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ORIGINAL RESEARCH

Gallic acid attenuates torsion/detorsion-induced testicular injury in rats through suppressing of HMGB1/NF-κB axis and endoplasmic reticulum stress

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Abstract

It was aimed to evaluate whether gallic acid (GA) have a beneficial effect in the testicular ischemia/reperfusion injury (IRI) model in rats for the first time. Testicular malondialdehyde, 8-hydroxy-2′-deoxyguanosine, superoxide dismutase, catalase, high mobility group box 1 protein, nuclear factor kappa B, tumor necrosis factor-alpha, interleukin-6, myeloperoxidase, 78-kDa glucose-regulated protein, activating transcription factor 6, CCAAT-enhancer-binding protein homologous protein and caspase-3 levels were determined using colorimetric methods. The oxidative stress, inflammation, endoplasmic reticulum stress and apoptosis levels increased statistically significantly in the IRI group compared with the sham operated group ($p < 0.05$). GA application improved these damage significantly ($p < 0.05$). Moreover, it was found that the results of histological examinations supported the biochemical results to a statistically significant extent. Our findings suggested that GA may be evaluated as a protective agent against testicular IRI.

Keywords

Apoptosis; Endoplasmic reticulum stress; Gallic acid; Inflammation; Oxidative stress; Testicular torsion

El ácido gálico atenúa la lesión testicular inducida por torsión/detorsión en ratas mediante la supresión del eje HMGB1/NF-κB y el estrés del retículo endoplásmico

Resumen

El objetivo era evaluar si el ácido gálico (GA) tenía un efecto beneficioso en el modelo de lesión por isquemia/reperfusión testicular (IRI) en ratas por primera vez. Malondialdehído testicular, 8-hidroxi-2′-desoxiguanosina, superóxido dismutasa, catalasa, proteína del grupo de alta movilidad caja 1, factor nuclear kappa B, factor de necrosis tumoral alfa, interleucina-6, mieloperoxidasa, proteína regulada por glucosa de 78 kDa, activadora el factor de transcripción 6, la proteína homóloga de la proteína de unión al potenciador de CCAAT y los niveles de caspasa-3 se determinaron mediante métodos colorimétricos. El estrés oxidativo, la inflamación, el estrés del retículo endoplásmico y los niveles de apoptosis aumentaron de manera estadísticamente significativa en el grupo IRI en comparación con el grupo operado de forma simulada ($p < 0.05$). La aplicación de GA mejoró significativamente estos daños ($p < 0.05$). Además, se encontró que los resultados de los exámenes histológicos respaldaron los resultados bioquímicos en un grado estadísticamente significativo. Nuestros hallazgos sugieren que GA puede evaluarse como un agente protector contra IRI testicular.

Palabras Clave

Apoptosis; Estrés del retículo endoplásmico; Ácido gálico; Inflamación; Estrés oxidativo; Torsión testicular

1. Introduction

Testicular torsion (TT) is a condition that results from the rotation of the spermatic cord around its own axis and requires emergency surgical intervention [1]. TT causes biochemical and histological changes in the tissue [2]. The severity of damage to the gonadal structures is directly related to the degree and duration of torsion, and delayed treatment leads to harmful consequences, such as subfertility and infertility [1, 3]. The only treatment for TT is surgical detorsion [4]. The testicular salvage rate of the surgical detorsion procedure performed within 6 h from the beginning of the torsion is 90%. Unfortunately, this rate decreases to 50% after 12 h and to 10% after 24 h [5]. However, as a result of increased perfusion after detorsion, a greater damage occurs in the tissue than ischemia-induced damage, and is known as ischemia/reperfusion injury (IRI) [2]. IRI can lead to testicular atrophy by increasing reactive oxygen species (ROS), lipid peroxidation, inflammation, endoplasmic reticulum (ER) stress and apoptosis [3]. Recent researches have therefore focused on antioxidant molecules that can prevent testicular IRI [2].

The ER is a central organelle involved in protein synthesis and folding [6]. Pathological conditions, such as hypoxia and nutritional deficiencies, trigger the accumulation of unfolded/misfolded proteins in the ER lumen, and this is termed "ER stress" [7]. Mild ER stress includes activation of the unfolded protein response (UPR) and works entirely to keep the cell alive. If UPR activation fails to overcome ER stress, the cell is driven to apoptosis [8]. Increasing evidence indicates that ER stress have a significant potential to induce IRI-induced tissue damage [9].

