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ORIGINAL ARTICLE

Protection by low-dose γ radiation on doxorubicin-induced nephropathy in rats pretreated with curcumin, green tea, garlic or L-carnitine

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Abstract The protective potentials of a single exposure to 0.3 Gy of γ radiation alone or with previous treatment with certain natural products with antioxidants activity, namely curcumin (50 mg/kg, i.p.), green tea (300 mg/kg, p.o.), garlic (100 mg/kg, p.o.) or L-carnitine (40 mg/kg, i.p.) against doxorubicin (DOX)-induced nephropathy in rats were studied. Natural products were administered daily for 14 successive days followed by single i.p. injection of DOX (5 mg/kg). Rats were subjected to whole body γ radiation, 1 day before DOX administration. Serum levels of creatinine, urea, uric acid, low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) cholesterols, total proteins and albumin as well as renal concentrations of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), nitric oxide (NO) and calcium (Ca) were determined. Irradiation provided significant protections against DOX-induced changes in all measured parameters, except renal Ca content. All the test natural products significantly improved radiation-induced protection against renal lipid peroxidation. L-Carnitine markedly augmented the protection toward changes in renal GSH, NO and Ca concentrations. Curcumin increased the protection toward changes in serum albumin and renal GSH and NO concentrations, while garlic increased the protection toward changes in serum LDL-C level. It could be concluded that low-dose γ radiation could provide prophylaxis against DOX-induced nephropathy which might be augmented by the use of certain natural antioxidants.

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1. Introduction

Doxorubicin (DOX) is an anthracycline antibiotic obtained from *Streptomyces peucetius*, although, a total chemical synthesis is now possible. Since being introduced for treatment of cancer in 1969, DOX has demonstrated high antitumor efficacy. However, the use of DOX has been limited largely due to possible diverse cardiac, renal, hematological and testicular

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toxicities.[1] The mechanism of DOX-induced cardio- and nephron-toxicities is most likely mediated by the formation of free radicals, which in turn, cause various oxidative damages on critical cellular components, plasma membrane lipids and mitochondria.[2] Researchers have expended great efforts trying to prevent or attenuate the side effects of DOX. In this sense, several strategies have been followed as dosage optimization, use of analogs or combined therapy. No promising results have been found with different routes and schedules of drug delivery.[3,4] Also, the use of several available DOX analogs did not show stronger antitumor efficacy or lower toxic effects than DOX.[5] The most immediate approach is the combination of the drug with an antioxidant in order to reduce oxidative stress.[6]

Experimentally, DOX induces rat nephropathy, which is characterized by massive proteinuria, hypoalbuminemia and dyslipidemia. The renal pathological changes are similar to those induced by daunomycin or puromycin animonucleoside, including increase in glomerular capillary permeability.[7]

High-dose radiation clearly leads to a progressive reduction in kidney function associated with concomitant glomerulosclerosis and/or tubulointerstitial fibrosis following chronic and persistent oxidative stress.[8] However, prior irradiation with low doses was shown to protect against damage induced by high challenge dose for many endpoints, including cell survival, mutation, chromosomal aberration induction and neoplastic transformation.[9] Low-dose radiation may reveal protection through stimulation of detoxification mechanisms for reactive oxygen species such as increase of free glutathione level and superoxide dismutase activity. These mechanisms appear to reach a maximum at about 4 h after irradiation and lasts for several hours or even weeks, depending on tissue and cell type.[10,11]

Curcumin is a yellow pigment obtained from *Curcuma longa*, commonly called turmeric. It has long been used as a spice, coloring agent in several foods as well as in cosmetics and drugs. Curcumin was shown to possess potent antioxidant activities both in vivo and in vitro as well as free radical quenching capabilities that are largely referred to its phenolic groups.[12,13]

Green tea, one of the naturally safe beverages all over the world, has antioxidant and free radical scavenging potencies. It contains a number of bioactive compounds most important of which is (−)-epigallocatechin-3-gallate which contributes to most of the green tea beneficial effects.[14]

Garlic, besides being used as a food, has been used in Egypt, India and China for clinical purposes for several decades. It is still being employed as folk medicine all over the world. Garlic is rich in organo-sulfur and organo-selenium compounds, which are responsible for its flavor, aroma as well as the potential biological and medical benefits.[15]

L-Carnitine is a natural amino acid obtained from food as well as biosynthesized from lysine and methionine in the liver, kidney and brain.[16] It acts as a cofactor for several enzymes of energy metabolism. L-Carnitine possesses a strong antioxidant activity and suppresses the mitochondrial release of free electrons that generate free radicals.[17]

The present study was aimed to investigate the possible protective potential of a single low dose of γ radiation against DOX-induced nephropathy in rats. The aim was extended to explore the possible added protection by the prior administration of curcumin, garlic, green tea or L-carnitine.

