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Modulatory Effect of Origanum Majorana L on Changes Induced By Bisphenol A in Female Pups Rats

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Abstract

Environmental contaminants play an important role in the inheritance of female reproductive dysfunction. Nutraceuticals are able to influence female reproductive function and could be considered a protective agent. The present study investigated the potential effect of the Origanum majorana L (OML) on the oxidative changes induced by Bisphenol A (BPA) in female offspring. 48 female albino rats were divided into 2 groups. Group I: female rats were given orally 50 mg/kg of BPA / day during gestation and/or lactation periods, and group II OML was given orally to dams in a dose of 250 mg /kg daily 3 weeks before BPA administration each as alone or in combination with BPA. At postnatal day 60 (PND 60) serum estradiol (E2), triacylglycerol (TAG), total cholesterol (TC), HDL –cholesterol (HDL-C), LDL- cholesterol (LDL-C), VLDL-cholesterol (VLDL-C)levels were assayed in female pups. Also malondialdehyde (MDA) and glutathione (GSH) contents, Superoxide dismutase(SOD), Catalase (CAT), and Glutathione–s- transferase (GST) activities were estimated in ovarian tissue of pups. BPA exposure resulted in decreased serum E2 and HDL-C levels, ovarian GSH content and SOD, CAT and GST activities. On the other hand BPA increased level of serum TAG,TC, LDL-C, VLDL-C and ovarian MDA content. The observed antioxidant and hypolipidemic effect of OML co-administration to dams modulated the adverse effects induced by BPA in a time- dependent manner in female pups. In conclusion; the modulatory effect of OML on pup's adverse oxidative changes induced by BPA showed its potential as a protective agent against female reproductive dysfunction.

Keywords: Origanum majorana L, Bisphenol A, Estradiol, Oxidative stress, Antioxidant enzymes.

Introduction

Exposure to an environmental contaminant in early life (prenatal) leads later to developmental disorders and disease manifesting in childhood, over the life course, or even transgenerationally. The primordial germ cells, embryo, and fetus are highly susceptible to epigenetic dysregulation by environmental chemicals, which can thereby exert multiple adverse effects **(Reamon-Buettner and Borlak, 2007).** The chemicals that interfere with the function of hormones by mimicking, blocking, or disrupting their synthesis, transport, or elimination are referred as endocrine disrupting chemicals (EDCs) **(Dolinoy et al., 2007).**

The developmental stage (embryonic, fetal and juvenile) is sensitive to EDCs. An oestrogenic EDCs can cause an alteration in the hypothalamus-pituitary-ovarian axis leading to the dysfunctional reproductive system later in adulthood. One of the EDCs found throughout the environment is bisphenol A (BPA). BPA is widely used in manufacturing polycarbonate and plastic materials **(Gurmeet et al., 2014).**

As the BPA-containing family of alkylphenols has estrogenic potential and the ability to disrupt hormone synthesis by acting directly on hormone receptors **(Mathur and D'Cruz, 2011)**. Apart from their endocrine disrupting effect, studies have shown that they cause cellular damage to protein and lipid structures through reactive oxygen species (ROS) in the tissues where BPA accumulates **(Chitra et al., 2003; Hasselberg et al., 2004)**. ROS, such as superoxide radicals and hydrogen peroxide, are generated as a result of the interaction of phenoxy radicals produced by phenol and metabolites of intermediate products, such as semiquinone and quinine, with biomolecules in the cells **(Michalowicz et al., 2007)**. Superoxide, hydrogen peroxide, and hydroxyl radicals cause oxidative damage to membrane lipids and result in lipid peroxidation. This oxidative stress results in necrosis, which leads to critical pathophysiological conditions. Various antioxidant mechanisms protect the cells from damage caused by free oxygen radicals **(Kabuto et al., 2003).**

Nutraceuticals is research focusing on identifying and understanding the molecular-level interaction between nutrients and other dietary bioactive with the genome **(Rawson, 2008).**