Investigation of various pharmacological action mechanisms of phenolic compounds obtained from natural products has attracted great interest in recent years [10]. Gallic acid (GA) is a phenolic acid and found in red wine, green tea, strawberry, pineapple, banana, lemon and saffron [11]. GA has been reported to exhibit many biological activities, including antimicrobial, anticancer, anti-inflammatory, antioxidant, antimutagenic and anti-apoptotic properties [12, 13]. Today, GA is therefore widely used drug, food and dyeing industries [12]. Although GA has been shown to protect kidney [14], brain [15], heart [16] and liver [17] tissues against IRI in experimental models, to our best knowledge, no study has been found examining the effect of GA against testicular IRI. Protecting male reproductive health against IRI is very important for the continuation of fertility. This study therefore aimed to evaluate whether GA had a beneficial effect in the testicular IRI model in rats for the first time.

2. Material and methods

2.1 Experimental design

The 18 rats were divided into three groups (n = 6): sham control, torsion/detorsion (T/D) and T/D + GA (50 mg/kg). The time-dependent procedures performed in the study were summarized in Table 1. In the sham control group, the left testicle was removed and placed back into the scrotum to create surgical stress. In both T/D and T/D + GA groups, the left testicle removed by incision was rotated 720° clockwise and fixed

to the scrotum using the method described previously [18]. The suture was removed after 4 h and testicular reperfusion was achieved for 2 h based on previously described methods [19–21]. In the GA treatment group, 30 min before detorsion, rats were given GA (50 mg/kg) *via* intraperitoneal route. GA dose (50 mg/kg) was determined considering previous studies and prepared by dissolving in sterile saline [17, 22, 23]. After 2 h of detorsion, orchiectomy was performed. The removed testicles were homogeneously divided longitudinally in two pieces, and one portion was frozen at −80 °C for biochemical analysis and other parts were immersed in Bouin's solution for histological evaluation.

TABLE 1. A summary of the procedures in the experimental groups.

	Groups		
	Sham Control	T/D	T/D + GA
Torsion 0 min	−	+	+
210 min after torsion	sterile saline	sterile saline	GA (50 mg/kg)
Detorsion (240 min after torsion)	−	+	+
Orchiectomy (360 min after torsion)	+	+	+

GA: gallic acid; T/D: torsion/detorsion.

2.2 Histological analysis

Routine histological tissue follow-ups were performed for testicular specimens fixed from Bouin's solution. Paraffin blocks were cut in 5 μm sections, stained with hematoxylin and eosin (H&E) and evaluated under a light microscope (Olympus BX51, Olympus Co., Tokyo, Japan) [24]. Later, seminiferous tubule architecture and the levels of spermatogenesis were graded with the scoring system defined by Johnsen [25]. After the first seminiferous tubule was chosen randomly, the remaining part was observed by moving it clockwise. Analyzes were performed by a histologist unfamiliar with the groups using the coding system.

2.3 Biochemical analysis

Tissue samples were homogenized in 2 mL of phosphate buffered saline at 9500 rpm using a homogenizer (IKA, T25 Ultra-Turrax, Staufen im Breisgau, Germany) and the homogenates were centrifuged at 1800×g for 10 min at 4 °C to obtain supernatants. Protein levels of the supernatants were determined using a commercial kit (Thermo Scientific, Pierce BCA Protein Assay Kit, Cat No: 23225, Rockford, IL, USA) according to the manufacturer's instructions. Bovine serum albumin was used as a standard, and the protein levels of the supernatants were calculated in mg/mL using the albumin standard graph. The biochemical parameters measured in the supernatants were proportioned to the amount of protein and expressed per mg of protein.

Testicular malondialdehyde (MDA) levels of all groups

were determined according to the method described previously [26], while total oxidant status (TOS) (Cat No: RL0024) and total antioxidant status (TAS) (Cat No: RL0017) levels were determined using commercial colorimetric kits (Rel Assay Diagnostics, Gaziantep, Turkey). The oxidative stress index (OSI) was determined using the following formula [27]:

$$OSI\ (arbitrary\ unit)\ =\ (TOS/TAS)\ \times\ 100$$

The tissue amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Cat No: ER1487-HS), superoxide dismutase (SOD) (Cat No: ER0332), catalase (CAT) (Cat No: ER0264), high mobility group box 1 (HMGB1) (Cat No: ER0291), nuclear factor kappa B protein 65 (NF-κB p65) (Cat No: ER1187), tumor necrosis factor-alpha (TNF-α) (Cat No: ER1393), interleukin-6 (IL-6) (Cat No: ER0042), myeloperoxidase (MPO) (Cat No: ER0142), 78-kDa glucose-regulated protein (GRP78) (Cat No: ER0562), activating transcription factor 6 (ATF6) (Cat No: ER1645), CCAAT-enhancer-binding protein homologous protein (CHOP) (Cat No: ER0694) and caspase-3 (Cat No: ER0143) were determined using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Finetest, Wuhan, China).

