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EVALUATION TOXIC EFFECT OF BISPHENOL A IN REPRODUCTIVE SYSTEM OF MALE MICE AND AMELIORATION ITS EFFECT BY GREEN TEA EXTRACT

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Abstract

Bisphenol A (BPA) is a chemical compound have chemical formula $(CH_3)_2C(C_6H_4OH)_2$. Bisphenol A (BPA) is use to synthesis various plastic and epoxy resin such as water bottle, many food and beverage cans, sports equipment, CDs, and DVDs. BPA could be hydrolyzed in rise temperature and basic or acidic conditions which major to leaching of BPA into drink and food containers. This study aim to evaluate toxic effect of Bisphenol A in testis of male mice and amelioration its toxic effect by co-administration of green tea extract. Healthy adult male albino laboratory mice (Mus musculus) treated with two doses of Bisphenol A (20 mg/kg and 40 mg/kg body weight per day) for thirty days. The Biochemical parameters of luteinizing hormone, testosterone and follicle stimulating hormone showed significant differences between different groups also histopathol-ogical of testis showed significant damage in groups that treat with Bisphenol A, in addition co administration of extract green tea showed significant mitigation in toxic effect of Bisphenol A.

***Keyword*:** Bisphenol A, Green Tea Extract, antioxidant, Amelioration.

Introduction

Bisphenol A (BPA) is a chemical compound have chemical formula $(CH_3)_2C(C_6H_4OH)_2$. Bisphenol A (BPA) is using to synthesis various plastic and epoxy resin such as water bottle, many food and beverage cans, sports equipment, CDs, and DVDs. BPA could be hydrolyzed in ride temperature and basic or acidic or conditions which can major to leaching of BPA into drink and food containers (Welshons *et al*., 2006). It is determination global product of BPA exceeds six billion pounds of BPA annually and expected that this amount will be increased in the coming years (Burridge, 2003). BPA is endocrine disruptor that can interferes with the hormonal system and contributes to adverse health effects in human specially woman including recurrent miscarriages , obesity, endometrial hyperplasia , polycystic ovarian syndrome and abnormal karyotypes (Monneret, 2017; Takeuchi & Tsutsumi, 2002). Several studies give an account the occurrence of oxidative toxicity after BPA exposure in mice (Chitra *et al*., 2003; Gong & Han, 2006). It was proposition that BPA chief to cause tissue injury in the kidney, liver, brain and other organs by the consistence of reactive oxygen species (ROS) (Bindhumol *et al*., 2003; Kabuto *et al*., 2004). Green tea is acquired from the tea plant Camellia silences which belongs to the family Thecae and its cultivated in at least 30 countries around the world, commonly consumed in India, China, Japan, Asian countries, some parts of North Africa, the United States, and Europe (Khan & Mukhtar, 2013). Several epidemiological and animal studies has studied the effects of green tea on surrogate risk factors associated with Cardiovascular disease, cancer and anti-inflammatory agent (Kolaczkowska & Kubes, 2013; Stangl *et al*., 2006). Such mechanisms including , lipid profile modification, endothelial function protection, antioxidant effects, anti-inflammatory, and anti-proliferative effects. The antioxidant nature of the polyphenol compounds in green tea is due to the ability of phenolic hydroxyl groups to scavenge reactive oxygen species(Rady *et al*., 2018). This study aims to evaluate toxic effect of Bisphenol A in testis of male mice and assessment the role of green tea extract as antioxidant to ameliorate toxic effect of Bisphenol A.

Material and Method

Animals

A sixty healthy adult male albino laboratory mice (*Mus musculus*) that weighting about (25-30 gm) was use in the study. They were purchased form animal house in Iraqi-Center for Cancer and Medical genetic research . All mice were acclimatized in the standard appropriate conditions 12 h/light and 12h/dark in 25 ±4 ºC for 1 weeks before experiments was starting.

