ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

EPIGENETIC REGULATION OF GENE EXPRESSION AND RESULTANT ENDOCRINE DISRUPTION BY BISPHENOL A

Jayalekshmi. G. Kurup[1], Francis Sunny[2] and V.S. Sugunan[3]

1. *Asst. Professor, Dept. of Biotechnology, RNC Arts, JDB Commerce & NSC Science College, Nasik Road, Nasik, Maharashtra*
2. *Principal (Retd), Govt. College, Attingal, Thiruvananthapuram, Kerala*
3. *Asst. Professor, Dept. of Zoology, University College, Thiruvananthapuram, Kerala*

ABSTRACT

Bisphenol A (BPA), a component monomer of polycarbonate plastics is considered as a potent endocrine disruptor. BPA monomers are held together by unstable ester linkages that can be easily disrupted by heat, acidic or alkaline conditions. This facilitates the leaching of BPA into the surroundings and also to the contents of polycarbonate plastics. BPA has been proved to cross placenta and exposure to even very low levels of BPA is associated with several endocrine dysfunctions, obesity and cancers. The exact mechanism by which BPA acts is not clearly understood. The present study reveals the ability of BPA to function as an epigenetic modulator of gene expression resulting in endocrine disruption. The effect of BPA on the expression of aromatase and cyp1a1 genes as well as the interaction of BPA with DNA was analyzed. Results prove that the epigenetic property of BPA plays a major role in regulating gene expression thereby leading to endocrine disruption.

Keywords: Bisphenol A, cyp1a1 genes, epigenetic property, gene expression

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

INTRODUCTION

Environmental pollution can be scientifically defined as the introduction of extraneous materials into a habitat causing harm, instability, disorder or discomfort to the living organisms in the habitat. The World Health Organization (WHO) estimates that prolonged exposure to environmental pollution is responsible for the occurrence of about a quarter of the diseases faced by mankind today. The predominant form of environmental pollution is aquatic pollution since majority of the pollutants enter seas, rivers, lakes and wetlands. The majority of aquatic pollutants include pesticides, fertilizers, metals like cadmium and mercury and other solid wastes. Among the solid wastes, the most important pollutants are plastics. In 2011, global plastic consumption worldwide was estimated at 5.5 million metric tons. Plastic is versatile, lightweight, flexible, moisture resistant, strong and relatively inexpensive, and these qualities boost the commercial usage of plastics. Once plastic debris enters the water, it becomes one of the most pervasive problems because of plastic’s inherent properties: buoyancy, durability, propensity to adsorb waterborne pollutants, its ability to get fragmented into microscopic pieces, and more importantly, its proven ability to leach Bisphenol A (BPA) and other toxins in the water.

HUMAN

BisphenolA(BPA)was investigated in the 1930’s as a possible synthetic estrogen to be used for miscarriages. It has become ubiquitous in the environment within the past 80 years because of its presence in a multitude of products including food and beverage packaging, flame retardants, adhesives, building materials and paper coatings [1]. Final products which use BPA as a monomer include powder paints, protective coatings, automotive lenses, protective window glazing, compact discs, optical lenses, thermal paper, paper coatings, as a developer in dyes and for encapsulation of electrical and electronic parts. BPA is also used in some polymers used in dental sealants or composites. Global consumption of BPA in 2011 exceeded 5.5 million metric tons [2]. BPA is a high-production volume, artificial, industrial, man-made molecule. It is a white powder and is a relatively small symmetric, organic compound with a molecular weight of 228. BPA (4,4’-dihydroxy-2,2-diphenylpropane) molecule comprises two phenol rings connected by a methyl bridge, with two methyl groups attached to the bridge. The structure of BPA shows strong resemblance with estradiol and

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

diethylstilbestrol (DES). With its two benzene rings and two (4,4)-OH substituents, BPA fits in the estrogen-receptor (ER) binding pocket. BPA functions as an estrogen mimetic and endocrine disruptor. It binds to estrogen-receptors in the cell in a haphazarded, uncoordinated way - disrupting the highly ordered working of the normal hormone, estrogen, especially during pregnancy[3]. Even though it was initially used in the medical field, its use as a pharmaceutical was abandoned when DES was determined to be much more potent and powerful estrogen substitute in humans than BPA [4].

