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Antioxidant role of zinc in PCB (Aroclor 1254) exposed ventral prostate of albino rats

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Abstract

The ability of zinc to retard oxidative processes has been recognized for many years. Polychlorinated biphenyls (PCBs) are persistent and bioaccumulative environmental toxicants. Previous study has indicated that PCBs can have deleterious effects, including oxidative stress, on various aspects of reproduction in male rats. The aim of this study was to determine the antioxidant role of zinc in PCB-exposed ventral prostate of albino rats. A group of 20 rats were treated with Aroclor 1254 (2 mg/kg body weight/day, i.p.) for 30 days. After the PCB treatment, 10 rats were treated as PCB control. The remaining 10 rats were given zinc (Zn SO_4) (200 mg/kg body weight/day, p.o.) for 10 days. Ventral prostatic enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were estimated in all the groups. Hydrogen peroxide (H_2O_2), lipid peroxidation (LPO) and ventral prostatic acid phosphatase (ACP) were also estimated. Serum hormonal profiles such as total tri-iodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH), testosterone, and estradiol were estimated. Ventral prostatic androgen and estrogen receptors, ventral prostatic zinc content, and serum zinc concentration were also quantified in all the groups. Antioxidant enzymes such as SOD, CAT, GPx, GST, and ACP were decreased while an increase in H_2O_2 and LPO were observed in PCB-treated animals. Decreased serum total T_3, T_4, testosterone, estradiol and increased TSH were observed in PCB-exposed rats. Ventral prostatic androgen and estrogen receptors were also decreased significantly in PCB-exposed rats. Zinc administration restored to previous levels all parameters except ventral prostatic ACP. These results suggest that PCB induces oxidative stress in rat ventral prostate by decreasing the levels of antioxidant enzymes; the effects could be reversed by the administration of zinc. The adverse effect of PCBs (Aroclor 1254) and zinc on ventral prostate might be due to indirect action through hormonal regulation.

Keywords: Oxidative stress; PCB (Aroclor 1254); Zinc; Serum hormones

1. Introduction

Many environmental contaminants increase the levels of reactive oxygen species (ROS) such as superoxide anions, hydroxy radicals, hydrogen peroxide, and hypochlorite. When the balance between ROS and antioxidant system is lost, oxidative stress results. Oxidative stress is the cytotoxic consequence of oxyradical and oxidant formation and the reaction with cellular constituents. The free radical $O_2^{-\cdot}$ is generated by multiple enzymatic and non enzymatic pathways and is often at the start of the oxidative stress cascade [1]. Antioxidant enzymes are involved in the defense system against free radical–mediated tissue or cellular damage. They metabolize either free radicals or reactive oxygen intermediates to nonradical products. These enzymes include a family of glutathione-dependent enzymes, superoxide dismutase (SOD), and catalase (CAT) [2]. Polychlorinated biphenyls (PCBs), a family of synthetic chlorinated organic compounds, are persistent environmental pollutants. Aroclor 1254 is a commercial mixture of polychlorinated biphenyls, which is defined as being 54% chlorine by weight [3]. They are used in transformers, capacitors, paints, pesticides, gas turbines, hydraulic systems, television, and air conditioners. They are absorbed through the skin, lungs, and GI tract [4]. PCBs are distributed throughout the entire ecosystem including soil, air, and water. Commercial PCB mixtures, such as Aroclor 1242 and 1254, as well as certain individual congeners cause hypothyroidism in animals exposed to these substances [5]. PCBs have also been shown to impair male fertility [6].

PCBs are endocrine-disrupting chemicals in that they can bind to hormone (usually estrogen or androgen) receptors to

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inactivate them. The ability of PCBs to affect the estrogen receptor is generally related to their degree of chlorination, with more lightly chlorinated PCBs acting in an estrogenic manner, and those PCBs with higher (>48%) chlorination acting as weak estrogens or as estrogen receptor antagonists [7]. In the neonatal brain, PCBs can affect levels of aromatase, the enzyme that is responsible for the conversion of testosterone to estradiol and that plays a critical role in the development of gender-appropriate sexual behavior [8]. We have observed thinning of the epithelial membrane in rat reproductive organs after PCB exposure [9]. We have also shown that administration of Aroclor 1254 decreased ventral prostatic acid phosphatase (ACP) and antioxidant enzymes [10]. Recent findings indicate that toxic manifestation induced by PCBs may be associated with the production of ROS [11].

