

Modulatory Effects of Lycopene and Ellagic Acid on Reproductive Dysfunction Induced by Polychlorinated Biphenyl (Aroclor 1254) in Male Rats

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(Received 27 August 2009; Accepted 18 October 2009)

Abstract: The present study was conducted to investigate the possible protective effects of lycopene (LP) and ellagic acid (EA) on aroclor (AR) 1254-induced testicular and spermatozoal toxicity associated with the oxidative stress and apoptosis in male rats. The control group was treated with placebo. LP (10 mg/kg/every other day), EA (2 mg/kg/every other day) and AR (2 mg/kg/day) groups were given alone LP, EA and AR respectively. One of the last two groups received AR + LP, and the other treated with AR + EA. Body and reproductive organ weights, epididymal sperm characteristics, testicular tissue lipid peroxidation levels, antioxidant enzyme activities, histopathological changes and apoptosis via Bax and Bcl-2 genes were investigated. AR administration caused statistically significant decreases in body-weight, epididymal sperm concentration, testicular superoxide dismutase activity, diameters of seminiferous tubules, germinal cell layer thickness and Johnsen's testicular score, and increases in relative weights of testis, epididymis and seminal vesicles, rates of abnormal sperm and apoptotic cell expression along with degeneration, desquamation and disorganization in spermatogenic cells, and interstitial oedema and congestion in testicular tissue. LP and EA treatments to AR-treated rats markedly decreased abnormal sperm rates, testicular thiobarbituric acid reactive substances level, and increased the glutathione (GSH) level, GSH-peroxidase, catalase activities and epididymal sperm concentration as compared with the alone AR group. Additionally, the AR-induced histopathological damages were totally or partially recovered by LP or EA administrations respectively. AR damages the testicular tissue and spermatozoa by impairing the oxidant/antioxidant balance and by increasing the apoptotic spermatogenic cell rates. However, both LP and EA have modulator effects on AR-induced reproductive dysfunction in male rats.

With the rapid development of industry and agriculture, environmental pollutants have drawn more and more concerns because of their potential health impacts on human beings and animals [1]. Among those, polychlorinated biphenyls are a group of widely dispersed environmental pollutants that disrupt normal endocrine functions in human beings and animals. Polychlorinated biphenyls are distributed throughout the entire ecosystem including soil, air and water. They are used in transformers and capacitors, in pesticides and additive in paints, copying paper, adhesives, sealants and plastics. Polychlorinated biphenyls are lipophilic and poorly metabolized and are absorbed through the skin, lungs and gastrointestinal tract, and are transported by blood to liver, muscles, adipose tissue, testes and other organs as well as to plasma membranes [2]. Main exposure in human beings is through consumption of meat, fish and dairy products. Infants are exposed through breastfeeding [3]. Indeed, polychlorinated biphenyls are still regarded as a major global

environmental problem, although most industrialized countries have strictly prohibited their use [1].

Aroclor (AR) 1254, a commercial mixture of polychlorinated biphenyls, has many adverse effects on male reproduction in human beings and animals. It has been reported that exposure to AR or other polychlorinated biphenyl congeners may result in decreased gonadotropin [follicle stimulating hormone and luteinizing hormone (LH)] and steroid (testosterone and oestradiol) hormone levels [4], disrupted Sertoli cell metabolic function [5], diminished Leydig cell LH receptor density and steroidogenic enzyme activity [6], reduced body, testis, epididymis [7] and accessory glands weights [8], reduced sperm count, sperm motility, increased abnormal sperm rate [7,9], degeneration in testicular histology [10], increased per cent of sperm DNA damage [11,12], altered testicular apoptosis-related *Fas*, *Bax*, *Bcl-2* and *p53* genes expression [13], increased proportion of Y-chromosome-bearing sperm [14], damaged spermatogenesis and spermatogenic cells [15].

The critical underlying mechanism of AR-mediated reproductive dysfunction in males is fully unknown; however, numerous studies have shown that AR can bind to hormone (usually oestrogen or androgen) receptors to inactivate them

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[4,6], and activate aryl hydrocarbon receptor [16], and also disrupt the redox balance of tissues, suggesting that biochemical and physiological disturbances may result from oxidative stress [1,4,9]. Free radicals are normally generated in subcellular compartments of testis, particularly mitochondria, which are subsequently scavenged by antioxidant defence systems of the corresponding cellular compartments [17]. However, this balance can be easily broken by chemicals, which disrupt the pro-oxidant–antioxidant balance, leading to cellular dysfunction [18]. Additionally, the mitochondrial membrane of spermatozoa is more susceptible to lipid peroxidation, as this compartment is rich in polyunsaturated fatty acids and has been shown to contain low amounts of antioxidants [19,20].

Recently, there is growing interest in understanding the role and mechanism of the carotenoids and phytochemicals as inhibitors of oxidative stress. Lycopene (LP) is the most abundant carotenoids in tomatoes with concentrations ranging from 0.9 to 4.2 mg/100 g depending on the variety. As a result of its extended system of conjugated double bonds, LP can quench singlet oxygen ($^1\text{O}_2$) and other free radicals and has been reported to be the most effective $^1\text{O}_2$ quencher among approximately 600 naturally occurring carotenoids. LP can function as an antioxidant against lipid peroxidation by several mechanisms, and one of the best documented mechanisms is through the quenching $^1\text{O}_2$ [21]. Phenolic phytochemicals such as ellagic acid (EA) are important components of fruits and vegetables and are partly responsible for their beneficial health effects against oxidation-linked chronic diseases such as cancer and cardiovascular diseases. It is believed that EA functions either by countering the negative effects of oxidative stress by directly acting as an antioxidant or by activating/inducing cellular antioxidant enzyme systems [22]. It contains four hydroxyl groups and two lactone groups in which the hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage [23]. In the light of the above information, the present study was designed to investigate whether LP or EA has a possible protective effect against AR-induced negative changes in epididymal sperm characteristics and testicular tissue associated with the oxidative stress and apoptosis in rats.

Materials and Methods

Chemicals. Aroclor was purchased from AccuStandard® (New Haven, CT, USA). LP 10% FS (Redivivo TM, Code 7803) was purchased from DSM Nutritional Products (İstanbul, Turkey). EA was purchased from Fluka (Steinheim, Germany) and the other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and experimental design. Forty-eight healthy adult male Sprague-Dawley rats (8 weeks old) were used in this study. The animals were received from Firat University, Experimental Research Centre (Elazığ, Turkey) and were housed under standard laboratory conditions (temperature $24 \pm 3^\circ\text{C}$, humidity 40–60%, a 12-hr light: dark cycle). A commercial pellet diet (Elazığ Food Company, Elazığ, Turkey) and fresh drinking water were given *ad libitum*. The protocol

for the animal use was approved by the Institutional Review Board of the National Institute of Health and Local Committee on Animal Research.

Aroclor was intraperitoneally given to the animals at the dose of 2 mg/kg/day. LP was suspended in corn oil and administered to the animals by gavage at the dose of 10 mg/kg/every other day. EA is hardly dissolved under natural condition. Therefore, it was dissolved in alkaline solution (0.01 N NaOH; approximately pH 12). The final solution (pH = 8) after addition of EA was administered to the animals by gavage at the dose of 2 mg/kg/every other day. The doses of AR [9], LP [24] and EA [25] used in this study were selected on the basis of the previous studies. All treatments were maintained for 8 weeks. As a result of the rats need a period of 48–52 days for the exact spermatogenic cycle including spermatocytogenesis, meiosis and spermiogenesis [26], the administration period was selected as 8 weeks. The animals were randomly divided into six experimental groups of 8 rats in each. These groups were arranged as follows:

Group 1 – Control: treated with placebo – received 0.5 ml/rat slightly alkaline solution + 0.5 ml/rat corn oil every other day.

Group 2 – LP: treated with 0.5 ml/rat slightly alkaline solution + 0.5 ml/rat LP.

Group 3 – EA: received 0.5 ml/rat corn oil + 0.5 ml/rat EA.

