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Studying the Efficiency of Irradiated *Ginkgo biloba* Leaves Against Nicotine Damage in Male Rats

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ABSTRACT

Exposure to nicotine leads to the activation of inflammatory factors, overproduction of free radicals, and hormonal disruption. *Ginkgo biloba* leaf extract can be used as a powerful antioxidant and free radical scavenger owing to its active compounds, but they may be contaminated by a variety of toxins that limit their efficiency. This study aimed to investigate the effect of two types of decontaminating-irradiation sources (gamma rays and electron beam irradiation (EBI)) at 5 kGy on the antioxidant contents (total phenolic and flavonoid contents) and antioxidant activities of *Ginkgo biloba* powder and investigate the potential protective effect of gamma-irradiated *G. biloba* aqueous extract (GGBE) against nicotine-induced cardiac and testicular toxicity in male rats. The HPLC chromatogram revealed twenty bioactive compounds in *Ginkgo biloba* (one phenolic acid, 15 flavonoid compounds, and 4 terpene lactones). Also, the data indicated that gamma rays were the most effective irradiation source for improving the antioxidant activity and increasing the level of phenolic compounds in *G. biloba* by percent change higher than that induced by electron-beam irradiation (EBI). The results of the biological study revealed that nicotine injection (1.0 mg/kg b.wt./ day/ 6 weeks) induced cardiac dysfunction, endocrine disruption on male hormone profile, inflammation, the elevation of lipid peroxidation products and lower the antioxidant status of male rats compared to the control group. Whereas administration of nicotine along with GGBE (150 mg/kg b.wt./day/6 weeks) significantly reduced the nicotine toxicity observed by a reduction in the level of cardiac markers, inflammatory cytokines, and the level of testicular and cardiac malondialdehyde, elevation of the serum level of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and the level of testicular and cardiac glutathione (GSH) and antioxidant enzymes (SOD and CAT) compared to NIC-group. In conclusion, this study suggested that gamma-ray irradiation by (5 kGy) can be utilized to boost biological effects (antioxidant properties and antioxidant activities) effectively. Also, the results concluded that gamma-irradiated *G. biloba* aqueous extract exhibit cardiac and testicular protective effects against nicotine toxicity.

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Key words
Nicotine, *Ginkgo biloba*, Gamma rays, Electron-beam irradiation, Inflammatory cytokines

INTRODUCTION

Cigarette, hookah, or pipe smoking is the worst addictive habit among teenagers and young adults and is considered a major public health problem (Jalili *et al*., 2021). Tobacco smoke is a mixture of more than 4000 components including carcinogens, oxidants, and aldehydes that can cause inflammation and damage to cells. Nicotine is the most addictive and toxic component in tobacco smoke as it can induce the overgeneration of reactive oxygen species (ROS) in tissues and the development of cardiovascular disorders, pulmonary diseases, lung cancer, and many other diseases (Jalili *et al*., 2021). Additionally, this toxic component adversely affects the male reproductive system and fertility, causes disturbances in the function of Leydig cells, and acts as an endocrine disturbance on the male hormone profile, specifically on luteinizing hormone (LH), testosterone, and prolactin levels (Ni *et al*., 2020). Several studies have shown that medicinal plants and their active ingredients have anti-peroxidative against nicotine toxicity in the testis and other tissues (Ni *et al*., 2020).

Living fossil (*Ginkgo biloba*) is a member of the Ginkgoaceae family and has been used as a traditional

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Chinese medicine for many years (Chen *et al*., 2020). This medicinal herb has several types of active constituents including flavonol glycosides (22–27%) (quercetin, kaempferol, and isorhamnetin) and terpene tri lactones (ginkgolides and bilobalide), proanthocyanidins, alkylphenols, flavones, simple phenolic acids, 6-hydroxy kynurenic acid, 4-O-methyl pyridoxine, and polyprenols (Xing *et al*., 2022). Therefore, *G. biloba* could be used as a potential therapy for a variety of diseases, such as diabetic cardiomyopathy, neurodegenerative diseases, myocardial lesion, cancer, obesity, and liver injury. Also, the study of Mansour *et al*. (2021) showed that *G. biloba* has an antioxidant protective effect against testicular oxidative damage and subsequent distortion of spermatogenesis induced by methotrexate.