2. Materials and methods

2.1. Animals

Male Wistar rats, purchased from the National Research Center (Giza, Egypt) weighing 150–200 g were used in this study. They were kept under appropriate conditions of temperature (25 ± 2 °C), humidity (60–70%) and light (12 h dark/light cycle). The animals were allowed free access to standard pellets obtained from El-Nasr Chemical Company (Cairo, Egypt) and water *ad libitum*. Rats were acclimatized in the animal facilities of the National Center for Radiation Research and Technology for one week before starting the experiments. The study was carried out according to the International Guidelines and approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

2.2. Drugs

Curcumin (Sigma Chemical Company, USA), green tea extract (MEPACO, Egypt), garlic powder (ATOS Pharma, Egypt) and L-carnitine (GLOBAL NAPI Pharmaceuticals, Egypt) were freshly dissolved in distilled water immediately before administration. Doxorubicin vials (Adriblastina vials; Pharmacia Italia S.P.A., Italy) were used for induction of nephropathy in rats. Each vial contains doxorubicin hydrochloride as a freeze-dried powder. The content of each vial was freshly dissolved in a sterile saline solution just before injection.

2.3. Experimental design

Three sets of experiments were carried out in the current investigation; the first one was designed to select the lowest dose of DOX that induces nephropathy in rats and the onset of induction. Four groups of rats ($n = 12$) were used in this set of experiment and were allocated as following:

Group 1: received saline and served as normal.
Groups 2–4: received a single i.p. injection of DOX in dose of 5, 10 or 15 mg/kg, respectively.

Serum levels of creatinine, urea and uric acid were determined 3, 10 and 15 days following DOX administration. Based on the obtained results (Table 1), DOX (5 mg/kg) was selected to induce nephropathy in the subsequent sets of experiments and 15 days following DOX administration was selected to be the time at which parameters should be measured.

The second set of experiment was carried out to select the proper time of γ radiation before DOX administration that provides the most prophylactic effect against DOX-nephropathy. Five groups of rats ($n = 6$) were used in this set of experiment and were allocated as following:

Group 1: received saline and served as normal.
Group 2: received a single i.p. injection of DOX (5 mg/kg) and served as control.
Group 3–5: received a single i.p. injection of DOX (5 mg/kg), 1, 3 or 7 days after exposure to a single dose (0.3 Gy) of γ radiation, respectively.

Table 1 Effect of a single i.p. injection of DOX (5, 10 and 15 mg/kg) on rat serum levels of creatinine, urea and uric acid measured 3, 10 or 15 days following administration.

Treatments	Creatinine (mg/dl)			Urea (mg/dl)			Uric acid (mg/dl)		
	Time after treatment			Time after treatment			Time after treatment		
	3 days	10 days	15 days	3 days	10 days	15 days	3 days	10 days	15 days
Normal (saline)	0.64 ± 0.02	0.72 ± 0.02	0.61 ± 0.03	24.52 ± 0.96	17.48 ± 0.58	21.34 ± 1.05	1.72 ± 0.23	1.92 ± 0.46	1.97 ± 0.20
DOX (5 mg/kg)	0.78 ± 0.03	1.23* ± 0.10	1.24* ± 0.07	35.02* ± 1.21	22.67* ± 1.23	30.48* ± 0.94	1.92 ± 0.36	3.72 ± 0.74	4.07* ± 0.33
DOX (10 mg/kg)	0.86 ± 0.08	1.45* ± 0.15	1.48* ± 0.14	39.28* ± 0.93	24.98* ± 1.08	33.52* ± 1.29	2.27 ± 0.47	4.52* ± 0.29	4.30* ± 0.53
DOX (15 mg/kg)	0.95* ± 0.06	1.65* ± 0.12	1.70* ± 0.25	43.64* ± 1.54	29.86* ± 1.04	57.73* ± 1.69	3.52* ± 0.57	4.90* ± 0.39	4.87* ± 0.47

Each value represents mean ($n = 12$) ± S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test.
* $p < 0.05$ *vs. normal value.*

Serum levels of creatinine, urea and uric acid were determined 15 days following DOX administration. Based on the obtained results (Table 2), irradiation 1 day before DOX administration provided the most protective effect against DOX-nephropathy, accordingly was selected to be the proper time for irradiation before DOX administration in the subsequent experiment.

