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RESEARCH ARTICLE

Ameliorative effect of fractionated low-dose gamma radiation in combination with ellagic acid on nicotine-induced hormonal changes and testicular toxicity in rats

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Abstract

Nicotine is an active pharmacological ingredient in cigarette smoke, which may negatively influence the male reproductive system and fertility. This study aims to investigate the effect of fractionated low-dose radiation (fractionated-LDR) and/or ellagic acid (EA) on nicotine-induced hormonal changes and testicular toxicity in rats. Nicotine was administrated orally (1 mg/kg) for 30 days, afterward, rats were treated with LDR (2 × 0.25 Gy/1-week interval), EA (10 mg/kg, 14 consecutive days p.o.), or a combination of both fractionated-LDR and EA. Rats were sacrificed 24 h after the last dose of treatment, then testes were dissected for histopathology examination, along with some biochemical parameters in serum and testicular tissue were evaluated. Nicotine-induced oxidative stress was evidenced by an increase in testicular thiobarbituric acid reactive substances (TBARS) and a decrease in reduced glutathione (GSH) content. Additionally, the activities of testicular androgenic enzymes were decreased, and the activity of serum lactate dehydrogenase (LDH) was significantly increased. The hormonal changes were verified by a noticeable reduction in follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone serum levels. Histological evaluation revealed that the testicular seminiferous tubules structure was distorted. On the contrary, fractionated-LDR plus EA attenuated the negative changes caused by nicotine observed through biochemical and histological findings. Accordingly, the exposure to fractionated-LDR combined with EA may be a promising candidate for treating hormonal changes and testicular toxicity caused by nicotine.

Keywords Nicotine · Low-dose radiation · Ellagic acid · Oxidative stress · Hormones · Androgenic enzymes

Introduction

Nicotine probably accounts for 90% of the overall alkaloid of cigarette smoke and is considered to have a detrimental effect on male reproductive organs (Kavitharaj and Vijayammal 1999; Aydos et al. 2001). Numerous studies have shown that nicotine adversely affects spermatogenesis, epididymal sperm count, motility, and the fertilizing potential of sperms (Oyeyipo et al. 2011; Ukwenya et al. 2020). Besides, nicotine leads to various histopathological changes in testicular tissue revealing atrophy, degenerative alterations, and sperm disturbance in several seminiferous tubules, along with increased

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interstitial spaces and a reduced number of Leydig cells (Mosbah et al. 2015). Disruptions in the Leydig cells thus reduce the biosynthesis of testosterone (Segarra and Strand 1989; Sarasin et al. 2003), luteinizing hormone (LH) secretion (Funabashi et al. 2005), and testicular androgenic enzymes (Yamamoto et al. 1998). Nicotine's deleterious toxic effects are at least partly due to increased reactive oxygen species (ROS) production. ROS caused damages to DNA, proteins, carbohydrates, and lipids and affects the activity of enzymes (Bandyopadhyay et al. 2008; Sudheer et al. 2008). Moreover, nicotine is oxidized to its main metabolite, cotinine, which has a long biological half-life and negatively affected sperm production, epididymal sperm count, motility, and fertility (Aydos et al. 2001).

Numerous studies have investigated the beneficial effects of low-dose radiation (LDR) namely "radiation hormesis" (Luckey 1982; Feinendegen 2005; Liu 2010). Hormesis induced by LDR was extensively observed in various biological systems including the reproductive, immune, and hematopoietic systems (Li et al. 2004; Ina et al. 2005; Liu et al. 2006). Besides, LDR-induced adaptive response can render cells resistant to DNA or chromosome damage from exposure to high-dose radiation (Olivieri et al. 1984; Feinendegen 2005; Yang et al. 2016). Such beneficial effects of LDR tend to reach the peak at around 4 h post-irradiation and persist for several hours or even weeks depending upon the type of cell and tissue (Yamaoka et al. 1991).

Ellagic acid (EA) is considered to be one of the natural polyphenolic compounds that are present in nuts, multiple fruits like pomegranate, berries, kiwi, grapes, apples, and assorted vegetables (Devipriya et al. 2007). EA has earned the greatest attention of all phytochemicals because of the variety of its physiological functions including, anti-carcinogenic (Umesalma and Sudhandiran 2011), antioxidant, anti-inflammatory, and anti-apoptotic properties (Chen et al. 2018).

The beneficial effects of fractionated-LDR in combination with EA against nicotine-induced testicular toxicity have rarely been discussed in researches. The aim of this study is therefore to elucidate the mechanisms by which nicotine causes hormonal changes and testicular dysfunction. In addition to investigate the ameliorative effects of either fractionated-LDR, EA, or their combination on these changes. By assessing different biochemical parameters including testicular oxidative stress content, androgenic key enzymes, and lactate dehydrogenase (LDH) activities, as well as the levels of reproductive hormones, in addition to histological changes.

Material and methods

Animals

Experiments were performed on male Wistar rats, weighing 120–180 g, purchased from the National Research Center's animal breeding unit (Dokki, Giza, Egypt). The rats were permitted to acclimatize 1 week prior to the experiment under the laboratory conditions of the National Center for Radiation Research and Technology (NCRRT)–Atomic Energy Authority (Nasr City, Cairo, Egypt). Rats were fed a standard pellet diet obtained from the National Research Center (Dokki, Cairo, Egypt) and allowed free access to water ad libitum. All experiments were performed following the guidelines set by the Environment-European Commission (EEC) (revised directive 86/609/EEC) and an ethical approval (Permit no: PT 1175) was granted from the Ethical Committee for Animal Experimentation, Faculty of Pharmacy, Cairo University.

Irradiation protocol

Whole-body irradiation of rats was carried out at the NCRRT using the Gammacell®-40 biological irradiator with a Cesium-137 source (Atomic Energy of Canada Limited; Sheridan Science and Technology Park, Mississauga, Ontario, Canada). The Gammacell®-40 provides a radiation dose level at the rate of 0.46 Gy/min during the time of the experiment. Accordingly, per required irradiation exposure, non-anesthetized rats were put in a plastic sample tray and left for the necessary period to reach the required radiation dose level.

Chemicals and reagents

All chemicals and reagents were purchased either from Sigma-Aldrich (Saint Louis, Missouri, USA) or of the purest analytical grade available. The enzyme-linked immunosorbent assay (ELISA) kit for measuring hormones of follicle-stimulating hormone (FSH) was obtained from LifeSpan BioSciences, Inc. (Seattle, Washington, USA), luteinizing hormone (LH) from Elabscience Biotechnology Inc. (Houston, Texas, USA) and that for testosterone from DRG® International, Inc. (Mountain Ave, Springfield Township, USA). Besides, lactate dehydrogenase (LDH) was measured by using a kinetic kit purchased from BioSystems (Barcelona, Spain).

Drugs and dosage

Nicotine hydrogen tartrate (95% nicotine); was purchased from Sigma-Aldrich Co. and 1 mg/kg was freshly prepared in normal saline and administered to rats once daily for 30 days. The selection of nicotine dose and/or duration of nicotine administration was based on numerous studies, for instance, Oyeyipo et al. (2010), Kolawole et al. (2019), Mohamed and Abdelrahman (2019), and Ukwenya et al. (2020). Moreover, the nicotine dose was also chosen after carrying out a preliminary experiment where rats received nicotine at a dose of 0.5 or 1 mg/kg orally showed the extent of testicular damage, but the oxidative stress in the testes was significantly shown at dose 1 mg/kg (the results are not shown).

The cigarette smoke contains an average of 0.5–1.6 mg of nicotine per cigarette and 60 mg dose would correspond to an oral LD_{50} of around 0.8 mg/kg in human, a dose that is considerably smaller than the values determined for laboratory animals (Hayes 1982; Okamoto et al. 1994; Bose et al. 2007). Therefore, 1 mg of nicotine/kg was used to make it relevant to human exposure, though the LD_{50} of nicotine is 50 mg/kg in rats (Okamoto et al. 1994).

Ellagic acid (EA) was obtained from Alfa Aesar (Karlsruhe, Germany) and dissolved in saline. According to Türk et al. (2008); Celik et al. (2013), Girish et al. (2014), and Georgy and Maher (2017), rats were given EA orally for 14 days at a dose of 10 mg/kg.

The rationale for choosing 14 days for treatment, a preliminary study was carried out to assess the persistence of testicular damage after stopping the administration of nicotine, where a group of rats received 1 mg/kg of nicotine for 30 days and then remained untreated for another 14 days. On day 45, the rats were sacrificed and the testes were excised for histopathological studies and measuring the testicular contents of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH). The outcome of this study was that the testicular damage was persistent until day 14. Consequently, the treatment of adverse effects caused by nicotine administration started on day 31 and continued for a further 14 days (the results are not shown).