2.4 Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical differences between groups were assessed by analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. $p <$ 0.05 is considered statistically significant.

3. Results

The MDA, TOS, OSI and 8-OHdG levels were significantly higher in the T/D group compared with sham control group ($p =$ 0.0001, $p = 0.003$, $p = 0.001$ and $p = 0.0001$, respectively). GA application reduced the MDA, TOS, OSI and 8-OHdG levels significantly compared with T/D group ($p = 0.0001$, $p = 0.002$, $p = 0.001$ and $p = 0.0001$, respectively) (Table 2).

The TAS, SOD and CAT levels were found to be significantly decreased in the testicular tissues of the T/D group compared to the sham control group ($p = 0.0001$, $p = 0.0001$ and $p = 0.007$, respectively). GA application increased TAS, SOD and CAT levels significantly compared with T/D group ($p = 0.0001$, $p = 0.0001$ and $p = 0.025$, respectively) (Table 2).

The HMGB1, NF-κB p65, TNF-α, IL-6 and MPO levels were significantly increased in the T/D group compared to the sham control group (all $p = 0.0001$). GA application decreased HMGB1, NF-κB p65, TNF-α, IL-6 and MPO levels significantly compared with T/D group (all $p = 0.0001$) (Table 2).

The GRP78, ATF6, CHOP and caspase-3 levels of the T/D group was significantly increased compared to the sham control group (all $p = 0.0001$). GA application decreased GRP78, ATF6, CHOP and caspase-3 levels significantly compared with T/D group (all $p = 0.0001$) (Table 2).

Johnsen scores were significantly lower in the T/D group compared to sham control group ($p = 0.0001$), and GA treatment increased these scores significantly compared to the T/D group (all $p = 0.0001$) (Table 2). Microscopic images were presented in Fig. 1. (A) Sham control group; testicular tissue showed normal seminiferous tubule germinal epithelial structure and normal intertubular space morphology (B) T/D group; irregularities, degeneration and decreased germinal epithelial cells were observed in the seminiferous tubule epithelium. The tubule structure with spermatozoa in the lumen was considerably reduced. Widespread severe edema were observed in the intertubular area (C) T/D + GA group; compared to the T/D group, testicular tissue was observed close to the control group, where the damage was largely eliminated. Although the degree of edema decreased compared to the T/D group, it still continued.

4. Discussion

The pathogenesis of IRI is associated with complex molecular processes, such as oxidative/nitrosative stress, inflammation and ER stress [2, 28]. Increased ROS cause damage to lipids, carbohydrates, proteins and DNA [2]. Peroxidation of lipids leads to the formation of unstable aldehyde compounds, including MDA, which are highly reactive for all biomolecules [22]. TAS and TOS are inexpensive and reliable cumulative markers to indirectly assess the degree of oxidative stress in a biological sample. TOS is an indicator of the total oxidant molecules in a sample, while TAS is a precise indicator of the total antioxidant capacity in a sample [17]. 8-OHdG is a biomarker of the oxidative DNA damage [29]. In this study, increased MDA, TOS, OSI and 8-OHdG levels and decreased TAS levels in the T/D group showed that testicular IRI is mediated by oxidative stress. Treatment with GA restored the levels of these parameters. Consistent with these results, GA has been reported to reduce IRI in various tissues, such as kidney, heart and brain, by reducing oxidative stress in various experimental models [14, 16, 22].

Under normal conditions, antioxidant enzymes play a very important role in maintaining fertility by protecting testicular tissue from free radical damage [30]. Various antioxidant enzymes work to eliminate the harmful effects of ROS in organisms. Among these enzymes, SOD forms the first line of defense and converts superoxide radicals to hydrogen peroxide (H_2O_2). H_2O_2 is then reduced to water by CAT [10]. Decreased levels of these enzymes cause an increase in ROS, and as a result, cell membranes lose their function and integrity over time [14]. The results showed that the protein levels of SOD and CAT were lower in the T/D group compared to the control group. It is known that increased ROS levels in the case of IRI decrease the expression of antioxidant enzymes [31–34]. This may be the main reason for the decrease in SOD and CAT protein levels in the T/D group. In order to reach a definite conclusion, SOD and CAT mRNA levels should also be determined in future studies. The levels of antioxidant enzymes in the T/D + GA group increased significantly and this may be due to the fact that GA protects antioxidant enzymes from attack by ROS. Similar with these results, GA has been reported to reduce IRI in heart, liver and kidney tissues *via* elevating the levels of antioxidant enzymes [10, 14, 16].

