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Effect of bisphenol-A- on reproductive system of female rats *(Rattus Norvegicus)*

تأثير البسفينول –أ-على النظام التكاثري لأناث الجر

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Abstract

Bisphenol-A-(BPA) is one of endocrine disruptor substances. The present study was conducted to investigate effect of BPA on reproductive system (reproductive hormones) such as Luteinizing hormone (LH), Follicle stimulating hormone(FSH) and Estradiol (E2)and (reproductive organs) such as ovary and uterus .Twenty four female rats were used and divided into three groups (8 animals for each group) randomly. Animals of first group was negative control group and second group was positive control group received normal saline and corn oil orally respectively, while female rat of third group were received BPA suspended with corn oil (250mg/kg B.W/day) orally as treatment group for 30 days. Results of the present study revealed significant increase ($P\leq0.05$) in serum level of (LH, FSH, and E2) while histopathological changes in ovary include presence cyst-like structure and histopathological changes in uterus including increase in uterine luminal epithelial height and increase in number of uterine glands in the endometrium in group administrated BPA in compared with all other groups.

الخلاصة

البسفينول -أ- هو أحد المواد المعرقلة لعمل الغدد الصم .الدراسة الحالية توصلت لكشف تأثير البسفينول -أ-على النظام التكاثري (الهرمونات التكاثرية) مثل الهرمون اللوتيني والهرهون المحفز للجريب وهرمون الاسترادايول و(الأعضاء التكاثرية) مثل المبيض والرحم . أربع وعشرون أنثى جرذ أستخدمت وقسمت الى ثلاث مجاميع (8 حيوانات لكل مجموعة) عشوائيا. حيوانات المجموعة الأولى هي مجموعة السيطرة السالبة والثانية هي مجموعة السيطرة الموجبة تناولت المحلول الملحي وزيت الذرة فمويا على التوالي، بينما أناث الجرذان للمجموعة الثالثة تناولت البسفينول -أ- المعلق بزيت الذرة (250 ملغم/كغم من وزن الجسم) فمويا وعملت كمجموعة معاملة ل 30 يوم. نتائج الدراسة الحالية عكست ارتفاع معنوي ($P\leq0.05$) في مستويات المصل للهرمون اللوتيني والهرهون المحفز للجريب وهرمون الاسترادايول بينما التغيرات النسجية المرضية في المبيض تتضمن وجود تركيب يشبه الكيس والتغيرات النسجية المرضية في الرحم تتضمن زيادة في ارتفاع الخلايا الطلائية الرحمية وزيادة في عدد الغدد الرحمية في الطبقة الرحمية -في المجموعة المعطية البسفينول-أ-بالمقارنة مع كل المجاميع الأخرى.

Introduction

Bisphenol-A- (BPA) is one of the most important industrial chemical introduced basically in polycarbonate plastics and in the epoxy resins, it also widely used in manufacture food and drink cans (1). Exposure to BPA as result of hydrolysis of the ester bonds that are link BPA molecules in polymer this hydrolysis of the ester bonds accelerated when temperature increases and in acidic or basic environment. BPA leeches from polycarbonate products significantly as aconsequence to repeat washing, exposure metal and plastic cans to heat and/or acidic or basic conditions (2) (3). Recently researches showed that BPA has ability to leach out of some products, include tableware, plastic lining of cans used for food, white dental fillings sealants and polycarbonate babies' bottles. The leaching was occured by exposure of the plastic to high temperatures (4). About 93% of urine samples in the US population contain on BPA (5). (1) BPA found in the fluid portion of many classes of vegetables such as green beans, mushrooms, mixed vegetables, peas, corn and artichokes,

which take from Cans with epoxy resin linings.(6) reported that BPA at low doses has action similar to female hormone (e.g.17b-estradiol, estrone or estriol), and BPA can causes biological effects. As a result, BPA belong to class of chemicals called "hormone disruptors" or "endocrine disruptors", that have ability to disturb the chemical messenger system in the body. (7) suggested that the BPA have a mechanistic effect on the local regulatory circuits of hypothalamus and pituitary. BPA exert its effects via interfering with either ESRα or ESRβ or with both the receptors that belong hypothalamic-pituitary-gonadal (HPG) axis (8). Some studies showed that BPA at high doses causes changes in ovary including necrosis, bleeding, presence of the large numbers of atretic follicles and failed in ovulation these considered indication on toxic effect for BPA (9).

