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Prophylactic role of β-carotene against acrylonitrile-induced testicular toxicity in rats: Physiological and microscopical studies

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Abstract Acrylonitrile (ACN) is an aliphatic nitrile product which is extensively used in various synthetic chemical industries. ACN is known to exert toxic actions to human beings as well as experimental animals. The present study was designed to examine the ability of β-carotene, a naturally occurring antioxidant, to attenuate ACN-induced testicular toxicity in adult albino rats. Daily oral administration of ACN at a dose level of 30 mg/kg b.w. (7.2 mg/animal) to male rats for a period of 5 days significantly reduced the levels of serum testosterone (T), androsterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which indicate injury to the testis function. Also, it decreased serum and testicular glutathione (GSH) content and glutathione-S-transferase (GST) activity. While, ACN induced lipid peroxidation as indicated by markedly increased malondialdehyde (MDA). ACN intoxication also induced marked alterations in most of the seminiferous tubules including germ cell depletion, tubular atrophy, maturation arrest, complete necrosis as well as multinucleated giant cell formation. Expansion of intertubular spaces and interstitial haemorrhage were also illustrated. Ultrastructural examination of the seminiferous tubules revealed thickened boundary tissue, pyknosis of Sertoli cell nuclei, damaged mitochondria and smooth endoplasmic reticulum-derived vacuoles. Spermatogenic cells also demonstrated altered cytoplasmic organelles, vacuoles of varying sizes and deformed spermatids. Mitochondrial disruption and a decrease in the amount of smooth endoplasmic reticulum were observed in Leydig cells. Compared to ACN-treated animals, pretreatment with β-carotene and its co-administration with ACN once daily at a dose of 40 mg/kg b.w. (9.6 mg/animal) for 30 days induced a remarkable degree of improvement in the levels of endocrine parameters including T, androsterone, FSH and LH. Also, it mitigates serum and testicular GSH content, GST activity and MDA level. Moreover, it protects testicular tissues and cell structures.

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In conclusion, the present results clearly demonstrate the prophylactic role of β-carotene against ACN-induced testicular toxicity in rats.

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Introduction

Acrylonitrile (CH═CH–CN, ACN), a highly reactive compound having active vinyl and cyanide groups, has been widely used in industry for the production of plastics, elastomers, and synthetic fibres and as an intermediate in the synthesis of industrial chemicals and pharmaceuticals (IARC, 1979). It is also used in the manufacture of soft prosthesis material (Parker and Braden, 1990), coating membranes for Langerhans islet implants (Kessler et al., 1992) and high permeable dialysis tubing (Ward et al., 1993). Human exposure to ACN could potentially occur during the manufacturing process, end product usage and transportation. Further, such exposure can also be possible in the general population through cigarette smoke and *via* contamination of drinking water (Byrd et al., 1990). ACN demonstrated acute toxicity in the testes of rats, mice, rabbits and guinea pigs having a high acute toxicity from inhalation and a high to extreme acute toxicity from oral or dermal exposure (Their et al., 2000). ACN is teratogenic in laboratory animals (rat and hamster) at high doses when maternal toxicity has been already manifested. ACN has been demonstrated to induce embryotoxic effects in rat (Saillenfait and Sabate, 2000). ACN-induced embryotoxic and teratogenic effects have also been found in ACN-exposed workers (Wu et al., 1995). According to environmental teratologic epidemiological study in inhabitants living in the surrounding region of an ACN factory, three congenital abnormalities (pectus excavatum, undescended testis and clubfoot) in 46,326 infants showed significant time–space clusters in the study region. There was a decrease in the risk of undescended testis with increasing distance from the ACN factory (Czeizel et al., 1999). Therefore, women not professionally exposed would appear to be at risk of teratogenic effects due to ACN toxicity.

ACN is rapidly absorbed and distributed to all major tissues in animals. Previous studies with 14C have shown that ACN covalently binds to thiol group of proteins (Ahmed et al., 1982) and tissue macromolecules and nucleic acids (Pilon et al., 1988). Therefore, estimation of free radical generation and antioxidant defence has become an important aspect of investigation in mammals. Carotenoids (β-carotene and lycopene) are naturally occurring antioxidants that play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant function of these micro-nutrients could, at least in part, enhance the immunity by maintaining the functional and structural integrity of important immune cells (Chew, 1995; El-Demerdash et al., 2004).

β-Carotene, aside from being a major source of vitamin A (retinol), an essential vitamin for spermatogenesis to proceed, has been reported to be a potent free radical quencher, singlet oxygen scavenger and lipid antioxidant (El-Missiry and Shalaby, 2000). Furthermore, Burton (1989) focused on the ability of β-carotene to function as a chain-breaking antioxidant in a lipid environment at physiological oxygen partial pressures that are considered most likely in mammalian cells. Therefore, the aim of the current study was to investigate the efficacy of β-carotene on ACN-induced functional and structural alterations related to oxidative stress in the testes of rats.

Materials and methods

Chemicals

Acrylonitrile (ACN) and β-carotene were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO, USA) and given by oral gavage at dose levels of 30 mg/kg b.w. (Takano et al., 2010) and 40 mg/kg b.w. (Sadir et al., 2007), respectively. All other chemicals and solvent used were of highest available commercial grade.