G. biloba may be exposed to a variety of toxins (heavy metals, pesticides, herbicides, biological contamination, and pesticide residues) and other environmental pollutants. To meet the demand and produce a safer and more effective herbal medicine, several techniques have been used to purify and preserve these medicinal plants. Food irradiation technology (Gamma-rays, electron-beams, and X-rays) is a non-thermal technique that becoming more widely accepted around the world for eliminating or reducing pests, parasites, and microorganisms without altering the food's chemical or organoleptic composition, being safe for consumers, or enabling a decrease in the use of chemical fumigants. The Food and Agriculture Organization (FAO) and the Codex Alimentarius Commission have approved gamma radiation from radioisotopes (Co^{60} or Cs^{137}) and electron beams produced by electricity from accelerators with energies less than 10 MeV as safe and efficient sources used in the application of food irradiation technology. Gamma irradiation is typically utilized for large volumes of food products because of its great dosage homogeneity and high penetrating strength. Otherwise, electron beam irradiation is used mainly for food products with low density (Ebrahim *et al*., 2022). This study aimed to investigate the effect of ionizing irradiation (gamma and electron beams) on antioxidant contents (total phenol and flavonoid contents) and antioxidant activities of *G. biloba* powder and to investigate the possible protective effect of gamma-irradiated *G. biloba* aqueous extract (GGBE) against nicotine-induced cardiac and testicular toxicity in male rats.

MATERIALS AND METHODS

Ginkgo biloba *leaves and their irradiation*

Leaves of *G. biloba* were purchased from the Egyptian local market. Leaves were manually cleaned and ground for 3 min using a mixer at the maximum speed setting. Ground leaves were passed through a 1 mm^2 sieve (Ali *et al*., 2019).

G. biloba powder was transferred into polyethylene bags and treated with gamma rays at the doses of 5 kGy, using Indian Gamma Cell (Ge 4000 A) Co^{60} source at a dose rate of 0.717 kGy/h (Duration= 83 Min) at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Nicotine ((S)-3-(1- Methyl-2-Pyrroli-dinyl) pyridine) and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Electron beam irradiation (EBI) at the doses of 5 kGy was carried out at the NCRRT using an industrial electron beam accelerator (model: ICT, VIVIRAD CO, France). This accelerator has a maximum energy of 3 MeV, a beam current of 30 MA, a beam power of 90 KW, a scan width of 90 cm, and a distance between the scanner and the conveyor system of 53.0 cm. Conveyer speed 10.8 m/min-max 16m/mi. Accelerator high voltage 2600 kv, max 3000 kv. The Department of Radiation Protection and Dosimetry at the National Center for Radiological Research and Technology (NCRRT) did extensive dose mapping in compliance with Egyptian requirements.

Preparation of aqueous extract of G. biloba

The aqueous extract of *G. biloba* leaves was produced by mixing 20 g of raw and gamma-irradiated ground leaves with 200 ml of distilled water (Kingsley *et al*., 2019). The mixture was refluxed in a water bath at 65°C for 1 h and filtered using Whatman filter paper No 1. The extracts were then filtered to eliminate particulate substances and to get clear solutions which were then stored in a refrigerator (4°C) for subsequent experiments.

Determination of total phenolic, total flavonoid contents, and antioxidant activity

The total phenolic contents of raw and irradiated *G. biloba* were determined based on a method described by Singleton *et al*. (1999). Phenolic contents were calculated based on the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per g of dry extract (mg GAE/g). The level of total flavonoid concentration was calculated using quercetin (QU) as a standard (Jia *et al*., 1999). The results were expressed as mg of quercetin equivalents per g of dry extract (mg QUE/g). The antioxidant activity of raw and irradiated *G. biloba* was determined based on the radical scavenging ability in reacting with a stable DPPH free radical according to Blois (2002).

