

## Protective Effect of *Morinda citrifolia* (Noni) on 3-methyl-4-nitrophenol-induced Injury in Rat Testes<sup>[1]</sup>

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<sup>[1]</sup> This study was supported by "Research Fund of Istanbul University with the grant number BAP-33141"

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Article Code: KVFD-2016-15163 Received: 04.02.2016 Accepted: 01.04.2016 Published Online: 01.04.2016

### Abstract

In this study, the protective effect of *Morinda citrifolia* (Noni) against 3-methyl-4-nitrophenol (PNMC) toxicity in rat testes was investigated with an experimental period of five days. Fifty-six adult male Sprague-Dawley rats were allocated into seven experimental groups and a control (n:7). One group received only Noni. Testicular tissue injury of six experimental groups was induced by subcutaneous injection of PNMC at three different doses (1, 10 and 100 mg/kg) and three received Noni treatment (2 ml per rat by gavage). On day six all rats were sacrificed and then blood samples and testis tissues were collected. Serum testosterone, FSH and LH levels were assessed. Testicular tissues were evaluated histomorphometrically in terms of tubular diameter, seminiferous epithelium density, luminal space and interstitial tissue and immunohistochemically labelled with inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) markers to assess oxidative damage. Histomorphometrically, most severe tissue injury was observed in the group of 10 mg/kg PNMC. Tissue injury improved significantly in its corresponding treatment group (with Noni). iNOS and eNOS levels increased in all PNMC groups and decreased with Noni treatments. Noni was most effective in the group of 100mg/kg PNMC in terms of oxidative damage. Serum hormone levels revealed no significant results. In conclusion, Noni reduced PNMC-induced tissue injury in rat testes.

**Keywords:** 3-methyl-4-nitrophenol, Rat, Noni, Testes, iNOS, eNOS

## Sıçan Testisinde 3-Metil-4-Nitrofenol İle Oluşturulan Hasara Karşı *Morinda citrifolia* (Noni)'nin Koruyucu Etkisi

### Özet

Bu çalışmada beş günlük deneysel bir periyotla *Morinda citrifolia* (Noni)'nin sıçan testislerinde 3-metil 4-nitrofenol (PNMC) toksitesine karşı koruyucu etkisi araştırıldı. Elli altı adet yetişkin erkek Sprague-Dawley sıçanı eşit sayıda yedi deneysel, bir kontrol grubuna ayrıldı (n:7). Bir gruba sadece Noni uygulandı. Altı deneysel grupta testiste doku hasarı farklı dozlarda (1, 10 and 100 mg/kg) subkutan PNMC enjeksiyonu ile indüklendi ve üç gruba Noni tedavisi uygulandı (her sıçan için 2ml gavaj ile). Altıncı günde tüm sıçanlar sakrifiye edilerek kan ve testis doku örnekleri toplandı. Serum testosteron, FSH ve LH seviyeleri değerlendirildi. Testis dokuları, tubuler çap, seminifer epitel yoğunluğu, lumen aralığı ve interstisyel doku açısından histomorfometrik olarak incelendi ve oksitativ hasarı değerlendirmek üzere indüklenebilir nitrik oksit sentaz (iNOS) ve endotelial nitrik oksit sentaz (eNOS) belirteçleri ile immunohistokimyasal olarak işaretlendi. Histomorfometrik olarak en şiddetli doku hasarı 10 mg/kg'lık PNMC grubunda gözlemlendi. Doku hasarı bu gruba karşılık gelen tedavi (Noni ile) grubunda anlamlı ölçüde iyileşti. iNOS ve eNOS düzeyleri tüm PNMC gruplarında yükseldi ve Noni tedavisi ile aynı değerler düşüş gösterdi. Noni'nin oksidatif hasar bakımından en çok 100mg/kg'lık PNMC grubunda etkin olduğu izlendi. Serum hormon değerleri anlamlı sonuçlar vermedi. Sonuç olarak, Noni, sıçan testislerinde PNMC ile indüklenecek doku hasarını azalttı.