Experimental animals

Forty male Sprague-Dawley rats, each weighing 240 ± 10 g, were obtained from the Breeding Unit of the Egyptian Organization for Biological and Vaccine production, A.R.E. The animals were housed in stainless steel cages after grouping in batches of five under standard animal house conditions of relative humidity (55 ± 5%), temperature (25 ± 2 °C) and a 12 h light/12 h dark cycle. Rats were allowed free access to standard commercial feed and tap water and were acclimatized to laboratory conditions for a period of one week before the onset of experimentation.

Experimental protocol

Animals were allocated into four groups each of ten rats as follows:

Group I: (Control) pre-treated with corn oil (2 ml/kg b.w.) once daily for 25 days and treatment continued with distilled water (2 ml/kg b.w.) once daily for additional 5 days i.e. from day 26 to day 30 of the experimental period of 30 days.
Group II: (ACN group) pre-treated with corn oil (2 ml/kg b.w.) once daily for 25 days and treatment continued with ACN in a dose of 30 mg in 2 ml distilled water per kg b.w. (7.2 mg/animal) once daily for additional 5 days.
Group III: (β-carotene group) pre-treated with β-carotene in a dose of 40 mg in 2 ml corn oil per kg b.w. (9.6 mg/animal) once daily for 25 days and treatment continued with distilled water (2 ml/kg b.w.) for additional 5 days.
Group IV: (β-carotene and ACN group) pre-treated with β-carotene (40 mg/kg b.w.) for 25 days and treatment continued with ACN (30 mg/kg b.w.) for additional 5 days.

At the end of the experimental period, the tested animal groups were sacrificed after 24 h of the last dose of different administrations and their blood were collected, by carotid

bleeding, in centrifuge tubes and serum was obtained from the blood after centrifugation at 3000 rpm for 10 min. The testes were immediately excised, cleared of adhering connective tissue and weighed. One of the testes was used for microscopical examination. Serum and other testis samples were stored at −20 °C until analysis studies.

Methods of analysis

Determination of luteinizing (LH), testosterone (T) and androsterone hormones in serum was carried out according to the method of Jaffe and Behrman (1974) and follicle stimulating hormone (FSH) was measured by radioimmunoassay (RIA) using the method of Rose (1998). Glutathione (GSH) was spectrophotometrically assayed in the serum and testes by the method of Sedlak and Lindsay (1968). Glutathione-S-transferase (GST) was assayed in serum and testes by the method of Habig et al. (1974). malondialdehyde (MDA) was determined in serum and testes by using the method of Mihara and Uchiyama (1978).

Microscopical examination of the testes

For qualitative analysis of testicular histology, tissue samples from the testes were immediately fixed in 10% neutral buffered formalin and processed routinely for paraffin embedding technique. Tissue sections of 5–7 μm thick were prepared using a rotary microtome and stained with haematoxylin and eosin (Humason, 1979). Testicular tissues were further processed for ultrastructural evaluation by transmission electron microscopy (TEM); the testis samples were cut into small pieces of about 1 mm^3 and fixed in 2.5% glutaraldehyde for 24–48 h. The specimens were then washed in 0.1 M phosphate buffer (pH 7.4) 3–4 times for 20 min every time and post-fixed in a buffered solution of 1% osmium tetroxide at 4 °C for 2 h. After dehydration in graded concentrations of ethyl alcohol, the tissue specimens were cleared in two changes of propylene oxide, and embedded in Epon resin. Semithin sections (∼1 μm thick) were stained with 1% toluidine blue and examined by using a light microscope. Areas of interest were selected and the blocks were trimmed accordingly. Ultrathin sections (60–70 nm) were cut with a diamond knife using an ultramicrotome (MT6000 – XL RMC, Inc.), mounted on copper grids and double-stained with uranyl acetate and lead citrate (Weakley, 1981). Grids were viewed and photographed using a transmission electron microscope (JEOL JEM-1200 EX II, Japan) operated at 60–70 kV (Faculty of Science, Ain Shams University).

Statistical analysis

Statistical analyses of the resulted data were done using InStat version 2.0 (Graph Pad, ISI, Philadelphia, PA, USA, 1993) computer software. The results were expressed as means (± SE). Multiple comparisons were done using one-way ANOVA followed by Tukey–Kramer as a post-ANOVA test. Statistical significance was accepted at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

Results

Analysis studies

Data listed in Table 1 show that treatment with ACN caused a significant ($P < 0.001$) decrease in the levels of T, androsterone, FSH and LH, as compared to the corresponding control group. Pre- and co-administration of β-carotene to ACN-challenged rats significantly improved ($P < 0.001$) the levels of these hormones as compared to ACN-treated group.

ACN exerts a significant ($P < 0.001$) decrease in serum and testis GSH content and GST activity, while the level of MDA in serum and testis significantly ($P < 0.001$) elevated as compared to the control values. β-Carotene treatment significantly ameliorated the ACN-induced decrease in these parameters (Tables 2 and 3).

Microscopical studies of the testes

At the light microscope level, normal morphology of the seminiferous tubules including all stages of the spermatogenic cycle was observed in the control group (Figs. 1 & 2). However, animals treated with ACN showed severely-damaged seminiferous tubules. The major deleterious effects of ACN in the affected tubules were germ cell depletion, tubular atrophy, maturation arrest, complete necrosis (Figs. 3 & 4) as well as multinucleated giant cell formation (Fig. 5). Congestion of blood vessels under tunica albuginea, expansion of intertubular spaces and interstitial haemorrhage were also illustrated (Figs. 3–5). Rats treated with ACN/β-carotene showed marked

Table 1 The effect of acrylonitrile (30 mg/kg b.w.) and/or β-carotene (40 mg/kg b.w.) on serum sex hormones of male albino rats.