Origanum majorana L(OML) is a member of the mint family Lamiaceae. Typically, identified OML products as the dried leaves and flowering tops of Origanummajorana L. is found throughout the world. It contains phenolic sterpenoids (thymol, carvacrol), flavonoids (diosmetin, luteolin, apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin, thymonin), triacontan, sitosterol, acids (oleanolic acid) and cis-sabinene hydrate .Crude extracts of spices rich in phenolics are now the mean interest in the food industry because they retard quality and nutritional value of food. This herb is beneficial to eliminate oxidative stress by virtue of its antioxidant properties. The hypolipidaemic activity of OML in rats could be attributed to the presence of valuable polyphenolic compounds, terpenoids, flavonoids, tannins, hydroquinone, phenolic glycosides and sabinene**(AL-Bandak, 2007; Shelbaya et al.,2014).**The antioxidant and antitumour activities of marjoram have recently been determined **(Zeytinoglu et al., 2003; Vagi et al., 2005).** Furthermore, marjoram extracts exhibited anti-hyperlipidaemic and anti-diabetic activity in rats. In addition, the herb is rich in flavonoids and phenolic compounds and its antioxidant activity is evident. Marjoram extracts were found to significantly elevate the cellular antioxidant capacity (**Haj-Husein et al., 2015**).

The objective of the current study is to evaluate the potential modulatory effect of OML against adverse oxidative stress induced by BPA in relation to its effects on the female reproductive system.

Materials and Methods

Medicinal Plant and extract preparation

Fresh Origanum majorana L (OML) leaves were extracted with 70 % (v/v) ethanol, lyophilized and stored at – 20 °C **(Mohamed et al., 2011).**

Animals

Total number of 48 healthy mature female and 16 healthy mature male albino rats weighing 100-150 grams were housed under normal laboratory hygienic conditions for adaption two weeks. Animals were kept and treated according to the guidelines of the ethics committee of Cairo University **(Approval number CU II S 12 16).** At the 14th week of age, each three mature female albino rats that were proved to be in estrous phase were kept with one mature male rat in a separate cage (**Cohen, 1966**). After mating a vaginal smear was taken. The presence of sperms indicated zero day of gestation (GD 0) **(Ayyed et al., 2009).** The pregnant female rats were divided into **Two** main groups as follows:

I. Control group: Female rats were sub-divided into **Four** subgroups:

IA. Rats were given daily 0.5 ml of corn oil orally as a vehicle for 8 weeks **(Manikkam et al., 2012).**

IB.BPA-treated group during pregnancy, and lactation: rats were given BPA (Sigma chemicals, St. Louis, Mo, USA) daily in a dose of 50 mg/kg b.w orally in corn oil from zero days of gestation until 30 days postnatal (8weeks).

IC. BPA-treated group during pregnancy: rats were given BPA daily in a dose of 50 mg/kg b.w orally in corn oil **(Manikkam et al., 2012)** from zero days of gestation until 30 days of gestation (4weeks).

ID.BPA-treated group during lactation: rats were given BPA daily in a dose of 50 mg/kg b.w orally in corn oil from zero day postnatal until 30 days postnatal (4weeks).

II. OML treated group: During three weeks before mating, all female albino rats in this group were given OML extract daily in a dose of 250 mg/kg b.w/ orally in sterile distilled water **(Ali, 2013)** from three weeks of pre-mating until 30 days of postnatal (12weeks). It subdivided into **Four** sub-groups:

II A.OML treated group: Did not received BPA.

II B.OML and BPA during pregnancy and lactation: rats were co-administrated 50 mg/kg b.w. of BPA daily (from zero day of gestation until 30 days postnatal (8weeks).

II C.OML and BPA during pregnancy: rats were co-administrated 50 mg/kg b.wof BPA daily (from zero days of gestation until 30days of gestation (4weeks)).

II D.OML and BPA during lactation: rats were co-administrated 50 mg/kg b.w of BPA daily (from zero day of postnatal until 30 days postnatal (4weeks).

