j.Egypt.net.med.Assoc 78, no 1. 135 – 148 (2018)

ANTIOXIDANT AND HYPOLIPIDEMIC EFFECTS OF *TRIGONELL A FOENUM-GRAECUML* ON THE CHANGES INDUCED BY BISPHENOL A IN FEMALE PUPS RATS

By

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ABSTRACT

Environmental contaminants in mammals play on important role in the development of female reproductive dysfunction. Nutraceuticals are able to influence female reproductive function and could be considered as chemopreventive agents. The present study investigated the potential effect of the *Trigonell a Foenum-graecum L* (*TFGL*) 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 *TFGL* extract was given orally to dams in a daily dose of 250 mg /kg for 3 weeks before BPA administration with or without BPA. At postnatal 60th day (PND 60), serum triacylglycerol (TAG), total cholesterol (TC), HDL - cholesterol (HDL-C), LDL- cholesterol (LDL-C), VLDL-cholesterol (VLDL-C) and Estradiol (E2) concentrations were assayed. The Malondialdehyde (MDA) and glutathione (GSH) contents, as well as activities of superoxide dismutase (SOD), Catalase (CAT), and Glutathione -s-transferase (GST) enzymes were also determined in ovarian tissue. BPA exposure resulted in an increased level of serum TAG, TC, LDL-C, VLDL-C and ovarian MDA. On the other hand, BPA decreased the levels of serum E_2, HDL-C. The BPA also decreased the level of GSH, and activities SOD, CAT and GST enzymes in ovarian tissue. The observed antioxidant and hypolipidemic effect of *TFGL* co-administration to dam's modulated the adverse effects induced by BPA in different stages of reproductive in female pups. In conclusion; the modulatory effect of *TFGL* on dam's adverse oxidative changes induced by BPA showed their potential as a protective agent against female reproductive dysfunction.

Keywords:

Trigonell a Foenum-graecum L,Bisphenol A,Estradiol,Oxidative stress, Antioxidant enzymes.

INTRODUCTION

Exposure to an environmental contaminant in early life (prenatal) leads to the developmental disorders and disease manifestation in late childhood, over the life course, or even transgenetically appeared. 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 is known as endocrine disrupting chemicals (EDCs) **(Dolinoy *et al.,* 2007**).

The developmental stage (embryonic, fetal and juvenile) is sensitive to EDCs. 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)**.

The alkylphenols containing BPA has estrogenic potential with 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 by increasing 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 phenoxyradicals 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, followed by critical pathophysiological conditions. Various antioxidant mechanisms protect the cells from damage caused by free oxygen radicals **(Kabuto *et al.,* 2003)**.

Nutraceuticals is a research focusing on identifying and understanding the molecular-level interaction between nutrients and other dietary bioactive with the genome **(Rawson, 2008).** *Trigonell a Foenum - graecum L* (*TFGL*) is anannual herb of Leguminosae family. The effective medicinal part of the plant is their driedripe seeds, which possess estrogen and / or progesterone-like effect. It possesses galactogogue activity, hypolipidemia, hypoglycemic, anti-inflammatory and an antifertility agent. It is also used for treatment of

wounds, gastrointestinal ailments and can reduce the severity of dysmenorrhea **(Sakran *et al.*, 2016). Veronika *et al.*, (2015)** showed that *TFGL* has antitumor activity in vivo. The chemical composition of fenugreek includes alkaloids (Trigonelline, Choline, Gentianine and Caprine), diosgenin, saponins, steroid sapogenins, trigoneosideIa, Ib, IIa, IIb, IIIa and IIIb, glycoside and trifoenoside A, flavonoids, tannins, including quercetin,vitexin, fixed oils. Seeds contain amino acid 4-hydroxyisoleucine (4-OH-Ile), (4, 5-dimethyl-3-hydroxy-2 [5H]-furanone) **(Flammang *et al.*, 2004)**. The objective of the current study is to evaluate the potential modulatory effects of *TFGL* against the adverse changes induced by BPA in relation to its effects on the female reproductive system.

