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RESEARCH ARTICLE

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Effects of sinapic acid on lead acetate-induced oxidative stress, apoptosis and inflammation in testicular tissue

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Abstract

In this study, the effect of lead acetate (PbAc) and sinapic acid (SNP) administration on oxidative stress, apoptosis, inflammation, sperm quality and histopathology in testicular tissue of rats was tried to be determined. PbAc was administered at a dose of 30 mg/kg/bw for 7 days to induce testicular toxicity in rats. Oral doses of 5 and 10 mg/kg/bw SNP were administered to rats for 7 days after PbAc administration. According to our findings, while PbAc administration increased MDA content in rats, it decreased GPx, SOD, CAT activity and GSH content. NF-kB, IL-1β, TNF-α, and COX-2, which are among the inflammation parameters that increased due to PbAc, decreased with the administration of SNP. Nrf2, HO-1, and NQO1 mRNA transcript levels decreased with PbAc, but SNP treatments increased these mRNA levels in a dose-dependent manner. RAGE and NLRP3 gene expression were upregulated in PbAc treated rats. MAPK14, MAPK15, and JNK relative mRNA levels decreased with SNP treatment in PbAc treated rats. While the levels of apoptosis markers Bax, Caspase-3, and Apaf-1 increased in rats treated with PbAc, the level of Bcl-2 decreased, but SNP inhibited this apoptosis markers. PbAc caused histopathological deterioration in testis tissue and negatively affected spermatogenesis. When the sperm quality was examined, the decrease in sperm motility and spermatozoon density caused by PbAc, and the increase in the ratio of dead and abnormal spermatozoa were inhibited by SNP. As a result, while PbAc increased apoptosis and inflammation by inducing oxidative stress in testicles, SNP treatment inhibited these changes and increased sperm quality.

KEYWORDS
apoptosis, inflammation, lead acetate, oxidative stress, sinapic acid

1 | INTRODUCTION

Toxic metals are among the most important causes of environmental pollution.[1–5] Lead (Pb) is among the environmentally polluting and toxic metals.[6–10] Humans and animals are exposed to Pb in ways such as drinking water, ambient air, food, electronic waste, industrial materials, and anthropogenic activities, such as industrial sites and consumer products.[11,12] It is accepted that lead can cause health problems in different body tissues such as kidney, lung, liver, brain, and hematopoietic, nervous, and reproductive systems in animals and humans.[13–21] Lead-induced deterioration of male reproductive health may occur.[22] It is stated that Pb can reduce sperm quality, impair spermatogenesis by negatively affecting the hypothalamus-pituitary-testis axis, inhibit testosterone release, trigger the formation of excessive reactive oxygen species, and potentially affect spermatozoa viability, motility, DNA fragmentation, and chemotaxis for spermatozoa-oocyte fusion.[23–25] Lead acetate (PbAc) causes various types of damage to the testicular architecture of Kunming mice, resulting in irregularities

of the seminiferous tubules.[26] It has been reported that the hypothalamic–pituitary-testis axis of male rats administered lead acetate is adversely affected, resulting in impaired steroidogenesis, decreased sperm parameters and testicular antioxidant enzyme activity.[27,28]

Oxidative stress occurs as a result of the interaction of lead with critical biomolecules. Therefore, it is necessary to strengthen the antioxidant defense of the cell.[29–34] Sinapic acid (SNP) is an important active ingredient in traditional Chinese medicines,[35] that reduces the effect of oxidants or scavenges excess free radicals.[36] It is stated that SNP has an anti-inflammatory effect in in-vivo[37,38] and in-vitro studies.[39] In addition, SNP has antidiabetic,[40] cardioprotective,[41] anti-anxiety,[42] gastro protective,[43] hepatoprotective,[44] and neuroprotective effects.[45] SNP inhibited inflammation and apoptosis by maintaining the oxidant balance in ischemia/reperfusion injury of testicular tissue in rats.[46] In another study, it was stated that SNP administration against testicular toxicity caused by cisplatin improved testicular histopathology and decreased apoptosis.[47]

The aim of this study was to determine the protective effects of SNP on testicular toxicity caused by the environmental toxicant PbAc by biochemical, histopathological and semen analysis.

2 | MATERIALS AND METHODS

2.1 | Animals

In this study, 35 male Sprague–Dawley rats with an average weight of 250–300 g, aged 10–12 weeks, obtained from Atatürk University Medical Experiment Application and Research Center, were used. During the study, animals were housed in standard laboratory conditions (24 ± 1°C, 45 ± 5% humidity and 12/12 light/dark cycles). They were fed standard feed and water ad libitum. Before the study, a week of adaptation was provided for them to get used to.

In the study, 35 Sprague Dawley rats, 7 rat each group, were used and the groups were formed as follows;

Control: Physiological saline was given to the rats by oral gavage for 7 days.
SNP: SNP was administered to rats by oral gavage at a dose of 10 mg/kg for 7 days.
PbAc: 30 mg/kg PbAc was given to the rats by oral gavage for 7 days.
PbAc+ SNP 5: The rats were given 30 mg/kg PbAc via oral gavage and 5 mg/kg SNP after 30 min for 7 days.
PbAC+ SNP 10: The rats were given 30 mg/kg PbAc via oral gavage and 10 mg/kg SNP after 30 min for 7 days.

