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Pharmacology

Bisphenol A: Investigation and Prevention of Endocrine Disrupting in Albino Mice

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Original Research Article

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Abstract: Bisphenol A (BPA) is an organic compound with the chemical formula $C_{15}H_{16}O_2$ and is made from phenol and acetone.It is a colorless solid that is soluble in organic solvents, but poorly soluble in water, having two phenol functional groups, it is used to make polycarbonate polymers and epoxy resins, along with other materials used to make plastics.Polycarbonate plasticis made by reacting BPA with phosgene. The mitochondrial toxicity was estimated by the assay of mitochondrial marker enzymes, by measuring the level of lipid peroxidation, GSH levels and levels of other antioxidant enzymes such as GPx, GR and SOD.Respiratory function of testicular mitochondria appears particularly susceptible to xenobiotic actions, which can contribute to a decrease in mitochondrially produced ATP and even to predispose cells to undergo mitochondria-mediated cell death. Our study showed that exposure to BPA induces significant oxidative stress in testicular mitochondria in mice and melatonin scavenges the free radicals. Human exposure to BPA is due to its widespread use, along with reproductive and developmental effects reported in animal study has generated considerable attention on this chemical in recent years.These aspects need further investigation in properly conducted studies with a wide dose range of BPA.
Keywords: Bisphenol A, Lipid Peroxidation, Antioxidant enzymes, Endocrine disrupting chemicals.

INTRODUCTION

A challenge to the field of endocrine disruption is that these substances are diverse and may not appear to share any structural similarity other than usually being small molecular mass (<1000 Daltons) compounds. Thus, it is difficult to predict whether a compound may or may not exert endocrine-disrupting actions. Nevertheless, in very broad terms, EDCs such as dioxins, PCBs, PBBs, and pesticides often contain halogen group substitutions by chlorine and bromine. They often have a phenolic moiety that is thought to mimic natural steroid hormones and enable EDCs to interact with steroid hormone receptors as analogs or antagonists. Even heavy metals and metalloids may have estrogenic activity, suggesting that these compounds are EDCs as well as more generalized toxicants.

Several classes of EDCs act as antiandrogens and as thyroid hormone receptor agonists or antagonists, and more recently, androgenic EDCs have been identified [1-3]. The sources of exposure to EDCs are diverse and vary widely around the world. The situation is constantly evolving because some EDCs were banned decades ago and others more recently, with significant differences between countries. In this respect, migrating people provide a model to study cessation and/or onset of exposure depending on contamination of the original and new milieus. There are also several historical examples of toxic spills or contamination from PCBs and dioxins that show a direct causal relationship between a chemical and the manifestation of an endocrine or reproductive dysfunction in humans and wildlife. However, these types of single exposures are not representative of more common widespread persistent exposure to a broad mix of indoor and outdoor chemicals and contaminants.

Industrialized areas are typically characterized by contamination from a wide range of industrial chemicals that may leach into soil and groundwater [4-6]. These complex mixtures enter the food chain and accumulate in animals higher up the food chain such as humans, American bald eagles, polar bears, and other predatory animals. Exposure occurs through drinking contaminated water, breathing contaminated air, ingesting food, or contacting contaminated soil. People who work with pesticides, fungicides, and industrial chemicals are at particularly high risk for exposure and

thus for developing a reproductive or endocrine abnormality.

Bisphenol A (BPA) is a key building block of polycarbonate plastic and a precursor for the manufacturing of monomers of epoxy resins. The BPA exposure of the general population is via food as a result of the use of BPA in food packaging and via skin as a result of contact with thermal paper. The vast majority of the population (91–99%) has detectable levels of BPA-conjugates in their urine. BPA is also present in medical devices including implants, catheters, tubing, and some dental materials. This Opinion describes the risk assessment of exposure to BPA via medical devices that are manufactured with materials that potentially leach BPA leading to oral (via dental material), subcutaneous and intravenous (e.g. during heamodialysis) routes of exposure. After oral exposure BPA is readily absorbed from the gastro-intestinal tract and due to the first pass effect in the liver and the small intestine is rapidly conjugated to non-toxic metabolites.

By the oral route BPA has a low systemic bioavailability (1-10% in humans) and has a half life time of a few hours. For parenteral routes of exposure (intravenous, intraperitoneal, subcutaneous), BPA can be considered 100% systemically bioavailable. However, BPA will also be conjugated in the liver and the clearance of free BPA from the circulation appears to be relatively fast. Toxicity studies indicate that the kidney and the liver are relevant target organs for BPA toxicity. The lowest NOAEL after oral repeated exposure identified in several studies, including multigeneration reproductive toxicity studies, was approximately 5 mg/kg b.w./day. By applying the benchmark dose (BMD) approach, a BMDL10 of 8.96 mg/kg b.w./day was derived (EFSA 2015), based on the alteration in kidney weight. BPA is not likely to pose a genotoxic hazard to humans and has no carcinogenic activity, although there are some effects observed in the mammary gland, which currently are of unknown significance to human health [7, 8]. Neither reproductive nor prenatal developmental toxicity are critical end-points in BPA toxicity, though BPA is associated with reproductive toxicity at doses higher than those causing liver and kidney damage. There are several indications that BPA might have biological effects below the recently determined BMDL10. However, the evidence is inconsistent, mainly obtained in dedicated studies focussing on different selected health effects and dose-response relationships could not be established. Regarding possible low dose effects, some concern remains for effects on mammary gland, metabolism and adiposity and neurobehaviour.

Bisphenol A [Bis(4-hydroxyphenyl)propane] (BPA) is a high-production volume industrial chemical, being a key building block of polycarbonate (PC) plastic and a precursor for the manufacturing of monomers of epoxy resins. Currently, many scientific discussions are ongoing on possible adverse effects of BPA. The general public is mostly exposed to BPA via food as a result of the use of BPA in food packaging and via skin as a result of contact with thermal paper. Polycarbonate is used in medical devices production (e.g. implants, catheters, tubing) for its balance of toughness, dimensional stability optical clarity, high heat resistance and electrical resistance. They may contain and release residual BPA during actual condition of use [8]. In addition to PC medical devices, various dental materials are fabricated from monomers such as bisphenol A glycidyl methacrylate (Bis-GMA) and bisphenol A dimethacrylate (Bis-DMA), derived from BPA.