Blood and tissue sample collection: At 60 days of postnatal, blood samples were taken from ten female pups rats (1St generation (F 1) after overnight fasting from each group then cervical dislocated for ovaries collection and kept at – 80°C. Serum samples were separated in sterile Eppindorff's tubes and kept at –20°C for estimation of estradiol and lipid profile.

Serum level of triacylglycerol (TAG) **(Schettler and Nussel, 1975)**, total cholesterol (TC)**(Richmond, 1973; Allain et al.,1974)**, HDL –cholesterol (HDL-C) **(Burstein et al.,1970)** were assayed. LDL-

cholesterol (mg/dl) and VLDL -cholesterol (mg/dl) were calculated according to **Friedewald, et al., (1972).** Serum estradiol (E2) concentration was measured using ELISA kit (DRG International, Inc., (USA).

The ovaries were homogenized according to the methods of **Shrilatha and Muralidhara, (2007); Zhao et al., (2014).** Malondialdehyde level (MDA) (**Ruiz-Larrea et al., 1994**), Glutathione (GSH) content (**Beutler et al., 1963**), and activities of Superoxide dismutase (SOD) (**Marklund and Marklund., 1974**), Catalase (CAT) (**Bergmeyer et al., 1985**), and Glutathione-S-transferase (GST) (**Habig et al., 1974**) were assayed.

Data are presented as the mean ± standard error (SE).A P-value of <0.05 was considered statistically significant .Differences among groups were analyzed using two-way analysis of variance (ANOVA) followed by LSD test **(Berly and Lindegren, 1990)**

Results and Discussion

The objective of this study was to determine if the exposures of endocrine disruptors (BPA) have the capacity to promote inheritance of a disease phenotype, and not to do the risk assessment of the exposures. The phenotypes observed may vary with time of exposure to BPA. This study investigates and compares the effect of OML either alone and/or in combination with BPA on lipid profile, hormonal status, and markers of oxidative stress in ovaries.

The evaluation of estradiol (E_2) level in female offspring rats from dams exposed to BPA during gestational and/or lactation period showed a significant reduction compared to control group (**figure 1**).The pronounced reduction in the above-mentioned traits was observed in pups from dams exposed to BPA during the gestational and lactation period. There is a paucity of human data. Experimental animal and in vitro studies have consistently observed decreased ovarian E2 synthesis with higher BPA levels of exposure **(Xu et al., 2002;Mlynarcikova et al., 2005; Zhou et al., 2008; Peretzet al., 2011)**. An in vitro study in rat ovarian granulosa cells demonstrated a dose-dependent inhibitory effect of BPA on E2 production as well as decreased expression of Cyp19 messenger RNA (mRNA), and cytochrome P450 aromatase **(Zhou et al., 2008)**. **Peretz et al. (2011)** used an in-vitro mouse follicle culture system and administered BPA. They found decreased E2 production in ovarian antral follicles with corresponding decreased mRNA expression of steroidogenic acute regulatory protein and cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc). **Xu et al. (2002)** showed that murine ovarian granulosa cells cultured with 100 μM BPA for 24–72 hr had decreased viability and increased apoptosis in a dose and time dependent relationship. They demonstrated that BPA administration (100 μM) increased the protein expression and mRNA levels of Bcl-2 associated X protein (Bax/pro-apoptotic) and decreased those of Bc1-2 (anti-apoptotic) genes. An imbalance of these genes could result in an increased cell death, resulting in granulosa cell apoptosis and follicular atresia **(Hughes and Gorospe, 1991; Yu et al., 2004)**. These results suggested that BPA may antagonize the anti-apoptotic effect of endogenous estrogens synthesized by granulosa cells **(Ehrlich et al., 2012)**.

