Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.196

RESEARCH ARTICLE

Naringenin Attenuates Testicular Toxicity and Apoptosis in Rats Chronically Exposed to Mercury Chloride

Merve Kahramanoğullari [1*], Mine Erişir [1], Mine Yaman[2], Şeyma Özer Kaya[3] and Tuba Parlak Ak[4]

[1*]Department of Biochemistry, Faculty of Veterinary Medicine, Fırat University, Elazığ, Türkiye; [2]Department of Histology-Embryology, Faculty of Veterinary Medicine, Fırat University, Elazığ, Türkiye; [3]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Fırat University,Elazığ, Türkiye; [4]Department of Nutrition and Dietetics, Faculty of Health Sciences, Munzur University, Tunceli, Türkiye
*Corresponding author: mervenyr.13@gmail.com

ARTICLE HISTORY (25-253)

Received: March 23, 2025
Revised: June 6, 2025
Accepted: June 10, 2025
Published online: June 30, 2025

Key words:
Apoptosis
Mercury (II) Chloride
Naringenin
Oxidative Damage
Reproductive Toxicity

ABSTRACT

Mercury is an industrial toxin with detrimental effects on the reproductive system. This study explored the impact of naringenin (NAR) on testicular toxicity and apoptosis in rats chronically exposed to mercury chloride (MC). The research involved 41 adult male Wistar-Albino rats aged about 2.5 months, which were allocated into 5 groups: group 1 was a control group, group 2 received 100mg/kg of NAR, group 3 was exposed to MC, group 4 received both MC and 50mg/kg of NAR, and group 5 received both MC and 100mg/kg of NAR. For the treatments, the control group took 1mL/kg saline via intraperitoneal (ip) injection and 1mL/kg corn oil via gavage. MC was administered at 0.4mg/kg/day to the MC groups via ip, and NAR was delivered in corn oil at 50 and 100mg/kg/day to the respective NAR groups via gavage. All treatments were performed over a period of 20 days, with MC administration starting 1 hour after NAR administration. The irregularity and loss in spermatogenic cell organization, degeneration and vacuolization in tubules, edema, and congestion in interstitial areas of the testicular tissue of the MC group were alleviated by NAR administration. In addition, the increase in Caspase 3, iNOS, and eNOS immunoreactivities of the MC group were alleviated by NAR administration. In conclusion, it was found that NAR had protective and therapeutic effects against oxidative damage, histopathological changes, apoptosis, and altered spermatological parameters resulting from MC exposure in the testis, and 100mg/kg NAR provided positive effects on altered histopathological analysis, spermatological parameters, and apoptosis.

To Cite This Article: Kahramanoğullari M, Erişir M, Yaman M, Kaya SO and Ak TP 2025. Naringenin attenuates testicular toxicity and apoptosis in rats chronically exposed to mercury chloride. Pak Vet J, 45(2): 791-798. http://dx.doi.org/10.29261/pakvetj/2025.196

INTRODUCTION

Heavy metals are very risky environmental pollutants considering the environmental conditions. Mercury, one of the heavy metals, has been used by humans for a long time to treat many ailments. Mercury-containing water and foodstuffs are the main sources of mercury exposure in humans and animals (Pavithra *et al.,* 2023). Mercury accumulation in different body tissues may cause numerous cellular and physiological disorders including hepatotoxicity, nephrotoxicity, neurotoxicity, lung damage, and reproductive disorders (Alina *et al.,* 2012). Exposure to mercury chloride (MC) has been declared to negatively effect the reproductive performance of rats (Pavithra *et al.,* 2023). Mercury was found to have negative effects on spermatogenesis, sperm viability, and sex hormone levels. Mercury exposure disrupts circulating androgen levels, luteinizing hormone (LH), progesterone, inhibin, estrogen, and follicle-stimulating hormone (FSH), adversely affects reproductive activity by altering the hypothalamic-pituitary-adrenal and gonadal axis (Rice *et al.,* 2014). MC decreases reproductive performance by causing cellular deformation in Leydig and seminiferous tubules and testicular degeneration (Gaber *et al.,* 2013).

Oxidative stress occurs when the body's oxidant-antioxidant balance is disrupted by an abnormal rise in reactive oxygen species (ROS). Oxidative stress may trigger pathologic events in the reproductive system (Bernhoft, 2012). Apoptosis, or programmed cell death, is

an important component of normal cellular development and function in organisms. Apoptosis can be initiated by two distinct, but interconnected, molecular signaling pathways. These two pathways consist of exogenous and endogenous signals (Fink and Cookson, 2005). Caspases from the group of proteases are effective in the occurrence or maintenance of apoptosis. Caspase-3 is among the most effective mechanisms in the maintenance of apoptosis in this group (Chen *et al.,* 2022).

Flavonoids are the most abundant natural polyphenolic compounds in plants. Naringenin (NAR) is widely found in citrus fruits, with a particularly high concentration in grapefruit. It also exhibits antiapoptotic properties by modulating the amounts of apoptotic regulatory proteins. Moreover, NAR was reported to significantly enhance levels of FSH, gonadotropin-releasing hormone (GnRH), LH, and testosterone, and to improve sperm function parameters in cases of cadmium exposure (Wang *et al.,* 2021).

The reproductive dysfunction caused by mercury is likely due to a compromised antioxidant defense system. Consequently, antioxidant treatment could potentially mitigate or alleviate the negative impacts of mercury on reproduction. As there are no existing studies on the effects of NAR on mercury-induced reproductive toxicity in male rats, this study aimed to evaluate NAR's protective effects on sperm parameters including sperm motility, density, and the ratio of abnormal spermatozoa along with lipid peroxidation (LP), antioxidant levels, and apoptosis in the reproductive system of rats chronically exposed to MC.