Chemicals Material

Bisphenol A (BPA) powder Purity 99% was purchased From Sigma (U.S.A) by Iraqi Biotechnology laboratory (Baghdad-Iraq). In addition, all histological processes of samples was carried out in Iraqi - Center for Cancer and Medical genetic research (Baghdad –Iraq) .

Biochemical assay kit

All biomedical assay kits(follicle stimulating hormone(FSH), Luteinizing hormone (LH), and Testosterone) was carry out using Cobas e411 depending on Roche diagnostic assay kit -Japan.

Green tea Leaves

Freshly green tea leaves was purchased from marketplace in Samwah city –Iraq, they washing three tie with running water to remove any odd matter and after that

washing distilled water , green tea were ground into a fine powder in a mill and kept in dark plastic container for next steps.

Preparation of the extract green tea leaves :

10 gram fine powder of green tea leaves added into 1000 ml of water and boiled in 80 ºC for 8 h with stirring, the mixture was cooled at room temperature and filtrated by using Whatman No.1 filter paper, supernatant was kept in dark plastic container for next steps (Wei *et al*., 1999; Chandra & De, 2010)

Experimental design and treatment schedule :

A sixty animals were divided randomly into six groups (10 mice per group) as following :

First group: Animals were not exposed to any treatment and were served as negative control (NC)

Second group: animal were treated with dimethylsulfoxide (DMSO) in concentration 40% in sterile water and act as positive control (PC)

Third group: animal were treated with low dose of Bisphenol A (20 mg /kg of body weight) (LD)

Forth group: animal were treated with high amount of Bisphenol A (40 mg/kg of body weight) (HD)

Fifth group: animal were treated with low dose of Bisphenol A (20 mg/kg of body weight) in addition extract green tea was co-administrated orally to this group. (LD+ plant extract).

Sixth group: animal were treated with high amount of Bisphenol A (40 mg/kg of body weight) in adding extract green tea was co-administrated orally to this group. (HD+ plant extract).

All treatment with Bisphenol A was carry out by intraperitoneal administration through dissolve Bisphenol A in 40 % DMSO and in range 100 µl per day for one month. Aqueous extraction of Green tea was co-administrated orally to fifth and sixth groups instead of water.

Preparation the blood and histological samples

After thirty days of treatment , animal were anesthetized through use Ether then 1 ml blood was collected by cardiac puncture and preserve in tube with using 130 mM anticoagulant sodium citrate in ratio 9:1 (blood : sodium citrate). The blood samples were centrifuged Immediately in 1800 rpm for 15 min and plasma was transferred into new tube and kept in -20 ºC until assay (Welshons *et al*., 2006).

Animal Testis were isolated and kept in formalin (10%), histological prepared sample was carried out by using sections stained with haematoxylin and eosin (H&E) (Adebayo *et al*., 2009).

Statistical Analysis

All obtained results from all parts of the study will be analyzed using graph Pad prism version 6.01 depending on one way A NOVA test to compare between different concentration of samples.

Results

The results of testosterone show in figures (1), there is no significant difference between negative (mean =5.382 ng/ml) and positive control (mean= 5.444 ng/ml). Result show significant decreased in testosterone value ($p < 0.05$) in group treated with low dose of Bisphenol A (mean= 4.108 ng/ml) compared with positive control, also significant decreased in testosterone value in group treated with high dose of Bisphenol A (mean= 2.046) compared with positive control, whereas using green tea extract show increase in testosterone value in group treated with little amount of Bisphenol A and extract green tea (mean=4.928 ng/dl) compared with LD group. In addition co administration of extract green tea showed significant increased testosterone value in group treated with high amount of Bisphenol A and extract green tea compared with group treated with high amount of Bisphenol A (HD group).

