Effects of vitamin E on ovarian tissue of rats following treatment with p-nonylphenol: A stereological study

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Abstract

**Background:** Para-Nonylphenol (p-NP) is one of the environmental pollutants which cause reproductive system disorders.

**Objective:** The effects of vitamin E on ovary structure during its development in rats treated with p-NP.

**Materials and Methods:** 32 Wistar female rats after mating were divided into 4 groups; control, vitamin E (100mg/kg/day), p-NP (250mg/kg/day) and p-NP + vitamin E. The rats were treated from the day 7 of pregnancy till 21⁴ day of postnatal through suckling period. After weaning, the female pups were treated by gavages for 120 days. The total volume of ovary, number of follicles, volume of oocyte, follicular cells and their nuclei and the thickness of zona pellucida were estimated stereologically. The results were analyzed using one way ANOVA and p<0.05 was considered significant.

**Results:** The ovary weight, mean total volume of ovary and cortex, number of antral and graafian follicles and body weight were decreased significantly (p<0.05) in the p-NP treated rats compared to control and other groups, while the number of atretic follicles was increased significantly (p<0.05). A significant reduction (p<0.05) in volume of oocyte, follicular cells and their nuclei in antral and graafian follicles was found in p-NP group. In addition, treatment with only vitamin E showed an improving effect on folliculogenesis due to a highly significant increase (p<0.01) in the number of primordial follicles.

**Conclusion:** Vitamin E could compensate the adverse effects of p-NP on the ovary structure during its development.

**Key words:** Ovary, p-Nonylphenol, Rat, Stereology, Vitamin E.

Introduction

Para-Nonylphenol (p-NP) is one of the endocrine disrupting compounds, which has estrogenic effects and is able to impair the endocrine system (1-3) through diverse mechanisms, such as receptor-mediated enzyme inhibition (1) and mimics the estrogen or androgen action (1, 2, 4). This chemical is used in the preparation of lubricating oil additives, plasticizers and surface active agents and also has been found in polyvinyl chloride which is used in the food processing and packaging industries (1). Investigations have shown that the p-NP causes dysfunction in male and female reproductive system (5-7) specially during the early phases of animal development (1). In addition it also produces oxidative stress (3, 8) through generating
free radicals and impairing estrous cycle as well as folliculogenesis (9). Some investigators have reported that the p-NP exposure have caused the reduction of body and ovary weight as well as reproductive impairment. Cunny et al, showed that the treatment of female rats with 139mg/kg body weight/day of p-NP for a period of 90 days caused significant reduction of body and ovary weight (10).

Treatment of rats for 20 days with 50 and 100mg/kg body weight/day of p-NP caused imbalance in estrous cycle whereas 100mg/kg body weight/day showed a significant reduction in ovary weight (11). Chapin et al, showed a significant reduction of the number of antral and graafian follicles after treatment of the rats with 350mg/kg body weight/day of p-NP in F2 generation (12). In another investigation, treatment of pregnant female rats from the day 7 of pregnancy till the maturation of the pups with 400mg/kg body weight/day of p-NP, the F1 generation have lost the fertility potential (13).

On the other hand, antioxidants such as vitamin E can prevent the adverse effects of oxidative stress by inhibiting reactive oxygen species (ROS) (14,15). Investigation showed that the co-administration of vitamin E with Methidathion and Organophosphate insecticides (16) prevents the harmful effects of these chemical in the reproductive organs. Considering the above, we aimed to investigate the preventive effects of vitamin E as a strong antioxidant on structural parameters of ovary during its development in the rats treated with p-NP, as an oxidative agent, using stereological methods.

