

Morinda citrifolia protective effects on paclitaxel-induced testis parenchyma toxicity: An experimental study

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ABSTRACT

The current study aimed to investigate the sensitivity of male testis parenchyma cells to chemotherapy agents and the protective effects and mechanisms of *Morinda citrifolia* (Noni) administration against structural and functional changes before and after chemotherapy (Paclitaxel (PTX)). For this purpose, rats were randomly assigned into four groups (Control = G1, PTX 5 mg/kg = G2; PTX + Noni 10 mg/kg = G3, PTX + Noni 20 mg/kg = G4). PTX was injected intraperitoneally for 4 consecutive weeks, at a dose of 5 mg/kg to all groups except the control group. Then noni was administered in 10 (G3) and 20 (G4) mg/kg groups orally (gavage) for 14 days. Biochemical analyses, Real-Time Polymerase Chain Reaction (PCR), and immunohistochemical analyses were performed. According to our results, Total Oxidative Stress (TOS) and Malondialdehyde (MDA) were significantly increased in the PTX group ($P < 0.01$). Superoxide Dismutase (SOD) enzyme activity and Total Antioxidant Capacity (TAC) levels were decreased ($P < 0.01$). The changes in the rats treated with PTX + Noni 20 mg/kg were noteworthy. The increased levels of IL1- β (Interleukin 1 beta) and TNF α (tumor necrosis factor-alpha) with PTX were down-regulated after treatment with PTX + Noni 20 mg/kg ($P < 0.01$) (9 % and 5 % respectively). In addition, Noni restored the testicular histopathological structure by reducing caspase-3 expression and significantly (61 %) suppressed oxidative DNA damage and apoptosis (by regulating the Bax (bcl-2-like protein 4)/Bcl-2 (B-cell lymphoma gene-2) ratio). In conclusion, Noni reduced cellular apoptosis and drastically changed Caspase 8 and Bax/Bcl-2 levels. Furthermore, it considerably decreases oxidative damage and can be used in testicular degeneration.

1. Introduction

Paclitaxel (PTX) is a widely used antineoplastic agent against various types of cancer. It is the first microtubule stabilizing agent identified in the taxane family [1,2]. It stops mitosis by stimulating the phosphorylation of Bcl-2 through microtubule stabilization [3], thereby inducing

cellular apoptosis [4]. PTX is an effective drug against cancers that are resistant to conventional chemotherapy such as endometrial cancer, malignant brain tumors, breast cancer, non-small cell lung cancer, and bladder cancer [5,6]. Despite its potent antitumor activities, PTX is toxic to many systems, including the reproductive system [2,7]. PTX can effectively penetrate all testis parenchyma cells in the testis and traverse

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the blood-testicular barrier within Sertoli cells [6]. PTX exerts a detrimental impact on parenchyma cells, resulting in the depletion of spermatozoa, degeneration of the testis parenchyma cell layer, and collapse of the testis parenchyma cell layer [8,9]. Many investigations have shown that the PTX injection generates oxidative stress, disturbing the tissue redox homeostasis. The exact mechanism of how it damages testicles is not fully understood [6,9–11]. Hypoxia-induced reactive oxygen species (ROS) production leads to DNA damage, endothelial damage, oxidative stress, apoptosis, and necrosis of testis parenchymal cells [12]. By increasing in the formation of ROS, (decreased antioxidants/oxidant ratio) plays a crucial role in inducing apoptosis and cellular oxidative damage [13,14]. Furthermore, ROS damages the mitochondrial membrane, promotes mitochondrial permeability, and causes of cytochrome C (Cyt-c) release from the mitochondria to the cytosol, initiating the caspase cascade and inducing the apoptotic process [15,16]. Death receptors on the cell membrane thereby trigger the extrinsic route, which then causes the successive activation of initiator caspases (caspases 8 or 2, 12), and effector caspases (caspases 3 and 7) [17]. Throughout testis parenchyma cell apoptosis, the testis initiates an active mechanism of apoptosis to control the damage, allowing for self-renewal of the seminiferous epithelium and normal spermatogenesis to continue.

Nevertheless, if the extent of damage becomes significantly severe, the seminiferous epithelium may experience irreversible harm. Additionally, numerous Leydig cells, and Sertoli cells that would typically not undergo the apoptotic process may succumb to cell death, ultimately leading to the cessation of spermatogenesis [18].

Natural antioxidants are increasingly being used to counteract the side effects of chemotherapeutic agents on the male reproductive system [19]. *Morinda citrifolia*, has been proven to exhibit many pharmacological features, including antioxidant, anti-inflammatory, and anti-tumor effects [20,21]. Malondialdehyde (MDA), a byproduct of lipid peroxidation, is a notable marker used to indicate increases in the production of free radicals in tissue [22]. Moreover, 8-hydroxy-2-deoxyguanosine (8-OHdG) identifies DNA damage resulting from oxidative stress [23].

Nevertheless, antioxidants such as Noni increase superoxide dismutase (SOD) and Glutathione (GSH), so it acts by chelating superoxide and other peroxides [24,25]. Studies have also indicated that Noni possesses anti-inflammatory activity by stimulating the release of specific cytokines such as *TNF- α* , *IL-1 β* , and *IL-10* [26,27]. In addition, the phytochemicals in Noni increase the immune response and suppress inflammation, playing a role in regulating the *Bax/Bcl-2* ratio [28]. Flavonoids, coumarins, anthraquinones, iridoids, and polysaccharides are the primary ingredients of Noni [29,30]. Based on research findings, Scopoletin is acknowledged as the primary component of Noni (*Morinda citrifolia* L), which contributes to its antioxidant and other therapeutic properties [30]. However, further investigation is required to ascertain the precise mechanisms by which Noni safeguards testicular tissue against the oxidative stress and apoptosis triggered by paclitaxel. Consequently, the present study was conducted with the following objectives: 1) to assess the protective effects of Noni on the susceptibility of male testis parenchyma cells to Paclitaxel, 2) to compare the differential impact of Noni administration before and after chemotherapy, and 3) to explore whether Noni's mechanism of action is linked to apoptotic, inflammatory, or oxidative stress markers.