Group 4 – AR: received 0.5 ml/rat AR + a mixture of slightly alkaline solution and corn oil (0.5 ml/rat).

Group 5 – AR + LP: treated with 0.5 ml/rat AR + 0.5 ml/rat LP.

Group 6 – AR + EA: treated with 0.5 ml/rat aroclor + 0.5 ml/rat EA.

Sample collection and homogenate preparation. The rats were killed under slight ether anaesthesia at the end of 8 weeks. Testes, epididymides, seminal vesicles and ventral prostate were removed, cleared of adhering connective tissue and weighed. Blood samples were collected from V. cava via sterile injector containing heparin and centrifuged at $3000 \times g$ for 10 min. to obtain plasma. One of the testes was fixed in 10% neutral-formalin solution for histopathological and immunohistochemical examinations. The other testis and plasma samples were also stored at -20°C until biochemical analyses. Testis tissues were taken from deep-freezer and weighed and thereafter they were immediately transferred to the cold glass tubes. Then, the tissues were diluted with a nine times volume of phosphate buffer (pH 7.4). For the enzymatic analyses, testicular tissues were minced in a glass and homogenized by a teflon–glass homogenizator for 3 min. in cold physiological saline on ice.

Sperm analyses. Epididymal sperm concentration. The epididymal sperm concentration was determined with a haemocytometer using a modified method [24,25]. The right epididymis was finely minced by anatomical scissors within 1 ml of isotonic saline in a Petri dish. It was completely squashed by a tweezers for 2 min., and then allowed to incubate at room temperature for 4 hr to provide the migration of all spermatozoa from epididymal tissue to fluid. After incubation, the epididymal tissue–fluid mixture was filtered via strainer to separate the supernatant from tissue particles. The supernatant fluid containing all epididymal spermatozoa was drawn into the capillary tube up to 0.5 lines of the pipette designed for counting red blood cells. The solution containing 0.595 M sodium bicarbonate, 1% formalin and 0.025% eosin was pulled into the bulb up to 101 lines of the pipette. This provided a dilution rate of 1:200 in this solution. Approximately 10 μl of the diluted sperm suspension was transferred to both counting chamber of Improved Neubauer (Deep 1/10 mm; LABART, Darmstadt, Germany) and allowed to stand for 5 min. The spermatozoa in both chambers were counted with the help of light microscope at $200\times$ magnification.

Sperm motility. Freshly isolated left epididymal tissue was used for the analysis of sperm motility. The per cent of sperm motility was evaluated using a light microscope with heated stage [27]. For this

process, a slide was placed on a light microscope with a heated stage warmed up to 37°C, and then several droplets of Tris buffer solution [0.3 M Tris (hydroxymethyl) aminomethane, 0.027 M glucose and 0.1 M citric acid] were dropped on the slide and a very small droplet of fluid collected from left cauda epididymis with a pipette was added to the Tris buffer solution and mixed by a cover-slip. The per cent of sperm motility was evaluated visually at 400× magnification. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score.

Sperm morphology. To determine the per cent of morphologically abnormal spermatozoa, the slides stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400× magnification. A total of 300 spermatozoa were examined on each slide (2400 cells in each group), and the head, tail and total abnormality rates of spermatozoa were expressed as per cent [24,25].

Biochemical analyses. Lipid peroxidation. The testicular tissue lipid peroxidation levels were measured according to the concentration of thiobarbituric acid reactive substances (TBARS) [28]. Briefly, one volume of the test sample and two volumes of stock reagent (15%, w/v trichloroacetic acid in 0.25 N HCl and 0.375%, w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated in boiling water for 15 min. After cooling, the precipitate was removed by centrifugation at $1500 \times g$ for 10 min., and then absorbance of the supernatant was read at 532 nm against a blank containing all reagents except test sample on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan). The TBARS level was expressed as nmol/ml.

Glutathione. The samples were precipitated with 50% trichloroacetic acid, and then centrifuged at $1000 \times g$ for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris–EDTA buffer (0.2 M; pH 8.9) and 0.1 ml of 0.01 M 5,5'-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min., and then read at 412 nm on the spectrophotometer. The level of glutathione (GSH) was expressed as nmol/ml [29].

Glutathione peroxidase and protein concentration. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN_3), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 IU/ml oxidized GSH (GSSG)-reductase, 1 mM GSH and 0.25 mM H_2O_2 . Enzyme source (0.1 ml) was added to 0.8 ml of the above mixture and incubated at 25°C for 5 min. before initiation of the reaction with the addition of 0.1 ml of peroxide solution. The absorbance at 340 nm was recorded for 5 min. on a spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value [30]. The protein concentration was also measured [31]. The GSH-peroxidase activity was expressed as IU/g protein.

Catalase. The testicular tissue catalase (CAT) activity was determined by measuring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm and was expressed as $k\text{U/g}$ protein, where k is the first-order rate constant [32].

Superoxide dismutase. The testicular tissue superoxide dismutase (SOD) activity was measured using xanthine and xanthine oxidases to generate superoxide radicals which react with nitroblue tetrazolium [33]. Briefly, each sample was diluted 1:10 with phosphate buffer (50 mM, pH 7.5). The assay solution containing sodium-carbonate buffer (50 mM, pH 10), 0.1 mM xanthine, 0.025 mM nitroblue tetrazolium, 0.1 mM EDTA, xanthine oxidase (0.1 U/ml in ammonium sulphate 2 M) and sample were mixed in a cuvette. One unit of SOD activity was defined as the amount of enzyme

required to cause inhibition of nitroblue tetrazolium. SOD activity was then read at 560 nm by the degree of inhibition of this reaction on a spectrophotometer and expressed as U/ml.

Testosterone. The plasma testosterone level was measured by ELISA method using DRG Elisa testosterone kit (ELISA EIA-1559, 96 Wells kit; DRG Instruments, GmbH, Marburg, Germany) according to the kit manufacturer's instructions and expressed as ng/dl.

Histopathology and immunohistochemistry. Blind evaluator assessed the histopathological and immunohistochemical evaluations. The testicular tissues were fixed in 10% neutral-formalin, embedded in paraffin, sectioned at 5 μm and were stained with haematoxylin and eosin [34]. Light microscopy was used to measure diameters of seminiferous tubules and germinal cell layer thicknesses and to evaluate the damages in testicular tissue. Johnsen's testicular score [35] was performed for control and treatment groups. All cross-sectioned tubules were evaluated systematically, and a score between 1 (very poor) and 10 (excellent) was given to each tubule according to Johnsen's criteria. Twenty-five tubules were evaluated for each animal.

Avidin–Biotin–Peroxidase method was used for the immunohistochemical analyses [36]. Testis tissues, which were embedded in paraffin and sectioned at 4 μm , were deparaffinized with xylene and dehydrated with alcohol series. Testicular sections were incubated in 0.01 M Na-citrate for 20 min. to bring into the open the antigenic receptors. They were washed with phosphate buffer solution and were then incubated in 3% H_2O_2 , which was prepared with phosphate buffer solution for 10 min. to inactivate endogenous peroxidase activity. Non-specific bindings were blocked by incubation with 1% untreated goat serum for 1 hr. After that, testicular tissues were incubated with primary rabbit polyclonal antibodies directed against Bax (proapoptotic protein) and Bcl-2 (antiapoptotic protein) at dilutions 1:200 and 1:400, respectively, in phosphate buffer solution containing 0.1% goat serum at 37°C for 1 hr. Testicular sections were washed again in phosphate buffer solution and were incubated with biotinylated secondary antibodies, which were diluted at the rate of 1:1000 in phosphate buffer solution containing 0.1% goat serum (secondary biotinylated goat anti-rabbit IgG), for 30 min. and thereafter tissues were washed with phosphate buffer solution and were incubated with avidin-conjugated horseradish peroxidase for 1 hr. 3-Amino-9-ethylcarbazole was used as colour-determining substrate. The reaction was stopped when colour change occurred after addition of this solution to the testicular tissues. At the last stage, testicular tissues were washed with tap water for 2 min. after they were stained with Mayer's haematoxylin for 15 sec. Stained tissues were covered with immune-mount and then Bax- and Bcl-2 positive spermatogenic cells (from spermatogonia to elongated spermatid) were evaluated under light microscope and scored as follows [37]:

Score 0: negative stained cells.