2.2 | Oxidative stress analysis

Testicular tissue was homogenized in a homogenizer (Tissue Lyser II, Qiagen, The Netherlands) using 1.15% potassium chloride solution to obtain a 1:10 (w/v) homogenate.[48] Glutathione (GPx) activity was determined by the method described by Matkovics.[49] Superoxide dismutase (SOD) activity was measured according to the method used by Sun et al.[50] Catalase (CAT) activity was evaluated by the method specified by Aebi.[51] Glutathione (GSH) content was determined by the method used by Sedlak and Lindsay.[52] Malondialdehyde (MDA) levels were determined by Placer et al.[53] The protein content of testicular tissues was measured by the method of Lowry et al.[54]

2.3 | RT-PCR analysis

Total RNAs were obtained from testicular tissues with QIAzol Lysis Reagent (79 306; Qiagen). Total RNA concentrations were measured with NanoDrop (BioTek Epoch) and the final RNA concentration of all experimental groups was equalized to 1000 ng/μL. With the iScript cDNA Synthesis Kit (Bio-Rad), cDNA synthesis was performed from RNAs according to the manufacturer's user manual. The mRNA transcript levels of Nrf2, HO-1, NQO1, NF-κB, IL-1A, TNF-α, COX-2, RAGE, NLRP3, MAPK14, MAPK15, JNK, Bax, Bcl-2, Apaf-1, and Caspase-3 genes whose sequences are given in Table 1 were analyzed using iTaq Universal SYBR Green Supermix (BIORAD) with cDNAs. RT-PCR analyzes were performed on the Rotor-Gene Q (Qiagen) instrument and under the conditions specified by the manufacturer. At the end of the procedures, normalization was performed according to B-actin using the $2^{-\text{eltadeltaCT}}$ method.[55]

2.4 | Histopathological analysis

At the end of the experiment, rat testicles were fixed in 10% neutral buffered formalin for 48 h. The fixed tissues were turned into blocks after dehydration, cleaning with xylol and paraffin treatment by passing through increasing grade alcohols. Sections of 5 μm thickness were taken from the blocks by means of microtome and the sections were stained with Hematoxylin–Eosin (H&E). The stained sections were examined using a Binocular Olympus Cx43 light microscope (Olympus Inc., Tokyo, Japan) and photographed with the EP50 brand camera (Olympus Inc., Tokyo, Japan) attached to the microscope.[48]

2.5 | Semen analysis

After the rats were sacrificed, the excised testicular tissue was separated from the epididymis. The cauda epididymis was trimmed in 5 mL of physiological saline heated to 35°C and incubated for 5 min. The obtained sperm fluid was used in semen analysis.[56]

To determine sperm total motility, a slide was placed on a light microscope (Primo Star; Carl Zeiss) with a heating plate. 20 μL of the obtained sperm liquid was dropped on the slide and covered with a coverslip. At 400× magnification, three different microscope fields were examined and the final score was scored as a percentage.[57]

For sperm density determination, after 10 μL of semen sample was taken into an Eppendorf tube, 990 μL of eosin solution was added and the mixture was vortexed at 1000 g for 15 s. 10 μL of the

TABLE 1 Primer sequences.

Gene	Sequences (5′-3′)	Length (bp)	Accession No
Nrf2	F: TTTGTAGATGACCATGAGTCGC R: TCCTGCCAAACTTGCTCCAT	161	NM_031789.2
HO-1	F: ATGTCCCAGGATTTGTCCGA R: ATGGTACAAGGAGGCCATCA	144	NM_012580.2
NQO1	F: CTGGCCAATTCAGAGTGGCA R: GATCTGGTTGTCGGCTGGAA	304	NM_017000.3
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106	NM_001276711.1
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197	NM_031512.2
TNF-α	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	139	NM_012675.3
COX-2	F: AGGTTCTTCTGAGGAGAGAG R: CTCCACCGATGACCTGATAT	240	NM_017232.3
RAGE	F: CTGAGGTAGGGCATGAGGATG R: TTCATCACCGGTTTCTGTGACC	113	NM_053336.2
NLRP3	F: TCCTGCAGAGCCTACAGTTG R: GGCTTGCAGCACTGAAGAAC	185	NM_001191642.1
MAPK14	F: GTGGCAGTGAAGAAGCTGTC R: GTCACCAGGTACACATCGTT	170	NM_031020.2
MAPK15	F: TGTTTGAGTCCATGGACACC R: GCATCCAATAGAACGTTGGC	169	NM_173331.2
JNK	F: GAATCAGACCCATGCTAAGC R: CCATGAGCTCCATGACTATG	149	NM_053829.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	214	NM_016993.2
Apaf-1	F: ACCTGAGGTGTCAGGACC R: CCGTCGAGCATGAGCCAA	192	NM_023979.2
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

mixture was transferred to the Thomas slide. Sperm counts were calculated using a light microscope (Primostar, Zeiss Co.) set at 400× magnification.[58]

For the rate of dead spermatozoa and abnormal sperm rate, 10 μL of semen and 10 μL of eosin dye (5%) were mixed with a coverslip on a slide then the smear was taken and dried. For the rate of dead spermatozoa, 200 spermatoza per slide were examined with a light microscope. Sperm cells were classified as dead according to the staining status of the head. For abnormalities of sperm cells, a total of 200 spermatoza were evaluated on the same slide and abnormality rates were calculated as percentages.[59]

2.6 | Statistical analysis

Statistical analysis results of the data obtained from the study were given as mean ± standard deviation. SPSS program (version 26.0; SPSS, Chicago, IL) was used for data analysis. Analysis of the data was performed with Tukey's post hoc test and one-way analysis of variance (ANOVA) for multiple comparisons. ($P < .05$) was considered statistically significant.

3 | RESULTS

3.1 | Analysis results of lipid peroxidation level and antioxidant enzyme activities in testicular tissues

MDA level, GPx, CAT, and SOD activities and GSH levels in testicular tissue are shown in Table 2. Compared with the control group, rats given PbAc showed significantly increased MDA levels ($p.001$). However, the MDA level decreased in the SNP treatment groups. In addition, GSH level, SOD, CAT, and GPx activity were found to be lower in the PbAc group compared to the control group ($P < .001$). However, antioxidant activity increased in a dose-dependent manner in the SNP treatment groups ($P < .001$).