BPA-resins are also used in inks and adhesives. In addition to BPA itself, polymers produced using BPA like polysulfone (PSU) used in medical devices (e.g. membrane in hemolysis dialyzers) are considered because they can release BPA. Some BPA-containing medical devices may have direct and/or indirect contact with patients (e.g. hemodialyzer apparatus, filters, bypasses, tubing, pumps, instruments, surgical equipment, blood pathway circuits and respiratory tubing circuits). This Opinion describes the risk assessment of exposure to BPA via medical devices made up of materials potentially leaching BPA, for which the exposure routes are not limited to oral applications (i.e. for dental material) as exposure via other applications such as subcutaneous and intravenous (e.g. during haemodialysis) routes may occur [9,10].

Exposure from medical devices Medical devices based on PC and polysulfone due to their chemistry can contain BPA residues, whereas others like PVC-based medical devices may or may not contain BPA residues depending on their production method. In addition, some other BPA-derivatives (such as epoxy resins) are used specifically in dental materials. The major factor influencing the residual amount of BPA levels is the employment of incorrect operating. The safety of the use of bisphenol A in medical devices 10 conditions during the processing step. Moreover, breakdown or hydrolysis of the PC polymer after manufacturing can occur, thus giving rise to the free monomer from the polymer available for exposure. In PC articles used for food contact, the residual content is usually less than 10 µg/g of PC (ECB, 2003). Exposure can be estimated by either measuring the BPA content of the medical devices or by extraction assays for potential release [11,12].

For dental materials, the leakage is limited to resins composed of Bis-DMA (Bisphenol A dimethylacrylate) which has an ester linkage that can be hydrolysed to BPA, whereas the ether linkage in Bis-GMA (Bisphenol A glycidyl methacrylate) was found to be stable. Dental materials release BPA especially shortly (0- 1 h) after placement: the levels of BPA

detected in saliva ranged from0.64 ng/mL to 30 µg/mL. This wide range in BPA measurements may indicate a continuous reduced leaching of BPA from dental materials, as well as reflect variations in the analytical methodology used. For BPA exposure resulting from the use of medical devices, little information is available. For the placing of dental composite resin restorations, measurements have shown that the release of BPA mainly occurs during the few hours directly after application while the BPA level is back to pretreatment levels at 24 hours. Values measured were up to 30 µg/mL saliva, and 931 µg in total saliva volume produced in one hour [13,14].

Calculations based on the actual amount of material used in clinical practice and a median 4-year life-time of a composite restoration; suggest a maximum exposure of 0.06 µg BPA/day from fissure sealants, and a maximum exposure of 0.36 µg BPA/day from composite restorations. Contact with dental materials gave an estimated acute (90%), but the systemic bioavailability of free BPA after oral exposure is reduced by the first pass effect. Based on the analysis of oral versus intravenous toxicokinetic data [15], the oral systemic bioavailability of unconjugated BPA is 2.8%, 0.2%,0.9% and less than 1% in rats, mice, monkeys, and dogs, respectively. The systemic availability of unconjugated BPA in humans has not been evaluated experimentally, however, modelled data as well as controlled biomonitoring studies indicated that internal exposure in humans to unconjugated BPA is very low (1-10%). The conjugates are readily excreted in urine; as a consequence the half-life of BPA in humans is very short, ranging from 1 to 3.5 h. After dermal exposure, the absorption fraction can be considered approximately 25-30% of the applied dose, which is directly systemically bioavailable [16].

Experimental Work

These include analysis procedures of biochemical estimations, mitochondrial enzyme estimations, and immunohistochemistry.

MATERIALS AND METHODS

Chemicals

Chemicals and other reagents used in this study were of high quality and purity grade available commercially. Some of the important chemicals are mentioned below. Bisphenol A, Benzylamine, diaminobenzidine, Glutathione reduced (GSH), Glutathione reductase (GR), Glutathione oxidized (GSSG), Isocitrate, Lead citrate, Mannitol, Melatonin, Nicotinamide adenine dinucleotide reduced (NADH), Nicotinamide adenine dinucleotide phosphate (NADP), Nicotinamide adenine dinucleotide phosphate reduced (NADPH), Sodium succinate, Xanthine, Xanthine oxidase, Uranyl acetate,Copper sulphate, Cyclohexane, Cytochrome c, Diaminobenzidine, 2, 6-Dicholorophenol indophenol, Disodium hydrogen phosphate, Ethyl acetate, Folin-Ciocalteu reagent (FCR), Formalin, Gluteraldehyde, Magnesium chloride, Malonic acid, Nitroblue tetrazolium (NBT), Oxaloacetic acid, Paraformaldehyde, Potassium chloride, Sodium azide, Sodium phosphate, Sodium potassium tartarate, Sodium carbonate, Trichloroacetic acid (TCA), Tris HCl.*Bovine serum albumin (BSA), epoxy resin araldite CY212, Ethylene diamine tetracetic acid (EDTA), HEPES Buffer,* Potassium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium chloride, *Sucrose, Tris Buffer, Dodecyl succinic anhydride, Osmium tetroxide, Sodium hydroxide (E. Merck. Germany). Thiobarbituric acid (TBA), tertiary butyl Hydroperoxide (Spectrochem Pvt. Ltd. Mumbai). Acetone, eosin, hematoxylin, hydrochloric acid, hydrogen peroxide, Metaphosphoric acid (HPO_3), perchloric acid, paraffin, toluidine, xylene (S D Fine Chem Ltd. Mumbai). Ethyl alcohol, methanol, o-phthalaldehyde (Jiansu Huaxi International Trade Co. Ltd. China).*DMP 30 (TAAB Laboratories Berks, UK). Anti-caspase 3 antibody (Biogenesis Inc. Poole, UK), anti- cytochrome c antibody (Santacruz Biotechnology Inc. USA), anti-BCl_2 antibody (Thermo Fisher Scientific, USA) and anti ER α antibody (Santacruz Biotechnology Inc. USA). Biotinylated antimouse IgG (Boehringer Mannheim, Germany), Streptavidin-peroxidase (Dako CA, USA.

Animals

The study was conducted in male Swiss albino mice (25 ± 2 g). The animals were maintained under the standard laboratory conditions (temperature 25 ± 2 ºC; photoperiod of 12 h). Commercial pellet diet and water were given *ad libitum.*

Necropsy

After termination of treatment animals were sacrificed under mild anesthesia and their testes were taken out and washed with ice cold saline (0.9 % NaCl). After washing in ice cold isolation medium (0.25 M sucrose) and 1 mM EDTA (pH 7.4) testes were gently blotted between the folds of filter paper and weighed.

