Original Research Article

Bisphenol A Disrupt endogenous estrone,estriolandestradiol levels in female albino wistarrats

ABSTRACT:

Bisphenol A (BPA) is a contaminant with increasing exposure and exerts both toxic and estrogenic effects on cellsThe general population is potentially exposed to many chemicals that can affect the endocrine system. These substances arecalled endocrine disruptors (EDs), and among them bisphenol A (BPA) is one of the most widely used and well-studied. Available literature presents inconsistent and in some cases incomplete data on associated effects of BPA. This study investigates the possibility of blood serum endogenous estrogens levels perturbations at prevailing low exposure rates of BPA in albino Wistar rats. To eleven (11) experimental groups each containing five (10) female rats were administered graded doses of BPA/kgbw/day. To the firstcontrol group was given water. Animals blood were collected weekly for twelve weeksand serum sample specimens analyzed by routine diagnostic procedures for estrogens assay using Chemwell Chemical Analyzer. Significantly increased concentrations of estrone and estradiol were observed, alongside significant decreased estriol concentrations, at all concentrations of BPA exposure at different time suggesting that bisphenol A upsets endogenous esrogens and causes perturbation of estradiol concentrations. These findings point to the overall disruption of estrogen metabolism.

Keywords: Bisphenol A, Estrone, Estrial, Estradiol, Hormone,

INTRODUCTION

There is still an ongoing debate whether environmental levels of BPA are harmful for the population or not. Bisphenol A (BPA) is a endocrine disruptor (ED), that still receives attention from the global scientific community and the general public, due its ubiquity in our environment and uncertainties about its effects on humans. It leaks from polycarbonate plastics, which are used in food and drink containers. BPA enters the body by the ingestion of contaminated food and beverages, through the skin by contact with thermal receipts (Ehrlich *et al.* 2014,) and inhalation of cigarette smoke or dust (Braun *et al.* 2011). BPA is a weak estrogen when considering its binding activities to the estrogen receptor (ER) (Welshons*et al.* 2003). On the other hand, it can act with the same potency as endogenous estradiol (E2) on the non-classical membrane estrogen receptor (Alonso- Magdalena *et al.* 2012). Its mode of action, however, is much more complex. It may act through other nuclear receptors including the estrogen related receptor (Delfosse*et al.* 2014), androgen receptor (Teng *et al.* 2013), thyroid receptor (Moriyama

et al. 2002), glucocorticoid receptor (Sargis*et al.* 2010), peroxisome proliferator activated receptor γ (PPARγ) (Pereira-Fernandes *et al.* 2013, Wang *et al.* 2010) and pregnane X receptor (Sui *et al.* 2012). BPA is capable of inducing toxic effect on non-reproductive vital organs; *several studies* have *reported that* absorption of BPA has cause extensive damage to the liver and kidney (Ezeonu*et al* 2015, Oguazu*et al* 2015), formation of multinucleated giant cells in liver hepatocytes, DNA adduct formation and induced the production of free radicals in hepatocytes *in vitro* (LaKind*et al* 2012). Indeed, this chemical compound may be involved in adipose tissue dysfunction, metabolic/endocrine dysfunctions, cancer and fertility problems (Wang *et al* 2012,), impaired plasma glucose (Teppala*et al* 2012,), involved in insulin resistance, (Alonso-Magdalena *et al* 2010), causes permanent chromosomal damage linked to recurrent miscarriage and birth defects (Vom and Hughes., 2005), spur both the formation and growth of fat cells, (Braun *et al*, 2011). An interaction of BPA with the expression and activity of steroidogenic enzymes has also been reported (Gilibili*et al.* 2014, Ye *et al.* 2014). Moreover, BPA exerts a non-monotonic dose response at low physiologically relevant concentrations, with tissue-specific effects (Wetherill *et al.* 2007). Endogenous estrogens are thought to have an important role in the developing and maintaining female sexual characteristics. The three major endogenous estrogens in females that have estrogenic hormonal activity are;estrone, estradiol, and estriol. And they are the three major naturally occurring forms of estrogen in females.
Estradiol(E2 estrogen) produced in the ovaries primarily by the follicles and corpus luteum, is the most potent and abundant. It is essential for formation of secondary sexual characteristics such as pubic hair, breastsand healthy menstrual cycle.
These disturbing facts raise questions about the extent to which current, widespread exposures to BPA are contributing to the burden of infertility and reproductive health challenges. The aim of this study is to unveil/establish the possible effects of Bisphenol A on sex hormones in female wistar albino rats.