The result of luteinizing hormone (LH) are showed no significant change among negative control (mean= 2.764) and positive control (mean= 2.740) whereas result are showed significant decrease in LH value in group treated with little amount of Bisphenol A (mean =1.758 ng/ml) compared to positive control and significant decreased in LH value in group treated with high amount of Bisphenol A (mean = 1.160 ng/ml) compared to positive group. In addition groups treated with green tea extract showed increased in LH value (mean =2.250 ng/ml and 1.718 ng / ml) for both high and low dose groups with green tea extract respectively.

Result of follicle stimulating hormone (FSH) are showed no significant difference between negative (mean= 2.764 ng/ml) and positive control 2.740 ng/ml) whereas groups treated with little amount and high amount of Bisphenol A showed significant decreased into FSH value (mean =1.758 ng/ml and 1.160 ng/ml respectively) compared to positive group whereas groups treated with Bisphenol A low and high dose with green tea extract showed increased in FSH value (2.250 ng/ml and 1.718 ng/ml respectively) compared to low and high dose groups respectively.

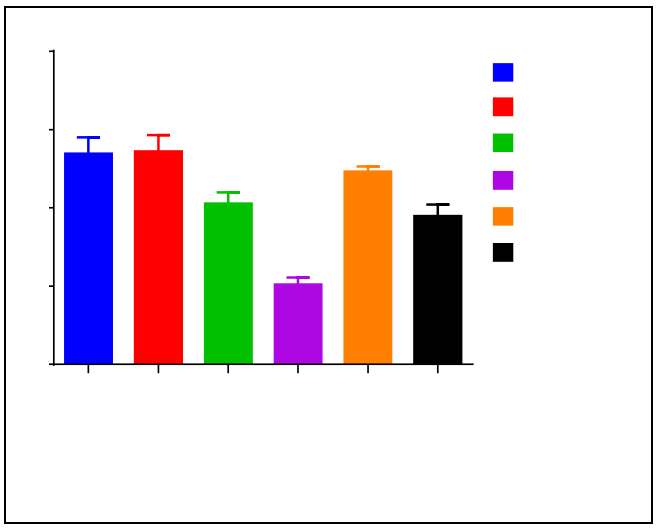

Fig. 1 : Serum testosterone level of male mice treated for 30 days with Bisphenol A and amelioration its effect by green tea extract .P value < 0.0001 (****) and n=10

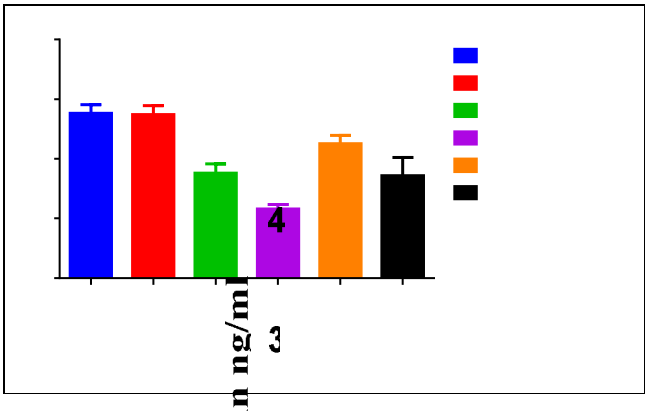

Fig. 2 : Serum luteinizing hormone level of male mice treated for 30 days with Bisphenol A and amelioration its effect by green tea extract .P value < 0.0001 (****) and n=10

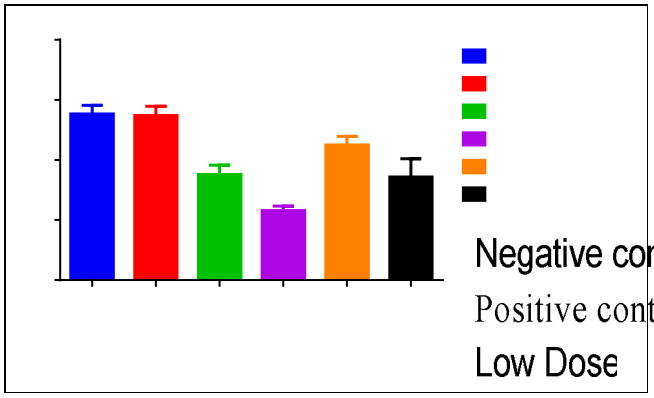

Fig. 3 : Serum follicle stimulating hormone level of male mice treated with Bisphenol A for 30 days and amelioration its effect by green tea extract . P value < 0.0001 (****) and n=10