Aims of the study

The current study was planned to estimate harmful effects of the exposure to BPA and the possible protective effect of red grape skin against harmful effect of BPA in female rats by study the following objectives:

1. Toxic effect of BPA on some blood and biochemical parameters and probable protective role of grape skin.
2. Investigate the effect of BPA and grape skin on some immunological parameters.
3. Study of histological changes caused by BPA and ameliorate effect of grape skin.
4. Evaluate effect of BPA on female reproductive system (hormones and organs) and probable improvement of grape skin.

Materials and Methods

Animals of the Study

The present study was conduct at College of Veterinary Medicine –University of Karbala. Twenty four mature *female Ratus Norvegicus* rats were purchased from care center and medicinal researches in Baghdad, Iraq. They were 14 to 16 weeks old with an average body weight (200-250gm).

The animals were clinically healthy, kept under hygienic conditions, metal cages and glassy bottles were used to avoid exposure to BPA from old polycarbonate cages. Water and feed were giving *ad –libitum* throughout the experimental period.

Female albino rats (24females) divided into three main groups (8animals) of each group as following:

1- Negative control group: Eight female rats that received only normal saline orally as vehicle (0.5ml/kg BW).
2-Posative control group: Eight female rats that received only corn oil orally as vehicle (0.5ml/kg BW).
3- Treatment group: Eight female albino rats, orally administer BPA 250mg/kg BW. /day (1/20 LD50) suspended in corn oil via gavage as high dose **(10).**

Female rats' of each group were sacrificed at the end of the experiment after 30 days; the rats before sacrifice were first anaesthetized by placing them in a closed jar containing cotton sucked with chloroform anesthesia.

Blood samples were collected by heart puncture the blood was put in plane tube to be centrifuged (6000) rpm for 10 minutes to obtain the serum which is then transferred to epndrofe tubes, for the hormonal estimation all tubes were stored at (-20c) until analyzed.

Ovary and uterus were isolated and trimmed of their fat. The organs were fixed in 10 % formalin for histological examination.

Hormonal parameters

Serum levels of Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and Estradiol (E2) were determined by RIA using the kit provided by the NIDDK as previously described **(11).**

Histopathological technique

Ovary and uterus of each animal were rapidly removed, then prepared for histological estimate according to **(12).**

Statistical analysis:

The data were presented as mean ±SE and subjected to analysis of variance by using one way ANOVA Post hoc test was used LSD to specify the significant difference among means the software package IBM SPSS Program version 20 was used for the analysis of data **(13)**.

Results

Effect of BPA on some serum hormones levels in mature female rats

Data explored presence of significant (P≤0.05) increased in the mean of LH, FSH and Estradiol serum level of female rat which was exposed to BPA (250 mg/Kg B.W) compared with control groups.

Table (4-4) the effect of BPA on some serum hormones levels in mature female rats (Means ± SE)

Parameters / Groups	LH μIU/ml	FSH μIU/ml	Estradiol (pg/ml)
Normal saline group (Negative control group) (0.5ml/kg/B.W)	CD 3.56±0.11	B 4.93±0.21	C 54.93±1.21
Corn oil group (Positive control group) (0.5ml/kg/B.W)	D 3.15±O.18	B 4.69±0.16	C 54.99±1.16
Bisphenol-A- group (Treatment group) (250 mg/kg/B.W)	A 6.56±0.20	A 7.40±0.11	A 73.49±1.40

N=8

Different letters represent a significant difference at (p≤0. 05)

Effect of BPA on histopathological changes in ovary and uterus in mature female rats

1-The ovary

The microscopic finding of ovaries in normal saline, corn oil shows normal follicle growth (figures1and2).On the other hand, histological changes in ovary of female rats exposed to 250mg/kg/B.W of BPA include presence cyst-like structure (figure 3).

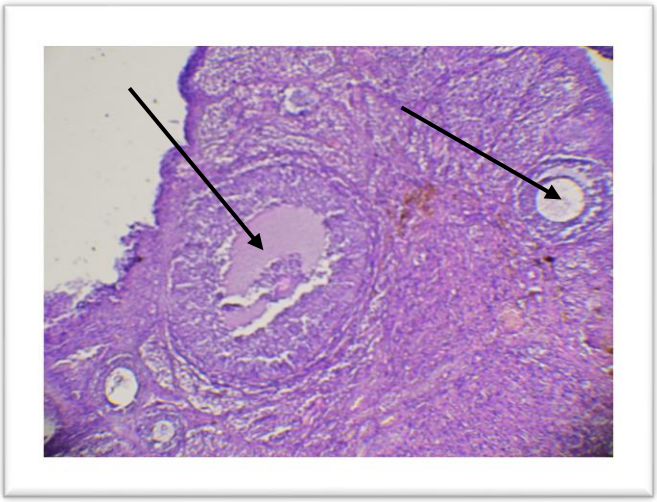

Figure 1: Micrograph of ovary of normal saline group in rat shows follicles in different developmental stages (thin arrows).H&S, 100x.