Score 1: <25% positive stained cells.

Score 2: 26–50% positive stained cells.

Score 3: 51–75% positive stained cells.

Score 4: >75% positive stained cells.

Statistical analysis. All values were presented as mean $\pm$ S.E.M. Differences were considered to be significant at $p < 0.05$. One-way ANOVA and *post hoc* Tukey-high significant difference (HSD) test were used to determine differences between groups. The SPSS/PC Program (Version 10.0; SPSS, Chicago, IL, USA) was used for the statistical analysis.

Results

Body and reproductive organ weights.

The mean values of body, absolute and relative reproductive organ weights at the end of the administration period are

shown in table 1. Alone LP or EA treatments had no significant effects on body-weight in comparison with the control group. While alone AR administration caused a statistically significant ($p < 0.001$) decrease in body-weight as compared with the control group, LP or EA administrations to AR-treated rats could not increase the decreased body-weight when compared with the alone AR group. There were no statistically significant differences among any of the groups in terms of absolute organ weights. However, the relative weights of testis, epididymis and seminal vesicles increased significantly ($p < 0.001$) after AR administration. Both LP and EA administration to AR-treated animals had no significant effects on relative organ weights in comparison with the alone AR group.

Epididymal sperm characteristics.

Epididymal sperm characteristics are shown in table 2. While alone LP and EA treatments did not affect all sperm parameters, only AR administration caused statistically significant decreases in sperm concentration ($p < 0.01$), and increases in tail and total abnormality of sperm ($p < 0.01$) as compared with the control group. A marked increase in AR + EA group was observed in sperm concentration ($p < 0.01$), and decrease in tail abnormality of sperm ($p < 0.01$) compared

with the alone AR group. Increments in sperm concentration and reductions in tail abnormality of sperm in AR + LP group were not significant when compared with the alone AR group. Similarly, although the values of total abnormality were brought near values to control by LP or EA administrations to AR-treated rats, these administrations could not increase significantly this sperm parameter when compared with the alone AR group. Simultaneous or alone administrations of AR, LP and EA had no significant effect on sperm motility and head abnormality parameters in comparison with the control group.

Biochemical parameters.

Testicular tissue lipid peroxidation levels, antioxidant enzyme activities and plasma testosterone levels are shown in table 3. While alone AR administration caused numerical but not statistical increase in TBARS levels when compared with the control group, both LP and EA administrations to AR-treated rats significantly ($p < 0.05$) reduced the increased TBARS levels in comparison with the only AR group. Although alone AR treatment did not affect the GSH level when compared with the control group, EA administration along with AR provided a statistically significant ($p < 0.05$) increase in GSH level as compared with the AR and the

Table 1.

Mean $\pm$ S.E.M. values of body, absolute and relative reproductive organ weights (LC, lycopene; EA, ellagic acid; AR, aroclor).

Groups	Body-weight (g)	Reproductive organs							
		Absolute weight (mg)				Relative weight (mg/g body-weight)			
		Testis	Epididymis	Seminal vesicles	Ventral prostate	Testis	Epididymis	Seminal vesicles	Ventral prostate
Control	275.6 $\pm$ 17.2 ^a	1345.0 $\pm$ 25.7	454.2 $\pm$ 9.5	901.7 $\pm$ 32.2	455.0 $\pm$ 26.1	4.99 $\pm$ 0.33 ^a	1.66 $\pm$ 0.12 ^a	3.42 $\pm$ 0.27 ^a	1.72 $\pm$ 0.14
LC	263.3 $\pm$ 8.4 ^a	1353.3 $\pm$ 119.8	473.7 $\pm$ 7.2	918.3 $\pm$ 64.7	463.3 $\pm$ 11.2	5.10 $\pm$ 0.11 ^a	1.80 $\pm$ 0.07 ^{ab}	3.75 $\pm$ 0.42 ^{ab}	1.74 $\pm$ 0.06
EA	263.5 $\pm$ 4.8 ^a	1348.3 $\pm$ 93.3	456.0 $\pm$ 6.1	985.0 $\pm$ 47.5	483.3 $\pm$ 10.2	5.12 $\pm$ 0.08 ^a	1.73 $\pm$ 0.02 ^{ab}	3.76 $\pm$ 0.23 ^{ab}	1.84 $\pm$ 0.03
AR	200.8 $\pm$ 4.3 ^{bc}	1290.0 $\pm$ 24.1	440.0 $\pm$ 14.1	1050.0 $\pm$ 78.3	414.0 $\pm$ 33.1	6.44 $\pm$ 0.20 ^b	2.19 $\pm$ 0.07 ^{cd}	5.24 $\pm$ 0.40 ^{bc}	2.07 $\pm$ 0.19
AR + LC	179.4 $\pm$ 4.6 ^b	1292.0 $\pm$ 45.4	446.0 $\pm$ 10.8	996.0 $\pm$ 121.8	410.0 $\pm$ 27.2	7.20 $\pm$ 0.20 ^b	2.49 $\pm$ 0.09 ^d	5.53 $\pm$ 0.61 ^c	2.29 $\pm$ 0.17
AR + EA	220.3 $\pm$ 6.9 ^c	1371.7 $\pm$ 36.5	458.3 $\pm$ 14.0	1028.3 $\pm$ 59.5	428.3 $\pm$ 38.2	6.26 $\pm$ 0.30 ^b	2.09 $\pm$ 0.10 ^{bc}	4.70 $\pm$ 0.34 ^{abc}	1.95 $\pm$ 0.19

The mean differences between the values bearing different superscript letters within the same column are statistically significant (a, b, c and d; $p < 0.001$).

Table 2.

Mean $\pm$ S.E.M. values of sperm parameters (LC, lycopene; EA, ellagic acid; AR, aroclor).

Groups	Parameters				
	Sperm motility (%)	Epididymal sperm concentration (million/g tissue)	Abnormal sperm rate (%)		
			Head	Tail	Total
Control	77.77 $\pm$ 2.94	347.5 $\pm$ 11.5 ^{ab}	2.28 $\pm$ 0.31	3.78 $\pm$ 0.78 ^{ac}	6.06 $\pm$ 2.01 ^a
LC	85.53 $\pm$ 1.86	344.6 $\pm$ 9.7 ^{ab}	2.16 $\pm$ 0.39	1.83 $\pm$ 0.37 ^a	3.99 $\pm$ 1.67 ^a
EA	82.76 $\pm$ 3.15	351.3 $\pm$ 11.5 ^b	1.89 $\pm$ 0.41	2.55 $\pm$ 0.31 ^{ac}	4.44 $\pm$ 0.72 ^a
AR	73.33 $\pm$ 3.80	237.1 $\pm$ 8.8 ^c	3.24 $\pm$ 1.15	7.08 $\pm$ 1.82 ^b	10.32 $\pm$ 2.95 ^b
AR + LC	78.64 $\pm$ 3.75	281.6 $\pm$ 5.6 ^{cd}	3.33 $\pm$ 0.56	5.20 $\pm$ 0.25 ^{bc}	8.53 $\pm$ 0.65 ^{ab}
AR + EA	79.97 $\pm$ 3.34	295.6 $\pm$ 14.9 ^{ad}	3.59 $\pm$ 0.80	3.93 $\pm$ 0.36 ^{ac}	7.52 $\pm$ 0.66 ^{ab}

The mean differences between the values bearing different superscript letters within the same column are statistically significant (a, b, c and d; $p < 0.01$).

Table 3.

Mean $\pm$ S.E.M. values of testicular tissue thiobarbituric acid reactive substances (TBARS), glutathione (GSH) levels and GSH-peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) activities and plasma testosterone levels (LC, lycopene; EA, ellagic acid; AR, aroclor).