Inflammation is an important biological process in response to external stimuli and it is the another major pathway that

T A B L E 2. The levels of biochemical parameters and Johnsen scores in testicular tissues of control and experimental rats (n = 6 rats/per group).

	Control	T/D	T/D + GA
MDA (nmol/mg protein)	5.67 ± 0.58	17.94 ± 2.49^a	5.81 ± 0.34^b
TOS (μM H_2O_2 equivalent/L)	21.62 ± 3.74	51.71 ± 11.61^a	20.40 ± 10.08^b
TAS (mM trolox equivalent/L)	1.24 ± 0.03	0.68 ± 0.21^a	1.22 ± 0.04^b
OSI (arbitrary unit)	1.75 ± 0.32	8.34 ± 3.56^a	1.68 ± 0.86^b
8-OHdG (ng/mg protein)	4.40 ± 1.18	12.28 ± 2.56^a	4.70 ± 0.85^b
SOD (ng/mg protein)	2.02 ± 0.57	0.48 ± 0.10^a	1.83 ± 0.48^b
CAT (mIU/mg protein)	15.80 ± 3.99	5.88 ± 0.74^a	14.30 ± 7.62^b
HMGB1 (pg/mg protein)	55.57 ± 8.16	172.18 ± 6.90^a	60.40 ± 2.80^b
NF-κB p65 (pg/mg protein)	90.03 ± 17.11	234.22 ± 18.10^a	84.42 ± 24.81^b
TNF-α (pg/mg protein)	0.26 ± 0.15	1.05 ± 0.16^a	0.23 ± 0.22^b
IL-6 (pg/mg protein)	40.47 ± 6.10	262.01 ± 19.74^a	45.25 ± 9.36^b
MPO (ng/mg protein)	0.57 ± 0.09	1.54 ± 0.29^a	0.61 ± 0.13^b
GRP78 (pg/mg protein)	21.65 ± 3.84	84.90 ± 18.28^a	19.82 ± 3.99^b
ATF6 (pg/mg protein)	36.38 ± 9.24	99.28 ± 15.54^a	34.10 ± 4.36^b
CHOP (pg/mg protein)	159.80 ± 29.90	353.90 ± 76.14^a	160.78 ± 16.86^b
Caspase 3 (ng/mg protein)	0.40 ± 0.13	1.70 ± 0.25^a	0.48 ± 0.26^b
Johnsen scores	9.48 ± 0.32	6.68 ± 0.83^a	8.68 ± 0.54^b

T/D: torsion/detorsion; GA: gallic acid; MDA: malondialdehyde; TOS: total oxidant status; TAS: total antioxidant status; OSI: oxidative stress index; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; SOD: superoxide dismutase; CAT: catalase; HMGB1: high mobility group box 1; NF-κB: nuclear factor kappa B; TNF-α: tumour necrosis factor alpha; IL-6: interleukin-6; MPO: myeloperoxidase; GRP78: 78-kDa glucose-regulated protein; ATF6: activating transcription factor 6; CHOP: CCAAT-enhancer-binding protein homologous protein.

p-values according to one-way ANOVA test, post-hoc Tukey test. Data were expressed as mean $\pm$ SD. $^a p < 0.05$ compared with control group, $^b p < 0.05$ compared with T/D group.
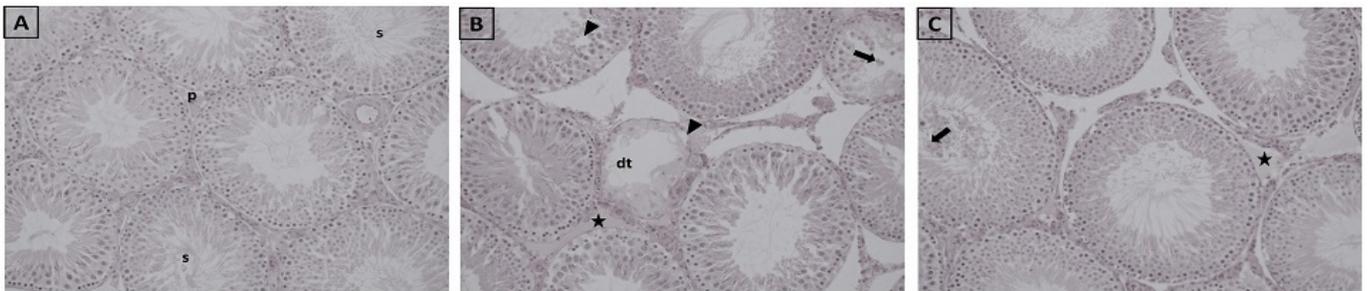
F I G U R E 1. Histopathological images of testicular tissues of groups ($\times$200, H&E staining). Control Group (A) P: normal peritubular tissue, S: spermatozoon in the lumen of the seminiferous tubule. T/D Group (B) DT: degenerative seminiferous tubule structure, black arrow: spilled immature germinal cells into the lumen of the seminiferous tubule, arrowhead: vacuolization in tubule epithelium, black star: edema in peritubular tissue. T/D + GA Group (C) Black arrow: spilled immature germinal cells into the lumen of the seminiferous tubule, black star: edema in peritubular tissue.