Histopathological examination result :

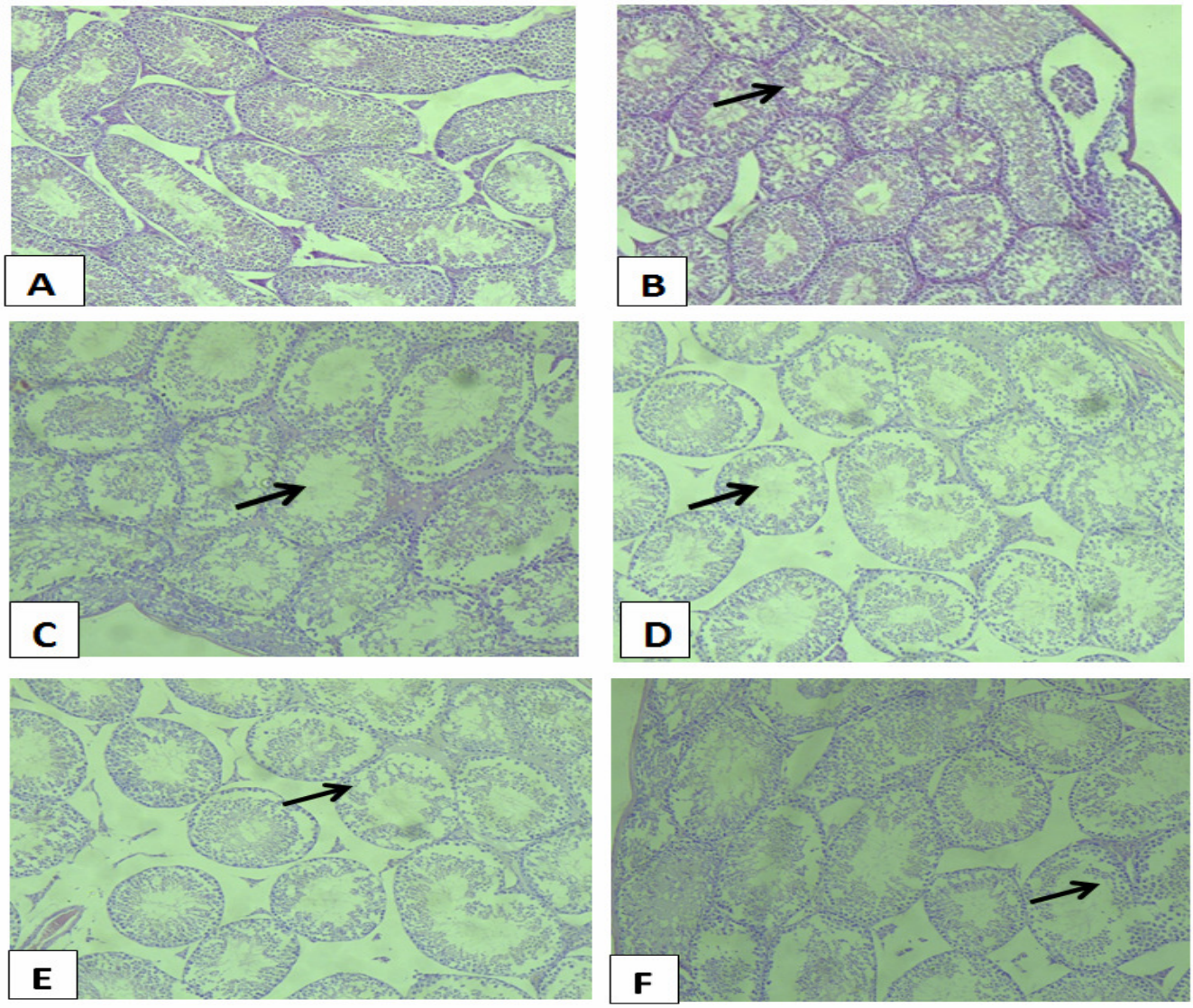

Fig. 4 : Histopathological sections of testicle mice treated with Bisphenol A and green tea extract for 30 days with A-Negative control showed no clear lesions , B-Positive control showed mild vacoulation of seminiferous tubules and dilated lumen, C-Treated with low dose Bisphenol A. appeared circled seminiferous tubules with decreased spermatogenesis , D-Treated with high dose of Bisphenol A showed vacoulation of spermatogonia and seminiferous tubules are totally devoid sperms, E-Treated with low dose of Bisphenol A and co-administration of green tea extract showed normal arranged seminiferous tubules with complete spermatogenesis. F- Group treated with green tea extract plus high dose of Bisphenol A figure (4F) showed increased cellular debris/sloughed germ cells, decreased luminal content.

Table 1 : Analysis results data of serum testosterone value for male mice using graphPad prism version 6.01 depending on one way A NOVA. P value < 0.0001 (****)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant	Summary
NC vs. PC	-0.06200	-0.4691 to 0.3451	No	ns
PC vs. LD	1.336	0.9289 to 1.743	Yes	****
PC vs. HD	3.398	2.991 to 3.805	Yes	****
LD vs. LD+ green tea extract	-0.8200	-1.227 to -0.4129	Yes	****
HD vs. HD+ green tea extract	-1.744	-2.151 to -1.337	Yes	****

Table 2 : Analysis results data of serum luteinizing hormone value for male mice using graphPad prism version 6.01 depending on one way A NOVA. P value < 0.0001 (****)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant	Summary