HPLC analysis of the phenolic compound contents

HPLC analysis was carried out according to Sati *et*

al. (2019) by using HPLC Hewllet Packared (series 1050) equipped without sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm, and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Peaks were identified by congruent retention times and UV spectrum and compared with those of the standards. The results were expressed in mg/g of water extract.

Nicotine preparation

Nicotine was supplied as a white powder containing 100 mg nicotine which was dissolved in 100 ml saline (1mg nicotine/ ml saline) and stored in a foil-wrapped glass bottle at 4°C for no longer than 10 days (Autifi *et al*., 2017).

Animals

Sprague Dawley male rats (200-230g body weight) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. For two weeks the rats were acclimated to controlled laboratory conditions. Rats were maintained on a stock rodent diet and tap water that was allowed *ad libitum*.

The 28 animals were randomly divided into 4 groups, each consisted of 7 rats: Control group: Rats fed on a balanced diet and served as control. Group NIC: Control was injected intraperitoneal (IP) with nicotine (1.0 mg/ kg b.wt) for 6 weeks (Autifi *et al*., 2017). Group NIC & RGBE: Rats were treated with an oral dose of raw *G. biloba* aqueous extract (RGBE; 150 mg/kg b.wt) daily for 6 weeks along with NIC injection (Khattab, 2012). Group NIC & GGBE: Rats were treated with an oral dose of gamma-irradiated *G. biloba* aqueous extract (GGBE; 150 mg/kg b.wt) daily for 6 weeks along with NIC injection (Khattab, 2012).

At the end of the experimental period (6 weeks), after 24 h from the last dose animals from each group were sacrificed. Blood samples were withdrawn by cardiac puncture after slight anathesation of rats using diethyl ether and allowed to coagulate and centrifuged to get serum for biochemical analysis. Also, testes and heart tissues were removed for biochemical investigation.

Biochemical analysis

The levels of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined by the method of King (1965). Troponin I (cTnI) and creatinine kinase-MB (CK-MB) were performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. Detection of serum tumor necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6) was performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. The concentration of testosterone was measured by using DRG Diagnostics testosterone kit, Germany, while follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were analyzed using a Monobind CA kit, USA. The hormone analysis was done according to the manufacturer's protocol. Testicular and cardiac tissues (100 mg tissue/ml buffer) were homogenized in 50 mM phosphate buffer (pH 7.2; St. Louis, MO, USA); the homogenates were then centrifuged at 1,200 x g for 15 min and the supernatant was used for determination of the concentration of malondialdehyde (MDA) according to Yoshioka *et al*. (1979), GSH content by Beutler *et al*. (1963), superoxide dismutase activity (SOD) by the method of Minami and Yoshikawa (1979) and Catalase activity (CAT) by Johansson and Borg (1988).

Statistical analysis

Results were presented as mean±SE (n=7). Experimental data were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Data were statistically analyzed with the aid of Statistical Package of the Social Sciences, SPSS version 25 (copyrighted by IBM SPSS software, USA). Differences between means were considered significant at $P < 0.05$.

RESULTS

The results revealed that the total phenolic and total flavonoid contents and antioxidant activity in *G. biloba* were significantly increased by irradiation treatment (Table I). The percent elevation induced by gamma

Table I. Total phenolic and total flavonoid contents and antioxidant activity of raw and irradiated *G. biloba*.