play critical role in the pathophysiology of testicular IRI [4, 5]. HMGB1 is a non-chromosomal nuclear protein involved in the initiation of the inflammatory cascade in the tissue damage response, including IRI [35]. After IRI, extracellular HMGB1 operates as a damage-associated molecular pattern (DAMP) through two major signaling pathways. It interacts with toll-like receptor 4 (TLR4) and receptor for advanced glycation end product (RAGE) receptors, inducing the activation of NF-κB. NF-κB activation increases the expression TNF-α and IL-6

[36]. Interestingly, it is reported that blocking HMGB1 can alleviate tissue damage caused by IRI [37, 38]. Neutrophil infiltration is another characteristic of IRI-induced inflammatory tissue damage [13]. In this study, increased levels of HMGB1, NF-κB p65, TNF-α, IL-6 and MPO in the T/D group showed that the testicular IRI is mediated by inflammation. Elevated levels of inflammatory markers as a result of T/D may be due to impaired antioxidant capacity, increased ROS amount and lipid peroxidation [36]. Treatment with GA significantly

restored these inflammation parameters significantly. This may be due to the anti-inflammatory activity of GA resulting from its strong antioxidant property. Consistent with these results, GA has been reported to reduce IRI in various tissues, such as kidney and liver, by reducing inflammation [17, 39].

Recent studies highlight that ER stress comes to the forefront as an important molecular mechanism in IRI-induced tissue damage [9]. Mild ER stress induces the UPR to activate cytoprotective mechanisms. In contrast, severe ER stress induces apoptosis to protect the organism [40]. There are three sensor proteins that detect ER stress and initiate the UPR pathway: ATF6, inositol requiring enzyme 1 (IRE-1) and protein kinase RNA-activated-like ER kinase (PERK) [6]. These sensors interact with GRP78 under non-stressed conditions and are kept inactive state. In cases of increased ER stress, sensor proteins are separated from GRP78 and thus UPR is activated [9]. ATF6 is activated by dissociation from GRP78 when ER stress is triggered. The released ATF6 is then transferred to the Golgi, where it is activated. Activated ATF6 migrates into nucleus where it elevates the expression level of proteins involved in optimizing protein folding, maturation and elimination of missense proteins. Therefore, there is no doubt that ATF6 is an important mediator during IRI [8]. In the case of excessive ER stress, ATF6 induces CHOP expression, resulting in apoptosis and elimination of the cell whose ER stress cannot be reduced. Under increased ER stress conditions, increased CHOP suppresses B-cell lymphoma 2 (Bcl-2) and glutathione levels, increases Bcl-2-associated X protein (Bax) and ROS levels, and causes caspase-3 activation [41]. Therefore, GRP78, ATF6 and CHOP levels were evaluated in testicular tissues to reveal ER stress, and caspase-3 levels were determined to show the level of apoptosis in this study. The results showed that increased ER stress and apoptosis contribute to testicular IRI, and GA attenuated this damage by ER stress inhibitor and anti-apoptotic activities. Similarly, Obafemi *et al.* [23] demonstrated that GA protects liver and pancreas tissues against diabetes-induced damage *via* alleviating the levels of ER stress, while Blas-Valdivia *et al.* [41] reported that GA exhibits neuroprotective effect against hypothyroid-induced oxidative and ER stress *via* decresing PERK, ATF6 and CHOP levels.

Histological evaluation is a very important method in evaluating tissue damage caused by IRI and revealing the degree of therapeutic effect of the investigated molecule [20, 42]. Histological data revealed the presence of pathological findings in T/D group and showed that GA application could eliminate these pathological findings in parallel with the biochemical results. Consistent with these results, previous studies have shown that GA can exhibit a testicular protective effect against damage caused by various chemicals by modulating oxidative stress and inflammation [13, 43–47].