Experimental design

The design of this study involved the following steps:

Step 1: A preliminary study was carried out to determine the appropriate dose and regimen of LDR to treat nicotine-induced testicular toxicity

The rats were randomly classified into five groups; 8 rats/group. Group I (normal control): Rats received oral saline solution daily. Group II (nicotine group): Rats orally received nicotine (1 mg/kg) once daily for 30 days. Groups III–IV (nicotine + LDR): After 30 days of nicotine administration, rats were exposed to single-dose levels of 0.25 and 0.5 Gy, respectively. Group V: After 30 days of nicotine administration, rats were exposed to fractionated dose delivered as 2 fractions (each of 0.25 Gy) at a 1-week interval. One week after the last radiation exposure, the rats were sacrificed and the testes were dissected for measuring TBARS and GSH contents. The outcome of this step was the selection of a fractionated-LDR to treat nicotine-induced testicular toxicity in rats (Supplemental figure 1).

Step 2: Study of the effect fractionated-LDR and/or EA on testicular toxicity induced by nicotine

The rats were randomly divided into five groups; 8 rats/group. Group I (normal control): Rats received normal saline solution orally. Group II (nicotine group): Rats were given nicotine orally for 30 consecutive days at a dose of 1 mg/kg. Group III (nicotine + LDR): After 30 days of nicotine administration, rats received the first and second radiation fraction of 0.25 Gy on day 31 and 37, respectively. Group IV (Nicotine + EA): After 30 days of nicotine administration, rats were given EA (10 mg/kg, p.o.) for a further 14 days. Group V (nicotine + LDR + EA): Rats received nicotine for 30 days followed by treatment with both LDR and EA. On day 45, rats were sacrificed and blood samples were withdrawn from the heart for serum separation under light ether anesthesia, and testes were excised. The right testis was used for histopathological studies and the left one was used to prepare 10% homogenate in different media according to the parameters to be measured using a Glass-Col homogenizer (Terre Haute, IN, USA). The homogenate was centrifuged using a Mikro 22R centrifuge (Hettich GmbH, Tuttlingen, Germany).

Evaluation of oxidative stress biomarkers

For the determination of testicular lipid peroxidation, the testes were homogenized in a buffer containing 1.5% potassium chloride. According to Uchiyama and Mihara (1978), the concentration of lipid peroxidation was calculated as TBARS and measured colorimetrically using a Unicam 8625 UV/V spectrophotometer (Cambridge, UK) at 535 nm. On the other side, testes homogenization in meta-phosphoric acid was used to assess the concentration of GSH according to Beutler et al. (1963), where 5,5`-dithio-bis (2-nitrobenzoic acid (DTNB) was added to the supernatant to give a yellow derivative that was spectrophotometrically measured at 412 nm.

Evaluation of androgenic enzymes activities

For measuring the activities of androgenic enzymes, the testes were homogenized in 15% glycerol containing 5 mmol potassium phosphate and 1 mmol ethylenediaminetetraacetic acid at a tissue concentration of 100 mg/ml. After centrifugation at 10,000×*g* for 30 min at 4 °C, the supernatant was divided into two parts, one for measurement 3 beta-hydroxysteroid dehydrogenase (3 β-HSD) according to Talalay (1962), and the other part for measuring 17 beta-hydroxysteroid dehydrogenase (17β-HSD) according to Jarabak et al. (1962). One unit of enzyme activity is equal to a change in absorbance of 0.001/min at 340 nm.

Hormonal study

According to the manufacturer's instructions, serum FSH, LH, and testosterone levels were measured by an ELISA kit. The optical density of each sample was measured by using an

ELISA plate reader (Dynatech® MR5000, Guernsey, Channel Islands, UK) set at 450 nm.

Evaluation of lactate dehydrogenase activity

The activity of serum LDH was determined according to the instructions from the manufacturer's kit.

Histopathological examination

The rats' testes were excised per group, washed, and then fixed in Bouin's solution for 24 to 48 h (Bilinska et al. 2018). The testes were dehydrated in graded ethanol series, then cleared in xylene, embedded in paraffin blocks, and cut to a thickness of 4–6 μm. The specimens were deparaffinized using xylol and stained using hematoxylin and eosin (H&E) for light microscopical examination (Bancroft and Stevens 1996). The testicular damage severity score was assessed according to Tuglu et al. (2015). Briefly, different parameters were computed for each testicular section and the microscopic score was graded on a scale of mild (+), moderate (++), and severe (+++). These parameters comprised desquamation in germinal cells, disorganization in germinal cells, interstitial edema, degeneration in germinal cells, and reduction in germinal cell counts.

Statistical analysis

IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA) was used for data management. Data were checked for normality using the Shapiro-Wilk test and were presented as the mean ± standard error of the mean (SEM). Comparison of means was done using one-way analysis of variance (ANOVA). Because ANOVA is robust for small departures from normality but not for heterogeneity of variances, it was followed by Games-Howell post hoc test, when the test of homogeneity of variances was significant and followed by Duncan's Multiple range tests when variances were not different. Probability values less than 0.05 ($P < 0.05$) was considered significant.

Results

Effect of fractionated-LDR and/or EA on oxidative stress biomarkers

Figure 1a showed that the administration of nicotine-induced a significant elevation in the testicular TBARS content by 62% as compared to the normal control ($P < 0.05$). Neither exposure to LDR (2 × 0.25 Gy) nor administration of EA (10 mg/kg) guarded against the increase in TBARS content in comparison to the nicotine group. However, the combination of fractionated-LDR and EA tended to normalize the testicular content of TBARS. Moreover, the administration of nicotine led to a severe drop in GSH content by 88% as compared to the normal control ($P < 0.05$). Both fractionated-LDR and EA tended to prevent the decrease in GSH content as it reached 74% and 62%, respectively, compared to normal ($P < 0.05$). On the other hand, exposure to fractionated-LDR combined with EA markedly guarded against the reduction in GSH content which then reached only 36%, after nicotine administration (Fig. 1b).

Effect of fractionated-LDR and/or EA on androgenic key enzymes activities

The activities of 3β-HSD (Fig. 2a) and17β-HSD (Fig. 2b) in testicular tissue were decreased significantly ($p < 0.05$) in the nicotine group reaching 69% and 45%, respectively of normal control. Whereas, treatment with either LDR (2 × 0.25 Gy) or EA (10 mg/kg) has amended the inhibitory responses to these testicular enzyme activities. In comparison, combination treatment (fractionated-LDR + EA) showed more propensity to defend against the decline in androgenic key enzyme activities induced by nicotine than the individual treatment.

Effect of fractionated-LDR and/or EA on reproductive hormone levels

Nicotine-induced hormonal changes evidenced by a noticeable drop in FSH serum levels (Fig. 3a), LH (Fig. 3b), and testosterone (Fig. 3c) relative to normal control reaching 55%, 59%, and 65%, respectively. Treatment with either LDR (2 × 0.25 Gy) or EA (10 mg/kg) reversed the decline in these hormones, in comparison to the normal control ($p < 0.05$). On the other hand, combined therapy (fractionated-LDR + EA) showed a more pronounced effect than individual therapy in restoring hormone levels after nicotine administration.

Effect of fractionated-LDR and/or EA on LDH activity

Lactate dehydrogenase (LDH) serum activity was assessed to further examine the influence of LDR and/or EA on testicular damage caused by the nicotine. As illustrated in Fig. 4, nicotine induced a substantial rise ($P < 0.05$) in serum LDH activity reaching 63% above normal. Treatment with either LDR (2 × 0.25 Gy), EA (10 mg/kg) alone, or in combination tended to normalize LDH activity.

