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Vitamin E ameliorates the toxic effect of nonyphenol on reproductive performance of male rats

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Abstract
Nonylphenol (NP) has been found to exert estrogenic effects on organisms and thus interfere male reproductive behavior. Therefore, the present study was conducted to evaluate the effects of nonylphenol on defense antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione reductase (GR). Moreover, its effect on testosterone and gonadotropin hormones (FSH, LH) on testies of rats was studied. Also the effect of nonylphenol on sperm characteristics was investigated. Adult male rats were divided into four groups: one group was gavaged with NP dissolved in corn oil, second one is the control group rats which were gavaged with corn oil alone, vit. E. alone was administrated to the third group and the last group was gavaged with a combination of vit. E. and NP. These treatments lasted 30 days. The activity levels of the SOD, CAT, GR and testosterone were significantly decreased in NP treated rats when compared to the control group, while the MDA and gonadotropins were significantly increased. Moreover, sperm count, motility and viability were significantly decreased in this treated group. Vit. E caused significant recovery on the different measured parameters once combined with the nonylphenol. In conclusion, this study revealed that NP treatment impairs spermatogenesis and has estrogenic potential which disrupt antioxidant balance which leads to oxidative stress. This study also supports the significance of vit. E. as an antioxidant.

Keywords: estrogen; nonylphenol; male rats; gonadotropins; vitamine E

Introduction
Many environmental pollutants have received great attention due to their potential estrogenic action, so these pollutants are referred as environmental estrogens. These environmental estrogens are classified as endocrine disrupting compounds (EDCs). Endocrine disrupter is defined as an exogenous substance that alters one or more functions of the endocrine system, and consequently causes adverse health effects in intact organisms [1, 2]. Determination of EDCs in environmental samples is an analytical challenge because these compounds exhibit different physiochemical characteristics, they occur in the aquatic environment in relatively low concentrations and furthermore environmental samples are considered as complex matrices [3].
Alkylphenol ethoxylates (APEs) and related compounds have been reported to be estrogenic [4]. APEs are non ionic surfactants that have been used over 50 years, primarily in the manufacture of plastics, elastomers, agricultural chemicals, and pulping and industrial detergent formulations. They consist of a branched-chain alkylphenol (AP) which has been reacted with ethyleneoxide, producing an ethoxylate chain. The main APs used are nonylphenol (NP) with nonylphenol ethoxylates (NPnEO) taking approximately 80% of the world market [5]. NP is by far the most commercially important AP in Europe, with an annual production of about 75000 tons [6].
Several studies have reported the adverse effects of NP on adult and developing male reproductive system as it induces oxidative stress like other environmental toxicants [2]. Exposure of male albino rats to NP at low concentrations induces oxidative stress in testes and epididymis of adult rats. They demonstrated that indices of testes and epididymis were significantly decreased in NP treated rats than controls [7, 8]. Also, the activity levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase levels were decreased significantly in NP treated rats when compared to the control group. It has been documented that vitamin E (vit.E) is a potent scavenger of free radicals and is able to prevent the membrane damage mediated by free radicals [9]. In addition, the antioxidant's role of this vitamin has been reported in reducing testicular oxidative stress [10]. Moreover, Reza Momeni *et al*. (2009) [11] examined the effect of NP on sperm parameters and reproductive hormones on rats and also studied the effect of vit. E. The results demonstrated that NP-treated rats presented a significant decrease in testis weight, sperm number and sperm motility compared to control and NP+vit. E groups. Also, a significant increase was also found in sperm viability in vit. E group compared to both NP and control groups. Moreover, rats treated with NP showed a significant decrease in FSH level. However, testosterone and LH level remained constant.
The present study aimed to evaluate the effect of p-nonylphenol and vit. E on some enzymatic activities in testes, sperm parameters and the level of gonadotropin hormones and testosterone in developing albino rats.