MATERIALS AND METHODS

In the study, 41 Wistar-Albino adult male rats aged about 2.5 months were preferred. Suitable environmental conditions were provided to the rats throughout the treatment. The Fırat University ethics committee approved the study (Approval No: 2024/01-07-21302).

The rats were allocated into five groups: Control, Naringenin (NAR), Mercury (II) chloride (MC), MC+NAR 50 and MC+NAR 100. The first two groups each consisted of 7 rats, while the remaining groups each included 9 rats. The control group took 1ml/kg saline via intraperitoneal injection (ip) and 1mL/kg corn oil via gavage. MC was solved in saline and administered via ip at 0.4mg/kg/day for 20 days (Agarwal and Behari, 2007). NAR was solved in corn oil and given by gavage at doses of 50mg/kg/day and 100mg/kg/day for 20 days (Wali *et al.*,2020). MC administration commenced 1 hour following NAR administration.

Biochemical Analyses: After termination of the experiment, the rats were euthanized without anesthesia. Samples were preserved (-60°C) for the measurement of glutathione (GSH), malondialdehyde (MDA) levels and glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activities. Tissue samples were weighed, diluted (1.15% KCl), homogenized and centrifuged (3500rpm for 15min). Another portion of homogenate in Eppendorf tubes was centrifuged at 11000rpm for 20min and protein concentrations and GSH-Px activities were determined in the supernatants obtained.

MDA and GSH concentrations were determined according to the method described by Placer *et al.* (1966) and Chavan *et al.* (2005), respectively. GSH-Px, SOD and CAT activities were determined according to the methods of Matkovics *et al.* (1988), Aebi (1984) and Sun *et al.* (1988). Protein levels were also determined by the modified Lowry method (Lowry *et al.*, 1951).

Spermatological Analyses: Epididimal sperm parameters such as sperm motility, morphology and density were analyzed using the methods of previous studies (Turk *et al.*, 2008). Sperm level in the epididymis of the right cauda epididymis was determined by hemocytometer. Sperm motility was determined from the left cauda epididymis tissue. Total sperm motility was evaluated using a light microscope with a heating table (400X magnification). For morphologic examination of abnormal spermatozoa, samples were stained with eosin-nigrosin solution (1.67% eosin, 10% nigrosin and 0.1M sodium citrate) and then examined by light microscopy. A total of 200 spermatozoa were evaluated per slide, with the percentage of sperm exhibiting head and tail abnormalities being documented. Organ weights were measured using a precision scale.

Histological evaluation: For histopathological examinations, samples were taken from the testicular tissues of the experimental groups. Subsequently, it was fixed in a solution containing 10% formalin for 24 hours. Then, the tissue tracking procedure was applied to the samples, and paraffin blocks were prepared. Sections taken at 5µm thickness were stained with hematoxylin-eosin (H&E) and Periodic acid-Schiff (PAS) methods and evaluated under a light microscope (Gur and Aktas, 2021). All sections were evaluated and photographed with a light microscope (Olympus BX-51, Olympus Optical Co., Ltd., Tokyo, JAPAN). Histopathological changes in the testicular tissue were evaluated and the scoring used an assessment that was carried out as follows.(-) score (null): no structural change, (+) score (mild): slight, (++) score (moderate): medium, (+++) score (severe): a significant structural change (Sengul *et al.,* 2023). Spermatogenesis categorization was performed according to Seven *et al.*, (2020). For the calculation of scoring according to Johnsen criteria, each of the 25 seminiferous tubules examined in each section was given a score ranging from 1 (very poor) to 10 (excellent) according to spermatogenesis level (Johnsen, 1970).

Immunohistochemical examination: Immunoreactivities for cysteine aspartic protease-3 (Caspase 3) (E-AB-66940, Elabscience, USA), inducible nitric oxide synthase (iNOS) (bs-22924R, Bioss, USA), and endothelial nitric oxide synthase (eNOS) (bs-0163R, Bioss, USA) in testicular tissues were performed using the Avidin-Biotin-Peroxidase Complex method. Immunoreactivity was computed by the formula area x intensity (intensity; none (0), very little (0.5), little (1), moderate (2), severe (3) x area; 0.1 (<25%), 0.4 (26–50%), 0.6 (51–75%), 0.9 (76–100%) (Kahramanoğullari *et al.,* 2024).

Statistical analysis: Each of the analyses were realized by using SPSS software (SPSS for Windows, version 22.0).

The standard error (SE) and mean (±) were used to present the data. To find significant differences between the groups, analysis of variance (ANOVA) was applied, followed by the Duncan test. A significance level of 5% was used to determine the presence of any differences.

RESULTS

Biochemical findings: In the biochemical analyses, rats exposed to mercury showed a statistically significant increase in MDA levels (P<0.001) and a significant reduce in GSH levels (P<0.001), as well as reduced GSH-Px and CAT activities (P<0.01) in testicular tissue. SOD activity in testicular tissue did not significantly change (P>0.05) (Table 1).

Treatment with NAR alongside MC significantly reduced the elevated MDA levels and significantly increased the reduced GSH levels in the MC group (P<0.001). The GSH increase was also greater than that in the control group, with the MC+NAR 100 group showing the highest increase. Additionally, NAR treatment restored CAT and GSH-Px levels, which were decreased by MC administration, to control values (P<0.01) (Table 1).

Spermatological findings: The alterations in spermatological parameters are detailed in Table 2. Sperm motility rates and sperm density were significantly reduced in the MC group compared to the control group (P<0.001). Moreover, the rates of sperm with abnormal heads and tails were significantly increased in the MC group (P<0.01) (Fig. 1).