MATERIALS AND METHODS

Non-pregnant female rats of age 5 weeks were acclimatized in the laboratory for seven days and randomly divided into eleven (11) groups experimental of 10 rats each and respectively administered 0.05, 0.1, 00.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg of BPA/kgbw/day. The first group which served as control did not receive any treatment but distilled water instead. The graded doses of BPA were dissolved in distilled water and administered by oral gavage using intubation canular. Blood were obtained from the tail of the various groups by capillary action weekly, after BPA administration for thirteen (13) weeks. Blood samples were processed for clinical assay.

Animals were housed in aluminum wire-mesh cages in a well-ventilated animal house with a 12 h dark/light cycle and at room temperature and were provided commercial rat pellets (Vital feed from Vital group of Company, Nigeria) and water *ad libitum*.

At the end of the experiments serum FSH and LH were assayed weekly using Chemwell 2910 Auotanalyser. All reagents were commercially obtained as already prepared kits. The kits for FSH and LH were purchased from Egyptian company for Biotechnology (SAE) Cairo Egypt. Individual tests were carried out according to the kit specifications

The data obtained from each set of study were subjected to statistical analysis using the IBM Statistics software, version 20. Differences between obtained values (mean ± SD) were carried out by one-way analysis of variance (ANOVA) using SPSS software version 20.0 followed by the Tukey-Kramer multiple comparison test. A $P \leq 0.05$ was taken as a criterion for a statistically significant difference.

RESULTS

EFFECT ON ESTRADIOL (ESTROGEN (E2)

It was observed that BPA induced a dose dependent increase in estrogen concentration, which increased with sustained administration as shown in fig 2. The higher concentrations of BPA caused a spike in estrogen concentration which is maintained for all weeks, on the other hand, lower concentration appeared to maintaine aslight but steady increase in estrogen concentrations over time, with sustained administration, which did not differ significantly from the estrogen level in the control group for week 1 to 3 for the dose groups that received 0.05mg/kg to 0.5mg/kg of BPA. Across the weeks, there is significant increase ($p \leq 0.05$) in the serum estrogen level, In each week, within the groups there is a significant increase that is dose dependent especially at dose group 0.6mg/kg to dose group 1mg/kg. There is significant increase in estradiol at groups 0.6mg/kg, 0.7mg/kg, 0.8mg/kg, 0.9mg/kg and 1mg/kg at all time interval. The group perfomance profile revealed tht BPA enhanced estrogen production in dose dependent manner. Higher doses of BPA induced higher estrogen production. However, there is noticeable reversion in estrogen profile with time after the BPA dose of 0.8mg/kg group (see fig 1). there was instant estrogen spike, which steadily rise as time progressed for group 0.9mg/kg and 1mg/kg but the observed rise was reversed time dependent.

In all instances, a dose dependent increase was observed for group 0.8mg/kg, 0.9mg/kg and 1mg/kg. The group the received 1mg BPA per kg body weight showed the highest level of estradiol, these was followed by 0.9mg/kg. These groups recorded highest value at week-1 and gradually decrease over time; the concentration of estradiol for these groups was lowest at week-13 (fig 1). Groups 0.6mg/kg and 0.7mg/kg are significantly higher than those of control and week-0, at $p \leq 0.05$. These group recorded the lowest value at week-1, and showed steady increase over time with their highest value obtained at week-13. Between week-3 to week-13, a significant dose dependent increase was observed for group 0.05mg/kg, 0.1mg/kg and 0.2 mg/kg at $p \leq 0.05$; which was consistent over time. Groups 0.05mg/kg to 0.7 mg/kg recorded their highest value at week-13 and lowest value for estradiol at week-1. While group 0.8mg/kg to 1 mg/kg recorded their highest value at week-1 and lowest value at week-13 (fig 1).