**Materials and methods**

**Animals and treatments**

Male and female Wistar rats with average weight of 200±10g were purchased from Pasteur Institute, Iran and kept in the animal house of Arak University under standard conditions of temperature, light and food. After mating, pregnant rats were divided into 4 groups (n=6): control, p-NP (250mg/kg/day) (Acros Company, New Jersey, USA), vitamin E (VE) (100mg/kg/day) (Aldrich Company, USA) and p-NP+VE. The treatment was carried out from the 7th day of pregnancy till 21st day of postnatal through sucking period (17-19). From this stage, female pups (F1 generation) were divided into the same groups similar to the mothers and the treatments were continued orally by gavage till maturation (120 day) (1). Considering the high viscosity of p-NP and vitamin E, corn oil was used as a carrier (5,20), with the concentration of 50mg p-NP/0.1ml corn oil (21), and similar vehicle was employed for vitamin E.

**Tissue preparation**

At the end of the treatments period, the rats were weighed, anesthetized by diethyl ether (Merck company, Germany) and their right ovary were taken out, weighed and fixed in 10% neutral buffered formalin. After tissue processing, the samples were blocked in cylindrical paraffin blocks.

**Stereological study**

The orientator method was used to obtain isotropic uniform random (IUR) sections (22, 23). For this purpose, the cylindrical paraffin blocks containing ovaries were randomly placed on the φ-clock which each half of it was divided into 9 equal parts. By choosing a random number from 1 to 9, an appropriate cut was made along the selected number. The block was then placed on the 0-clock, each half of it was divided into 9 unequal sine-weighted parts, along its cut surface on the 0-0 axis and then the random number was selected and the cut was made along the selected number. Consecutive 5 and 20μm thick sections were prepared using a microtome and stained with Hematoxylin and Eosin (H&E) (Merck company, Germany) method.

**The volume of ovary, cortex, medulla and corpus luteum (mm³)**

To estimate the mean total volume of ovary, the images of 5μm thick sections were transferred on the working table using the micro-projector (NeoPromar Leitz Germany) with the magnification of 80. Counting probe was randomly superimposed on the images, then the points were counted and the total volume of the ovary was estimated using the Cavalieri methods applying the following formula (22): 

\[ V_{total\ ovary} = \sum_{i=1}^{p} \times a(p) \times t, \]

in which \( \sum_{i=1}^{p} \) is the total number of points superimposed on the image, (t) is the thickness of the section and a (p) is the area associated with each point. To obtain the volume of the ovary compartments, the volume density for each was calculated as:

\[ V_{Vortex} = \frac{\sum_{i=1}^{p} p_{cortex}}{\sum_{i=1}^{p} p_{total}} (22), \]

where
\( \sum_{i=1}^{n} p_{\text{total}} \) is the total number of counted points
and \( \sum_{i=1}^{n} p_{\text{cortex}} \) is the total number of points
superimposed on the cortex. The volume of cortex was then obtained through multiplying the volume
density (Vv) by the total volume of ovary.

The number of follicles
To estimate the number of follicles, the optical
disector method was used. The average of 12
sections was selected from 20μm thick sections
using systematic random sampling. The sections
were studied using the Olympus microscope
(BX41TE model) with 100x magnification and the
microator (ND 221 B, Heidenhain, Germany)
connected to a computer. The nuclei of follicular
cells were sampled by an unbiased counting frame
superimposed on the monitor. Identification of the
stage of follicles was carried out based on the
Mayer et al classification (24). Any nucleus that
laid in the frame and did not touch the left and
bottom lines of the frames was selected. The number density (Nv) of different types of follicles
was estimated as:

\[ N_v = \frac{\sum_{i=1}^{n} Q}{a \times f \times h \times \sum_{i=1}^{n} p} \]  

(22, 24), in

which \( \sum_{i=1}^{n} Q \) is the total number of counted follicles,
ah is the tissue thickness considered for counting,
\( a/f \) is the frame area in the true tissue scale and
\( \sum_{i=1}^{n} p \) is the total number of the points superimposed
on the selected fields. The result of the equation is
then multiplied by the total volume of the ovary
to obtain the total number of follicles (24, 25).