**Anahtar sözcükler:** 3-metil 4-nitrofenol, Sıçan, Noni, Testis, iNOS, eNOS



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## INTRODUCTION

Nowadays, accelerating air pollution due to industrialization has become a serious threat for environmental health. With the increasing use of motor vehicles, diesel exhaust particles (DEP) found in motor vehicle emissions have posed a worldwide health-threatening problem <sup>[1]</sup>.

Miscellaneous compounds found in DEP are among endocrine impairing chemicals, which exhibit estrogenic and anti-androgenic activities <sup>[2-5]</sup>. DEP compounds were reported to have adverse effects on reproductive system and impair both reproductive and endocrine functions in male mice and rats <sup>[6,7]</sup>. Watanabe and Kurita <sup>[8]</sup> indicated a decrease in the Sertoli cell count of rats, which were exposed to diesel exhaust in the fetal period while testosterone levels of the animals were found to have increased.

3-methyl-4-nitrophenol (PNMC) is a nitrophenol derivative of DEP and an important metabolite of fenitrothion, which is a widely used organophosphate insecticide in agriculture. This pesticide, however, was shown to have accumulated in water, air and soil <sup>[9,10]</sup>, which inevitably has become a growing health threatening problem for all living things <sup>[11,12]</sup>.

Nitric oxide (NO) is a free radical which is synthesized from L-arginin by nitric oxide synthase (NOS). There are 3 isoforms of NOS: inducible (i) NOS, endothelial (e) NOS and neuronal (n) NOS. They are found at high levels in tissues during inflammation <sup>[13]</sup>. NO is known to exhibit cytotoxic effects since it interacts with superoxide which results in tissue damage <sup>[14]</sup>. In previous experimental studies NO was shown to induce testicular tissue damage, as well <sup>[15]</sup>. In a study it was reported that NO exerted an inhibitory effect on testicular steroidogenesis. Although NO was reported to have shown preventive effect on testicular steroidogenesis, the specific site of action or its mechanism of action was not elucidated. Nonetheless, NO expression at high levels reduced testosterone production in testis <sup>[16]</sup>.

*Morinda citrifolia* L. (Rubiaceae), which is an endemic and widespread plant species in the Pacific and tropical regions of Asia, is commonly named as Noni <sup>[17]</sup>. The fruit itself and its juice have been used in conventional medicine for prophylactic purposes and for the cure of miscellaneous diseases for many years <sup>[18]</sup>. Noni contains a number of compounds and enzymes which show antioxidant activity by supporting cellular functions <sup>[19,20]</sup>.

Since there are limited numbers of studies with respect to utilizing antioxidants against PNMC-associated tissue injury, we designed this experimental model, in which tissue damage was induced in rat testes with PNMC, to investigate the putative protective effect of Noni owing to its antioxidant properties, by means of histomorphometric and immunohistochemical methods.

## MATERIAL and METHODS

### Animals

Fifty-six sexually-mature Sprague-Dawley male rats were purchased from the Institute of Experimental Medical Research, Istanbul University. These animals were housed in polypropylene cages with a 12-h light/dark cycle at 22-24°C with 50% humidity. They were provided with standard laboratory chow and tap water ad libitum for at least 7 days of acclimation. All experiments were carried out according to the protocols approved by the Animal Care and Use (2013/53).

### Administration of PNMC and Noni

3-methyl-4-nitrophenol (4-nitro-m-cresol, PNMC) was purchased from Sigma Chemical Co. (St. Louis, MO, USA.) and was dissolved in phosphate buffered saline (PBS) containing 0.05% Tween 80 (Merck) before injection. Noni juice (99.5%) was provided from Alnoni Ltd. (Antalya, Turkey).

The animals were randomly divided into eight groups (n: 7). Noni juice was administered at a dose of 2 ml per rat, regardless of body weight via gastric gavage. The group designs were as follow:

Group 1: (G1; Control) received PBS containing 0.05% Tween 80 subcutaneously (s.c.)

