0.001$
	IR	10	2.50 (2.00-3.00)	23.75	**$p = 0.011$
	IR+Daidzein	10	1.00 (1.00-3.00)	16.65	
Dilatation	Sham	10	0.00 (0.00-1.00)	6.55	*$p < 0.001$
	IR	10	2.00 (2.00-3.00)	24.40	**$p =0.002$
	IR+Daidzein	10	1.00 (1.00-3.00)	15.55	
Caspase-6 expression	Sham	10	0.00 (0.00-1.00)	6.70	*$p <0.001$
	IR	10	3.00 (2.00-3.00)	23.90	**$p = 0.009$
	IR+Daidzein	10	1.00 (1.00-3.00)	15.90	
Ki-67 expression	Sham	10	1.00 (0.00-2.00)	9.00	*$p <0.035$
	IR	10	2.00 (2.00-3.00)	16.85	**$p = 0.003$
	IR+Daidzein	10	1.00 (0.00-2.00)	15.50	

*Sham *vs*. IR; **IR *vs*. IR+Daidzein.

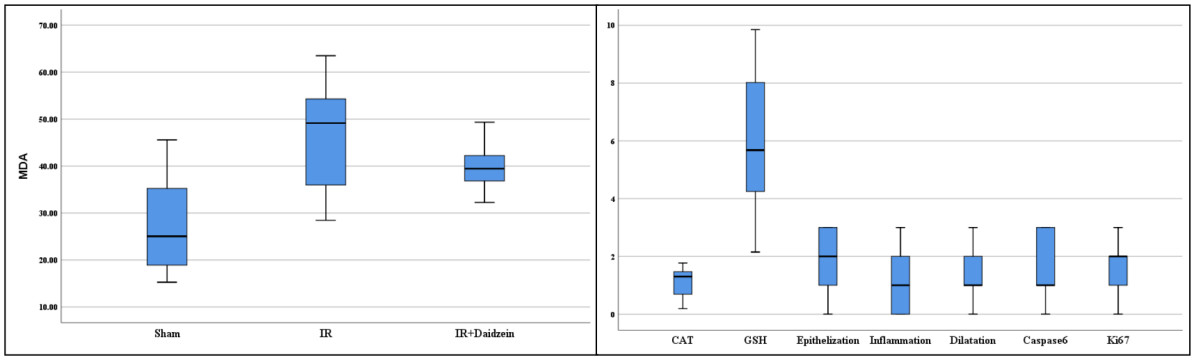

Figure 1. Graphical illustration of mean of MDA, CAT, GSH, epithelization, inflammation, vascular dilatation, Caspase6 and Ki-67 expression.