The volume of oocyte, follicular cells and their
nuclei
To estimate the volume of oocyte, follicular
cells and their nuclei the nucleator method was
applied. This is a method for estimating the
number-weighted mean volume (\( V_v \)). An average
of 12 sections from 20μm thick sections was
randomly selected and the selected follicles were
then studied using the Olympus microscope with
100x magnification. On the selected cells with an
unbiased counting frame, the distance from the
center of the nucleolus to the oocyte membrane
was measured (\( L_v \)) to estimate the oocyte volume.

To estimate the volume of oocyte nucleus the
distance from the center of the nucleolus to the
nucleus membrane was measured. The same
measurements were carried out for the follicular
cells and their nuclei. The volume of each was
calculated using the equation

\[ V_v = \frac{4}{3} \pi (L_v^3) \]  

(22, 26).

Zona pellucida thickness (μm)
To estimate the mean thickness of zona
pellucida (ZP), an average of 12 sections from 5μm
thick sections was randomly selected and studied
with 100x magnification. To identify measurement
sites, the specific line grid (3 parallel lines) was
randomly superimposed on the sampled fields. The
ZP thickness was measured using the orthogonal
intercept method, in brief by measuring the length
of a line extended perpendicularly from the inner
membrane to outer surface of ZP at each intercept
of the line of the grid with zona membrane, and
was considered as orthogonal intercept (oi). An
average of 110 measurements was made to
calculate the harmonic mean thickness using
following formula (27):

\[ \text{Harmonic mean thickness} = 8\pi /3 \times \text{Harmonic mean of orthogonal intercepts}, \text{where harmonic mean} = \text{number of measurements / sum of the reciprocal of orthogonal intercepts lengths} = \text{number of measurements} \times (\frac{1}{o_{i1}} + \frac{1}{o_{i2}} + \frac{1}{o_{i3}} + \frac{1}{o_{i4}} + \ldots) \]

Statistical analysis
The results were analyzed by one-way ANOVA, Tukey test, using the SPSS V11/0
software and the means were considered
significantly different at p<0.05.

Results
The volume of ovary, cortex, medulla and
 corpus luteum
The mean total volume of ovary and the volume
of cortex showed a high significant reduction in p-
NP group compared to the others (p<0.01), while
co-administration of p-NP with vitamin E
increased the volume of ovary and cortex to the
control level. Comparing the mean volume of
corpus luteum, a significant reduction was found in
the p-NP group compared to vitamin E and p-NP +
vitamin E groups (p<0.05) (Table I).

The number of follicles
The mean number of antral (p<0.04) and
graafian follicles (p<0.04) reduced while the mean
number of atretic follicles increased significantly
(p<0.001) in p-NP group when compared to the
other groups. In p-NP+vitamin E group, variations
due to p-NP exposure were compensated to the normal level. In the group treated with only vitamin E, the mean total number of follicles (p<0.02) and the mean total number of primordial follicles (p<0.001) significantly increased compared to the other groups (Table II).

The thickness of zona pellucida (μm)
Comparing the mean thickness of zona pellucida in the secondary follicles (p<0.03), antral (p<0.001) and graafian (p<0.002) a significant reduction was found in the p-NP group, while simultaneous treatment of rats with p-NP + vitamin E compensated these reductions (p>0.05) to the level of control group. In addition, treatment with only vitamin E caused considerable increased in the zona pellucida thickness of secondary follicles (p<0.05) (Table III).

The volume of oocyte (μm³)
The mean volume of oocyte in antral and graafian follicles reduced significantly in p-NP group compared to other groups (p<0.04), while in the p-NP + vitamin E group, vitamin E compensated the reducing effect of p-NP to the normal level (p>0.05). The result also showed treatment with only vitamin E caused a considerable increase in oocyte volume of the mentioned follicles (p<0.001). The mean volume of oocyte in primordial, primary and secondary follicles showed no significant difference in any of groups (p>0.05) (Table IV).

