

## Histo-morphological and Functional alterations in the Ovary of Adult Mice following Phenol Administration

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**Abstract:** Phenol (C<sub>6</sub>H<sub>5</sub>OH) is one of the most common representatives of toxic organic compounds. In addition, recently the most detrimental health effects attributed to phenol in the humans or animals include renal or hematopoietic toxicity and also neurological disorders. For this experiment, a total of 60 adult female Balb/C mice aged 9 to 10 weeks were randomly divided into one control group and three experimental groups. The control group received only distilled water, whereas experimental groups were received phenol as 80, 180 and 320 mg/kg of body weight intraperitoneally for 49 consecutive days. At the end of the experiment, all animals were anesthetized; the ovaries were washed by saline solution and then were weighed by using digital electronic scales. Tissue sections were taken and stained with hematoxyline-eosin method and prepared slides were studied under light microscope. For ovarian function assay, the blood samples were obtained via cardiac puncture and serum levels of FSH, LH, estradiol and progesterone were measured. The results showed a significant reduction in the number of ovarian follicles but also increase in the number of atretic follicles in the treated animals when compared with controls. In addition, in the treated animals the thickness of the tunica albuginea increased, but the relative and absolute ovarian weights were reduced significantly. Finally, the serum levels of FSH and estrogen hormone concentrations in the experimental groups were significantly lower than of control mice. These findings suggested that administration of phenol affected ovarian structural and functional integrity.

**Key words:** Phenol • Ovary • Histology • Follicle.Hormone

### INTRODUCTION

Phenol (C<sub>6</sub>H<sub>5</sub>OH), a monohydroxy derivative of benzene, is used to synthesize resins and plastics [1]. Also, large amounts of phenol produced in industry and from other natural sources cause this chemical to be an important environmental and occupational hazard [1, 2]. Phenol is one of the most common representatives of toxic organic compounds. Most of the industrial effluents with high phenol levels come from petroleum refineries, phenolic resin production, plastic and coke oven industries [3].

There has been an increasing interest and necessity to have more information concerning the possible side effects of phenol on the various organs. The most detrimental health effects attributed to phenol in the humans or animals include renal toxicity [4], hematotoxicity [5, 6] and neurological disorders [7]. Previous works demonstrated that phenol has been shown to cross the placenta and introduced in the fetus body [1, 8]. It has been reported that cigarette smoke components include phenol could be a main inhibitor for ciliary beat frequency in the hamster oviduct tissue [9].

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Since data is very little about possible detrimental effects of phenol on the ovary; therefore, the current work was taken to examine histo-morphological and functional alterations in the ovary of adult mice.

**MATERIALS AND METHODS**

Phenol (C<sub>6</sub>H<sub>5</sub>OH) was obtained from Biochem Chemical Company (Tehran, Iran) and dissolved in the distilled water. Deionized water was used as the vehicle for phenol. Solution of chemical tested at concentrations of 80, 180 and 320 mg/kg were prepared to provide the appropriate dilution when administered by injection.

A total number of 60 female Balb/C mice aged 9 to 10 weeks with weight average of 29-35g were purchased from Razi Institute (Karaj, Iran). The animals were maintained in a controlled environment at a temperature of 23±1°C; a humidity of 45±5% and natural 12:12 h light-dark cycle and had ad-lib access to drinking water and food. Mice were allowed to be acclimatized to the laboratory environment at least 6 days before commencement of testing. Mice were randomly divided into one control group and three experimental groups, each comprising of 15 mice. The control group received only distilled water, whereas experimental groups were received phenol as 80, 180 and 320 mg/kg concentration intraperitoneally for 49 consecutive days. Estimations of the various stages of the estrous cycle of animals was performed by daily vaginal sample taken and staining with Giemsa method. For this purpose, sample of vaginal discharge was placed on a slide and then fixed with ethanol. Slides prepared by this method were used for determination of the type of cells of the various stages of the sexual cycle.