Materials and Method
Chemicals and experimental animals
The para-nonylphenol (p-NP, CAS: 8485-2-15-3) used in the present study was purchased from Sigma-Aldrich Inc. (St.Louis, MO, USA). It contains several isomers (a mixture of isomers with differently branched nonyl side chain), with a purity greater than 98%, while the main impurities are traces of 1,000 µg.
Adult male albino rats (n=40, body weight ranges from 150 to 200 g) were obtained from the Animal House, Medical Technology Center, Medical Research Institute, Alexandria University, Egypt. Animals were housed in plastic cages at an

environmentally controlled room (constant temperature 25-27 °C, with 12 h light/dark cycle) for two weeks prior to beginning of the experiment for adaptation to the laboratory conditions. They were provided with tap water and standard rat diet (protein 24%, fat 5%, fiber 4%, carbohydrates 55%, calcium 0.6%, moisture 10% and ash 9%). The animals were randomly divided into 4 equal groups (10 rats each). Each group was housed in separate cage. Oral administration was applied for the four groups as follow: corn oil (0.5 ml, control), vitamin E (100 mg/kg body weight), para-nonyl-phenol (0.5 mg/kg body weight), and vitamin E with a p-NP, respectively.

At the end of the experiment which lasted 30 days, rats were sacrificed by cervical decapitation and dissected. Testis were collected, cleaned from adhering fats and connective tissues, washed with saline solution, blotted and weighed.

Preparation of testis homogenates

A portion of testis tissues from each rat was stored at -20°C. The reminder portion were minced and homogenized in 5-10 ml cold buffer (i.e, 50 mM potassium phosphate, pH 7.4, 1 mM ethylene diamine tetracetic acid (EDTA) for the determination of biochemical and enzymatic parameters). Homogenates were centrifuged at 10,000 rpm for 20 minutes at 4°C [12] and the clear supernatants were used to determine some enzymatic and non-enzymatic antioxidant in testis: Catalase (CAT), Glutathione reduced assay (GSH), and Superoxide dismutase assay (SOD). Also, this homogenates were used to determine lipid peroxidation level by malondialdehyde (MDA) assay.

Assessment of enzymic antioxidants

Superoxide dismutase (SOD) was assayed by the method of Nishikimi *et al*. (1972) [13]. Briefly, this assay relies on the ability of the enzyme to inhibit the phenazine metho sulphate mediated reduction of nitro blue tetrazolium dye. The assay mixture contained 50 mM phoshate buffer, 1 mM nitroblue tetrazolium (NBT), 1 mM/l NADH, 0.1 mM/l phenazine metho sulphate (PMS) and extraction reagent. Measure the increase in absorbance at 560 nm for 5 min. for control ($\Delta A_{control}$) and for sample (ΔA_{sample}) at 25 ºC.

Calculation:

$$\text{Percent inhibition} = \frac{\Delta A_{control}}{\Delta A_{control} - \Delta A_{sample}} \times 100$$

$$\text{SOD activity (U/g tissue)} = \frac{\% \text{ inhibition} \times 3.75 \times 1}{\text{g tissue used}}$$

Catalase (CAT) was assayed as previously mentioned by Aebi (1984) [14]. Briefly, the assay mixture contained 2.40 ml of phosphate buffer (100 mM, pH 7.0), 500 mM/l hydrogen peroxide and peroxidas enzyme source and preservative. Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. Incubate 10 min. at 37ºC, read sample (A_{sample}) against sample blank and standard ($A_{standard}$) against standard blank at 510 nm.

Glutathione reductase (GR) was assayed by the method of Beutler *et al*. (1963) [15]. Briefly, the method based on the reduction of 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Lipid peroxidation (LPO), malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm. A break down product of LPO, thiobarbituric acid reactive substance was measured by the method of Ohkawa *et al*., (1979) [16]. Briefly, the stock solution contained equal volumes of trichloroacetic acid 15% (w/v) in 0.25 N HCl and 2-thiobarbituric acid 0.37% (w/v) in 0.25 N HCl. One volume of the test sample and two volumes of stock reagent were mixed in a screw-capped centrifuge tube, vortexed and heated for 15 min on a boiling water bath. After cooling on ice the precipitate was removed by centrifugation at 1000 g for 15 min and absorbance of the supernatant was measured at 532 nm against blank containing all the reagents except test sample. The value is expressed as µmol of malondialdehyde formed/min per mg protein.

Hormones Assay

Testosterone (T), luteinizing hormone (LH), and and follicle – stimulating hormone (FSH*)* were assayed as follow:-

Determination of testosterone (T)

According to Saknma (2009) [17] the microtiter plate has been pre-coated with an antibody specific to testosterone. Standards or sample are then added microtiter plate wells with a HRP- conjugated testosterone and antibody then a TMB (3.3, 5, 5\ tetramethhyl – benzidine) substrate solution is added to each well. The enzyme substrate reaction is terminated by the addition of asulphuric acid solution and the color change is measured spectropotometrically at 450 nm. The concentration of testosterone in the samples is then determined by comparing the optical density of the samples to a standard curve. Average the duplicate readings for each standard, Blank, and sample and subtract the optical density of blank, construct a standard curve.