The volume of oocyte nucleus (μm³)
The mean volume of the oocyte nucleus (μm³) in secondary, antral and graafian follicles showed a highly significant reduction in p-NP group compared to the other groups (p<0.001). Meanwhile the mean volume of oocyte nucleus in these follicles was increased highly significant following vitamin E treatment (p<0.001) in the p-NP + vitamin E group. In the vitamin E group, an increase in the volume of the oocyte nucleus (μm³) in antral and graafian follicles was seen (p<0.05). The volume of the oocyte nucleus of primordial and primary follicles showed no significant difference between the groups (p>0.05) (Table V).

The volume of follicular cells (μm³)
The mean volume of follicular cells in primary, secondary, antral and graafian follicles significantly decreased in p-NP group when compared to the others (p<0.0001), while simultaneous treatment of vitamin E and p-NP compensated this reducing effect of p-NP in the above mentioned follicles (p<0.05). The treatment of rats with only vitamin E resulted in a significant increase in the mean volume of follicular cells in antral and graafian follicles (Table VI).

The volume of nucleus of follicular cells (μm³)
The mean volume of the follicular cells nucleus in primary, secondary, antral and graafian follicles showed a highly significant reduction in the p-NP group compared to the other groups (p<0.001).

The simultaneous treatment of rats with vitamin E + p-NP compensated the reduction of volume of follicular cells nucleus due to p-NP in the above follicles. Meanwhile the treatment of rats with only vitamin E led to a highly significant increase in the mean volume of follicular cells nucleus in antral and graafian follicles (p<0.001) (Table VII).

The body and ovary weight (g)
The mean body weight showed a significant reduction in the p-NP and p-NP + vitamin E groups compared to the other groups at the end of treatment (p<0.01). In the p-NP group compared to the other groups, a highly significant reduction in ovary weight was observed (p<0.001), while in p-NP + vitamin E group, it was seen that treatment with vitamin E could compensate the reduction of ovary weight caused by p-NP (Table VIII).

The histopathological findings
An increase in the number of atretic follicles was found in p-NP group compared to the other groups. However, simultaneous treatment of p-NP and vitamin E seemed to normalize the number of atretic follicles (Figure 1).

Figure 1. Micrographs of the ovary tissue (5μm thick section with H&E staining) in different groups of rats: a) representing the normal structure of ovary in control group, b) Showing an increase in the number of atretic follicles in the rats exposed to p-NP. In p-NP + vitamin E and vitamin E groups the ovarian tissue structure was same as the control group (photos not shown).
Table I. Comparing the mean total volume of ovary, cortex, medulla and corpus luteum (mm³) in different groups of rats 120 days after treatment.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>Volume of ovary (mm³)</th>
<th>Volume of cortex (mm³)</th>
<th>Volume of medulla (mm³)</th>
<th>Volume of corpus luteum (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.1±1.2</td>
<td>7.8±0.8</td>
<td>1.2±0.4</td>
<td>3.5±0.8</td>
</tr>
<tr>
<td>VE</td>
<td>10.8±0.9</td>
<td>9.6±0.9</td>
<td>1.1±0.2</td>
<td>3.8±0.6</td>
</tr>
<tr>
<td>p-NP</td>
<td>7.2±0.1</td>
<td>5.9±0.9</td>
<td>1.2±0.3</td>
<td>0.8±0.6</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>9.0±0.8</td>
<td>7.7±0.8</td>
<td>1.1±0.3</td>
<td>3.8±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Table II. Comparing the mean total number of follicles, the number of primordial, primary, secondary, antral, graafian and atretic follicles in different groups of rats 120 days after treatment.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>Total number of follicles</th>
<th>Primordial follicles</th>
<th>Primary follicles</th>
<th>Secondary follicles</th>
<th>Antral follicles</th>
<th>Graafian follicles</th>
<th>Atretic follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5789±820</td>
<td>2855±392</td>
<td>1168±263</td>
<td>993±336</td>
<td>569±64</td>
<td>205±56</td>
<td>9±1</td>
</tr>
<tr>
<td>VE</td>
<td>7135±458</td>
<td>4007±454</td>
<td>1098±313</td>
<td>1156±234</td>
<td>579±179</td>
<td>228±118</td>
<td>9±1</td>
</tr>
<tr>
<td>p-NP</td>
<td>4676±593</td>
<td>2693±480</td>
<td>804±284</td>
<td>839±358</td>
<td>296±88</td>
<td>49±22</td>
<td>14±1</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>6449±956</td>
<td>3288±426</td>
<td>1092±263</td>
<td>1312±351</td>
<td>581±61</td>
<td>175±58</td>
<td>10±2</td>
</tr>
</tbody>
</table>