	Raw	Ionizing radiation (5 kGy)	
		Gamma-rays	Electron beam irradiation (EBI)
Total phenolic (mg GAE/g)	74.8± 0.82^{c}	96.9± 0.86 ^{a}	87.1± 0.69 ^{b}
Total flavonoid (mg QUE/g)	85.32± 1.57^{c}	99.13± 1.63 ^{a}	93.94± 1.74 ^{b}
Antioxidant activity (%)	76.32 ± 1.21^{c}	88.76± 1.32 ^{a}	83.59± 1.27 ^{b}

Values are means of three replicates (± SD). Values in the same row with different superscript are significantly different at P<0.05.

Table II. Bioactive phenolic compounds (mg/g of extract) of raw and irradiated *G. biloba.*

Compound	Raw	Irradiated (5 kGy)	
		Gamma rays	Electron beam irradiation (EBI)
Protochatchuic acid	1.41 ± 0.09	4.42 ± 0.11	3.11± 0.08
Bilobalide	NQ	NQ	NQ
Ginkgolide A	NQ	NQ	NQ
Ginkgolide B	NQ	NQ	NQ
Kaempferol-3-O-rhamnosylhexoside-7-O-glucoside	0.37 ± 0.04	1.18 ± 0.07	1.03 ± 0.05
Isorhamnetin-3-O-rhamnosylhexoside-7-O-glucoside	0.30 ± 0.04	1.11 ± 0.13	1.05 ± 0.02
Kaempferol-O-rhamnosyl-glucoside	0.13 ± 0.02	0.56 ± 0.10	0.47 ± 0.07
Quercetin 3-O-2″,6″-dirhamnosylglucoside	0.61 ± 0.03	1.49 ± 0.09	1.27 ± 0.06
Myricetin-3-O-rutinoside	0.10 ± 0.01	0.52 ± 0.04	0.39 ± 0.03
Ginkgolide C derivative	NQ	NQ	NQ
Kaempferol-3-O-dirhamnosylglucoside	1.25 ± 0.02	3.46 ± 0.05	3.22 ± 0.03
Isorhamnetin-3-O-dirhamnosylglucoside	0.40 ± 0.01	1.32 ± 0.05	1.26 ± 0.04
Quercetin-3-O-rutinoside	0.83± 0.06	2.77 ± 0.06	2.53 ± 0.05
Patuletin-3-O-rutinoside	0.53 ± 0.02	1.87 ± 0.04	1.69 ± 0.03
Quercetin-3-O-glucoside	0.10 ± 0.01	0.51 ± 0.03	0.42 ± 0.02
Quercetin-3-O-glucosyl-(1,2)-rhamnoside	0.19 ± 0.03	0.72 ± 0.05	0.63 ± 0.04
Kaempferol-3-O-rutinoside	1.39 ± 0.05	4.27 ± 0.07	3.98 ± 0.06
Isorhamnetin-3-O-rutinoside	1.10 ± 0.02	3.15 ± 0.05	2.73 ± 0.04
Quercetin-3-O-rhamnoside	0.19 ± 0.03	0.69 ± 0.06	0.57 ± 0.05
Isorhamnetin-3-O-glucoside	0.16 ± 0.03	0.48 ± 0.04	0.37 ± 0.03

NQ, not quantified.

Table III. Effect of raw and γ-irradiated *G. biloba* along with NIC on cardiac function TNFα, IL-6, on testosterone, LH and FSH in rats.

Parameters	Control	NIC	NIC & RGBE	NIC & GGBE (5 kGy)
Cardiac function lists				
CPK (U/L)	252.43±5.36^{d}	485.38±8.74^{a}	352.66±7.45^{b}	307.59±6.47^{c}
CK-MB (ng/mL)	3.42 ±0.81^{d}	7.57±0.91^{a}	5.64 ±0.62^{b}	4.62±0.81^{c}
CTnI (ng/mL)	27.11±1.16^{d}	58.48±1.83^{a}	43.62±1.54^{b}	33.69±1.22^{c}
LDH (U/ml)	232.14±15.12^{d}	542.43±18.84^{a}	382.11±16.37^{b}	301.95±13.42^{c}
Inflammatory cytokines				
TNF-α (pg/mL)	681.52 ± 48.71^{d}	917.22±60.52^{a}	769.96 ± 42.47^{b}	721.39 ± 38.78^{c}
IL-6 (pg/mL)	330.26 ± 22.18^{d}	532.18±30.10^{a}	423.21 ± 28.42^{b}	359.79 ± 30.18^{c}
Endocrime hormones				
T (n mol/L)	7.39±1.72^{a}	4.69±1.83^{d}	6.06±1.75^{c}	6.78±1.66^{b}
LH (mU/ml)	0.81±0.15^{a}	0.49±0.09^{d}	0.61±0.13^{c}	0.72±0.12^{b}
FSH (mU/ml)	0.52±0.16^{a}	0.41±0.14^{d}	0.46±0.12^{c}	0.49±0.17^{b}