Group 2: (G2) received Noni alone via gastric gavage

Group 3: (G3) received PNMC (1 mg/kg, s.c.)

Group 4: (G4) received PNMC (10 mg/kg, s.c.)

Group 5: (G5) received PNMC (100 mg/kg, s.c.)

Group 6: (G6) received Noni + PNMC (1 mg/kg, s.c.)

Group 7: (G7) received Noni + PNMC (10 mg/kg, s.c.)

Group 8: (G8) received Noni + PNMC (100 mg/kg, s.c.)

### Sample Collection

Animals in all groups were weighed and then sacrificed with an overdose of diethyl ether anesthesia 24 h after the last treatment. Blood samples were collected from each rat via cardiac puncture just prior to sacrifice and centrifuged at 2.500 x g for 15 min. Each serum sample was allocated into 1.5 mL microcentrifuge tube and stored at -80°C until further analysis. All samples were measured together centrally to avoid inter-assay variation. The right and left testes were excised, weighed separately and then processed for histological examination.

### Hormone Assays

Rat serum follicle stimulating hormone (FSH) concentrations were determined by a commercial ELISA kit using a double antibody sandwich enzyme immunoassay technique (Cat. No. YHB0436Ra; Shanghai Yehua Biological Technology Co. Ltd, China). Each ELISA analysis was carried out according to the manufacturer's instructions. All tests showed intra-

assay and inter-assay coefficients of variations (CVs) below 10% and 12%, respectively. The analytical sensitivity of the test was 0.12 mIU/mL.

Rat luteinizing hormone (LH) levels were measured by a commercial ELISA kit (Cat. No. YHB0686Ra; Shanghai Yehua Biological Technology Co. Ltd, China). The analytical sensitivity was 0.051  $\mu$ IU/mL. The intra- and inter-assay CVs were below 10% and 12%, respectively.

A commercial ELISA kit was used for rat testosterone measurements (Cat. no. YHB1031Ra; Shanghai Yehua Biological Technology Co. Ltd, China). The lowest limit of the assay was 0.25 nmol/L. The intra- and inter-assay CVs were under 10% and 12%, respectively.

### **Histomorphometric Evaluations**

The testes were fixed in neutral buffered formalin (10%) for 24 h, routinely processed and embedded in paraffin. Paraffin blocks were sectioned at 4-5  $\mu$ m thickness and then placed onto poly-L-lysine-coated slides. Finally, all slides were stained with hematoxylin and eosin (H&E) and examined by light microscopy for morphometric analyses.

For morphometric analysis, each testis of each animal was divided into four equal pieces. Fifteen randomly selected microscopic fields were evaluated on the sections obtained from each piece. For this purpose, we chose an area fraction approach with an area of an unbiased counting frame of 1.000  $\mu$ m x 1.000  $\mu$ m. Meander sampling of each section was done in a 2.000  $\mu$ m x 2.000  $\mu$ m step size in a systematic-random manner.

The density of testicular tissue components was determined measuring by the density occupied by seminiferous epithelium, tubular lumen and interstitial tissue. The counting was performed by the standard point counting method. For this purpose, a 100-point grid printed on a transparency was placed over each unbiased counting frame field and the number of grid points over the seminiferous epithelium, tubular lumen and interstitial tissue was counted by a software program (stereo investigator, MBF Bio-science, version 9) associated to an Leica, DM400B microscope at x40 magnification. The arithmetic means of the values obtained were expressed as percentage values in 1 mm<sup>2</sup> area <sup>[21]</sup>.

The diameter of seminiferous tubules was measured with a x100 magnification, using the software program (stereo investigator, MBF Bio-science, version 9) associated to light microscope (Leica, DM400B). Thirty tubular profiles that were round or nearly round were chosen randomly and measured for each animal <sup>[21,22]</sup>.