Values are means ± SD. The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Table III. Comparing the mean thickness of ZP (μm) in different groups of rats 120 days after treatment. Values are means ± SD.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>ZP thickness of secondary follicles (μm)</th>
<th>ZP thickness of antral follicles (μm)</th>
<th>ZP thickness of graafian follicles (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.8±0.8</td>
<td>13.3±2.2</td>
<td>14.3±0.6</td>
</tr>
<tr>
<td>VE</td>
<td>11.6±1.3</td>
<td>12.1±0.8</td>
<td>13.4±1.5</td>
</tr>
<tr>
<td>p-NP</td>
<td>7.2±0.8</td>
<td>9.9±0.9</td>
<td>11.9±0.6</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>9.3±0.9</td>
<td>12.0±0.9</td>
<td>13.2±2.1</td>
</tr>
</tbody>
</table>

The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Table IV. Comparing the mean volume of oocyte (μm³) in different types of follicles in the groups of rats 120 days after treatment.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>Primordial follicles</th>
<th>Primary follicles</th>
<th>Secondary follicles</th>
<th>Antral follicles</th>
<th>Graafian follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1596.0±75.9</td>
<td>3653.3±265.2</td>
<td>45429.7±3070.3</td>
<td>166983.7±6740.7</td>
<td>209667.7±8273.0</td>
</tr>
<tr>
<td>VE</td>
<td>1599.0±29.4</td>
<td>3781.9±280.0</td>
<td>53975.6±7477.6</td>
<td>201609.0±7933.0</td>
<td>238230.3±10344.8</td>
</tr>
<tr>
<td>p-NP</td>
<td>1565.1±84.7</td>
<td>3392.5±246.4</td>
<td>40004.9±3837.7</td>
<td>146749.0±10324.8</td>
<td>187795.3±12854</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>1609.4±81.4</td>
<td>3495.8±173.8</td>
<td>47706.0±4619.3</td>
<td>170312.5±9548.8</td>
<td>216893.5±18644.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Table V. Comparing the mean volume of oocyte nucleus (μm³) in different types of follicles in the groups of rats 120 days after treatment. Values are means ± SD.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>Primordial follicles</th>
<th>Primary follicles</th>
<th>Secondary follicles</th>
<th>Antral follicles</th>
<th>Graafian follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>291.7±14.9</td>
<td>516.5±50.6</td>
<td>1612.6±138.2</td>
<td>2395.4±139.4</td>
<td>3311.2±176</td>
</tr>
<tr>
<td>VE</td>
<td>301.8±9.8</td>
<td>527.2±11.9</td>
<td>1716.5±99.1</td>
<td>3174.9±45.8</td>
<td>3780.3±160.9</td>
</tr>
<tr>
<td>p-NP</td>
<td>285.7±14.4</td>
<td>531.5±24.4</td>
<td>1105.9±18.4</td>
<td>1827.8±134</td>
<td>2156.2±200.4</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>299.0±9.9</td>
<td>521.7±34.3</td>
<td>1678.4±91.9</td>
<td>2473.0±343.3</td>
<td>3460.3±187.7</td>
</tr>
</tbody>
</table>