2. Material methods

2.1. Animals

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Ataturk University, Erzurum, Turkey (protocol code no. E-42190979-000-2200327979). Sprague Dawley male rats (4 groups, $n = 6$, 24 rats in total) were obtained from the Medical Experimental Application and Research Center (Erzurum, Turkey). The rats' body weight was

250 ± 10 g. Throughout the research, the rats were kept in a controlled environment with a 12-hour light/dark cycle, 22 ± 3 °C and 55 ± 5 % humidity. All experimental methodologies were carried out in accordance with the instructions of Ataturk University Medical Experimental Application and Research Center (Turkey, Erzurum).

2.2. Experimental groups

Rats were randomly assigned into four groups, Group 1 (G1 = control); Group 2 (PTX = G2): 5 mg/kg; Group 3 (PTX + Noni 10 mg/kg = G3) [31]; and Group 4 (PTX + Noni 20 mg/kg = G4) [31–34]. Nono fruit extract capsule (1000 mg) was purchased from Full Life Reuma-Art® (New York, USA). The 10 and 20 mg/kg doses were dissolved in distilled water and administered by oral gavage. Paclitaxel taxol® was purchased from Mediexa Pharma, Mumbai, India. PTX was injected intraperitoneally (4 consecutive weeks) at a dose of 5 mg/kg diluted in 1 ml of normal saline to all experimental rats except the control ones [35]. Then, for 14 days, Groups 3 and 4 were treated orally with Noni (iherb; NOW Foods, Product Code: NOW-04712, USA). At the end of the experiment, the rats were anesthetized with ketamine and xylazine (90/10 mg/kg intramuscularly (i.m.)) and sacrificed [36].

Testicular tissues were kept at -80 °C degrees to be analyzed biochemically and molecularly (in RNA stabilization reagent). Testicular tissues were preserved for histological and immunohistochemical analyses in 10 % buffered formalin solution (NBF) [11,18].

2.3. Biochemical analysis

Testicular tissues were ground in liquid nitrogen using the Tissue-lyser II instrument (Qiagen, Germany). Then, 1 ml of phosphate-buffered saline (PBS) and 50 mg of pulverized tissue were homogenized and centrifuged at $5000 \times g$ for 5 minutes to obtain the supernatant. The supernatants were collected and used for analysis. Total antioxidant capacity (TAC) (Elabscience, United States, Cat no. E-BC-K801-M), Total oxidant status (TOS) (Elabscience, United States, Cat no. E-BC-K802-M), Lactate Dehydrogenase (LDH) (Elabscience, United States, Cat no. E-EL-H0843), Glutathione (GSH) (Elabscience, United States, Cat no. EN-E-EL-0026), SOD, 8-Hydroxyguanosine (8-OHDG) (Elabscience, United States, Cat no. EN-E-EL-0028), and Malondialdehyde (MDA) levels (Elabscience, United States, Cat no. E-BC-K801-M) were assessed using the according to the manufacturer's manual. The optical densities of each sample were measured (Multiskan™ GO Microplate Spectrophotometer reader Thermo Scientific, Canada) at 450 nm [37,38].

2.4. Molecular analysis

2.4.1. Real-time qPCR for testicular tissue

Testicular tissue samples were subsequently treated with RNAlater Solutions for RNA Stabilization and Storage (RNAlater™, Thermo Fisher Scientific - USA) and then stored at -80 °C. RNA extraction was carried out using the Light Cycler® (Roche, Germany). The High Pure RNA Isolation Kit (Roche; Mannheim, Germany) was used to isolate total RNA, and the RNA samples were stored at -80 °C. The RNA samples were subsequently transformed into cDNA using the High-Capacity complementary DNA (cDNA) Reverse Transcription Kit (Roche; Mannheim, Germany, 5081955001). The concentration of the obtained cDNA samples was measured by nanodrop spectrophotometry (Qubit 4 Fluorimeter, Thermo Fisher Scientific – USA) [39].

The Polymerase Chain Reaction (PCR) was performed using Rotor-Gene Q (QIAGEN) and specific primers. Their sequences are listed in Table 1 (Roche; Darmstadt, Germany). The results are expressed as relative changes in mRNA expression (fold-changes), using Beta actin as a housekeeping gene. We normalized the mRNA expression of the target genes to beta-actin using the $2^{-\Delta\Delta Ct}$ method [39,40].

Table 1
Specific primers forward and reverse.

Gene	Forward	Reverse	LENGTH and Recycle Temp
β actin:	5'-CCAACCGCGAGAAGATGA-3'	5'-CCAGAGGCGTACAGGGATAG-3'	1866 bp/62 °C
Bcl-2:	5'-GAGTACCTGAACCGGCATCT-3'	5'-GAAATCAAACAGAGGTCGCA-3'	241 bp/60 °C
Bax	5'-TTGCTACAGGGTTTCATC CA-3'	5'-GAGTACCTGAACCGGCATCT-3'	538 bp/60 °C
IL-1 β :	5'-CTCAATGGACAGAACATAAGCC-3'	5'-GGTGTGCCGTCTTTCATCA-3'	116 bp/58 °C
Caspase 8:	5'-CGGGATCCGCCATGGACTTCAGCAGAAATC-3'	5'-TCCCCCGGGCACCATCAATCAGAAGGG-3'	815 bp/60 °C
TGF- β :	5'-AAGTGGATCCACGCGCCAAGG-3'	5'-GCAGGAGCGCACGATCATGTTGGAC-3'	299 bp/60 °C

2.5. Histopathological examination

After performing regular tissue follow-up procedures, testicular tissue samples were collected at the end of the examination and fixed in paraffin blocks. Sections with a thickness of 4 μ m were produced from each block. The preparations for histopathological analysis were stained with Hematoxylin-Eosin (HE) and analyzed under a light microscope (Olympus BX 51, JAPAN). A semiquantitative scoring system was used for the histopathological evaluation as follows: -, no staining; +, mild staining; ++, moderate staining; +++, strong staining [41].