Histopathological findings

Light microscopic analysis of normal control rats' testicular tissue revealed closely packed seminiferous tubules lined by stratified germinal epithelium. Spermatogonia and Sertoli cells rested on intact basement membranes (Fig. 5 a1). The

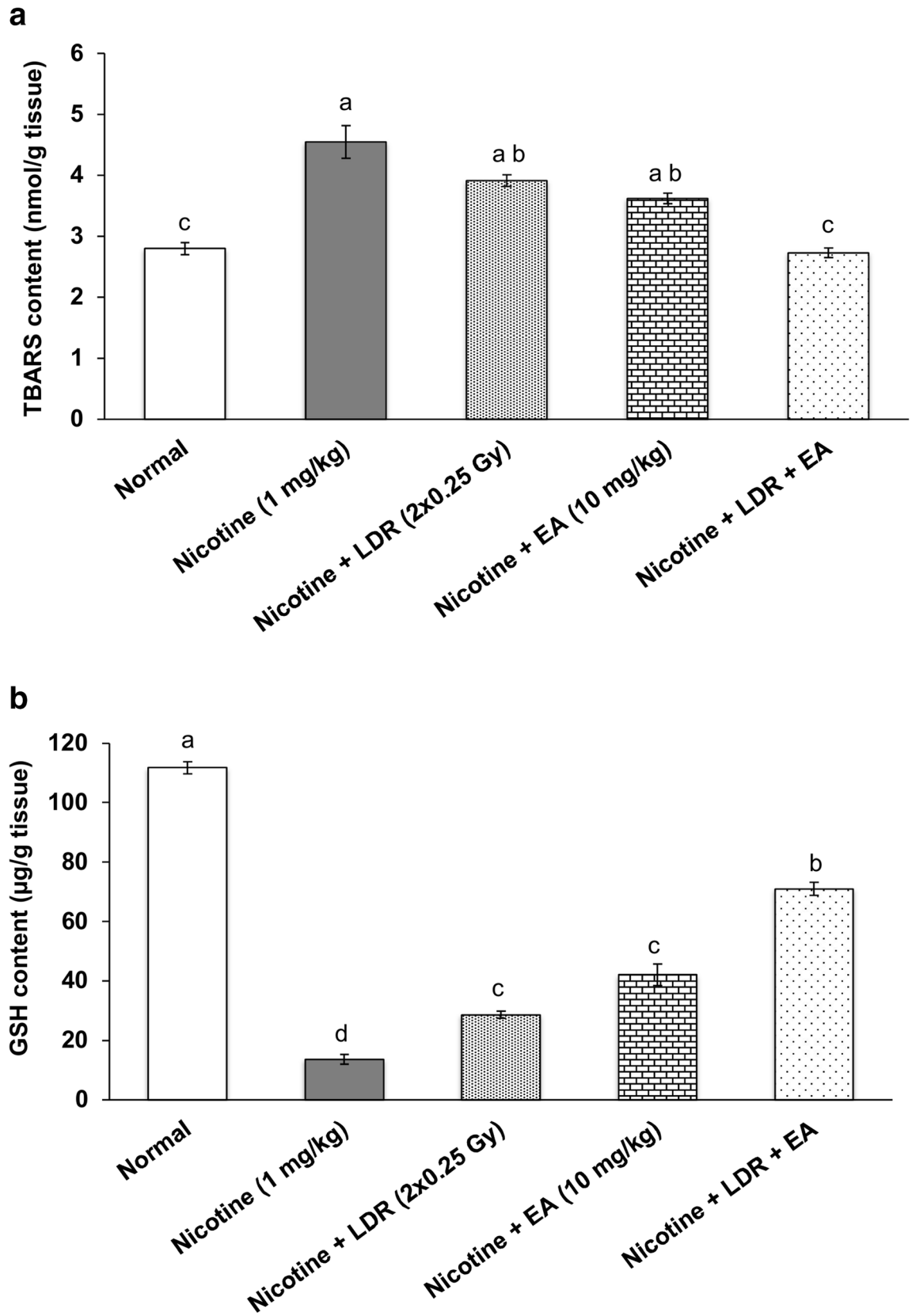

Fig. 1 Effect of oral administration of nicotine (1 mg/kg) and treatment with low-dose radiation (LDR, 2 × 0.25 Gy) and/or ellagic acid (EA, 10 mg/kg) on testicular **a** thiobarbituric acid reactive substances (TBARS) and **b** reduced glutathione (GSH) content in rats. Results are represented as mean ± SEM ($n = 8$/group). Groups with different superscript letters above column are significantly different ($P < 0.05$). Groups sharing the same superscript letters above column are non-significantly different ($P < 0.05$)

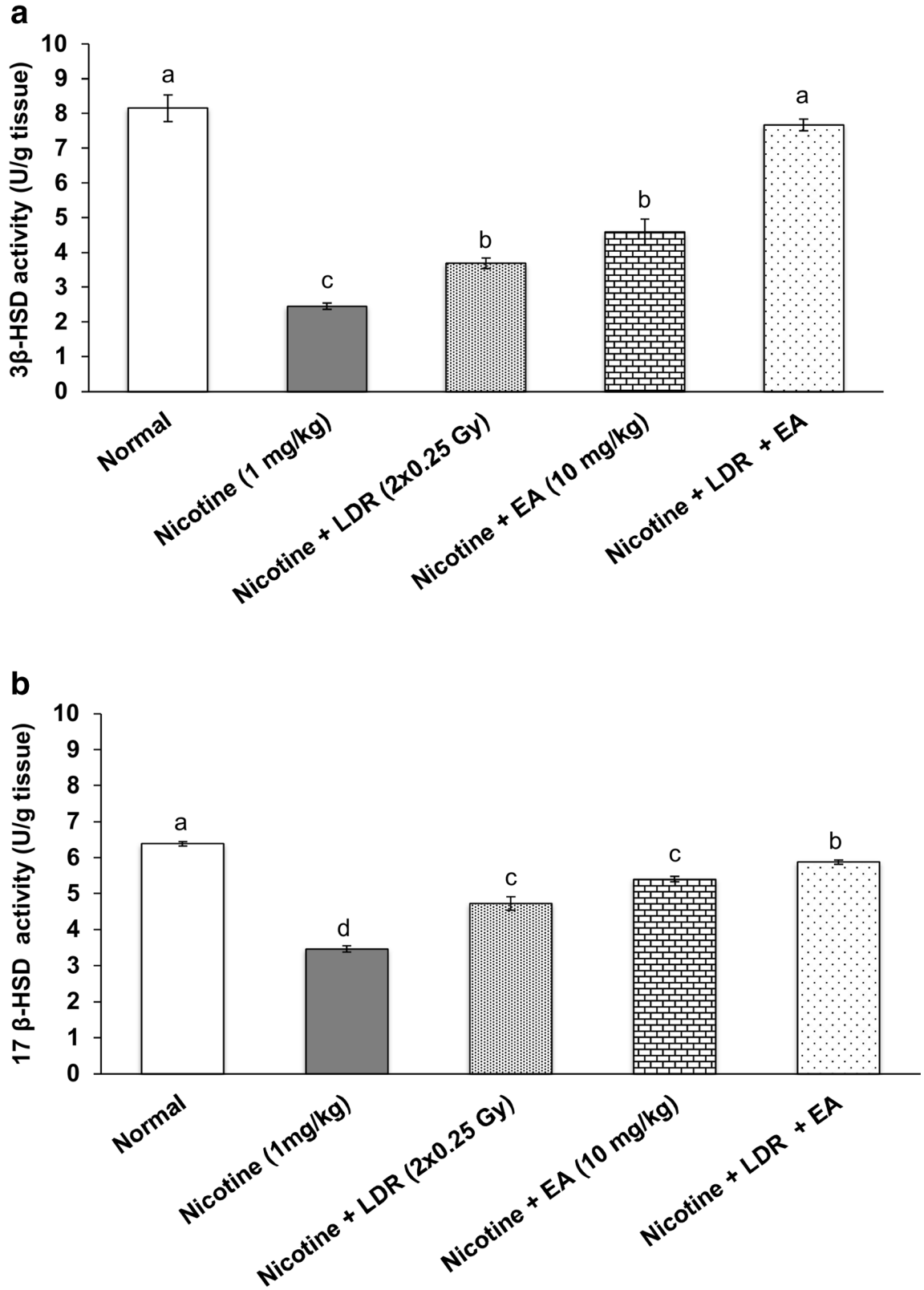

Fig. 2 Effect of oral administration of nicotine (1 mg/kg) and treatment with low-dose radiation (LDR, 2 × 0.25 Gy) and/or ellagic acid (EA, 10 mg/kg) on testicular **a** 3 beta-hydroxysteroid dehydrogenase (3 β-HSD) and **b** 17 beta-hydroxysteroid dehydrogenase (17β-HSD) activities in rats. Results are represented as mean ± SEM ($n = 8$/group). Groups with different superscript letters above column are significantly different ($P < 0.05$). Groups sharing the same superscript letters above column are non-significantly different ($P < 0.05$)

