Ameliorative Effect of Honey Bee Propolis on the Nonylphenol Induced- Reproductive Toxicity in Male Albino Rats

Kamal A. Shalaby, Eman M. Saleh

Biochemistry Department, Faculty of Science, Ain Shams University, Abbassia, 11566, Cairo, Egypt.

Abstract: Nonylphenol (NP) has been considered an endocrine disrupting substance causing reproductive dysfunction and increase in reactive oxygen species production in different organs including testes. Propolis is a honeybee product that has an antioxidant property. The aim of this study was to elucidate the protective effects of propolis against reproductive toxicity induced by NP in male rats. Five experimental groups receiving a combination of NP (100 mg/ kg body weight/day) and/or propolis (50 mg/ kg body weight/day) for 6 weeks were divided as follows: no treatment (control); NP alone (Group 2), propolis alone (Group 3), NP plus propolis (Group 4; prophylactic), and NP for 6 weeks followed by propolis for 6 weeks (Group 5; curative). NP caused a decrease in prostate and testes relative weights, testicular 17-ketosteroid reductase (17-KSR), SOD, CAT GST, and total proteins, accompanied by a decrease in blood testosterone, LH, total antioxidant capacity, total proteins, albumin, and globulin - while testicular MDA and DNA fragmentation rate were augmented, compared to control group. Administration of propolis either alone or combined with NP ameliorated these toxic effects. Similarly, histopathological results revealed that NP caused alterations in the testes. In conclusion, propolis reduced the oxidative stress toxicity induced by NP in the reproductive system of male albino rats.

Key words: propolis; nonylphenol; reproductive system; antioxidant enzymes.

INTRODUCTION

Several common pollutants have profound effects on reproduction and growth of animals because they mimic or suppress the actions of sex hormones. Of particular concern, alkylphenol-polyethoxylates (APEOs) are a large number of nonionic surfactants in commercial production of lubricating oil additives, resins, plasticizers, detergents, herbicides, paints and cosmetics (Hernandez-Rodrigues et al., 2007). These compounds enter the aquatic environment mainly from sewage treatment (Trudeau et al., 2002). Nonylphenol (NP) and octylphenol (OP), formed as hydrophobic metabolites of APEOs, act as estrogenic compounds (Blake et al., 2004), and can accumulate within the internal organs of animals in concentrations enough to disrupt the reproductive and endocrine system (Ying et al., 2002). Nonylphenol (para-nonylphenol, 4-NP) metabolites, including linear and branched isomers of nonylphenol (n-NP and t-NP, respectively), have been considered as endocrine disrupting substance causing reproductive dysfunction, and increasing production of reactive oxygen species in testis, liver, kidney, and brain in many animal (Hsieh et al., 2009; Zha et al., 2007). Male mice exposed to NP (200mg/kg) during sexual maturation period suffered from damaged reproductive development (El-Dakdoky and Helal, 2007).

Free radicals are reactive oxygenated compounds (ROS) that have unpaired electrons with deleterious effects on macromolecules (DNA, proteins and lipids). They are continually produced by the body during cell metabolism, cellular respiration, and also as a consequence of some pathological events. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs), and their oxidative destruction by free radicals is known as lipid peroxidation, which causes tissue injury. Cells are able to defend themselves from the destructive potential of ROS through their own antioxidant mechanisms, which includes enzyme systems, vitamins, elements, and some antioxidant molecules. Normally there is an exquisite balance between production and destruction of ROS, but once this equilibrium is disturbed, ROS are produced excessively and all tissues are exposed to oxidative injury (Bindhumol et al., 2003). Many reports suggest that NP and OP caused tissue injury by enhancing ROS generation (Chitra et al., 2003; Kabuto et al., 2004; Okai et al., 2004), where their administration in fish and rodents profoundly impaired testicular function - as evidenced by reduced testis size, low circulating testosterone, disturbed testicular structure and suppressed spermatogenesis (Nagao et al., 2001; Tan et al., 2003; Cardinali et al., 2004).