Isolation of mitochondria

All procedures were carried out on ice or at 4 ºC. Testes were minced in fresh isolation medium and gently homogenized manually (10 % w/v in isolation medium) using a homogenizer with a loose fitting pestle. The homogenate was centrifuged at 500 x *g* for 10 min. The supernatant fraction was retained and the pellet was washed with fresh isolation medium and recovered by the initial supernatant fraction. The pooled fractions were centrifuged at 500 x *g* for 10 min. The supernatant thus obtained was centrifuged at 5000 x *g* for 15 min to obtain the mitochondrial pellet. The pellet was washed first with isolation medium and then with respiration reaction buffer (70 mM sucrose, 220 mM mannitol, 2 mM HEPES, 0.5 mM EDTA, 2.5 mM $MgCl_2$, 0.5 mM KH_2PO_4, 2 mM K_2HPO_4, (pH 7.4). The purifed mitochondria were obtained by centrifugation at 12,000 x *g* for 10 min. The final mitochondrial pellet

was re-suspended in respiration reaction buffer (1 ml/g tissue) to produce a suspension containing 25-40 mg of mitochondrial pellet/ml. Mitochondria were isolated according to the method of Sayeed *et al*. [17].

Measurement of antioxidants

Enzymatic antioxidant

Measurement of glutathione peroxidase (GPx)

Freshly isolated mitochondria (0.1 ml) were suspended in 1 ml of buffer (50 mM Tris HCl, 5 mM EDTA, 1 mM GSH, 0.22 mM NADPH and 0.4 U GR (pH 7.6). Reaction was started by adding tertiary butyl hydroperoxide to a final concentration of 0.22 mM. The enzyme activity was calculated as nmol NADPH oxidized/min mg protein using molar extinction coefficient of 6.22 x 10^3 M^{-1} cm^{-1} [18-19].

Measurement of glutathione reductase (GR)

Assay mixture preheated to 25 °C prior to assay consisted of sodium phosphate buffer (0.2 M, p H- 7.6), containing 1 m M GSSG and 0.1 m M NADPH. An aliquot of 30 µl homogenizing buffer (250 mM sucrose, 80 mM Tris HCl, 5 mM $MgCl_2$, 250 mM KCl, 1 mM EDTA (pH 7.4) was dispensed in 6 wells of 96 plate to determine background rate of reaction which was substracted from enzymatic rate prior to calculation. Sample in aliquots of 20, 25 and 30µl were placed in triplicate in remaining wells. Homogenizing buffer was added to bring the volume in each well to 30 µl. Reaction was initiated by addition of 200µl of assay mixture per well. Reading was taken at 2 min interval for 10 min. Plate returned to incubator between readings. The enzyme activity was quantitated as NADPH disappeared at 340 nm and calculated as nmol NADPH consumed/min mg protein using molar extinction coefficient of 6.22 x 10^3 M^{-1} cm^{-1}. GR activity was measured by 96-well plate method of McFarland *et al*.[20].

Measurement of manganese Superoxide dismutase (Mn SOD)

Manganese superoxide dismutase (Mn SOD) was measured by xanthine/xanthine oxidase mediated ferricytochrome c reduction method of Flohe and Otting [21]. Briefly, mitochondrial pellet (1 mg) suspended in (50 mM phosphate buffer and 0.1 mM EDTA, pH 7.8) and disrupted by sonication. Supernatant was collected after centrifugation and assayed for MnSOD activity. The assay mixture consisted of 50 µl sample in 2.9 ml reaction buffer (0.5 M xanthine, 0.1 mM NaOH and 2M of cytochrome c in (50 mM phosphate buffer, 0.1 mM EDTA, pH 7.8). The reaction was initiated by adding 50 µl of xanthine oxidase (0.2 U/ml in 0.1 mM EDTA) and change in absorbance was monitored for 3 min at 438 nm. The SOD activity was calculated as its ability to inhibit 50 % reduction of ferricytochrome c and expressed as U/min mg protein.

Non-enzymatic antioxidant

Measurement of reduced glutathione (GSH)

Mitochondrial glutathione was estimated using the method of Hissin and Hilf [22]. Mitochondrial preparation (1 mg protein) was suspended in 25 µl of 25% HPO_3 and 90 µl of sodium phosphate buffer (0.1 M, pH 8.0, with 5 mM EDTA). The samples were centrifuged at 15,000 x *g* for 10 min at 4 °C and the supernatant was collected for measurement of GSH. Supernatant (100 µl) was incubated with 100 µl of o-phthalaldehyde (0.1% in methanol) and 1.8 ml of 0.1 M phosphate buffer (pH 8.0) for 15 min at room temperature in the dark. Fluorescence was measured with a fluorescence spectrophotometer (PerkinElmer, USA) at an excitation wavelength of 350 nm and an emission wavelength of 420 nm. The GSH values are expressed as nmol/mg mitochondrial protein.

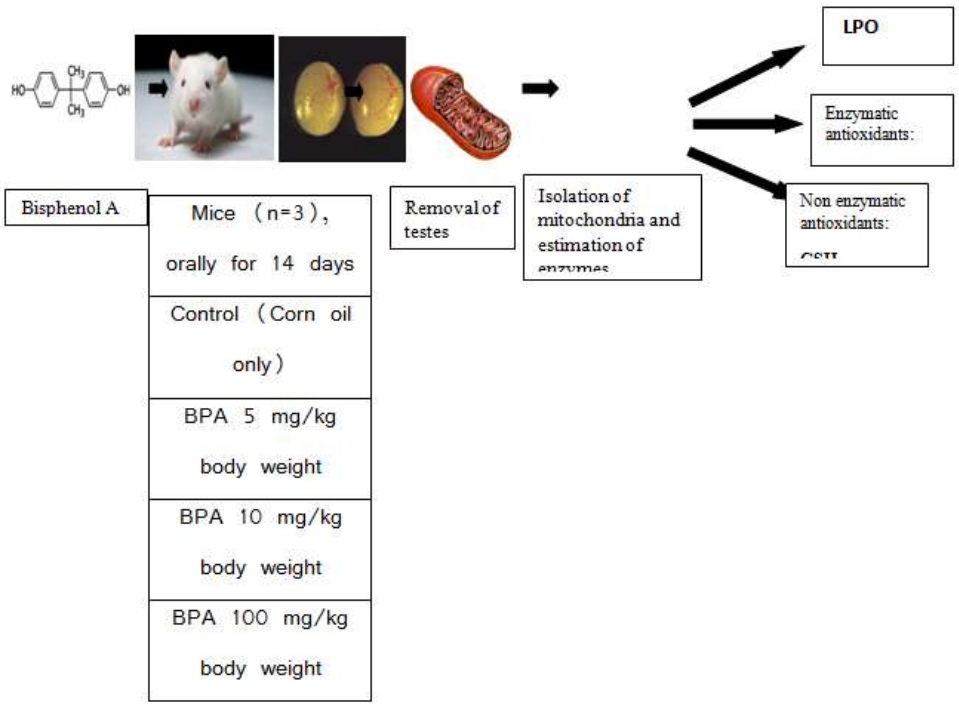
Estimation of mitochondrial lipid peroxidation (LPO)