The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).
Table VI. Comparing the mean volume of follicular cells (μm³) in different types of follicles in the groups of rats 120 days after treatment. Values are means ± SD.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>Volume of follicular cells (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary follicles</td>
</tr>
<tr>
<td>Control</td>
<td>183.4±12.4</td>
</tr>
<tr>
<td>V E</td>
<td>196.5±6.9</td>
</tr>
<tr>
<td>p-NP</td>
<td>163.7±2.7</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>188.3±7.6</td>
</tr>
</tbody>
</table>

The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Table VII. Comparing the mean nucleus volume of follicular cells (μm³) in different types of follicles in the groups of rats 120 days after treatment.

<table>
<thead>
<tr>
<th>Groups (N=6)</th>
<th>Volume of nucleus of follicular cell (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary follicles</td>
</tr>
<tr>
<td>Control</td>
<td>59.3±5.2</td>
</tr>
<tr>
<td>V E</td>
<td>59.3±1.8</td>
</tr>
<tr>
<td>p-NP</td>
<td>35.0±2.1</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>68.8±4.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Table VIII. Comparing the mean body and ovary weight (g) in different groups of rats 120 days after treatment. Values are means ± SD.

<table>
<thead>
<tr>
<th>Group (N=6)</th>
<th>Body weight (g) (at the end of weaning)</th>
<th>Body weight (g) (at the end of treatment)</th>
<th>Weight of ovary (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.6±10.0</td>
<td>218.5±14.2</td>
<td>0.034±0.06</td>
</tr>
<tr>
<td>V E</td>
<td>44.0±1.2</td>
<td>219.5±7.4</td>
<td>0.037±0.04</td>
</tr>
<tr>
<td>p-NP</td>
<td>39.0±3.8</td>
<td>176.7±3.2</td>
<td>0.023±0.04</td>
</tr>
<tr>
<td>p-NP + VE</td>
<td>42.6±5.5</td>
<td>175.8±18.3</td>
<td>0.032±0.05</td>
</tr>
</tbody>
</table>

The means with the same code do not differ significantly (one-way ANOVA, Tukey test, p>0.05).

Discussion

As the results showed a significant reduction in the weight of the ovary in the rats treated with p-NP was found which is in agreement with other investigations (28-29) and also it is believed that this reduction is dose-dependent (10, 12). Atrophy of the ovary along with the lack of corpus luteum and reduction in the number of follicle could be considered as parameters involved in ovary weight reduction (20, 30). In addition, treatment with p-NP can reduce the level of gonadotrophins (29), known as an inhibiting factors of apoptosis in granulosa cells (31), therefore following p-NP treatment granulosa cells may undergo apoptosis and shrinkage which will finally lead to a reduction in ovary weight and volume. In another study the reduction in mitosis of granulosa and theca cells during folliculogenesis as a result of p-NP treatment was shown (32), which can affect the follicle growth and may also cause the reduction of ovary weight and volume. Co-administration of p-NP with vitamin E compensated the reduction in ovary weight and volume. This could be due to the fact that vitamin E can stimulate the secretion of Gonadotrophins (33, 34) which had been reduced as a consequence of p-NP. In addition it should be noted that vitamin E as a strong antioxidant (15, 16) may prevent weight and volume reduction in the ovary, due to the prevention of oxidative stress caused by p-NP (9).

The results showed that the mean thickness of zona pellucida significantly reduced due to p-NP treatment. Follicular atresia and reduction in mitosis in granulosa and theca cells following treatment with p-NP has been reported by other investigators (1, 20, 32). Since it is believed that the follicular cells and oocytes are involved in production of zona plllucida (35) and in this study a significant reduction in the volume of oocyte nucleus, volume of follicular cells and its nucleus in secondary, antral and graafian follicles as well as the volume of oocyte in antral and graafian follicles following treatment with p-NP was found, therefore the reduction in the thickness of zona pellucida is expected. In addition, oxidative stress caused by p-NP (9) could be involved in the degradation of lipid and protein components of zona pellucida, as this effect has been reported on the chorion layer in zebra fish (36). As an outstanding result of this study, vitamin E not only...
compensated the reduction of zona pellucida thickness when given simultaneously with p-NP, but also normalized the reducing effects of p-NP on the other mentioned structural parameters.