Alternatively, low doses of NP (below 100 ng/ml), were reported to enhance apoptosis in different cell lines such as PC12 cells (Aoki et al., 2004), murine neural stem cells (Kudo et al., 2004), and the human T lymphocyte cell line Jurkat cells (Yao et al., 2007), mainly through DNA fragmentation.

Propolis is a honeybee product with a very complex chemical composition. It has an antioxidant property owing to its high content of polyphenolic composites including flavonoids, tannins, terpenoids and phenolic

Corresponding Author: Kamal A. Shalaby, Biochemistry Department, Faculty of Science, Ain Shams University, Abbassia, 11566, Cairo, Egypt.
Tel: +202 26335549; Mob: +2 (0106) 8346747, Fax: +202 26853561,
E-mail: kamal_shalabi@sci.asu.edu.eg
compounds which have free-radical scavenging activity. Numerous biological and pharmacological properties of propolis have been noted, including antibacterial, antifungal, anti-inflammatory, antioxidant, immunomodulatory, antiviral and anticarcinogenic properties (Ramos and Miranda, 2007; Sabuncuoglu et al., 2007). Propolis can also reduce the levels of ROS; such as H$_2$O$_2$ and NO, that might be responsible for its anti-inflammatory effects (Tan-No et al., 2006). Another compound in the structure of propolis, caffeic acid phenethyl ester, blocks the production of ROS (Hosnutter et al., 2004).

The current work aimed to determine the toxic effects of NP on male albino rat reproductive system and to evaluate the ability of propolis to reduce or neutralize the toxicity induced by NP.

**MATERIALS AND METHODS**

**Chemicals:** NP was purchased from Sigma-Aldrich (Sigma-Aldrich chemical Co. St. Louis, MO, USA). Propolis was obtained from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, USA. All other chemicals were of analytical grade.

**Experimental design:** Fifty male albino rats (average weight 150-180g) were used in this study. Animals were obtained from the Egyptian Holding Company for Vaccines and Antisera, Cairo, Egypt. All care and uses of animals were performed according with institutional and governmental guiding principles. After two weeks of acclimation, animals were divided into five groups (n = 10). Group 1(control); rats were fed on standard diet, group 2 (NP); rats were orally administered NP by gavage at a dose of 100 mg kg$^{-1}$ day$^{-1}$ for 6 weeks (Chapin, et al., 1999), group 3 (propolis); rats were orally administered propolis by gavage at a dose of 50 mg kg$^{-1}$ day$^{-1}$ for 6 weeks (Yousef and Salama, 2009), group 4 (prophylactic); animals were orally administered with NP at concentration of 100 mg kg$^{-1}$ day$^{-1}$ and propolis at a concentration of 50 mg kg$^{-1}$ day$^{-1}$ for 6 weeks, group 5 (curative); rats were orally administered with NP at a concentration of 100 mg kg$^{-1}$ day$^{-1}$ and propolis at a concentration of 50 mg kg$^{-1}$ day$^{-1}$ for 6 weeks followed by oral administration of propolis at a concentration of 50 mg kg$^{-1}$ day$^{-1}$ for 6 weeks.

At the end of the experiment, animals were sacrificed using ether anesthesia and decapitation after 24 hours fasting period from the final administration. Blood samples were collected and divided into 2. Samples were left to clot, and then centrifuged at 3000 rpm for 15 minutes to separate blood serum, or collected on EDTA, centrifuged at 3000 for 20 minutes and stored at -80°C as plasma samples. Testes were immediately excised, washed using chilled saline solution, blotted, weighed and processed for biochemical studies.

**Testicular homogenate:** The testicular tissues were homogenized in Mechanika Precyzyjna Warszawa Universal Laboratory Aid type homogenizer MPW-309, Poland in 10-volume 0.25 M-sucrose containing 0.05 mM-EDTA and 5 mM mercaptoethanol, buffered with 0.05 M potassium phosphate (pH 7.4). The homogenate was centrifuged at 4°C in a cooling centrifuge, CRU-5000 centrifuge, DAMON/IEC Division, USA.