At the end of the experiment, all animals were anesthetized; the ovaries were removed from body and washed with saline solution, then were weighed by using digital electronic scales. For tissue assessment; the specimens from ovaries were immersion imprisoned

overnight in 10% neutral buffered formalin to be fixed. Then the specimens were mounted to allow 5-µm sections. Sections were stained via Hematoxylin and Eosin (H and E) method and photographed directly using a stereo microscope in 400 high power fields with Microsoft system. For exact description of the structural changes in the ovarian tissues a histometrical analyze was performed. For this purpose the numbers of primordial, primary, secondary, antral and atretic follicles were recorded. In addition, the thickness of the albuginea was measured.

For ovarian function assay, the blood samples were obtained via cardiac puncture and serum was separated by centrifuging at 2500 rpm for 20 minutes. Serum levels of FSH, LH, estrogen and progesterone were measured by using a commercial radioimmunoassay kit and gammacounter instruments (LKB, Sweden).

All statistical analyses were carried out using Statistical Package for Social Scientist (SPSS version 13, Chicago, IL, USA). Results were tested for normal distribution (Shapiro-Wilks) and homogeneity of variances (Bartlett test) and then expressed as standard error of the mean (SEM). The analysis of variance (ANOVA) was used to test the overall significance of differences among the means. Tukey-Kramer's Multiple Comparison Test was applied for post hoc comparison. A probability level of less than 5% (P < 0.05) was considered as significant.

**RESULTS**

It was not found any outstanding differences in the histological results between three experimental groups.

The results showed a significant reduction in the number of ovarian follicles but also increase in the number of atretic follicles in the treated animals when compared with controls. There are no significant changes in the number and size of the ovarian corpus luteum after administration of phenol when compared with control animals (Table 1).

Table 1: Mean±standard error characteristics of ovarian histometrical mice Balb/C control group and groups treated with phenol.

Ovarian parameters	Groups				Significance
	Control	80 mg/kg phenol	180 mg/kg phenol	320 mg/kg phenol	
The number primordial follicles	13.58±2.1	11.24±1.4	10.28±3.2	11.88±3.3	*
The number of primary follicles	9.21±1.5	5.35±2.6	4.78±0.1	5.05±0.4	**
The number of secondary follicles	10.26±1.6	5.08±0.9	5.99±0.8	4.38±0.1	**
The number of antral follicles	8.47±2.9	4.23±0.2	4.35±1.7	4.89±0.6	*
The number of atretic follicles	1.58±0.9	7.55±0.6	8.03±1.3	7.69±1.9	**
The number of corpus luteum	2.03±0.4	2.05±0.3	2.04±0.8	2.01±0.5	-
Tunica albuginea thickness (µ)	0.89±0.08	3.37±0.7	4.59±0.1	3.38±0.7	*
Absolute ovarian weight (g)	0.008± 0.0001	0.003± 0.0004	0.002± 0.0005	0.003± 0.0003	*
Relative ovarian weight (%)	0.8	0.3	0.2	0.3	*

(-: not significant, \*: P<0.05, \*\*: P<0.01 in comparison with control).

Table 2: Mean  $\pm$  standard error of the hormones FSH, LH, Estrogen and Progesterone in mice Balb/C control group and groups treated with phenol

Ovarian parameters	Groups				Significance
	Control	80 mg/kg phenol	180 mg/kg phenol	320 mg/kg phenol	
FSH (U/L)	0.4 $\pm$ 0.09	0.09 $\pm$ 0.02	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	*
LH (U/L)	0.09 $\pm$ 0.002	0.06 $\pm$ 0.002	0.07 $\pm$ 0.005	0.08 $\pm$ 0.001	-
Estrogen (pg/ml)	31.11 $\pm$ 0.3	14.19 $\pm$ 2.1	13.5 $\pm$ 0.6	12.6 $\pm$ 0.5	*
Progesterone (ng/ml)	35.4 $\pm$ 0.6	35.9 $\pm$ 0.5	34.7 $\pm$ 0.9	35.2 $\pm$ 0.6	-

(-: not significant, \*: P<0.05, \*\*: P<0.01 in comparison with control).