Zinc is a homeostatically regulated essential mineral. It is a component of numerous metalloenzymes, and it is important for cell growth and replication, osteogenesis, and immunity [12]. Zinc has close interrelationships with endocrine system and is essential for reproductive and thyroid function. Zinc deficiency causes delayed sexual maturation, hypogonadism, and thyroid function [13,14]. Possible involvement of zinc in the oxidative defense system that protects cells from oxidant-mediated damage has been studied in different in vitro and in vivo models. Chronic zinc deficiency is associated with a condition of oxidative stress characterized by increased oxidation of lipids, proteins, and DNA, and by alterations in components, enzymes and substances of the oxidative defense system [15]. Zinc content serves as one of the biochemical markers for the function of prostate gland. Costello and Franklin [16] have proposed that zinc inhibits mitochondrial aconitase activity and citrate oxidation in prostate epithelial cells.

Thus, the present study was designed to determine whether zinc could reverse the PCB-mediated effects in the ventral prostatic antioxidant system.

2. Methods and materials

2.1. Animal experimentation

Healthy adult male albino rats of the Wistar strain *Rattus norvegicus,* 90 days of age and weighing 150–180 g, were used in the present study. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house with a constant 12-hour light, 12-hour dark schedule. Animals were maintained according to the guidelines of our institutional ethical committee (Ref. no. IAEC no. 03/005/02). All animals underwent deworming with albendazole (10 mg/kg body weight, p.o.) before initiation of the experiments. A group of 20 rats were treated with Aroclor 1254 (2 mg/kg body weight/day/i.p.) for 30 days. After PCB treatment, 10 rats were treated as PCB control. The remaining 10 rats were given zinc in the form of $ZnSO_4$ (200 mg/kg body weight/day/p.o.) for 10 days. Separate controls were also maintained. The dose levels and dosing period of PCB were selected according to our previous studies [10]. The dose of ZnSO4 was selected according to Salgueiro et al. [17]. Aroclor 1254 and chloramine-T were purchased from Sigma Chemical (St. Louis, MO). ZnSO4 was purchased from SISCO Research Laboratories (Mumbai, India) and RIA Kits were obtained from Diagnostics Products Corp. (Los Angeles, CA, 90045 USA). ^{3}H-testosterone and ^{3}H estradiol were purchased from Amersham Pharmacia Biotech Ltd. (London, UK).

2.2. Collection of blood and tissue

At 24 hours after the last treatment, rats were killed. Blood from the trunk was collected in clean, dry test tubes, allowed to clot at room temperature, and then centrifuged at $1500 \times g$ for 10 minutes. The clear serum was removed and used for zinc and hormone assay.

Ventral prostate was removed from the adhering connective tissue, washed in ice-cold physiological saline repeatedly, and accurately weighed. After thorough washing, ventral prostate at the known weight was blotted, wrapped in aluminum foil, and stored at −80°C for biochemical assays.

2.3. Preparation of tissue homogenate for biochemical assay

The ventral prostatic tissue was homogenized in 0.1 mol/L Tris–HCl buffer, pH 7.4, and used for determining the biochemical parameters described below.

Protein was estimated by the method of Lowry et al. [18]. Estimation of SOD [19], CAT [20], GPx [21], GST [22], ACP [23], LPO [24], and H_2O_2 generation assay [25] were performed.

2.4. Radioimmunoassay of hormones

2.3.1. Serum total tri-iodothyronine and thyroxine

Serum total tri-iodothyronine (T_3) and thyroxine (T_4) were assayed by solid phase technology by making use of a commercial kit obtained from DiaSorin (Italy). The results were expressed as μg/mL. Sensitivity of the assays was 30 pg/mL for total T_4 and 700 pg/mL for total T_3. Interassay coefficients of variation were as follows: Total T_4: 4.5–14.5%; total T_3: 5.7–10%; intra-assay coefficients of variation were as follows: total T_4: 2.7–3.8%, total T_3: 3.1–8.9%, respectively.