The leaching out of BPA from polycarbonates and other solid wastes that are wantonly disposed into the aquatic ecosystems pose severe health hazards to the aquatic life forms. Moreover, the effects are transferred to members of the higher trophic levels through the process of bioconcentration and biomagnifications. BPA has widespread effects on the control of various reproductive events including the time of onset of puberty, prostate weight, sperm production, estrous cycle and prolactin release. It also causes changes in fertility, sexual and general behavior of mammals and other organisms. It is therefore certain that it has profound influence on the regulation of expression of various genes that are involved in various reproductive pathways. BPA can accumulate along food chains and has been detected in tissues of both wildlife and humans [5]. Trans-placental transport of BPA has been demonstrated in both rodents and humans [6]. Other than the adverse effects of BPA on developmental and reproductive processes in rodents and primates [7], health hazards such as abnormal increase in prostate weight, decrease in epididymis weight, reduction in sperm production and decrease in concentrations of LH and testosterone in blood serum [8] have been reported. Although the adverse effects of BPA on reproduction have been studied extensively, most of the studies addressed the impacts only at the levels of conventional histology and the gross comparison of anatomical features or tissue mass. The mechanistic actions of BPA on animal reproductive health at the molecular level have not yet been fully elucidated.

One of the key players in the reproductive physiology is the hypothalamus-pituitary-gonadal (HPG) axis.The HPG axis is a critical part in the development and regulation of a number of the body systems, including the reproductive and immune systems. Variations in the

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

hormones produced by one gland in the axis cause changes in the hormones produced by another gland, which in turn have various widespread and local effects on the body. Analysis of the effect of BPA on the HPG axis hormones will give a closer picture on how BPA modulates reproductive function at the molecular level. This was achieved by analyzing the effect of BPA on the expression of aromatase, a major enzyme involved in the steroidogenic pathway.Steroidogenesis the complex multienzyme process by which cholesterol is converted to biologically active steroid hormones. The major enzymes required for the synthesis of steroid hormones belongs to the cytochrome P450 (CYP) superfamily. Aromatase, the product of cyp 19 gene, is a member of the P450 cytochrome superfamily of enzymes that catalyzes the formation of estrogen from androgen. cyp19 is expressed in multiple tissues, including gonads, brain, adipose tissue, skin and placenta. More recently, the ability of several xenoestrogens to modulate aromatase gene expression and enzymatic activity has been demonstrated. Loss of function mutations in cyp19 results in a lack of estrogen production and a failure of women to develop properly at puberty. Conversely, overproduction of cyp19 gene product in males may lead to abnormal feminization. In this regard, estrogenic compounds and synthetic estrogens have been shown to strongly up-regulate cyp19b gene expression in the zebrafish [9].

CYP1A1 (Cytochrome P450, family 1, subfamily A, polypeptide 1)

Proteins in the CYP1A subfamily are prominent in the metabolism and activation of many hydrocarbon carcinogens that are environmental contaminants [10] and so the occurrence and functions of CYP1A forms in diverse organisms are being investigated vigorously. There are two CYP1A forms in mammals, CYP1A1 and CYP1A2, thought to have diverged between 65 and 250 million years ago [11]. These proteins preferentially activate polynuclear aromatic hydrocarbon and aromatic amine procarcinogens respectively. They also metabolize endogenous compounds, including steroids. Both CYP1A1 and CYP1A2 are induced by polynuclear and planar halogenated aromatic hydrocarbons, including the chlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls,which are aryl hydrocarbon receptor (AhR) agonists. Mammalian CYP1A1 and CYP1A2 proteins differ in their patterns of chemical

induction and in the number and location of 5' non-coding sites identified as regulatory sequences binding the Ah receptor (xenobiotic response elements)[12].

Even though there are several members in the cytochrome P450 gene superfamily, two members - cyp19a1 and cyp1a1 were investigated to study the effects of BPA on their expression.This paper describes the epigenetic influence of BPA in regulating gene expressionof aromatase and cyp1a1 and the resultant endocrine disruption using the eukaryote experimental model *Anabastestudineus*. The interaction between BPA and DNA was also looked into to establish the epigenetic nature of action of BPA.

MATERIALS AND METHODS

A. testudineus acclimatized to laboratory conditions was used as the experimental model. The fish were exposed to different sub-lethal concentrations, 2.5, 5.0 and 7.5mg/l for 30 days.