In the MC+NAR 50 and MC+NAR 100 groups, sperm motility rates and sperm densities were significantly higher compared to the MC group, with the MC+NAR 100 group showing a greater increase in sperm motility (P<0.001). The MC+NAR 100 group also had significantly lower rates of sperm with abnormal heads and tails compared to the MC group (P<0.01), whereas the reduction in the MC+NAR 50 group was less significant. The group receiving NAR alone exhibited higher sperm density than all other groups (P<0.001).

The weights of the right testis (P<0.01), right cauda (P<0.001), and prostate (P<0.05) were significantly lower in the MC group. No significant changes were found in the weights of the right epididymis and seminal vesicles. In the MC+NAR 50 and MC+NAR 100 groups, the weights of the right testis and right cauda were significantly higher compared to the MC group (P<0.01).

Histopathologic findings: In the testicular tissues stained with H&E of control and NAR groups, normal and functional seminiferous tubules containing well-arranged spermatogenic cells showing all stages were observed. In addition, the interstitial areas filling the intertubular space were in normal morphology. In the MC group, there was irregularity and loss in germinal epithelial spermatogenic cell organization, degeneration in some spermatogenic cells, vacuolization, and atrophic structure in the tubules. There were also edematous interstitial areas and congested blood vessels. Testicular tissues of the MC+NAR 50 and MC+NAR 100 groups showed germ cell structures representing normal stages of spermatogenic cells in the germinal epithelium compared to the MC group. It was found that the restoration of spermatogenesis in the seminiferous tubules was especially evident in the MC+NAR 100 group and the histopathological changes in the interstitial areas were alleviated (Fig. 2A). Table 3 displays the histopathologic score of each group's testicular tissues.

In the control and NAR groups of testicular tissues stained with PAS, seminiferous tubule structures and basement membrane appeared normal. In the testicular tissue of the MC group, a significant decrease and displacement of spermatogenic cells in the seminiferous tubules and their partial separation from the thickened, sometimes irregular, and discontinuous basement

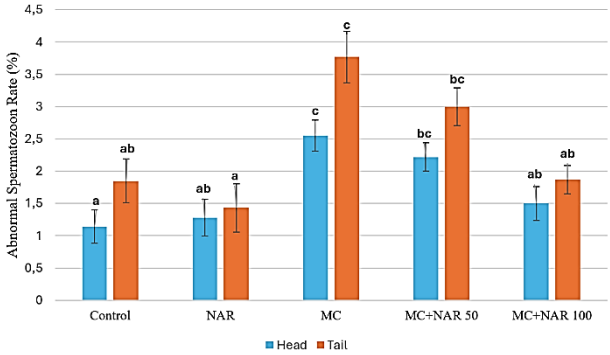

Fig. 1: Abnormal Spermatozoon Rate (%), Head and Tail.

Table 1: Impacts of NAR on oxidant and antioxidant parameters in testicular tissue of rat chronically exposed to MC

Parameters	Control	Naringenin	MC	MC+NAR 50	MC+NAR 100	P-Value
MDA (nmol/g tissue)	7.10±1.06^{a}	5.49±0.60^{a}	10.53±0.53^{b}	5.04±0.52^{a}	5.17±0.48^{a}	P<0.001
GSH (µmol/g tissue)	0.45±0.01^{b}	0.45±0.01^{b}	0.28±0.01^{a}	0.60±0.02^{c}	0.72±0.02^{d}	P<0.001
CAT (k/g protein)	18.33±1.70^{b}	17.57±1.41^{b}	12.25±1.03^{a}	18.55±2.17^{b}	19.00±1.85^{b}	P<0.01
GSH-Px (U/g protein)	18.86±1.37^{b}	19.57±0.29^{b}	14.83±1.01^{a}	18.78±1.01^{b}	19.93±1.98^{b}	P<0.01
SOD (U/g protein)	125.67±35.73	136.65±25.99	130.43±13.42	128.94±16.56	105.86±12.48	P>0.05

Note: a, b, c, d: The difference between means with different letters in the same row is significant.

Table 2: Impacts of NAR on sperm motility and density, abnormal spermatozoon rate, right cauda epididymis, seminal vesicles, epididymis, testis, and prostate weights in rats chronically treated with MC

	Control	Naringenin	MC	MC+NAR 50	MC+NAR 100	P-Value
Motility (%)	72.85±2.85^{ab}	78.57±2.31^{a}	37.50±4.78^{d}	50.00±2.28^{c}	62.50±2.50^{b}	P<0.001
Density (million/cauda)	71.71±5.17^{b}	96.28±4.28^{a}	36.66±1.76^{c}	54.55±4.09^{b}	56.50±4.77^{b}	P<0.001
Testis (g)	1.50±0.05^{b}	1.75±0.04^{a}	1.26±0.03^{c}	1.57±0.03^{b}	1.47±0.06^{b}	P<0.01
Epididymis (g)	0.50±0.01^{ab}	0.53±0.02^{a}	0.45±0.01^{b}	0.51±0.01^{ab}	0.48±0.02^{ab}	P<0.01
Right Cauda Epididymis (g)	0.20±0.01^{ab}	0.22±0.01^{a}	0.15±0.01^{c}	0.19±0.01^{b}	0.19±0.01^{b}	P<0.001
Seminal vesicles (g)	1.29±0.12	1.31±0.06	1.16±0.08	1.19±0.04	1.22±0.11	-
Prostate (g)	0.45±0.03^{a}	0.42±0.02^{ab}	0.35±0.06^{b}	0.41±0.02^{ab}	0.38±0.02^{ab}	P<0.05

Note: a, b, c, d: The difference between means with different letters in the same row is significant.