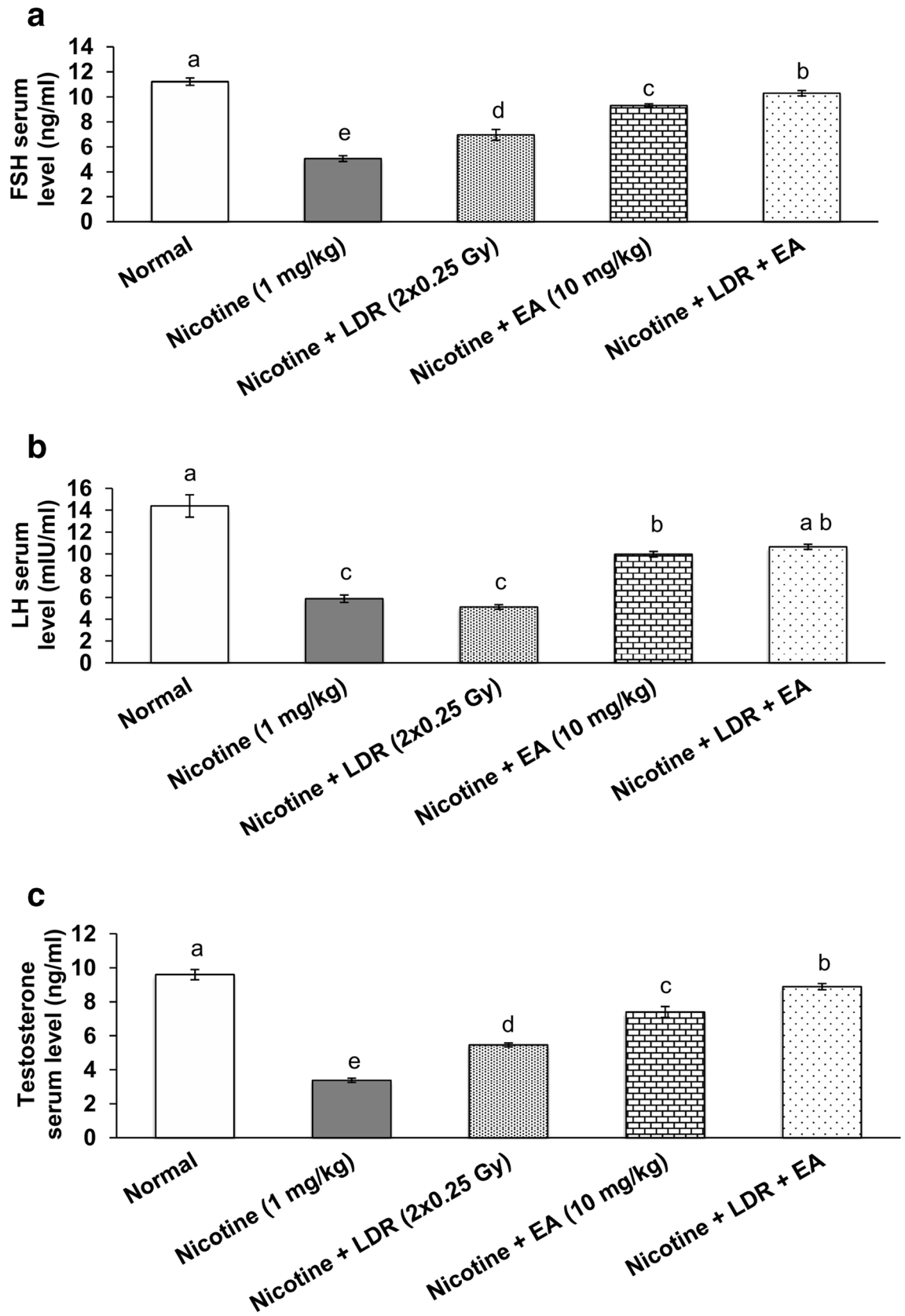

Fig. 3 Effect of oral administration of nicotine (1 mg/kg) and treatment with low-dose radiation (LDR, 2 × 0.25 Gy) and/or ellagic acid (EA, 10 mg/kg) on serum **a** follicle-stimulating hormone (FSH), **b** luteinizing hormone (LH), and testosterone **c** levels in rats. Results are represented as mean ± SEM ($n = 8$/group). Groups with different superscript letters above column are significantly different ($P < 0.05$). Groups sharing the same superscript letters above column are non-significantly different ($P < 0.05$)

spermatogenic cells consisted of several layers, namely the spermatogonia, primary and secondary spermatocytes, and spermatids. Narrow interstitium in-between the tubules contained clusters of a few Leydig cells with an acidophilic

the normal tubular lumen contained spermatids (Fig. 5 d1). However, the interstitial connective tissue showed edema which appeared as deeply eosinophilic homogenous areas in-between seminiferous tubules with a reduction of intersti-

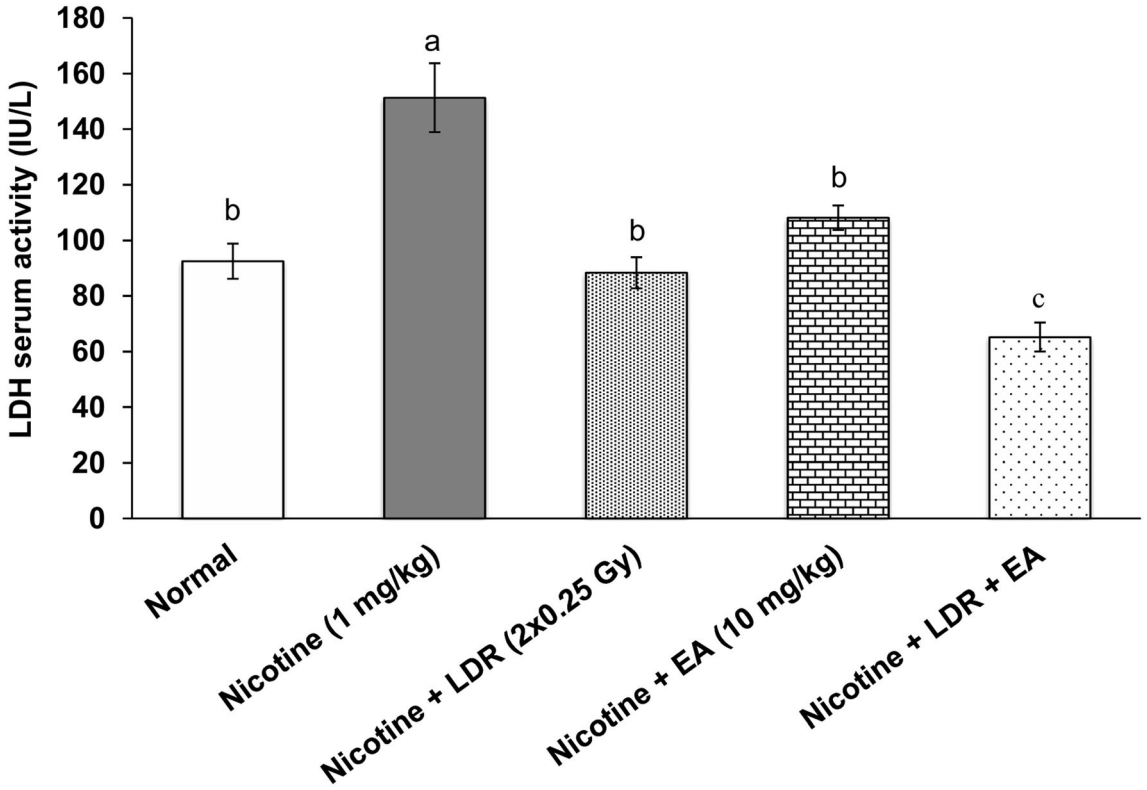

Fig. 4 Effect of oral administration of nicotine (1 mg/kg) and treatment with low-dose radiation (LDR, 2 × 0.25 Gy) and/or ellagic acid (EA, 10 mg/kg) on serum lactate dehydrogenase (LDH) activity in rats. Results are represented as mean ± SEM ($n = 8$/group). Groups with different superscript letters above column are significantly different ($P < 0.05$). Groups sharing the same superscript letters above column are non-significantly different ($P < 0.05$)

cytoplasm scattered between the seminiferous tubules and blood vessels (Fig. 5 a2). After nicotine administration, Fig. 5 b1 showed disorganization of spermatogenic cells within distorted shrunken seminiferous tubules with wide interstitium in-between. Additionally, the seminiferous tubules showed a reduction in the thickness of the germinal epithelium and wide empty lumina. In Fig. 5 b2, the spermatogenic cells had different histological alterations including pyknotic nuclei, necrotic cells, and vacuolated cytoplasm. The number of the cells in the spermatogenic series showed a marked decline in spermatogonia, primary and secondary spermatocytes numbers. Besides, Sertoli cells with vacuolated cytoplasm were seen, as a result, the damage severity score reached 3 (Table 1). Fractionated-LDR resulted in the disappearance of most of the histopathological lesions induced by nicotine taking score 2 as illustrated in Fig. 5 c and Table 1. Similarly, seminiferous tubules were protected by EA administration, and the spermatogenic cells appeared regularly arranged and tial cell number. A mild degree of germinal cell degeneration and less orderly, non-cohesive germinal cells and closely packed seminiferous tubules were seen (Fig. 5 d2). The best protective effects emerged in the combination group (LDR + EA) which revealed features approximately like those of the normal control (Fig. 5e). Accordingly, there was no change in the damage severity score from normal (Table 1).