- **Analysis of the effect of BPA on the expression of aromatase (cyp19a1) and cyp1a1 gene**

Isolation of RNA: Total RNA was isolated from the liver and gonads of both control and fish exposed to BPA. 100mg of each tissue sample was homogenised separately using 1ml Trizolreagent [13] and centrifuged at 12000g for 10min at 4°C. To the homogenate, 0.2ml chloroform was added and kept at room temperature for 5min, and for phase separation. The samples were vigorously shaken for 15 seconds, incubated for 10min at room temperature and centrifuged at 12,000g for 15min at 4°C. The aqueous phase was collected and transferred to a fresh tube. RNA was precipitated by adding isopropyl alcohol (0.5ml per millilitre Trizol used in the initial homogenization), incubated at room temperature for 10min and centrifuged at 12,000g for 10min at 4°C to precipitate the RNA. The RNA pellet was washed once with 75% ethanol, air-dried and finally re-dissolved in RNase-free water. The purity of extracted total RNA was determined spectrophotometrically (A_{260}/A_{280} ratio) and total RNA was diluted to 1µg μl^{-1} in RNase-free water.

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR):

Total RNA extracted from the gonads of both sexes of control and exposed fish was subjected to RT-PCR analysis. 2μg total RNA was converted to cDNA using reverse primer for aromatase (antisense 5’TGGCTGATGCTCTGCTGAGG3’). β-actin was chosen as the reaction control and so reverse transcription using reverse primer of β-actin (antisense 5’AGGGACAACACTGCCTGGAT3’) was also done according to the manufacturer’s instructions. Reverse transcription was performed in a 20μl reaction mixture with a final concentration of 2X RTPCR buffer (500mM KCl, 100mM Tris-HCl (pH 9.0), 15mM MgCl2), 3.5mM MgCl2, 1mM dNTP, 1U RNase inhibitor, 20U reverse transcriptase and 0.75mM of the aromatase reverse primer in one tube but with 0.75mM β-actin reverse primer in the other tube. Reverse transcription was done at 42°C for 30min followed by inactivation of the enzyme at 94°C for 15min. Gene specific primer pairs were based on the cDNA sequences of carp aromatase. The forward and reverse primers for aromatase gene were sense, 5’ATCGGATCCCTGCCTGTGAC3’ and antisense, 5’TGGCTGATGCTCTGCTGAGG3’ respectively. Forward and reverse for β-actin were sense 5’ CCAAAGCCAACAGGGAGAA3’ and antisense 5’AGGGACAACACTGCCTGGAT3’ respectively. PCR amplification was carried out in mastercycler (Eppendorf, Germany) in a 20μl reaction using 1.25U of Taq DNA Polymerase, 1X PCR Buffer, 1.5mM MgCl2, 0.2mM dNTP, 0.1μM of each primer, and 2-5μl of template cDNA. PCR conditions consisted of initial denaturation at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 68.9^{o}C for aromatase primers and 65.45^{o}C for β-actin primers for 30s and extension at 72°C for 30s. A final elongation step was performed at 72°C for 5min.

Aliquots from the total RNA isolated from the liver of control and exposed fish were subjected to RT-PCR analysis as described above, with the difference that specific primers for cyp1a1 (sense, 5’TACCCTGAGATCCAGGAGCA3’ and antisense 5’TCGAGAAGTGGCAAGTCCGT3’) were used according to the manufacturer’s instructions. PCR conditions consisted of initial denaturation at 94°C for 5min, followed by

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

35 cycles of denaturation at 94°C for 30s, annealing at 66°C for 30s and extension at 72°C for 30s. A final elongation step was performed at 72°C for 5min.

The PCR products were resolved by electrophoresis in 1% agarose gel using a Mini VE complete cell (Amersham Biosciences, USA). The PCR products were visualized using ethidium bromide and photographed using Gel-doc (Gelstan, Medicare, Germany).

- **Analysis of the interaction between BPA and DNA**

Isolation and electrophoretic detection of DNA from hepatic tissue:

Isolation of DNA from liver tissue and electrophoresis was carried out according to the method of with some modifications. The homogenized liver tissues were lysed with buffer containing 100mM Tris-HCl, 25mM EDTA, 0.5% SDS and 1mg/ml proteinase K and kept in a water bath for 12 hours at 37°C. DNA was extracted with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). To the aqueous phase, sodium acetate was added and the DNA was precipitated with chilled isopropanol. Following a 70% ethanol wash, the precipitated DNA was re-suspended in Tris-EDTA buffer. 5µg of DNA per sample were electrophoretically resolved on 1% agarose gel containing 0.5µg/ml ethidium bromide in submarine gel electrophoresis system. The images of the stained gels were captured using Gel-doc (Gelstan, Medicare, Germany).The integrity of bands was used as the measure of DNA damage.