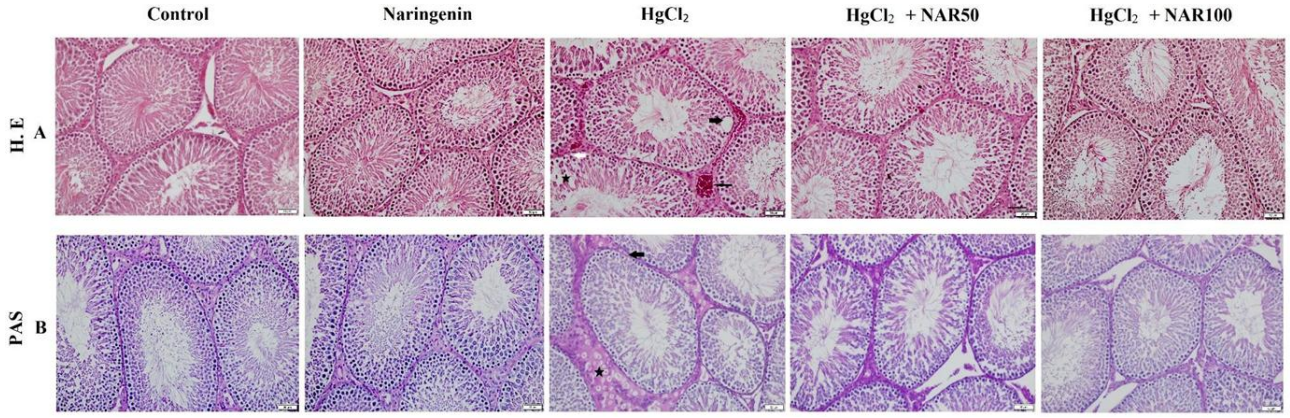

Fig. 2: Photomicrographs of histopathological changes on the testicular tissues of all groups. (A) Black thick arrow: vacuolization, white thick arrow: degeneration, black thin arrow: congested blood vessel, black star: irregularity and loss in germinal epithelial spermatogenic cell organization. H&E. (B) Black thick arrow: irregular and discontinuous basement membrane, black star: hyalinized interstitial areas. PAS.

Table 3: Histopathologic score results of all groups

	Control	Naringenin	MC	MC+NAR 50	MC+NAR 100
Disorganization in spermatogenic cells	-	-	+++	++	+
Degeneration in spermatocytes	-	-	+++	++	+
Vacuolization in tubules	-	-	+++	++	+
Edema in interstitial areas	-	-	+++	++	-
Congested blood vessels	-	-	+++	++	-
Irregular basement membrane	-	-	+++	++	+

Note: (-): null, (+): mild, (++): moderate, (+++): severe.

membrane were noted. Additionally, hyalinized interstitial areas were detected. Testicular tissues of the MC+NAR 50 and MC+NAR 100 groups showed better seminiferous tubule morphology compared to the MC group. It was found that the thickening of the basement membrane, which had a regular appearance, decreased and the expansion in the interstitial areas alleviated. These restorative findings were more striking in the MC +NAR 100 group (Fig. 2B). The MC+NAR 50 and MC+NAR 100 groups had significantly higher Johnsen testicular score values than the MC group ($P<0.05$). Table 4 displays the Johnsen's testicular score findings of all groups.

Immunohistochemical findings: In the control and NAR groups, Caspase 3 (Fig. 3A), iNOS (Fig. 3B), and eNOS (Fig. 3C) immunoreactivities were observed to be negative to weak. In contrast, a significant increase in Caspase 3, iNOS, and eNOS immunoreactivities was found in the spermatogenic cells, sperm, and Sertoli cells within the seminiferous tubules of the MC group. These immunoreactivities were found to decrease significantly in the MC+NAR 50 and MC+NAR 100 groups. The Caspase 3, iNOS, and eNOS immunohistochemical histoscores for testicular tissues across all groups are given in Table 5.

DISCUSSION

Mercury promotes the production of peroxides and superoxide anion radicals, leading to membrane LP, protein denaturation, DNA damage, and overall cellular damage through oxidative stress. MDA, a primary product of peroxidized polyunsaturated fatty acids, serves as a key marker of LP. Previous research has demonstrated that MC elevates MDA levels in testicular tissue (Boujbiha *et al.,* 2009 and Jahan *et al.,* 2019). In the presented study, a significant increase of MDA contents was determined in the testis tissue of rats subjected to chronic MC exposure, confirming that such exposure induces oxidative damage.

Several mechanisms were suggested to elucidate the biological toxicity of MC. The primary mechanism of damage caused by MC is that it causes changes in the inner membrane structure of mitochondria, decreases mitochondrial GSH levels, and leads to increased H_2O_2 production (Boujbiha *et al.,* 2009; Jahan *et al.,* 2019). This study observed a significant reduction in GSH contents in the testis of rats exposed to mercury. Mercury reduced GSH content, making spermatogenic cells more vulnerable to oxidative damage, particularly during elevated free radical formation.

The SOD-CAT system forms the first line of defense against oxidative stress, with these two enzyme systems functioning together to eliminate free radicals (Boujbiha *et al.,* 2009). In addition, GSH-Px inactivates H_2O_2 in the environment where GSH is converted to GSSG and thus prevents the formation of hydroperoxides. Therefore, any change in the activity of antioxidant enzymes may cause some harmful influences such as the accumulation of superoxide radicals and hydrogen peroxide (Al-Othman *et al.,* 2011). In various studies, it was found that MC caused oxidative stress in testicular tissue by decreasing CAT, GSH-Px, and SOD activities (Boujbiha *et al.,* 2009; Kalender *et al.,* 2013). This study, a significant decrease detected in CAT and GSH-Px activities in the testis tissue of rats exposed to mercury. Reduced removal of ROS by scavenging mechanisms is the cause of mercury-induced oxidative damage.