Discussion

In the present study, nicotine-induced oxidative stress was evidenced by a significant change in the concentrations of testicular TBARS and reduced GSH. These findings are in line with earlier studies (Jana et al. 2010; Oyeyipo et al. 2014; Ray and Majumder 2018). Various and interrelated mechanisms may underlie the testicular toxicity induced by nicotine. For instance, the induction of oxidative stress is

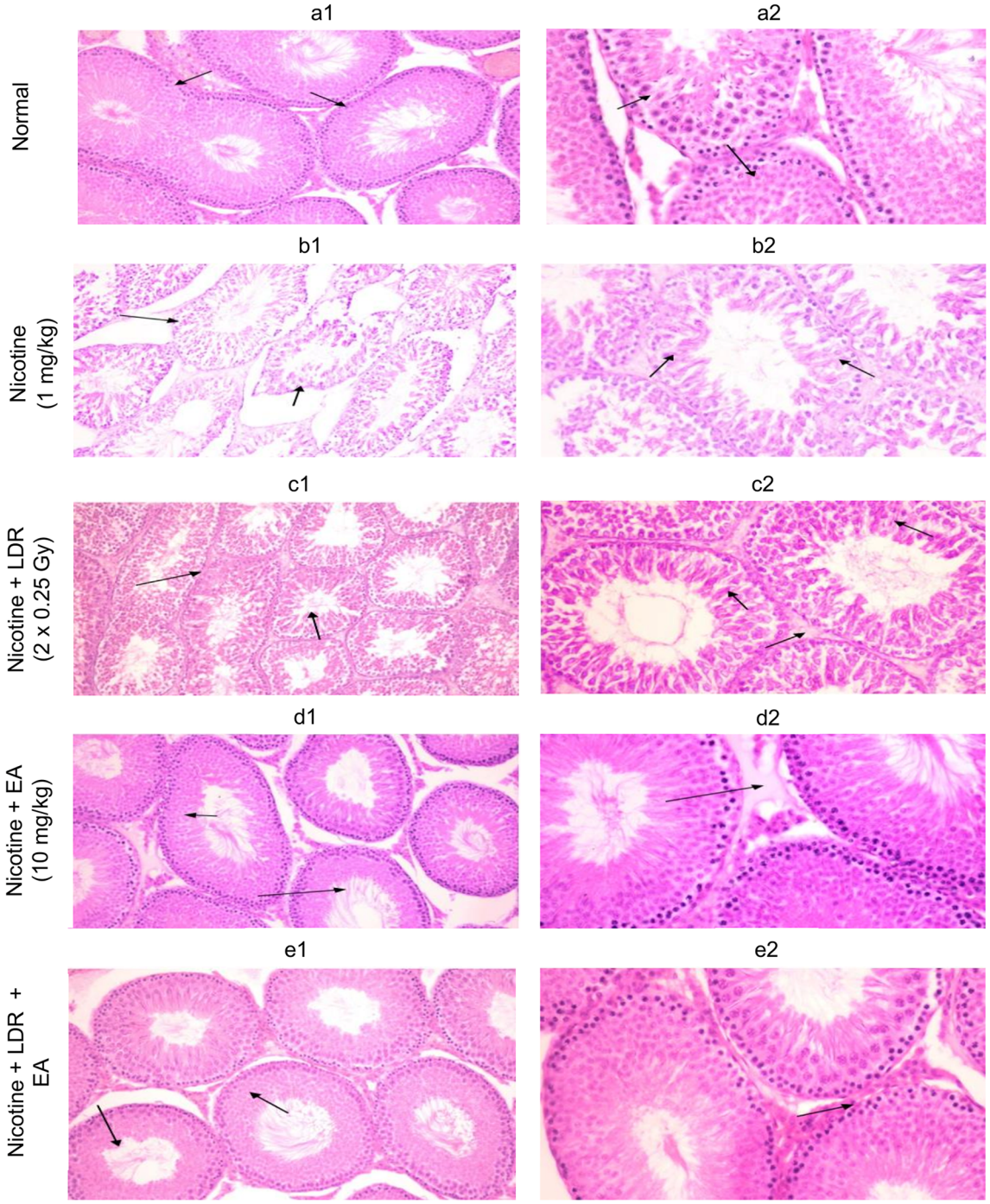

Fig. 5 Representative photomicrographs of testes sections stained with hematoxylin and eosin (HE) at different magnifications [× 200 (a1, b1, c1, d1, e1) and × 400 (a2, b2, c2, d2, e2)]. **a** The testicular histological sections of normal rats show (a1) active spermatogenesis in normal-size seminiferous tubules with thin basement membranes (arrow), and (a2) the spermatogenic cells consisted of many layers, primary and secondary spermatocytes and spermatids (arrow). **b** In the nicotine group, the testicular tissue shows (b1) distorted shrunken seminiferous tubules with wide interstitium (arrow), and (b2) the spermatogenic cells show pyknotic nuclei, necrotic cells, and vacuolated cytoplasm (arrow). **c** The testicular tissue of rats exposed to fractionated low-dose radiation (LDR) show (c1) sloughed germinal cells with shrunken pyknotic nuclei and less distinct seminiferous tubule borders (arrow), (c2) disorganization of spermatogenic cells, and interstitial edema (arrow). **d** Oral treatment with ellagic acid (EA) at a dose of 10 mg/kg, show (d1) spermatogenic cells appeared regularly arranged within the seminiferous tubules and normal tubular lumen contained spermatids (arrow) (d2) interstitial edema and reduction of interstitial cells number (arrow). **e** Treatment with fractionated-LDR in combination with EA show (e1) normal architecture of seminiferous tubules (arrow), and (e2) narrow interstitium in-between the tubules (arrow)

Table 1 Effect of oral administration of nicotine (1 mg/kg) and treatment with fractionated low-dose radiation (LDR, 2 × 0.25 Gy) and/or ellagic acid (EA, 10 mg/kg) on testicular damage severity score

Parameters groups	Desquamation in germinal cells	Disorganization in germinal cells	Interstitial edema	Degeneration and necrosis germinal cells	Reduction in germinal cells
Normal (control)	ND	ND	ND	ND	ND
Nicotine (1 mg/kg)	+++	+++	++	+++	+++
Nicotine + LDR (2×0.25 Gy)	++	++	+	+	+
Nicotine + EA (10 mg/kg)	+	+	ND	ND	+
Nicotine + LDR + EA	ND	ND	ND	ND	ND

Mild (+), moderate (++), severe (+++), not detected (ND)

expected to affect the sperm cells because they are rich in polyunsaturated fatty acids that make them vulnerable to ROS attacks (Rajpurkar et al. 2000; Aitken and Baker 2006; Bisht et al. 2017). As a result, it can induce lipid peroxidation, alter DNA, RNA, and protein functions in spermatozoa and other testicular cells (Darbandi and Darbandi 2016). Moreover, the ROS can decrease the level of male reproductive hormones, and directly or indirectly disrupts the hormonal balance by inducing oxidative stress or by acting on the release of the hormones from the hypothalamic axis, leading to infertility (Appasamy et al. 2007; Spiers et al. 2015). That mechanisms were elucidated in the findings of this study, in which the activities of 3β-HSD and 17β-HSD which are the key enzymes responsible for the synthesis of male reproductive hormones were decreased after nicotine administration (Seema et al. 2007; Jana et al. 2010). Besides, the anterior pituitary gonadotropic hormones (FSH and LH) were reduced attributable to either the activation of oxidative stress or the harmful effect of nicotine on the central nervous system by inhibiting the neuronal stimulus required for pituitary gonadotropins release (Reddy et al. 1995). Furthermore, serum testosterone levels were significantly reduced as a consequence of many factors: the decrease in FSH and LH levels that maintain testosterone levels via the hypothalamic-pituitary-testicular axis (Funabashi et al. 2005; Tweed et al. 2012; Oyeyipo et al. 2013), disruption of testicular cytoarchitecture which adversely affects the number and function of Leydig cells (Oyeyipo et al. 2010), and/or the cholinergic nicotine agonist activity which was reported to inhibit testosterone secretion (Kasson and Hsueh 1985). The observed reduction in testosterone along with a decline in FSH and LH following administration of nicotine is compatible with several previous studies (Jana et al. 2010; Kolawole et al. 2019). Another study, on the other hand, reported that the reduction in testosterone serum levels is concomitant with the rise in FSH and LH serum levels. They assumed that nicotine has local testicular damaging effects and explained the rising of FSH and LH by compensatory feedback mechanisms after testosterone reduction (Ramlau-Hansen et al. 2007).