Analysis of DNA fragmentation by diphenylamine method:

The hepatic tissue of fish exposed to 2.5, 5.0 and 7.5mg/l of BPA for 30 days was tested for DNA fragmentation using DPA method [14]. Intact DNA was separated from fragmented DNA by centrifugal sedimentation followed by precipitation and quantification using DPA. To minimize formation of oxidative artifacts during isolation, 2, 2, 6, 6-tetramethyl piperidinoxyl was added to all solutions and all procedures were performed on ice. The hepatocytes (1 x 10^6 in 1ml PBS) were put in a 1.5ml centrifuge tube (tube B) and centrifuged (200g, 4°C, 10min) to obtain a cell pellet. The supernatants were transferred to fresh tubes (tube S). The pellet obtained (tube B) was suspended in 1ml TTE buffer and centrifuged at 20,000g (4°C,

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

10min). The supernatant obtained was transferred to fresh tubes (tube T) and the resulting pellets were re-suspended in TTE buffer. TCA was added to tubes T, B and S and vortexed vigorously. Tubes were kept overnight at 4°C followed by centrifugation at 20,000g (4°C, 10min). The supernatant was discarded and the pellet was hydrolyzed by the addition of 160µl of 5% TCA followed by heating at 90°C for 15 minutes. This was followed by addition of 320µl of freshly prepared DPA. The color was developed by incubation at 37°C for 4 hours. Optical density of the solution was determined at 600nm using UV-visible spectrophotometer (Perkin Elmer, USA).

Percentage DNA fragmentation was calculated using the following formula:

% Fragmented DNA = [S + T/S+T+B] x 100

- **Statistical analyses**

Statistical analysis of data was done by ANOVA. The differences in means were tested by using Duncan analysis. The level of significance was set at $P<0.05$. All the statistical analyses were performed using the software SPSS 10.0 for Windows.

HUMAN

RESULTS AND DISCUSSION

- **Analysis of the effect of BPA on the expression of cyp19a1 (aromatase) and cyp1a1gene**

Isolation of RNA:

Total RNA was isolated from the hepatic tissue of both the control and exposed fish and from the gonadal tissues of both sexes of control and exposed fish. The RNA isolated from the hepatic tissue had A_{260}/A_{280} ratio 1.00 to 1.08 and that from the gonadal tissue had A_{260}/A_{280} ratio 0.96 to1.06.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR):

The electrophoregram of RT-PCR product of aromatase gene as compared to the standard reference gene β-actin is shown in Fig 1. The RT-PCR product of aromatase gene was of 224

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

kbp.It was found that the expression of aromatase is higher in females than in males. Expression of aromatase was found up-regulated in the gonads of both male and female fishexposed to 7.5mg/l BPA for 30 days. The electrophoregram of RT-PCR product of cyp1a1 gene compared to the standard reference gene β-actin is shown in Fig 2. The exposureto 7.5mg/l of BPA for 30 days increased the expression of cyp1a1 in the hepatic tissue. cyp1a1 is involved in the detoxification of xenobiotics and increase in cyp1a1 expression in the liver is indicative of the detoxification of BPA in the exposed models.

- **Analysis of interaction between BPA and DNA**

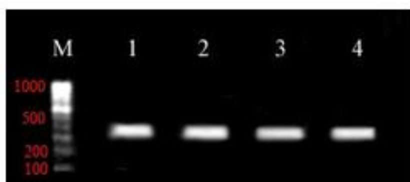
Isolation and electrophoretic detection of DNA from hepatic tissue:

Experiments were conducted to analyze the interaction between BPA and DNA.Fragmentation of DNA strands should have resulted in bands with molecular weight less than the control DNA strands. No such bands were seen in the DNA strands of test samples exposed to BPA. Hence it was evident that exposure to BPA did not result in fragmentation. On the contrary, bands of irregular (higher) molecular weights were seen above the bands of interest(Fig 3) indicating the formation of complexes - possibly with BPA- shifting those strands up. These bands were seen as minor streaks above the major DNA bands, suggestive of non-specific irregular binding or interaction.

Analysis of DNA fragmentation by diphenylamine method:

As several reports vouch that endocrine disruptors cause DNA fragmentation[15], an effort to analyze and quantify the extent of DNA fragmentation, if any, was made by employing the DPA method. The results (Table a, Fig 4) showed that the percentage of DNA fragmentation was insignificant at 18-20% in exposed sampleswhich was not different from that of unexposedcontrolswhich showed19% fragmentation. This indicates that BPAdid not fragment the DNA at the sub-lethal concentrations studied.