### **Immunohistochemical Analysis**

Testicular tissue samples were immunohistochemically

examined for endothelial Nitric Oxide Synthase (eNOS) and inducible Nitric Oxide Synthase (iNOS) using the Streptavidin-Biotin Complex (Strep-ABC) method. Briefly, tissue sections from paraffin blocks were mounted on positively charged slides, deparaffinized and then subjected to antigen retrieval using citrate buffer solution, and endogen peroxidase and protein blocking procedures. They were incubated with commercially available, ready to use rabbit polyclonal primary antibodies for iNOS (clone RB-9242-R7; Thermo Scientific) and eNOS (clone RB-9279; Thermo Scientific) overnight at 4°C. Then they were treated with a commercial kit for secondary antibody (Thermo Scientific) and the reaction was visualized via AEC chromogen (3-amino-9-ethyl carbazole, Cat. No. TA-060-HA, Thermo Scientific). Finally, the sections were counterstained with Mayer's hematoxylin. The negative control sections were incubated with PBS instead of the primary antibody.

Immunohistochemical iNOS and eNOS staining were quantified using a histological scoring system (HSCOREs), which is a semiquantitative measurement of staining intensity and distribution. For this purpose, tissue sections were stained with antibodies against eNOS and iNOS and then observed under an Olympus microscope equipped with a special ocular grid. Cells were counted in at least 8-10 different regions at x400 magnification by two blinded observers. Based on staining intensity, positively stained cells were scored as: 0, no staining; 1, weak staining; 2, distinct staining; 3, intense staining. For each tissue, an HSCOREs was calculated using the equation:  $HSCOREs = \sum (i + 1) \cdot P_i$  where "i" is the intensity score and "P<sub>i</sub>" is the corresponding percentage of stained cells with that score <sup>[23,24]</sup>.

### **Statistical Analysis**

All of the variables were analyzed using Kruskal -Wallis test. All calculations were carried out using the SPSS statistical software (version 13.0 for Windows, Chicago, IL, USA).

## **RESULTS**

### **Body Weights and Testes Weights**

Body weights, absolute weights of left and right testes and relative weights of both testes (testes /body weights) were shown in *Table 1*. There were no significant differences among the groups in terms of body weights, absolute testes weights and relative weights of both testes (P>0.05).

### **Histology and Histomorphometric Findings**

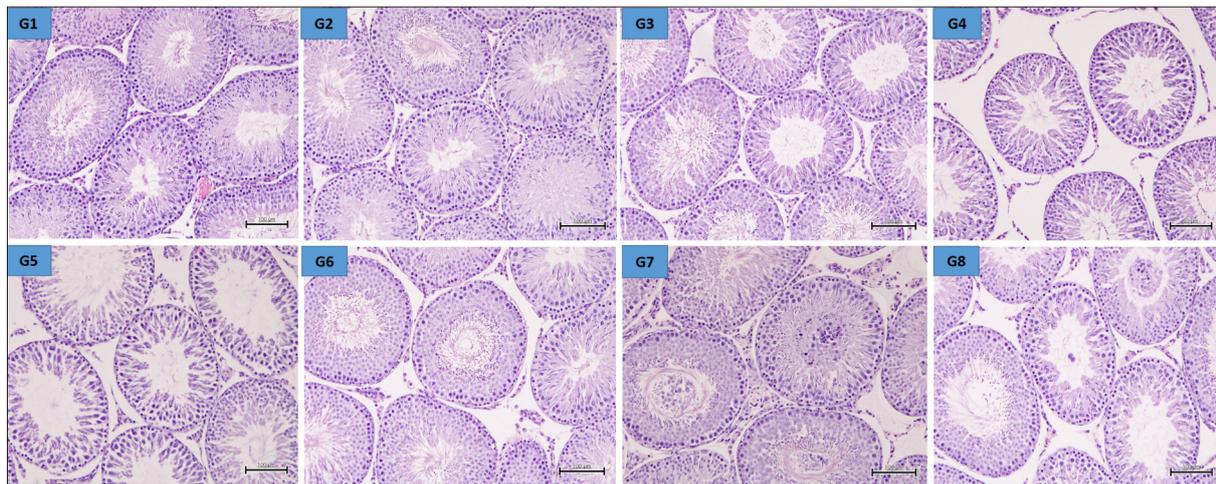
There were no histopathological finding in the H&E stained sections of control and other groups (*Fig. 1*).