2.6. Immunohistochemical examination

All sections were mounted on adhesive (poly-L-Lysine coated) slides. Prior to the immunoperoxidase stage, the slides underwent a series of xylene and alcohol treatments. The primary antibody (Caspase 3 Cat No: sc-56053, Dilution Ratio: 1/100, US) was applied to the tissues (present on the slides) and incubated as per the manufacturer's instructions of the primer (Abcam HRP/DAB Detection IHC kit). 3-3' Diaminobenzidine (DAB) was utilized as the chromogen. Hematoxylin was employed for counterstaining. The stained sections were observed using a light microscope (Zeiss AXIO, Germany). For quantitative analysis of immunohistochemistry (IHC) and immunofluorescence staining, the average immunoreactivity intensity was determined using ImageJ software (ImageJ). Following color deconvolution, the images were inverted, and the average pixel density was measured using ImageJ. Five areas from each group were assessed [42,43].

2.7. Statistical analysis

All data were displayed as means \pm standard deviation (mean \pm SD). After conducting a one-way analysis of variance (one-way ANOVA), Tukey's least significant difference (LSD) test was employed to evaluate

the results. The statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA). P- Value $p < 0.05$ and $p < 0.001$ were considered indicative of statistical significance. The Kruskal-Wallis method was utilized to assess the density of immunoreactivity among nonparametric groups.

3. Results

3.1. Biochemical analysis

3.1.1. Redox status in testicular tissue of rats treated with Noni after PTX toxicity

Oxidative stress was investigated with TOS, TAC, MDA, 8-OHdG, and SOD. Our study found the lowest TOS levels in the control group (9.65 ± 1.81). The TOS level was determined to be 21.93 ± 1.08 in the PTX group ($P < 0.001$). The TOS levels in the PTX group were substantially higher than those in the control group. Oxidative damage induced by PTX was found to be decreased in the Noni groups ($P < 0.05$ and $P < 0.001$, respectively). The Noni 20 mg/kg group showed a decrease in oxidative stress and was almost close to the control group ($P < 0.001$) (Fig. 1a).

The control group was found to have the highest TAC value, in contrast to the TOS level. Despite the decrease in TAC level in the PTX group (18.87 ± 1.98 , $P < 0.001$), there was a significant increase in TAC level at the maximum dose of Noni 20 mg/kg (Fig. 1b).

Similar to the TOS assay result, MDA and 8-OHdG levels dramatically increased in the PTX 5 mg/kg group ($P < 0.001$). The MDA level in this group was determined to be 297.82 ± 3.48 . However, the Noni 20 mg/kg group showed a reduction of approximately two times, with a value of 190.69 ± 9.19 ($P < 0.001$) (Fig. 2a). The amount of 8-OHdG, which was found to be high in the PTX group and was reversed by Noni, decreased in a dose-dependent manner, similar to the MDA and TOS levels ($P < 0.05$; $P < 0.01$) (Fig. 2b).

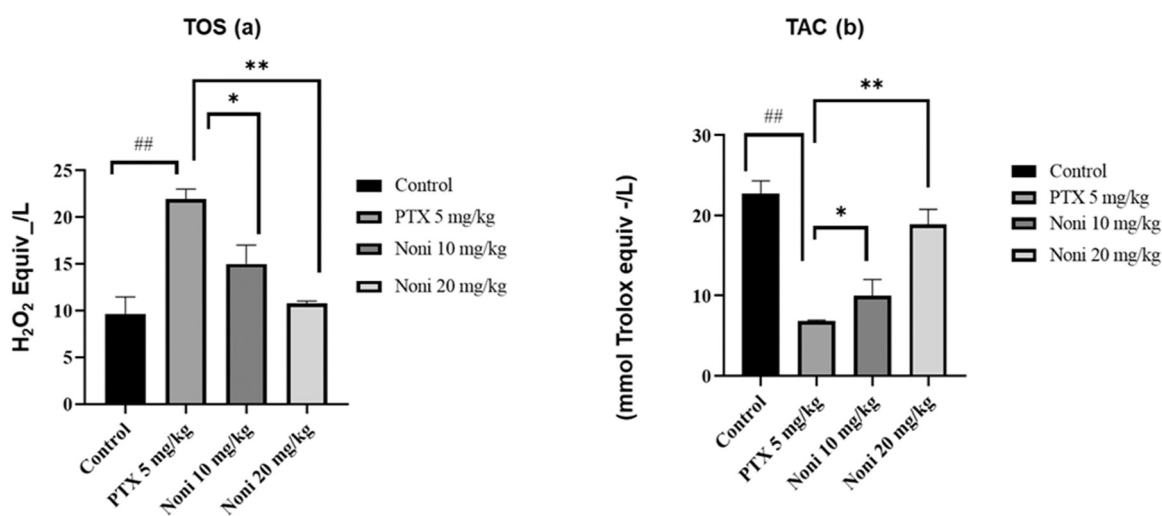


Fig. 1. The effect of Noni on testicular tissue's TOS (a) and TAC (b) (n = 6). Rats were exposed to PTX 5 mg/kg and treated with Noni 10 mg/kg and 20 mg/kg. The outcomes are the means of three different experiments. Statistical significance: ## $P < 0.001$ compared to the control group. * $P < 0.05$; ** $P < 0.001$ compared to the PTX.