Co-administration of OML (group II) as protective treatment showed significant defend in the E2 level of female pups born to BPA-exposed dams during gestation and/or lactation period nearly into its normal value. Possible explanation for this observation could be due to OML, is one of herbs that known to be phytoestrogenic and can act as substitutes for estrogen in the body **(http://www.newhealthguide.org/Normal-EstrogenLevels.html).** Additionally, phytoestrogens may influence development and trigger life-long effects. Mice and rats exposed before or right after birth to several phytoestrogens, develop adverse reproductive function later in life. The studies report altered ovarian development, altered estrous cycles, problems with ovulation, and subfertility (fewer pregnancies; fewer pups per litter), and infertility **(Jefferson et al. 2006)**. Other studies recorded that exposure of rats to phytoestrogens alters pituitary responses that contribute to the ovulation problems **(Levy and Faber,1995 ;http ://e.hormone. Tulane.edu/learning/phytoestrogens.html#healthrisks).**The determination of TAG, TC, LDL-C, VLDL-C concentrations in serum of female offspring from dams treated with BPA during gestation and/ or lactation, showed a significant increase (the highest level was observed in offspring from the dam treated with BPA during gestation and lactation period, group IB), with a significant decrease in HDL-C concentration **(Table 1)**. Also the attained data showed that BPA exposure during lactation increased serum lipid profile higher than exposure during pregnancy.

Our results coincide with those reported by **Grasselli et al. (2010)**, who found that PBA treatment altered steroid hormone production in rat ovary. The precise mechanism remains unclear **(Caserta et al., 2014)**, but they speculated that steroidogenic acute regulatory protein (StAR) and aromatase cytochrome P450 appeared to be targeted by BPA. Moreover, **Peretz et al. (2011)**, concluded that BPA may interfere with the steroidogenesis by inhibiting cholesterol uptake, which consistent with our study concerning the results of lipid profile picture. Also, our data of lipid profile came in harmony with those recorded by **Jiang et al. (2014)**, who found that Wister rats treated with BPA up–regulated hepatic lipid metabolism and up-regulated genes involved in lipogenesis pathway**(Moustafa and Ahmed, 2016).**

Co-administration of OML (group II) as protective treatment maintained TAG,TC, HDL-C, LDL-C, VLDL-C levels in pup's from dams exposed to BPA during gestation and/or lactation within normal range **(Table 2)**.

OML extract is a rich source of terpenoids, asthymol, carvacrol**(Roth, 2001)**, terpinen-4-ol, a-terpinene and α-terpinene **(El-Hosseiny et al., 2014)**. Among these, the monoterpenoid carvacrol was appointed as an activator of PPARα and γ. An effect that may be related to the observation that carvacrol supplementation tended to reduce body weight gain, visceral fats and plasma lipids compared with control, probably through regulating adipose tissue genes expression and proteins associated with the signaling cascades that lead to adipogenesis **(Cho et al., 2012)**. As such, it can suggest that treated obesity status and associated hyperlipidemia by OML presented here is mainly dependent on the dual activation of PPARα and PPARγ by terpenoid constituents abundantly present in OML.These findings were consistent with those of **El-Wakf et al. (2015),** where OMLextracts lead to a significant decrease of serum TC, TAG,LDL-C, VLDL-C.

In this respect, **Vagi et al.(2005)** and **Amarowicz et al. (2008)** found that OML ethanolic extract contain considerable amounts of total phenolics compounds and have antioxidant activity and free radical-scavenging capacity. The hypocholesterolemic effect of OML could be attributed to presence of isoflavones which prevent intestinal absorption of cholesterol by competition for its absorption sites (**Ahmed et al.,2009**). These results also in accordance with **Nagm (2002),** who found that marjoram extract lead to significantly lower TAG than control group .This effect of lowering TAG may be due to decrease of fatty acids synthase.

Administration of BPA (group I) caused significant decrease in activities of antioxidant enzymes (SOD, CAT, and GST) and GSH content and – on the contrary- significant increase of MDA concentration in ovarian tissue of female offspring from dams treated with BPA during gestation and/ or lactation, especially during gestation and lactation period(Group IB), **(Table 2)**. BPA may cause oxidative stress through generation of highly reactive membrane toxic intermediates in the ovary of rats by disturbing the redox status in cells. Increased lipid peroxidation may indicate an increased oxygen free radical generation where BPA induces reactive oxygen species (ROS) production and significantly compromises mitochondrial function (**Kabuto et al., 2004).** These data are in agreement with the previous results illustrated that treatment of rats with BPA increases levels of ROS production in tissue (**Kabuto et al., 2003; Hizb Ullah et al., 2016). Avci et al. (2014),** reported an increased level of H_2O_2 and lipid peroxidation in offspring from the dams exposed to BPA eliciting depletion of the antioxidant defense systems and induced oxidative stress in ovaries of rats, and exhausted antioxidant defense enzymes in the ovarian cells.