3.2 | The expression profiles of Nrf2, HO-1 and NQO1

The expression profiles of NRF-2, HO-1, and NQO1 genes in testicular tissue in all experimental groups are shown in Figure 1. The group receiving PbAc had reduced expression levels of NRF-22, HO-1, and NQO1 compared to the control and SNP groups. Compared to the SNP group, Nrf2, HO-1, and NQO1 gene expression levels were higher in the PbAc + SNP 5 and PbAc + SNP 10 groups ($P < .05$).

3.3 | Effect of PbAc and SNP treatment on inflammatory markers

The effects of PbAc and SNP treatment on mRNA transcript levels of NF-κB, IL1B, TNF-6, and COX-2 in rat testis tissue are presented in Figure 2. When the control group and SNP were compared, it was seen that the gene expressions were almost identical. It was observed that PbAc administration increased NF-κB, IL1B, TNF-6, and COX-2 levels, but SNP administration decreased these gene expression levels in a dose-dependent manner ($P < .001$).

3.4 | Effect of PbAc and SNP treatment on RAGE and NLRP3 mRNA transcription level

RAGE and NLRP3 gene expression levels are shown in Figure 3. In the presented study, it was determined that PbAc exposure significantly increased the levels of RAGE and NLRP3 gene expression and thus the level of mRNA transcripts ($P < .001$). In addition, SNP administration after PbAc exposure decreased these gene expressions in testicular tissue.

TABLE 2 The effects of PbAc and SNP treatments on MDA and GSH levels and SOD, CAT, and GPx activities in testis tissue.

Parameters	Control	SNP	PbAc	PbAc+SNP 5	PbAc+SNP 10
MDA (nmol/g tissue)	26.96 ± 1.96	26.26 ± 2.18$^{###}$	65.45 ± 2.86***	54.24 ± 2.38$^{***/###/+++}$	44.64 ± 2.40$^{***/###}$
GSH (nmol/g tissue)	2.36 ± 0.13	2.51 ± 0.10$^{###}$	1.19 ± 0.08***	1.47 ± 0.09$^{***/###/+++}$	1.92 ± 0.11$^{***/###}$
GP × (U/g protein)	17.03 ± 1.45	18.15 ± 1.42$^{###}$	9.37 ± 0.79***	13.05 ± 1.06$^{***/###/+}$	15.22 ± 1.02$^{###}$
SOD (U/g protein)	14.65 ± 1.08**	16.18 ± 0.88$^{###}$	4.75 ± 0.44***	6.64 ± 0.55$^{***/##/+++}$	9.36 ± 0.73$^{***/###}$
CAT (catal/g protein)	10.29 ± 0.92	11.06 ± 0.87$^{###}$	2.85 ± 0.31***	4.44 ± 0.54$^{***/##/+++}$	7.42 ± 0.68$^{***/###}$

Note: Control versus others: $^{*}P < .05$, $^{**}p < .01$, $^{***}p < .001$, PbAc versus others: # $P < .05$, ## $p < .01$, ### $p < .001$, PbAc+SNP 5 versus PbAc+SNP 10: ✦ $P < .05$, ✦✦ $p < .01$, ✦✦✦ $p < .001$.