Groups	Testosterone (ng/ml)	Androsterone (pg/ml)	FSH (mIU/ml)	LH (mIU/ml)
Control	7.05 ± 0.04	49.41 ± 0.29	10.64 ± 0.12	8.96 ± 0.10
ACN	3.00 ± 0.03 (−57.45%)a**	30.41 ± 0.22 (−38.45%)a**	4.09 ± 0.26 (−61.65%)a**	4.51 ± 0.06 (−49.67%)a**
β-Carotene	7.63 ± 0.09 (8.23%)a**b**	52.40 ± 0.27 (6.05%)a**b**	12.61 ± 0.16 (18.52%)a**b**	9.26 ± 0.15(3.35%)b**
β-Carotene + ACN	4.62 ± 0.16 (−34.47%)a**b**c**	41.61 ± 0.83 (−15.79%)a**b**c**	8.32 ± 0.22 (−21.80%)a**b**c**	7.48 ± 0.12 (−16.52%)a**b**c**

Data are expressed as means ± SE ($n = 10$ in each group).
Values between parentheses are the difference % of each parameter with respect to control value.
a: Significant change at $P < 0.05$ with respect to control group.
b: Significant change at $P < 0.05$ with respect to ACN-group.
c: Significant change at $P < 0.05$ with respect to β-carotene-group.
** Very high significant change exists at $P < 0.001$.

Table 2 The effect of acrylonitrile (30 mg/kg b.w.) and/or β-carotene (40 mg/kg b.w.) on serum GSH content, GST activity and MDA level of male albino rats.

Groups	GSH (mmol/l)	GST (mmol/l)	MDA (nmol/ml)
Control	4.23 ± 0.08	4.49 ± 0.11	37.78 ± 0.07
ACN	1.41 ± 0.12 (−66.67%)a**	2.26 ± 0.05 (−49.67%)a**	104.67 ± 0.09 (177.05%)a**
β-Carotene	4.79 ± 0.13 (13.24%)ab**	4.83 ± 0.07(7.57%)ab**	31.53 ± 0.09 (−16.54%)a**b**
β-Carotene + ACN	3.37 ± 0.16 (−20.33%)a**b**c**	3.49 ± 0.08 (−22.27%)a**b**c**	57.42 ± 0.12 (51.99%)a**b**c**

Data are expressed as means ± SE (n = 10 in each group).
Values between parentheses are the difference % of each parameter with respect to control value.
a: Significant change at $P < 0.05$ with respect to control group.
b: Significant change at $P < 0.05$ with respect to ACN-group.
c: Significant change at $P < 0.05$ with respect to β-carotene-group.
** Very high significant change exists at $P < 0.001$.

Table 3 The effect of acrylonitrile (30 mg/kg b.w.) and/or β-carotene (40 mg/kg b.w.) on testicular GSH content, GST activity and MDA level of male albino rats.

Groups	GSH (mmol/g)	GST (mmol/g)	MDA (nmol/g)
Control	3.93 ± 0.12	5.05 ± 0.16	17.22 ± 0.43
ACN	1.51 ± 0.12 (−61.58%)a**	2.46 ± 0.09 (−51.29%)a**	43.35 ± 1.06 (151.74%)a**
β-Carotene	4.30 ± 0.07 (9.41%)b**	5.78 ± 0.58 (14.46%)a**b**	18.13 ± 0.58 (5.28%)b**
β-Carotene + ACN	3.28 ± 0.15 (−16.54%)a*b**c**	3.39 ± 0.10 (−32.87%)a**b**c**	30.91 ± 0.23 (79.50%)a**b**c**

Data are expressed as means ± SE (n = 10 in each group).
Values between parentheses are the difference % of each parameter with respect to control value.
a: Significant change at $P < 0.05$ with respect to control group.
b: Significant change at $P < 0.05$ with respect to ACN-group.
c: Significant change at $P < 0.05$ with respect to β-carotene-group.
* *Highly significant change exists at $P < 0.01$.
** Very high significant change exists at $P < 0.001$.

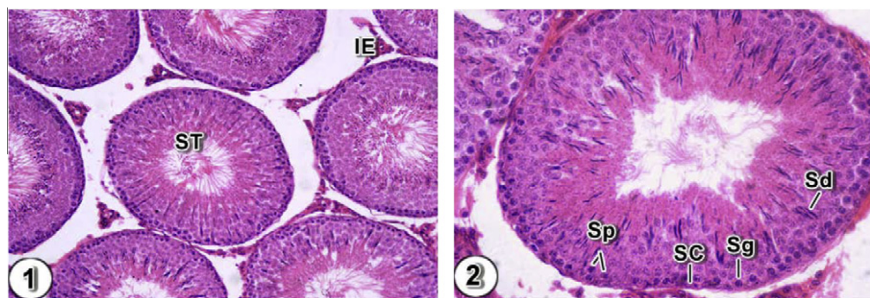

Figure 1 and 2 Light micrographs of control rat testes; Fig. (1): showing regularly arranged seminiferous tubules (ST) and interstitial elements (IE) in between (H&E; ×200); Fig. (2): showing different stages of germ cell development: the Spermatogonia (Sg), spermatocytes (Sp) and spermatids (Sd). The supporting Sertoli cells (SC) are resting on the basal lamina (H&E; ×400).

restoration of spermatogenesis in most of the seminiferous tubules; however, pyknosis of some Sertoli and germ cell nuclei and expansion of intertubular space were still observed (Fig. 6).