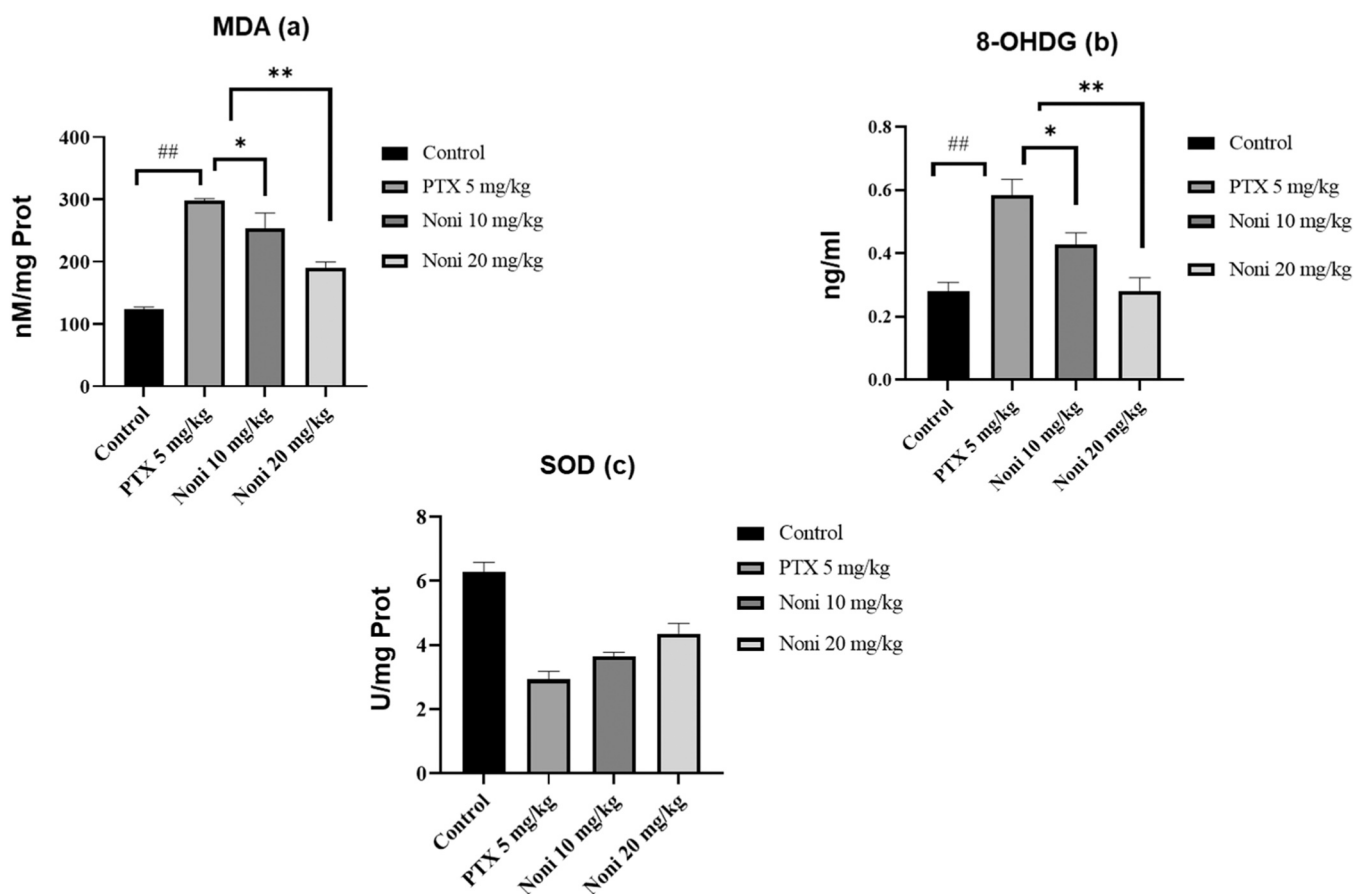


Fig. 2. The effect of Noni on testicular tissue’s MDA (a), 8-OHDG (b), and SOD © (n = 6). Rats were exposed to PTX 5 mg/kg and treated with Noni 10 mg/kg and 20 mg/kg. The outcomes are the means of three different experiments. Statistical significance: ## P < 0.001 compared to the control group. * P < 0.05; ** P < 0.001 compared to the PTX.

A tendency toward an increase of SOD activity was seen in both Noni groups compared to PTX, however, these differences were not statistically significant (p > 0.05) (Fig. 2C). As a result of these observations, it has been established that Noni can reduce the damage caused by free radicals induced by PTX and protect cells from free radicals by enhancing their antioxidant activity.

3.1.2. Noni treatment increase GSH and decrease LDH levels in testicular tissue in a PTX toxicity model of rats

In the PTX group, the levels of GSH in testicular tissues dramatically dropped to 15.71±5.98 mM/mg compared with the control group (55.80±2.12 mM/mg) (P < 0.001). The GSH level was considerably higher in the Noni (46.23±2.71 mM/mg) compared to the positive

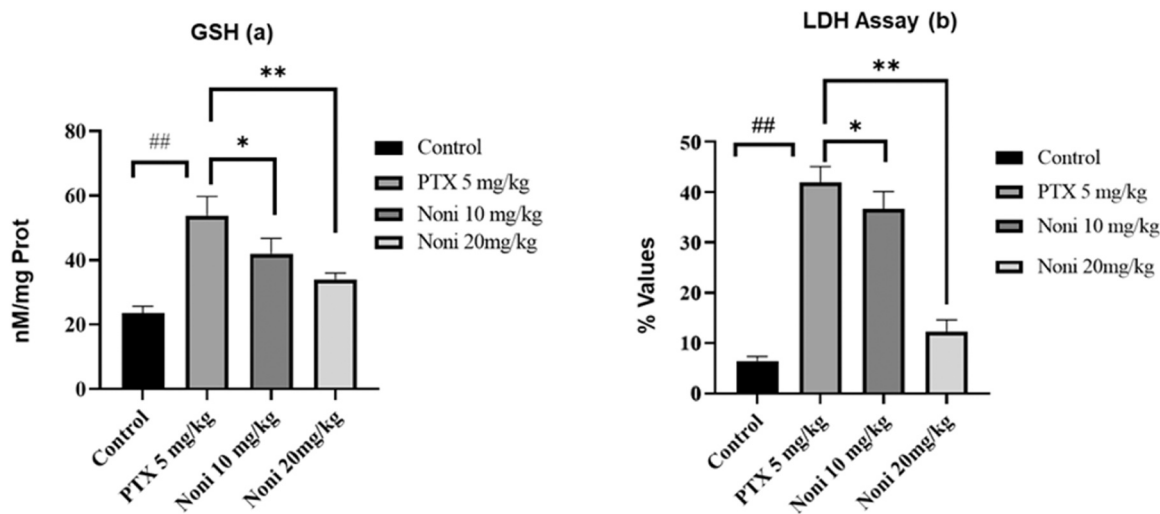


Fig. 3. The effect of Noni on testicular tissue’s GSH and LDH (n = 6). Rats were exposed to PTX 5 mg/kg and treated with Noni 10 mg/kg and 20 mg/kg. The outcomes are the means of three different experiments. Statistical significance: ## P < 0.01 compared to the Control group. * P < 0.05; ** P < 0.01 compared to the PTX.

control groups (PTX) ($P < 0.05$; $P < 0.001$) (Fig. 3a).