2.3.2. Serum TSH assay

For assay of serum TSH, NIDDK-rTSH was iodinated using the chloramine-T method with carrier free ^{125}I and used for radioimmunoassay (RIA) of serum TSH with specific rat antibody (NIDDK–anti-rTSH–s–s) and reference preparation (NIDDK-rTSH RP_2). Antirabbit gammaglobulin (ARGG) was used as the second antibody to precipitate

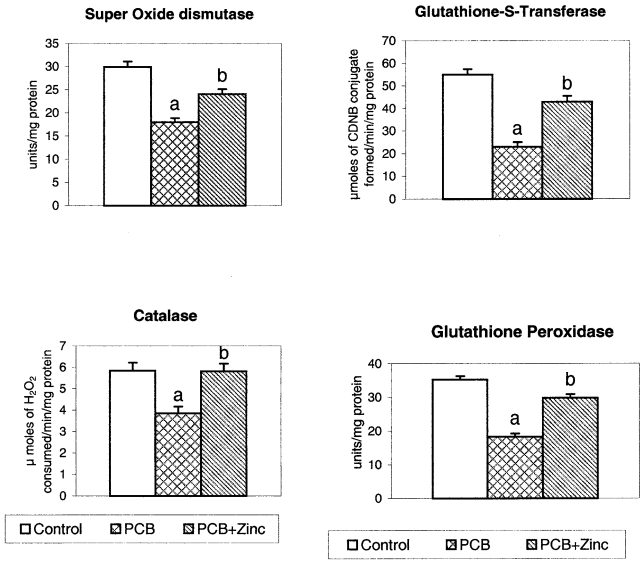

Fig. 1. Effect of zinc on ventral prostatic antioxidant enzyme activities in albino rats exposed to polychlorinated biphenyls (PCBs). Each bar represents the mean and vertical lines above denote the SEM of 10 animals. Letters a and b denote the statistical significance of the data at the level of $P < 0.05$: letter a, control animals vs. those treated with PCB; letter b, PCB control animals vs. PCB + zinc treated animals. Open bars represent control group; bars with large cross-hatching, PCB-exposed group; bars with small cross-hatching, PCB+Zinc group. CDNB:1 chloro 2,4 dinitrobenzene.

the antibody bound TSH. Radioactivity was counted on the Pharmacia liquid scintillation system, and the results were expressed as ng/mL. The sensitivity of the TSH assay was 0.01 ng/mL, and the intra- and interassays coefficients of variation were 5–8.2% and 4–6%, respectively. ^{125}I was purchased from the Board of Radiation and Isotope Technology (Mumbai, India). TSH antigen, antibodies, and references for rat TSH were obtained from the NIDDK, Pituitary Distribution Programme (Baltimore, MD).

2.3.3. Serum estradiol and testosterone

Serum estradiol and testosterone were assayed by solid phase radioimmunoassay, Coat-A-Count procedure, by using a commercial kit obtained from Diagnostic Products Corp. (USA). The results were expressed as pg/dL and ng/mL, respectively. The sensitivity of the estradiol assay was 0.08 pg/dL and the cross-reactivity of the antiserum was 0.1% with estrone, 0.32% with estriol, 0.017% with 17β-estradiol, 0.004% with DHT, and 0.001% with testosterone. The inter- and intra-assay variations were 6–8% and 4–6%, respectively. Sensitivity of the testosterone assay was 0.3 pg/mL, and cross-reactivity of the antiserum was 14% with 5α androstenediol and 0.001% with cortisol.

2.4. Androgen and estrogen receptor assay

The estrogen receptor and antrogen receptor binding assays were performed as described by Re et al. [26] and