**Steriodogenic enzyme 17-ketosteroid reductase:** Aliquots of the resulting supernatant were used as a crude preparation of 17-ketosteroid reductase according to the method described by Katryna and Anita (1980).

**Testicular antioxidant enzymes and malondialdehyde:** The activity of glutathione S-transferase (GST) was measured according to the previously described method of Habig et al. (1974). The absorbance was measured spectrophotometrically at 340 nm using UV-Double Beam spectrophotometer UNICAM (UVE 054103), Helios Epsilon, USA. The enzyme catalase (CAT) activity was measured spectrophotometrically according to Xu et al. (1997). The SOD activity was determined as previously described by Minamai and Yoshikawa (1979). Testicular malondialdehyde (MDA) levels were determined according to the method described by Draper and Hadley (1990).

**Plasma testosterone and luteinizing hormone:** Testosterone was determined using the ARCHITECT Chemiluminescence Microplate Immunoassay (CMIA) system I 2000 (B7k730), Abbott Diagnostic Division Lissamuck, Longford Co. Longford Ireland. Luteinizing hormone (LH) was determined using the ARCHITECT Chemiluminescence Microplate Immunoassay (CMIA) system (34-4522/R8), Abbott Diagnostic Division, Abbott Park, IL 60064 USA. Plasma total antioxidant capacity was measured using the commercial kit of Biodiagnostic Company according to Koracevic et al. (2001).

**Proteins:** The protein content of testicular tissue and serum was determined according to the method described by Lowry et al. (1951). Albumin was measured according to Doumas et al. (1971).

**DNA fragmentation:** Testes samples were collected immediately after sacrificing the animals. The proportion of fragmented DNA was determined according to the method described by (Gibb et al., 1997).

**Histopathology:** Tissues taken from testes of rats were cut, immediately fixed into 10% formaldehyde, and processed for preparation of paraffin blocks as described by Drury and Wallington (1980).

**Statistical analysis:** Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. The criterion for statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS Inc., USA).
Results:

Serum total proteins, albumin and globulin levels in the different groups showed a decline in the NP group, which was normalized after treatment with propolis as depicted in Figure 1.

![Figure 1](image-url)

*Significant difference from the control group at $p<0.05$

# Significant difference from the nonylphenol group at $p<0.05$

Oxidative stress: NP caused a significant decrease in plasma total antioxidant capacity, SOD, CAT, and GST, which were significantly elevated after treatment with propolis; as prophylactic or curative, but were still lower than control levels (Table 1). Conversely, MDA activity was elevated in NP group, and improved significantly after treatment with propolis, but the decrease did not reach the normal level.

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>Group 1 (Control)</th>
<th>Group 2 (Nonylphenol)</th>
<th>Group 3 (Propolis)</th>
<th>Group 4 (Prophylactic)</th>
<th>Group 5 (Curative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total antioxidant capacity</td>
<td>1.09±0.06</td>
<td>0.59±0.03*</td>
<td>1.19±0.02*</td>
<td>0.83±0.01**</td>
<td>0.76±0.02**</td>
</tr>
<tr>
<td>MDA</td>
<td>125.63±5.26</td>
<td>477.86±11.35*</td>
<td>100.00±5.10*</td>
<td>192.29±8.29** 250.92±4.96**</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>48±1.28</td>
<td>17.68±2.09*</td>
<td>49.15±2.27</td>
<td>40.84±1.99**</td>
<td>36.27±1.14**</td>
</tr>
<tr>
<td>CAT</td>
<td>0.38±0.01</td>
<td>0.12±0.01*</td>
<td>0.40±0.01*</td>
<td>0.30±0.01**</td>
<td>0.27±0.01*</td>
</tr>
<tr>
<td>GST</td>
<td>2.99±0.26</td>
<td>1.69±0.32*</td>
<td>3.75±0.42*</td>
<td>2.44±0.27**</td>
<td>1.97±0.28*</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD; n=10 for each treatment group.