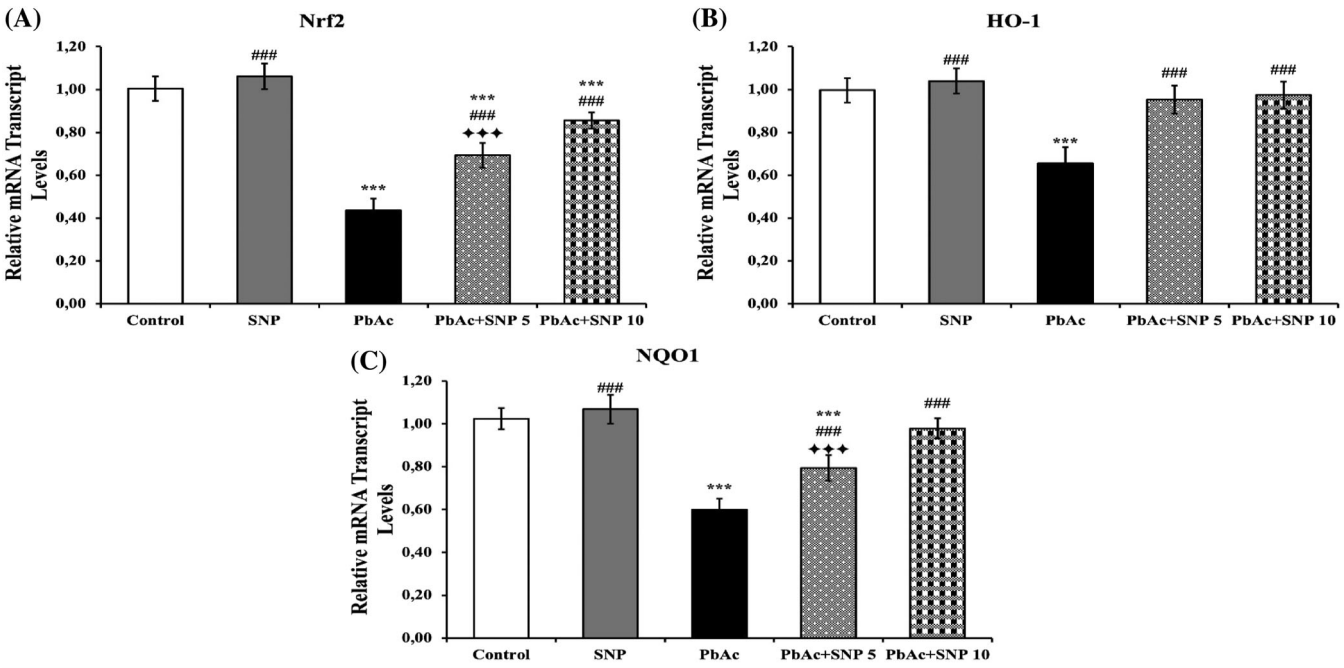

FIGURE 1 The effects of SNP and PbAc applications on Nrf-2, HO-1 and NQO1 mRNA expression levels in testicular tissue. (A) Nrf-2 mRNA transcript levels, (B) HO-1 mRNA transcript levels, (C) NQO1 mRNA transcript levels. The different symbols in the columns differ statistically. One symbol ($P < .05$), two symbols ($P < .01$) three symbols ($P < .001$).

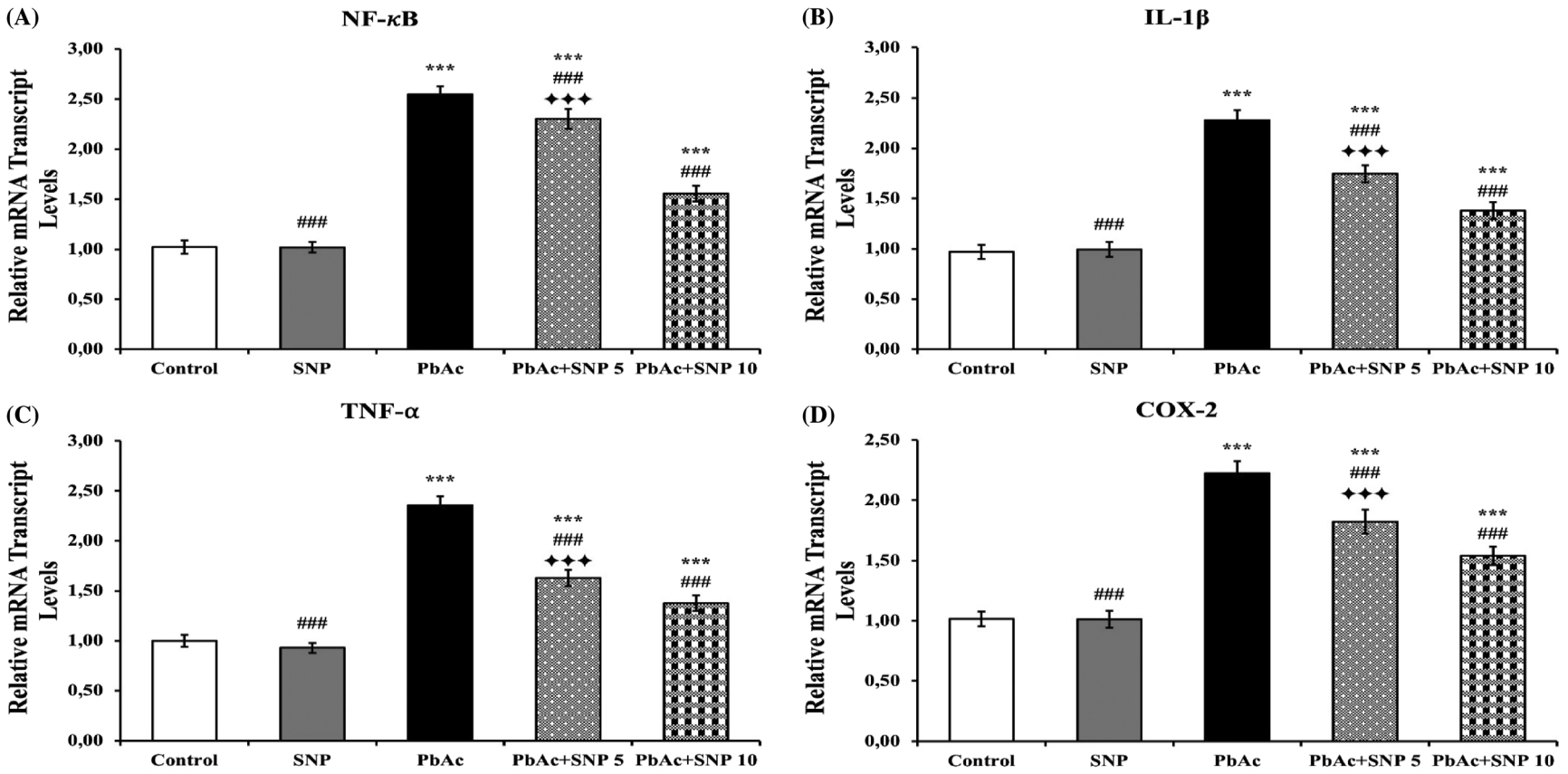

FIGURE 2 Effects of PbAc and SNP applications on inflammation in testicular tissues (A) NF-κB mRNA transcript levels, (B) IL-1β mRNA transcript levels, (C) TNF-α mRNA transcript levels, (D) COX-2 protein levels. The different symbols in the columns differ statistically. One symbol ($P < .05$), two symbols ($P < .01$) three symbols ($P < .001$).

3.5 | Effect of PbAc and SNP administration on MAPK14/MAPK15/JNK pathway in testicular tissue

The results of mitogen-activated protein kinase 14 (MAPK14), mitogen-activated protein kinase 15 (MAPK15) and c-Jun N-terminal kinase (JNK) analysis results are presented in Figure 4. The obtained data showed that PbAc triggered MAPK14, MAPK15, and JNK expressions. SNP treatment appeared to suppress the expression of related genes in the pathway ($P < .001$).