A review of the literature revealed no studies specifically examining the impact of NAR on reproductive toxicity induced by chronic mercury exposure in rats. NAR, a flavanone, is known for its various effects on health, including antioxidant, anti-inflammatory, antidiabetic, and anti-neurodegenerative properties (Renugadevi and Prabu, 2009). It was demonstrated that

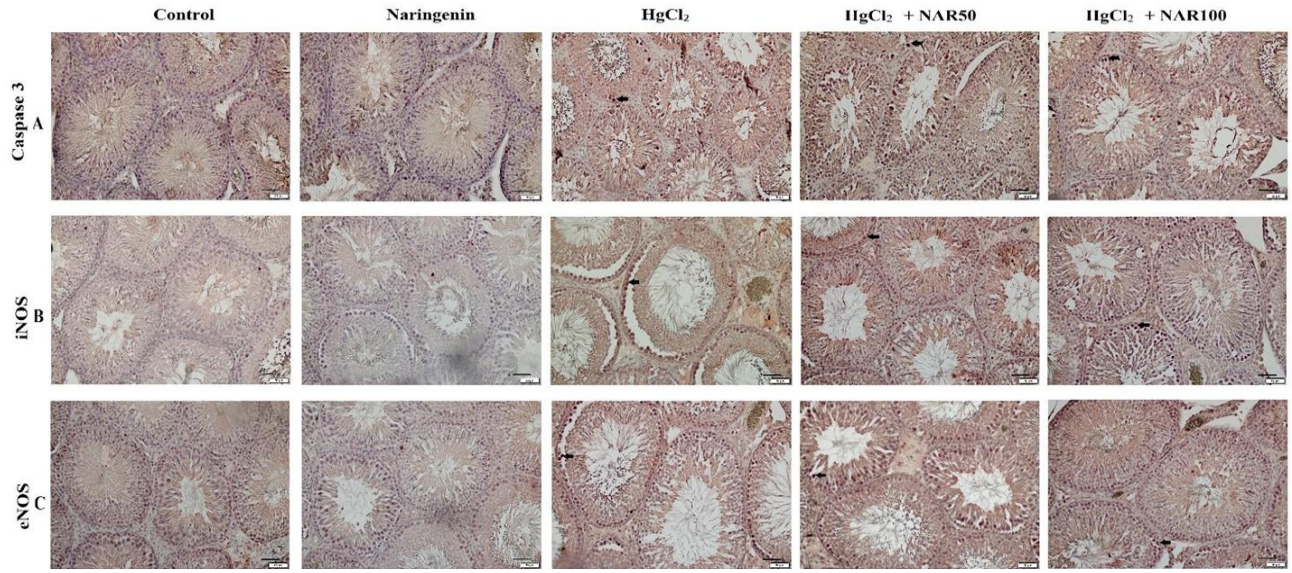

Fig. 3: Photomicrographs of immunohistochemical changes on the testicular tissues of all groups. (A) Caspase 3 immunoreactivity (arrows) (B) iNOS immunoreactivity (arrows) (C) eNOS immunoreactivity (arrows). Mayer's hematoxylin.

Table 4: Histomorphometric analysis findings of all groups

	Control	Naringenin	MC	MC+NAR 50	MC+NAR 100
Johnsen's testicular score (1-10)	10.00±0.00^{a}	10.00±0.00^{a}	4.80±0.53^{c}	6.00±0.48^{bc}	7.10±0.51^{b}

Note: (P<0.05); a, b, c: Different superscripts in the same row indicate significant differences between groups.

Table 5: Histoscore results of Caspase-3, iNOS, and eNOS immunoreactivities of all groups

Immunoreactivity	Control	Naringenin	MC	MC+NAR 50	MC+NAR 100
Caspase 3	0.15±0.08^{c}	0.14±0.05^{c}	2.80±0.14^{a}	1.98±0.06^{ab}	1.03±0.07^{b}
iNOS	0.11±0.06^{c}	0.10±0.05^{c}	2.57±0.19^{a}	1.85±0.13^{ab}	0.93±0.11^{b}
eNOS	0.14±0.09^{c}	0.13±0.03^{c}	2.15±0.16^{a}	1.98±0.14^{ab}	0.95±0.10^{b}

Note: (P<0.05); a, b, c: Different superscripts in the same row indicate significant differences between groups.

NAR effectively neutralizes free radicals due to the 4'-hydroxyl group on the β-ring, which can donate electrons and act as a radical target. This action helps protect cell membranes from free radical damage and inhibits LP (Chandran *et al.*, 2019).

In studies addressing oxidative stress-related changes in the testis of rats treated with hydrogen peroxide, aluminum, and cadmium, NAR treatment successfully restored elevated MDA levels and normalized reduced GSH-Px, CAT, and SOD activities, as well as GSH concentrations (Rai *et al.*, 2013; Sahin *et al.*, 2017; Wang *et al.*, 2021). Furthermore, it was determined that this was an indication of the ROS scavenging property of NAR. Thus, it was conclusion was reached that the activities of antioxidant enzymes were improved and tissue damage was reduced. In the present study, the GSH increase observed with the addition of NAR was also higher than the control group values and this increase was higher in the MC + NAR 100 group. In a study researching the influence of aluminum on testicular toxicity in rats, it was reported that NAR increased testicular GSH content and GSH has particular roles in protecting the body from heavy metal toxicity as both a heavy metal binder and an antioxidant agent (Rai *et al.*, 2023). Flavanoids were found to modulate the Nrf-2/Keap-1 pathway in rats (Ijaz MU *et al.*, 2023). It has been reported that naringenine may inhibit prooxidant enzymes and modulate the levels of intracellular antioxidants through transcriptional regulation. Naringenin may maintain intracellular GSH concentrations attributable to Nrf2-mediated signaling processes and the presence of a 4'-hydroxyl group in the β-ring of naringenin may also contribute to the efficient quenching of free radicals (Chandran *et al.*, 2019).