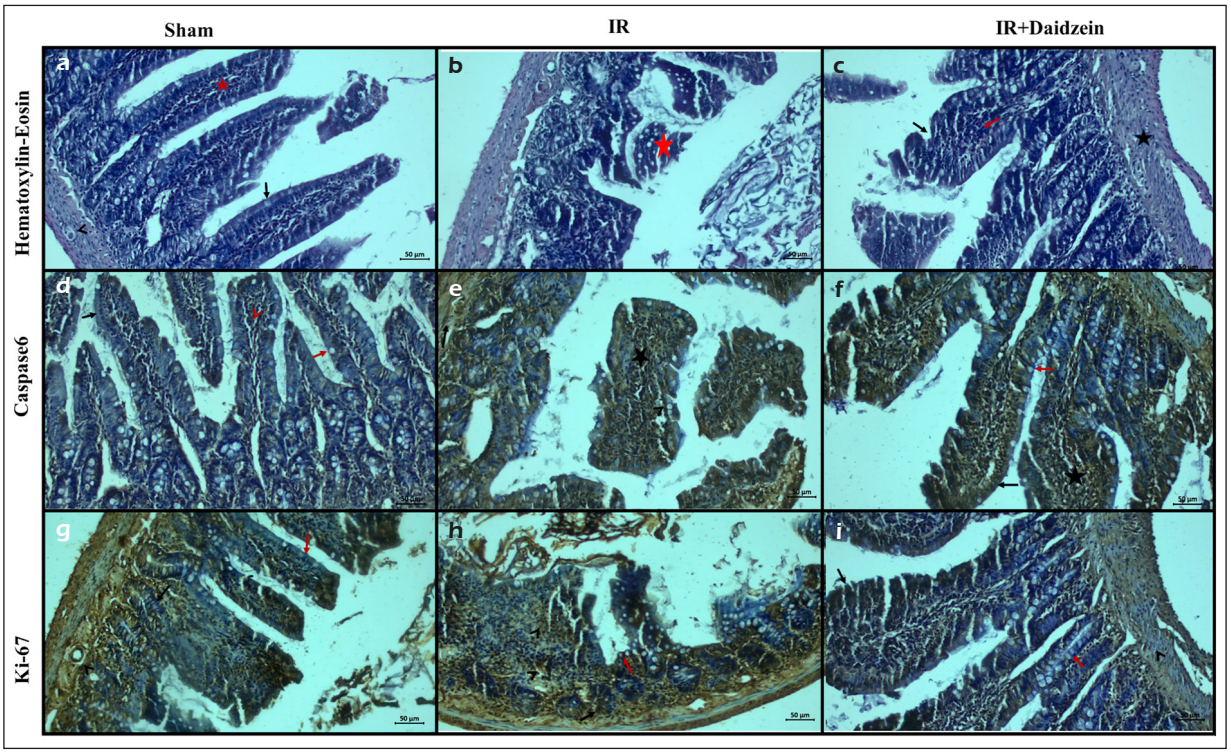

Figure 2. Hematoxylin eosin staining (a-c), Caspase6 (d-f) and Ki67 (g-i) immunostaining of intestinal sections of sham, IR and IR+Daidzein groups. **a,** The regular villi (*red star*), oval-shaped goblet cells (*arrow*), circular muscles in the muscular layer (*arrowhead*). **b,** Ruptured villi (*red star*), mononuclear cell infiltration, dilatation and congestion. **c,** Restored epithelial structures (*black arrow*), decreased inflammation in the lamina propria (red arrow), regular muscle layer (*star*). **d,** The negative caspase6 reaction in epithelial cells (*black arrow*), in goblet cells (*red arrow*), in lamina propria (*arrowhead*). **e,** The positive caspase6 reaction in the lamina propria (*star*). **f,** Positive Caspase6 in epithelial cells (*black arrow*) and in lamina propria (*star*), negative expression in goblet cell nuclei (*red arrow*). **g,** Negative Ki67 expression in glands and epithelium (*arrow*). **h,** Positive ki67 reaction in the regions of the deep glands (*arrowheads*), in some goblet cell nuclei (*red arrow*) and lamina propria (*black arrow*). **i,** Positive Ki67 expression in the desquamated epithelial cells (*black arrow*), negative expression in goblet cells (*red arrow*) and in muscle layers (*arrowhead*). Scale Bar: 50 µm, magnification: 20×.

IR group: Ruptures and degeneration in the villi, loss of epithelial cells, increase in mononuclear cell infiltration and dilated vascular structures were observed. Edema and hyperplasic muscle cells were detected (Figure 2b).

IR+Daidzein group: Villi with epithelial cells and goblet cells were improved. There was a decrease in inflammation compared to the ischemia group in the lamina propria. Muscular layers seemed to be restored (Figure 2c).

Caspase-6 immune staining in the Sham group: Caspase-6 reaction in epithelial and goblet cells was generally negative. Caspase-6 reaction in plasma cells was found to be moderately positive (Figure 2d).

IR group: Caspase-6 reaction was especially positive in goblet cells, in the lamina propria and in the basement membrane (Figure 2e).