Groups	Biochemical parameters					
	TBARS (nmol/ml)	GSH (nmol/ml)	GSH-Px (IU/g protein)	CAT (kU/g protein)	SOD (U/ml)	Testosterone (ng/dl)
Control	93.4 $\pm$ 12.0 ^a	6.54 $\pm$ 1.15 ^a	10.24 $\pm$ 1.04 ^a	6.20 $\pm$ 0.35 ^{ab}	1.72 $\pm$ 0.28 ^a	392 $\pm$ 65.1
LC	106.6 $\pm$ 8.9 ^a	10.4 $\pm$ 0.64 ^b	8.45 $\pm$ 1.10 ^a	13.35 $\pm$ 0.89 ^d	1.79 $\pm$ 0.26 ^a	289 $\pm$ 65.9
EA	105.8 $\pm$ 15.4 ^a	8.74 $\pm$ 0.67 ^{ab}	10.24 $\pm$ 2.44 ^a	6.50 $\pm$ 0.32 ^b	1.60 $\pm$ 0.29 ^a	354 $\pm$ 73.7
AR	112.4 $\pm$ 4.9 ^a	7.60 $\pm$ 1.53 ^{ab}	7.89 $\pm$ 1.16 ^a	5.01 $\pm$ 0.24 ^a	0.38 $\pm$ 0.05 ^b	279 $\pm$ 57.2
AR + LC	56.7 $\pm$ 6.6 ^b	9.98 $\pm$ 1.18 ^{ab}	21.37 $\pm$ 2.80 ^b	8.59 $\pm$ 0.68 ^c	0.48 $\pm$ 0.04 ^b	324 $\pm$ 109.0
AR + EA	60.5 $\pm$ 10.4 ^b	17.59 $\pm$ 1.78 ^c	21.44 $\pm$ 3.42 ^b	7.12 $\pm$ 0.37 ^b	0.56 $\pm$ 0.07 ^b	420 $\pm$ 94.7

The mean differences between the values bearing different superscript letters within the same column are statistically significant (a, b, c and d: $p < 0.05$).

other groups. Although alone AR treatment decreased the GSH-peroxidase (GSH-Px) and CAT activities at the rate of 22.9% and 19.2%, respectively, compared with the control group, these reductions were statistically insignificant. However, both LP and EA administrations to AR-treated animals provided statistically significant ($p < 0.05$) increments in GSH-Px and CAT activities in comparison with the alone AR group. Alone AR treatment caused decreases in SOD activity (significant, $p < 0.05$) and plasma testosterone level (insignificant) as compared with the control group. However, both LP and EA administrations to AR-treated rats provided numerical but not statistical increases in these two biochemical parameters as compared with the alone AR group.

Testicular histopathology and immunohistochemistry.

When the structure of testes was histopathologically examined; it was observed that histological appearances of testicular tissues of control (fig. 1D), LP (fig. 1E) and EA (fig. 1F) groups were normal. The histopathological changes such as degeneration, desquamation, disorganization and reduction in germinal cells, interstitial oedema and congestion were observed in alone AR group (table 4; fig. 1A). Although LP administration to AR-treated rats caused a pivotal amelioration in testicular histological view (table 4; fig. 1B), simultaneous administration of EA and AR improved markedly a great many of damages induced by AR except germinal disorganization, interstitial oedema and congestion (table 4; fig. 1C). In other words, EA administration to AR-treated rats provided a moderate improvement. Significant ($p < 0.05$) decreases in diameters of seminiferous tubules, germinal cell layer thickness and Johnsen's testicular score were observed in alone AR group compared with the control group. However, both LP and EA administrations to AR-treated animals significantly ($p < 0.05$) prevented the AR-induced decreases in these parameters (table 5).

There were no immunohistochemically significant differences among control (fig. 2D), LP (fig. 2E) and EA (fig. 2F) groups in terms of Bax positive staining. However, Bax positive cells were observed more frequently in the alone AR-treated (fig. 2A) rat testis sections than in the control group rat testis sections. The intense staining was observed

in almost all the spermatogenic cell types (from spermatogonia to elongated spermatid) in alone AR-treated rat testis sections. The decrease in intense staining was observed in both AR + LP (fig. 2B) and AR + EA (fig. 2C) groups when compared with the alone AR group. When the Bax positive apoptotic cell scores were examined in table 5; AR administration significantly ($p < 0.05$) increased the Bax positive cells compared with the control group. The mean values of Bax positive apoptotic cell scores of AR + LP and AR + EA groups were not statistically different from control and AR groups. In other words, improvements in Bax positive apoptotic cell scores provided by LP or EA administrations to AR-treated rats were in moderate style.

With respect to Bcl-2 positive staining, there were no immunohistochemically significant differences among control (fig. 3D), LP (fig. 3E) and EA (fig. 3F) groups. Similarly, alone AR administration caused no significant differences in terms of staining in comparison with the control group (fig. 3A). LP administration to AR-treated animals did not affect the immunohistochemical staining of testicular cells in comparison with the alone AR group (fig. 3B). A slight increase in staining of almost all spermatogenic cells was observed in AR + EA (fig. 3C) group when compared with the alone AR group. When the Bcl-2 positive antiapoptotic cell scores were examined in table 5, no significant differences were found with respect to Bcl-2 antiapoptotic cell scores between the groups.

Discussion

Polychlorinated biphenyls lead to partial or total reproductive dysfunction in human beings [3] and different species of animals such as chicken [1], fish [38], rat [4,9], mouse [10], bear [39], goat [11] and monkey [8]. It is known that monitoring body-weight provides information on the general health level of animals, which can be important to interpretation of reproductive effects [40]. While some authors have reported that exposure of AR or other polychlorinated biphenyls result in reduced body [7,41] and reproductive organ weights [7], other authors [13,42] have alleged that polychlorinated biphenyls have no effect on these parameters

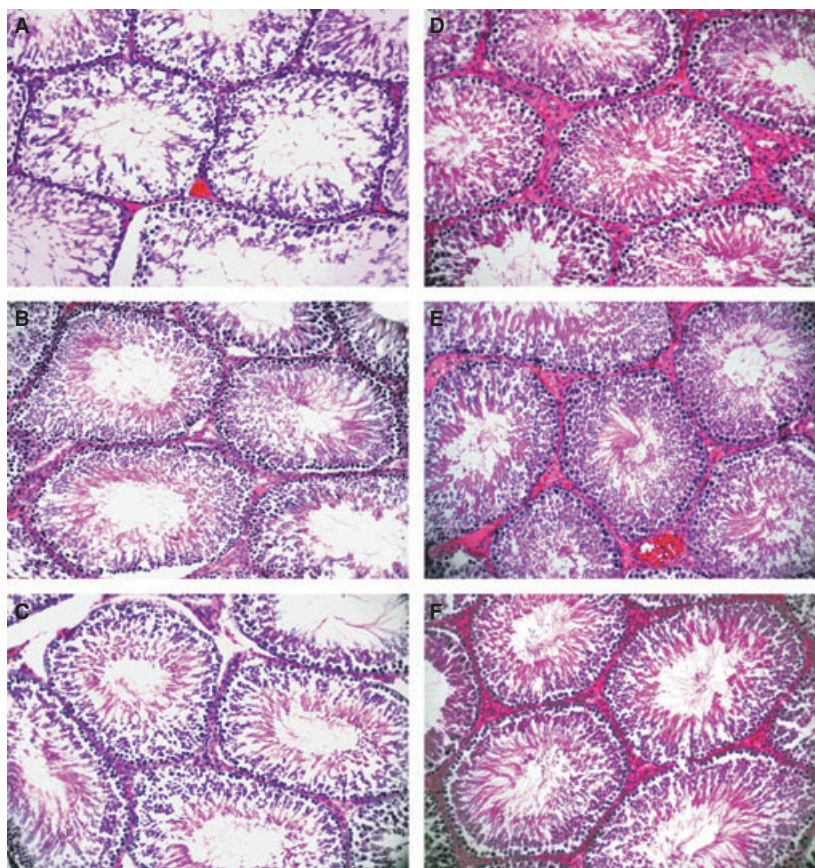


Fig. 1. (A) Disorganization, degeneration and reduction in germinal cells along with interstitial oedema and capillary congestion in alone AR group. (B) Pivotal amelioration in testicular view and normal spermatogenesis in AR + LC (aroclor + lycopene) group. (C) Normal spermatogenesis along with slightly disorganization and interstitial oedema AR + EA (ellagic acid) group [haematoxylin and eosin stain (H&E), 100 $\times$]. (D) Normal histological appearance of seminiferous tubules in control group. (E) Normal histological appearance of seminiferous tubules in alone LC group. (F) Normal histological appearance of seminiferous tubules in alone EA group (H&E, 100 $\times$).