Our results came in harmony with those reported by **Kabuto et al., (2003),** who found that administration of 50 mg/kg b.wt. of BPA reduced the activity of detoxifying enzymes in tissue. Also, **Popa et al. (2014),** recorded increased lipid peroxidation and decreased activity of some antioxidant enzymes such as SOD, CAT, GSH-PX, GSH-R and GST in female rats treated with BPA.

The cells have various defense mechanisms against oxidative stress, including enzymatic scavengers (such as SOD, CAT and GST) that protect the system from deleterious effects of ROS. Our data revealed that BPA caused marked oxidative impact by decreasing the activities of antioxidant enzyme compared to their activities in the control group. These data are in agreement with the previous results of **Chitra et al. (2003)** who reported that treatment of rats with BPA increased levels of ROS production. Also, other results of **Karafakioglu et al. (2010)** evidenced that concentrations and activities of antioxidant enzymes significantly decreased in rats after nonylphenol (as BPA) administration.

Co-administration of OML with BPA (group II) maintained activities of antioxidant enzymes (SOD, CAT, and GST), GSH and MDA contents in pup's ovarian tissue from dams exposed to BPA during gestation and/or lactation within the range of control group, **(Table 2)**. That observed ameliorating antioxidative effect of OML extract against oxidative effect of BPA is attributed to its anti-oxidant constituents (flavonoids and steroid saponins).

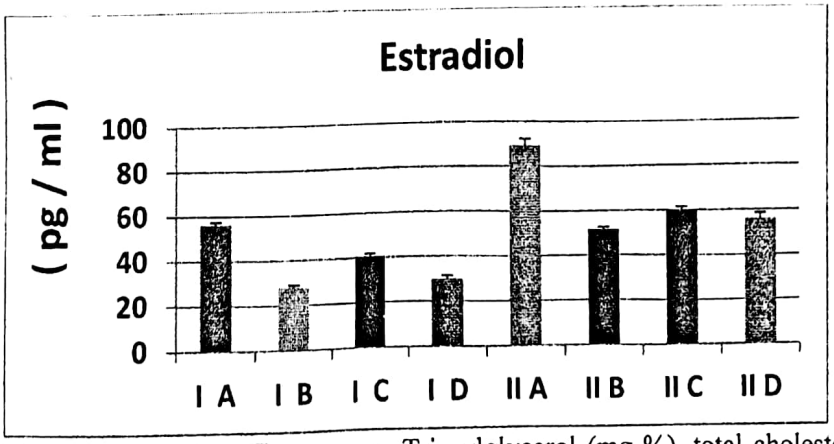
The phenolic content of OML mainly caffeic acid derivatives, such as rosmarinic acid, as well as glycosides of luteolin and hydroquinone are responsible for its powerful antioxidant activity **(Kosin , 2008; Haj-Husein et al., 2015)**.

The phenolic content of OML can play an important role in the hydrogen-donating abilities and the total scavenging capacities**(Vagi et al., 2005)** and adsorbing and neutralizing free radicals as well as protect antioxidant defenses mechanism of the cell **(Nichenametla et al., 2006). Pimple et al.,(2012)** reported that oral administration of OML extract resulted in decreasing the levels of oxidative stress markers in the tissue which shown that some flavonoids can boost the activity of some free-radical-fighting mechanisms of anti-oxidants enzymes. Accordingly, flavonoids can lower the occurrence of lipid peroxidation and lower levels of MDA in the tissue. OML contains considerable amounts of total phenolics compounds that have antioxidant activity and free radical-scavenging capacity**(Amarowicz et al., 2008)**.