Determination of luteinizing hormone (LH)

According to Wakabayashin (1998) [18], standards and samples are incubated in monoclonal anti-LH and antibody-coated wells to capture LH. After 2 hours of incubation and washing, biotin-labeled anti-LH β antibody is added and incubated further for 1 hour to bind with captured LH. After washing HRP (horse radish peroxidased)-labeled avidin is added, and incubated for 30 minutes. After washing, HRP complex remained in wells are reacted with a chromogenic substrate (TMB) for 20 minutes, and reaction is stopped by addition of acidic solution, and absorbance of yellow product is measured by microplate reader at 450 nm.

Determination of follicle – stimulating hormone (FSH)

In rat FSH ELISA kit, biotin – conjugated anti-FSH and antibody-Coated wells. After 15 – 18 hours incubation and washing HRP (horse radish peroxidase) – (HMB) for 20 minutes, and reaction is stopped by addition of acidic solution, and absorbance of yellow product is measured spectrophotometrically at 450 nm standard curve is prepared by plotting absorbance against standard FSH concentrations.

FSH concentration in unknown samples are determined using a standard curve [19].

Sperm quality analysis

Collection of epididymal sperms

At the end of the experimental period, epididymis was removed immediately from sacrificed animals and the caudal epididymis was used for sperm collection. This was done by slicing the caudal epididymis according to Gray *et al*. (1988) [20]. Epididymis was chopped with the help of sharp razor blade in 5 ml physiological solution and incubated for 5 mins at 35 ºC. After several washing, the collected sperms in the medium were used for sperm motility, count and abnormalities. These parameters were carried out using a computer assisted semen analysis - sperm Vision ™ CASA System (Eclipse E-200 Nikon Co., Japan) [21].

Sperm count

The sperm count was determined with a hemocytometer using a modified method described by Turk *et al.* (2007) [22]. Briefly, one drop of the supernatant fluid containing all spermatozoa was transferred to counting chamber (depth 10 μm, Sefi-Medical Instruments, Germany) and allowed to stand for 5 min. The sperm cells in chambers were evaluated as million sperm cells per ml of suspension under 200x magnification using light microscope.

Sperm Morphology

Sperm morphological study was done by using a part of sperm suspension for preparing smears to evaluate the sperm morphological abnormalities. Abnormalities were assessed using the giemsa stain. Following liquefaction, 10 µL of semen was spread onto a glass slide and allowed to air-dry at room temperature. The smears were then stained with Giemsa stain and sperm morphology was assessed according to WHO criteria (1992) [23]. Two different examiners counted 200 cells *per* smear using bright field illumination at final magnification of 1000x and oil immersion. According to WHO criteria, a morphologically normal spermatozoon has an oval head and an acrosome covering 40%–70% of the head area. A normal spermatozoon has no neck, midpiece, tail abnormalities nor cytoplasmic droplets larger than 50% of the sperm head [24].

Sperm the unknown, DS = optica density of the standard, 0.1 = mg. of glucose in 2 ml. of standard solution, **otility**

One drop of the suspension was placed on a slide, covered by 24x24 mm cover slips and evaluated under light microscope. Sperm motility was categorized into “motile” or “immotile”. The percentage of forward progressive sperm motility was evaluated visually at 400x using a light microscope with heated stage by Sonmez *et al*. (2005) [25]. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score.

Determination of glycogen

A mixture of anthrone reagent, trichloroacetic acid and ethanol was used for such determination according to a method described by Nicholas *et al*. (1956) [26]. This method is done in cold water then immersion of solution in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 minutes and then removed to a cold water bath and cooled to room temperature. The tubes and stoppers are wiped dry and the contents of each tube are transferred to a calorimeter tube and read at 620 mp after adjusting the calorimeter with the reagent blank. Glycogen content (mg/100 g of tissue) is calculated as follows:

$$\frac{DU}{DS} * 0.1 * \frac{volume\ of\ extract}{gm.of\ tissue} * 100 * 0.9$$

where DU = optical density of the unknown, DS = optica density of the standard, 0.1 = mg. of glucose in 2 ml. of standard solution, 0.9 = factor for converting glucose value to glycogen value.