The highest serum concentration of estradiol was recorded in week-13 for groups 0.05mg/kg to 0.7mg/kg; groups 0.8mg/kg to 1mg/kg recorded low estradiol levels at week-13 and high estradiol levels at week-1. In fig 1, a time dependent effect was observed, as the duration of exposure to graded doses of BPA continued, the serum concentration of estradiol continued to rise, up-to group 0.8mg/kg to 1mg/kg where the reverse effect was obtained with the estradiol level decreasing as the duration of the exposure to BPA extends. Between group 0.7mg/kg and 0.8mg/kg was the point of reversal of the trend (fig 1).

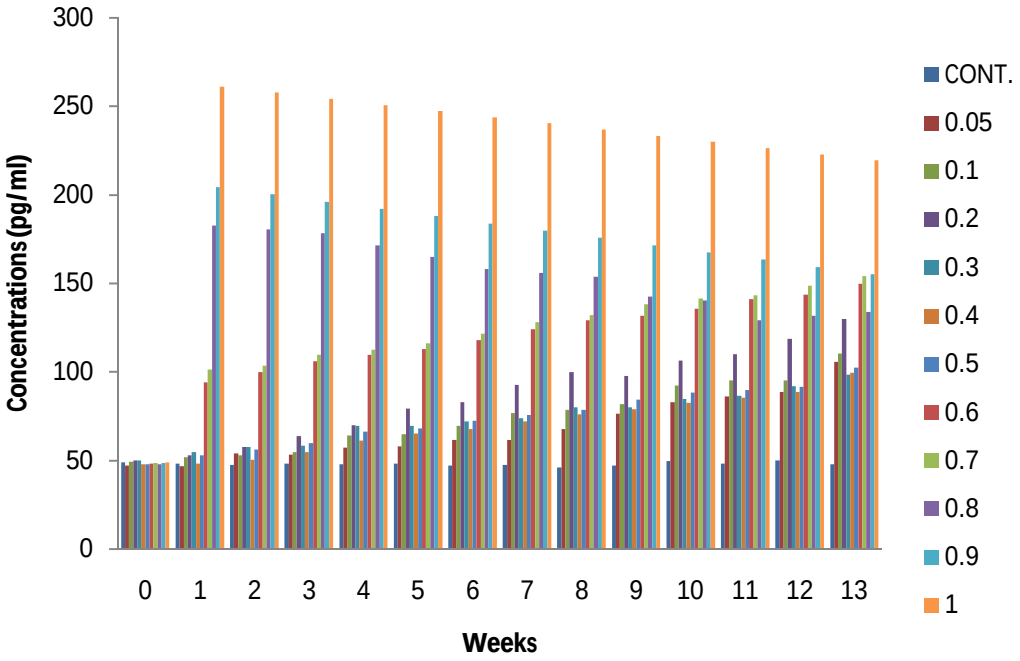

Fig. 1; Chart of concentration against weeks (durations) for change in Estradiol E2 (Estrogen).

EFFECT ON CONJUGATED ESTRADIOL

It was observed that BPA induced a dose a significant decrease in the conjugated estradiol concentration when compared with control throughout the period of exposure (fig 2).