Histomorphometric measurements of testes (diameter

**Table 1.** Body weights, absolute testes weights, relative testes weights, and testicular morphometric parameters in the control and experimental groups  
**Tablo 1.** Kontrol ve deney gruplarında vücut ağırlığı, absolut ve rölatif testis ağırlığı ve testisin morfometrik parametreleri

Parameters	G1 Mean±SE	G2 Mean±SE	G3 Mean±SE	G4 Mean±SE	G5 Mean±SE	G6 Mean±SE	G7 Mean±SE	G8 Mean±SE	P Values
Body weight(g)	248±17	259±22	264±10	260±10	253±10	224±28	236±23	259±14	N.S
Left testis weight (mg)	1367±48	1538±82	1448±45	1516±44	1455±65	1338±58	1451±77	1467±56	N.S
Right testis weight(mg)	1375±34	1483±60	1418±55	1541±32	1413±64	1303±66	1456±104	1465±71	N.S
Relative testis weight (left) (mg/g b.w.)	5.5±0.2	6±0.3	5.4±0.2	5.8±0.3	6±0.1	6.3±0.5	6.3±0.4	5.7±0.1	N.S
Relative testis weight (right) (mg/g b.w)	5.6±0.2	5.7±0.3	5.4±0.3	5.9±0.2	5.8±0.2	6.1±0.5	6.3±0.5	5.7±0.1	N.S
Tubular diameter (µm)	278±5.5	285±3.1	282±5.5	282±2.8	267±6.1	278±6.9	273±5.5	274±7.2	N.S
Seminiferous epithelium (%)	65.84±1.4 <sup>ab</sup>	64.53±1.5 <sup>abc</sup>	61.04±3.2 <sup>bc</sup>	59.33±0.7 <sup>c</sup>	62.56±3.3 <sup>bc</sup>	66.31±0.9 <sup>ab</sup>	70.38±1.1 <sup>a</sup>	63.05±1.1 <sup>bc</sup>	<0.01
Luminal space (%)	13.74±0.6	14.27±0.9	15.08±0.8	14.53±0.6	15.56±1.2	14.71±1	15.07±1	15.07±0.9	N.S
Interstitial tissue (%)	20.42±1.7 <sup>b</sup>	20.20±1.8 <sup>b</sup>	23.88±1.8 <sup>ab</sup>	26.14±0.4 <sup>a</sup>	21.88±1.3 <sup>ab</sup>	18.98±0.9 <sup>bc</sup>	14.55±1.5 <sup>c</sup>	21.88±3 <sup>ab</sup>	<0.01

<sup>a-c</sup> Different superscripts within the same line demonstrate significant differences (P<0.01), NS: Not significant (P>0.05)



**Fig 1.** Histological architecture of the testes in all groups. G1) Control group, G2) Noni, G3) 1 mg/kg PNMC, G4) 10 mg/kg PNMC, G5) 100 mg/kg PNMC, G6) Noni + PNMC (1 mg/kg), G7) Noni + PNMC (10 mg/kg), G8) Noni + PNMC (100 mg/kg) H&E stain, Magnification x200.

**Şekil 1.** Tüm gruplarda testisin histolojik yapısı. G1) Kontrol grup, G2) Noni, G3) 1 mg/kg PNMC, G4) 10 mg/kg PNMC, G5) 100 mg/kg PNMC, G6) Noni + PNMC (1 mg/kg), G7) Noni + PNMC (10 mg/kg), G8) Noni + PNMC (100 mg/kg). H&E stain, x200 Büyütme

of seminiferous tubules, percentage values for density of seminiferous epithelium, luminal space and interstitial tissue) were given in *Table 1*.