Determination of fructose

Fructose colorimetric and fluorometric method is done by using Fructose Assay Kit, In this kit, free fructose is enzymatically converted to β-glucose, which is then specifically converted to a product that reacts with OxiRed Probe to generate color (λ=570 nm) and fluorescence (Ex/Em=535/587 nm). The kit provides a rapid, simple, sensitive, and reliable method suitable for high throughput assay of D-fructose.

Statistical Analysis

The values are expressed as mean±SE. The results were computed statistically using statistical Package for Social sciences (SPSS software package, version 15) using one-way analysis of variance (ANOVA). Post hoc testing was performed for inter-group comparison on using the LSD. $P<0.05$ was considered as significant [27].

Results

The activites of superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) were measured in testis of male rat treated with p-NP, vitamin E and their combination. Table 1 indicates that antioxidant enzymes levels decreased significantly ($p <0.05$) in NP treated group compared to those of the control group. In vitamin E treated group, no significant difference was detected between the groups. The combination of p-NP and vit.E. caused recovery of the enzymes levels but there is still significant differences compared with the control group.

The concentration of lipid peroxidation end product (malondialdehyde, MDA) in testis of male rate is also presented Table 1. The results indicate that treatment with p-NP significantly ($p<0.05$) increased MDA level in testis compared with the control group. While treatment with vitamin E alone significantly ($p<0.05$) decreased the MDA level compared with the control group. Treatment with vitamin E and p-NP in combination group caused significant ($p<0.05$) decrease of MDA compared with the p-NP treated group.

Table 2 shows the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone in different treated groups. Treatment with p-NP alone caused significant ($p<0.05$) increase in LH and FSH and decrease in testosterone compared to the control group. Additionally, treatment with vitamin E alone caused significant ($p<0.05$) increase in LH

and FSH and decrease in testosterone level compared with the control group. On the other hand treatment with vitamin E and p-NP in combination group, caused significant ($p<0.05$) decrease in LH and FSH and increase in testosterone compared to p-NP treated group.

The effect of p-NP, vitamin E, and their combination on glycogen and fructose levels in testis is shown in Table 3. Treatment with p-NP significantly ($p<0.05$) decreased the glycogen and fructose compared to the control group. Treatment with vitamin E alone had no significant ($p<0.05$) effect on glycogen and fructose levels compared with the control group. Treatment with vitamin E and p-NP in combination group caused significant ($p<0.05$) increase in glycogen and fructose compared to p-NP group.

A summary of sperm parameters and morphological abnormalities from smear of spermiating males in control, vit. E. and NP exposed groups are presented in Table 4. The parameters evaluated are sperm count, sperm motility and sperm abnormality. Treatment with p-NP alone caused significant ($p<0.05$) decrease in sperm count and sperm motility and increase in the percentage of abnormality compared with the control group. Treatment with vitamin E alone showed no significant ($p<0.05$) effect on sperm count, sperm motility and sperm abnormality, presenting values close to the normal levels presented in the control group. However, treatment with vit. E. combined with NP has recured the value but does not reach the control value. Conformingly, Fig. 1. shows the cytology of spermiating male rats showed that sperm consists of three regions, a round head without acrosome, a short mid-piece and a flagellum. Exposing males to NP result in increase the incidence of sperm morphological abnormalities and decrease in the number of sperms compared to that of the control or vit. E. treated groups. The combined treated group is still significantly different than the control group even if the number of sperms has been increased and percentage of abnormal sperms has been decreased. The abnormality end points detected involved heads without tails and coiled tails (Fig. 1).

Table 1: The (mean ±SE) antioxidant enzymes (glutathione reductase, superoxide dismutase, malondialdehyde and catalase) in testis for different groups of male rats exposed to vit. E, p-NP, and their combination for 30 days.

Antioxidant enzyme	Experimental groups			
	Control	Vitamin E	p-NP	p-NP +Vitamin E
Glutathione reductase (U/g tissue)	219.61±39.17 [a]	232.6237.72 [a]	157.17±11.76 [b]	177.84±30.09[c]
Superoxide dismutase (U/g tissue)	2680.85±390.23 [a]	2490.79±350.26 [a]	2054.92±192.13[b]	2191.34±122.03[b]
Malondialdehyde (U/g tissue)	11.16±1.04 [a]	7.02±0.99[a]	25.64± 1.02 [b]	11.49±0.66 [a]
Catalase (U/g tissue)	1.81±0.27 [a]	1.54±0.18 [a]	1.15 ±0.12 [b]	1.38±0.2 [b]

Means followed by different superscript in the same row are significantly different, $p<0.05$

Table 2: The (mean ±SE) gonadotropine hormones (lutanizing hormne, follicle stimulating hormone) and testosterone hormone in testis for different groups of male rats exposed to vit. E, p-NP, and their combination for 30 days.