3.6 | Status of apoptosis markers after PbAc and SNP treatments

Relative mRNA transcript levels of Bax, Bcl-2, Caspase-3, and Apaf-1 biomarkers were analyzed for detection of apoptotic status in testicular tissue. According to the results shown in Figure 5, PbAc up-regulated the expression of Bax, Caspase-3, and Apaf-1 in testicular tissue and down-regulated the expression of Bcl-2. On the other hand, it was determined that Bax, Caspase-3, Apaf-1, and JNK

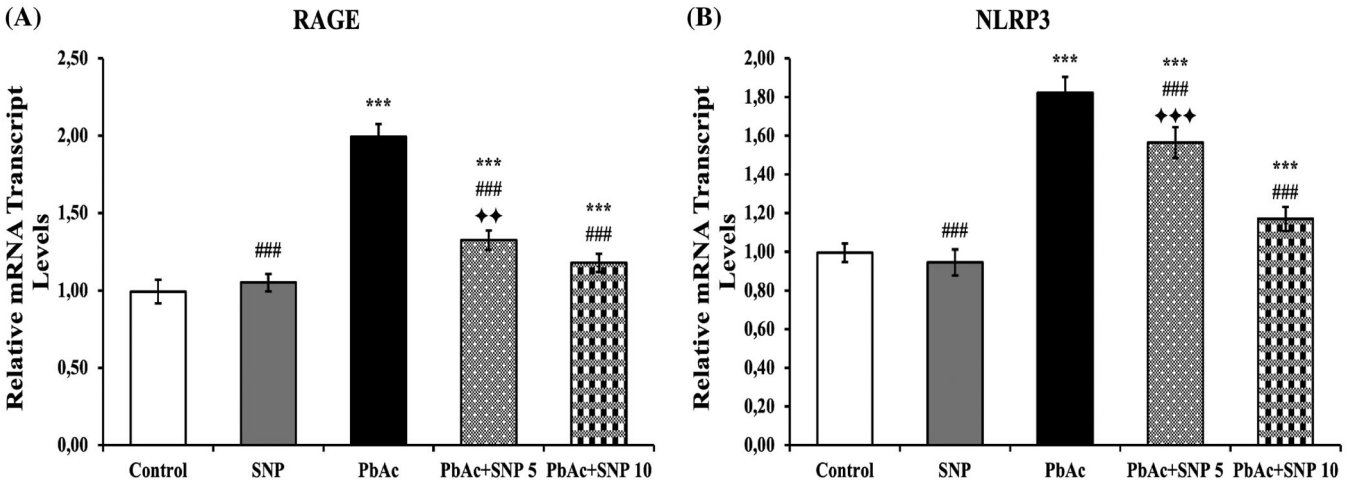

FIGURE 3 The effects of PbAc and SNP applications on RAGE and NLRP3 gene levels in testicular tissues. (A) RAGE mRNA transcript level, (B) NLRP3 mRNA transcript level. The different symbols in the columns differ statistically. One symbol ($P < .05$), two symbols ($P < .01$) three symbols ($P < .001$).

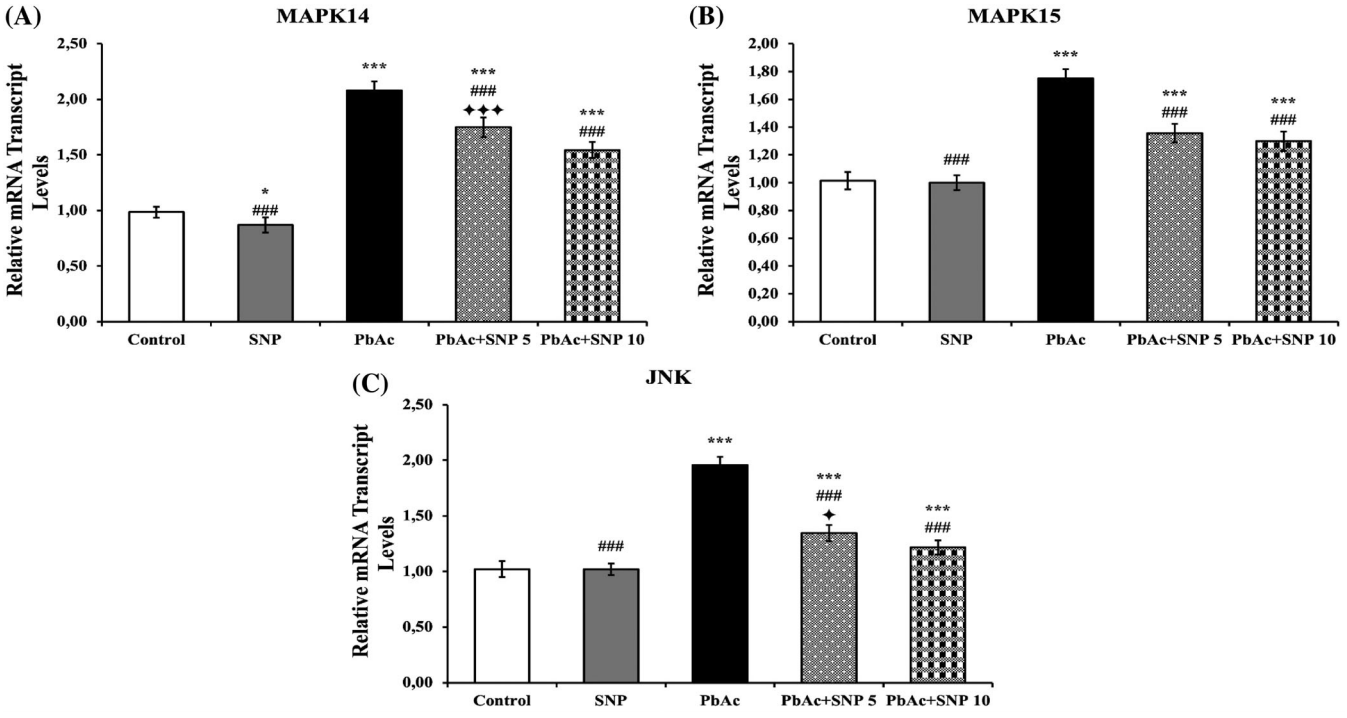

FIGURE 4 Effects of PbAc and SNP administrations on inflammation in testis tissues (A) MAPK14 mRNA transcript level, (B) MAPK15 mRNA transcript level, (C) JNK, mRNA transcript level. The different symbols in the columns differ statistically. One symbol ($P < .05$), two symbols ($P < .01$) three symbols ($P < .001$).