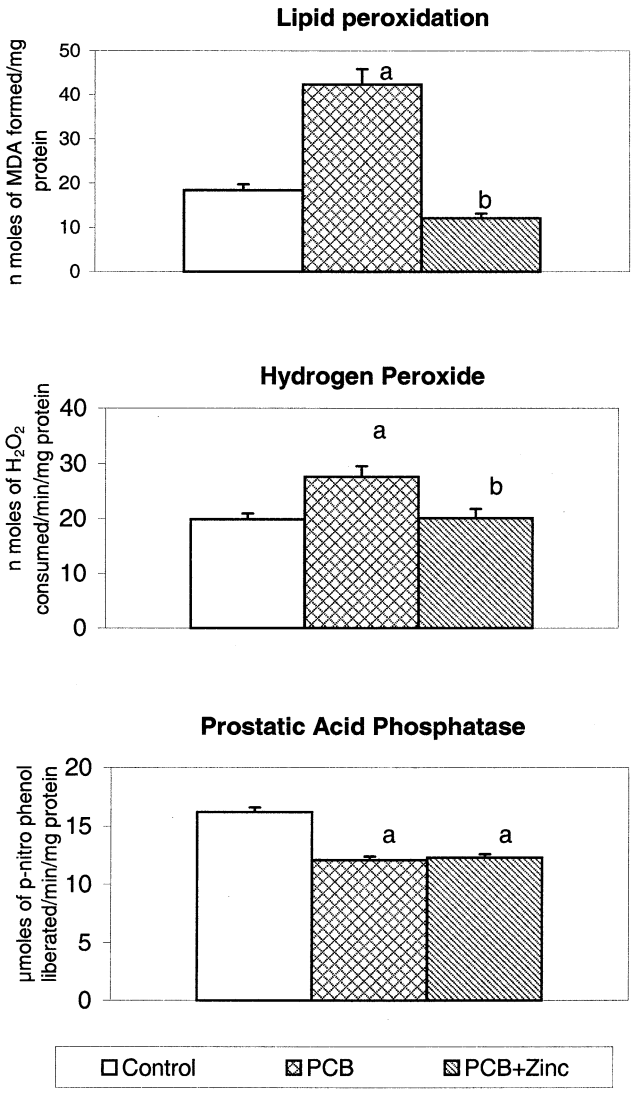

Fig. 2. Effect of zinc on ventral prostatic lipid peroxidation (LPO), hydrogen peroxide (H_2O_2), and acid phosphatase (ACP) activity in albino rats exposed to polychlorinated biphenyls (PCBs). Open bars represent control group; bars with large cross-hatching, PCB-exposed group; bars with small cross-hatching, PCB+Zinc group. MDA: malondialdehyde.

Leake and Habib [27], respectively, with minor modifications. Briefly, aliquots of cytosol (200 µL) were incubated overnight in triplicate at 4°C with 50 µL of increasing concentrations of (^3H) estradiol (0.1–2.8 nmol/L) or (^3H) testosterone (0.13–2.6 nmol/L). To estimate nonspecific binding, two parallel sets of tubes containing a 250-fold molar excess of nonlabeled diethylstilbesterol as an estrogen competitor and a 500-fold molar excess of nonlabeled testosterone as an androgen competitor were prepared. In the androgen receptor assay, to avoid possible binding of testosterone to glucocorticoid receptors, 100 nmol/L triamcinolone acetonide was added to each incubation tube. The cross binding of testosterone and estradiol with cortisol and progesterone receptors was blocked by the addition of triamcinolone acetonide to the assay vial. After absorption of free hormones on dextran-coated charcoal and precipitation,

Table 1
Effect of zinc on PCB-exposed rat serum hormonal profiles and ventral prostatic androgen and estrogen receptor concentrations

Parameter	Control	PCB	PCB+Zinc
Body weight (g)	171.00 ± 6.5	138.00 ± 5.2*	168.00 ± 6.3†
Ventral prostatic weight (g)	0.118 ± 0.008	0.089 ± 0.005*	0.135 ± 0.004†
Serum Total T_3 (μg/mL)	6.99 ± 0.32	4.00 ± 0.12*	5.88 ± 0.56†
Serum Total T_4 (μg/mL)	710.00 ± 25	590.00 ± 11*	690.00 ± 14†
Serum TSH (ng/ml)	11.00 ± 0.80	13.95 ± 0.60*	10.99 ± 0.51†
Serum testosterone (ng/mL)	4.90 ± 0.22	3.35 ± 0.16*	4.87 ± 0.24†
Serum Estradiol (pg/dL)	10.21 ± 0.38	8.05 ± 0.27*	11.31 ± 0.50†
Ventral prostatic androgen receptor (fmol/mg protein)	38.82 ± 2.02	29.11 ± 1.21*	41.90 ± 3.18†
Ventral prostatic estrogen receptor (fmol/mg protein)	20.03 ± 0.89	14.91 ± 0.81*	21.86 ± 1.98†

Values are mean ± SEM of 10 animals.
* Control vs PCB.
† PCB vs PCB+Zinc.