Moreover, LDH is a cytoplasmic marker enzyme and plays a major role in the process of spermatogenesis (Kaur and Bansal 2004). In the current study, nicotine administration caused an increase in LDH serum activity which was taken as a reflection for testicular degeneration. According to Yildiz et al. (1999), the increment in LDH activity may be due to oxidative stress induced by nicotine, which can cause significant changes in the molecular organization of lipids, resulting in increased membrane permeability and infiltration of cytoplasmic markers (such as LDH) into the circulation (Wetscher et al. 1995; Madole et al. 2016).

Additionally, evidence of testicular toxicity was reflected histologically by changes in the focal detachment of the basal spermatogenic cells from their basement membrane and frequent deficiency of elongated mature spermatozoa in seminiferous tubules. Such histopathologic results were compatible with Ray and Majumder (2018), who reported that the histological examination of testes revealed atrophy, degenerative change in seminiferous tubules.

Various studies have been conducted on the beneficial effects of LDR in different models, for instance, El-Ghazaly et al. (2015, 1985), Chen et al. (2000), and Nowosielska et al. (2009). The study of Liebmann et al. (2004) showed that the therapeutic effect of LDR is time and fractionation-dependent. In the same concept, El-Ghazaly et al. (2020), showed that the fractionated-LDR (2 × 0.25 Gy) in the treatment of arthritis was more effective than the single exposure (0.5 Gy) could be due to the timing effect as the intervals between the endpoint and the last dose of irradiation was not equal (8 days vs. 1 day). On the same line, the present study showed that fractionated-LDR at a total dose level of 0.5 Gy (2 × 0.25 Gy/1-week interval) was capable to reduce the testicular toxicity induced by nicotine administration. Since, it has been reported that LDR has antioxidant properties (Kojima et al. 2002; Yoshimoto et al. 2012; Lee et al. 2013). Therefore, it is possible to postulate that the beneficial effects of LDR in this study may be partially mediated by its antioxidant properties and counteraction to oxidative stress. The observed decrease in testicular content of TBARS was accompanied by an increase in reduced GSH. As a result, the levels of reproductive hormones (FSH, LH, and testosterone), androgenic enzyme (3β-HSD, 17β-HSD) activities were increased,

along with a reduction in LDH activity and histopathological improvements. Our observations are in agreement with Zhao et al. (2010), who reported that repetitive exposures to LDR alleviate testicular apoptotic cell death, hormones serum levels (FSH, LH, and testosterone), and testicular oxidative damage and antioxidant contents (superoxide dismutase, catalase, and GSH) induced by diabetes. Besides, the studies by Kojima et al. (1998a,b), showed that exposure of mouse to LDR stimulates antioxidant production and protection from oxidative damage as the levels of reduced GSH, glutathione reductase (GR), γ- glutamylcysteine synthetase (γ-GCS), and thioredoxin (TRX) elevated in either liver or brain shortly post-irradiation with 50 cGy of gamma rays.

In earlier studies, EA demonstrated a defensive response against testicular damage induced by various toxic agents such as cisplatin (Türk et al. 2008, 2011), cyclophosphamide (Türk et al. 2010), and adriamycin (Çeribaşı et al. 2012). Regarding the present study, EA was considered to be effective at a dosage of 10 mg/kg in ameliorating the nicotine-induced testicular toxicity and hormonal change. The treatment with EA after nicotine administration was found to guard against all the deranged biochemical parameters such as the oxidative stress, androgenic enzyme, reproductive hormones, LDH, besides the histological changes evoked by nicotine administration. The precise mechanism of EA has not been fully detected. Numerous in vivo and in vitro studies have demonstrated, as reviewed by Izquierdo-Vega et al. (2019), that the beneficial impact of EA on male fertility may be due in part to its antioxidant properties. Where the EA alleviates oxidative damage through its radical-clearing properties (García-Niño and Zazueta 2015; Akkoyun and Karadeniz 2016) as well as potentiation of biological antioxidants and antioxidant enzymes activities (Kannan and Quine 2011; Kaya et al. 2017; Chen et al. 2018). In parallel with the aforementioned studies, it has been speculated that the observed improvement in the testicular toxicity and hormonal change induced by nicotine might be related to the antioxidant effect of EA.

Although the current study relied on the estimation of testicular contents of TBARS and reduced GSH as one of the representative markers of oxidative stress. This may, however, be considered as a limitation of our study, and further evaluation of broader panels of biomarkers of oxidative stress is required to reflect the prominent role of oxidative stress as a contributor to hormonal changes and testicular toxicity induced by nicotine and to demonstrate the antioxidant effect of EA.

In conclusion, our study showed that oxidative stress may have a contributed role in nicotine-induced hormonal changes and testicular toxicity. Additionally, the current study elicited that fractionated-LDR combined with EA might represent a promising candidate for the amelioration of nicotine detrimental effects on rat testes, which is possibly attributable to their combined antioxidant properties.

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Author' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Aliaa H. Ashoub, Doaa H. Abdel-Naby, Marwa M. Safar, Mona A. El-Ghazaly, and Sanaa A. Kenawy. The first draft of the manuscript was written by Aliaa H. Ashoub and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript to be submitted.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical approval All experiments were performed following the guidelines set by the Environment-European Commission (EEC) (revised directive 86/609/EEC), and an ethical approval (Permit no: PT 1175) was granted from the Ethical Committee for Animal Experimentation, Faculty of Pharmacy, Cairo University.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Aitken RJ, Baker MA (2006) Oxidative stress, sperm survival and fertility control. Mol Cell Endocrinol 250:66–69. https://doi.org/10.1016/j.mce.2005.12.026

Akkoyun HT, Karadeniz A (2016) Investigation of the protective effect of ellagic acid for preventing kidney injury in rats exposed to nicotine during the fetal period. Biotech Histochem 91:108–115. https://doi.org/10.3109/10520295.2015.1078910

Appasamy M, Muttukrishna S, Pizzey A, Ozturk O, Groome N, Serhal P, Jauniaux E (2007) Relationship between male reproductive hormones, sperm DNA damage and markers of oxidative stress in infertility. Reprod BioMed Online 14:159–165. https://doi.org/10.1016/s1472-6483(10)60783-3

Aydos K, Guven MC, Can B, Ergun A (2001) Nicotine toxicity to the ultra-structure of the testis in rats. BJU Int 88:622–626. https://doi.org/10.1046/j.1464-4096.2001.02384.x

Bancroft JD, Stevens A (1996) Theory and practice of histological techniques, 4th edn. Churchill Livingstone, New York, p 766

Bandyopadhyay G, Sinha S, Chattopadhyay BD, Chakraborty A (2008) Protective role of cucumin against nicotine induced genotoxicity on rat liver under restricted dietary protein. Eur J Pharmacol 588:151–157. https://doi.org/10.1016/j.ejphar.2008.04.023

Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888

Bilinska B, Hejmej A, Kotula-Balak M (2018) Preparation of testicular samples for histology and immunohistochemistry. Methods Mol Biol 1748:17–36. https://doi.org/10.1007/978-1-4939-7698-0_3

Bisht S, Faiq M, Tolahunase M, Dada R (2017) Oxidative stress and male infertility. Nat Rev Urol 14:470–485. https://doi.org/10.1038/nrurol.2017.69

Bose M, Debnath D, Chen Y, Bose HS (2007) Folding, activity and import of steroidogenic acute regulatory protein into mitochondria changed by nicotine exposure. J Mol Endocrinol 39:67–79

Celik G, Semiz A, Karakurt S, Arslan S, Adali O, Sen A (2013) A comparative study for the evaluation of two doses of ellagic acid on hepatic drug metabolizing and antioxidant enzymes in the rat. Biomed Res Int 2013:1–9. https://doi.org/10.1155/2013/358945