*Significant difference from the control group at $p<0.05$

# Significant difference from the nonylphenol group at $p<0.05$

Plasma testosterone and LH: The levels of plasma testosterone and LH were significantly lower in NP group compared to control group, prophylactic and curative groups (Figure 2). When propolis was orally administered alone, their levels were significantly increased compared to control group.

Activity of testicular 17-ketosteroid reductase (17-KSR) and protein content: As shown in Table 2, the activity of 17-KSR and protein content were significantly decreased in NP group compared to control group and NP + propolis groups. Propolis alone caused increase in these two parameters compared to control group.

DNA fragmentation: The rate of testicular DNA fragmentation was significantly higher in NP group compared to control group. Both prophylactic and curative groups showed that propolis exerted a protective influence against NP-induced toxicity, reducing the damage of testicular DNA (Figure 3).

Reproductive organs damage: Results indicated significant decrease in the relative weights of testes and prostate in animals treated with NP compared to control and propolis alone (Table 3). Propolis showed ameliorative effect in both prophylactic and curative groups and the weights of these tissues reached the control values.
Fig. 2: Plasma level of testosterone (ng/ml) and LH (mIU/ml) of male albino rats treated with nonylphenol, propolis and combination of both.

*Significant difference from the control group at $p<0.05$.

# Significant difference from the nonylphenol group at $p<0.05$.

Table 2: Changes in the concentrations of testicular 17-ketosteroid reductase (U/min/mg protein) and testicular protein content (mg/g tissue) of male albino rats treated with nonylphenol, propolis and combination of both.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Group 1 (Control)</th>
<th>Group 2 (Nonylphenol)</th>
<th>Group 3 (Propolis)</th>
<th>Group 4 (Prophylactic)</th>
<th>Group 5 (Curative)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17-KSR</td>
<td>18.2±0.94</td>
<td>10.4±0.68*</td>
<td>23.4±1.19*</td>
<td>15.2±0.43**</td>
<td>12.2±0.30**</td>
</tr>
<tr>
<td></td>
<td>Total protein content</td>
<td>88.8±1.77</td>
<td>61.9±1.54*</td>
<td>133.3±4.94</td>
<td>79.8±0.76**</td>
<td>72.6±1.05**</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD; n=10 for each treatment group.

*Significant difference from the control group at $p<0.05$.

# Significant difference from the nonylphenol group at $p<0.05$.

Fig. 3: Rate of DNA fragmentation of rat testes, effect of nonylphenol (NP) and propolis, alone and combined with NP.

Values are expressed as means±SD; n=10 for each treatment group.

*Significant difference from the control group at $p<0.05$.

# Significant difference from the nonylphenol group at $p<0.05$.

Table 3: Relative weights (g/100g body weight) of testes and prostate treated with NP and propolis, alone and combined with NP.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Group 1 (Control)</th>
<th>Group 2 (Nonylphenol)</th>
<th>Group 3 (Propolis)</th>
<th>Group 4 (Prophylactic)</th>
<th>Group 5 (Curative)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testes</td>
<td>0.66±0.011</td>
<td>0.49±0.025*</td>
<td>0.75±0.040</td>
<td>0.53±0.046</td>
<td>0.59±0.022*</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>0.18±0.039</td>
<td>0.13±0.016*</td>
<td>0.19±0.027</td>
<td>0.17±0.029*</td>
<td>0.17±0.031*</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD; n=10 for each treatment group.

*Significant difference from the control group at $p<0.05$.