GA is a natural phenolic acid and has powerful antioxidant activity due to chain breaking, hydrogen-donating, metal chelating and radical scavenging potential [12, 13]. It is also stated that GA functions by strengthening antioxidant enzymes [11, 29]. In addition, it has been demonstrated that it can exhibit anti-inflammatory activity by reducing the expression of inflammatory molecules [17, 39]. Therefore, we speculated that the protective effect of GA against testicular IRI is mainly

due its antioxidant and anti-inflammatory potential.

There were also some limitations of our study. First, only the one dose of GA was evaluated based on previous literature data within the scope of this study. Second, the efficacy of GA at different times or in chronic T/D conditions was not evaluated in this study. Third, the effect of GA on sexual behavior and fertility level of rats were not evaluated in the study. We believe that demonstrating the protective efficacy of GA against testicular IRI in long-term studies together with physiological fertility behavior experiments will shed light on clinical stages.

5. Conclusions

The results showed that GA reduced testicular IRI in an experimental model for the first time. This protective effect of GA is thought to be due its antioxidant, anti-inflammatory, anti-apoptotic and ER stress inhibitor properties. The suitability of GA for clinical use needs to be supported by more detailed studies.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

SD, IOK, GK and AM—contributed to study design, data collection and research evaluation; IOK and FC—contributed to perform torsion/detorsion induction; SD, NTA and AM—contributed to perform the biochemical analysis; GK and TA—contributed to perform the histological analysis; SD, IOK, AM and YA—performed data analysis and drafted the manuscript. All authors accomplished the edition and approved the ultimate version of this manuscript for submission, contributed to the completion of the manuscript and affirmed the ultimate draft.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics committee approval was received for this study from the Animal Experiments Local Ethics Commitee of Karadeniz Technical University (Protocol number: 2019/53).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Shamsi-Gamchi N, Razi M, Behfar M. Cross-link between mitochondrial-dependent apoptosis and cell cycle checkpoint proteins after experimental torsion and detorsion in rats. Gene. 2021; 795: 145793.

[2] Arena S, Iacona R, Antonuccio P, Russo T, Salvo V, Gitto E, et al. Medical perspective in testicular ischemia-reperfusion injury. Experimental and Therapeutic Medicine. 2017; 13: 2115–2122.

[3] Vaos G, Zavras N. Antioxidants in experimental ischemia-reperfusion injury of the testis: where are we heading towards? World Journal of Methodology. 2017; 7: 37.

[4] Osumah TS, Jimbo M, Granberg CF, Gargollo PC. Frontiers in pediatric testicular torsion: an integrated review of prevailing trends and management outcomes. Journal of Pediatric Urology. 2018; 14: 394–401.

[5] Karaguzel E, Kadihasanoglu M, Kutlu O. Mechanisms of testicular torsion and potential protective agents. Nature Reviews Urology. 2014; 11: 391–399.

[6] Chong WC, Shastri MD, Eri R. Endoplasmic reticulum stress and oxidative stress: a vicious nexus implicated in bowel disease pathophysiology. International Journal of Molecular Sciences. 2017; 18: 771.

[7] Xin Q, Ji B, Cheng B, Wang C, Liu H, Chen X, et al. Endoplasmic reticulum stress in cerebral ischemia. Neurochemistry International. 2014; 68: 18–27.

[8] Su Y, Li F. Endoplasmic reticulum stress in brain ischemia. International Journal of Neuroscience. 2016; 126: 681–691.

[9] Lin C. Attenuation of endoplasmic reticulum stress as a treatment strategy against ischemia/reperfusion injury. Neural Regeneration Research. 2015; 10: 1930.

[10] Bayramoglu G, Kurt H, Bayramoglu A, Gunes HV, Degirmenci İ, Colak S. Preventive role of gallic acid on hepatic ischemia and reperfusion injury in rats. Cytotechnology. 2015; 67: 845–849.

[11] Asci H, Ozmen O, Ellidag HY, Aydin B, Bas E, Yilmaz N. The impact of gallic acid on the methotrexate-induced kidney damage in rats. Journal of Food and Drug Analysis. 2017; 25: 890–897.

[12] Gao J, Hu J, Hu D, Yang X. A role of gallic acid in oxidative damage diseases: a comprehensive review. Natural Product Communications. 2019; 14: 1934578X1987417.

[13] Abarikwu SO, Mgbudom-Okah CJ, Njoku RC, Okonkwo CJ, Onuoha CC, Wokoma AFS. Gallic acid ameliorates busulfan-induced testicular toxicity and damage in mature rats. Drug and Chemical Toxicology. 2022; 45: 1881–1890.

[14] Canbek M, Bayramoglu G, Senturk H, Oztopcu Vatan AP, Uyanoglu M, Ceyhan E, et al. The examination of protective effects of gallic acid against damage of oxidative stress during induced-experimental renal ischemia-reperfusion in experiment. Bratislava Medical Journal. 2014; 115: 557–562.