LPO was measured by the method of Buege and Aust [23]. Mitochondrial preparation (0.5 ml) was mixed with equal volume of Buege and Aust reagent (TCA 15% w/v in 0.25 M HCl and TBA 0.37 % w/v in 0.25 M HCl) and heated for 15 min in boiling water. After cooling the precipitate was removed by centrifugation at 1000 x *g* for 10 min at room temperature. Absorbance was measured at 532 nm. TBARS were quantified using an extinction coefficient of 1.56 $X10^5$ M^{-1} cm^{-1} and expressed as nmol of TBARS/ mg protein.

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was applied to determine significant differences in results of various groups. P values < 0.05 were considered significant. Subsequently Tukey's post hoc test was applied to determine significant changes between different treatment groups. The values were expressed as mean ± S.E. GraphPad Prism 3 software (GraphPad Software, Inc. San Diego, USA) was used for statistical analysis.

Experimental design



BPA is a potent ligand of human estrogen-related receptor γ (ERRγ) with an EC50 value of 13.1 nM [24]. Although most studies have focused on the estrogenic effects of BPA, this compound also disrupts thyroid hormone action [25], acts as an AR antagonist and disrupts testosterone synthesis. BPA can be transferred to the fetuses from pregnant mice and alter postnatal development and sexual maturity. Perinatal or pubertal exposure of mice to BPA increases ERα and ERβ expression in the mouse forebrain and hypothalamus in rats [26]. BPA affects not only the receptors but also other cellular components such as plasma membrane, chromosomes etc amongst which mitochondria is of specific concern as complex I of the mitochondrial respiration chain is an important site of ROS [27].

ROS such as superoxide anion, hydrogen peroxide and hydroxyl radical are generated *in vivo* from the incomplete reaction of oxygen during aerobic metabolism or in stimulated host phagocytes, as well as from exposure to environmental agents such as redox cycling agents and radiations [28]. Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defense mechanisms causing damage to macromolecules such as DNA, proteins and lipids [29]. At low concentration ROS have been implicated in the regulation of several physiological processes such as proliferation, differentiation, apoptosis and senescence. At high concentrations ROS are very toxic to cells and causes DNA damage, protein degradation and lipid peroxidation [30].

To counteract the damaging effect of ROS, aerobic cells are provided with extensive antioxidant enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase etc) [31] and non-enzymatic antioxidants such as glutathione, vitamin E, vitamin C etc. EDC results in damage to the plasma membrane disrupted mitochondrial membranes as well as nuclear membranes with ROS generation and altered Ca^{2+} homeostasis. The damage to mitochondria by EDCs not only leads to a pathophysiological condition but also leads to developmental toxicity in general. EDCs impair mitochondrial function in β-cells and induced remarkable swelling of mitochondria with loss of distinct cristae structure within the membrane, which was accompanied by disruption of mRNA expression of genes playing a key role in β-cell function (Glut2), (Slc2a2), Gck, Pdx1, Hnf1α, Rab27a, and Snap25) and mitochondrial function (Ucp2 and Ogdh) [32].

BPA caused a significant mitochondrial dysfunction including ROS production, mitochondrial membrane hyperpolarization, lipid accumulation, lipoperoxidation and the release of pro-inflammatory cytokines in hepatic cells triggered by disturbances in mitochondrial function, alterations in lipid metabolism and by inflammation [33]. With emerging data about the delirious effects of EDCs on mitochondria, it will be of worth to study the effects of BPA on the mitochondria in gonadal tissues. Doses were calculated and selected according to Tachibana et al. [34].

Table-1: Bispinol A and other control group

Number of Group	Dose and Control
Group I	Corn Oil (Control)
Group II	BPA (5 mg/kg body weight)
Group III	BPA (10 mg/kg body weight)
Group IV	BPA (100 mg/kg body weight)

RESULTS & DISCUSSION

Results

There are following effect on antioxidant enzymes as below. a) Glutathione Peroxidase (GPx): Group 1 (5 mg/kg body weight) of BPA shown significant decrease ($P< 0.01$) in GPx activity in the compare of Group 1 (Control group). Group II, BPA treated animal showed result as 21 % decrease in GPx activity in the compare of Group 1 (Control group). As likely Group III and Group IV BPA treated (10 mg/kg and 100 mg/kg body weight respectively) obtained significant decrease ($P< 0.01$) in GPx activity in the compare of Group 1 (Control group). Group III and Group IV (10 and 100 mg/kg body weight) BPA resulted in 27 % and 66 % decrease in GPx activity, respectively when compared with control animals (Table 2).

b) Glutathione Reductase (GR): Group II in compare to Group 1 was observed a significant decrease ($P< 0.05$). Group III and Group III were resulted 19 % and 35% decrease in GR activity, respectively ($P< 0.01$) (Table 2). High dose of BPA (100 mg/kg body weight) caused about 65 % decrease in activity of GR as compared with control group of animals.

c) Superoxide Dismutase (SOD): The activity of SOD was decrease as the dose of BPA increased. Decrease in activity of SOD in Group II and Group III was 34 % and 60 % as compared to control group of animals. Group IV (100 mg/kg body weight) treated with BPA caused a significant 76 % decrease in activity of SOD as compared to control group (Table 2).

Lipid Peroxidation (LPO)

The levels of LPO increased as the dose of BPA increased and testicular mitochondrial LPO levels were significantly ($P< 0.01$) higher in BPA exposed groups as compared to Group 1 (Control group). Lipid peroxidation increased about 51 % on treatment of Group 2 (5 mg/kg body weight) of BPA as compared with Group 1 (control group). Group III (10 mg/kg body weight) BPA was caused significantly high about 113 % increase in LPO when compared with control group. Group IV (100 mg/kg body weight) of BPA caused LPO to increase almost 4 times over the controls.