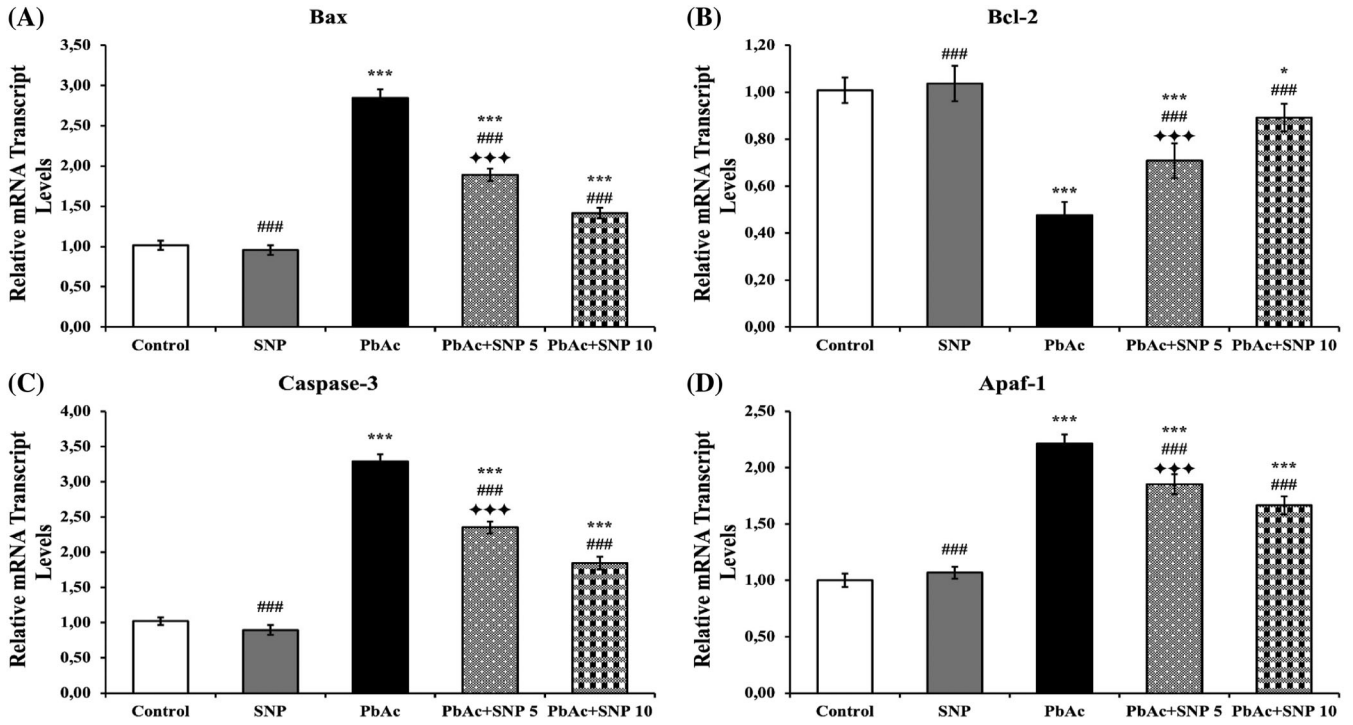

FIGURE 5 Apoptotic markers in testis tissue. (A) Bax mRNA transcript level, (B) Bcl-2 mRNA transcript level, (C) Caspase-3 mRNA transcript level, (D) Apaf-1 mRNA transcript level. The different symbols in the columns differ statistically. One symbol ($P < .05$), two symbols ($P < .01$) three symbols ($P < .001$).

expressions were suppressed and Bcl-2 expression was triggered in rats given SNP ($P < .001$).

3.7 | Histopathological result

When the control and SNP groups were examined in H&E stained testicular sections, it was observed that smooth-looking seminiferous tubules, narrow interstitium, germinal epithelium and basal membrane preserved their normal histomorphological order. It was observed that there were a large number of sperm in the lumens of the seminiferous tubules and the spermartogenic cell lineage preserved its normal structure (Figure 6Aa,Bb). After administration of lead acetate to the rats, it was determined that the normal structure of the testis was impaired. Particularly, the seminiferous tubules exhibited amorphous, wide and irregular appearance, and the germinal epithelium was observed to be disrupted. In this group, no sperm cells were observed in most tubule lumens and desquamation was observed in the series of germ cells. Areas with vacuoles in the seminiferous tubules were particularly striking (Figure 6Cc). When the groups given SNP after PbAc were examined, it was seen that SNP could prevent the damage. In the PbAc+SNP5 and PbAc+SNP10 groups, the pathological changes were decreased, the seminiferous tubules preserved their morphological integrity and the germinal epithelium was regular. In these groups, germ cells were regularly distributed and sperm cells were increased in the tubule lumens. In addition, the formed vacuole areas were found to be minimal (Figure 6Dd,Ee).