[15] Sun J, Li Y, Ding Y, Wang J, Geng J, Yang H, et al. Neuroprotective effects of gallic acid against hypoxia/reoxygenation-induced mitochondrial dysfunctions in vitro and cerebral ischemia/reperfusion injury in vivo. Brain Research. 2014; 1589: 126–139.

[16] Badavi M, Sadeghi N, Dianat M, Samarbafzadeh A. Effects of gallic acid and cyclosporine a on antioxidant capacity and cardiac markers of rat isolated heart after ischemia/reperfusion. Iranian Red Crescent Medical Journal. 2014; 16: e16424.

[17] Basuguy E, Okur MH, Arslan S, Zeytun H, Aydogdu G, Ekinci A. Effect of gallic acid on liver injury during obstructive cholestasis after bile duct ligation in rat. Experimental Biomedical Research. 2021; 4: 47–57.

[18] Turner TT, Tung KS, Tomomasa H, Wilson LW. Acute testicular ischemia results in germ cell-specific apoptosis in the rat. Biology of Reproduction. 1997; 57: 1267–1274.

[19] Kutlu O, Mentese A, Turkmen S, Turedi S, Gunduz A, Yulug E, et al. Investigation of the possibility of using ischemia-modified albumin in testicular torsion: an experimental study. Fertility and Sterility. 2011; 95: 1333–1337.

[20] Kazaz IO, Mentese A, Demir S, Kerimoglu G, Colak F, Bodur A, et al. Berberine inhibits the ischemia-reperfusion induced testicular injury through decreasing oxidative stress. The American Journal of Emergency Medicine. 2020; 38: 33–37.

[21] Demir S, Kazaz IO, Aliyazicioglu Y, Kerimoglu G, Teoman AS, Yaman SO, et al. Effect of ethyl pyruvate on oxidative state and endoplasmic reticulum stress in a rat model of testicular torsion. Biotechnic & Histochemistry. 2020; 95: 317–322.

[22] Praveen Kumar P, D M, Siva Sankar Reddy L, Dastagiri Reddy Y, Somasekhar G, Sirisha NVL, et al. A new cerebral ischemic injury model in rats, preventive effect of gallic acid and in silico approaches. Saudi Journal of Biological Sciences. 2021; 28: 5204–5213.

[23] Obafemi TO, Jaiyesimi KF, Olomola AA, Olasehinde OR, Olaoye OA, Adewumi FD, et al. Combined effect of metformin and gallic acid on inflammation, antioxidant status, endoplasmic reticulum (ER) stress and glucose metabolism in fructose-fed streptozotocin-induced diabetic rats. Toxicology Reports. 2021; 8: 1419–1427.

[24] Kazaz IO, Demir S, Kerimoglu G, Colak F, Alemdar NT, Akman AU, et al. Effect of chrysin on endoplasmic reticulum stress in a rat model of testicular torsion. Journal of Investigative Surgery. 2022; 35: 1106–1111.

[25] Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. Hormones. 1970; 1: 2–25.

[26] Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry. 1978; 86: 271–278.

[27] Demir EA, Mentese A, Kucuk H, Alemdar NT, Demir S. p-Coumaric acid alleviates cisplatin-induced ovarian toxicity in rats. Journal of Obstetrics and Gynaecology Research. 2022; 48: 411–419.

[28] Demir, S, Kazaz IO, Kerimoglu G, Ayazoglu Demir E, Colak F, Yilmaz S, et al. Astaxanthin protects testicular tissue against torsion/detorsion-induced injury via suppressing endoplasmic reticulum stress in rats. Journal of Investigative Surgery. 2022; 35: 1044–1049.

[29] Ayazoglu Demir E, Mentese A, Livaoglu A, Turkmen Alemdar N, Demir S. Ameliorative effect of gallic acid on cisplatin-induced ovarian toxicity in rats. Drug and Chemical Toxicology. 2023; 46: 97–103.

[30] Kazaz IO, Demir S, Kerimoglu G, Colak F, Turkmen Alemdar N, Yilmaz Dogan S, et al. Chlorogenic acid ameliorates torsion/detorsion-induced testicular injury via decreasing endoplasmic reticulum stress. Journal of Pediatric Urology. 2022; 18: 289.e1–289.e7.

[31] Mard SA, Azad SM, Ahangarpoor A. Protective effect of crocin on gastric mucosal lesions induced by ischemia-reperfusion injury in rats. Iranian Journal of Pharmaceutical Research. 2016; 15: 93–99.