With the transmission electron microscope, Sertoli, germinal and Leydig cell morphology of testes of the control rats revealed normal ultrastructural features (Figs. 7–10). Ultrastructural examination of the testes of ACN-treated rats confirmed the light microscopic findings and demonstrated thickened boundary tissue (Fig. 11), pyknosis of Sertoli cell nuclei, damaged mitochondria and many irregular-shaped vacuoles derived from the endoplasmic reticulum (Fig. 12). Disorganized spermatogenic cells with altered cytoplasmic organelles and vacuoles of various sizes were illustrated in Fig. 11. Also, deformed spermatids and necrotic debris were observed in the lumina of the seminiferous tubules (Fig. 13. Leydig cells revealed ill defined cell membrane, irregular nuclear envelope, mitochondrial disruption with loss of cristae and a decrease in the amount of smooth endoplasmic reticulum (Fig. 14. In rats treated with ACN/β-carotene, testicular tissues retained nearly their normal ultastructural features (Figs. 15–18). However, in addition to mild oedema of the boundary tissues in some seminiferous tubules (Fig. 15), a decrease in the amount of smooth endoplasmic reticulum was still observed in the cytoplasm of Leydig cells (Fig. 18).

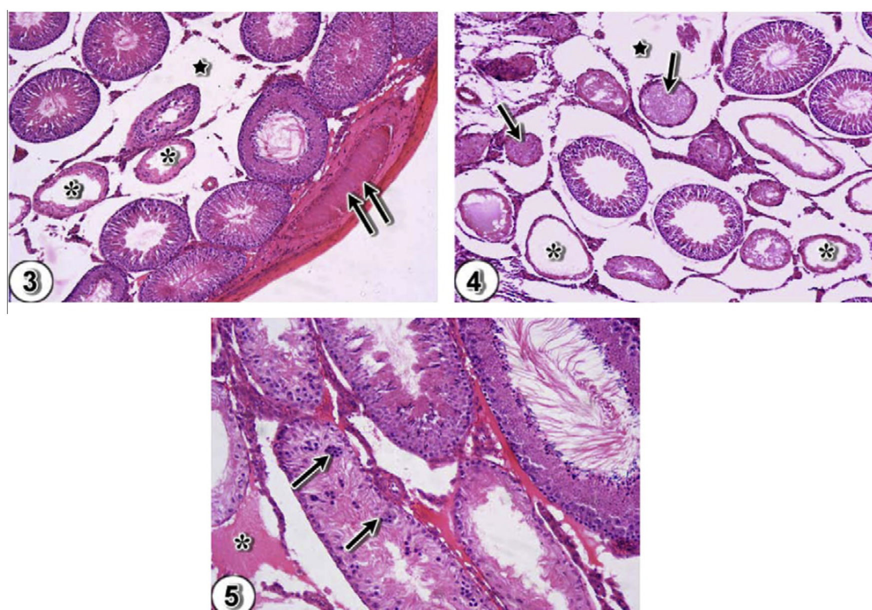

Figure 3–5 Light micrographs of rat testes from ACN-treated group; Figs. (3 & 4): showing severely damaged seminiferous tubules (asterisks) where they exhibit germ cell depletion, tubular atrophy and maturation arrest. Necrosis of almost all the spermatogenic cells is also visible in some tubules (arrows). Note the congestion of the blood vessel under tunica albuginea (double arrows) and expansion of the intertubular space (stars) (H&E; 3 & 4 ×100); Fig. (5): showing a multinucleated giant formation (arrows) and interstitial haemorrhage (asterisk) (H&E; ×200).

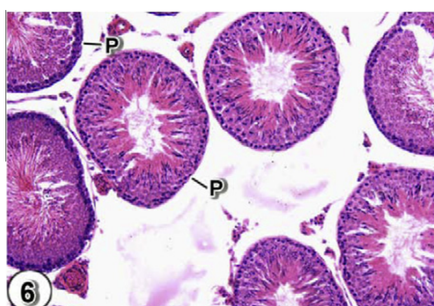

Figure 6 Light micrograph of rat testis from ACN/β–carotene-treated group showing restoration of spermatogenesis in most of the seminiferous tubules, however, pyknosis (P) of some Sertoli and germ cell nuclei and expansion of intertubular space are still observed (H&E; ×200).

Discussion

Acrylonitrile has been demonstrated to induce male reproductive toxicity in laboratory animals (Ahmed et al., 1992; Liu et al., 2004) and also in ACN-exposed workers (Xu et al., 2003). Whole body autoradiography and toxicokinetic studies showed that the brain is a target organ for ACN toxicity (Ahmed et al., 1982). Mc Lachlan et al. (2002) reported that endocrine support is essential for normal spermatogenesis and disturbance can lead to altered spermatogenesis in both humans and rodents. Therefore, the decrease in the levels of serum T, androsterone, FSH and LH could be explained in the current study. The present results are in accordance with the study of Ivanescu et al. (1990) who reported that ACN decreases testosterone synthesis and/or secretion in humans. In this study, the observed decrease in the levels of T and androsterone following ACN administration may be attributed to the increase in oxidative stress. This finding is consistent with that of Diemer et al. (2003) who showed that H_2O_2 is a potent oxidant that could inhibit steroidogenesis (reduce testosterone synthesis) in Leydig cells. Similarly, Yang et al. (2005) reported that serum T level and Leydig cell viability were decreased in rats treated with the structurally similar vinyl monomer, acrylamide (ACA). Their interpretation is that the decreased viability of Leydig cells caused by ACA treatment lowered the testosterone level, which in turn, reduced spermatogenesis in the rat testes. Furthermore, the results of El-Yamany (2009) showed a significant decline in serum levels of T, FSH, LH and prolactin (PRL) of rats fallowing ACA administration. The author attributed the decline of T, FSH, LH and PRL levels to the dysfunction of pituitary gland and also demonstrated that ACA affects the testes directly and/or indirectly through its effect on pituitary gland and decreases the secretion of FSH and LH. Also, the present results are in accordance with the study of Gunnarsson et al. (2003) who found that cadmium caused a decrease in T production through the decrease in LH receptor messenger ribonucleic acid (mRNA) levels as well as cyclic adenosine monophosphate (cAMP) levels in rats.