Çeribaşı AO, Sakin F, Türk G, Sönmez M, Ateşşahin A (2012) Impact of ellagic acid on adriamycin-induced testicular histopathological lesions, apoptosis, lipid peroxidation and sperm damages. Exp Toxicol Pathol 64:717–724. https://doi.org/10.1016/j.etp.2011.01.006

Chen P, Chen F, Zhou B (2018) Antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid in liver and brain of rats treated by D-galactose. Sci Rep 8:1465. https://doi.org/10.1038/s41598-018-19732-0

Chen SL, Cai L, Meng QY, Xu S, Wan H, Liu SZ (2000) Low-dose whole-body irradiation (LD-WBI) changes protein expression of mouse thymocytes: effect of a LD-WBI-enhanced protein RIP10 on cell proliferation and spontaneous or radiation-induced thymocyte apoptosis. Toxicol Sci 55:97–106. https://doi.org/10.1093/toxsci/55.1.97

Darbandi S, Darbandi M (2016) Lifestyle modifications on further reproductive problems. Cresco J Reprod Sci 1:1–2

Devipriya N, Srinivasan M, Sudheer AR, Menon VP (2007) Effect of ellagic acid, a natural polyphenol, on alcohol-induced prooxidant and antioxidant imbalance: a drug dose dependent study. Singap Med J 48:311–318

El-Ghazaly M, Kenawy S, Khayyal MT, Roushdy H, Saleh S (1985) Effect of exposure to radiation on the inflammatory process and its influence by diclofenac. Br J Pharmacol 85:45–50. https://doi.org/10.1111/j.1476-5381.1985.tb08829.x

El-Ghazaly MA, Fadel NA, Abdel-Naby DH, Abd El-Rehim HA, Zaki HF, Sanaa A, Kenawy SA (2020) Amelioration of adjuvant-induced arthritis by exposure to low dose gamma radiation and resveratrol administration in rats. Int J Radiat Biol 9:1–11. https://doi.org/10.1080/09553002.2020.1748911

El-Ghazaly MA, Sadik NA, Rashed ER, Abd-El-Fattah AA (2015) Neuroprotective effect of EGb761® and low-dose whole-body γ-irradiation in a rat model of Parkinson's disease. Toxicol Ind Health 31:1128–1143. https://doi.org/10.1177/0748233713487251

Feinendegen LE (2005) Evidence for beneficial low level radiation effects and radiation hormesis. Br J Rad 78:3–7. https://doi.org/10.1259/bjr/63353075

Funabashi T, Sano A, Mitsushima D, Kimura F (2005) Nicotine inhibits pulsatile luteinizing hormone secretion in human males but not in human females, and tolerance to this nicotine effect is lost within one week of quitting smoking. J Clin Endocrinol Metab 90(3):908–913. https://doi.org/10.1210/jc.2005-0041

García-Niño WR, Zazueta C (2015) Ellagic acid: pharmacological activities and molecular mechanisms involved in liver protection. Pharmacol Res 97:84–103. https://doi.org/10.1016/j.phrs.2015.04.008

Georgy GS, Maher OW (2017) Ellagic acid and rosmarinic acid attenuate doxorubicin-induced testicular injury in rats. J Biochem Mol Toxicol 31:9. https://doi.org/10.1002/jbt.21937

Girish C, Shweta O, Raj V, Balakrishnan S, Varghese RG (2014) Ellagic acid modulates sodium valproate induced reproductive toxicity in male Wistar rats. Indian J Physiol Pharmacol 58:416–422

Hayes WJ (1982) Pesticides studied in man. Lippincott Williams & Wilkins, Baltimore, London pp 86–91

Ina Y, Tanooka H, Yamada T, Sakai K (2005) Suppression of thymic lymphoma induction by life-long low-dose-rate irradiation accompanied by immune activation in C57BL/6 mice. Radiat Res 163:153–158. https://doi.org/10.1667/rr3289

Izquierdo-Vega JA, Madrigal-Santillán EO, Chavez-Pagola JT, Valadez-Vega CM, Sánchez-Gutiérrez M (2019) The activity of ellagic acid in male reproduction: a mini-review. Int J Med Rev 6:135–139. https://doi.org/10.30491/ijmr.2019.101966

Jana K, Samanta PK, De DK (2010) Nicotine diminishes testicular gametogenesis, steroidogenesis, and steroidogenic acute regulatory protein expression in adult albino rats: possible influence on pituitary gonadotropins and alteration of testicular antioxidant status. Toxicol Sci 116:647–659. https://doi.org/10.1093/toxsci/kfq149

Jarabak J, Adams JA, Williams-Ashman HG, Talalay P (1962) Purification of a 17β-hydroxysteroid dehydrogenase of human placenta and studies on its transhydrogenase function. J Biol Chem 237:345–357

Kannan MM, Quine SD (2011) Ellagic acid ameliorates isoproterenol induced oxidative stress: evidence from electrocardiological, biochemical and histological study. Eur J Pharmacol 659:45–52. https://doi.org/10.1016/j.ejphar.2011.02.037

Kasson BG, Hsueh AJ (1985) Nicotinic cholinergic agonists inhibit androgen biosynthesis by cultured rat testicular cells. Endocrinology 117:1874–1880. https://doi.org/10.1210/endo-117-5-1874

Kaur P, Bansal MP (2004) Influence of selenium induced oxidative stress on spermatogenesis and lactate dehydrogenase-X in mice testis. Asian J Androl 6:227–232

Kavitharaj NK, Vijayammal PL (1999) Nicotine administration induced changes in the gonadal functions in male rats. Pharmacol 58:2–7. https://doi.org/10.1159/000028262

Kaya N, Ozan G, Dabak DO, Gur S, Ozan IE (2017) Antioxidant effects of ellagic acid on testicular tissue of rats exposed to tobacco smoke metabolite-acetic acid. J Turgut Ozal Med Cent 24:381–386. https://doi.org/10.5455/jtomc.2017.05.068

Kojima S, Ishida H, Takahashi M, Yamaoka K (2002) Elevation of glutathione induced by low-dose gamma rays and its involvement in increased natural killer activity. Radiat Res 157:275–280. https://doi.org/10.1667/0033-7587(2002)157[0275:eogibl]2.0.co;2

Kojima S, Matsuki O, Nomura T, Kubodera A, Honda Y, Honda S, Tanooka H, Wakasugi H, Yamaoka K (1998a) Induction of mRNAs for glutathione synthesis-related proteins in mouse liver by low doses of γ-rays. Biochem Biophys Acta 1381:312–318. https://doi.org/10.1016/s0304-4165(98)00043-9

Kojima S, Matsuki O, Nomura T, Shimura N, Kubodera A, Yamaoka K, Tanooka H, Wakasugi H, Honda Y, Honda S, Sasaki T (1998b) Localization of glutathione and induction of glutathione synthesis-related proteins in mouse brain by low doses of γ rays. Brain Res 808:262–269. https://doi.org/10.1016/s0006-8993(98)00832-4

Kolawole T, Adienbo O, Dapper V (2019) Ameliorative effects of hydromethanolic extract of citrullus lanatus (watermelon) rind on semen parameters, reproductive hormones, and testicular oxidative status following nicotine administration in male wistar rats. Niger J Physiol Sci 34:83–90

Lee EK, Kim JA, Kim JS, Park SJ, Heo K, Yang KM, Son TG (2013) Activation of de novo GSH synthesis pathway in mouse spleen after long term low-dose γ-ray irradiation. Free Radic Res 47:89–94. https://doi.org/10.3109/10715762.2012.747678

Li W, Wang G, Cui J, Xue L, Cai L (2004) Low-dose radiation (LDR) induces hematopoietic hormesis: LDR-induced mobilization of hematopoietic progenitor cells into peripheral blood circulation. Exp Hematol 32:1088–1096. https://doi.org/10.1016/j.exphem.2004.07.015

Liebmann A, Hindemith M, Jahns J, Madaj-Sterba P, Weisheit S, Kamprad F, Hildebrandt G (2004) Low-dose X-irradiation of adjuvant-induced arthritis in rats. Efficacy of different fractionation schedules. Strahlenther Onkol 180:165–172

Liu G, Gong P, Zhao H, Wang Z, Gong S, Cai L (2006) Effect of low-level radiation on the death of male germ cells. Radiat Res 165:379–389. https://doi.org/10.1667/rr3528.1