Parameters	Experimental groups			
	Control	Vitamin E	p-NP	p-NP+ Vitamin E
LH	0.35±0.03 [a]	0.56±0.06 [b]	1.05±0.07 [c]	0.75±0.07[d]
FSH	0.87±0.07 [a]	1.12±0.20 [a]	2.74±0.36 [b]	1.69±0.26 [c]
Testosterone	5.02±0.34[a]	4.31±0.50 [a]	1.18±0.22[b]	2.95±0.44[c]

Means followed by different superscript in the same row are significantly different, $p<0.05$

Table 3: The (mean ±SE) of glycogen and fructose in testis for different groups of male rats exposed to vit. E, p-NP, and their combination for 30 days.

Parameters	Experimental groups			
	Control	Vitamin E	p-NP	p-NP+ Vitamin E
Glycogen	2.30±0.19 [a]	2.27±0.17 [a]	1.09±0.14[b]	1.75±0.10 [c]
Fructose	315.20±20.47 [a]	325.60±12.01 [a]	159.80±16.30 [b]	261.40±20.2[c]

Means followed by different superscript in the same row are significantly different, $p<0.05$

Table 4: The (mean ±SE) sperm count, motility and abnormality in testis for different groups of male rats exposed to vit. E, p-NP, and their combination for 30 days.

Parameters	Experimental groups			
	Control	Vitamin E	p-NP	p-NP+ Vitamin E
Sperm Count	81.20±3.99 [a]	83.80±3.38 [a]	14.80±2.30[b]	50.80±4.39[c]
Sperm Motility	72.60±3.10 [a]	63.40±2.43 [a]	29.00 ±3.25 [b]	60.40±3.90 [c]
Abnormal sperms (%)	11.4±2.67 [a]	14.20±1.97 [a]	63.80 ±3.47[b]	35.00±3.1[c]

Means followed by different superscript in the same row are significantly different, $p<0.05$