NC vs. PC	0.02400	-0.2094 to 0.2574	No	ns
PC vs. LD	0.9820	0.7486 to 1.215	Yes	****
PC vs. HD	1.580	1.347 to 1.813	Yes	****
LD vs. LD+ green tea extract	-0.4920	-0.7254 to -0.2586	Yes	****
HD vs. HD+ green tea extract	-0.5580	-0.7914 to -0.3246	Yes	****

Table 3 : Analysis results data of serum follicle stimulating hormone value for male mice using graphPad prism version 6.01 depending on one way A NOVA. P value< 0.0001 (****)

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant	Summary
NC vs. PC	0.02400	-0.2094 to 0.2574	No	ns
PC vs. LD	0.9820	0.7486 to 1.215	Yes	****
PC vs. HD	1.580	1.347 to 1.813	Yes	****
LD vs. LD+ green tea extract	-0.4920	-0.7254 to -0.2586	Yes	****
HD vs. HD+ green tea extract	-0.5580	-0.7914 to -0.3246	Yes	****

Discussion

The hurtful effects of BPA on male reproductive job, following in utero exposure, have been vastly studied in laboratory animals (Vom Saal *et al*., 1998). It is lucid that environmental xenobiotic estrogens such as Bisphenol A affect reproductive functions in experimental animals, and it has newly been hypothesized that exposure to estrogenic substances might account for the increasing frequency of infertility and the associated disorders of the male reproductive system in humans. However, there is little information on the effects of BPA on reproductive functions in adult males. In the present study, BPA effect was determined effects on adult male mice reproductive purposes in vivo and investigated by measuring hormonal secretion from sex glands.