Table-2: Bispinol A effect on biochemical parameters of mitochondria

Groups	GPx	GR	SOD
Group I	371.5 ± 17.015	41.05 ± 3.79	61.37 ± 4.11
Group II	297.39 ± 4.024	35.21 ±1.07 *	31.17 ± 3.87 **
Group III	255.13 ± 3.41**	29.31 ± 1.89	23.51 ±1.48 **
Group IV	131.25 ± 7.33	15.01 ± 1.73 **	11.39 ± 1.01 **

Group I (Corn oil) is control, Group II (5 mg/kg body weight), Group III (10 mg/kg body weight), Group IV (100 mg/kg body weight). Values are expressed as Mean ± SE (n = 3). Significant is expressed as *$P< 0.05$ and **$P< 0.01$.

Discussion

The general public is mostly exposed to BPA via food as a result of the use of BPA in food packaging and via skin as a result of contact with thermal paper. Polycarbonate is used in medical devices production (e.g. implants, catheters, tubing) for its balance of toughness, dimensional stability optical clarity, high heat resistance and electrical resistance. They may contain and release residual BPA during actual condition of use. In addition to PC medical devices, various dental materials are fabricated from monomers such as bisphenol A glycidyl methacrylate (Bis-GMA) and bisphenol A dimethacrylate (Bis-DMA), derived from BPA. BPA-resins are also used in inks and adhesives. In addition to BPA itself, polymers produced using BPA like polysulfone (PSU) used in medical devices (e.g. membrane in hemolysis dialyzers) are considered because they can release BPA. Some BPA-containing medical devices may have direct and/or indirect contact with patients (e.g. hemodialyzer apparatus, filters, by passes, tubing, pumps, instruments, surgical equipment, blood pathway circuits and respiratory tubing circuits). BPA is an endocrine-disrupting chemical which is continuously released into the environment because of its extensive usage as plasticizer and other industrial applications. BPA radical, are formed by a reaction of radical oxygen [35-36].

Atkinson and Roy, have reported that BPA is oxidized to a reactive metabolite 4,5-bisphenol- O-quinone which forms DNA adduct in rat liver DNA in the presence of peroxidase activation [37]. Oxidative stress in any tissue results from an imbalance between the production of (ROS) and their efficient removal by available antioxidant systems. ROS are small, oxygen-

based molecules that are highly reactive because of unpaired electrons [38]. The predominant source of free radical generation is the mitochondrial respiratory chain and inhibition of this process is often connected to increased levels of free radicals [39]. These ROS result in increased lipid peroxidation and modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression and apoptosis.

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Toxicity studies indicate that the kidney and the liver are relevant target organs for BPA toxicity. The lowest NOAEL after oral repeated exposure identified in several studies, including multigeneration reproductive toxicity studies, was approximately 5 mg/kg b.w./day. By applying the benchmark dose (BMD) approach, a BMDL10 of 8.96 mg/kgb.w./day was derived, based on the alteration in kidney weight. BPA is not likely to pose a genotoxic hazard to humans and has no carcinogenic activity, although there are some effects observed in the mammary gland, which currently are of unknown significance to human health. Neither reproductive nor prenatal developmental toxicity are critical end-points in BPA toxicity, though BPA is associated with reproductive toxicity at doses higher than those causing liver and kidney damage. BPA administered orally to mice induced LPO and decreased various vital antioxidant enzymes such as SOD, GPx, GR and non-enzymatic antioxidant such as GSH in testicular mitochondria.

This suggests increase production of reactive oxygen species (ROS) in mitochondria of exposed group. In most of the *in-vivo* toxicity studies in mammals oral route of administration of BPA has been employed, as it is the main route of exposure in human [40]. BPA induced discernible oxidative stress response in mice. It is now recognized that the oxidative damage is the primary cause of sub-cellular effects of different classes of EDCs [41]. LPO is caused by the action of ROS. ROS also cause damage to DNA and proteins resulting in various harmful consequences have reported that BPA induced LPO in rats and role of mitochondria in testicular apoptosis [42].

LPO induction was found to be dose-dependent. GSH either alone or in conjugation with other proteins, can protect cells against LPO [43]. Mitochondrial GSH plays a critical role in maintaining cell viability through the regulation of mitochondrial inner membrane permeability by maintaining sulphydryl groups in reduced state [44].

However, in our study when the relative responses of LPO and GSH were compared effect of BPA was more pronounced in case of LPO induction than decrease in GSH. Moon et al., using mice model have shown that BPA (0.05 mg/kg body weight and 1.2 mg/kg body weight for 5 days i.p) led to increase in LPO in liver mitochondria in mice [45]. On the pattern of our observations, they also found that the oxidative stress and LPO occurs in mitochondria. Hassan et al., have also reported on LPO inducing effect of BPA in liver and decrease in activities of SOD, GPx and GR in liver in rat. Antioxidants are located throughout the cell and provide protection against ROS toxicity [46]. The lower activity of SOD in BPA exposed group may be explained by the inhibition of SOD by its product hydrogen peroxide or by hydroxyl radical. Our study also shows a dose dependent decrease in activities of GR, GPx, and SOD in the testicular mitochondria of mice. Decrease in the GSH level and disruption of activities of antioxidant enzymes as reported here might contribute to histological changes.

Findings of the present study suggest a role for free radicals, especially ROS and disruption of antioxidants in BPA induced toxicity in testicular mitochondria of mice.Oxidative stress can induce mitochondrial damage and damaged mitochondria can generate more ROS. Mitochondria are vulnerable to ROS due to impairment of the antioxidant and DNA repair enzyme systems [47]. Accumulation of oxidative damage in the mitochondria induces mitochondrial dysfunction, mitochondrial DNA depletion and cell apoptosis [48]. Deleterious effect of BPA includes its oxidative stress causing property, which generates highly reactive membrane toxic intermediates in liver, kidney, testes and brain of rats. BPA has been shown to induce ROS generation in the epididymal sperm of rats. BPA exposure causes enzyme inactivation by excess ROS production in mitochondria and microsomes [49]. In a tissue like the testis, with its high rates of metabolism and cell replication, oxidative stress can be especially damaging, which makes the antioxidant capacity of the tissue very important. ROS have been shown to play an important role in the defense mechanisms against pathological conditions but excessive generation of free oxygen radicals may damage tissues.

BPA is a known endocrine disruptor and studies have suggested that it causes developmental and reproductive toxicity. Endocrine disrupting chemicals cause adverse effects in target organs by interfering with the interaction of endogenous hormones and their receptors. Environmental estrogens can mimic the natural estrogen, 17 b-estradiol (E2) and disrupt the endocrine system of aquatic animals [50].The reproductive toxicity is caused by interaction with androgen and estrogen receptors [51]. Most developmental effects of estrogens are mediated by their binding to an intracellular steroid receptor protein, either ERα or ERβ. BPA also compete for 17β-E2 to ERβ and its binding to ERs results in changes in expression of related genes [52].