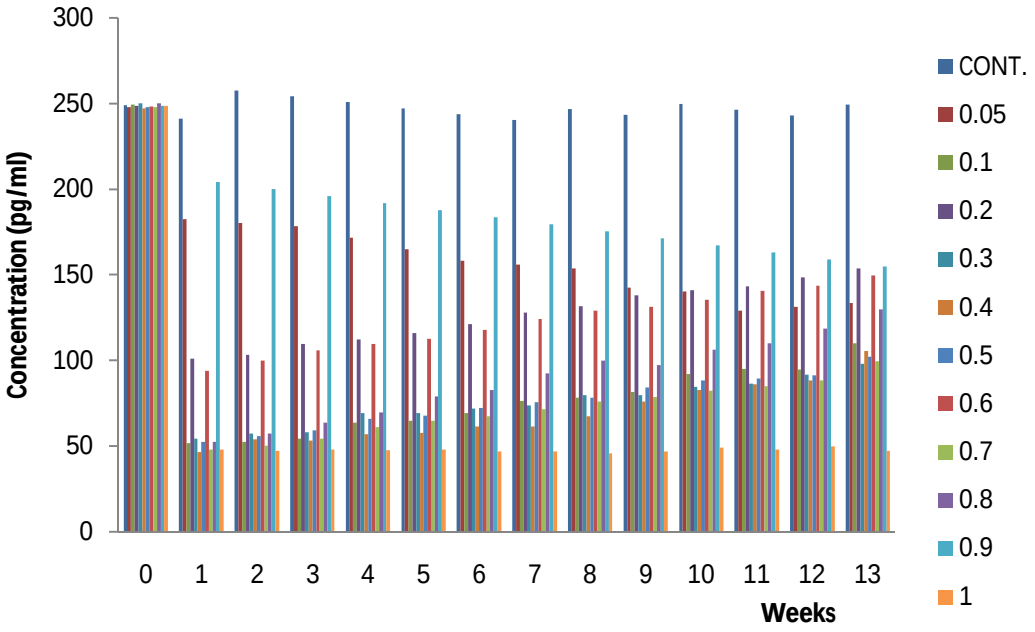

Fig. 2; Chart of concentration against weeks (durations) for change in conjugated Estradiol E2 (Estrogen).

EFFECT ON ESTRONE

At the onset of the experiment, it was observed that the estrone serum concentration were increase across the weeks (fig. 3) . Estrone levels were higher in week 1 for groups 0.8mg/kg to 1mg/kg. There is significant rise in estrone level when compared with the control at $p \leq 0.05$. Groups 0.05mg/kg to 0.5mg/kg showed a non signifiant difference at weeks-1 to week-3. As the duration of exposure increased, the level of estrone increased correspondingly (fig 3). At groups 0.8mg/kg to 1mg/kg, there is a turn around with the week 13 having the least rise in estrone level. The group performance profile reveal a dose dependent increase which is more pronounced at higher doses 1mg/kg, it was observed that the estrone serum concentration showed slight increase over time for group 0.05mg/kg to 0.5mg/kg, this was followed for a spike rise. Again from group 0.8mg/kg, there is decline in sterone in the time (see fig. 3) that is,. Estrone levels were higher in week 13 for groups 0.05mg/kg to 0.7mg/kg, but the reverse was the case in groups 0.8mg/kg to 1mg/kg.

In all instances, a dose dependent increase was observed for group 0.8mg/kg, 0.9mg/kg and 1mg/kg. The group the received 1mg BPA per kg body weight showed the highest level of estrone, these was followed by 0.9mg/kg. These groups recorded highest value at week-1 and gradually decrease over time; the concentration of estrone for these groups was lowest at week-13 (fig 3). Groups 0.6mg/kg and 0.7mg/kg are significantly higher than those of control and week-0, at $p \leq 0.05$. These group recorded the lowest value at week-1, and showed steady increase over time with their highest value obtained at week-13. Between week-3 to week-13, a

significant dose dependent increase was observed for group 0.05mg/kg, 0.1mg/kg and 0.2mg/kg at p≤ 0.05; which was consistent over time. Groups 0.05mg/kg to 0.7mg/kg recorded their highest value at week-13 and lowest value for estrone at week-1. While group 0.8mg/kg to 1mg/kg recorded their highest value at week-1 and lowest value at week-13 (fig 3).