We also found that treatment with p-NP caused reduction in the number of antral and graafian follicles, which is in agreement with Chapin and co-workers' study (12). In the control and p-NP groups, the number of primordial, primary and secondary follicles were nearly identical, and a significant increase in the number of atretic follicles was observed in the p-NP group which is in agreement with other investigators (1, 20); therefore this increase is mainly relevant to the decrease of antral and graafian follicles, so it is concluded that p-NP effects are more obvious in the later stages of follicular development. On the other hand, it is reported that the increase in the number of atretic follicles is due to the disturbance of folliculogenesis and oogenesis as a result of oxidative stress and estrogenic property of p-NP which leads to a decrease in the number of follicles in preovulation stage (9, 20). In the present study vitamin E as strong antioxidant (15, 16) could prevent follicular degeneration and atresia as a consequence of p-NP treatment and also compensate the reduction in the number of follicles. The same results were also reported when vitamin E and vitamin C were given as antioxidant following treatment with methiadiathon, a substance which cause lipid peroxidation in the ovary and increase the number of atretic follicles (16).

As mentioned above, in this study we also observed that the mean volume of oocyte and its nucleus in antral and graafian follicles decreased significantly in p-NP group. The same results were also obtained for the volume of follicular cells and their nucleus in primary, secondary, antral and graafian follicles. p-NP exposure can inhibit the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are vital for oocyte development (29, 37) and also causes apoptosis of granulosa cells which can be considered as another reason for FSH level reduction (31,38). Therefore, reduction in the level of these hormones retards the follicular phase and impairs oocyte maturation which leads to a decrease in the volume and the number of follicular cells. In addition, as a result of estrogenic property of p-NP (1), folliculogenesis impairment and retardation in oocyte maturation are expected as shown with other xenoestrogens (37, 39). Co-administration of vitamin E with p-NP could prevent the disturbance in the number of mentioned follicles caused by p-NP which is due to the antioxidant effect of vitamin E (15, 16) or the fact that vitamin E is able to stimulate FSH secretion(33, 34).

The presented data also revealed that the mean body weight significantly reduced in p-NP group. Investigations have shown that p-NP as an environmental toxicant can reduce body weight through its estrogenic property which seems to be dose-dependent (1, 20, 40, 41). This could be due to the disturbance of hypothalamus-pituitary-ovary axis which leads to deficiency in growth hormone secretion as seen following the treatment of rats with octyphenol as another estrogenic substance (42). We also observed that p-NP caused anorexia and reduction in food consumption which can be another influencing parameter in weight loss as reported by other investigators (10, 40). Following treatment with p-NP an increase of T	extsubscript{3} hormone level is also reported (29) which can be considered as another mechanism for reduction of body weight.

However, treatment of rats with p-NP+ vitamin E as a strong antioxidant (14, 43) had no effect on weight reduction. Therefore, the weight reduction following treatment with p-NP may not be due to its oxidative stress or may be, the used dosage of vitamin E had not been sufficient.

In conclusion, vitamin E could compensate the most adverse effects of p-NP treatment on ovary structure and also it was seen that the application of vitamin E only could increase the volume of ovary and cortex, the number of primary follicles and improved the main structural parameters of follicle in the late stages of follicular development. As the result of this study showed the consumption of vitamin E might be useful where ever the toxicity with p-NP is the matter of concern.

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References


41. Cooper S, Latendresse JR, Doerge DR, Twaddle NC, Fu X, Delcos KB. Dietary modulation of p-nonylphenol-induced
Ovary structure after p-nonylphenol & vitamin E treatments