Liu SZ (2010) Biological effects of low level exposures to ionizing radiation: theory and practice. Hum Exp Toxicol 29:275–281. https://doi.org/10.1177/0960327109363967

Luckey TD (1982) Physiological benefits from low levels of ionizing radiation. Health Phys 43:771–789. https://doi.org/10.1097/00004032-198212000-00001

Madole MB, Dilip B, Mamatha MT, Ankur P (2016) Study of serum lactate dehydrogenase and lipid profile in patients with chronic cough. Int J Clin Biochem Res 3:409–412

Mohamed DA, Abdelrahman SA (2019) The possible protective role of zinc oxide nanoparticles (ZnONPs) on testicular and epididymal structure and sperm parameters in nicotine-treated adult rats (a histological and biochemical study). Cell Tissue Res 375:543–558. https://doi.org/10.1007/s00441-018-2909-8

Mosbah R, Yousef MI, Mantovani A (2015) Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: the protective effects of green tea extract. Exp Toxicol Pathol 67:253–259

Nowosielska EM, Cheda A, Wrembel-Wargocka J, Janiak MK (2009) Immunological mechanism of the low-dose radiation induced suppression of cancer metastases in a mouse model. Dose-Response 8:209–226. https://doi.org/10.2203/dose-response.09-016.Nowosielska

Okamoto M, Kita T, Okuda H, Tanaka T, Nakashima T (1994) Effects of aging on acute toxicity of nicotine in rats. Pharmacol Toxicol 75:1–6

Olivieri G, Bodycote J, Wolff S (1984) Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 223:594–597. https://doi.org/10.1126/science.6695170

Oyeyipo IP, Raji Y, Bolarinwa AF (2013) Nicotine alters male reproductive hormones in male albino rats: the role of cessation. J Hum Reprod Sci 6:40–44. https://doi.org/10.4103/0974-1208.112380

Oyeyipo IP, Raji Y, Bolarinwa AF (2014) Antioxidant profile changes in reproductive tissues of rats treated with nicotine. J Hum Reprod Sci 7:41–46. https://doi.org/10.4103/0974-1208.130823

Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF (2010) Effects of oral administration of nicotine on organ weight, serum testosterone level and testicular pathology in adult male rats. Niger J Physiol Sci 25:81–86

Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF (2011) Effects of nicotine on sperm characteristics and fertility profile in adult male rats: a possible role of cessation. J Reprod Infertil 12:201–207

Rajpurkar A, Dhabuwala CB, Jiang Y, Li H (2000) Chronic cigarette smoking induces an oxidant antioxidant imbalance in the testis. J Environ Pathol Toxicol Oncol 19:369–373

Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP (2007) Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. Hum Reprod 22:188–196. https://doi.org/10.1093/humrep/del364

Ray D, Majumder S (2018) The protective effects of chlorophytum borivilianum on nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats. JIPBS 5:76–81

Reddy A, Sood A, Rust PF, Busby JE, Varn E, Mathur RS, Mathur S (1995) The effect of nicotine on in-vitro sperm motion characteristics. J Assist Reprod Genet 12:217–223. https://doi.org/10.1007/BF02211802

Sarasin A, Schlumpf M, Muller M, Fleischmann I, Lauber ME, Lichtensteiger W (2003) Adrenal-mediated rather than direct effects of nicotine as a basis of altered sex steroid synthesis in fetal and neonatal rat. Reprod Toxicol 17:153–162. https://doi.org/10.1016/s0890-6238(02)00119-3

Seema P, Swathy SS, Indira M (2007) Protective effect of selenium on nicotine-induced testicular toxicity in rats. Biol Trace Elem Res 120:212–218. https://doi.org/10.1007/s12011-007-8021-7

Segarra AC, Strand FL (1989) Prenatal administration of nicotine alters subsequent sexual behaviour and testosterone levels of male rats. Brain Res 480:151–159. https://doi.org/10.1016/0006-8993(89)91577-1

Spiers JG, Chen HJ, Sernia C, Lavidis NA (2015) Activation of the hypothalamic pituitary-adrenal stress axis induces cellular oxidative stress. Front Neurosci 8:456. https://doi.org/10.3389/fnins.2014.00456

Sudheer AR, Muthukumaran S, Devipriya N, Devraj H, Menon VP (2008) Influence of ferulic acid on nicotine induced lipid peroxidation, DNA damage and inflammation in experimental rats as compared to N-acetylcysteine. Toxicology 243:317–329. https://doi.org/10.1016/j.tox.2007.10.016

Talalay P (1962) Hydroxysteroid dehydrogenase. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol 5. Academic Press, New York, pp 512–516

Tuglu D, Yuvanc E, Yilmaz E, Gencay IY, Atasoy P, Kisa U, Batislam E (2015) The antioxidant effect of dexmedetomidine on testicular ischemia-reperfusion injury. Acta Cir Bras 30:414–421. https://doi.org/10.1590/S0102-865020150060000007

Türk G, Ateşşahin A, Sönmez M, Çeribaşı AO, Yüce A (2008) 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 89:1474–1481. https://doi.org/10.1016/j.fertnstert.2007.04.059

Türk G, Çeribaşi AO, Sahna E, Ateşşahin A (2011) Lycopene and ellagic acid prevent testicular apoptosis induced by cisplatin. Phytomedicine 18:356–361. https://doi.org/10.1016/j.phymed.2010.07.008

Türk G, Çeribaşi AO, Sakin F, Sönmez M, Ateşşahin A (2010) Antiperoxidative and anti-apoptotic effects of lycopene and ellagic acid on cyclophosphamide-induced testicular lipid peroxidation and apoptosis. Reprod Fertil Dev 22:587–596. https://doi.org/10.1071/RD09078

Tweed JO, Hsia SH, Lutfy K, Friedman TC (2012) The endocrine effects of nicotine and cigarette smoke. Trends Endocrinol Metab 23:334–342. https://doi.org/10.1016/j.tem.2012.03.006

Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278. https://doi.org/10.1016/0003-2697(78)90342-1

Ukwenya VO, Olawuyi TS, Adam AM, Ukwenya MU (2020) Hormonal changes and redox imbalance in nicotine-induced testicular toxicity: the mitigating influence of D-ribose L-cysteine JOBAZ 81:48.https://doi.org/10.1186/s41936-020-00173-z

Umesalma S, Sudhandiran G (2011) Ellagic acid prevents rat colon carcinogenesis induced by 1, 2 dimethyl hydrazine through inhibition of AKT-phosphoinositide- 3 kinase pathway. Eur J Pharmacol 660:249–258. https://doi.org/10.1016/j.ejphar.2011.03.036

Wetscher GJ, Bagchi D, Perdikis G, Bagchi M, Redmond EJ, Hinder PR, Glaser K, Hinder RA (1995) In vitro free radical production in rat oesophageal mucosa induced by nicotine. Dig Dis Sci 40:853–858. https://doi.org/10.1007/BF02064991

Yamamoto Y, Isoyama E, Sofikitis N, Miyagawa I (1998) Effects of smoking on testicular function and fertilizing potential in rats. Urol Res 26:45–48. https://doi.org/10.1007/s002400050022

Yamaoka K, Edamatsu R, Mori A (1991) Increased SOD activities and decreased lipid peroxide levels induced by low dose X irradiation in rat organs. Free Radic Biol Med 11:299–306. https://doi.org/10.1016/0891-5849(91)90127-O

Yang G, Li W, Jiang H, Liang X, Zhao Y, Yu D, Zhou L, Wang G, Tian H, Han F, Cai L, Cui J (2016) Low-dose radiation may be a novel approach to enhance the effectiveness of cancer therapeutics. Int J Cancer 139:2157–2168. https://doi.org/10.1002/ijc.30235

Yildiz D, Liu YS, Ercal N, Armstrong DW (1999) Comparison of pure nicotine and smokeless tobacco extract induced toxicities and oxidative stress. Arch Environ Contam Toxicol 37:434–439. https://doi.org/10.1007/s002449900537

Yoshimoto M, Kataoka T, Toyota T, Taguchi T, Yamaoka K (2012) Inhibitory effects of prior low dose X-irradiation on cold-induced brain injury in mouse. Inflammation 35:89–97. https://doi.org/10.1007/s10753-011-9293-9

Zhao H, Xu S, Wang Z, Li Y, Guo W, Lin C, Gong S, Li C, Wang G, Cai L (2010) Repetitive exposures to low-dose X-rays attenuate testicular apoptotic cell death in streptozotocin-induced diabetes rats. Toxicol Lett 192:356–364. https://doi.org/10.1016/j.toxlet.2009.11.011