Materials and Methods:

Medicinal Plant and extract preparation:

*TFGL*seeds were extracted with 70 % (v/v) ethanol, lyophilized and stored at - 20 ˚C **(Eidi *et al.*, 2007).**

Animals:

A total number of 48 healthy mature female albino rats weighing 100-150 grams and 16 healthy mature male albino rats were housed under normal laboratory hygienic conditions for adaption two weeks. Animals were kept and treated according to the guidelines of ethical committee of Cairo University (Approval number CU II S 12 16). At the 14 th week of age, each three mature female albino rat 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 group:

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 day 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 day 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 days postnatal until 30 days postnatal (4weeks).

II. *TFGL* treated group: During three weeks before mating, all female albino rats in this group were given *TFGL* extract daily in a dose of 250 mg/kg b.w/ orally in sterile distilled water **(Eidi *et al.,* 2007)** from three weeks of pre-mating until 30 days postnatal (12weeks). It subdivided into four sub-groups:

II A. *TFGL* treated group: Did not received BPA.

II B. *TFGL* 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. *TFGL* and BPA during pregnancy: Rats were co-administrated 50 mg/kg b.w of BPA daily (from zero day of gestation until 30 days of gestation (4weeks).

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

Blood and tissue sample collection: At 60 days postnatal, blood samples were taken from ten female pups rats (1^{ST} generation (F 1) after overnight fasting from each group then cervical dislocated for ovaries collection. **Blood and tissue sample collection:** At 60 days postnatal, blood samples were taken from ten female pups rats (1^{St} generation (F1) 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 before estimation of estrdiol 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 exposure 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 a *TFGL* either alone and/or in combination with BPA on lipid profile, estradiol status and oxidative stress markers in ovaries.

Evaluation of TAG, TC, LDL-C, and VLDL-C 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 *TFGL* (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 (Table 2). The nutrients present in *TFGL*, especially flavonoids and polyphenols are responsible for making them powerful antioxidant **(Prema *et al.*, 2017).** The administration of *TFGL* extract decreased serum TC, TAG, LDL-C, and VLDL-C concentrations as compared

with control rats. These findings were consistent with those of **Sharma *et al.* (2014),** where *TFGL* extract lead to a significant decrease of serum TC, TAG, LDL-C, and VLDL-C levels. Hypolipidemic effect of *TFGL* extract was partly related to the decrease in fat accumulation, up-regulation of LDL receptor and decrease in insulin, which could decrease the activity of pyruvate dehydrogenase and acetyl-CoA carboxylase involved in lipogenesis as well as activity of 3-hydroxy-3-methylglutaryl-CoA reductase involved in cholesterol synthesis **(Brownsey *et al.*, 2006;Abedinzade *et al.*, 2015).** The role of fenugreek seed in the management of dyslipidemia and decreased risk of cardiovascular disease during the post menopause period was proposed **(Vijayakumar *et al.*, 2010).**

The evaluation of estradiol (E_2) level in female offspring rats from dams exposed to BPA during gestational and/or locational period was showed a significant reduction compared to control group Fig. (1). the pronounced reduction in the above-mentioned traits was observed in pups from dams exposed to BPA during the gestational and locatation period. There is a paucity of human data, experimental animal and *in vitro* studies have consistently observed decreased ovarian E 2 synthesis with higher BPA levels **(Xu *et al*., 2002**; **Mlynarcikova *et al*., 2005; Zhou *et al*., 2008; Peretz *et 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)**. More recently, **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 h 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 increased cell death, resulting in granulosa cell apoptosis and follicular atresia **(Hughes and Gorospe, 1991; Yu *et al.*, 2004).** These results suggest that BPA may antagonize the anti-apoptotic effect of endogenous estrogens synthesized by granulosa cells **(Ehrlich *et al.*, 2012).**