The present result of the study establishes oxidative stress inducing effects of BPA in testicular mitochondria of Swiss albino mice. The present study focuses on the light microscopic and ultra-structural changes in the adult mice after treatment with BPA as compared with control group. In present study we are going to discuss the effects of BPA on testicular tissue in general and mitochondria in specific. Light microscopic observations showed that low doses of BPA (5 and 10 mg/kg body weight) exposure for 14 days caused reduction in the number of cells in the layers of seminiferous tubules. This reduction may be due to the apoptosis of cells by BPA treatment [53]. Highest dose of BPA treatment for 14 days caused much damage to the seminiferous tubules and the tubule (Fig. 1H) is in the spermatogenetic cycle. Portions of the tubule show disappearance of cells and prominent vacuolar change. This shows that a lot of cells turned apoptotic.

There were many ultra-structural changes found in the treated groups as compared to the control group. Studies suggest that BPA exposure causes ultrastructural changes in testis of rodents [54, 55]. Basement membrane is the membrane covering the seminiferous tubules of the testes. BPA treatment caused membrane damage of the seminiferous tubules. Membranes are made up of lipids and BPA causes LPO in the membranes. The damage increased as the dose of BPA increased. Another significant change which was observed was the detachment of the basal cells.

The decrease in number of spermatogenic cells shows that apoptosis may have occurred and cells might have died. Destruction of the spermatogonial complement of the testis results in sterility. Also vacuolization was observed in these cells. Study has also confirmed that BPA caused apoptosis in testicular cells. Spermatocyte and spermatogonia were affected by BPA treatment in our study. A study showed that low concentration of BPA caused spermatogenic and Sertoli cells to apoptosis, whereas a higher concentration tend to lead spermatogenic and Sertoli cells to necrosis in goat [56].

Apoptotic spermatogonia were characterized by a ruptured nucleus, shrinkage of the cytoplasm and nucleus, and still functioning cell organelles. Mitochondria are target of BPA and it causes mitochondrial membrane permeability transition has been shown by Kashiwagi *et al*. [57]. These ROS could also cause apoptosis of the Sertoli and spermatogenic cells in the seminiferous tubules of the testicular tissue. A study has shown that BPA and some related phenols are cytotoxic, most likely through the impairment of mitochondrial function and consequent decrease in the cellular level of ATP [58]. Also compound bisphenol A diglycidyl ether-induced apoptosis involves Bax/Bid dependent mitochondrial release of apoptosis-inducing factor (AIF), cytochrome c and Smac/DIABLO. Cristae of mitochondria were absent in many mitochondria of Sertoli cell in animals treated with BPA (Fig. 3N). This may be due to oxidative stress, Ca2+ overload, and ATP loss perhaps due to Bax-mediated effects and this shows mitochondria mediated apoptosis [59].

BPA negatively affected male germ cell number and hence may affect fertility. BPA causes release of ROS and oxidative stress in mitochondria of testes. These free radicals generated are having the potential to adversely affect the germ cell and is components. There are evidences that ROS can induce apoptosis via several mechanisms, including mitochondria-dependent or independent pathway in various cell lines and tissues [60]. Mitochondria provide the gateway through which apoptosis can be executed in a rapid and robust manner. BPA affected activities of vital enzymes of mitochondria which are part of mitochondrial bioenergetics as well as reduced glutathione levels. Ultra-structurally BPA causes apoptosis of spermatogenic cells in the testes.

Apoptosis caused by BPA may be manifested as a result of release of ROS in the testicular tissue, which is also inferred by the reduced levels of GSH. At molecular level BPA interferes with the development process of sperm by interacting with the estrogen receptors. BPA affected different type of cells in various stages of sperm development in the seminiferous tubules. It affected spermatogonia, spermatocyte, sertoli cell and developing spermatids. Infertility is a major problem now a days and by the results received by us we can infer that environmental endocrine chemical BPA is a potent endocrine disruptor and has potential to cause testicular toxicity. Human exposure to low level of BPA is continuous and BPA can bioaccumulate in some conditions, there is concern about human reproductive health especially for occupational workers exposed to higher levels of BPA. The result shown that BPA exposure causes testicular toxicity and histopathological alterations in the testes of mice. BPA induced alterations in mitochondria and causes germ cell apoptosis as seen by decrease number of cells in BPA exposed groups.

CONCLUSION

Most human exposures to BPA result from its use in food and beverage containers. BPA can leach into food from containers lined with epoxy resin coatings, and from polycarbonate plastic products. Warming the plastic, such as in a microwave, increases the leaching of BPA into liquids; temperature appears to be a more important factor in leaching than the age of the container. Human exposure to BPA is due to its widespread use, along with reproductive and developmental effects reported in animal studies have generated considerable attention on this chemical in recent years. BPA is continuously released into the environment because of its extensive usage as plasticizer and other industrial applications. Liver is the major site of metabolism of BPA. Metabolic pathway of BPA involves conjugation with glucuronic acid in liver microsomes to form BPA glucuronide. BPA glucuronide is the major metabolite in urine. The cyp P450 system is closely associated with the metabolism and clearance of BPA. BPA radical, are formed by a reaction of radical oxygen.

Human exposure to BPA is continuous there is concern about human reproductive health, exposed to low dose of BPA. Our study suggests that BPA induces toxicity in mitochondria and upsets the antioxidant status of the mitochondria. Also it causes apoptosis as determined by the expression pattern of proteins involved in apoptosis cascade. These findings suggest that BPA may cause testicular toxicity by impacting various testicular cell types and by activating the mitochondrial apoptotic pathway. Our study showed that exposure to BPA induces significant oxidative stress in testicular mitochondria in mice and melatonin scavenges the free radicals. Human exposure to BPA is due to its widespread use, along with reproductive and developmental effects reported in animal study has generated considerable attention on this chemical in recent years.These aspects need further investigation in properly conducted studies with a wide dose range of BPA.

In the present investigation the decrease in enzyme activity in exposed groups could be attributed to ROS production. Small inefficiencies in the mitochondrial ETC produce background levels of (ROS) that can lead to severe mitochondrial dysfunction and cell death. In mitochondria oxidative stress is caused when the balance between antioxidant enzymes such as GPx, GR and SOD and most importantly of GSH goes down. When GSH levels are greatly decreased, H_2O_2 accumulates, and this leads to extensive mitochondrial damage. Other antioxidants may be involved in the protection of mitochondria. Mitochondrial GSH homeostasis is essential to ensure the protection against the oxidative damage of mitochondria and thus of the whole cell.Oxidative stress in mitochondria may lead to mitochondrial dysfunction and can affect bioenergetics processes.