Values are expressed as means ± S.E. (n=7). Values in the same row with different superscripts are significantly different at P<0.05.NIC: Nicotine, RGBE: raw G. biloba aqueous extract, GGBE: gamma-irradiated G. biloba aqueous extract. CPK, creatine phosphokinase; CK-MB, creatine kinase-MB; CTnI, troponin 1; LDH, lactate dehydrogenase; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6; T, testosterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

irradiation is higher than that induced by electron-beam irradiation (EBI).

HPLC chromatographic profile of raw and irradiated *G.biloba* samples revealed that about twenty compounds such as phenolic acid, flavonoids (15 compounds), and 4 terpene lactones were detected (Table II). The most

Table IV. Effect of raw and γ-irradiated *G. biloba* on testicular and cardiac antioxidant status system and lipid peroxidation in NIC -treated rats.

Parameters		C	NIC	NIC & RGBE	NIC & GGBE (5 kGy)
MDA (n mol/g tissue)	Testes	227.35±5.82^{d}	496.42±8.11^{a}	360.32±6.73^{b}	288.76±5.15^{c}
	Heart	136.45±3.48^{d}	312.14±4.39^{a}	235.86±4.37^{b}	170.59±3.79^{c}
GSH (mg/g tissue)	Testes	36.27±0.64^{a}	19.75±0.86^{d}	25.75±0.69^{c}	29.28±0.73^{b}
	Heart	5.50 ± 0.27^{a}	2.97 ± 0.32^{d}	3.74 ± 0.26^{c}	4.85 ± 0.30^{b}
SOD (U/mg protein)	Testes	45.62±0.92^{a}	27.63±0.82^{d}	35.62±0.84^{c}	40.12±0.74^{b}
	Heart	28.66±0.83^{a}	17.16±0.90^{d}	19.93±0.85^{c}	22.47±0.81^{b}
CAT (U/mg protein)	Testes	48.83±0.72^{a}	32.46±0.74^{d}	39.25±0.78^{c}	43.23±0.72^{b}
	Heart	43.69±0.77^{a}	21.52±0.81^{d}	30.59±0.88^{c}	38.05±0.91^{b}

Values are expressed as means ± S.E. (n=7). Values in the same row with different superscript are significantly different at P<0.05.NIC: Nicotine, RGBE: raw *G. biloba* aqueous extract, GGBE: gamma-irradiated *G. biloba* aqueous extract. MDA, malondialdehyde; GSH, intracellular glutathione; SOD, superoxide dismutase; CAT, catalase

abundant compounds in the raw and irradiated samples were kaempferol-3-O-rutinoside and protocatechuic acid. The concentration of these active ingredients was significantly increased by gamma irradiation treatment more than by E-beam processing.

The results of the biochemical analysis revealed that nicotine caused a significant increase in the level of CPK, CK-MB, CTnI, LDH, inflammatory cytokines (TNF-α and IL-6), and the level of testicular and cardiac MDA compared to the control group, however, the serum level of testosterone, LH, FSH and the level of testicular and cardiac GSH and antioxidant enzymes (SOD and CAT) were significantly reduced by nicotine treatment compared to control rats (Tables III and IV).