ISSN 2349-7203
IJPPR
International Journal of Pharmacy & Pharmaceutical Research
An official Publication of Human Journals
HUMAN



β-actin- 409 kbp

Aromatase- 224 kbp

Fig 1: Up-regulation of cyp19a1 (aromatase) in the gonads of female and male fish exposed to 7.5mg/l of BPA for 30 days

Lane M- Ladder, Lane 1- Ovary of control fish, Lane 2- Ovary of exposed fish, Lane 3- Testis of exposed fish, Lane 4- Testis of control fish

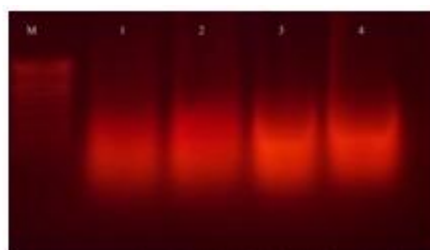


Fig 2: Up-regulation of cyp1a1 in the liver of fish exposed to 7.5mg/l of BPA for 30 days

Lane M- Ladder, Lane 1 and 2- Liver of control fish, Lane 3 and 4- Liver of exposed fish

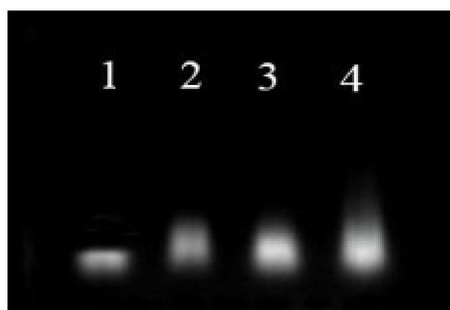


Fig 3: Electrophoregram of DNA isolated from hepatic tissue of fish exposed to7.5mg/l of BPA for 10, 20 and 30 days

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Lane 1- Liver of control fish, Lane 2- Liver of fish exposed to 7.5mg/l BPA for 10 days, Lane 3- Liver of fish exposed to 7.5mg/l BPA for 20 days, Lane 4- Liver of fish exposed to 7.5mg/l BPA for 30 days

Table A: Percentage of fragmentation of DNA quantified by DPA method

Sample	Percentage of fragmentation
Control	19.22±1.0212
7.5mg/l (10 days)	20.77±0.9895
7.5mg/l (20 days)	19.82±0.8967
7.5mg/l (30 days)	18.90±1.1105

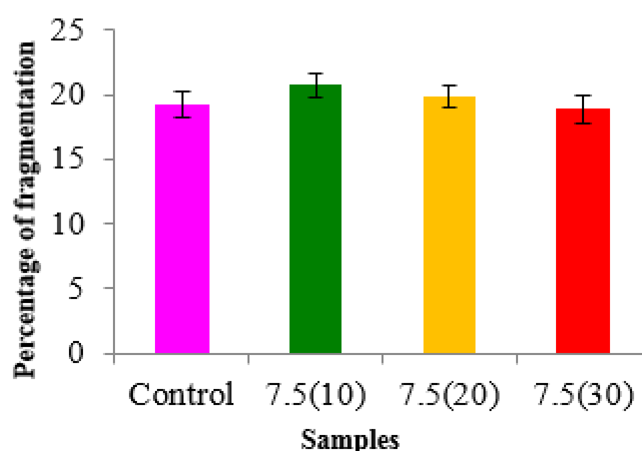


Fig 4: Percentage of fragmentation of DNA quantified by DPA method

DISCUSSION

Many natural and synthetic compounds present in the environment exert a number of adverse effects on the endocrine system, for which they are termed endocrine disrupting chemicals (EDCs). A decrease in reproductive success is one of the well-documented signs of endocrine disruption. Important reproduction-related processes, including sexual differentiation, maturation etc. are under the control of the steroid hormone estrogen. Careful spatial and temporal balance of estrogens in the body is crucial for proper functioning of the reproductive system. At the final step of estrogen biosynthesis, cytochrome P450 aromatase, encoded by

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

the cyp19 gene, converts androgens into estrogens. Therefore, modulation of aromatase CYP19 expression and function can dramatically alter the rate of estrogen production, disturbing the local and systemic levels of estrogens. Two cyp19 genes are present in most teleosts, cyp19a1 and cyp19a2, primarily expressed in the ovary and brain, respectively. Both aromatase CYP19 isoforms are involved in the sexual differentiation and regulation of the reproductive cycle and male reproductive behavior in diverse teleost species. Alteration of aromatase expression and/or activity, be it up-regulation or down-regulation, lead to diverse disturbances of the above mentioned processes.