200 μL of the supernatant were added to 4 mL of scintillation liquid. Radioactivity in the bound fraction was counted on an LKB Wallac microprocessor–based liquid scintillation counter for a minute. Results were expressed as fmol/mg protein.

2.5. Estimation of zinc

Ventral prostatic zinc content and serum zinc concentration were estimated by atomic absorption spectroscopy [28]. The results were expressed as μg/g tissue for ventral prostatic zinc and μmol/L for serum zinc.

2.6. Statistical analysis

Data were statistically analyzed using analysis of variance. When the F ratio was statistically significant, the data were subjected to Student-Newman-Keuls test according to the method of Zar [29]. Values were considered significant at $P < 0.05$.

3. Results

The body weight and ventral prostatic weight were significantly decreased in PCB-exposed rats. Zinc supplementation recovered the PCB-mediated effect. The specific activities of SOD, CAT, GST, GPx, and ACP were decreased, whereas lipid peroxidation and H_2O_2 were increased in the ventral prostate of PCB-treated rats when compared to controls. Administration of zinc restored the antioxidant enzymes only and decreased LPO and H_2O_2 generation (Figs. 1 and 2). Increased TSH and decreased serum total T_3, T_4, testosterone and estradiol, and ventral prostatic androgen and estrogen receptor concentrations were observed after PCB exposure. Zinc treatment restored the effects to control levels (Table 1). Decreased ventral prostatic zinc content and serum zinc concentration were observed in PCB exposure; these concentrations increased after the administration of zinc (Fig. 3).

4. Discussion

PCBs are environmental contaminants that have been classified by the World Health Organization as moderately hazardous [30]. There is much concern that exposure to PCB causes reproductive toxicity in humans and animals [6]. The dose selected in the present study (2 mg/kg body weight) has been shown to cause dysfunction in the rat ventral prostate [10]. In the present study, ventral prostate weight and body weight were decreased significantly, which might be due to decreased bioavailability and production of androgens in the present experiment. Increased level of androgen after zinc supplementation could have enhanced the body and ventral prostatic weight. Vijaybabu et al. [31] reported that PCBs (Aroclor 1254) increased ROS in rat ventral prostate. Recent findings also suggest that PCBs causes decrease activity of antioxidant enzymes such as SOD, CAT, GPx, and GST and increases H_2O_2 generation and LPO levels [10]. Decreased CAT activity could be associated with the oxidative stress in seminal plasma [32]. CAT is the main scavenger of H_2O_2 at high concentrations [33]. CAT activity is also linked SOD activity. The decrease in SOD activity in animals exposed to high dose of metals may result in more accumulation of $O_2^{\cdot -}$, which has been shown to inhibit CAT [34]. Along with CAT, GPx is also

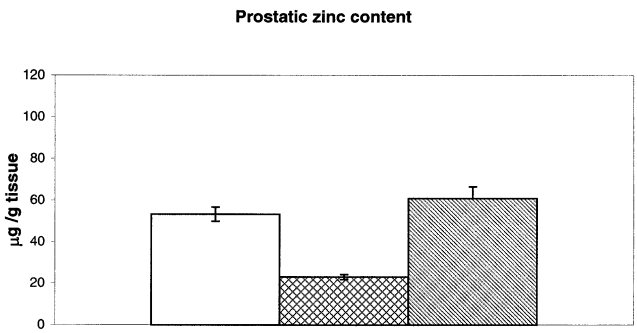

Fig. 3. Effect of zinc on ventral prostatic zinc content and serum zinc concentration in albino rats exposed to polychlorinated biphenyls (PCBs). Open bars represent control group; bars with large cross-hatching, PCB-exposed group; bars with small cross-hatching, PCB+Zinc group.

involved in the scavenging of H_2O_2. ROS induce tissue damage by initiating self-propagating LPO [35]. Therefore, the increase in prostatic LPO observed in the present study could be due to the concominant increase in the generation of free radicals such as H_2O_2 and OH^- in the prostate of PCB-treated rats. This may upset the pro-oxidant/antioxidant balance within the prostate, which could be one of the reasons for decreased ACP concentration.