On the basis of our findings no statistically significant difference was noted among groups in terms of tubular diameter and luminal space (P>0.05). Furthermore, the lowest value for tubular diameter was observed in G5. There was an increase in percentage value of luminal space in G3, G4 and G5 in comparison to those of G1 and G2 whereas there was a reduction in luminal density in G6, when compared with those of G3 and G5, which was not statistically significant (P>0.05).

In terms of seminiferous epithelium component, there was a statistically significant decrease (P<0.01) in G4 in comparison to G1 (*Table 1*). Percentage value of density of seminiferous epithelium of G7 was found to have

significantly increased (P<0.01) when compared with other groups (*Table 1*).

When the groups were compared in terms of interstitial tissue component, the highest value was detected in G4, and this increase (P<0.01) was statistically significant compared to G1 and G2. On the other hand, G7 exhibited the lowest value (P<0.01) and the difference was significant among groups except for G6 (*Table 1*).

### Biochemical Findings

Serum testosterone, LH and FSH levels were given in *Table 2*. Although serum testosterone levels increased in G3 when compared with other groups, this difference was far from being statistically significant (P>0.05).

FSH levels were observed to have increased in G4 in comparison to the control group while G5 revealed

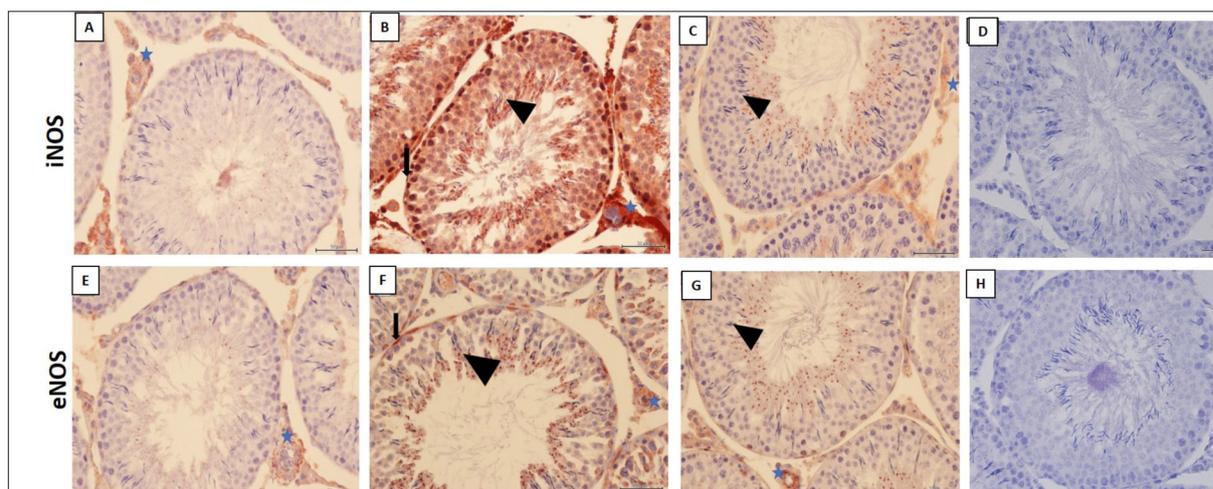
**Table 2.** Serum hormone levels for the control and experimental groups**Tablo 2.** Kontrol ve deney gruplarında serum hormon düzeyleri

Hormonal Parameters	G1 Mean±SE	G2 Mean±SE	G3 Mean±SE	G4 Mean±SE	G5 Mean±SE	G6 Mean±SE	G7 Mean±SE	G8 Mean±SE	P Values
Testosterone(nmol/L )	37.89±6.4	43.70±4.9	56.51±8.1	45.24±6.5	52.43±8.4	52.36±3.9	51.80±4.6	44.54±6.0	N.S
Follicle-stimulating hormone (mIU/mL.)	3.87±0.18	4.32±0.27	4.35±0.50	4.45±0.36	3.64±0.29	4.02±0.10	3.80±0.09	3.75±0.15	N.S
Luteinizing hormone (mIU/mL)	2.49±0.18	2.67±0.13	3.24±0.45	2.88±0.12	2.69±0.16	3.05±0.17	2.94±0.17	2.70±0.18	N.S