The group with the highest LDH level was determined to be the PTX group, in contrast to the GSH value ($P < 0.001$). The oxidative stress triggered by the administration of PTX in this group enhanced cellular cytotoxicity and forced the cells into apoptosis. Following PTX administration, Noni treatment significantly reduced LDH levels (%) and protected the cells from cytotoxicity. The LDH level of PTX groups was compared to the control group ($p < 0.001$). The treatments LDH levels were compared to the PTX group. PTX group rose LDH to 45.59 % (up to 4 fold) in comparison to the control group. Noni 10 and 20 mg/kg decreased LDH levels in comparison to the PTX group ($p < 0.05$ and $p < 0.001$, respectively). The highest decrease was seen in Noni 20 mg/kg up to 30.84 % in comparison to the PTX group ($p < 0.001$) (Fig. 3b).

3.2. Real-time PCR results

3.2.1. IL-1 β , TGF- β and Caspase 8 levels in testicular tissue

IL-1 β is an essential mediator of the inflammatory response, implicated in several biological processes, including cell proliferation, differentiation, and cell death [44,45]. The expression of IL-1 β was found upregulated by nearly 2-fold ($P < 0.001$) in the PTX group compared to the control, whilst both 10 and 20 mg/kg Noni decreased its expression compared to PTX (Fig. 4A, $P < 0.05$; $P < 0.001$, respectively). Similarly, TGF- β mRNA level increased by nearly 2.5-fold in the PTX group compared to control ($P < 0.001$), and oral supplementation with both Noni doses caused a significant reduction in testicular TGF- β expression (Fig. 4B, $P < 0.05$; $P < 0.001$, respectively).

Cellular Apoptosis increased in conjunction with increased inflammation. Caspase-8 level decreased after Noni treatments, with 20 mg/kg dose inducing the most remarkable effect (Fig. 4c) ($P < 0.001$).

3.2.2. Bax, BCL-2 and Bax/BCL-2 ratio levels in testicular tissue

The PTX group's Bax level increased due to an increase in caspase 8 activity, which caused the testicular tissue to undergo apoptosis. When

compared with the control group, there was a noticeable rise in the PTX group. Even though Noni considerably decreased the Bax level at low concentrations (10 mg/kg) ($P < 0.05$), the Noni 20 mg/kg group had a much higher reduction ($P < 0.001$) (Fig. 5a).

Contrarily, the level of Bcl-2 expression decreased by almost 50 % in the PTX group compared to control ($P < 0.05$). After the treatment, the Bcl-2 level in the Noni groups increased and got close to the control level (Fig. 5b). We found a nearly 3.5-fold increase in the Bax/Bcl-2 ratio in the PTX group compared to Control, indicative of a very high cellular damage. However, 20 mg/kg dose Noni administration restored this ratio to the control level, thus preventing apoptotic cell death (Fig. 5c).

3.3. Histopathologic analysis

Upon histopathological examination of testicular tissues, the control group exhibited a normal histological appearance (Fig. 6, Table 2). Conversely, in the PTX group, severe manifestations of tubular wall thinning, spermatocyte destruction and degeneration, intratubular edema, and prominent hyperemia in the veins were observed (Fig. 6, Table 1). In contrast to the damage observed in the PTX group, the Noni 10 mg/kg group exhibited moderate tubular wall thinning, spermatocyte degeneration, mild necrosis, moderate intratubular edema, and prominent hyperemia in the vessels (Fig. 6, Table 2). Furthermore, in the Noni 20 mg/kg group, a significant reduction in testicular tissue damage was noted.

3.4. Immunohistochemical findings

Immunohistochemical analysis of testicular tissues revealed negative Caspase 3 (Protein) levels in the control group (Fig. 7). In contrast, the PTX group exhibited severe cytoplasmic Caspase 3 (Protein) levels in spermatocytes (Fig. 7), indicating a significant increase in caspase 3 activity and highlighting the extent of immunohistochemical damage. Noni administration mitigated the PTX-induced damage associated with