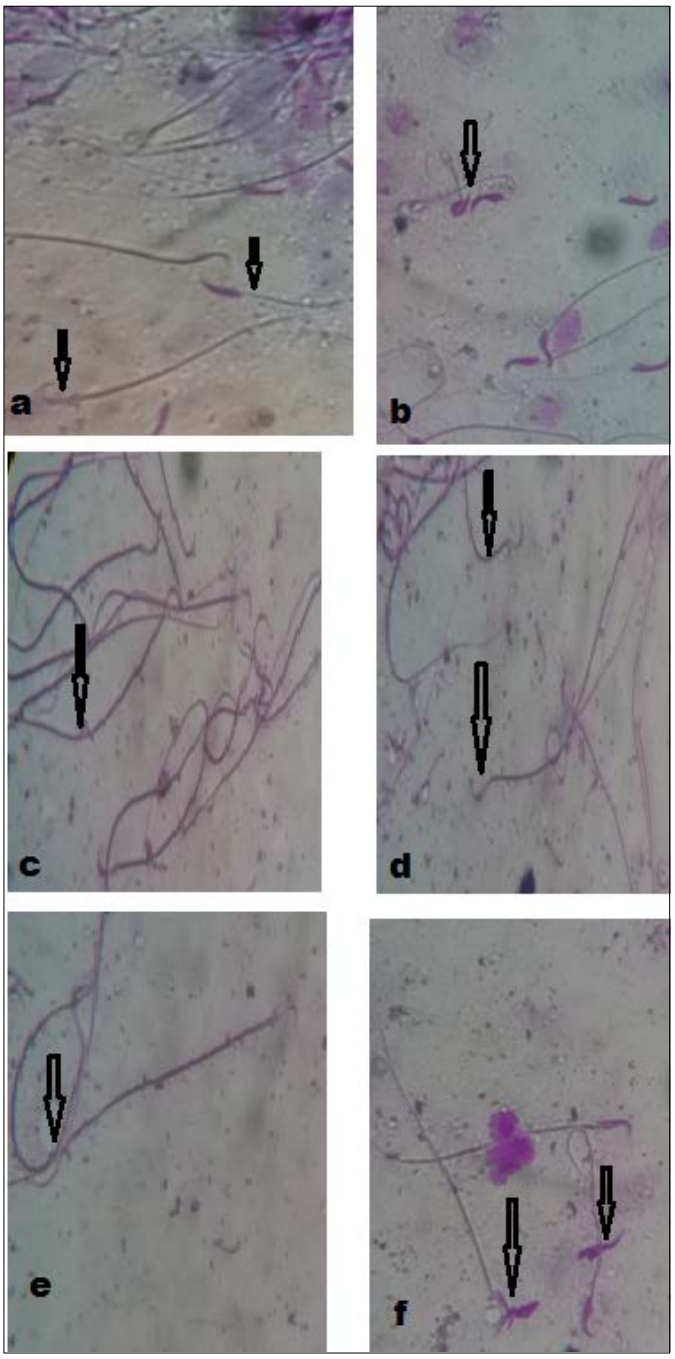

Fig 1: Photomicrographs of sperm morphology from smears of spermiating male rats in control and p-NP exposed groups at the end of experiment (stained with giemsa 7.5%) showing: a, control group rats. Note, normal sperm (arrows) with normal head and tail. (b – f), Nonylphenol administrated group showed abnormalities in their sperm morphology, sperm with both abnormal head and coiled tail (arrows), sperm without tail (f).

Discussion

Chronic and acute toxicities of nonylphenol on different terrestrial and aquatic organisms have been reviewed by several authors [28, 29, 1, 2, 30]. They reported that the degree of toxicity of NP varies according to the dose and exposure period. The present study revealed that chronic exposure to p-NP led to significant alterations in reproductive performance, physiological parameters of testis and sperms characteristics. However, such exposure being partially recovered once applying vit. E. as an antioxidant.

The estrogenic and non-estrogenic actions of phenolic compounds have been studied in several axes [31]. Alkyl phenol, nonylphenol, octylphenol and other derivatives and metabolites have shown the severity of such environmental pollutants on organisms especially the reproductive organs and may lead to gonads impairment.

Despite the wide distribution of nonylphenol (NP) in the environment and its great hazard to the reproductive health of human and animals, the detailed mechanism of NP toxicity has not been fully studied [32]. Nonylphenol has been illustrated to interfere with normal reproductive processes and lead to testicular abnormalities such as lack of differentiation of seminiferous tubules, decreased sperm count and motility [33].

This study examined the adverse effect of p-NP on epididymal sperm number, sperm motility and abnormality as well as serum hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone in developing rats. In addition, vit. E. showed to reverse the toxic effect of NP on the different examined parameters.

The effects of different environmental pollutants have been studied by many authors [7, 29, 34]. They have demonstrated that such pollutants decrease the antioxidant activity and increase MDA level in the testis tissue in mice. According to a certain theory, superoxide dismutase dismutate the superoxide anion into H_2O_2 and molecular oxygen [35]. Furthermore, the epididymis has possessed antioxidant defense system to protect spermatozoa from oxidative injury by producing scavengers of ROS [35, 7]. In order to frustrate the effects of ROS, spermatozoa are equipped with systems of antioxidant defense by glutathione peroxidase, superoxide dismutase and catalase to reduce cellular damage [36]. As it has been shown that catalase and glutathione peroxidase/reductase system catalyse the degradation of H_2O_2 and lipid hydroperoxides by using reduced glutathione [37]. Thus, the reduction in catalase activity reflects the inability of spermatozoa to eliminate H_2O_2 produced by the administration of nonylphenol. The levels of lipid peroxidation and H_2O_2 were increased in epididymal sperm of nonylphenol-treated rats. Certain study proposed that the toxicity of lipid peroxidation which induced by ROS plays a key role in the inhibition of sperm function and the physiology of male infertility [38]. Conformingly, de Jager *et al*. 1999 [39] showed that exposing rats to 10mg/kg body weight to p-NP/L leads to significant drop in antioxidant enzymes such as glutathione reductase, superoxide dismutase and catalase. Meanwhile a significant increase in MDA. The results suggest that NP elicit depletion of antioxidant defense system in rat systems, indicating NP induced oxidative stress in the testis of rats which could be reversed by the administration of vit. E. [40].