NS: Not significant (P&gt;0.05)

**Table 3.** iNOS and eNOS H-SCOREs values in the testicular tissue of the control and experimental groups**Tablo 3.** Kontrol ve deney gruplarının testis dokusunda iNOS ve eNOS H-SKOR değerleri

Parameters	G1 Mean±SE	G2 Mean±SE	G3 Mean±SE	G4 Mean±SE	G5 Mean±SE	G6 Mean±SE	G7 Mean±SE	G8 Mean±SE	P Values
iNOS	67.75±2.70 <sup>a</sup>	78.50±3.71 <sup>a</sup>	122.75±5.90 <sup>c</sup>	250.20±4.53 <sup>e</sup>	283.30±6.95 <sup>f</sup>	102.45±4.93 <sup>b</sup>	113.35±2.35 <sup>bc</sup>	190.75±3.48 <sup>d</sup>	<0.001
eNOS	45.50±2.90 <sup>a</sup>	57.40±2.74 <sup>b</sup>	70.70±1.58 <sup>c</sup>	145.40±1.67 <sup>f</sup>	177.95±2.73 <sup>g</sup>	63.05±2.81 <sup>b</sup>	87.30±2.27 <sup>d</sup>	132.90±2.02 <sup>e</sup>	<0.001

<sup>a-g</sup>Different superscripts in the same row indicate the significant difference (P<0.001)**Fig 2.** Immunohistochemical staining showing iNOS and eNOS expression in testes. A,E) Control grup, B,F) 100 mg/kg PNMC, C,G) Noni + PNMC (100 mg/kg), D) iNOS negative control, H) eNOS negative control. iNOS and eNOS immunostaining were seen within peritubular myoepithelial cells (arrow), Leydig cells (star) and spermatids (arrowheads), Magnification x400**Şekil 2.** Testiste İNOS ve eNOS ekspresyonu gösteren immunohistokimyasal boyama A,E) Kontrol grubu; B,F) 100 mg/kg PNMC, C,G) Noni + PNMC (100 mg/kg), D) iNOS negatif kontrol, H) eNOS negatif kontrol. iNOS ve eNOS immün boyama peritübuler miyoepitelyal hücrelerde (ok), Leydig hücrelerinde (yıldız) ve spermatidlerde (okbaşı) izlendi, x400 Büyütme

a reduction, which was not statistically significant (P>0.05).

Serum LH levels were higher in all experimental groups in comparison to the control. However, this change was statistically insignificant (P>0.05).

### Immunohistochemical Findings

iNOS and eNOS H-SCOREs values were summarized in Table 3. iNOS and eNOS H-SCOREs values in G3, G4 and G5 were significantly higher (P<0.001) than those of G1 and G2. The relevant values showed a significant reduction (P<0.001) in G6, G7 and G8 when compared with those of G3, G4 and G5. Intensity of immunohistochemical

staining for iNOS and eNOS was more prominent in the cytoplasm of peritubular myoepithelial cells, Leydig cells, and spermatids (Fig. 2).

## DISCUSSION

It is well known that DEP have serious adverse effects on male reproductive system. Numerous studies exhibited toxic effects of these particles on testicular tissue and spermatogenesis [6-8,25,26]. PNMC, one of the derivatives of DEP, is a degradation product of a widely used insecticide applied in agriculture and therefore it is quite likely to have been exposed to this chemical both in rural and residential environments [9,10]. Experimental animal models of PNMC-

induced tissue injury regarding male reproductive systems were carried out mostly with immature animals like rats, quails and mice and were mostly focused on serum hormone level changes of these animals [27-29].

Noni has been used in numerous animal models to demonstrate its protective and therapeutic effectiveness against cytotoxic agents including the chemicals which impair male reproductive system and its functions [30,31].