In the present study, zinc supplementation effectively reversed the PCB induced toxicity in antioxidant system of ventral prostate. Zinc is an essential trace mineral that acts as an antioxidant by neutralizing free radical generation [36]. Zinc could exert a direct antioxidant action by occupying iron or copper binding sites of lipids, proteins, and DNA [37]. Zinc deficient male rats had higher levels of LPO (33% higher thibarbituric acid reactive substance [TBARS] content), protein oxidation, and decreased SOD activity leading to reduced testicular growth and oxidative stress in cadmium exposed rats [38]. Zinc administration has been shown to increase antioxidant metallothionein [36], which protected retinal LPO. Similar changes could have occurred in the ventral prostate of zinc treated animals.

PCBs are endocrine-disrupting chemicals that bind to androgen and estrogen receptors to inactivate them [7]. In the present study, decreased levels of androgen and estrogen receptor concentrations in animals exposed to Aroclor 1254 may also be due to the decreased levels of testosterone and estradiol. Previous studies in our laboratory have shown that Aroclor 1254 exposure decreases serum testosterone, estradiol, and thyroid hormones level [10]. Khan and Thomas [40] also suggest that PCBs significantly reduce the concentration of 5-hydroxy tryptophan by inhibiting hypothalamic tryptophan hydroxylase and GnRH content in the preoptic anterior hypothalamic area of rats. Administration of zinc restored serum testosterone and estradiol levels. Zinc is essential for testosterone secretion and spermatogenesis [12]. Zinc also induces the activity of 3β-OH steroid dehydrogenase, which is the key enzyme used for testosterone biosynthesis [41]. Hafiez et al. [42] also showed that zinc deficiency causes decreased levels of serum testosterone. In the present study an increase in testosterone and estradiol was observed after zinc supplementation. Increased levels of both hormones stimulate receptor concentrations after zinc supplementation by mechanisms of up-regulation and down-regulation. ACP is an excellent marker for androgen-dependent functions in the prostate. Thus its decrease in the PCB-exposed rats may suggest androgen deprivation in the ventral prostate [23]. The activity of ACP in Aroclor 1254–exposed rats is not restored after the zinc supplementation. Rosenkrantz [43] suggested that estradiol decreases the secretion of ACP in the prostate. In the present experiment, increased levels of estradiol in animals treated with zinc could not retrieve the ACP level.

The PCB-induced decrease in thyroid hormones may be due to multiple mechanisms, such as induction of uridine diphosphate glucuronyl transferases and binding to thyroid transport protein transthyretin [44]. Kodavanti et al. [45] also suggested that polychlorinated biphenyls and related chemicals have been associated with decreasing circulating thyroid hormones in adult animals. The decreased level of T_4 and T_3 increase TSH levels in the pituitary by feedback mechanism in PCB-exposed rats. Zinc supplementation restored levels of T_3, T_4, and TSH in PCB-exposed rats. Zinc deficiency was shown to impair the metabolism of thyroid hormones. Kralik et al. [14] reported that male Sprague-Dawley rats fed a low-zinc diet for 40 days showed decreased serum concentrations of T_3 and T_4 by approximately 30% compared with those fed diets adequate in zinc.

Srivastava et al. [46] proposed that androgens stimulate prostatic zinc content and serum zinc concentration in rhesus monkey. Thus, decreased levels the serum testosterone in PCB-exposed rats yields decreased level of prostatic zinc content and serum zinc concentration. Sridhar et al. [10] shows ventral prostatic weight was decreased in hypothyroid rats. In the present study also, PCB exposure induced hypothyroidism, which leads to decreased ventral prostatic zinc content along with serum testosterone and estradiol. Decreased levels of testosterone and thyroid hormones were reversed after the administration of zinc and they stimulate the ventral prostatic zinc content and serum zinc concentration.

In conclusion, Aroclor 1254 (PCBs) alters ventral prostatic function by inducing oxidative stress, which could be reversed by administration of zinc.