The highest serum concentration of estrone was recorded in week-13 for groups 0.05mg/kg to 0.7mg/kg; groups 0.8mg/kg to 1mg/kg recorded low estrone levels at week-13 and high estrone levels at week-1. In fig 2b, a time dependent effect was observed, as the duration of exposure to graded doses of BPA continued, the serum concentration of estrone continued to rise, up-to group 0.8mg/kg to 1mg/kg where the reverse effect was obtained with the estradiol level decreasing as the duration of the exposure to BPA extends. Between group 0.7mg/kg and 0.8mg/kg was the point of reversal of the trend (fig 3).

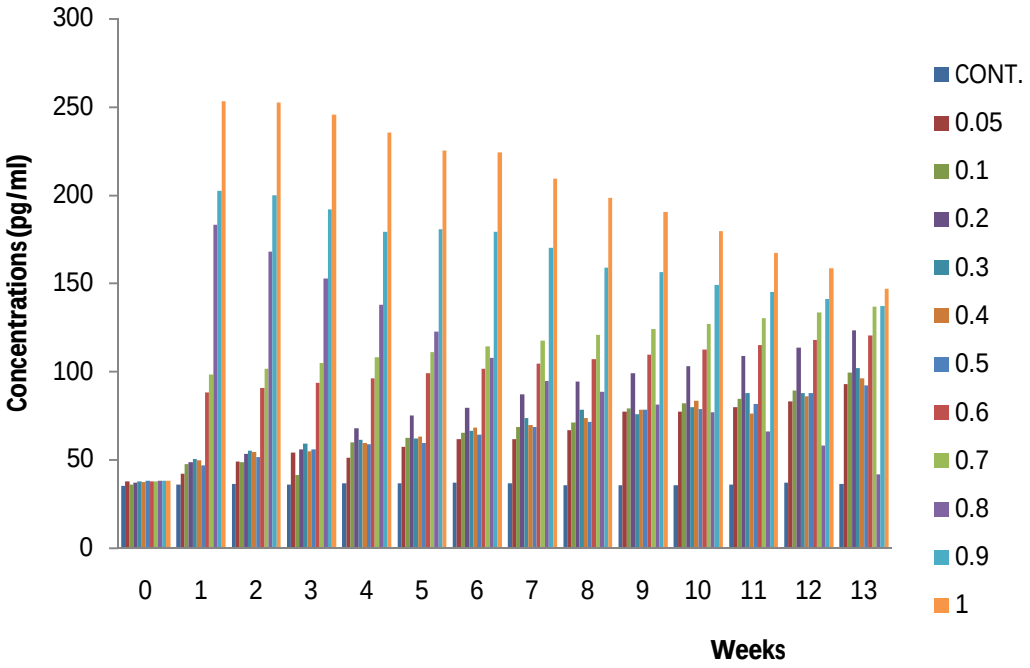

Fig. 3; Chart of concentration against weeks (durations) for change in serum estrone level.

EFFECT ON ESTRIOL

There is a significant decrease in serum estriol level when compared with the control at p≤0.05(see fig. 4). As the estriol descreases from group 0.05mg/kg to 0.3mg/kg, at group 0.4mg/kg, there is a rise peak; group 0.8mg/kg showed the lowest levels of estriol (fig.4). As the estriol descreases from group 1(0.05mg/kg) to 4(0.3mg/kg), at group 5(0.4mg/kg), there is a rise peak (fig.4); group 0.8mg/kg showed the lowest levels of estriol; the week effect observed was mild, as it appeared to be superimposed on each other (fig. 4). The trend observed for estradiol and estrone levels were almost similar. But the trend observed for estriol was different, in that there was a drastic and significant (p≤0.05) decrease in the estriol level in all the treated groups.

The BPA group 0.4mg/kg, gave a rise (peak) effect in the estriol which is still significantly lower than that of the control. There is an instant decrease in serum estriol level of all the treated rats within the first week(fig 4). This decrese which is significant ($p \leq 0.05$) is sustained virtually consistently at the same level throughout the thirteen (13) weeks of the study (fig 4). The decrease in estriol level appear to be dose dependent except for group 0.4mg/kg (400µg/kg) and group 0.7mg/kg (700µg/kg). The result suggests that the reduction in serum estriol level is influenced by dose of BPA administered but not by time.