3.8 | Reproductive parameters analysis results

The results of reproductive parameters of all experimental groups are shown in Table 3. According to these results, no statistical difference was found between the groups in terms of testicular weights. While the total motility value was the lowest in the PbAc group, the highest value was seen in the SNP group ($P < .001$). In addition, the total motility value increased in the PbAc groups in a dose-dependent manner. While the rate of dead sperm was highest in the PbAc group, it was the lowest in the SNP group ($P < .001$). Head and tail abnormal sperm ratio was similarly highest in the PbAc group ($P < .05$). While semen density had the highest value in SNP group, it had the lowest value in PbAc group. Sperm density increased in a dose-dependent manner in the treatment groups ($P < .001$).

4 | DISCUSSION AND CONCLUSION

As a result of industrial activities, the release of lead, which is toxic to organisms and the environment, is increasing.[60–63] Lead has a toxic effect on the female and male reproductive systems.[64,65] In this study, the effects of SNP on PbAc-induced testicular toxicity were investigated by semen analyses, oxidative stress, inflammation, ER stress, apoptosis, and histopathological examinations.

Antioxidant enzymes and compounds maintain the oxidant/antioxidant balance in the organism.[66] As a result of the decrease in antioxidant activity in the organism, cells are exposed to reactive

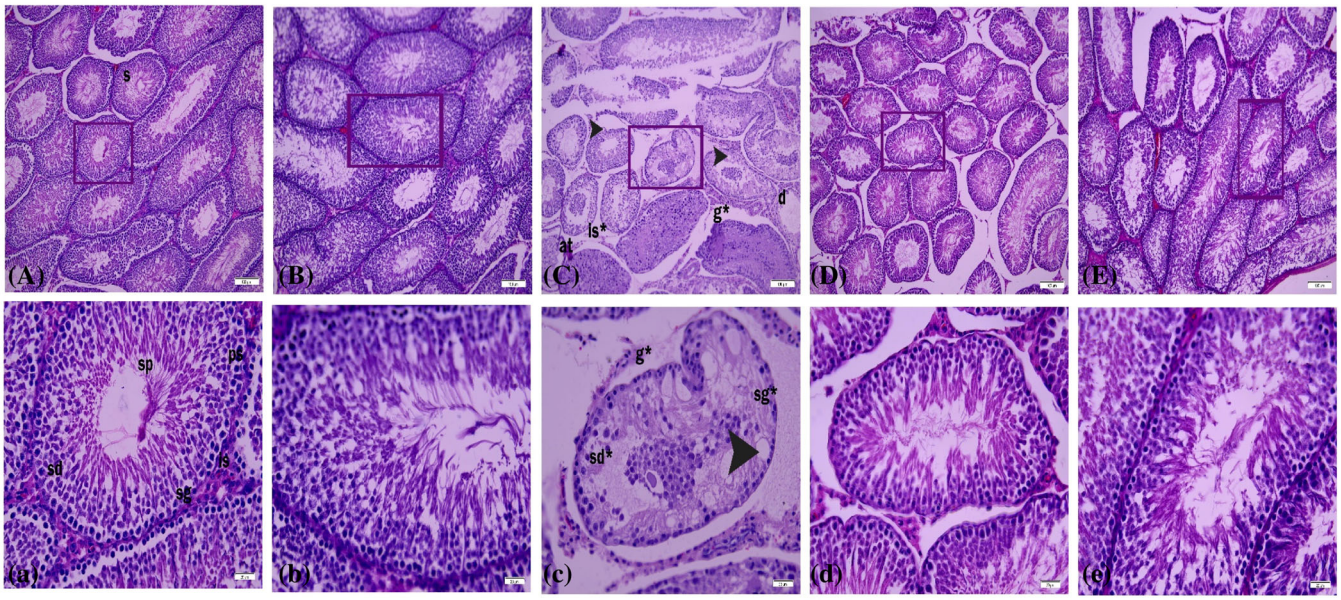

FIGURE 6 Photomicrographs of histological changes in testis tissue (H&E staining, original magnifaction; top row 100 μm; bottom row 20 μm). Control group (A,a); s: seminiferous tubule in normal morphology, Sinapic acid group (B,b); Pb group (C,c); arrowhead: vacuolization, d: desquamation, at: amorphous seminiferous tubule, g*: irregular germinal epithelium, sg*: loss of spermatogonium, sd*: spermatid loss, is*: irregular interstitial space, Pb + SNP5 group (D,d); Pb + SNP10 group (E,e).

TABLE 3 Reproductive parameters analysis results.

	Control	SNP	PbAc	PbAc+SNP 5	PbAc+SNP 10
R. T. W.	1464.57 ± 73.62	1488.42 ± 122.18	1434.28 ± 61.36	1508.57 ± 103.95	1571.42 ± 82.75
L. T. W.	1457.57 ± 77.22	1441.57 ± 96.65	1416.71 ± 79.80	1506.14 ± 118.46	1539.71 ± 83.03
Total motility	80.44 ± 3.56^{b}	83.31 ± 3.31^{b}	66.16 ± 4.90^{a}	74.87 ± 3.03^{a}	82.84 ± 6.21^{b}
Dead spermatozoa rate	11.86 ± 2.61^{b}	9.28 ± 1.11^{a}	17.01 ± 1.15^{c}	11.71 ± 0.95^{b}	11.85 ± 0.69^{b}
Head anomaly spermatozoa	5.42 ± 0.79^{a}	5.14 ± 0.69^{a}	7.57 ± 0.97^{b}	7.57 ± 0.78^{b}	7.01 ± 0.82^{b}
Tail anomaly spermatozoa	5.57 ± 0.78^{ab}	5.01 ± 0.82^{a}	7.14 ± 0.69c	6.29 ± 0.49^{ab}	6.01 ± 0.82^{bc}
Density	71.14 ± 3.93^{ab}	79.86 ± 5.18^{c}	69.43 ± 5.53^{a}	78.28 ± 4.92^{bc}	83.57 ± 3.59^{c}