Conclusion:

The study showed that OML could be used to prevent lipid peroxidation in ovarian tissue, which cause impairment in ovarian development and affecting female adult fertility. The polyphenol constituents, flavonoids, steroid saponins compounds in OML extracts exhibit antioxidant properties and hypolipidemic effects could ameliorate the alternations induced in female offspring of dams exposed to BPA.

Figure. (1) Effect of Bisphenol A and OML on serum Estradiol concentration (pg / ml) in F 1 female pups at PND 60.



Table(1). Effect of Bisphenol A and OML on serum Triacylglycerol (mg %), total cholesterol (mg %), serum HDL-cholesterol (mg %), LDL-cholesterol (mg %), and VLDL-cholesterol (mg %) concentrations in normal and different experimental groups of F 1 female pups at PND 60.

Groups		Triacylglycerol (mg %)	Total cholesterol (mg %)	HDL- cholesterol (mg %)	LDL- cholesterol (mg %)	VLDL- cholesterol (mg %)
I	A	$89.33^{e} \pm 2.68$	$110.00^{d} \pm 3.87$	$54.79^{ab} \pm 0.98$	$37.34^{c} \pm 3.88$	$17.87^{e} \pm 0.54$
	B	$195.07^{a} \pm 3.07$	$139.07^{a} \pm 5.41$	$24.97^{f} \pm 0.56$	$75.09^{a} \pm 5.91$	$39.01^{a} \pm 0.61$
	C	$95.21^{d} \pm 0.91$	$119.04^{c} \pm 1.71$	$45.44^{d} \pm 0.73$	$54.56^{b} \pm 1.63$	$19.04^{d} \pm 0.18$
	D	$178.74^{b} \pm 1.94$	$129.03^{b} \pm 1.81$	$32.93^{e} \pm 0.73$	$60.35^{b} \pm 1.73$	$35.75^{b} \pm 0.39$
II	A	$80.25^{f} \pm 1.65$	$101.33^{fg} \pm 1.88$	$57.25^{a} \pm 0.38$	$28.03^{d} \pm 2.29$	$16.05^{f} \pm 0.33$
	B	$117.77^{c} \pm 2.00$	$107.39^{def} \pm 1.12$	$49.42^{c} \pm 1.55$	$34.41^{cd} \pm 2.52$	$23.55^{c} \pm 0.40$
	C	$86.53^{e} \pm 0.87$	$100.51^{g} \pm 0.78$	$55.40^{ab} \pm 0.89$	$27.81^{d} \pm 1.15$	$17.31^{e} \pm 0.17$
	D	$88.39^{e} \pm 1.50$	$102.80^{efg} \pm 0.83$	$53.41^{b} \pm 1.33$	$31.72^{cd} \pm 1.81$	$17.68^{e} \pm 0.30$
LSD At P< 0.05		= 5.58	= 6.41	= 3.73	= 7.63	= 1.12

- Data represent as mean ±standard error (SE)
- The presence of the different superscripted small letters indicates significant variations at $P \leq 0.05$.

Table (2). Effect of Bisphenol A and OML on MDA concentration (n mol /g tissue), Glutathione (GSH) content (mg /g tissue), total SOD activity (U/ g tissue), CAT activity (U / mg protein), and GST activity (U / mg protein) in F 1 pups ovarian tissue at PND 60.