Whereas, in rats treated with both raw and gamma-irradiated *G. biloba* along with NIC, *G. biloba* seems to significantly counteract the elevation induced in the level of cardiac function, inflammatory cytokines and the level of testicular and cardiac MDA compared to NIC-group. In addition, treatment of rats with RGBE or GGBE along with NIC significantly increases the level of testosterone, LH, FSH, and the level of testicular and cardiac GSH and antioxidant enzymes (SOD and CAT) compared to NIC group (Tables III and IV).

DISCUSSION

The different parts of *Ginkgo* (fresh or dried leaves, and seeds) are known for their medicinal potential and can be used as an antioxidant agent due to the presence of chemical constituents such as terpenes tri-lactone (ginkgolides and bilobalide) and flavonoid glycosides (Ali *et al*., 2019). Food irradiation technology (Gamma-rays, electron beams) have been approved by FAO and Codex as a safe method for decontamination of medicinal plant (Hwang *et al*., 2021). Nicotine is one of the toxic components that can cause cardiovascular disease and male reproductive system disturbance (Ni *et al*., 2020). This study aims to investigate the possible protective effect of irradiated *G. biloba* aqueous extract on nicotine-induced cardiac and testicular toxicity in male rats.

The results in this study obtained that the *G. biloba* dried powder possesses a high amount of total phenolic (74.8 mg GAE/g) and total flavonoid contents (85.32 mg QUE/g) and high antioxidant activity (76.32%) in agreement with the study of (El-Beltagi and Badawi, 2013). The results also indicated that gamma-irradiation induced a significant increase in the total phenolic (29.54%) and total flavonoid contents (16.18%) and antioxidant activity (16.29%) by percent change higher than that induced by electron-beam irradiation (16.44%, 10.10%,9.52%, respectively). Fang and Wu (1998) reported that gamma irradiation has high efficiency and high penetration power than electron-beam (IAEA, 2008). In this study, the results of the HPLC chromatographic profile of *G. biloba* samples are in agreement with the results of El-Beltagi and Badawi (2013) and Pereira *et al*. (2015). *G. biloba* leaves extract is a complex product containing different active compounds with major components Kaempferol-3-O-rutinoside and Protochatchuic acid. It is difficult to detect and quantification of terpene tri-lactones by using UV detection because that type of compound presents as active substances in the complex matrix of *G. biloba* and has low UV absorption (Mesbah *et al*., 2005). In this study, gamma-irradiation and EBI significantly increased the concentration of phenolic and flavonoid contents which could be explained by the efficiency of irradiation to break chemical bonds that link polyphenols to other molecules, thereby releasing soluble phenols of low molecular weight and leading to an increase of antioxidant-rich phenolics

(Alothman *et al*., 2009). Pereira *et al*. (2015) suggested that the use of irradiation could improve the bioactive properties and extractability of phenolic contents. in the extracts obtained from *G. biloba*.

Since the results of this study showed that the gamma-irradiation had a more effective potential on the antioxidative properties of ginkgo leaves than electron-beams irradiation, the gamma-irradiated *G. biloba* powder were chosen to conduct a biological experiment and study its effect against the toxicity induced by nicotine in male rats.

The results of biological study obtained that nicotine injection induced cardiac and testicular dysfunction evidenced by reduction in the serum level of testosterone, LH and FSH. Galam *et al*. (2013) suggested that nicotine being a central nervous system stimulant also interferes with endocrine secretion such as the release of gonadotrophins (FSH, LH) and prolactin from the anterior pituitary and the administration of varied doses of nicotine caused a reduction in mean values of reproductive hormonal parameters (FSH, LH, prolactin, and testosterone). Egesie *et al*. (2013) reported that cigarette smoking or nicotine treatment results in testicular degeneration, deficiency of male sex hormone and reduction in sperm count, thus the low sperm count would probably be a result of decrease or absent androgens and FSH to adequately steer the process of spermatogenesis.