# Significant difference from the nonylphenol group at $p<0.05$. 
Histopathology: Histopathological examination revealed normal architecture of testicular parenchyma, normal functioning spermatogonial cells and spermiogenesis of normal control group (Fig. 4A). Lesions observed in NP group were severe and variable. Marked atrophy of seminiferous tubules associated with edema of the intertubular tissue was commonly observed (Fig. 4B). Some seminiferous tubules showed degeneration and pyknosis of spermatogonial cells and absence of spermiogenesis (Fig. 4C), others showed necrosis and desquamation of the spermatogonial cells (Fig. 4D). In both cases the tubules were widely separated by edema of the intertubular tissue. In severe cases, vacuolation, necrosis and desquamation of spermatogonial cells with accumulation of the necrotic debris in the tubular lumen were observed (Fig. 4E & F). Seminiferous tubules were almost similar to those of control group and no pathological alterations were observed in group 3 (Fig. 5A). Histopathological alterations were very mild compared with those observed in group 2. Some tubules revealed pyknotic and / or vacuolated spermatogonial cells and decreased spermiogenesis (Fig. 5B). Intertubular edema and congestion of intertubular blood vessels were also observed (Fig. 5C). Histopathological alterations were almost similar to those observed in group 4. Degeneration and pyknosis of spermatogonial cells and decreased spermiogenesis were more obvious (Fig. 5D & E).

Fig. 4: A. Testes of control group showing normal architecture of testicular parenchyma, normal functioning spermatogonial cells and spermiogenesis (H&E stain, x20). B. Testes of group 2 showing marked atrophy of seminiferous tubules associated with edema of the intertubular tissue (H&E stain, x10). C. Testes of group 2 showing degeneration and pyknosis of spermatogonial cells and absence of spermiogenesis (H&E stain, x20). D. Testes of group 2 showing necrosis and desquamation of the spermatogonial cells (H&E stain, x40). E. Testes of group 2 showing vacuolation, necrosis and desquamation of spermatogonial cells with accumulation of the necrotic debris in the tubular lumen (H & E stain, x20). F. Higher magnification of (E)(H&E stain, x40).
Fig. 5: A. Testes of group 3 showing normal testicular parenchyma (H & E stain, x 20). B. Testes of group 4 showing pyknotic and/or vacuolated spermatogonial cells and decreased spermiogenesis (H&E stain, x 20). C. Testes of group 4 showing Intertubular edema and congestion of intertubular blood vessels (H&E stain, x 20). D. Testes of group 5 showing Degeneration and pyknosis of spermatogonial cells and decreased spermiogenesis (H&E stain, x 20). E. Higher magnification of (D) (H & E stain, x 40).

Discussion:

This study aimed to evaluate whether the exposure to nonylphenol (NP) induces oxidative stress in reproductive organs of male rats and if co-administration or treatment with propolis after exposure to NP can reduce the toxic effect of NP. A wide variety of reactive oxygen species (ROS) are produced in the course of the normal metabolism in biological systems and they have several important physiological functions, but their accumulation beyond the needs of the cell can potentially damage macromolecules (Schulz et al., 2000). However, excessive generation of free radicals can occur due to endogenous biological or exogenous environmental factors, such as exposure to endocrine disruptors (Korkmaz et al., 2010). NP is one of the endocrine disruptors that are produced as a final degradation product of alkylphenol polyethoxylates, nonionic surfactants in many industrial commercial products. NP can cause tissue injury in many organs by leading to the formation of ROS (Chitra et al., 2003). It has been demonstrated that NP could interfere with reproduction in fish, reptiles and mammals; it can also induce cell death in gonads and changes to other reproductive parameters (Gong and Han, 2006).

Previous reports demonstrated that increased MDA concentration due to lipid peroxidation and decreased levels of antioxidant enzymes, resulting in oxidative cell damage (Vidyasagar et al., 2004). In the present study, level of testicular MDA, a marker for lipid peroxidation, significantly increased in NP group compared to control group, this result is in agreement with the previous results (Karafakioglu and Aslan, 2010). According to Katz et al., (1996), lipid peroxidation is often the first parameter analyzed for proving the involvement of free radical damage. Thus, the presence of MDA is considered as an indicator of free-radical damage through membrane lipid peroxidation. Conversely, MDA was significantly lower in propolis group compared to control group, and also in both prophylactic and curative groups compared to NP group. Both flavonoids and phenolic compounds that are found in high concentrations in propolis are known to be antioxidant substances with free...
radical scavenging activity (Galvao et al., 2007). This may explain the ability of propolis to ameliorate the oxidative stress toxicity induced by NP.