The present results demonstrated that ACN is capable of inducing oxidative stress in the testes of rats as indicated by a significant depletion of both enzymatic (GST) and non-enzymatic (GSH) antioxidant defence system. Furthermore, ACN significantly enhanced lipid peroxidation, as assessed by monitoring MDA production. These findings are in harmony with the known ability of ACN to induce oxidative stress *in vivo* and in astrocytes *in vitro* (Jiang et al., 1998; Kamendulis et al.,

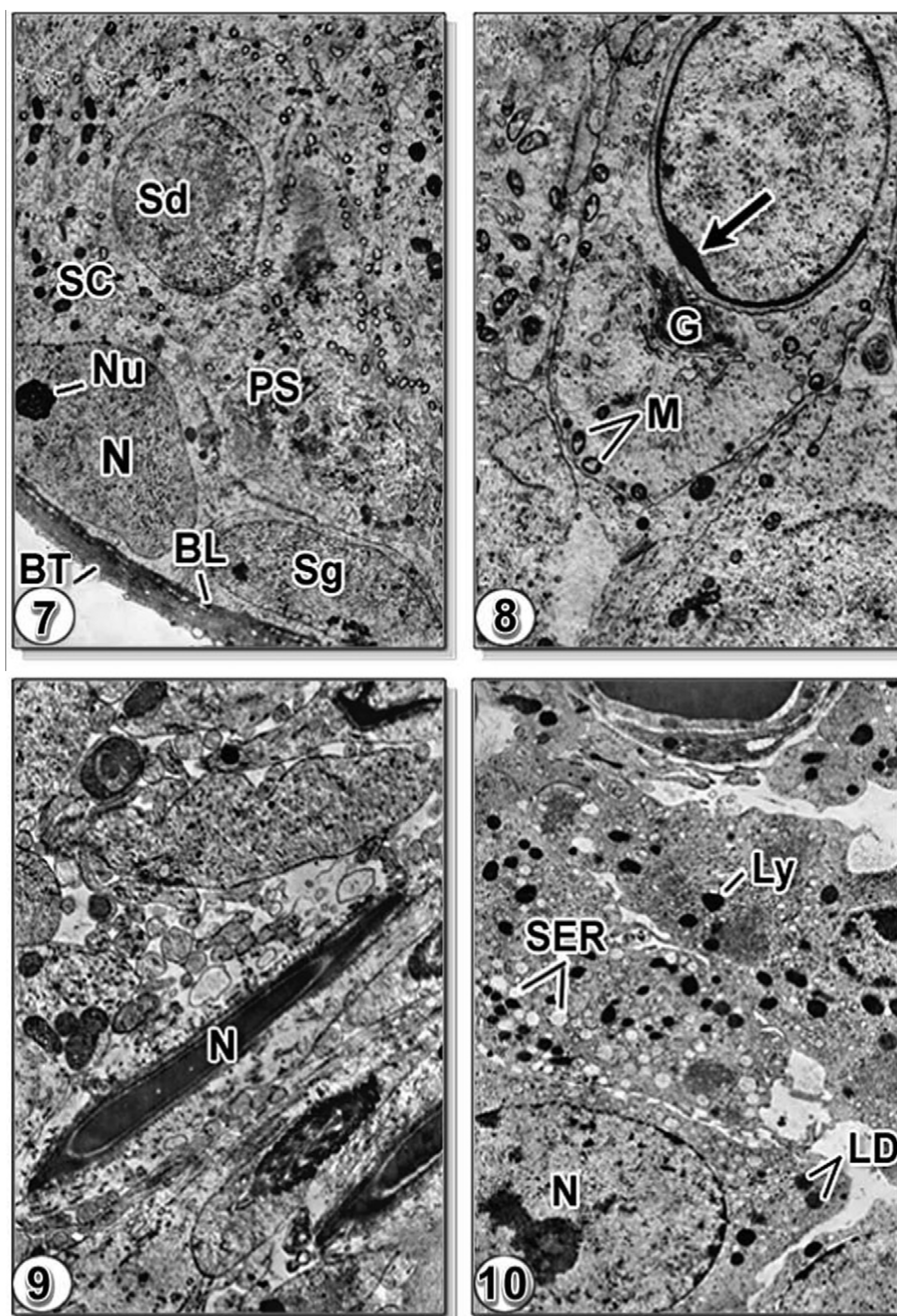

Figure 7–10 Transmission electron micrographs of control rat testes; Fig. (7): showing normal seminiferous tubules surrounded by a thin basal lamina (BL) followed by the boundary tissue (BT), Sertoli cell (SC) resting on the basal lamina and having nucleus (N) with prominent nucleolus (NU), type A spermatogonium (Sg) with ovoid nucleus, primary spermatocyte (PS) with spherical nucleus containing clumps of heterochromatin and early spermatid (Sd) (×2700); Fig. (8): showing early spermatid with acrosomal formation (arrow), peripheral arrangement of mitochondria (M) and well developed Golgi apparatus (G) (×5000); Fig. (9): showing late spermatids with elongated nuclei (N) (×8000); Fig. (10): showing Leydig cells with large spherical nuclei (N); their cytoplasm contains abundant smooth endoplasmic reticulum (SER), lysosomes (Ly) and lipid droplets (LD) (×5000).