IR+Daidzein group: Caspase-6 showed a positive reaction in most of the epithelial cells, while negative expression was detected in goblet cells. The positive expression continued in the connective tissue cells (Figure 2f).

Ki67 immune staining in the Sham group: Ki67 expression was negative in the glands and epithelium, but positive in lamina propria (Figure 2g).

IR group: Ki67 reaction was positive in inflamed epithelial cells and in some goblet cell nuclei (Figure 2h). IR+Daidzein: Ki67 expression was positive in epithelial cells and inflammatory cells in the lamina propria (Figure 2i).

Discussion

Ischemia causes the insufficient delivery of oxygen and other metabolites by the circulation to the tissues, leading to cell death and organ failure. Reperfusing the ischemic tissue sometimes may give more harm than ischemia itself. During IR injury, the production of reactive oxygen species increases[17]. MDA is an indicator of lipid peroxidation in tissues. High level of MDA is related to oxidative damage. The cell scavenges these harmful molecules by its antioxidant enzymes such as superoxide dismutase (SOD), GSH and CAT. During the intestinal IR, the oxidant/antioxidant balance may change. Ji et al[18] studied effect of intestinal IR on MDA and myeloperoxidase (MPO) levels. The authors state that MDA and MPO levels increased in the IR group. Similarly, Chen et al[19] studied MDA, SOD, GSH levels in their intestinal IR injury. They found that MDA level was increased in IR group compared to sham group, while SOD and GSH level was lower in IR group than in sham group. In our study, MDA and histological scores of inflammations, dilatation and Caspase-6 expression were increased in IR group. CAT, GSH levels and histological scores of epithelization and Ki67 expression were decreased in IR group. After Daidzein treatment, MDA and histological scores of inflammations, dilatation and Caspase-6 expression decreased and CAT, GSH levels and histological scores of epithelization and Ki67 expression increased in IR+Daidzein group (Table I, Figure 1).

After the reblooding of the tissue, some cellular functions were regained; however, it may cause more cellular damage. These changes affect the histology of the intestinal tissue. Keskin Çimen et al[20] performed intestinal IR on rats and showed that IR injury caused polymorphonuclear leukocyte infiltration, edema, hemorrhage, vascular dilatation and congestion in IR group. Terzi et al[21] conducted 60 min/60 min IR injury on rat intestine and showed that desquamation in epithelial layers, hemorrhage, edema are pathologies observed in IR group. In our study, sham group showed normal intestinal histology with regular villi, cylindrical epithelial cells, oval-shaped goblet cells and normal submucosa and muscle layers (Figure 2a). In IR group, ruptures and decrease in the villi structures, shortening of the villus length, increased mononuclear cell infiltration, edema and dilated vascular structure were observed (Figure 2b). In the IR+Daidzein group, epithelial structures were restored, the goblet cells were normal, and there was a decreased inflammation with normal muscle layers (Figure 2c).

Caspases are cysteine proteases involved in cell death. They play very important roles in embryonic development and cell homeostasis. Caspase 6 is a member of cysteine proteases family and involves in apoptosis. Caspase-6 activity can be observed in many diseases. It is executive proteases and plays role in inflammation[22]. Singh et al[23] studied expression of caspase-6 in renal ischemia reperfusion injury. The authors found that caspase-6 immune activity was more intense and diffused cytoplasmic staining with prominent nuclear staining in IR group. In an ischemic stroke study, caspase-6 activity was investigated. The author revealed that preventing activity of caspase-6 decreased the neuropathologies in cerebral or retinal in-

farction[24,25]. In our study, in sham group: the caspase-6 reaction was generally negative in epithelial cells and goblet cells (Figure 2d). In IR group, caspase-6 reaction was especially positive in the lamina propria, in some of the goblet cells, in connective tissue cells scattered in the lamina propria and some muscle cells (Figure 2e). In IR+Daidzein group, caspase-6 showed positive reaction in most of the epithelial cells and negative in goblet cell nuclei (Figure 2f).