In the present study, the potential of endocrine disruptor BPA to interfere with the mRNA expression of cyp19a in teleost fish was analyzed. Exposure to 7.5 mg/l of BPA for 30 days up-regulated the gonadal expression of aromatase mRNA in both the sexes of *A.testudineus*. The expression of aromatase is higher in females than in males. Up-regulation of aromatase in the exposed fish of both sexes is an indication that BPA interferes with the normal process of steroidogenesis. Expression levels of P450aromA in the vast majority of the species studied are higher in females (ovary) than in males (testis) (Blazquez and Piferrer, 2004). Increased expression of aromatase in fish exposed to BPA indicates the involvement of BPA in regulating the steroidogenic process.

HUMAN

In a study by, the mRNAs of steroidogenic enzyme CYP19A1was seen up-regulated and CYP19A1 (cytochrome P450 aromatase) activity were significantly increased in the uro-genital sinus of mouse exposedto BPA, indicating that BPA interacts with *in situ* steroidogenesis[16].

The studies by on the effect of BPA in rat testicular Leydig R2C cells showed that BPA induced a time and concentration dependent increase in aromatase expression[17]. They also reported that BPA up-regulated aromatase gene expression and increased the enzyme activity, but reduced testosterone synthesis in the cells. Their findings correlated aromatase activity with COX-2 up-regulation mediated by the CRE, PKA, Akt, and MAP kinase signaling pathways in rat testicular Leydig cells. Since exposure to BPA induces the over expression of

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

COX, its presence would strongly influence aromatase expression through COX-2 up-regulation.

Prediction of multiple transcriptional regulatory elements in the promoters of teleost cyp19 genes suggests the possibility that several EDC classes affect cyp19 expression at the transcriptional level. These sites include cAMP responsive elements, a steroidogenic factor 1/adrenal 4 binding protein site, an estrogen-responsive element (ERE), half-EREs, dioxin-responsive elements, and elements related to diverse other nuclear receptors (peroxisome proliferator activated receptor, retinoid X receptor, retinoic acid receptor). Diverse EDCs may affect the expression and/or activity of aromatase cyp19 genes through a variety of mechanisms, many of which need further characterization in order to improve the prediction of risks posed by a contaminated environment to teleost fish population.

In the present studyof *A.testudineus*, exposure to BPA was also found to up-regulatecyp1a1 expression in the liver in a dose-dependent manner. cyp1a1 is normally expressed at low levels in hepatic tissues, but it is highly inducible in the liver on exposure to xenobiotics. Living organisms are constantly exposed to harmful foreign chemicals and materials from dietary, therapeutic, environmental, and occupational sources. The cyp1a1 gene encodes a member of the cytochrome P450 superfamily of enzymes, which are monooxygenases that catalyse many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids (Coon, 2005). The enzyme's endogenous substrate is unknown; however, it is able to metabolize some polycyclic aromatic hydrocarbons to carcinogenic intermediates. cyp1a1 is expressed primarily in extra-hepatic tissues such as the lungs, lymphocytes and placenta while only low-level expression has been reported in liver tissue. In contrast, cyp1a2 is expressed primarily in the liver with little if any detectable expression in the extra-hepatic tissues [18].

Cytochrome P4501a(cyp1a) induction is mainly hepatic and it plays a key role in the elimination or detoxification of pollutants, such as dioxins, PCBs and PAHs. This is widely used as a biomarker both in vertebrates and invertebrates for environmental bio-monitoring, especially in marine bivalves and fish. Induction of cyp1a by PAHs is mediated through the

aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor. Binding of PAH to AhR elicits sequential signaling events leading to the activation of AhR and transcription of cyp1a genes through the dioxin response element located in the enhancers of the genes. In this framework, PAH, AhR, dioxin response element, and cyp1a1/2 form a receptor-mediated transcriptional loop that directly senses the concentrations of PAH in cells and increases the activities of the enzymes through transcription. Induction of such metabolizing proteins is initiated and maintained only as needed and subsides as the xenobiotic is metabolized. Therefore, induction of such proteins is not only necessary for clearance of chemicals in the body but also tightly regulated according to the cellular concentrations of xenobiotics. The biological impact of cyp1a induction can be 2-fold. Induction of cyp1a in general serves as a means of maintaining the homeostasis of the chemical environment in cells by increasing the metabolic clearance of substrates. Since CYP1A1/2 catalyzes the metabolic activation of PAHs and HAAs to carcinogens, induction of the enzymes may turn out to be detrimental in humans who are exposed to high levels of PAHs and HAAs. Furthermore, since CYP1A1/2 can metabolize a range of substrates; induction of the enzymes by one substrate may increase the metabolism of other chemicals (for instance, clinical drugs), resulting in unexpected drug-drug interactions (DDIs).

HUMAN

Demonstrated that the estrogenicity of either BPA or Bisphenol B [BPB; 2,2-bis(4-hydroxyphenyl)butane] was several times higher after it was incubated with rat liver S9 fraction[19]. This metabolic activation, requiring both microsomal and cytosolic fractions, was observed with not only rat liver, but also human, monkey, and mouse liver S9 fractions. The finding highlights the strong interaction between liver and the components of HPG axis. Any compound that affects the HPG axis is initially processed by the liver before manifesting its endocrine disrupting function. The up-regulation of cyp1a1 in the liver upon exposure to BPA indicates the detoxification mechanism carried out by the liver, and the metabolic products can deleteriously affect the HPG axis. These products can play crucial roles in the up-regulation of female-specific proteins such as aromatase in male fish exposed to BPA through initiating disturbances in the normal functioning of HPG axis. This in turn results in endocrine disruption and reproductive toxicity in the organisms exposed to BPA.

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Experiments to analyze the interaction between BPA and DNA showed that the integrity of DNA was not affected in the exposed fish. No sign of DNA fragmentation was evident. Instead, in the gel-shift assay, bands of irregular molecular weights were seen as minor streaks above the major DNA bands, indicating the formation of complexes - possibly with BPA- shifting those bands up. The absence of fragmentation was further confirmed by DPA analysis which showed no significant difference in the percentage of fragmentation between the DNA of control fish and fish exposedto BPA.

BPA has been investigated for its genotoxic and carcinogenic properties, but the results have been either inconclusive or controversial. Metabolically activated BPA has previously been shown to form DNA adducts both *in vitro* and in rat liver. Izzotti*et al*. (2009) showed that administration of BPA to mice caused the formation of DNA adducts in liver. In addition, they provided evidencesshowing that DNA adducts are formed in target mammary cells. Although DNA adducts do not necessarily evolve into tumors or other chronic degenerative diseases, the formation of these molecular lesions in target cells may bear relevance for the potential involvement of BPA in carcinogenesis.

Results of the experiments strongly confirmed the potential of BPA to cause reproductive toxicity as evident from the up-regulation of aromatase in the exposed male fish. The up-regulation of cyp1a1 in the hepatic tissue of exposed fish is an indicative of the ability of liver to metabolize BPA. Even though BPA does not fragment DNA, BPA or its metabolic products including those formed during detoxification could interact with DNA as was evident from the gel-shift assay of the exposed fish.The possible mechanism of action is that the products of metabolic activation can play crucial roles in initiating disturbances in the normal functioning of HPG axis, resulting in endocrine disruption and reproductive toxicity.

CONCLUSION

Adverse effects of BPA on animal reproductive health have been reported, however most of the studies relied on the approaches in the assessment of conventional histology and anatomical features. In the present part of the study, the molecular basis of toxicity caused by BPA was analyzed using the freshwater fish *A.testudineus*as the model. Exposure to 7.5mg/l

of BPA for 30 days resulted in the up-regulation of the expression levels of aromatase in both male and females. The results indicated that BPA caused reproductive dysfunction by affectingsteroidogenesis,thereby resulting in the anomalous release of endogenous steroid hormones. This non-ER-mediated effect is more potent in affecting the feedback regulatory circuits in the HPG-axis. BPA exposure also induces the expression of cyp1a1, which codes for the main xenobiotic metabolizing enzyme, in the liver of exposed fish. The induction of cyp1a1 in liver is associated with xenobiotic metabolism and hence it can be interpreted that BPA is metabolized by the liver. Analysis of the effect of BPA on DNA indicated that BPA, at the concentrations tested, did not cause fragmentation. However, BPA or its metabolic products could interact with DNA, thereby affecting the processes of DNA replication and protein synthesis. Up-regulation of aromatase in males and induction of cyp1a1 expression in hepatic tissueseem to be one of the major events that cause deviations in the normal molecular processes that occur as a result of exposure to BPA. The results of experiments strongly suggest the role of BPA as an epigenetic regulator of gene expression thereby leading to endocrine disruption in target species.