Note: a-c; Values indicated by different letters in the same column are significantly different from each other ($P < .05$). Abbrevations: LA, Lead Acetate; L.T. W, Left Testis Weight; R.T.W, Right Testis Weight; SA, sodiumm Arsenite.

oxygen species and increase oxidative stres.[67,68] PbAc suppresses the antioxidant defense system and causes an increase lipid peroxidation in testicles.[24,65,69] It has been reported that Pb reduces the activities of antioxidant enzymes by binding to SH- groups, decreases the level of GSH, which is a non-enzymatic antioxidant, and increases MDA, the end product of lipid peroxidation.[70] In our study, it was determined that the antioxidant defense system was weakened and the MDA content, which is a marker of lipid peroxidation, increased in the testicular tissue of rats treated with PbAc. SNP, known for its antioxidant properties, is a compound that maintains the oxidant balance in the testicles.[46] When our findings were examined, a dose-dependent decrease in MDA content and an increase in GPx, SOD, CAT activity and GSH levels were observed in the groups treated with PbAc+SNP ($P < .05$).

NRF-2 is a primary transcription factor activated by increased oxidative stress.[71] An increase in NRF-2 expression regulates oxidative and inflammatory responses through the induction of phase II detoxification enzymes such as HO-1 and NQO1.[66,72] In the present study, PbAc suppressed the NRF-2, HO1, and NQO1 genes. This is evidence that PbAc is suppressed after increased oxidative stress in testicular tissue. The significant decrease in NRF-2, HO-1, and NQO-1 mRNA expressions in the testes of SNP-treated rats can be interpreted as protecting endogenous antioxidants by reducing free radicals induced by PbAc.

The enhanced glycation end products receptor (RAGE) has an important role in the inflammatory process.[73] NLRP3 is activated in response to exogenous stimuli, resulting in the release of proinflammatory cytokines.[74] In our findings, the increase in RAGE and NLRP3 gene expression in the PbAc group was an indicator of inflammation, and SNP administration downregulated the expression of these inflammatory genes.

Inflammation in testicular tissue is an important factor in decreasing fertility.[24,75] Increased oxidative stress triggers inflammation in the

testicles.[6] Excessive ROS production activates the MAPK14 signaling pathway, a potent NF-κB activator.[76] The proinflammatory cytokines activated by NF-κB are IL-1β, TNF-α, and IL-6.[77] When our findings were examined, PbAc caused inflammation by increasing MAPK14, MAPK15, JNK, NF-κB, IL-6, TNF-α, and COX mRNA transcript levels in testis tissue. SNP administered in the treatment groups decreased the expression of these genes in a dose-dependent manner. This confirms that the SNP has anti-inflammatory properties.

Apoptosis is a type of programmed biological cell death required to maintain homeostatic balance.[78–81] Pro-apoptotic proteins, such as Bax, Caspase-3 and anti-apoptotic proteins, such as Bcl-2 regulate apoptosis.[82] PbAc triggers apoptosis by activating Caspas-3 protein levels.[24,83,84] In another study, PbAc increased the expression of Bax and Caspase-3 in the testicles, while it decreased the expression of Bcl-2.[85] In the present study, we evaluated apoptotic Bax, Caspase-3, and Apaf-1 and anti-apoptotic Bcl-2 genes by q RT-PCR method for the evaluation of apoptosis. In our study, the fact that PbAc administration increased the levels of apoptotic factors Bax, Caspase-3, and Apaf-1 in testicular tissue, while decreasing the levels of the antiapoptotic factor Bcl-2 is proof that PbAc induces apoptosis. SNP administration to the treatment group caused a decrease in Bax and Caspase-3 levels and an increase in Bcl-2 levels. This shows that the SNP has antiapoptotic properties and supports the view that flovanoids are antiapoptotic.[86]

It is known that there is a positive correlation between lead exposure and male reproductive dysfunction.[27,87] It is stated in a study that PbAc causes a decrease in testicular weight in rats.[88–90] In the present study, no significant difference was found between the experimental groups in terms of testicular weights. Epididymal sperm count was used as an important indicator giving information about testis.[91] In our study, the epididymal semen density was found to be the lowest in the PbAc group. It is thought that this condition originates from the affected seminiferous tubules in the histologically deteriorated testicular tissue. Motility is one of the main parameters used to determine sperm quality.[92] Previous studies report that PbAc reduces sperm motility in rats.[24,90,93] In our study, after PbAc administration, it was determined that sperm total motility decreased significantly, and the percentage of dead and abnormal sperm increased significantly compared to other groups. It was observed that SNP administration improved these values. Dead sperm rate and abnormal sperm rate increased in rats treated with PbAc similarly in our study. It was interpreted that this situation might be caused by the deterioration of the seminiferous tubules and desquamization.

In conclusion, the results of this study determined that PbAc administration decreased sperm quality in male rats by causing oxidative stress, upregulating apoptosis, inflammation, endoplasmic reticulum stress. However, it was observed that SNP treatment significantly inhibited PbAc-induced male reproductive toxicity. Therefore, according to the results of our study, it was suggested that SNP is a protective alternative treatment method against PbAc-induced male reproductive toxicity.

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CONFLICT OF INTEREST STATEMENT

We would like to declare that the presented study is original research. Our work has not been published anywhere before. All listed authors wish to be considered for publication in "Tissue and Cell". The authors declare that there is no conflict of interest regarding this work.

CONSENT TO PARTICIPATE

This research does not involve human experiments.

CONSENT FOR PUBLICATION

All authors have read and approved the manuscript.

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