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References

[1] Allen RG. Oxygen reactive species and antioxidant responses during development: the metabolic paradox of cellular differentiation. Proc Soc Exp Biol Med 1991;196:117–29.
[2] Chaudiere J, Ferrari-Illiou R. Intracellular antioxidants: from chemical to biochemical mechanisms. Food Chem Toxicol 1999;37:949–62.
[3] ASTDR. Selected PCBs (Aroclor 1260-1254-1248-1232-1221 and 1061). Washington, DC: Agency for Toxic Substances and Disease Registry. US Public Health Service 1987;1:45–63.
[4] Baird L. Toxic organic chemicals. In: Baird C, editor. Environmental Chemistry. New York: WH Freeman and company, 1995. pp 252–74.
[5] Li MH, Zhao YD, Hansen LG. Multiple dose toxicokinetic influence on the estrogenicity of 2,2,4,45,5′-hexachlorobiphenyl. Bull Environ Contam Toxicol 1994;53:583–90.
[6] Chitra KC, Latchomycandane, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology 2003;185:119–27.
[7] Kester MHA, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, Coughtrie MW, Bergman A, Safe SH, Kuiper GG, Schuur AG,

Brouwer A, Visser TJ. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 2000;141:1897–1900.
[8] Hany L, Lilenthal H, Sarasin A, Roth-Harer A, Fastabend A, Dunemann L, Lichtensteiger W, Winneke G. Developmental exposure of rats to a reconstituted PCB mixture or Aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol Appl Pharmacol 1999;158:231–43.
[9] Anbalagan J, Kanagaraj P, Srinivasan N, Aruldhas MM, Arunakaran J. Effect of polychlorinated biphenyl, Aroclor 1254 on rat epididymis. Indian J Med Res 2003;118:236–42.
[10] Sridhar M, Venkataraman P, Dhanammal S, Arunkumar A, Aruldhas MM, Srinivasan N, Arunakaran J. Impact of polychlorinated biphenyl (Aroclor 1254) and vitamin C on antioxidant system of rat ventral prostate. Asian J Androl 2004;6:19–22.
[11] Tharappel JC, Lee EY, Robertson LW, Spear BT, Glanet HP. Regulation of cell proliferation, apoptosis and transcriptional activities during the promotion of liver carcinogenesis by PCB. Toxicol Appl Pharmacol 2002;179:172–84.
[12] Kaji M. Zinc in Endocrinology [Review]. Int Pediatr 2001;16:1–7.
[13] Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev 1993;73:79–118.
[14] Kralik A, Eder K, Kirchgessner M. Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. Horm Metab Res 1996;28:223–6.
[15] Oteiza PI, Clegg MS, Zago MP, Keen CL. Zinc deficiency induces oxidative stress and AP-activation in 3T3 cells. Free Rad Biol Med 2000;28:1091–9.
[16] Costello IC, Franklin RB, Liu Y. Zinc causes a shift toward citrate at equilibrium of the m-aconitase reaction of prostate mitochondria. J Inorg Biochem 2000;161–5.
[17] Salguiero MJ, Zubilliga MB, Lysionek AE, Sarabia MI, Caro RA, De paoli T, Hager A, Ettlin E, Weill R, Boccio JR. Bio availability, bio distribution, and toxicity of bioZn-AAS(1): a new zinc source. Comparative studies in rats. Nutrition 2000;16:762–6.
[18] Lowry OH, Risebrough NJ, Farr, Randall RJ. Protein measurement with the folin-phenol reagent. J Biol Chem 1951;193:265–70.
[19] Marklund S, Marklund G. Involvement of superoxide anion in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469–74.
[20] Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47: 389–94.
[21] Rotruck JT, Pope AL, Ganther HE, Saunson AB, Hafeman G, Hoekstraw WG. Selenium; biochemical role as a component of glutathione peroxidase. Science 1973;179:588–90.
[22] Habig WH, Pabst MJ, Jacoby WB. Glutathione-S-transferase; the first step in mercapturic fermentation. J Biochem 1973;24:7130–9.
[23] Tenniswood M, Bird CE, Clark AF. Acid phosphatases: androgen dependent markers of rat prostate. Can J Biochem 1976;54:350–7.
[24] Devasagayam TP, Tarachand U. Decreased lipid peroxidation in rat kidneys during gestation. Biochem Biophys Res Comm 1987;145: 134–8.