1999). Also, in the same line, the study of Abdel-Wahab (2003) who showed that i.p. injection of structurally related compounds such as dibromoacetonitrile (DBAN) in mice significantly decreases the testicular content of GSH and increases the content of MDA.

ACN is metabolized *via* 2 major pathways (Burka et al., 1994; Sumner et al., 1999). The first pathway entails the direct conjugation of parent ACN with reduced glutathione (GSH). Subsequent degradation of this metabolite leads to the formation and urinary excretion of N-acetyl-S (2-cyanoethyl) cysteine (Fennell et al., 1991; Sumner et al., 1999). The second pathway involves epoxidation of ACN *via* cytochrome P450 2E1 (CYP 2E1) leading to the formation of the epoxide intermediate, 2-cyanoethylene oxide (CEO), a reactive and a

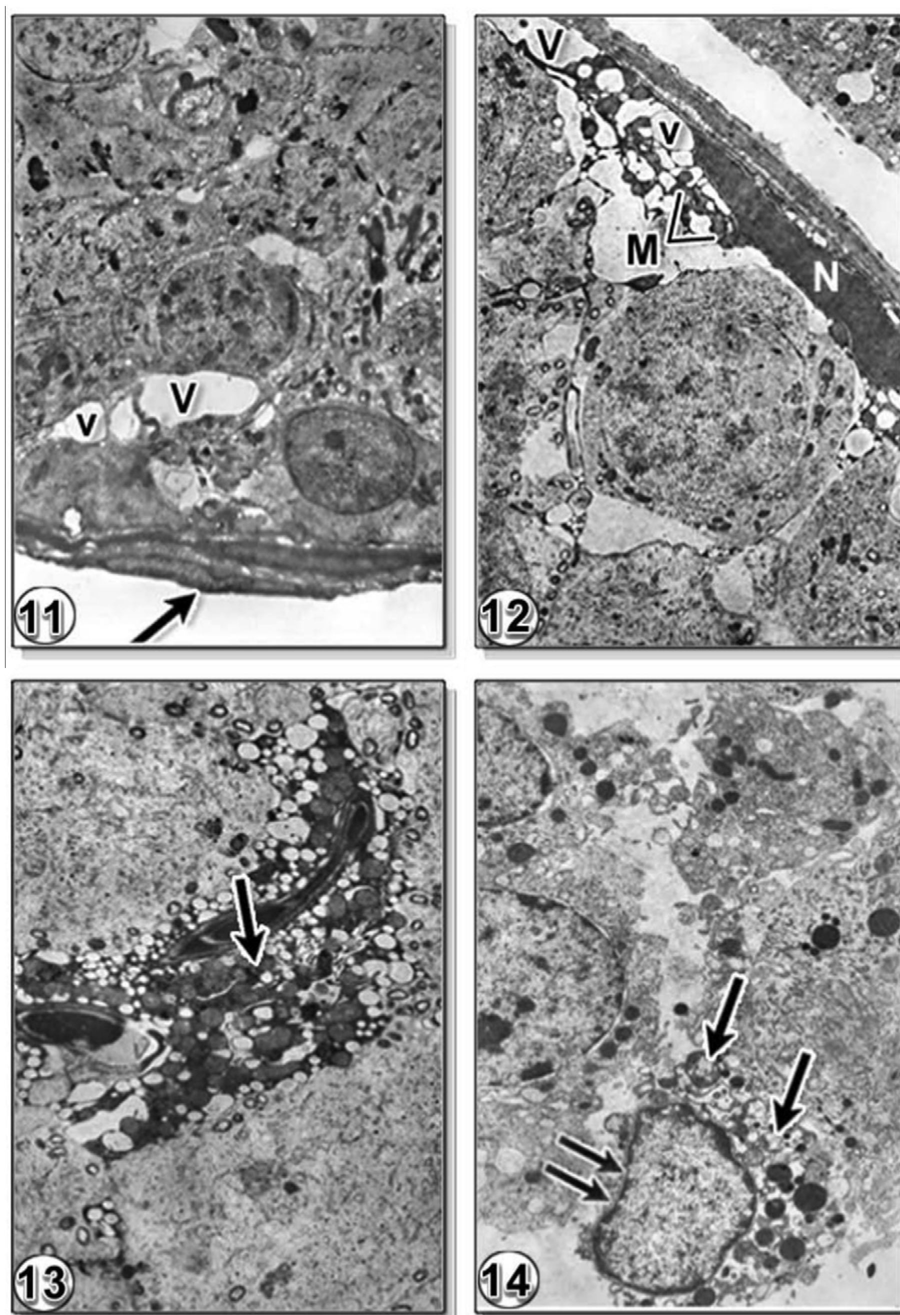

Figure 11–14 Transmission electron micrographs of the testes from ACN-treated rats; Fig. (11): showing thickened boundary tissue (arrow), disorganized spermatogenic cells with altered cytoplasmic organelles and vacuoles of various sizes (V) (×2700); Fig. (12): showing pyknosis of Sertoli cell nucleus (N), damaged mitochondria (M) and smooth endoplasmic reticulum-derived vacuoles (V) (×4000); Fig. (13): showing deformed spermatids and necrotic debris (arrow) in the lumina of the seminiferous tubules (×4000); Fig. (14): showing degenerative changes in Leydig cells where they exhibited ill defined cell membrane, irregular nuclear envelope (double arrows), mitochondrial disruption with loss of cristae (arrows) and a decrease in amount of the smooth endoplasmic reticulum (×4000).