Co-administration of *TFGL* (group II) as protective treatment showed a 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 dietary concentrations of phytoestrogens, *TFGL* extract has an estrogenic and androgenic effect that will lead to the development of sex organs in female, also *TFGL*has antioxidant property, which improves organ function **(Chaloob *et al.,* 2010)**. Supplementation of *TFGL* extract seem to have positive effects on body composition in combination with resistance training by decreasing body fat percentage **(Wilborn, 2010)** but its consumption can actually increase estrogen levels **(Sreejaa Anju, 2010; http://nutrientjournal.com/fenugreek-studies-show-no-anabolic-effect-but-affects-body-composition/).** BPA in group IB, IC and ID caused an oxidative stress status by increasing MDA content and decreasing GSH concentration and inhibiting the activities of antioxidant enzymes (SOD, CAT, and GST) in ovarian tissue, (Table 2). That oxidative stress was more pronounced in offspring from the dam treated with BPA during gestation and lactation period, group IB. 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 illustrated that treatment of rats with BPA increases 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. 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 **(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. Co-administration of *TFGL* (group II) as protective treatment maintained MDA and GSH level and also antioxidant enzyme activity of SOD, CAT, and GST in pup's ovarian tissue from dams exposed to BPA during gestation and/or lactation within the normal range (Table 2). The nutrients present in *TFGL*, especially flavonoids and polyphenols are responsible for making them powerful antioxidant **(Prema *et al.,* 2017)**. **Bhatia *et al.* (2006)** reported an antioxidant protective effect of *TFGL* against lipid peroxidation. The antioxidant effect of *TFGL* has been correlated to main compounds in its constituents, flavonoids, steroid saponins **(Basu and Srichamroen, 2010)**, and the phenolic contents particularly rutin, ferulic, myricetin, chlorogenicacid, p-coumaric acid and ferulic acid **(Petit *et al.,* 1995)**. Polyphenols can play an important role in adsorbing and neutralizing free radicals as well as protect antioxidant defenses mechanism of the cell **(Nichenametla *et al.,* 2006).**

CONCLUSION

The study showed that *TFGL* could be used to prevent lipid peroxidation in ovarian tissue, which causes impairment of ovarian development and affecting female fertility.

The polyphenol constituents, flavonoids, steroid saponinscompound in *TFGL* extracts exhibit antioxidant properties and hypolipidemic effects could ameliorate the alternations induced in female offspring of dams treated with BPA.

Table (1): Effect of Bisphenol A and *TFGL* on serum Triacylglycerol (mg %), total cholesterol (mg %), 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} ± 2.68	110.00 ^{d} ± 3.87	54.79 ^{a} ± 0.98	37.34 ^{d} ± 3.88	17.87 ^{e} ± 0.54
	B	195.07 ^{a} ± 3.07	139.07 ^{a} ± 5.41	24.97 ^{d} ± 0.56	75.09 ^{a} ± 5.91	39.01 ^{a} ± 0.61
	C	95.21 ^{d} ± 0.91	119.04 ^{c} ± 1.71	45.44 ^{b} ± 0.73	54.56 ^{b} ± 1.63	19.04 ^{d} ± 0.18
	D	178.74 ^{b} ± 1.94	129.03 ^{b} ± 1.81	32.93 ^{c} ± 0.73	60.35 ^{b} ±1.73	35.75 ^{b} ± 0.39
II	A	85.00 ^{e} ± 2.13	104.11 ^{d} ± 1.12	54.16 ^{a} ± 1.88	32.95 ^{de} ± 2.27	17.00 ^{ef} ± 0.43
	B	131.01 ^{c} ± 1.95	119.55 ^{c} ± 1.97	47.03 ^{b} ± 2.92	46.31 ^{c} ± 1.46	26.20 ^{c} ± 0.39
	C	88.62 ^{e} ± 1.20	104.58 ^{d} ± 0.99	54.15 ^{a} ± 1.83	32.71 ^{de} ± 2.37	17.72 ^{e} ± 0.24
	D	90.13 ^{de} ± 1.52	109.03 ^{d} ± 1.21	54.32 ^{a} ± 0.37	36.69 ^{d} ± 1.38	18.03 ^{de} ± 0.30
LSD value at 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 ≤ 0.05.