There are studies indicating that the effects of mercury on the reproductive system are negative (Kalender *et al.*, 2013; Gaber *et al.*, 2013). Pathologic events in the reproductive system can be triggered by oxidative stress and mercury is a pro-oxidant that causes oxidative stress. This decline in epididymal sperm concentration is consistent with histological findings of the seminiferous tubules, which revealed a decline in luminal spermatozoa in rats exposed to MC (Gaber *et al.*, 2013). In another study, rats exposed to MC exhibited a decrease in sperm count and motility, along with an increase in sperm abnormalities. It was concluded that MC might impact spermatogenesis by crossing the blood-testis barrier and affecting germinal cells directly. Significant adverse effects on testicular tissue were noted, including marked spermatogenic degeneration at the spermatocyte level (Vachhrajani *et al.*, 1988).

In the present study, it was found that sperm motility and sperm density rates decreased significantly, head and tail abnormal sperm rates increased and right testis, right cauda, and prostate weights decreased in the MC group compared to the control group. These changes were determined as evidence that chronic MC exposure had negative effects on the testis. In a study researching the effect of NAR in rats exposed to cadmium, decreased sperm count and motility, sperm dysfunction, and infertility were detected and this was associated with oxidative damage of sperm cells, whereas NAR significantly increased sperm motility and sperm viability

and improved sperm function parameters. It was reported that NAR played a positive role in preventing the decrease in testosterone levels with its antioxidative properties and in defense against sperm abnormalities and oxidative stress (Wang *et al.,* 2021). In the present study, sperm motility rate, sperm density, right testis, right cauda, and prostate weight, which decreased with MC administration, increased with the addition of NAR, and increased head and tail abnormal sperm rates decreased. Positive effects of NAR on sperm parameters were determined and the increase in sperm motility and decrease in abnormal spermatozoon rate were more pronounced in MC +NAR 100 group.

MC has been detected to induce an increase in hydrogen peroxide production, exacerbate LP, and deplete glutathione levels, which leads to oxidative damage and following testicular damage. Additionally, MC exposure in rats has been related with an increase in the count of macrophages (Heath *at al.,* 2012). Kalender *et al.* (2013) also found that MC caused degeneration, edema, and necrosis in the testicles, attributing these effects to oxidative stress. In our study, consistent with the above literature, it has been observed that MC exposure causes various histopathological changes such as disorganization, degeneration, and vacuolization in spermatogenic cells in the testicle, and edema and hemorrhage in the interstitial area. It was noted that in the groups administered NAR, there was normalization of spermatogenic series cells, and changes in the interstitial areas were alleviated. In a study in which NAR was used against cadmium toxicity, it was found that the decrease in the spermatogenic cell layer in the seminiferous tubules and the irregularity in the serial cells were significantly alleviated by the healing effect of NAR (Wang *et al.,* 2021).

ROS production is known to increase hydrogen peroxide levels and stimulate LP of the mitochondrial membrane, leads to disruption of membrane integrity in cell necrosis or apoptosis (Leonard *et al.,* 2004). MC is known to induce apoptosis by activating caspase-3 and caspase-9 through the release of cytochrome c (Cytc) from the mitochondria. Active caspase-3, following cytochrome c release, lead to the generation of proteolytic products from polypolymerase (PARP) (ADP-ribose) and DNA fragmentation during apoptosis. Research has demonstrated that MC triggers apoptosis via the mitochondrial pathway by activating caspase-3 and Bax (Venkatesan and Sadig, 2017). It has been determined that with MC application, the levels of Bax and caspase-3, which are apoptotic markers, show a significant increase in tissues such as the testis, liver, kidney, and heart (Ercal *et al.,* 2001). Similarly, Massanyi *at el.* (2007) also revealed that mercury triggered apoptosis in the testis.

In our study, consistent with the above literature, it was revealed that exposure to MC led to a rise in caspase-3 immunoreactivity in the testis. However, it was noted that this apoptotic increase caused by MC decreased with NAR application. It is known that NAR protects against various toxic chemicals through the regulation of Bcl-2, Bax, and caspase 3, thanks to its strong antioxidant activity. In a study researching the effect of NAR on oxidative stress and apoptosis, it was found that this agent was effective in suppressing oxidative stress and preventing apoptosis. Jin and Wang (2019) revealed the healing effects of NAR, used against testicular toxicity, on testicular structural damage and cell apoptosis. Another study investigated the effect of naringin on the induction of cell death by cadmium exposure through activation of the mitochondrial apoptotic pathway. The study found that cadmium exposure increased the production of the pro-apoptotic protein Bax and decreased the production of the anti-apoptotic protein Bcl-2, restored the activation of the caspase protease family, and activated caspase-3, resulting in apoptotic cell death. It has been reported that NAR reduces apoptosis and thus corrects testicular damage due to its modulating effect on the expression of the apoptosis-related Cytc, caspase 3, caspase 9, Bax, and Bcl-2 genes (Fouad *et al.,* 2020).

Additionally, in a study researching the anti-apoptotic properties of NAR, it was found that it blocked the increase in testicular caspase 3 by preventing the decrease in the Bcl-2/Bax ratio (Jin *et al.,* 2019). Some studies have also revealed the strong anti-apoptotic effects of NAR (Xingyuan *et al.,* 2021; Chen *et al.,* 2022). Therefore, it can be concluded that NAR possesses antiapoptotic effects by mitigating MC-induced apoptosis and cell damage, attributable to its anti-inflammatory and antioxidant properties.