HUMAN

REFERENCES

1. Coon M.J. (2005). Cytochrome P450: nature's most versatile biological catalyst. Annu. Rev. Pharmacol. Toxicol. 45: 1–25.
2. Hotchkiss A.K. Rider C.V. Blystone C.R. Wilson V.S. Hartig P.C. Ankley G.T. 2008. Fifteen years after Wingspread environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicol Sci. 105: 235–259.
3. Richter C.A. Birnbaum L.S. Farabollini F. Newbold R.R. Rubin, B.S. Talsness C.E. (2007). In vivo effects of bisphenol A in laboratory rodent studies. Reprod. Toxicol. 24, 199–224.
4. Arase S. Ishii K. Igarashi K. Aisaki K. Yoshio Y. Matsushima A. Shimohigashi Y. Arima K. Kanno J. Sugimura Y.(2011) . Endocrine disrupter bisphenolA increases in situ estrogen production in the mouse urogenital sinus. Biol.Reprod. 84, 734-742.
5. Balakrishnan B. Henare K. Thorstensen E.B. Ponnampalam A.P. Mitchell M.D. 2010. Transfer of bisphenol A across the human placenta. Am. J. Obstet. Gynecol. 202,393–397.
6. Blazquez M. Piferrer F.(2004). Cloning, sequence analysis, tissue distribution, and sex-specific expression of the neural form of P450 aromatase in juvenile sea bass (Dicentrarchuslabrax). Mol. Cell Endocrinol. 219, 83–94.
7. Chomczynski P. Sacchi N. (1987) Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. Analy. Biochem. 162, 156–159.
8. Gonzalez F.J.(1989). Pharmacol. Rev. 40, 243-287.
9. Honma S.Suzuki A. Buchanan D.L. Katsu Y. Watanabe H. Iguchi T.(2002). Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod. Toxicol. 16, 117–22.

10. Izzotti A. Kanitz S. D'Agostini F.Camoirano A. De Flora S.(2009). Formation of adducts by bisphenol A, an endocrine disruptor, in DNA in vitro and in liver and mammary tissue of mice. Mutat. Res. 679, 28-32.
11. Jaiswal A.K. Gonzalez F.J. Nebert D.W.(1985). Science. 228, 80-83.
12. Kazeto Y. Place A.R.Trant J.M.(2004). Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (Daniorerio) juveniles. Aquat. Toxicol. 69, 25–34.
13. Kim D. Guengerich F.P.(2005). Cytochrome P450 activation of arylamines and heterocyclic amines. Annu. Rev. Pharmacol. Toxicol.45,27–49.
14. Oehlmann J. Schulte-Oehlmann U. Kloas W. Jagnytsch O. Lutz I. Kusk K.O.(2009). A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. Lond.B. Biol. Sci. 364, 2047–2062.
15. Ramos J.G. Varayoud J. Sonnenschein C. Soto A.M. Munoz D.T. Luque E.H.(2001). Prenatal exposure to low doses of Bisphenol A alters the periductalstroma and glandular cell function in the rat ventral prostate. Biol. Reprod. 65, 1271–1277.
16. Schweikl H. Taylor J.A. Kitareewan S.Linko P. Nagorney D. Goldstein J.A.(1993). Pharmacogenetics. 3(5), 239-249.
17. Strom D.K. Postlind H. Tukey R.H. (1992). Arch. Biochem. Biophys. 294, 707-716.
18. Teuten E.L. Saquing J.M. Knappe D.R. Barlaz M.A. Jonsson S. Bjorn A.(2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 2027–2045.
19. Thompson R.C. Moore C.J. VomSaal F.S. Swan S.H.(2009). Plastics, the environment and human health: current consensus and future trends. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 2153–2166.
20. VomSaal F.S. Cooke P.S. Buchanan D.L. Palanza P. Thayer K.A. Nagel S.C.(1998). A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol. Ind. Health. 14, 239–260.
21. Welshons W.V. Nagel S.C. Thayer K.A. Judy B.M. VomSaal F.S.(1999). Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol. Ind. Health. 15,12–25.

HUMAN