REFERENCES

1. Cao Y, Calafat AM, Doerge DR, Umbach DM, Bernbaum JC, Twaddle NC, Ye X, Rogan WJ2009. Isoflavones in urine, saliva and blood of infants data from a pilot study on the estrogenic activity of soy formula. J Expo Sci Environ Epidemiol 19:223–234.
2. Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology. 2004 Feb 1;145(2):592-603.
3. Alexander HC, Dill DC, Smith LW, Guiney PD, Dorn P. Bisphenol A: acute aquatic toxicity. Environmental Toxicology and Chemistry. 1988 Jan 1;7(1):19-26.
4. Adam-Vizi V, Tretter L. The role of mitochondrial dehydrogenases in the generation of oxidative stress. Neurochemistry international. 2013 Apr 1;62(5):757-63.
5. Calafat AM, Needham LL. Human exposures and body burdens of endocrine-disrupting chemicals. InEndocrine-Disrupting Chemicals 2007 (pp. 253-268). Humana Press.
6. Gore AC, Crews D**.** Environmental endocrine disruption of brain and behavior. In: Pfaff DW, Arnold AP, Etgen A, Fahrbach S, Rubin R, eds. Hormones, Brain and Behavior. San Diego, Academic Press. 2009; pp. 1789–1816.
7. Aluru N, Leatherland JF, Vijayan MM. Bisphenol A in oocytes leads to growth suppression and altered stress performance in juvenile rainbow trout. PloS one. 2010 May 20;5(5):e10741.
8. Andrady AL, Neal MA. Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 2009 Jul 27;364(1526):1977-84.
9. Babu S, Uppu S, Claville MO, Uppu RM. Prooxidant actions of bisphenol A (BPA) phenoxyl radicals: implications to BPA-related oxidative stress and toxicity. Toxicology mechanisms and methods. 2013 May 1;23(4):273-80.
10. Babu S, Vellore NA, Kasibotla AV, Dwayne HJ, Stubblefield MA, Uppu RM. Molecular docking of bisphenol A and its nitrated and chlorinated metabolites onto human estrogen-related receptor-gamma. Biochemical and biophysical research communications. 2012 Sep 21;426(2):215-20.
11. Flint S, Markle T, Thompson S, Wallace E. Bisphenol A exposure, effects, and policy: a wildlife perspective. Journal of environmental management. 2012 Aug 15;104:19-34.
12. Brede C, Fjeldal P, Skjevrak I, Herikstad H. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Additives & Contaminants. 2003 Jul 1;20(7):684-9.

13. ASSESSMENT R. European Union Risk Assessment Report. Heal.(San Fr. 2008 May 29;77:107-259.
14. Lewis JB, Rueggeberg FA, Lapp CA, Ergle JW, Schuster GS. Identification and characterization of estrogen-like components in commercial resin-based dental restorative materials. Clinical oral investigations. 1999 Sep 1;3(3):107-13.
15. Doerge DR, Twaddle NC, Vanlandingham M, Brown RP, Fisher JW. Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague–Dawley rats. Toxicology and applied pharmacology. 2011 Sep 15;255(3):261-70.
16. Gillette JW. 1983. A comprehensive prebiological screen for ecotoxicological effects. Environ. Toxicol. Chem. 2:463-476.
17. Sayeed I, Parvez S, Winkler-Stuck K, Seitz G, Trieu I, Wallesch CW, Schönfeld P, Siemen D. Patch clamp reveals powerful blockade of the mitochondrial permeability transition pore by the D2-receptor agonist pramipexole. The FASEB journal. 2006 Mar;20(3):556-8.
18. Flohé L, Günzler WA. [12] Assays of glutathione peroxidase. InMethods in enzymology 1984 Jan 1 (Vol. 105, pp. 114-120). Academic Press.
19. Flohe L. [10] Superoxide dismutase assays. InMethods in enzymology 1984 Jan 1 (Vol. 105, pp. 93-104). Academic Press.
20. McFarland VA, Inouye LS, Lutz CH, Jarvis AS, Clarke JU, McCant DD. Biomarkers of oxidative stress and genotoxicity in livers of field-collected brown bullhead, Ameiurus nebulosus. Archives of Environmental Contamination and Toxicology. 1999 Aug 1;37(2):236-41.
21. Flohe L. [10] Superoxide dismutase assays. InMethods in enzymology 1984 Jan 1 (Vol. 105, pp. 93-104). Academic Press.
22. Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Analytical biochemistry. 1976 Jul 1;74(1):214-26.
23. Buege JA, Aust SD. [30] Microsomal lipid peroxidation. InMethods in enzymology 1978 Jan 1 (Vol. 52, pp. 302-310). Academic Press.
24. Takayanagi S, Tokunaga T, Liu X, Okada H, Matsushima A, Shimohigashi Y. Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor γ (ERRγ) with high constitutive activity. Toxicology letters. 2006 Dec 1;167(2):95-105.
25. Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, Hataya Y, Shimatsu A, Kuzuya H, Nakao K. Thyroid hormone action is disrupted by bisphenol A as an antagonist. The Journal of Clinical Endocrinology & Metabolism. 2002 Nov 1;87(11):5185-90.
26. Kawai K, Murakami S, Senba E, Yamanaka T, Fujiwara Y, Arimura C, Nozaki T, Takii M, Kubo C. Changes in estrogen receptors α and β expression in the brain of mice exposed prenatally to bisphenol A. Regulatory Toxicology and Pharmacology. 2007 Mar 1;47(2):166-70.
27. Nakagawa Y, Tayama S. Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. Archives of toxicology. 2000 Apr 1;74(2):99-105.
28. Babior BM. The respiratory burst oxidase. Adv Enzymol Relat Areas Mol Biol. 1992 Jan 1;65:49-95.
29. Bartsch H, Nair J. Ultrasensitive and specific detection methods for exocylic DNA adducts: markers for lipid peroxidation and oxidative stress. Toxicology. 2000 Nov 16;153(1-3):105-14.
30. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radical Biology and Medicine. 1990 Jan 1;8(6):583-99.
31. Kayanoki Y, Fujii J, Islam KN, Suzuki K, Kawata S, Matsuzawa Y, Taniguchi N. The protective role of glutathione peroxidase in apoptosis induced by reactive oxygen species. The Journal of Biochemistry. 1996 Apr 1;119(4):817-22.
32. Song L, Xia W, Zhou Z, Li Y, Lin Y, Wei J, Wei Z, Xu B, Shen J, Li W, Xu S. Low-level phenolic estrogen pollutants impair islets morphology and β-cells function in isolated rat islets. Journal of Endocrinology. 2012 Sep 3:JOE-12.
33. Huc L, Lemarié A, Guéraud F, Héliès-Toussaint C. Low concentrations of bisphenol A induce lipid accumulation mediated by the production of reactive oxygen species in the mitochondria of HepG2 cells. Toxicology in vitro. 2012 Aug 1;26(5):709-17.
34. Tachibana T, Wakimoto Y, Nakamuta N, Phichitraslip T, Wakitani S, Kusakabe K, Hondo E, Kiso Y. Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. Journal of Reproduction and Development. 2007;53(3):509-14.
35. Sajiki J, Yonekubo J. Leaching of bisphenol A (BPA) from polycarbonate plastic to water containing amino acids and its degradation by radical oxygen species. Chemosphere. 2004 May 1;55(6):861-7.
36. Sajiki J. Decomposition of bisphenol-A (BPA) by radical oxygen. Environment international. 2001 Oct 1;27(4):315-20.
37. Atkinson A, Roy D. In vitro conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite (s). Biochemical and biophysical research communications. 1995 May 16;210(2):424-33.
38. Papa S, Skulachev VP. Reactive oxygen species, mitochondria, apoptosis and aging. InDetection of Mitochondrial Diseases 1997 (pp. 305-319). Springer, Boston, MA.
39. Madathil KS, Karuppagounder SS, Haobam R, Varghese M, Rajamma U, Mohanakumar KP. Nitric oxide synthase inhibitors protect against rotenone-induced, oxidative stress mediated