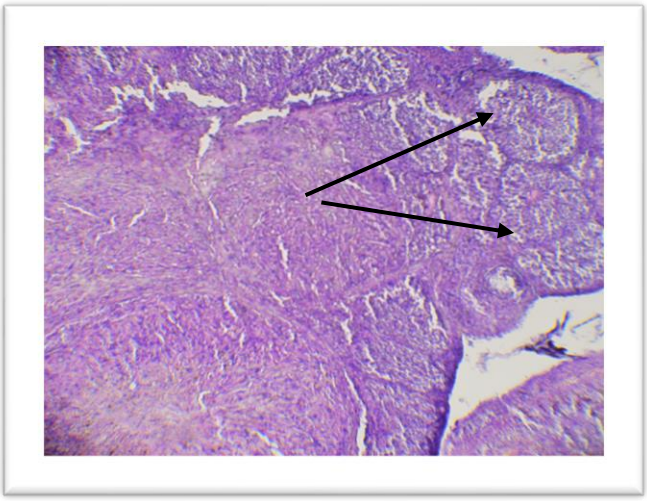

Figure 2: Micrograph of ovary of corn oil group in rat shows normal follicle growth (thin arrows) H&S, 100x.

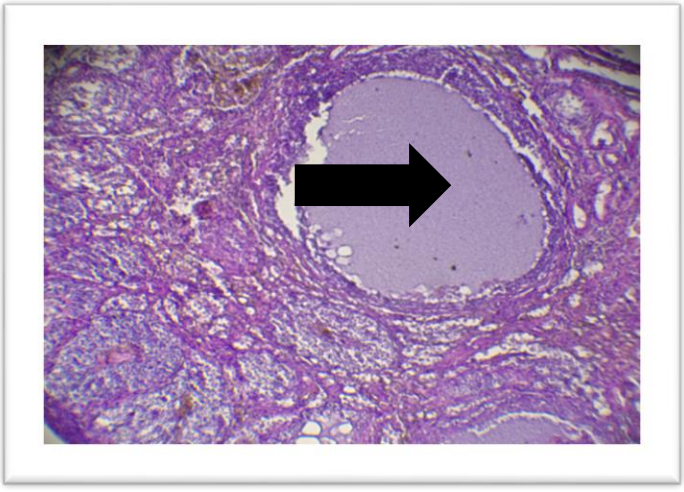

Figure 3:Micrograph of rats ovary exposed to BPA 250mg/kg B. W. shows presence cyst-like structure (thick arrow).H&E,100 x.

2-The uterus

Microscopic examination of uterus in rats treated with normal saline and corn oil groups showed normal distribution of uterine glands in the endometria with normal epithelial height (figs.4 and 5).

While the uterus of rats exposed to 250mg/kg B.W of BPA revealed histopathological changes including increase in uterine luminal epithelial height and increase in number of uterine glands in the endometrium (fig 6).

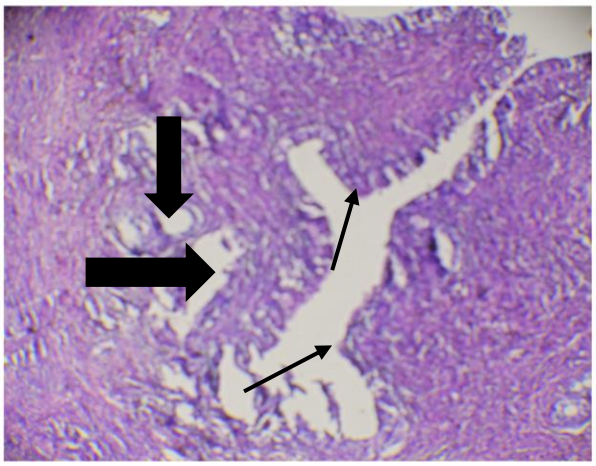

Figure 4: Micrograph of uterus of normal saline group in rat shows normal distribution of uterine glands (thick arrow) in the endometria with normal epithelial height (thin arrows) H&E, 100x.