In fig. 4, it was observed that following the administration of BPA, a pattern of behaviour was established by the dose groups throughout the duration of the experiment. There was a dose dependent decrease in estriol levels from 0.05mg/kg to 0.3mg/kg, then a spike increase by 0.4mg/kg, which decrease at 0.5mg/kg to 0.6mg/kg, another rise was observed at group 0.7mg/kg, this was followed by a decrease at group 0.8mg/kg from which dose dependent rise in estriol through 0.9mg/kg to 1mg/kg (fig 4). The test dose groups showed a time dependent effect which was mild. All the weeks peaks at test dose 0.4mg/kg group (fig. 4).

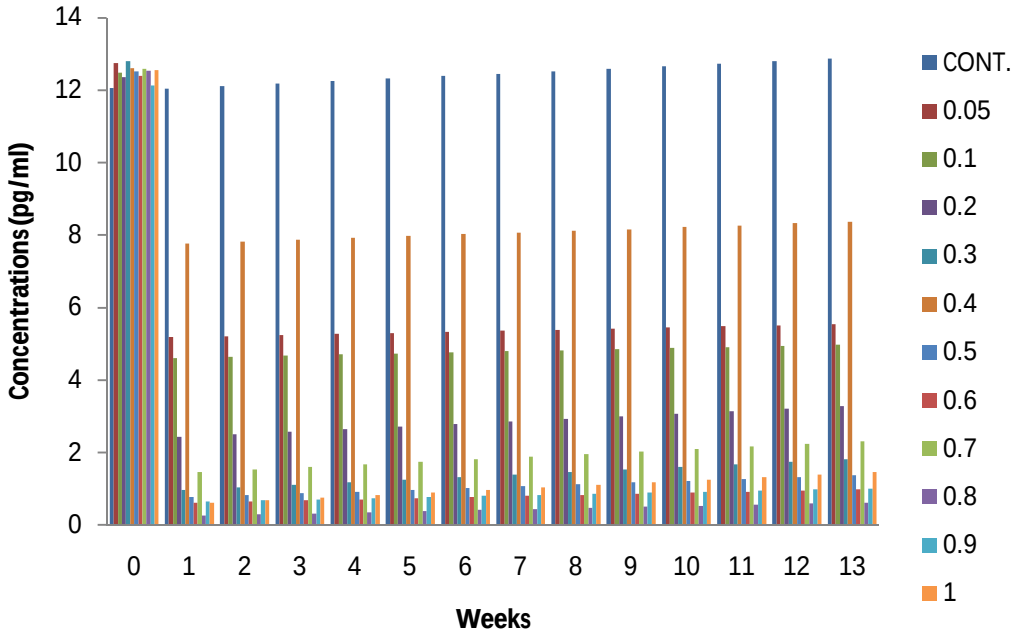

Fig. 4; Chart of concentration against weeks (durations) for serum estriol concentration.

DISCUSSION

The present study examined the effect of BPA exposure on serum estrone, estradiol, conjugated estradiol and estriol hormones concentrations. The result obtained from the study revealed significant high estrone and estradiol concentrations; and significant low estriol and conjugated estradiolconcentration. A significant positive association was found between BPA level and serum PROG, testosterone, androstenedioneand endogenous estrogens concentration.

Endogenous estrogens are thought to have an important role in the developing and maintaining female sexual characteristics. The three major endogenous estrogens in females that have **estrogenic** hormonal activity are;estrone, estradiol, and estriol. And they are the three major naturally occurring forms of estrogen in females.All of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzymearomatase.
Low dose BPA decreased progesterone levels in adult mice during early pregnancy (Berger et al. 2008 and Zhou et al. 2008) but tends to increase serum progesterone level in non pregnantwistar rats (oguazu et al 2021). Additionally, in adult rats, low dose BPA (below 0.1 mg/kg/day) decreased estradiol (Lee SG et al. 2013) which contravene the finding of this study.