parkinsonism in rats. Neurochemistry international. 2013 Apr 1;62(5):674-83.
40. Komada M, Asai Y, Morii M, Matsuki M, Sato M, Nagao T. Maternal bisphenol A oral dosing relates to the acceleration of neurogenesis in the developing neocortex of mouse fetuses. Toxicology. 2012 May 16;295(1-3):31-8.
41. Tiwari D, Kamble J, Chilgunde S, Patil P, Maru G, Kawle D, Bhartiya U, Joseph L, Vanage G. Clastogenic and mutagenic effects of bisphenol A: an endocrine disruptor. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2012 Mar 18;743(1):83-90.
42. Chitta KR, Figueroa JA, Caruso JA, Merino EJ. Selenium mediated arsenic toxicity modifies cytotoxicity, reactive oxygen species and phosphorylated proteins. Metallomics. 2013;5(6):673-85.
43. Tirmenstein MA, Reed DJ. Characterization of glutathione-dependent inhibition of lipid peroxidation of isolated rat liver nuclei. Archives of biochemistry and biophysics. 1988 Feb 15;261(1):1-1.
44. Cadenas E. Mitochondrial free radical production and cell signaling. Molecular aspects of medicine. 2004 Feb 1;25(1-2):17-26.
45. Moon MK, Kim MJ, Jung IK, Koo YD, Ann HY, Lee KJ, Kim SH, Yoon YC, Cho BJ, Park KS, Jang HC. Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. Journal of Korean medical science. 2012 Jun 1;27(6):644-52.
46. Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, AlOlayan EM. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxidative Medicine and Cellular Longevity. 2012 Jul 24;2012.
47. Turrens JF. Superoxide production by the mitochondrial respiratory chain. Bioscience reports. 1997 Feb 1;17(1):3-8.
48. Ott M, Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria, oxidative stress and cell death. Apoptosis. 2007 May 1;12(5):913-22.
49. Pigeolet E, Corbisier P, Houbion A, Lambert D, Michiels C, Raes M, Zachary MD, Remacle J. Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. Mechanisms of ageing and development. 1990 Feb 15;51(3):283-97.
50. Maclatchy DL, Vanderkraak GJ. The phytoestrogen β-sitosterol alters the reproductive endocrine status of goldfish. Toxicology and applied pharmacology. 1995 Oct 1;134(2):305-12.
51. Ashby J, Tinwell H. Uterotrophic activity of bisphenol A in the immature rat. Environmental health perspectives. 1998 Nov;106(11):719.
52. Fernandez MF, Rivas A, Pulgar R, Olea N. Human exposure to endocrine disrupting chemicals: the case of bisphenols. InEndocrine Disrupters 2001 (pp. 149-169). Springer, Dordrecht.
53. Wang Q, Zhao XF, Ji YL, Wang H, Liu P, Zhang C, Zhang Y, Xu DX. Mitochondrial signaling pathway is also involved in bisphenol A induced germ cell apoptosis in testes. Toxicology letters. 2010 Nov 30;199(2):129-35.
54. Toyama Y, Suzuki-Toyota F, Maekawa M, Ito C, Toshimori K. Adverse effects of bisphenol A to spermiogenesis in mice and rats. Archives of histology and cytology. 2004;67(4):373-81.
55. Toyama Y, Yuasa S. Effects of neonatal administration of 17β-estradiol, β-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis. Reproductive toxicology. 2004 Dec 1;19(2):181-8.
56. Andriana BB, Tay TW, Hiramatsu R, Awal MA, Kanai Y, Kurohmaru M, Hayashi Y. Bisphenol A-induced morphological alterations in Sertoli and spermatogenic cells of immature Shiba goats in vitro: An ultrastructural study. Reproductive medicine and biology. 2004 Dec;3(4):205-10.
57. Kashiwagi A, Utsumi K, Kashiwagi K, Ohta S, Sugihara K, Hanada H, Kitamura S. Effects of endocrine disrupting chemicals on amphibian metamorphosis and mitochondrial membrane permeability transition. Journal of health science. 2008;54(3):273-80.
58. Nakagawa Y, Tayama S. Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. Archives of toxicology. 2000 Apr 1;74(2):99-105.
59. Perkins GA, Scott R, Perez A, Ellisman MH, Johnson JE, Fox DA. Bcl-xL-mediated remodeling of rod and cone synaptic mitochondria after postnatal lead exposure: electron microscopy, tomography and oxygen consumption. Molecular vision. 2012;18:3029.
60. Arunachalam C, Doohan FM. Trichothecene toxicity in eukaryotes: Cellular and molecular mechanisms in plants and animals. Toxicology letters. 2013 Feb 27;217(2):149-58.