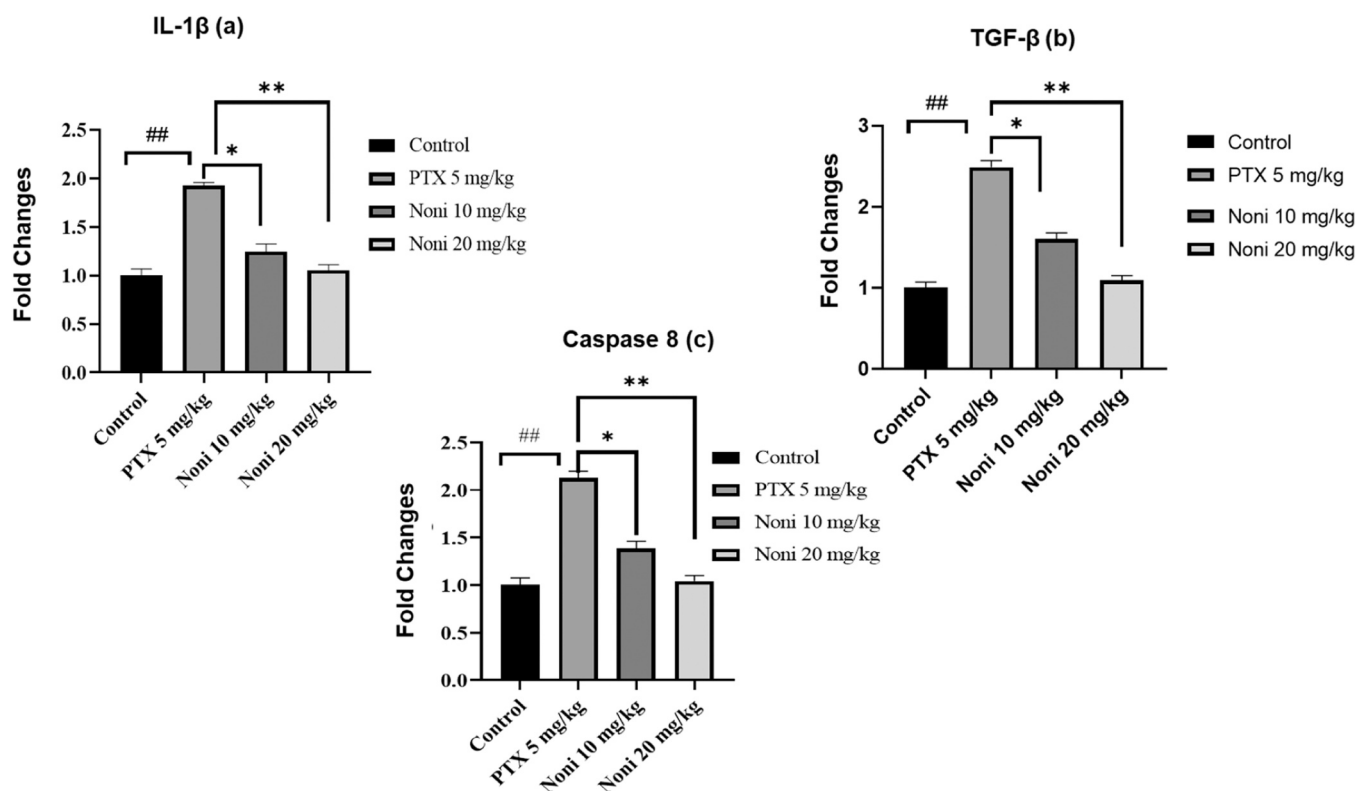


Fig. 4. Noni improves PTX-induced testicular inflammation (a), testicular mRNA expression levels of IL-1 β (b), TGF- β , and (c), Caspase 8. The outcomes are the means of three different experiments. Statistical significance: ## $P < 0.001$ compared to the Control group. * $P < 0.05$; ** $P < 0.001$ compared to the PTX.

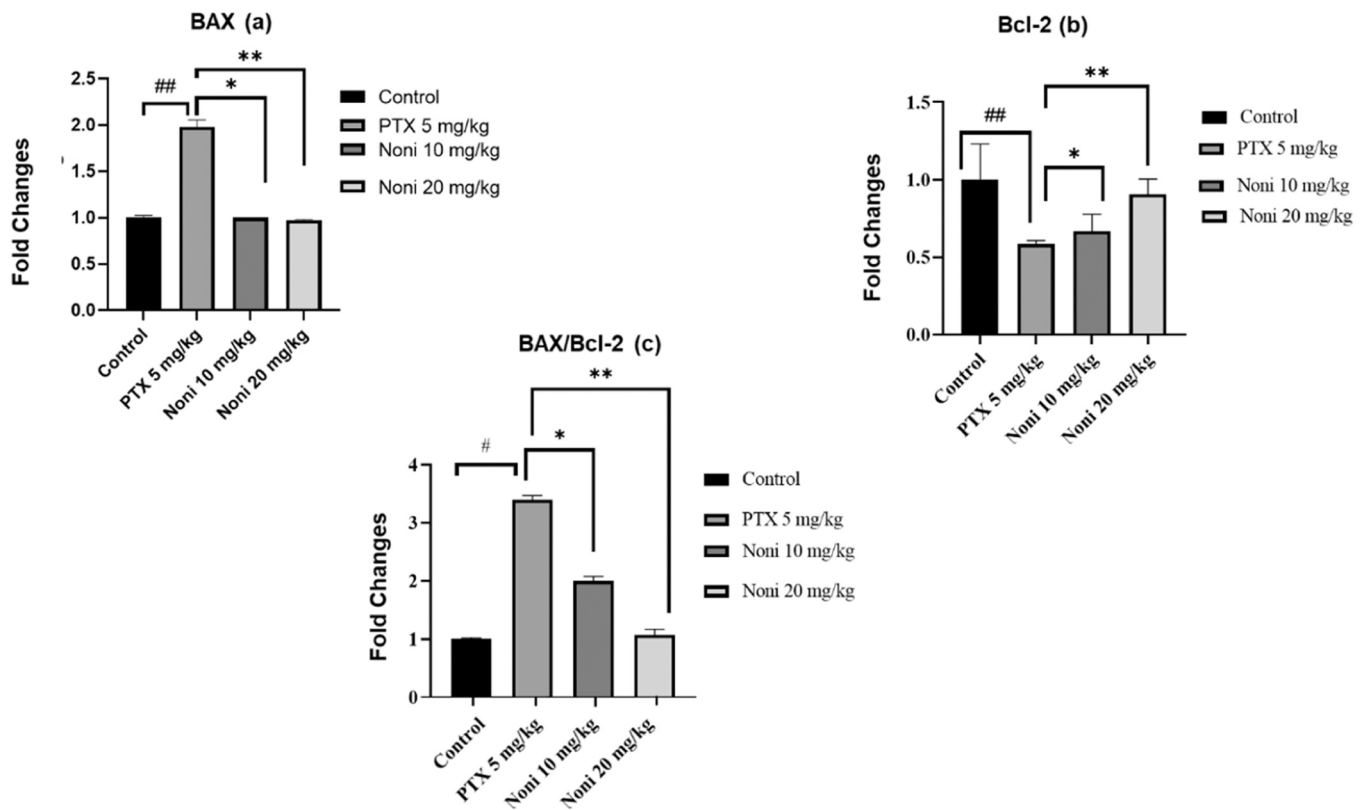


Fig. 5. Noni improves PTX-induced testicular damage (a), testicular mRNA expression levels of Bax (b), Bcl-2, and (c), Bax/Bcl-2. The outcomes are the means of three different experiments. Statistical significance: ## $P < 0.001$ compared to the Control group. * $P < 0.05$; ** $P < 0.001$ compared to the PTX.