Table 4.

The existence of some pathological lesions in testicular tissues of different treatment groups (LC, lycopene; EA, ellagic acid; AR, aroclor).

Parameters	Groups					
	Control	LC	EA	AR	AR + LC	AR + EA
Degeneration in germinal cells	–	–	–	+	–	–
Desquamation in germinal cells	–	–	–	+	–	–
Reduction in germinal cell counts	–	–	–	+	–	–
Disorganization in germinal cells	–	–	–	+	–	+
Interstitial oedema and capillary congestion	–	–	–	+	–	+

in rats. Androgens stimulate the growth by inducing the protein synthesis. Reactive oxygen species, one of the major types of free radicals, can attack and inactivate or alter the biological activity of molecules such as lipids and proteins that are essential for cell function [17]. Previous studies have shown that decreased level of testosterone and increased level of reactive oxygen species leads to reduced body-weight in AR-exposed animals [4,41]. In the present study, AR treatment caused significant decreases in body-weight and significant increases in relative weights of testes, epididymides and seminal vesicles. Additionally, numerical but not statistically

significant decreases in testosterone levels and increases in reactive oxygen species-induced lipid peroxidation by-products were observed after AR exposure. AR-induced reduced body-weight might be as a result of decreased bioavailability and production of androgens and increased lipid peroxidation found in this study. Although AR treatment decreased very significantly the body-weight, it had no effect on absolute organ weights. The relative reproductive organ weights were calculated by dividing the absolute reproductive organ weights to body-weight in this study. The significant increment in relative organ weights observed in this study may be

Table 5.

Mean $\pm$ S.E.M. values of DST, GCLT, Johnsen's testicular and immunohistochemical scores (DST, diameter of seminiferous tubules; GCLT, germinal cell layer thickness; LC, lycopene; EA, ellagic acid; AR, aroclor).

Groups	Parameters				
	DST (μm)	GCLT (μm)	Johnsen's testicular score	Bax positive cell score	Bcl-2 positive cell score
Control	223.6 $\pm$ 2.20 ^a	76.40 $\pm$ 0.98 ^a	9.67 $\pm$ 0.21 ^{ac}	0.33 $\pm$ 0.21 ^{ab}	0.33 $\pm$ 0.21
LC	225.1 $\pm$ 2.00 ^a	75.67 $\pm$ 1.19 ^a	10.00 $\pm$ 0.00 ^a	0.17 $\pm$ 0.17 ^b	0.50 $\pm$ 0.22
EA	224.5 $\pm$ 1.90 ^a	74.73 $\pm$ 0.99 ^a	10.00 $\pm$ 0.00 ^a	0.67 $\pm$ 0.21 ^{ab}	0.50 $\pm$ 0.22
AR	208.4 $\pm$ 2.26 ^b	50.33 $\pm$ 0.59 ^b	8.33 $\pm$ 0.21 ^b	1.50 $\pm$ 0.22 ^c	0.50 $\pm$ 0.22
AR + LC	230.4 $\pm$ 2.51 ^a	63.20 $\pm$ 0.82 ^c	8.83 $\pm$ 0.31 ^{bc}	1.17 $\pm$ 0.17 ^{ac}	0.50 $\pm$ 0.22
AR + EA	230.3 $\pm$ 3.13 ^a	68.33 $\pm$ 0.73 ^d	9.39 $\pm$ 0.13 ^{ac}	1.00 $\pm$ 0.00 ^{ac}	0.66 $\pm$ 0.33

The mean differences between the values bearing different superscript letters within the same column are statistically significant (a, b, c and d: $p < 0.05$).

explained by the significant decrease in body-weight and unchanging of absolute reproductive organ weights after AR exposure.

The structure of mature sperm plasma membrane is consistent throughout, in that it is composed of three layers or zones: lipid bilayer, phospholipid–water interface and glycocalyx. A major part of plasma membrane consists of lipid bilayer and phospholipid–water interface layers. Because

sperm plasma membranes contain large quantities of lipids (polyunsaturated fatty acids) and their cytoplasm contains low concentrations of scavenging enzymes, they are particularly susceptible to the damage induced by excessive reactive oxygen species [19,20]. Reactive oxygen species can attack the unsaturated bonds of the membrane lipids in an autocatalytic process, with the genesis of peroxides, alcohol and lipidic aldehydes as by-product of the reaction. Thus, the

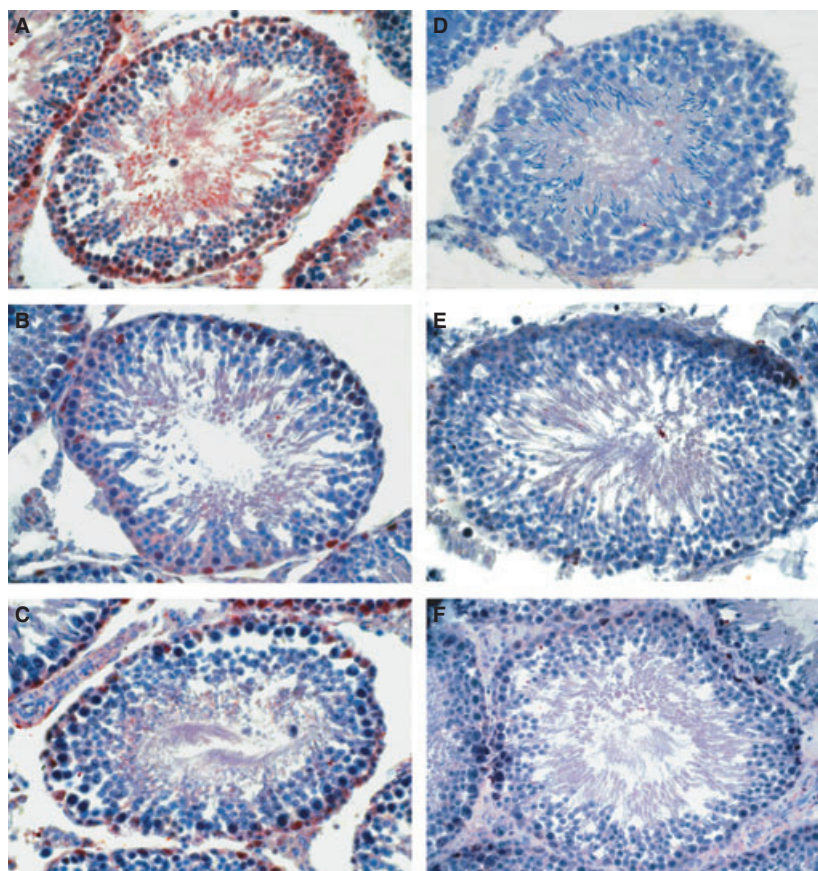


Fig. 2. (A) Bax positiveness in seminiferous tubules in AR group. (B) Bax positiveness in seminiferous tubules in AR + LC (aroclor + lycopene) group. (C) Bax positiveness in seminiferous tubules in AR + EA (ellagic acid) group. (D) Bax positiveness in seminiferous tubules in control group. (E) Bax positiveness in seminiferous tubules in alone LC group. (F) Bax positiveness in seminiferous tubules in alone EA group.