Nitric oxide (NO) is crucial for numerous physiological processes in the body and is released in multiple organs. The expression and activity of nitric oxide isoforms (iNOS and eNOS) are regulated through distinct signaling pathways in response to diverse physiological and pathological stimuli (Serreli *et al.,* 2023). While it has been detected that NO serves as an important effector molecule in the regulation of spermatogenesis (Ren *et al.,* 2019), it is known that iNOS and eNOS play a complementary role in the apoptosis of spermatogonial progenitors (Dutta *et al.,* 2022). Almeer *et al.* (2020) reported a significant increase in iNOS mRNA expression associated with MC-induced testicular damage. Similarly, our study found that MC exposure led to increased iNOS and eNOS immunoreactivities in the testis. However, it was found that the increased iNOS and eNOS immunoreactivities caused by MC decreased with the application of NAR. Similarly, a study reported that NAR application inhibited the increased iNOS expression in the testis following cisplatin exposure (Fouad *et al.,*2019).

Experimental evidence has noted that NAR polyphenols, among other possible therapeutics, have a wide range of diverse pharmacological activities (Emran *et al.,* 2022). These polyphenol-containing natural components have been reported to have positive effects like inducing or inhibiting NO release (Serreli *et al.,* 2023). In this scope, it can be indicated that NAR shows changes in iNOS and eNOS activities through its regulatory effects on NO release. There are some limitations in the current study. We determined the effect of NAR on the reproductive system of rats chronically exposed to MC in the light of spermatogenic and biochemical parameters, histopathological and immunohistochemical evaluations. However, considering the potential role of NAR on hormone profiles in MC exposure, we hope to include it in our future studies.

Conclusions: In conclusion, because of its strong antioxidant properties, NAR was found to have protective impacts against MC-induced oxidative damage,

histopathologic alterations, apoptosis, and spermatological abnormalities in the testis. 100 mg/kg NAR had more positive effects on histopathologic analysis, spermatological parameters, and apoptosis.

Funding statement: Financial support for this article was provided by Fırat University Scientific Research Projects Unit (FUBAP), Elazığ, Türkiye, The Research Supporting Project number: VF.21.21.

Declaration of interest: None of the researchers expressed a negative opinion.

Authors contribution: MK was involved in the design of the study, the experimental process, the analysis of samples and data. ME was involved in study design, evaluation and interpretation of biochemical analyses, MY and TPA were involved in histopathological and immunohistochemical analyses, SKÖ was involved in examination and evaluation of spermatological parameters. All authors interpreted and reviewed the data and approved the final version.

REFERENCES

Aebi H, 1984. Catalase In vitro. Methods Enzymol 105: 121-126.

Agarwal R and Behari JR, 2007. Effect of Selenium Pretreatment in Chronic Mercury Intoxication in Rats. Bull Environ ContamToxicol 79(3):306-310.

Alina M, Azrina A, Mohd Yunus A, *et al.,* 2012. Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. Int Food Res J 19(1):135-40.

Almeer RS, Albasher G, Kassab RB, *et al.,* 2012. Ziziphusspina-christi leaf extract attenuates mercury chloride induced testicular dysfunction in rats. Environ Sci Pollut Res 27:3401-12.

Al-Othman AM, Al-Numair KS, El-Desoky GE, *et al.,* 2011. Protection of α -tocopherol and selenium against acute effects of malathion on liver and kidney of rats. Afr J Pharm Pharmaco 10:1263-71.

Bernhoft RA, 2012. Mercury toxicity and treatment: a review of the literature. J Environ Public Health 2012(1):1-10.

Boujbiha MA, Hamden K, Guermazi F, *et al.,* 2009. Testicular toxicity in mercuric chloride treated rats: Association with oxidative stress. Reprod Toxicol 28:81-89.

Chandran AMK, Christina H, Das S, *et al.,* 2019. Neuroprotective role of naringenin against methylmercury induced cognitive impairment and mitochondrial damage in a mouse model. Environ Toxicol Pharmacol 71:103224.

Chavan S, Sava L, Saxena V, *et al.,* 2005. Reduced Glutathione: Importance of specimen collection. I J of Clin Biochem 20(1):150-52.

Chen H, Chen J, Shi X, *et al.,* 2022. Naringenin protects swine testis cells from bisphenol-induced apoptosis via Keap1/Nrf2 signaling pathway. Biofactors 48(1):190-203.

Emran TB, Islam F, Nath N, *et al.,* 2022. Naringin and Naringenin Polyphenols in Neurological Diseases: Understandings from a Therapeutic Viewpoint. Life 13:99.

Ercal N, Gurer-Orhan H and Aykin-Burns N, 2001. N. Toxic metals and oxidative stress part I: Mechanisms involved in metal induced oxidative damage. Curr Top Med Chem 1:529-39.

Dutta S, Sengupta P, Das S *et al.,* 2022. Reactive Nitrogen Species and Male Reproduction: Physiological and Pathological Aspects. Int J Mol Sci 23:10574.

Fink SL and Cookson BT, 2005. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun 73(4):1907-16.

Fouad AA, Refaie MM and Abdelghany MI, 2019. Naringenin palliates cisplatin and doxorubicin gonadal toxicity in male rats. Toxicol Mech Methods 29(1):67-73.

Fouad AA, Amin EF and Ahmed AF, 2020. Naringenin and Vanillin Mitigate Cadmium-Induced Pancreatic Injury in Rats via Inhibition of JNK and p38 MAPK Pathways. Pharmacogn J 12(4):742-48.

Gaber E, Samir ED, Bashandy A, *et al.,* 2013. Improvement of mercuric chloride-induced testicular injuries and sperm quality deteriorations by Spirulina platensis in rats. PLoS One 8(3):59-177.