In biochemical alteration, there was significant decrease in protein and glycogen levels due to the absence of the spermatogenic stages and there was no formation of new cells [41].

Testosterone is the key hormone that maintains reproductive organ morphology and physiology, but the threshold levels of required hormone might be specific for different functions. The reduced testosterone level might have effects on reproductive functions of male rats and cellular structures [42], [43]. The results obtained in the current study show significant decrease in testosterone level when rats were subjected to the given dose of NP. Moreover, the decrease in testosterone

level was associated with an increase in the cholesterol level in the blood (unpublished data). Additionally, Duan *et al*. (2016) [44] studied the effect of 60mg/kg NP on prague-Dawley male rats and the results showed a significant reduction in fructose level.

For better understanding and to shed the light on the actual effects of NP, hormones levels should be assayed. Gonadotropins (LH and FSH) have been significantly increased compared with the control groups but the testosterone level decreased significantly. Moreover, administration of NP resulted in decreased plasma testosterone level, suggesting that NP has an inhibitory effect on testosterone production. Han *et al*. (2004) [45] reported that NP significantly decreased serum testosterone at a dose of 250 mg/kg/day. On the other hand, Wu *et al*. (2010) [46] reported that NP has a differential effect on testosterone synthesis. The decrease in plasma testosterone concentration is associated with changes in plasma LH concentration which may due to changes in the anterior pituitary, thus altering the normal secretion of testosterone by the testis [47]. NP, a weak estrogenic and endocrine disrupting compound, can regulate the secretion of gonadotropins (FSH and LH) and interfering in the normal balance of hypothalamus pituitary-gonad axis [48, 1, 30]. Vitamin E is essential for normal reproduction in animals as it is an antioxidant present in all mammalian cells. Vitamin E stimulated both LH [49] and FSH [50] release. The results from the current study demonstrate that vitamin E at 100 mg/kg when combined with NP decreased both LH and FSH levels.

With administration of nonylphenol at 10 and 100 g/kg to rats, significant decrease in sperm counts was detected, and this may be due to the lack of testicular differentiation and spermatogenesis as reported byde jager *et al*. (1999) [39]. In the current study, decreased sperm count may be due to inhibition of spermatogenesis, therefore reduction in the production of sperms as well as number of sperms in the epididymal lumen. Such decrease in spermatogenesis may be due to the inhibition in steroidogenic enzyme activity [51]. Such changes in sperm count may be due to an adverse effect of NP on spermatogenesis. This result was also confirmed with the findings of Lukac *et al*. (2013) [52] who observed a decrease in sperm number and reduction in its motility in NP treated group. The reduction in sperm count and motility was associated with decreased plasma testosterone level [53]. The decrease in sperm count may be attributed to the severity of p-NP in inducing oxidative stress on organs like testis [40]. If this hypothesis is true, vit.E should have reversed hazardous effect of p-NP on sperm number. Interestingly, it was observed that in p-NP+vit.E group, vit.E significantly ameliorated p-NP mediated decrease in sperm number. Motility and abnormality of sperm appear to be the most important parameters for the assessment of sperm quality and fertilization capacity. The change in sperm motility pattern induced by p-NP could be due to the ability of this toxicant in the induction of oxidative stress by lipid peroxidation (MDA). To support this idea, this study showed that vit.E significantly reversed motility pattern in p-NP+vit.E group compared to p-NP group.

The integrity of sperm plasma membrane may also play an important role in assessing sperm quality. Sperm plasma membrane contains a high percentage of unsaturated fatty acids which is easily susceptible to lipid peroxidation caused by oxidative stress [54, 11]. The change in sperm motility pattern induced by NP may be due to the ability of this toxicant in the induction of oxidative stress by lipid peroxidation. To emphasize on this idea, the results of the present study showed that vit.E significantly reversed motility pattern in p-NP+vit.E group compared to p-NP group. An interesting finding in sperm abnormality assay was that vit.E alone decreased the percentage of abnormal sperms compared to control group.

Effect of vit. E. may also interpret the sperm characteristics in p-NP+vit.E treated group compared to p-NP group. p-NP as an endocrine disruptor chemical is an estrogen like component which mimics the effect of estrogen to induce hazardous effects on male reproductive performance [33].

Generally, the results obtained in the current study concerning the sperm properties and hormonal suppression confir the estrogenic property of p- NP. More investigations are required to determine the mechanism by which NP affects the level of different enzymes and hormones.

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