The cell proliferation antigen Ki67 is constitutively expressed in dividing mammalian cells. Therefore, it is widely used as a cell proliferation marker[26]. Ki67 is generally a necessary protein for cell proliferation. Ki67 index is used as a diagnostic, prognostic and predictive tool in the fields of pathology. Immunohistochemical scoring of Ki67 is a gold standard for many diseases for prognosis[27]. Khan et al[27] studied role of Ki67 in mouse renal IR injury as a proliferation marker. The authors found that Ki67 immune activity was low in IR group but was increased after a synthetic oligopeptides' treatment. Liu et al[28] conducted IR injury in transgenic mouse kidney. The authors showed that after IR, Ki67 expression was increased in medulla and papilla of the kidney in the cells repairing. In our study, Ki67 immune staining was negative in the general glands and in the epithelium in sham group (Figure 2g). In IR group, Ki67 expression was positive in inflammatory cells, deep glandular cells and in some goblet cell nuclei (Figure 2h). In IR+Daidzein group, Ki67 expression was positive in degenerated epithelial cells and in some inflammatory cells (Figure 2i).

Limitations

A quantitative method would be better to support the immunohistochemical study.

Conclusions

The apoptotic pathway induced due to increased oxidative stress and inflammation in ischemia-reperfusion. We suggest that Daidzein reduces oxidative stress and is effective in the inflammation process and helps to prevent apoptotic progression by regulating Ki-67 expression.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Conceptualization, formal analysis, investigation, writing - review and editing, supervision, project administration, data curation, writing - original draft: CD, FA; Methodology, validation, resources, visualization: FA.

Informed Consent

Not applicable.

Ethics Approval

Ethical Approval was taken from Animal Experimentation Ethics Committee, Siirt University, with record number 21/2022.

Availability of Data and Materials

All generated data in this study were presented.

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References

1) Klempnauer J, Grothues F, Bektas H, Pichlmayr R. Long-term results after surgery for acute mesenteric ischemia. Surgery 1997; 121: 239-243.
2) Wilcox MG, Howard TJ, Plaskon LA, Unthank JL, Madura JA. Current theories of pathogenesis and treatment of nonocclusive mesenteric ischemia. Dig Dis Sci 1995; 40: 709-716.
3) Kassahun WT, Schulz T, Richter O, Hauss J. Unchanged high mortality rates from acute occlusive intestinal ischemia: six year review. Langenbecks Arch Surg 2008; 393: 163-171.
4) Rawat S, Pathak S, Gupta G, Singh SK, Singh H, Mishra A. Recent updates on daidzein against oxidative stress and cancer. Excli J 2019; 18: 950-954.
5) Alshehri MM, Sharifi-Rad J, Herrera-Bravo J, Jara EL, Salazar LA, Kregiel D, Uprety Y, Akram M, Iqbal M, Martorell M, Torrens-Mas M, Pons DG, Daştan SD, Cruz-Martins N, Ozdemir FA, Kumar M, Cho WC. Therapeutic Potential of Isoflavones with an Emphasis on Daidzein. Oxid Med Cell Longev 2021; 2021: 6331630.
6) Zheng M, Karki R, Vogel P, Kanneganti TD. Caspase-6 Is a Key Regulator of Innate Immunity, Inflammasome Activation, and Host Defense. Cell 2020; 181: 674-687.

7) Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma 2018; 127: 175-186.
8) Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421-431.
9) Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.
10) Brown JM, Grosso MA, Terada LS, Whitman GJ, Banerjee A, White CW, Harken AH, Repine JE. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. Proc Natl Acad Sci 1989; 86: 2516-2520.
11) Aşır F, Nergiz Y, Pala A. Vitamin E protected the mouse adrenal gland against immobilization stress. Pol J Vet Sci 2022; 25: 447-454.
12) Taş F, Erdemci F, Aşır F, Maraşlı M, Deveci E. Histopathological examination of the placenta after delivery in pregnant women with COVID-19. J Health Sci Med 2022; 5: 868-874.
13) Tasin C, Ermis I, Deveci E. Endothelin-1 and APAF-1 Expression in the Umbilical Cord of Placenta Previa Cases. Anal Quant Cytopathol Histpathol 2021; 43: 439-445.
14) Deveci S, Deveci E. Histopathological changes in incisive teeth of the newborn pups of cadmium-applied female rats during pregnancy. Int J Morphol 2010; 28: 1131-1134.
15) Özevren H, İrtegün S, Deveci E, Aşır F, Pektanç G, Deveci Ş. Ganoderma Lucidum Protects Rat Brain Tissue Against Trauma-Induced Oxidative Stress. Korean J Neurotrauma 2017; 13: 76-84.
16) Özgökçe Ç, Öcal A, Ermiş IS. Expression of NF-κB and VEGF in normal placenta and placenta previa patients. Adv Clin Exp Med 2023. [ahead of print].
17) Beskisiz S, Asir F. Hesperidin May Protect Gastric Tissue Against Immobilization Stress. Anal Quant Cytopathol Histpathol 2021; 43: 399-405.
18) Ji YY, Wang ZD, Wang SF, Wang BT, Yang ZA, Zhou XR. Ischemic preconditioning ameliorates intestinal injury induced by ischemia-reperfusion in rats. World J Gastroenterol 2015; 21: 8081-8088.
19) Chen R, Zhang YY, Lan JN, Liu HM, Li W, Wu Y, Leng Y, Tang LH, Hou JB, Sun Q, Sun T, Zeng Z, Xia ZY, Meng QT. Ischemic Postconditioning Alleviates Intestinal Ischemia-Reperfusion Injury by Enhancing Autophagy and Suppressing Oxidative Stress through the Akt/GSK-3β/Nrf2 Pathway in Mice. Oxid Med Cell Longev 2020; 2020: 6954764.
20) Keskin Çimen F, Çimen O, Altuner D, Çekiç AB, Kurt N, Süleyman H. Effect of rutin on experimentally induced small intestinal ischemia reperfusion injury in rats: A biochemical and histopathological evaluation. J Surg Med 2021; 5: 26-30.
21) Terzi A, Coban S, Yildiz F, Ates M, Bitiren M, Taskin A, Aksoy N. Protective effects of Nigella sativa on intestinal ischemia-reperfusion injury in rats. J Invest Surg 2010; 23: 21-27.
22) Dagbay KB, Hardy JA. Multiple proteolytic events in caspase-6 self-activation impact conformations of discrete structural regions. Proc Natl Acad Sci 2017; 114: E7977-E7986.
23) Singh AB, Kaushal V, Megyesi JK, Shah SV, Kaushal GP. Cloning and expression of rat caspase-6 and its localization in renal ischemia/reperfusion injury. Kidney Int 2002; 62: 106-115.
24) Shabanzadeh AP, D'Onofrio PM, Monnier PP, Koeberle PD. Targeting caspase-6 and caspase-8 to promote neuronal survival following ischemic stroke. Cell Death Dis 2015; 6: e1967.
25) MacCallum DE, Hall PA. The biochemical characterization of the DNA binding activity of pKi67. J Pathol 2000; 191: 286-298.
26) Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 2015; 11: 1566-1572.
27) Khan NA, Susa D, van den Berg JW, Huisman M, Ameling MH, van den Engel S, Henk R. Amelioration of renal ischaemia-reperfusion injury by synthetic oligopeptides related to human chorionic gonadotropin. Nephrol Dial Transplant 2009; 24: 2701-2708.
28) Liu X, Liu H, Sun L, Chen Z, Nie H, Sun A, Liu G, Guan G. The role of long-term label-retaining cells in the regeneration of adult mouse kidney after ischemia/reperfusion injury. Stem Cell Res Ther 2016; 7: 68.