In this study, nicotine administration significantly increased level of testicular and cardiac oxidative stress products (MDA), inflammatory cytokines (TNF-α and IL-6), level of heart markers (CPK, CK-MB, CTnI, LDH) and reduced the antioxidant status by reducing the level of GSH and activity of antioxidant enzymes (SOD and CAT). It has been suggested that nicotine exposure has been shown to negatively affect heart function and reproductive health by causing disruption in oxidant–antioxidant balance and increasing the level of ROS and lipid peroxidation (Saad *et al*., 2020). Also, Aprioku (2013) reported that nicotine exposure caused over production of ROS and oxidative stress that induced lipids in the cell membrane resulting in the release of the unsaturated reactive aldehyde product (MDA) which is an important indicator of oxidative stress. Moreover, Oztekin *et al*. (2020) found that ROS and inflammatory cytokines (TNF-α and IL-6) were significantly higher in rats with nicotine exposure that may affect spermatozoal functions by increasing nitrite oxide production. Additionally, the increased of heart markers (CPK, CK-MB, CTnI, LDH) could be due to the bad effect of nicotine and generation of free radicals inducing cell necrosis or heart tissues damage which lead to release more of CPK, CK-MB, CTnI, LDH to the blood stream (Kim *et al*., 2017).

Whereas administration of nicotine along with either RGBE or GGBE significantly reduced the nicotine-induced toxicity evidenced by reduction in the level of cardiac function, inflammatory cytokines and the level of testicular and cardiac MDA and elevation of the serum level of testosterone, LH, FSH and the level of testicular and cardiac GSH and antioxidant enzymes (SOD and CAT) compared to NIC-group. The effectiveness of GGBE against nicotine could be attributed to flavonoids, terpene lactones and other active components that can scavenge free radicals and maintain redox homeostasis (Mansour *et al*., 2021). The study of Mansour *et al*. (2021) concluded that concurrent treatment with *G. biloba* alleviated methotrexate-induced testicular insult evidenced by elevating the serum LH, FSH and testosterone levels, amelioration of oxidative stress biomarkers, energy functions, seminal sperms abnormalities and spermatogenesis status. Ahmed *et al*. (2016) revealed that *G. biloba* treatment effectively attenuated the testicular tissue damages, oxidative stress and apoptosis induced by ischemia/perfusion. In addition, Singh *et al*. (2012) reported that the organic acids of *G. biloba* extract were responsible for its antioxidant, antiallergic, anti-inflammatory, antiproliferative, antianxiety, and anticarcinogenic effects. Moreover, *G. biloba* might protect the tissues against free radicals-induced tissue damage due to its bioflavonoid's contents. It could be effective on cardiovascular, respiratory, and central nervous system and renal systems as it can prevent cell damage by inhibiting the platelet activating factor (Ahmed *et al*., 2016).

CONCLUSION

The results of this study concluded that food-irradiation (gamma-rays and electron-beam) could be an effective way not only in improving the antioxidant activity but also increasing the level of phenolic compounds in *G. biloba*. Also, the results supported that gamma rays (5 kGy) were the most effective irradiation source for improving the antioxidant activity compared to electron-beam.

Owing to the high antioxidant and high phenolic content of gamma-irradiated *G. biloba* aqueous extract (GGBE), GGBE enhanced the antioxidant status of cardiac and testicular- tissues, ameliorated the nicotine-induced endocrine disruption on male hormone profile (LH, FSH and testosterone) in addition GGBE reduced inflammation and oxidative damage induced by nicotine toxicity.

DECLARATIONS

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Ethical consideration

All animal procedures were carried out following the Research Ethics Committee for experimental studies (Human and Animal subjects) at the National Centre for Radiation Research and Technology (REC-NCRRT), Egyptian Atomic Energy Authority (Cairo, Egypt). Conformed to the CIOMS and ICLAS International Guiding Principles for Biomedical Involving Animals 2012.

Statement of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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