[25] Pick E, Keisari Y. Superoxide anion and H_2O_2 production by chemically elicited peritoneal macrophages induced by multiple nonphagocytic stimuli. Cell Immunol 1981;59:301–18.
[26] Re G, Badino P, Dacasto M, Di Carlo F, Girardi C. Regulation of uterine estrogen receptors by β adrenergic stimulation in immature rats. J Vet Pharmacol Ther 1993;16:328–34.
[27] Leake RE, Habib F. Androgen receptor assay. In: Green B, Leake RE, editors. Steroid Hormones: a Practical Approach. Oxford: IRL Press, 1987. pp. 76–82.
[28] Wong YC, Wang YZ, Lee JS, Tam NN. Changes in serum and tissue zinc levels in the sex hormone induced prostatic carcinogenesis in the noble rat. Tumour Biol 2000;21:328–36.
[29] Zar JH. Biostatistical Analysis. Englewood Cliffs, NJ: Prentice-Hall, 1974.
[30] WHO report on international programme on chemical safety. In: Dobson S, Van Esch GJ, editors. Environmental Health Criteria—Polychlorinated Biphenyls and Terphenyls. Geneva: World Health Organization, 1993.
[31] Vijaybabu MR, Arunkumar A, Srinivasan N, Aruldhas MM, Arunakaran J. Role of quercetin in Aroclor 1254 induced oxidative damage in prostate of male albino rats. Proceedings of the XX Symposium on Reproductive Biology and Comparative Endocrinology 2002; Bharathidasan University, Tiruchirapplai, 111–2.
[32] Jeulin C, Soutir JC, Weber P, Laval-Martin D, Calavayrac R. Catalase activity in human spermatozoa and seminal plasma. Gamete Res 1989;24:185–96.
[33] Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;74:139–62.
[34] Kono Y, Fridorich I. Superoxide radical inhibits catalase. J Biol Chem 1982;257:5751–4.
[35] Mylonas C, Kouretas D. Lipid peroxidation and tissue damage. In Vivo 1999;13:209–85.
[36] Powell SR. Antioxidant properties of zinc. J Nutr 2000;130:1447s–54s.
[37] Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic Biol Med 1990;8:281–91.
[38] Oteiza PI, Adonaylo VN, Keen CL. Cadmium-induced testes oxidative damage in rats can be influenced by dietary zinc intake. Toxicology 1999;137:13–22.
[39] Miceli MV, Tate DJ, Alcock NW, Newsome DA. Zinc deficiency and oxidative stress in the retina of pigmented rats. Invest Ophthalmol Vis Sci 1999;40:1238–44.
[40] Khan A, Thomas P. Disruption of neuroendocrine control of luteinizing hormone secretion by Aroclor 1254 involves inhibition of hypothalamic tryptophan hydroxylase activity. Biol Reprod 2001;64: 955–64.
[41] Mansour MM, Hafiez AA, el-Kirdassy ZH, el-Malkh MN, Halawa FA, el-Zayat EM. Role of zinc in regulating the testicular function. Part 2. Effect of dietary zinc deficiency on gonadotrophins, prolactin and testosterone levels as well as 3β-OH steroid dehydrogenase activity in testes of male albino rats. Nahrung 1989;33:941–7.
[42] Hafiez AA, el-Kirdassy ZH, Mansour MM, Sharada HM, el-Zayat EM. Role of zinc in regulating the testicular function. Part 1. Effect of dietary zinc deficiency on gonadotrophins, prolactin and testosterone in male albino rats. Nahrung 1989;33:935–40.
[43] Rosenkrantz H. Physiology of prostatic phosphohydrolase secretion. Ann NY Acad Sci 1969;166:466–81.
[44] Cheek OA, Kow K, Chen J, Lachlan JA. Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptors, transthyretin and thyroid binding globulin. Environ Health Perspect 1999;107:273–8.
[45] Kodavanti PRS, Derr-Yelin EC, Mundin WR, Shafer TJ, Herr DW, Barone S, Choksi NY, Macphail RC, Tilson HA. Repeated exposure of adult rats to Aroclor 1254 causes brain region-specific changes in intracellular Ca^{2+} buffering and protein kinase C activity in the absence of changes in tyrosine hydroxylase. Toxicol Appl Pharmacol 1998;153:186–98.
[46] Srivastava A, Chowdhury AR, Setty BS. Zinc content in the epididymis, vas deferens, prostate and seminal vesicles of juvenile rhesus monkey (*Macca mulata*); effect of androgen and estrogen. Prostate 1984;5:153–8.