The results of *in vitro* studies on the effects of BPA on steroidogenesis are also equivocal. BPA exposure inhibited estradiol, testosterone, androstenedione, estrone, dehydroepiandrosterone, and progesterone production in cultured intact murine antral follicles (Peretz et al. 2011; Ziv-Gal et al. 2013), However, BPA exposure caused an increase in estradiol, testosterone, androstenedione, estrone,and progesterone production female rats (oguazu et al 2021 and oguazu et al 2020) with decrease in estriol, conjugated estradiolconcentration. Also, in isolated rat theca-interstitial cells, BPA (100 nM to 100 μM) had an increasing testosterone synthesis effect (oguazu et al 2020; Zhou et al. 2008). In a separate study using porcine granulosa cells, 0.1 μM BPA increased estradiol levels (Grasselli et al. 2010) aligned the findings of this present study.

Further, if all of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzymearomatase andif low dose BPA decreased expression of estrogen and progesterone receptors (Berger et al. 2010), this might be the reason for observed high estradiol concentration and account for the observed low conjugated estradiol concentration and implicative to the high testosterone and androstenedione concentration observed in a separate study (oguazu et al 2020).

These studies indicate that BPA adversely affects on estrogenic hormonal activity, but the effects depend on BPA concentration, and that different species model used (intact follicles, isolated cells in cultures, mice or rats).

References

Berger RG, Foster WG, DeCatanzaro D. (2010). Bisphenol-A exposure during the period of blastocyst implantation alters uterine morphology and perturbs measures of estrogen and progesterone receptor expression in mice. ReprodToxicol**30**:393-400.

Berger RG, Shaw J, deCatanzaro D. (2008). Impact of acute bisphenol-A exposure upon intrauterine implantation of fertilized ova and urinary levels of progesterone and 17betaestradiol. ReprodToxicol**26**:94-99.

Braun JM, Kalkbrenner AE, Calafat AM, Bernert JT, Ye X, Silva MJ, Barr DB, Sathyanarayana S, Lanphear BP,(2011). Variability and predictors of urinary bisphenol A concentrations during pregnancy. *Environ Health Perspect***119**: 131-137.

Delfosse V, Grimaldi M, Le Maire A, Bourguet W, Balaguer P, (2014). Nuclear receptor profiling of bisphenol-a and its halogenated analogues. *VitamHorm***94**: 229-251.

Ehrlich S, Calafat AM, Humblet O, Smith T, Hauser R. (2014). Handlign of thermal receipts as a source of exposure to bisphenol A. JAMA **311**:859-60.

Gilibili RR, Vogl AW, Chang TK, Bandiera SM (2014). Localization of cytochrome P450 and related enzymes in adult rat testis and downregulation by estradiol and bisphenol A. *Toxicol Sci* **140**: 26-39.

Grasselli F, Baratta L, Baioni L, Bussolati S, Ramoni R, Grolli S, (2010). Bisphenol A disrupts granulosa cell function. Domest. Anim Endocrinol **39**:34-39.

LaKind JS, Goodman M, Naiman DQ (2012). Use of NHANES data to link chemical exposures to chronic diseases: a cautionary tale. PLoS One. **7**:e51086.

Lee SG, Kim JY, Chung JY, Kim YJ, Park JE, Oh S, Yoon YD, Yoo KS, Yoo YH, Kim JM. (2013). Bisphenol A exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17beta-estradiol synthesis via downregulation of aromatase in rat ovary. Environ Health Perspect**12**:663-669.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A.., Kuzuya, H and Nakao, K (2002). Thyroidhormone action is disrupted by bisphenol A as an antagonist. Journal of Clinical Endocrinology and Metabolism**87(11)**:5185-5190.

Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors Tl, JorensPg, Blust R, Vanparys C, (2013). Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. *PLoS One* **8**: e77481.

Peretz J, Gupta RK, Singh J, Hernandez-Ochoa I, Flaws JA. (2011). Bisphenol A impairs follicle growth, inhibits steroidogenesis, and downregulates rate-limiting enzymes in the estradiol biosynthesis pathway. Toxicol Sci **119**:209-217.