We designed this experimental model in mature rats since there is a limited number of studies with respect to PNMC toxicity on testicular tissue of adult animals. We evaluated its adverse effects with histomorphometric aspects which were also demonstrated by oxidative stress parameters with the anticipation that Noni might have been utilized in testicular injury, as well owing to its well-known therapeutic properties as an antioxidant.

On the basis of our findings PNMC administered at different doses did not have a significant impact on body weights. Likewise, no statistically significant difference was noted among groups in terms of testes weights. It was found that PNMC particularly at high doses decreased testes weights in immature rats [28,32]. Tsukue et al. [33] observed the adverse effects of DEP especially on accessory organs of male adult rats rather than on testes and they used fisher 344 rats in their research model unlike our design in which we used Sprague Dawley rats.

The difference in terms of tubular diameter and percentage value of luminal space was not statistically significant among our groups. Yue et al. [32] reported that tubular diameter increased significantly in immature rats. Bu et al. [34] showed that PNMC at low doses did not affect tubular diameter while high doses of the substance (100 mg/kg PNMC) increased tubular diameter and caused germ cell loss, which was compatible with our findings.

There is no data available with respect to the effects of PNMC on percentage values of seminiferous epithelium and interstitial tissue. In our study PNMC significantly reduced seminiferous epithelium component in the group which received PNMC at an intermediate dose (10 mg/kg) while yielding an increase in the density of interstitial tissue. This increase was considered to be associated with the thickening of interstitial region due to lymphatic dilatation and edema developed as a result of PNMC administration [35]. On the other hand, percentage value of seminiferous epithelium density increased while that of interstitial tissue decreased in G7 (Noni + 10 mg/kg PNMC). These findings revealed the effectiveness of Noni against the adverse effects of PNMC on seminiferous epithelium and interstitial tissue. It is clear that the inconsistency between the results of our histomorphometric measurements and those of the previous studies, in general, was associated with the age of our animals since most previous studies

were performed on immature or growing animals of different breeds.

Testosterone and estradiol levels were shown to have significantly increased in the male offspring born from the females that were exposed to exhaust gas during pregnancy while FSH and LH levels were reduced [6]. Testosterone levels were increased in PNMC-treated castrated immature male rats while LH and FSH levels were decreased [27]. Li et al. [28] reported that PNMC decreased LH levels in immature male rats. Tsukue et al. [32] observed a marked and statistically significant increase in FSH, LH and testosterone levels in the male fisher 344 rats exposed to PNMC compared with the control group. On the basis of our findings these three hormone levels were observed to have increased though the difference was not statistically significant. The inconsistency in our findings when compared with those of the previous researches regarding the effects of PNMC on hormone levels might be associated with animal species, age, the duration of the experimental period and the individual susceptibility.

NOS, which is expressed also in male reproductive system under physiological conditions is essential for the maintenance of spermatogenesis and testicular androgen concentrations [36,37]. Pathogenic agents cause an enormous increase in both iNOS and eNOS activities, which lead to oxidative stress and thus increase apoptotic activity in germ cells [15,38]. PNMC was shown to induce oxidative stress by increasing free radical production [39]. No data is available with respect to elevation of iNOS and eNOS activities in testicular tissue due to tissue injury induced by PNMC. In our study, the expression of both iNOS and eNOS was increased in PNMC-treated groups. The immunoreactivity was manifested by intense staining with iNOS and eNOS antibodies. On the contrary, immunoreactivity for iNOS and eNOS was reduced in germ cells and Leydig cells of testes in Noni-treated animals, which was an evidence of beneficial effect of Noni against testicular tissue injury.

On the basis of histomorphometric and immunohistochemical findings, we may conclude that PNMC caused adverse effects on testicular tissue of adult male rats, which was markedly alleviated by the administration of Noni.

In conclusion, our findings suggest that Noni treatment would be able to alleviate the oxidative damage caused by the PNMC in the kidney of rats

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