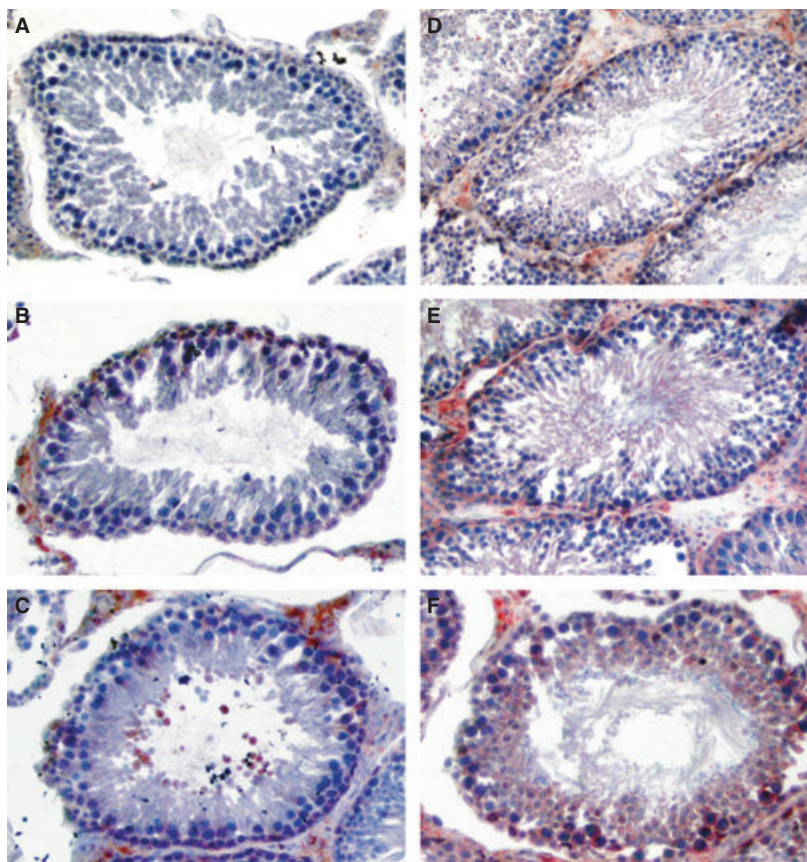


Fig. 3. (A) Bcl-2 positiveness in seminiferous tubules in AR group. (B) Bcl-2 positiveness in seminiferous tubules in AR + LC (aroclor + lycopene) group. (C) Bcl-2 positiveness in seminiferous tubules in AR + EA (ellagic acid) group (200 $\times$). (D) Bcl-2 positiveness in seminiferous tubules in control group. (E) Bcl-2 positiveness in seminiferous tubules in alone LC group. (F) Bcl-2 positiveness in seminiferous tubules in alone EA group (200 $\times$).

increase of free radicals in cells can induce the lipid peroxidation by oxidative breakdown of polyunsaturated fatty acids in membranes of cells. Obviously, peroxidation of sperm lipids destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability and increased mid-piece morphological defects, and even it completely inhibits spermatogenesis in extreme cases [24,25]. Many authors [7,9,13,42] have reported that AR or other polychlorinated biphenyl congeners cause decreased daily sperm production, epididymal sperm count and motility in rats [12]. It has been reported that human dietary polychlorinated biphenyl exposure might have a negative impact on sperm chromatin integrity. Similarly, in a study by Hsu *et al.* [43], it was demonstrated that polychlorinated biphenyl-exposed men had a higher oligospermia, abnormal sperm morphology and reduced sperm capability of binding and penetration to oocytes. In this study, AR-exposed animals had lower sperm motility (insignificant) and count (significant), and higher abnormal sperm rate than the corresponding control group. Our findings are in agreement with the above reports. It has been reported that AR inhibits basal and LH-stimulated testosterone concentration and increases lipid peroxidation [4]. The negative changes observed in

sperm quality after AR exposure in the present study may be attributed to the peroxidation of polyunsaturated fatty acids in plasma membranes of spermatozoa, loss of ATP and damaged flagellum which is important machinery for the sperm motility, decreased daily sperm production because of the reduced testosterone concentration, impaired spermatogenesis and DNA.

Aroclor and some polychlorinated biphenyls cause various histopathological damages in testes such as disorganization of lobules and spermatogenic elements, inhibition of spermatogenesis, fibrosis of lobule walls, fatty necrosis [44], degenerative seminiferous tubules, fewer layers of seminiferous epithelium, increase in intercellular spaces, impaired spermiogenesis, appearance of pyknotic nuclei [10] and accelerated spermatogenic senescence [15]. However, some authors have alleged that polychlorinated biphenyl 132 [13], polychlorinated biphenyl 126 and polychlorinated biphenyl 153 [11] have no effects on testicular histology. Reduced diameters of seminiferous tubules, germinal cell layer thickness and Johnsen's testicular score along with degeneration, desquamation, disorganization and reduction in germinal cells, interstitial oedema and congestion were observed in the histological structure of AR-treated rats in the present study. The damages observed in the histological structure of testis

in this study may be elucidated with the decreased testosterone which stimulates spermatogenesis especially spermiogenesis or increased oxidative stress and lipid peroxidation which is a chemical mechanism capable of disrupting the structure and function of testis.

Testicular germ cell apoptosis (programmed cell death) occurs normally and continuously throughout life. Bax and Bcl-2 are members of a growing family of genes that are involved in promoting either cell survival or cell death via apoptosis. Proapoptotic (Bax) and antiapoptotic (Bcl-2) proteins exist in culmination of apoptosis after the onset of cellular stress. The ratio of these molecules has been implicated to be a critical determinant of cell fate, such that elevated Bcl-2 favours extended survival of cells and increasing levels of Bax expression accelerates cell death [45]. Oskam *et al.* [11] have reported that male offsprings of pregnant goats fed with polychlorinated biphenyls during pregnancy and goats not fed with polychlorinated biphenyls have similar apoptotic cell rate in their testicular tissue. However, Hsu *et al.* [13] found that expression of testicular *Fas*, *Bax*, *Bcl-2* and *p53* genes that are indicators of apoptosis in cells decreased after polychlorinated biphenyl 132 exposure. In this study, Bax positive cells were observed more frequently in the alone AR-treated rat testis sections than in the control group. Additionally, AR administration significantly elevated the Bax positive apoptotic cell scores compared with the control group. With respect to Bcl-2 positive staining and Bcl-2 positive cell scores, there were no immunohistochemically significant differences between control and AR groups. H_2O_2 , is one of reactive oxygen species, induces testicular germ cell apoptosis by extrinsic and intrinsic mechanisms as well other regulatory pathways [46]. Elevated apoptotic cell rates after exposure to AR observed in this study may be explained by increased reactive oxygen species and lipid peroxidation levels in testicular tissue or direct DNA and chromatin damages of germ cells.

Reactive oxygen species include superoxide anion ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$), peroxy ($ROO\cdot$) and alkoxy ($RO\cdot$) radicals, as well as non-radical species such as 1O_2 , ozone (O_3) and hydrogen peroxide (H_2O_2), which act either as oxidizing agents or can be easily converted to radicals. The $O_2^{\cdot-}$ can be generated from oxygen during mitochondrial respiration by single electron transfer and is a major source of $\cdot OH$ radicals [21,47]. In addition, $O_2^{\cdot-}$ is converted either spontaneously or by the enzyme SOD to H_2O_2 which can be transported across the nuclear membrane where it can react with metal ions to produce $\cdot OH$ radicals. In the presence of $O_2^{\cdot-}$ transition metals can be reduced and then catalyse the formation of $\cdot OH$ from H_2O_2 by a Fenton-type reaction. Iron and copper are the most likely promoters of $\cdot OH$ *in vivo*. Finally, 1O_2 is another highly reactive oxygen species which can be formed by photooxidation, enzymatically or in the process of lipid peroxidation of biomembranes [21,48]. Cells have mechanisms to combat partially or totally reactive oxygen species production through antioxidant mechanisms. In general, antioxidant defence can be through enzymatic and non-enzymatic systems. Enzymes such as SOD and

CAT react with radicals $O_2^{\cdot-}$ and H_2O_2 respectively. GSH-Px scavenges alkyl ($R\cdot$), $RO\cdot$ and $ROO\cdot$ radicals that may be formed from oxidized membrane components. Inhibition of oxidation pathways can equally be achieved via molecules such as vitamin C, vitamin E, GSH and co-enzyme Q. This antioxidant consortium is a network of different elements that do work in a cooperative manner, very efficient in removing (most) radicals and preventing most somatic and germinative cells from massive oxidative damage [49]. It is generally accepted that the increased lipid peroxidation is one of the toxic manifestations of AR administration in testis. It has been reported that AR treatment results in elevated lipid peroxidation levels because of the excessive generation of free radicals, and reduced enzymatic (SOD, CAT and GSH-Px) and non-enzymatic (GSH) antioxidants [6,9]. It was observed that alone AR treatment leads to alterations in oxidant/antioxidant balance in this study. The reason for these alterations is because of the AR-induced excessive production of free radicals and consequently elevated lipid peroxidation, and as well as excessive utilization of enzymatic antioxidants to scavenge the free radicals.