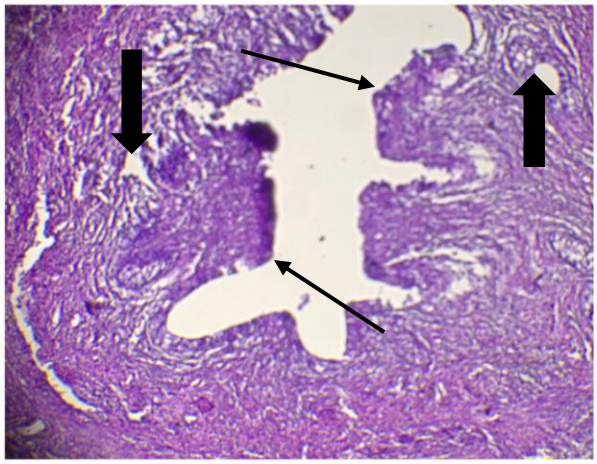

Figure 5: Micrograph of uterus of corn oil group in rat shows normal distribution of uterine glands (thick arrow) in the endometria with normal epithelial height (thin arrows) H&E, 100x.

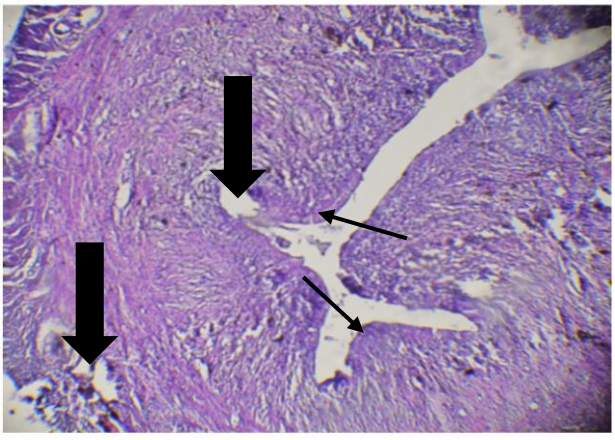

Figure 6: Micrograph of histological changes in uterus of female rats exposed to 250 mg/kg B.W. of BPA shows increase in uterine luminal epithelial height (thin arrow) increase number of uterine gland in the endometrium (thick arrow). H&E, 100x.

Discussion

Results of the present study showed there were significant increase in FSH, LH and Estradiol serum level group administrated BPA (250 mg/Kg B.W) compared with all other groups. These results came in matched with previous studies **(14) (15) (9) (16) (17).** Some studies appeared that increased in levels LH hormone in females indicator on in fertility because its secretion interfere with hypothalamic pituitary gonadal axis therefore raise of LH level in blood stream indicator for decrease produce sexual steroids from ovary as in the case of premature ovarian failure and conjugate increased in LH level with poly cystic ovarian symptom and then lead to decrease in fertility **(15)** and supported by results of histology of ovary in the present study.**(14)** showed that BPA lead to increase in level of plasma LH is probably due to a reduction in the negative feedback regulation by estrogen. Some studies appeared that there are increase in FSH level which is matched with raise in estradiol concentration with the histological alterations in ovaries which showed increase the texture of ovary beside the cystic follicles **(18)** in our histological study. The present results revealed that there is increased E2 level resulted from interfering of BPA with E2 synthesis pathways as mentioned by **(19) (20) (21) (22)** via increasing expression level of Cytochrome P450 19a (CYP19a) as recorded by **(23).** Increasing of serum E2 level in the present study explained by possible interference of BPA mechanisms of local regulatory circuits of hypothalamus and pituitary. The outcomes results of microscopic examination appeared different histopathological changes in rats ovary treated with BPA at high dose include different abnormal conditions such as presence cystic-like structure. Bisphenol A effect on FSH receptors in ovary and changes in granular oocytes therefore BPA effect on regulation of transcription process and hormonal activity that play important role in replication of cells and occur essential conditions for growth of follicles **(24).** Histological finding of other study had shown necrosis, hemorrhage and presence large number of ataractic follicles with no indication of ovulatory process in rats exposed to high dose of BPA that indicate the ovo-toxic nature of BPA **(15).**The microscopic finding in present study of uterus of rats treated with high doses of BPA (250mg/kg B.W) revealed histopathological changes including increase in uterine luminal epithelial height and increase number of uterine gland in the endometrium these results agreement with previous studies **(25)** that showed presence effect of BPA on reproductive organs such as uterus. Previous study done on the ovariectomized mouse showed that both BPA and estrogen caused significant increase in the luminal epithelial cell height and the thickness of uterus in both layers (stromal and myometrial) **(26).**

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