relatively long-lived epoxide (Kedderis et al., 1995). Subsequent metabolism of CEO occurs *via* conjugation with GSH or *via* hydrolysis to yield cyanide (CN^-) and other metabolites. Production of CN^- induces oxidative stress by enhancing hydroperoxide generation and lipid peroxidation. Also, CN^- inhibits enzymes of biological system, the most important being cytochrome oxidase with subsequent cessation of energy production, as indicated by depletion of ATP production. Cessation of energy-dependent pathways could also augment oxidative stress (Esmat et al., 2007). Thus, GSH-depleting properties of ACN could be attributed to enzymatic conjugation and/or direct binding with thiol group, which in turn resulted in enhanced lipid peroxidation. Several studies have reported the implication of free radicals such as hydroxyl radial and superoxide anion in the testicular toxicity of ACN (Mashino and Fridovich, 1987; Rashba-step et al., 1993;

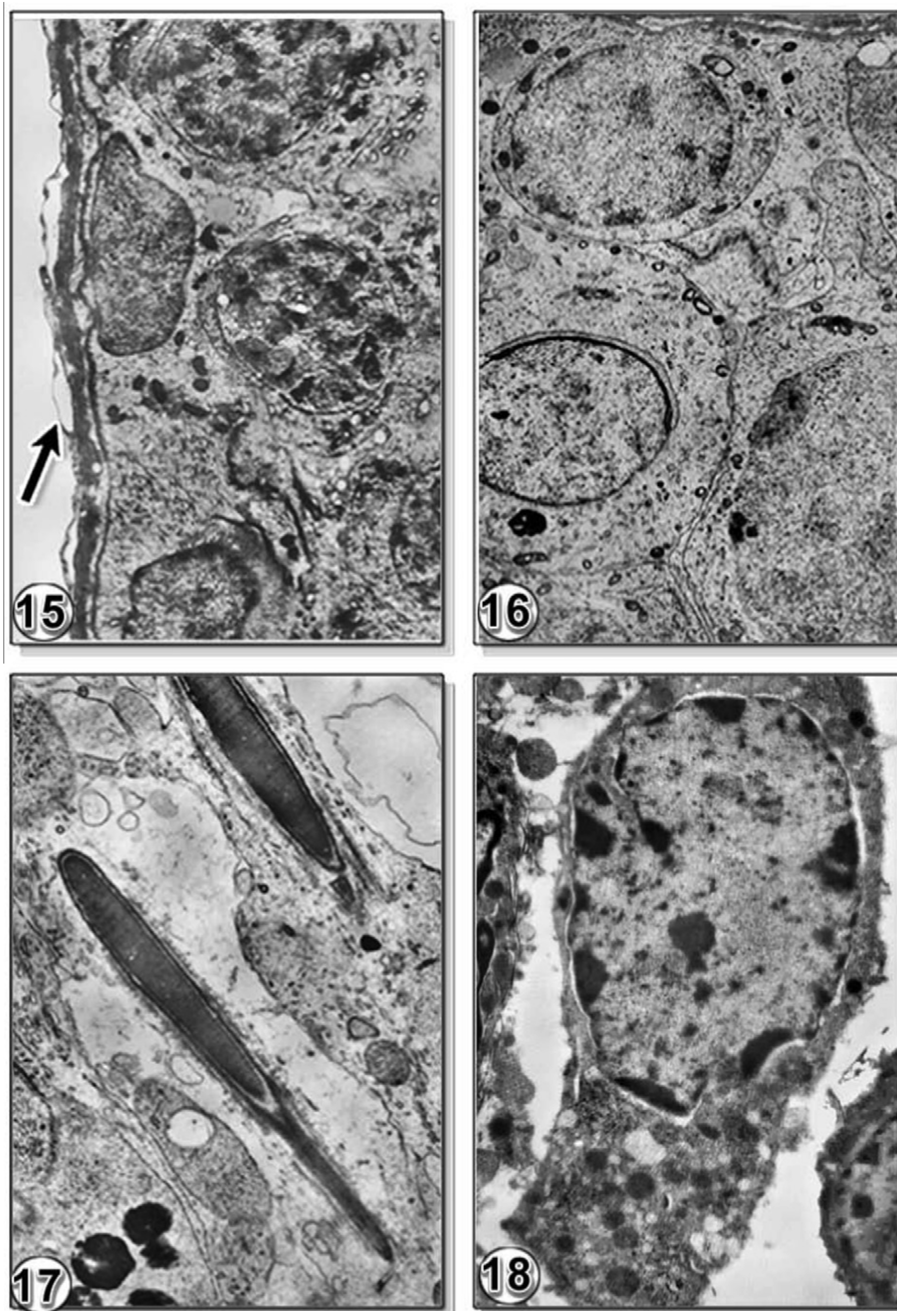

Figure 15–18 Transmission electron micrographs of the testes from ACN/β-carotene-treated rats; Figs. (15-17) showing nearly normal structure of Sertoli and spermatogenic cells, however, mild oedema of the boundary tissue was evident (arrow) (Fig. 15: x2700; Fig. 16: x4000; Fig. 17: x8000); Fig. (18): showing almost normal Leydig cell with its characteristic cell organelles; however, a decrease in the amount of smooth endoplasmic reticulum is still observed in its cytoplasm (x6000).