Testosterone is essential for spermatogenesis and is produced by Leydig cells. The synthesis of testosterone in Leydig cells is dependent on the expression of highly regulated genes such as StAR protein, cytochrome P450 scc, 3β - and 17β -HSD. Elumalai *et al.* [41] have reported that the cultured Leydig cells from adult rats exposed to AR resulted in lowered synthesis of testosterone. In addition, it has been reported [6] that the AR-exposed rats had decreased Leydig cellular antioxidant enzyme activities, and increased levels of reactive oxygen species and lipid peroxides. However, Anbalagan *et al.* [50] have alleged that AR treatment had no significant effect on testosterone level. In the present study, AR administration caused a decrease at the rate of 28.8% in plasma testosterone level as compared with control, but the effect was not statistically significant. The insignificant decrease in plasma testosterone level in AR-exposed rats may be attributed to the inhibition of testicular steroidogenesis and increased level of lipid peroxides.

Lycopene, the most effective antioxidant among the carotenoids, is known as highly efficient scavengers of 1O_2 and other excited species. During 1O_2 quenching, energy is transferred from 1O_2 to the LP molecule, converting it to the energy-rich triplet state. Trapping of other reactive oxygen species, like $\cdot OH$, NO_2 or peroxynitrite, in contrast, leads to oxidative breakdown of the LP molecule. Thus, LP may protect *in vivo* against oxidation of lipids, proteins and DNA [21]. Being a strong antioxidant, EA attenuates the damaging effect of H_2O_2 , scavenge $O_2^{\cdot-}$ and $\cdot OH$ by its metal-chelating property, thus providing protection against lipid peroxidation [22]. Many exogenous antioxidants such as vitamins E and C [9], quercetin [1] and zinc [51] have potential improvement effects on AR-induced increased lipid peroxidation and decreased SOD, GSH, GSH-Px and CAT activities. Additionally, it has been reported that LP [41], vitamins E and C [4], as well as zinc [51] have a potential protective role on the damaged steroidogenesis in Leydig cells and testosterone

synthesis induced by AR. In our earlier studies, we found that LP- and EA-protected lipid peroxidation-induced testicular and spermatozoal toxicity [26,52,53]. Yu *et al.* [54] have reported that EA reduces oxidative stress and apoptosis in hiperlipidaemic rabbits.

In the present study, co-administrations of LP and EA with AR caused significant decrease in TBARS levels, and significant increase in GSH contents, GSH-Px and CAT activities, diameters of seminiferous tubules, germinal cell layer thickness and Johnsen's testicular score, and insignificant increase in SOD activity and testosterone levels when compared with the alone AR group. Simultaneous administration of EA with AR but not LP provided significant increase in sperm concentration and decrease in tail abnormality in comparison with the alone AR group. Improvements in Bax and Bcl-2 positive staining and proapoptotic and antiapoptotic cell scores provided by LP or EA administrations to AR-treated rats were in moderate style. These improvements in testicular tissue, sperm quality and oxidant/antioxidant balance after LP or EA administrations may be explained with their free radical scavenging and antioxidant capacity, and increased Leydig cell steroidogenesis activity.

In conclusion, this study apparently suggests that LP and EA have modulatory effects against testicular and spermatozoal toxicity induced by AR. These modulatory effects of LP and EA seem to be closely involved with the suppressing of lipid peroxidation and enhancing of antioxidant enzyme activities. Therefore, antioxidants from food consumed by human beings and animals, such as LP and EA, can attenuate the negative effects of environmental pollutants.

Acknowledgements

The authors acknowledge the financial support from The Scientific and Technological Research Council of Turkey (TÜBİTAK); Project number: 106O123.

References

- Mi Y, Zhang C. Protective effect of quercetin on aroclor 1254-induced oxidative damage in cultured chicken spermatogonial cells. *Toxicol Sci* 2005;**88**:545–50.
- Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. *Crit Rev in Toxicol* 1995;**25**:133–63.
- Pflieger-Bruss S, Hagemann S, Körner W, Hanf V, Köhn F-M, Müller C *et al.* Effects of single non-ortho, mono-ortho, and di-ortho chlorinated biphenyls on human sperm functions *in vitro*. *Reprod Toxicol* 2006;**21**:280–4.
- Murugesan P, Muthusamy T, Balasubramanian K, Arunakaran J. Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells. *Toxicology* 2007;**232**:170–82.
- Krishnamoorthy G, Murugesan P, Muthuvel R, Gunadharini DN, Vijayababu MR, Arunkumar A *et al.* Effect of aroclor 1254 on Sertoli cellular antioxidant system, androgen binding protein and lactate in adult rat *in vitro*. *Toxicology* 2005;**212**:195–205.
- Murugesan P, Kanagaraj P, Yuvaraj S, Balasubramanian K, Aruldas MM, Arunakaran J. The inhibitory effects of polychlorinated biphenyl Aroclor 1254 on Leydig cell LH receptors, steroidogenic enzymes and antioxidant enzymes in adult rats. *Reprod Toxicol* 2005;**20**:117–26.
- Aly HAA, Domenech O, Abdel-Naim AB. Aroclor 1254 impairs spermatogenesis and induces oxidative stress in rat testicular mitochondria. *Food Chem Toxicol* 2009 (In press). doi: 10.1016/j.fct.2009.03.019.
- Ahmad SU, Tariq S, Jalali S, Ahmad MM. Environmental pollutant Aroclor 1242 (PCB) disrupts reproduction in adult male rhesus monkeys (*Macaca mulatta*). *Environ Res* 2003;**93**:272–8.
- Krishnamoorthy G, Venkataraman P, Arunkumar A, Vignesh RC, Aruldas MM, Arunakaran J. Ameliorative effect of vitamins (α -tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm. *Reprod Toxicol* 2007;**23**:239–45.
- Alston DA, Tandler B, Gentles B, Smith EE. Testicular histopathology in deer mice (*Peromyscus maniculatus*) following exposure to polychlorinated biphenyl. *Chemosphere* 2003;**52**:283–5.
- Oskam IC, Lyche JL, Kroghenæs A, Thomassen R, Skaare JU, Wiger R *et al.* Effects of long-term maternal exposure to low doses of PCB126 and PCB153 on the reproductive system and related hormones of young male goats. *Reproduction* 2005;**130**:731–42.
- Spano M, Toft G, Hagmar L, Eleuteri P, Rescia M, Rignell-Hydbom A *et al.* Exposure to PCB and *p,p'*-DDE in European and Inuit populations: impact on human sperm chromatin integrity. *Hum Reprod* 2005;**20**:3488–99.
- Hsu P-C, Pan M-H, Li L-A, Chen C-J, Tsai S-S, Guo YL. Exposure in utero to 2,2',3,3',4,6'-hexachlorobiphenyl (PCB 132) impairs sperm function and alters testicular apoptosis-related gene expression in rat offspring. *Toxicol Appl Pharm* 2007;**221**:68–75.
- Tiido T, Rignell-Hydbom A, Jonsson B, Giwercman YL, Rylander L, Hagmar L *et al.* Exposure to persistent organochlorine pollutants associates with human sperm Y:X chromosome ratio. *Hum Reprod* 2005;**20**:1903–9.
- Wakui S, Takagi F, Muto T, Yokoo K, Hirono S, Kobayashi Y *et al.* Spermatogenesis in aged rats after prenatal 3,3',4,4',5-pentachlorobiphenyl exposure. *Toxicology* 2007;**238**:186–91.
- Kerkvliet NI. Immunological effects of chlorinated dibenzop-dioxins. *Environ Health Perspect* 1995;**103**(Suppl. 9):47–53.
- Agarwal A, Cocuzza M, Abdelrazik H, Sharma RK. Oxidative stress measurement in patients with male or female factor infertility. In: Popov I, Lewin G (eds). *Handbook of Chemiluminescent Methods in Oxidative Stress Assessment*. Transworld Research Network, Kerala, 2008;195–218.
- Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer I* 2005;**34**:12–7.
- Aitken RJ, McLaughlin EA. Molecular mechanisms of sperm capacitation: progesterone-induced secondary calcium oscillations reflect the attainment of a capacitated state. *Soc Reprod Fertl Suppl* 2007;**63**:273–93.
- Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. *Am J Reprod Immunol* 2008;**59**:2–11.
- van Breemen R, Pajkovic N. Multitargeted therapy of cancer by lycopene. *Cancer Lett* 2008;**269**:339–51.
- Vattem DA, Shetty K. Biological functionality of ellagic acid: a review. *J Food Biochem* 2005;**29**:234–66.
- Pari L, Sivasankari R. Effect of ellagic acid on cyclosporine A-induced oxidative damage in the liver of rats. *Fundam Clin Pharm* 2008;**22**:395–401.
- Türk G, Ateşsahin A, Sönmez M, Yüce A, Çeribaşı AO. Lycopene protects against cyclosporine A-induced testicular toxicity in rats. *Theriogenology* 2007;**67**:778–85.
- Türk G, Ateşsahin A, Sönmez M, Çeribaşı AO, Yüce A. Improvement of cisplatin-induced injuries to sperm quality, the

- oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. *Fertil Steril* 2008;**89**(S):1474–81.
- 26 Bennett JP, Vickery BH. Rats and mice. In: Hafez ESE (ed.). *Reproduction and Breeding Techniques for Laboratory Animals*. Lea and Febiger, Philadelphia, PA, 1970;299–315.
 - 27 Sönmez M, Türk G, Yüce A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male wistar rats. *Theriogenology* 2005;**63**:2063–72.
 - 28 Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal Biochem* 1966;**16**:359–64.
 - 29 Sedlak J, Lindsay RH. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968;**25**:192–205.
 - 30 Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* 1976;**71**:952–8.
 - 31 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *J Biol Chem* 1951;**193**:265–75.
 - 32 Aebi H. Catalase. In: Bergmeyer HU (ed.). *Methods in Enzymatic Analysis*. Academic Press, New York, 1983;276–86.
 - 33 Flohe L, Otting F. Superoxide dismutase assay. *Method Enzymol* 1984;**195**:93–104.
 - 34 Bancroft JD, Stevens A. *Theory and Practice of Histological Techniques*. Churchill Livingstone, London, 1990.
 - 35 Johnsen SG. Testicular biopsy score count a method for registration of spermatogenesis in human normal values and results in 335 hypogonadal males. *Hormones* 1970;**1**:2–25.
 - 36 Jahnukainen K, Chrysis D, Hou M, Parvinen M, Eksborg S, Söder O. Increased apoptosis occurring during the first wave of spermatogenesis is stage-specific and primarily affects midpachytene spermatocytes in the rat testis. *Biol Reprod* 2004;**70**:290–6.
 - 37 Kandi Coşkun B, Çobanoğlu B. Bazal hücreli karsinoma ile skuamoz hücreli karsinomunun histokimyasal özelliklerinin Bax, Bcl-2 ve Ki-67 ile belirlenmesi. *Türkderm* 2005;**39**:185–8.
 - 38 Njiwa JRK, Müller P, Klein R. Binary mixture of DDT and Aroclor 1254: effects on sperm release by *Danio rerio*. *Ecotox Environ Safe* 2004;**58**:211–9.
 - 39 Sonne C, Leifsson PS, Dietz R, Born EW, Letcher RJ, Hyldstrup L *et al.* Xenoendocrine pollutants may reduce size of sexual organs in East Greenland polar bears (*Ursus maritimus*). *Environ Sci Technol* 2006;**40**:5668–74.
 - 40 Fernandes GSA, Arena AC, Fernandez CDB, Mercadante A, Barbisan LF, Kempinas WG. Reproductive effects in male rats exposed to diuron. *Reprod Toxicol* 2007;**23**:106–12.
 - 41 Elumalai P, Krishnamoorthy G, Selvakumar K, Arunkumar R, Venkataraman P, Arunakaran J. Studies on the protective role of lycopene against polychlorinated biphenyls (Aroclor 1254) induced changes in StAR protein and cytochrome P450 scc enzyme expression on Leydig cells of adult rats. *Reprod Toxicol* 2009;**27**:41–5.
 - 42 Hsu P-C, Li M-H, Guo YL. Postnatal exposure of 2,2',3,3',4,6'-hexachlorobiphenyl and 2,2',3,4',5',6-hexachlorobiphenyl on sperm function and hormone levels in adult rats. *Toxicology* 2003;**187**:117–26.
 - 43 Hsu P-C, Huang W, Yao WJ, Wu MH, Guo YL, Lambert GH. Sperm changes in men exposed to polychlorinated biphenyls and dibenzofurans. *J Am Med Assoc* 2003;**289**:2943–4.
 - 44 Sangalang GB, Freeman HC, Crowell R. Testes abnormalities in cod (*Gadus morhua*) fed aroclor 1254. *Arch Environ Con Tox* 1981;**10**:617–26.
 - 45 Sinha Hikim AP, Swerdloff RS. Hormonal and genetic control of germ cell apoptosis in the testis. *Rev Reprod* 1999;**4**:38–47.
 - 46 Maheshwari A, Misro MM, Aggarwal A, Sharma RK, Nandan D. Pathways involved in testicular germ cell apoptosis induced by H₂O₂ *in vitro*. *FEBS J* 2009;**276**:870–81.
 - 47 Halliwell B. Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Am J Med* 1991;**91**:14S–22S.
 - 48 Halliwell B, Gutteridge MC. Biologically relevant metal ion-dependent hydroxyl radical generation. *FEBS Lett* 1992;**307**:108–12.
 - 49 Silva P. Physiology of Peroxidation Processes in Mammalian Sperm. PhD thesis, Ridderprint, Ridderkerk, Utrecht, 2006.
 - 50 Anbalagan J, Kanagaraj P, Srinivasan N, Aruldas MM, Arunakaran J. Effect of polychlorinated biphenyl, Aroclor 1254 on rat epididymis. *Ind J Med Res* 2003;**118**:236–42.
 - 51 Venkataraman P, Sridhar M, Dhanammal S, Vijayababu MR, Srinivasan N, Arunakaran J. Antioxidant role of zinc in PCB (Aroclor 1254) exposed ventral prostate of albino rats. *J Nutr Biochem* 2004;**15**:608–13.
 - 52 Ateşşahin A, Karahan İ, Türk G, Gür S, Yılmaz S, Çeribaşı AO. Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. *Reprod Toxicol* 2006;**21**:42–7.
 - 53 Ateşşahin A, Türk G, Karahan İ, Yılmaz S, Çeribaşı AO, Bulmuş Ö. Lycopene prevents adriamycin-induced testicular toxicity in rats. *Fertil Steril* 2006;**85**(Suppl. 1):1216–22.
 - 54 Yu Y-M, Chang W-C, Wu C-H, Chiang S-Y. Reduction of oxidative stress and apoptosis in hyperlipidemic rabbits by ellagic acid. *J Nutr Biochem* 2005;**16**:675–81.