[32] Zhou F, Wang M, Ju J, Wang Y, Liu Z, Zhao X, et al. Schizandrin A protects against cerebral ischemia-reperfusion injury by suppressing inflammation and oxidative stress and regulating the AMPK/Nrf2 pathway regulation. American Journal of Translational Research. 2019; 11: 199–209.

[33] Arfian N, Wahyudi DAP, Zulfatina IB, Citta AN, Anggorowati N, Multazam A, et al. Chlorogenic acid attenuates kidney ischemic/reperfusion injury via reducing inflammation, tubular injury, and myofibroblast formation. BioMed Research International. 2019; 2019: 5423703.

[34] Almoiliqy M, Wen J, Xu B, Sun Y, Lian M, Li Y, et al. Cinnamaldehyde protects against rat intestinal ischemia/reperfusion injuries by synergistic inhibition of NF-κB and p53. Acta Pharmacologica Sinica. 2020; 41: 1208–1222.

[35] Dong LY, Chen F, Xu M, Yao LP, Zhang YJ, Zhuang Y. Quercetin attenuates myocardial ischemia-reperfusion injury via downregulation of the HMGB1-TLR4-NF-κB signaling pathway. American Journal of Translational Research. 2018; 10: 1273–1283.

[36] Zhang B, Zhong Q, Chen X, Wu X, Sha R, Song G, et al. Neuroprotective effects of celastrol on transient global cerebral ischemia rats via regulating HMGB1/NF-κB signaling pathway. Frontiers in Neuroscience. 2020; 14: 847.

[37] Yang C, Yang W, He Z, He H, Yang X, Lu Y, et al. Kaempferol improves lung ischemia-reperfusion injury via antiinflammation and antioxidative stress regulated by SIRT1/HMGB1/NF-κB axis. Frontiers in Pharmacology. 2020; 10: 1635.

[38] Lai HJ, Zhan YQ, Qiu YX, Ling YH, Zhang XY, Chang ZN, et al. HMGB1 signaling-regulated endoplasmic reticulum stress mediates intestinal ischemia/reperfusion-induced acute renal damage. Surgery.

2021; 170: 239–248.

[39] Singh JP, Singh AP, Bhatti R. Explicit role of peroxisome proliferator-activated receptor gamma in gallic acid-mediated protection against ischemia-reperfusion-induced acute kidney injury in rats. Journal of Surgical Research. 2014; 187: 631–639.

[40] Gao X, Xu Y. Therapeutic effects of natural compounds and small molecule inhibitors targeting endoplasmic reticulum stress in Alzheimer's disease. Frontiers in Cell and Developmental Biology. 2021; 9: 745011.

[41] Blas-Valdivia V, Franco-Colín M, Rojas-Franco P, Chao-Vazquez A, Cano-Europa E. Gallic acid prevents the oxidative and endoplasmic reticulum stresses in the hippocampus of adult-onset hypothyroid rats. Frontiers in Pharmacology. 2021; 12: 671614.

[42] Kazaz IO, Demir S, Yulug E, Colak F, Bodur A, Yaman SO, *et al*. N-acetylcysteine protects testicular tissue against ischemia/reperfusion injury *via* inhibiting endoplasmic reticulum stress and apoptosis. Journal of Pediatric Urology. 2019; 15: 253.e1–253.e8.

[43] Oyagbemi AA, Omobowale TO, Saba AB, Adedara IA, Olowu ER, Akinrinde AS, *et al*. Gallic acid protects against cyclophosphamide-induced toxicity in testis and epididymis of rats. Andrologia. 2016; 48: 393–401.

[44] Owumi SE, Adedara IA, Akomolafe AP, Farombi EO, Oyelere AK. Gallic acid enhances reproductive function by modulating oxido-inflammatory and apoptosis mediators in rats exposed to aflatoxin-B_1. Experimental Biology and Medicine. 2020; 245: 1016–1028.

[45] Altındağ F, Meydan İ. Evaluation of protective effects of gallic acid on cisplatin-induced testicular and epididymal damage. Andrologia. 2021; 53: e14189.

[46] Hosseinzadeh A, Mehrzadi S, Siahpoosh A, Basir Z, Bahrami N, Goudarzi M. Gallic acid ameliorates di-(2-ethylhexyl) phthalate-induced testicular injury in adult mice. Human & Experimental Toxicology. 2022; 41: 096032712210788.

[47] Owumi SE, Bello SA, Najophe SE, O Nwozo S, O Esan I. Coadministration of gallic acid abates zearalenone-mediated defects in male rat's reproductive function. Journal of Biochemical and Molecular Toxicology. 2022; 36: e22940.