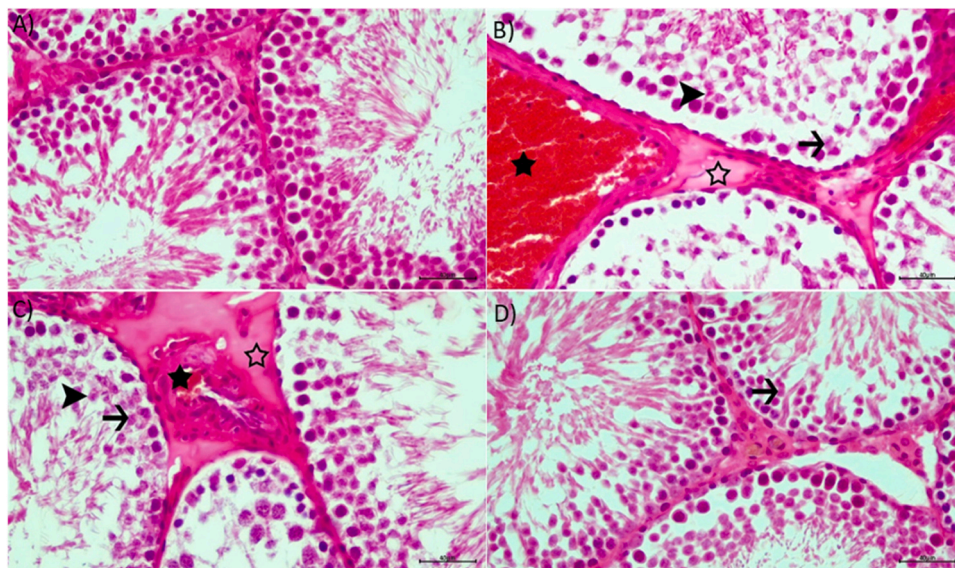


Fig. 6. Testicular tissue, Control, PTX, Noni 10 mg/kg, and Noni 20 mg/kg (A, B, C, D) show spermatocytes degeneration (indicated by an arrow) and necrosis (indicated by an arrowhead), edema in intertubular areas (indicated by an empty star), and hyperemia in veins (indicated by a star). The staining method used is Hematoxylin and Eosin (H&E), and the scale bar represents 40 μm .

Caspase 3 activity, as shown by the moderate intracytoplasmic Caspase 3 level observed in spermatocytes in the Noni 10 mg/kg group (Fig. 7). Notably, the Noni 20 mg/kg group exhibited a substantial elimination of this toxic effect. In the examination, mild intracytoplasmic Caspase 3 (Protein) levels were observed in the spermatocytes of this group (Fig. 7). A statistically significant difference ($P < 0.05$) was detected when comparing all treated groups (PTX alone or with Noni) vs control

group (Table 3).

4. Discussion

In our study, the PTX group exhibited a significant increase in 8-oHdG levels, indicating DNA damage. Antioxidants possess the capability to counteract the DNA damage caused by 8-oHdG. Our findings

Table 2
Testicular tissue, histopathological findings, and scoring.

Treatment	Control	PTX	Noni 10 (mg/kg)	Noni 20 (mg/kg)
Thinning of the tubular wall	-	+++	++	+
Degeneration of spermatocytes	-	+++	++	+
In spermatocytes necrosis	-	+++	+	-
Edema	-	+++	++	+
Hyperemia	-	+++	+++	+

Legend: -, no staining; +, mild staining; ++, moderate staining; +++, strong staining.

revealed that Noni exhibited potent antioxidant properties, effectively safeguarding cells against PTX-induced DNA damage. In the study conducted by Ghafuri-fard et al. (2021) Effects of chemotherapeutic agents on male germ cells and the possible ameliorating impact of antioxidants were evaluated. They show cisplatin, doxorubicin, paclitaxel, 5-fluorouracil, and cyclophosphamide have toxicity to testicular tissue. In addition, they show antioxidants have the potential to recover oxidative damage [46]. In correlation with our study, Wang et al. (2022) showed that PTX induced damage in a testicular structure, and significantly affected germ cell proliferation, meiosis, and subsequent sperm function. The damage induced apoptosis and necrosis to testis tissue. In addition, melatonin in this study, an efficient antioxidant, showed significant protection against oxidative damage caused by PTX [47]. In another study, Hesperidin, an antioxidant, showed a protective effect on PTX-induced damage by correcting the deterioration in Apoptosis marker (BAX, BCL2), NF- κ B, IL-1 β , and TNF- α levels [48].

Lajos Pusztai et al. (2004) showed that PTX administration increased the release of IL-1 β and TNF α in cancer patients in comparison to healthy people [47]. A. Mielgo et al. (2009) shows Paclitaxel promotes a caspase 8-mediated apoptosis through death effector domain association with microtubules in primer and cancer cell lines [48]. Caspase-8 is an initiator of death receptor-induced apoptosis. In addition, caspase-8 is known to be crucially involved in the inflammatory response [49]. In the PTX control group by inducing inflammation, in correlation, our result shows an increase in IL-1 β , TGF- β , and caspase 8 levels.

Treatment with Noni (phenolic properties) decreased inflammation and in contrast IL-1 β , TGF- β , and caspase 8 levels. The major decrease

was seen in noni 20 mg/kg ($p < 0.001$).

It is believed that the significant increase in 8-OHdG levels observed in rats given PTX results from oxidative stress caused by PTX, which causes 8-OHdG to enter the DNA chain. Additionally, reactive aldehydes such as MDA, which can damage DNA and produce exocyclic DNA products, can be produced due to peroxidized phospholipids caused by ROS [49]. SOD, an antioxidant enzyme expressed in the testicles, plays a crucial role in inhibiting the oxidation chain by scavenging primary free radicals. By controlling ROS levels, SOD limits their potential toxicity [42,50]. Previous studies have shown that chemotherapeutic drugs decrease SOD activity in testicular tissue, but this can be counteracted by administering antioxidant-containing substances [51]. In this study, it was observed that Noni substantially elevated the levels of superoxide dismutase (SOD) compared to the decreased SOD levels induced by paclitaxel. The significant increase in SOD levels played a crucial role in protecting testicular tissue against the toxic effects of PTX. In addition, many studies revealed that TOS levels in testicular tissue increased in toxicity groups [42,43,52]. In our investigation, paclitaxel caused an increase in TOS levels. However, Noni significantly decreased these levels after administration of 20 mg/ml. This result shows that a high dose of Noni (20 mg/ml) protects against paclitaxel-induced oxidative damage. This investigation also discovered that high-dose Noni considerably raised the TAC level, whereas paclitaxel drastically lowered it.