Groups		MDA	GSH	SOD	CAT	GST
I	A	$34.78^{ef} \pm 1.88$	$17.46^{bc} \pm 0.92$	$57.40^{b} \pm 1.21$	$8.42^{b} \pm 0.42$	$1.55^{b} \pm 0.03$
	B	$76.92^{a} \pm 2.67$	$9.30^{e} \pm 0.40$	$21.97^{e} \pm 1.30$	$3.86^{f} \pm 0.35$	$0.47^{f} \pm 0.02$
	C	$49.36^{c} \pm 0.94$	$13.30^{d} \pm 0.40$	$31.77^{d} \pm 0.46$	$5.97^{e} \pm 0.20$	$0.89^{d} \pm 0.04$
	D	$62.98^{b} \pm 2.13$	$10.50^{e} \pm 0.32$	$25.03^{e} \pm 1.56$	$5.05^{e} \pm 0.07$	$0.72^{e} \pm 0.07$
II	A	$26.60^{g} \pm 2.30$	$19.66^{a} \pm 0.12$	$73.43^{a} \pm 3.57$	$9.80^{a} \pm 0.43$	$1.93^{a} \pm 0.03$
	B	$37.34^{de} \pm 2.12$	$13.28^{d} \pm 0.46$	$48.72^{c} \pm 1.08$	$7.00^{d} \pm 0.55$	$0.91^{d} \pm 0.03$
	C	$32.05^{f} \pm 1.26$	$17.66^{bc} \pm 0.70$	$57.53^{b} \pm 1.30$	$8.67^{b} \pm 0.34$	$1.49^{b} \pm 0.05$
	D	$35.10^{def} \pm 1.70$	$17.06^{bc} \pm 0.76$	$55.85^{b} \pm 3.67$	$8.07^{bc} \pm 0.42$	$1.24^{c} \pm 1.05$
LSD At P< 0.05		= 5.12	=1.54	= 5.16	= .80	= .14

- Data represent as mean ±standard error (SE)
- The presence of the different superscripted small letters indicates significant variations at $P \leq 0.05$.
- IA= Control, IB= BPA-treated group during pregnancy, and lactation, IC= BPA-treated group during pregnancy, ID=BPA-treated group during lactation
- II A= OML treated group, II B= OML and BPA during pregnancy and lactation, II C= OML and BPA during pregnancy, II D= OML and BPA during lactation

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الملخص العربى

اجريت الدراسه الحاليه لدراسة مدى التأثير السمي نتيجة التعرض لمادة البسفينول – أ اثناء فترة الحمل و/ او الرضاعة على الكفاءة التناسلية لاناث جرذان الجيل الاول وتقيم الدور الوقائى للنباتات الطبية (البردقوش) لمنع التاثير العكسي لمادة البسفينول – أ . استخدمت هذه الدراسه (48) انثى جرذ حامل قسمت بالتساوي الى مجموعتين ،المجموعة الاولى تم اعادة تقسيمها بالتساوى الى اربعة مجاميع المجموعة (A1) : مجموعة ضابطة سالبة (اعطيت زيت الذره النقى عن طريق الفم)، ،بينما الثلاث مجاميع الأخرى (B1 ، C1 ، و D1) جرعت عن طريق الفم مادة البسفينول – أ المعلقة بمحلول زيت الذرة وبالجرعة الاتية 50 ملجم لكل كجم من وزن الجسم خلال فترة الحمل و/ او الرضاعة ،اما المجموعة الثانية المعالجة بالبردقوش تم اعادة تقسيمها بالتساوى ايضا الى اربعة مجاميع بالجرعة الاتية 250 ملجم لكل كجم من وزن . وعند عمر 60 يوم كشفت النتائج أن البسفينول – أ أدى الى ارتفاع الدهون الثلاثية، الكوليسترول الكلي ، الكوليسترول منخفض الكثافة، الكوليسترول منخفض الكثافة جدا وانخفضت تركيزات كلا من الكوليسترول عالى الكثافة وهرمون الاستراديول فى الدم .وأيضا البسفينول – أ أدى الى زيادة فى تركيز مالون داى ألدهيد فى خلايا المبيض وأنخفاض معنوى فى تركيزات كلا من الجلوتاثيون المختزل، ونشاط الأنزيمات المضادة للأكسدة فى خلايا المبيض كأنزيم السوبر أوكسيد ديسميوتاز، الكاتلاز ،والجلوتاثيون – أس – ترانسفيراز. أعطاء البردقوش قبل البسفينول – أ أدى الى تغير ملحوظ في معظم القياسات التي سبق ذكرها إلى معدلاتها الطبيعية. لذلك أستخدام هذه النباتات الطبية (البردقوش) قد يحمى من الآثار الضارة التى تحدثها مادة البسفينول – أ على صحة الاناث.