Table (2): Effect of Bisphenol A and TFGL on MDA concentration (n mol /g tissue), Glutathione (GSH) content, 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} ± 1.88	17.46^{a} ± 0.92	57.40 ^{b} ± 1.21	8.42 ^{a} ± 0.42	1.55 ^{b}± 0.03
	B	76.92 ^{a} ± 2.67	9.30^{d} ± 0.40	21.97 ^{e} ± 1.30	3.86 ^{d} ± 0.35	0.47 ^{f}± 0.02
	C	49.36 ^{c} ± 0.94	13.30^{c} ± 0.40	31.77 ^{d} ± 0.46	5.97 ^{c} ± 0.20	0.89 ^{d}± 0.04
	D	62.98 ^{b} ± 2.13	10.50^{d} ± 0.32	25.03 ^{e} ± 1.56	5.05 ^{c} ± 0.07	0.72 ^{e}± 0.07
II	A	31.25 ^{f} ± 1.15	18.38^{a} ± 0.46	69.83 ^{a} ± 1.98	8.94 ^{a} ± 0.28	1.78 ^{a}± 0.02
	B	40.07 ^{d} ± 1.69	12.52^{c} ± 0.52	45.13 ^{c} ± 1.26	7.27 ^{b} ± 0.43	0.80 ^{de}± 0.03
	C	34.30 ^{ef} ± 0.76	17.08^{a} ± 0.28	57.03 ^{b} ± 1.61	8.55 ^{a} ± 0.23	1.59 ^{b}± 0.07
	D	36.06 ^{def} ± 1.21	16.74 ^{b} ± 0.68	54.83 ^{b} ± 2.61	8.32 ^{a} ± 0.44	1.19 ^{c}± 0.07
LSD value at 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 ≤ 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= *TFGL* treated group, II B= *TFGL* and BPA during pregnancy and lactation, II C= *TFGL* and BPA during pregnancy, II D= *TFGL* and BPA during lactation.

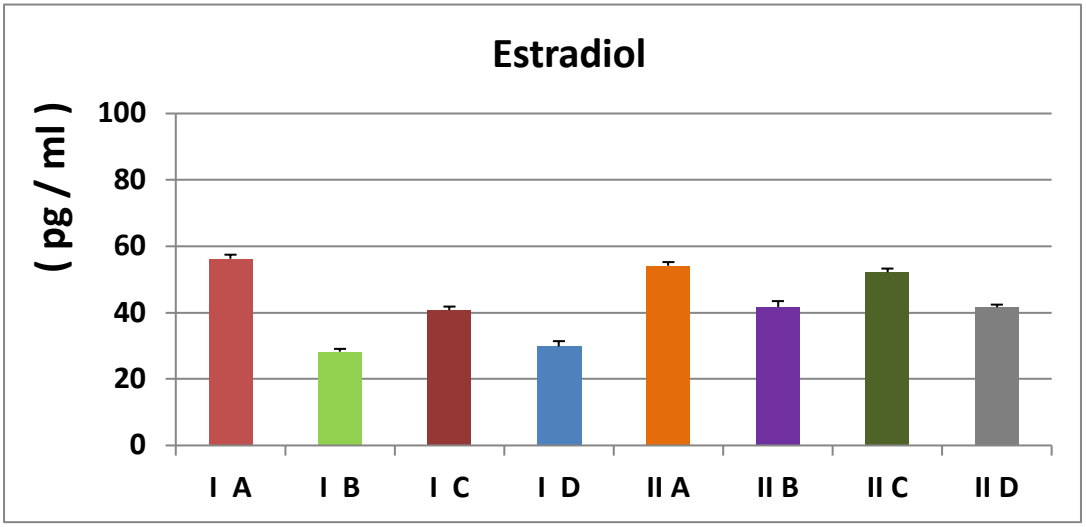

Fig. (1): Effect of Bisphenol A and *TFGL* on serum Estradiol concentration (pg. / ml) in F 1 female pups at PND 60.

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