Another mechanism suggested to be involved in paclitaxel-induced testicular toxicity is the activation of the apoptotic pathway [53].

Intracellular accumulation of reactive oxygen species (ROS) can trigger the release of cytochrome c from the mitochondria's inner membrane. Subsequently, cytochrome c participates in the formation of the apoptosome, a protein complex that activates pro-caspase 9.

Table 3
Analysis of data and statistical findings from immunohistochemical studies.

Treatment	Caspase 3 expression levels
Control	19.26 \pm 0.31 ^a
PTX	82.93 \pm 1.29 ^b
Noni 10 mg/kg	60 \pm 1.13 ^c
Noni 20 mg/kg	37.52 \pm 0.89 ^d

Legend: Different letters (a, b, c, d) in the same column represent statistical differences in comparison with the control group ($P < 0.05$).

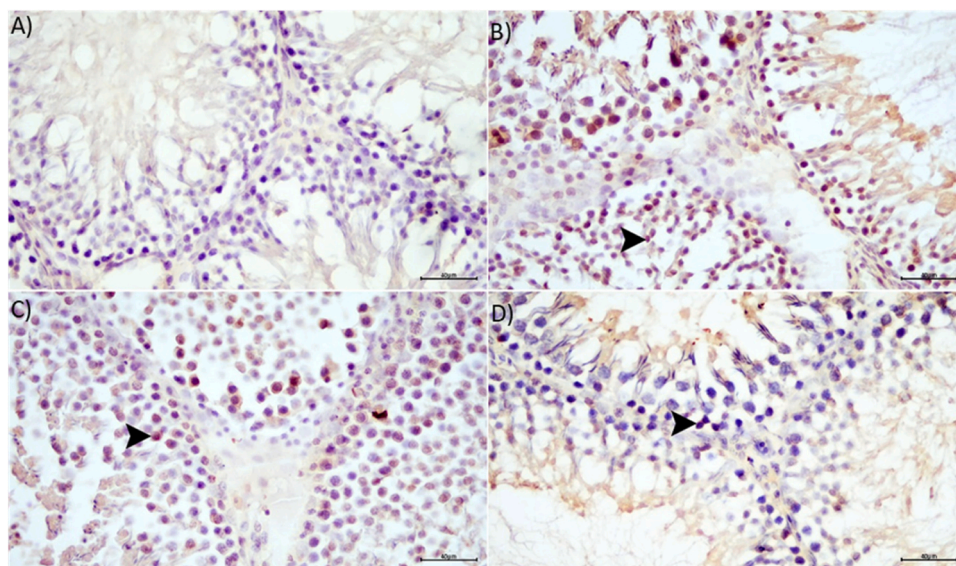


Fig. 7. Testicular tissue, Control, PTX, Noni 10 mg/kg, and Noni 20 mg/kg (A, B, C, D) show intracytoplasmic Caspase 3 protein levels in spermatocytes (indicated by an arrowhead). The staining method used is Immunohistochemistry-Paraffin (IHC-P), and the scale bar represents 40 μ m.

Additionally, it can activate caspase-3 and caspase-7, which are classified as effector caspases [54]. This sequential process leads to the initiation of apoptosis. The internal apoptotic pathway is directly regulated by two groups of proteins: pro-apoptotic proteins (Bax, Bid, Bad) and nitric oxide molecules [55]. These molecules and genes activate the cytochrome c release pathway from the mitochondrial inner membrane. Another controlling protein of the intracellular apoptotic pathway is Bcl-2 and Bcl-XL, known as the anti-apoptotic group, which inhibit cytochrome c release [49,56].

Limitations of the study: This study has some limitations; in particular, (i) sex hormones were not evaluated, (ii) phytochemical analysis of Noni was not performed, which could help to assess Noni's antioxidant activity, and (iii) sperm parameters were not measured.

5. Conclusion

Our study's findings showed that Noni significantly reduced the PTX-induced inflammation. Furthermore, it was found that Noni considerably altered the levels of Caspase 8 and Bax/Bcl-2, inhibiting cellular apoptosis. However, it dramatically decreased oxidative damage. A limitation of our study is the lack of specific analysis conducted on sperm motility, mitochondrial dysfunction, and sperm acrosome. In future studies, our research team plans to investigate the effects of Noni on these parameters.

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Authors' contributions

Sidika Genc, Mehmet Kuzucu, Ahmet Hacimuftuoglu, Ismail Bolat, and Serkan Yildirim substantially contributed to the design of this study. Sidika Genc, Yesim Yeni, Betul Cicek and Himasadat Zaker carried out laboratory work. Athanasios Zachariou, Nikolaos Sofikitis, and Ali Taghizadehghalehjoughi collected the data. Charalampos Mamoulakis, Aristidis Tsatsakis, and Ali Taghizadehghalehjoughi analyzed and interpreted data. Sidika Genc, Betul Cicek, and Himasadat Zaker mainly drafted the manuscript. Charalampos Mamoulakis, Aristidis Tsatsakis, and Ali Taghizadehghalehjoughi and Nikolaos Sofikitis critically revised. All authors agreed to be authors and approved the final manuscript.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Ataturk University, Erzurum, Turkey (protocol code no. E-42190979-000-2200327979).

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Not applicable.

CRedit authorship contribution statement

Mehmet Kuzucu: Writing – original draft, Methodology, Investigation, Formal analysis. **Ahmet Hacimuftuoglu:** Writing – original draft, Validation, Software, Methodology. **Ali Taghizadehghalehjoughi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Betul Cicek:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Yesim Yeni:** Writing – original draft, Methodology, Formal analysis, Data curation. **Charalampos Mamoulakis:** Writing – original draft, Supervision, Data curation. **Aristidis Tsatsakis:** Writing – original draft, Visualization, Supervision. **Sidika Genc:** Writing – original draft, Formal analysis, Data curation. **Nikolaos Sofikitis:** Writing – original draft, Visualization, Investigation, Formal analysis. **Himasadat Zaker:** Writing – original draft, Visualization, Methodology. **Athanasios Zachariou:** Writing – original draft, Visualization, Methodology. **Ismail Bolat:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Serkan Yildirim:** Writing – original draft, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Not applicable.

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