Hodnick et al., 1994). The formation of reactive oxygen species ($OH^{\cdot}$, $O_2^{\cdot-}$ and H_2O_2) is done through bioactivation of cyanide-containing compounds (acrylonitrile, dibromoacetonitrile and choloroacetonitrile) by hepatic microsomal enzymes such as cytochrome-P450 or peroxidases leading to the liberation of CN ions which interact with certain compounds inducing free radicals formation (Aust et al., 1993).

The testicular toxicity induced by ACN is further confirmed by histological and ultrastructural changes. In the ACN-group, histopathological examination of the affected tubules showed germ cell depletion, tubular atrophy, maturation arrest and multinucleated giant cell formation. In addition to this, the electron micrographs showed thickened boundary tissue and altered cytoplasmic organelles in the three main testicular types of cells (Sertoli, germinal and Leydig cells). This is in accordance with a similar observation given by Nilsen et al. (1980) who observed an impaired spermatogenesis in the exposed male rats to ACN. Also, Tandon et al. (1988) revealed degeneration of the germinal epithelium and seminiferous tubules and decreased sperm count in mice administered ACN.

Furthermore, oral administration of ACN to rats decreases sperm count and motility and inhibits the activity of pachytene spermatocyte marker LDH-X, a unique isoenzyme of lactate dehydrogenase and its activity is associated with spermatogenesis and male testicular development (Ahmed et al., 1993). More recently, Abdel-Wahab (2003) reported that i.p. injection of dibromoacetonitrile caused marked pathological changes in mice testes. Several studies suggest that ACN alters DNA structure in testes, a phenomenon which could have a major impact on reproductive behaviour. According to Ahmed et al. (1992), covalent binding of ACN to testicular tissue DNA was observed in the testes of rats following a single oral dose (46.5 mg/kg) of ACN. Further, a significant decrease in DNA synthesis (80% of control) was observed at 0.5 h after treatment. At 24 h following ACN administration, testicular DNA synthesis was severely inhibited (38% of control). Testicular DNA repair was increased 1.5-fold at 0.5 h and more than 3.3-fold at 24 h following treatment with ACN. These results suggest that ACN can act as a multipotent genotoxic agent by alkylating DNA in testicular tissue and may affect the male reproductive function by interfering with testicular DNA synthesis and repair processes.

To protect spermatogenesis from toxicant exposure, many clinical and experimental trials of antioxidant agents have been attempted. Carotenoids as potential antioxidant are well known as highly efficient scavengers of singlet molecular oxygen ($^1O_2$), and other excited species. The present study indicates the beneficial effects of β-carotene against ACN-induced testicular toxicity. β-Carotene treatment improved the levels of endocrine parameters including T, androsterone, FSH and LH. Also, β-carotene treatment mitigates serum and testicular GSH content, GST activity and MDA level. Furthermore, it protects the germ cell integrity as indicated by microscopical studies. These results are in agreement with those obtained by Livera et al. (2002) who found that in adult rats, retinoids increased basal testosterone secretion in Leydig cell primary cultures. Also, Hanukoglu (2006) reported that the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase are parallel to steroidogenesis and the antioxidant β-carotene exerted a protective role on Leydig cell steroidogenesis to produce testosterone; thus it stimulated the development of reproductive organs through the growth of Leydig and Sertoli cells and the promotion of spermatogenesis. El-Missiry and Shalaby (2000) indicated that co-treatment with β-carotene produces a significant reduction in $CdCl_2$-induced increase in lipid peroxidation in rat brain and testis. This finding is paralleled by modulation of SOD and GST activities and GSH content in both tissues. Also, Silva et al. (2001) suggested that pretreatment with another carotenoid, bixin reduced the total number of chromosome aberrations and inhibited the increase in lipid peroxidation induced by cisplatin. Furthermore, Gupta and Kumar (2002) elucidated that the effect of oral lycopene, a naturally occurring carotenoid in tomatoes, therapy in men with idiopathic infertility and found improvement in male infertility and especially in sperm characteristics. More recently, Atessahin et al. (2006) reported that pre- and post-treatment with lycopene significantly inhibited the increase in MDA and GSH depletion in the testes induced by cisplatin exposure. A rational mechanism for the protective effects of β-carotene is its potential antioxidant activity. Because β-carotene is a lipophilic substance, it exerts its action in hydrophobic environment such as the lipid core of membranes. Thus, it is anticipated that natural β-carotene, a chain breaking antioxidant, can contribute to protecting cell membranes from lipid peroxidation (Krinsky, 1998). β-Carotene can function as an effective antioxidant not only against $^1O_2$ but also against lipid peroxidation and the highly destructive, hydroxyl radical ($OH^•$) that is implicated in many diseases such as cancer and heart disease (O'Neill and Thurnham, 1998).

In conclusion, this study clearly showed the potential antioxidant usefulness of β-carotene in managing testicular toxicity induced by ACN in rats.

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