Pavithra KG, SundarRajan P, Kumar PS *et al.,* 2023. Mercury sources, contaminations, mercury cycle, detection and treatment techniques. Chemosphere 312:137-314.

Gur FM and Aktas I, 2021. The ameliorative effects of thymoquinone and beta aminoisobutyric acid on streptozotocin-induced diabetic cardiomyopathy. Tissue Cell 71:101582.

Heath J, Abdelmageed C, Braden Y, *et al.,* 2012. The effects of chronic ingestion of mercuric chloride on fertility and testosterone levels in male Sprague Dawley rats. J Biomed Biotechnol 2012: 815186.

Ijaz MU, Kalsoom A, Hamza A, *et al.,* 2023. Sciadopitysin attenuates paraquat induced renal toxicity by modulating Nrf-2/Keap-1 pathway in male albino rats. Asian J Agric Biol (4):2023110.

Jahan S, Azad T, Ayub A, *et al.,* 2019. Ameliorating potency of Chenopodium album Linn. and vitamin C against mercuric chloride-induced oxidative stress in testes of Sprague Dawley rats. Environ Health Prev Med 24(1):23-62.

Jin Y, Wang H, *et al.,* 2019. Naringenin inhibit the hydrogen peroxide induced SH-SY5Y cells injury through Nrf2/HO-1 pathway. Neurotox Res 36:796-805.

Johnsen SG, 1970. Testicular biopsy score count - A method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. Hormones 1:2-25.

Kahramanoğullari M, Erişir M, Yaman M *et al.,* 2024. Effects of naringenin on oxidative damage and apoptosis in liver and kidney in rats subjected to chronic mercury chloride. Environ Toxicol 39:2937-47.

Kalender S, Uzun FG, Demir F, *et al.,* 2013. Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem Toxicol 55:456-62.

Leonard SS, Harris GK, and Shi XL 2004. Metal-induced oxidative stress and signal transduction. Free Radic Biol Med. 37:1921-42.

Lowry OH, Rosenbrough NJ, Farr AL, *et al.,* 1951. Protein measurement with the folin-phenol reagent. J Biol Chem. 193(1):265-57.

Massanyi P, Lukac N, Slivkova J, *et al.,* 2007. Mercury-induced alterations in rat kidneys and testes in vivo. J Environ Sci Health A Tox Hazard Subst Environ Eng. 42:865-70.

Matkovics B, Szabo I and Varga I, 1988. Determination of enzyme activities in lipid peroxidation and glutathione pathways. Laboratiumi Diagnosztika. 15:248-49.

Placer ZA, Cushman L and Johnson BC, 1966. Estimation of products of lipid peroxidation in biological fluids. Anal Biochem 16(2):359-64.

Rai R, Jat D, Mishra SK, *et al.,* 2023. Naringenin ameliorates aluminum toxicity-induced testicular dysfunctions in mice by suppressing oxidative stress and histopathological alterations. Syst Biol Reprod Med 69(5):347-53.

Ren Y, Shao W, Zhao W, *et al.,* 2019. Mechanism of cadmium poisoning on testicular injury in mice. Oncol Lett 18:1035-42.

Renugadevi J and Prabu SM, 2009. Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. Toxicology 256(1-2):128-34.

Rice KM, Walker EM Jr, Wu M, *et al.,* 2014. Environmental mercury and its toxic effects. J Prev Med Public Health 47(2):74-83.

Sahin Z, Ozkay A, Cuce G, *et al.,* 2017. Investigation of the effect of naringenin on oxidative stress-related alterations in testis of hydrogen peroxide-administered rats. J Biochem Mol Toxicol e21928.

Sengul E, Gelen V, Yildirim S *et al.,* 2023. Effects of naringin on oxidative stress, inflammation, some reproductive parameters, and apoptosis in acrylamide-induced testicular toxicity in rat. Environ Toxicol 38:798-808.

Serreli G and Deiana M 2023. Role of Dietary Polyphenols in the Activity and Expression of Nitric Oxide Synthases: A Review. Antioxidants 12:147.

Seven I, Tatli Seven P, Gul Baykalir, *et al.,* 2020. Bee glue (propolis) improves reproductive organs, sperm quality and histological changes and antioxidant parameters of testicular tissues in rats exposed to excess copper. Andrologia 52:1-11.

Sun Y, Oberley WL and Li Y, 1988. A simple method for clinical assay of superoxide dismutase. Clin Chem 34 (3):497-500.

Turk G, Sonmez M, Aydin M, *et al.,* 2008. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin Nutr 27(2):289-96.

Xingyuan S, Xueping L, Ting C. *et al.,* 2021. Naringenin inhibits migration, invasion, induces apoptosis in human lung cancer cells and arrests tumour progression in vitro. J Hücre Mol Med 25 (5):2563-71.

Vachhrajani K, Makhija S, Chinoy N, *et al.,* 1988. Structural and functional alterations in testis of rats after mercuric chloride treatment. J Reprod Biol Compara Endocrinol 8:97-104.

Venkatesan RS and Sadig AM, 2017. Effect of morin-5'-sulfonic acid sodium salt on the expression of apoptosis related proteins caspase 3, Bax and Bcl 2 due to the mercury induced oxidative stress in albino rats. Biomed Pharmacother 85:202-8.

Wali AF, Rashid S, Rashid SM, *et al.,* 2020. Naringenin Regulates Doxorubicin-Induced Liver Dysfunction: Impact on Oxidative Stress and Inflammation. Plants 24 9(4):550.

Wang A, Zhu H, Lin S, *et al.,* 2021. Protective effect of naringenin against cadmium-induced testicular toxicity in male SD rats. J Inorg Biochem 214:111-310.