Sargis RM, Johnson DN, Choudhury RA, Brady MJ, (2010). Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. *Obesity (Silver Spring)* **18**:1283-1288.

Teng C, Goodwin B, Shockley K, Xia M, Huang R, Norris J, Merrick Ba, Jetten Am, Austin Cp, Tice Rr, (2013). Bisphenol A affects androgen receptor function via multiple mechanisms. *Chem Biol Interact* **203**: 556-564.

Teppala S, Madhavan S, Shankar A. (2012). Bisphenol A and metabolic syndrome: results from NHANES. Int J Endocrinol. **5**:98180.

vom Saal FS, Hughes C, (2005). An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect**113**: 926–933.

Wang HX, Zhou Y, Tang CX, Wu JG, Chen Y, Jiang QW, (2012). Association between bisphenol A exposure and body mass index in Chinese school children: a cross-sectional study. Environ Health. **11**:79.

Wang YF, Chao HR, Wu CH, Tseng CH, Kuo YT, Tsou TC, (2010). A recombinant peroxisome proliferator response element-driven luciferase assay for evaluation of potential environmental obesogens. *Biotechnol Lett* **32**:1789-1796.

Wetherill,Y.B., Akingbemi, B.T., Kanno,J., McLachlan, J.A., Nadal, A.,Sonnenschein, C., Watson, C.S Zoller, R.T and Belcher, S.M (2007). *In vitro* molecular mechanisms of bisphenol A action. Reprod Toxicol.**24**:178-198.

Zhou W, Liu J, Liao L, Han S, Liu J. (2008). Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. Mol Cell Endocrinol **283**:12-18.

Ziv-Gal A, Craig ZR, Wang W, Flaws JA. (2013). Bisphenol A inhibits cultured mouse ovarian follicle growth partially via the aryl hydrocarbon receptor signaling pathway. ReprodToxicol. **42C**:58-67.

Ezeonu F.C, Oguazu C.E, Ubaoji K.I, and Anajekwu B, (2015). Bisphenol A Causes Blood Electrolyte Imbalance and Upsets Kidney Functions in Albino Wistar Rats. J Pharm Sci Bioscientific Res, **5(6)**:547-550

Oguazu C.E, Ezeonu F.C, Ubaoji K.I, and Anajekwu B, (2015). Bisphol A Exerts a Transient Perturbation of Liver Function in Wistar Albino Rats at Acute and Sub-chronic Exposure Doses. J Pharm Sci Bioscientific Res, **5(3)**:274-278.

Alonso-Magdalena P, Ropero AB, Soriano S, García-Ar_evalo M, Ripoll C, Fuentes E, et al. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. Mol Cell Endocrinol 2012;355:201–7.

Welshons W, Nagel S, vom Saal F. Large effects from small exposures. III. Endrocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology. 2006;147 Suppl:S56–69.

Oguazu, C.E, Ezeonu, F.C., Ani, N. O., Anajekwu B. A., Ikimi G. C., Dike C. C., Nwobodo, V.O., Ubaoji, K.I. (2020). Bisphenol A Triggers Abnormal Production of Testosterone and Androstenedione Causing Imbalance in the Hormonal Functions in Female Albino Wistar Rats. IOSR Journal Of Pharmacy And Biological Sciences 15(3): 10-14. DOI: 10.9790/3008-1503051014

Chinenye E. Oguazu, Francis C. Ezeonu, Enemali, M.O, Kingsley I. Ubaoji, Dike C.Charles (2021): Bisphenol A exposure causes prolactin imbalance and alters progesterone functions in rats. Scholars International Journal of Biochemistry, 4(9): 102-107 DOI: 10.36348/sijb.2021.v04i09.002

SUI Y, AI N, PARK SH, RIOS-PILIER J, PERKINS JT, WELSH WJ, ZHOU C: Bisphenol A and its analogues activate human pregnane X receptor. *Environ Health Perspect***120**: 399-405, 2012.

YE L, GUO J, GE RS: Environmental pollutants and hydroxysteroid dehydrogenases. *VitamHorm***94**: 349-390, 2014.