As it shown in Figure (1) and Table (1) there are no significant change among negative and positive group which are treated with DMSO, whereas there are significant decreased in testosterone level in groups treated with low dose and high amount of Bisphenol A comparative to positive group and this decreased in testosterone level due to effect of Bisphenol A as xenobiotic estrogens which prevent synthesis of testosterone is synthesized from cholesterol in Leydig cells. testosterone synthesis decreased by BPA which agreed with other genetic study about effect of Bisphenol A on the expression of gene which is responsible of enzymes which play important role in convert cholesterol to testosterone, BPA generate ROS by increasing oxidative stress in testis and decreasing the activities of antioxidant enzymes (Nakamura *et al*., 2010; Hauet *et al*., 2005).

Green tea is rich in anti-oxidants compounds such as polyphenol and vitamin C. in Table (1) and Figure (1) which showed amelioration effect of Bisphenol A through increased in testosterone level compared to little dose and high dose groups which is referred to prevent stress oxidation of Bisphenol A by green tea extract (Suthar *et al*., 2014).

Luteinizing hormone (LH) is a hormone produced by gonadotropic cells in the anterior pituitary gland. In male it stimulates Leydig cell production of testosterone. It acts synergistically with FSH (Ujihara *et al*., 1992). Follicle-stimulating hormone (FSH) is a gonadotropin and consider as a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland. In males, FSH induces Sertoli cells to secrete androgen-binding proteins (ABPs), regulated by inhibin's negative feedback mechanism on the anterior pituitary. Specifically, activation of Sertoli cells by FSH sustains spermatogenesis and stimulates inhibin B secretion and stimulates the maturation of primordial germ cells (Haggstrom, 2014). As show in Table (2) and Figure (2), there is no significant difference in LH value between negative and positive control and small but significant decrease in group treated with little dose of Bisphenol A ,this decreasing is extra significant in group treated with high amount of Bisphenol A and this due to BPA levels had adverse effects on testicular function by decreasing pituitary LH secretion and reducing Leyden cell steroidogenesis. proved that BPA binds with LH receptor ligand binding, and releasing of LH from the receptor may cause reduction of steroidogenic activity. LH value was increase in group treated with Bisphenol A plus extract green tea compared to group treated with only Bisphenol A and that proved role of green tea against Bisphenol A and role it in prevent binding of Bisphenol A to receptor that can lead to inhibit secreted of LH as negative feedback mechanism . FSH also show results as showed in Figure (3) and Table (3) which are similarity to LH results and this due to FSH acts synergistically with LH.

Spermatogenesis is exact dynamic and orchestrated process, in which germ cells undergo mitotic and meiotic

divisions to produce ESs. Germ cells are vulnerable to external pollutants such as chemicals, drugs and radiation (Willhite *et al*., 2008).

The histopathological results of treated mice of this study showed damages of the spermatogonial cells upon exposure to BPA at different level from few damage in LD group (Figure 4 ºC) to sever damage in HD group (Figure 4D) and degenerative changes in the form of vacuolation, of the seminiferous tubules, degenerative changes in Leydig cells. BPA induced seminiferous tubule degeneration, necrosis, wide interstitial tissues, desquamation of germinal cells and the deceleration of spermatogenesis (Takahashi & Oishi, 2001). He seminiferous tubules are avascular, all oxygen and nutrients have to pass out of the interstitial space, then through the peri-tubular myoid cells, and lastly out of the Sertoli cells to reach the germ cells. This venue them on the boundary of hypoxia and may makes them very susceptible to BPA (Kamel *et al*., 2018). Leydig cells synthesize androgens that promote spermatogenesis as well as maintain secondary sexual characteristics and sexual function after getting signals from luteinizing hormone. During the process of spermatogenesis, meiosis and sperm differentiation are facilitated by androgens (Cai *et al*., 2000) These degenerative changes in Leydig cells may interfere with normal function and inhibit synthesis of androgens, which may further affect the spermatogenesis process.

On other hand the histological results of groups treated with Bisphenol A and green tea Figures (4E and 4F) showed a amelioration compared with LD and HD groups which treated with same dose of Bisphenol A respectively and that refer to strong action of the green tea which act as anti-oxidant and its ability to amelioration the adverse effect of BPA (Cabaton *et al*., 2010)

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