The antioxidant defense system includes small molecular antioxidants, antioxidant enzymes and metal chelating agents. The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, ensuing in oxidative cell damage (Vidyasagar et al., 2004). The present study revealed that plasma total antioxidant capacity was significantly decreased in NP group, this result is in agreement with previous data shown by Chitra et al., (2002) who reported that activities of antioxidant enzymes SOD, CAT, GSH and GPX decreased significantly while the levels of H$_2$O$_2$ generation and lipid peroxidation increased significantly in the animals treated with NP when expressed in terms of milligram protein and milligram DNA. However the concentration of total antioxidant enzymes as well as serum total proteins were significantly increased when propolis was administered in both prophylactic and curative groups compared to NP group. Administration of propolis alone induced significant increase in total antioxidant capacity compared to control group. This may be due to the free radical scavenging property of flavonoids in propolis that are known to have antioxidant benefits more than vitamins C and E. The antioxidant capacity can prevent the free radicals acting on the cell lipids, proteins and even the DNA (Matsushige et al., 1996).

Enzymatic scavengers like SOD, CAT and GST protect the system from deleterious effects of ROS. The present data revealed that NP caused marked oxidative impact as evidenced by the increase in testicular lipid peroxidation (MDA level) as well as the decrease in testicular antioxidants activities including SOD, CAT and GST compared to their activities in control group. This might reflect the oxidative damage and inhibitory effect of NP on testes tissues affecting enzymatic and non-enzymatic antioxidants in testes. This data is in agreement with the previous results of Chitra and Mather (2004) who showed that treatment of rats with NP increased the level of ROS production. Also, other results of Karafakioglu and Aslan (2010) showed that antioxidant enzymes concentrations and activities significantly decreased in rat blood by NP intoxication. However, when propolis was used in both prophylactic and curative doses, the activities of SOD, CAT and GST were significantly increased compared to NP group. This result is supported by the fact that propolis has antioxidant activity and is rich in flavonoids and phenolic compounds that have free radical scavenging activity (Galvao et al., 2007) that relief the intoxication induced by NP. Ability of propolis to reduce the testicular oxidative stress induced by NP was supported by Yousef and Salama (2009) a similar study, which proved that propolis attenuated the testicular toxicity induced by aluminum by decreasing level of thiobarbituric acid-reactive substances and increasing the activity of CAT, GST and level of GSH.

DNA fragmentation is used to detect cell apoptosis (Zhan et al., 2001). The present results showed that NP induced significant testicular DNA fragmentation compared to control group. This result is in agreement with previous reports on different cells, such as PC12 cells (Aoki et al., 2004), murine neural stem cells (Kudo et al., 2004) and Jurkat cells (Yao et al., 2007). When propolis was administered either in prophylactic or curative groups, it significantly reduced the level of DNA fragmentation due to its antioxidant ability that could reduce the toxic effect of NP on testes. Propolis can have a double benefit of protecting healthy cells whilst killing cancer cells (Chen et al., 2003). Fitzpatrick et al., (2001) also showed that propolis could protect healthy DNA and restrict macrophage activity.

Protein content of testicular tissue is considered as a marker of tissue injury, damage and rewound healing (Yousef and Salama, 2009). The present results showed significant decrease in testicular protein content in animals treated with NP compared to control group, another indication to the oxidative damage induced by NP on testicular tissue. Serum total proteins, albumin and globulin were significantly decreased in NP group compared to control group. Propolis was able to restore testicular protein, serum total proteins, albumin and globulin contents. Our results indicated significant decrease in relative weights of testes and prostate in animals treated with NP compared to control and propolis alone. However, in both prophylactic and curative groups, propolis alleviated the toxicity induced by NP and the weight of these tissues reached the control values.