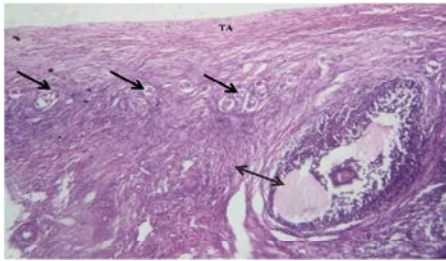


Fig. 1: Ovarian transverse sections of the control mice. In this figure TA indicate the normal thin thickness of the tunica albuginea. In addition, a number of various follicles (arrows) are noticeable in the ovary tissue (Haematoxylin and Eosine stain) ( $\times$  400)

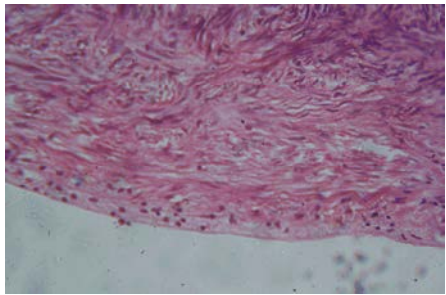


Fig. 2: Ovarian transverse sections of the mice treated with phenol at 80 mg/kg concentration. The figure shows an increasing in the tunica albuginea thickness as well as a decreasing in the number of various ovarian follicles. (Haematoxylin and Eosine stain) ( $\times$  400)

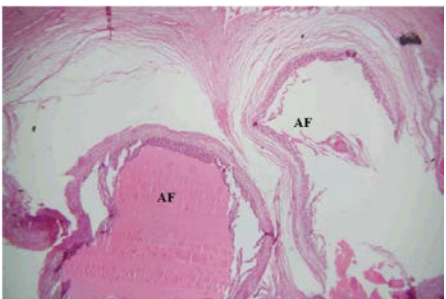


Fig. 3: Ovarian transverse sections of the mice treated with phenol at 180 mg/kg concentration. The figure shows an increase in the number of atretic follicles (AF) in ovary (Haematoxylin and Eosine stain) ( $\times$  400)

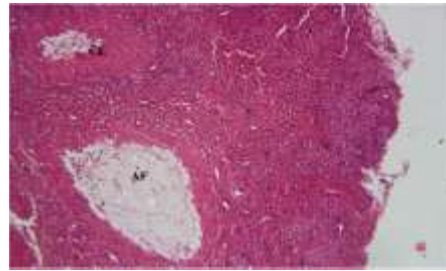


Fig. 4: Ovarian transverse sections of the mice treated with phenol at 320 mg/kg concentration. The figure shows an increase in the number of the atretic follicles (AF) as well as a reduce in the number of various ovarian follicles (Haematoxylin and Eosine stain) ( $\times$  400)

In addition, in the treated animals the thickness of the tunica albuginea increased, but the relative and absolute ovarian weights were reduced significantly (Table 1).

Finally, the serum levels of FSH and estrogen hormone concentrations in the experimental groups were significantly lower than of control mice (Table 2).

## DISCUSSION

The histo-morphologic as well as serum levels of reproductive hormones assay from present study showed a significant detrimental alteration in the both structure and function of the ovary in the treated mice with various phenol solutions.

There are limited studies in the literature which shown that phenol and its derivatives had destructive reproductive effects [10]. In line with our results, previous studies have been shown that phenol administration for 30 consecutive days could reduce the ovarian index and also prevents maturation and development of ovarian structure in the common carp [11]. It has been demonstrated that any reduction in the steroidogenesis of the ovarian tissue following treatment with phenol may be attributed to prevention of maturity and development of various ovarian cells [11].

Although mechanism(s) of toxic effects of phenol on the ovarian integrity is not clear, but its hydrophobicity properties, formation of phenoxyl radicals [12] and oxidative stress phenomena [13] noted as possible mechanisms of phenolic cytotoxicity. Structural changes seen in the present study may, resulting in the formation of free radicals phenoxyl origin of phenol and also the ability of these compounds to destruction in the plasma membrane of the ovarian cells [12].

As a result, these findings suggested that administration of phenol affected ovarian structural and functional integrity.

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