The present study showed that NP caused significant decline in the activity of testicular 17-KSR compared to control group. The significant decrease of this enzyme in NP intoxicated rats interprets the decreased level of testosterone in such group. However, administration of propolis caused increase in the activity of this enzyme and subsequently increased testosterone level in propolis, prophylactic and curative groups. The low activity of 17-KSR is in accordance to the nature of the enzyme as a protein, that was damaged like other proteins by NP-induced oxidative toxicity, and treatment with propolis attenuated the damage effect on the tissue protein content and hence on the enzyme, i.e. as a sort of repairing effect of propolis. Previous studies reported similar effects on 17-KSR and testosterone in rats due to oxidative toxicity induced by cadmium (Lafuente et al., 2001; Salama and El-Bahr, 2007). In fact, this is additionally confirmed by our results, where rats treated with propolis only showed an increase in their testicular protein, which in turn enhanced the 17-KSR enzyme activity and consequently blood testosterone and LH concentrations, as opposed by the toxic NP effect that caused low testosterone and LH plasma levels.
The low concentrations of testosterone in NP group can be attributed to the low LH level, given that the action of LH is mediated by the intracellular secondary messenger cAMP, which enhances the conversion of acetate to squalene, the precursor for cholesterol synthesis, and enhances the conversion of cholesterol to pregnenolone, a necessary step in the formation of testosterone. The low level of release and synthesis of testosterone can also be due to the decline in the activity of the testicular 17-KSR which converts androstenedione to testosterone. This is in agreement with the previously presented data by Wu et al. (2010) who reported decreased testosterone concentrations in rat plasma after intravenous injection of NP (100 μg/kg) at different time intervals. Propolis was able to reduce the toxicity induced by NP as indicated by increase in the activity of 17-KSR and concentration of testosterone, LH and protein content in both prophylactic and curative groups and when it was administered alone. Testicular oxidative stress appears to be a common feature in much of what underlies male reproductive system toxicity, which suggests that there may be benefits of developing better antioxidant therapies for relevant cases. The anti-oxidative property of flavonoids and phenolic compounds of propolis inhibited the formation of free radicals and reduced the oxidative stress, which might explain our results, which is in line with the findings of Nirala et al., (2008).

The histological examination showed that testes of rats treated with NP exhibited variable lesions, marked atrophy, edema, absence of spermiogenesis, together with degeneration, pyknosis and necrosis of spermatogonial cells, these observations are in agreement with the findings of Jobling et al. (2009). On the other hand, testes of rats treated with NP plus propolis showed somewhat healthy appearance as revealed by the normal seminiferous tubules.

**Conclusion:**

The present study shows that NP was able to induce testicular oxidative stress toxicity which is reflected by increased MDA concentration and reduced activity of 17-KSR, SOD, CAT and GST and reduced protein content. NP intoxication also induced low testosterone and LH concentration. Testicular damage was also proved by the decrease in testes relative weight, increased DNA fragmentation and the histological results. Moreover, the results showed that propolis could provide advantages against NP intoxication due to its antioxidant activity by restoring the activity of testicular antioxidant enzymes and 17-KSR. Propolis was also able to enhance the levels of protein content, testosterone and LH and to reduce the level of DNA fragmentation. The results prove the antioxidant activity and the free radical scavenging ability of propolis. Consequently, propolis could be used as a potential antioxidant against NP intoxication.

**ACKNOWLEDGMENTS**

The authors thank Biochemistry Department, Ain Shams University, Faculty of Science for laboratory facilities and instrumental support of this work. Also, thanks for Research and Training Center of Ain Shams University for providing the suitable housing, care and handling for animals. We also thank Professor Elshazly M.O. (professor of pathology-Faculty of veterinary medicine-Cairo university) for his help in histopathologic examination.

**Abbreviations:**

(NP): Nonylphenol  
(SOD): Superoxide dismutase enzyme  
(CAT): Catalase enzyme  
(GST): Glutathione S-transferase enzyme  
(MDA): Malondialdehyde  
(LH): Luteinizing hormone  
(ROS): Reactive oxygen species

**Conflict of Interest:**

The authors declare that there are no conflicts of interest.

**REFERENCES**