The third set of experiment was performed to study the possible effects of curcumin, green tea, garlic or L-carnitine on γ radiation-induced protection against DOX-nephropathy. Seven groups of rats ($n = 6$) were allocated as following:

Group 1: received saline and served as normal.
Group 2: received a single i.p. injection of DOX (5 mg/kg) and served as control.
Group 3: received a single i.p. injection of DOX (5 mg/kg), 1 day after exposure to a single dose (0.3 Gy) of γ radiation.
Group 4: received curcumin (50 mg/kg, i.p.).
Group 5: received green tea (300 mg/kg, p.o.).
Group 6: received garlic (100 mg/kg, p.o.).
Group 7: received L-carnitine (40 mg/kg, i.p.).

Groups 4–7 received their corresponding treatments daily for 14 successive days followed (at day 15) by a single i.p. injection of DOX (5 mg/kg). They were exposed to a single dose (0.3 Gy) of γ radiation, 1 day before DOX administration.

2.4. Irradiation of animals

Rats were exposed to whole body γ radiation at a single dose level of 0.3 Gy delivered at a dose rate of 0.46 Gy/min. The radiation source was ^{137}Cs using a Canadian gamma cell-40 biological irradiator. Radiation was performed in the National Center for Radiation Research and Technology, Cairo, Egypt.

2.5. Measured parameters

Rats were anesthetized with urethane (1.2 g/kg, i.p.), blood samples were withdrawn from the retro-orbital venous plexus and sera were separated. Serum level of creatinine was measured using the specific test reagent kit (BIO ADWIC, Egypt), while serum levels of urea and uric acid were determined using the corresponding test reagent kit (Diamond Diagnostics, Egypt). Serum low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) cholesterols, total proteins and albumin were determined using the corresponding test reagent kit (Biodiagnostic, Egypt).

Rats were killed by cervical dislocation, the kidneys were immediately excised, washed with cold-saline, blotted, weighed and homogenized in ice-cold saline to prepare 20% w/v homogenate. Renal contents of nitric oxide (NO) and calcium (Ca) were determined using the corresponding test reagent kit (Biodiagnostic, Egypt). Renal contents of glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) were performed according to the methods of Ellman[18] and Yoshioka et al.,[19] respectively.

2.6. Statistical analysis

Values were expressed as means ± S.E. Comparisons between means were carried out using one-way ANOVA followed by

Table 2 Effect of a single i.p. injection of DOX (5 mg/kg) administered 1, 3 or 7 days after a single exposure to γ radiation (0.3 Gy) on serum levels of creatinine, urea and uric acid in rats.

Treatments	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Normal (saline)	0.66 ± 0.13	18.55 ± 1.41	1.69 ± 0.20
Control (DOX; 5 mg/kg)	1.29[*] ± 0.16	33.63[*] ± 1.75	3.85[*] ± 0.49
γ radiation (0.3 Gy) → (1 day) → DOX (5 mg/kg)	0.78[@] ± 0.08	22.06[@] ± 2.00	1.88[@] ± 0.32
γ radiation (0.3 Gy) → (3 day) → DOX (5 mg/kg)	0.83[@] ± 0.05	25.86[*,@] ± 2.29	2.17[@] ± 0.34
γ radiation (0.3 Gy) → (7 day) → DOX (5 mg/kg)	1.12[*] ± 0.09	29.75[*] ± 0.84	3.43[*] ± 0.55

Irradiated rats were exposed to whole body γ radiation at a single dose level of 0.3 Gy delivered at a dose rate of 0.46 Gy/min. Irradiation was carried out 1, 3 or 7 days before DOX administration in the 3rd–5th groups, respectively. Serum levels of creatinine, urea and uric acid were determined 15 days following DOX administration.
Each value represents mean ($n = 6$) ±S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test.
[*] $p < 0.05$ *vs. normal value*.
[@] $p < 0.05$ *vs. control value*.

the Tukey–Kramer multiple comparisons test using Instat software, version 2 (Graphpad Software, Inc., San Diego, USA). In description of results, percentage of protection was calculated according to the following formula:% protection = (Treatment value – Control value/Normal value – Control value) × 100.

3. Results

The first set of experiment was carried out to select the lowest single i.p. dose of DOX that induce nephropathy in rats as manifested by elevations of serum levels of creatinine, urea and uric acid. The endpoint was the time at which all the three parameters were elevated. DOX in the highest studied dose; 15 mg/kg significantly elevated the serum levels of creatinine, urea and uric acid starting from 3 days following administration. On the other hand, DOX in doses of 5 and 10 mg/kg significantly elevated the serum levels of these three parameters after 15 and 10 days of administration, respectively (Table 1). Accordingly, single i.p. injection of DOX in a dose of 5 mg/kg was selected to induce rat nephropathy in the subsequent sets of experiments. Also, serum and kidney samples collection was designed to be after 15 days from DOX administration, since this period allowed the elevation of all of the three parameters measured to indicate nephropathy.

The second set of experiment was performed to study the protective effect of a single low dose (0.3 Gy) of whole body γ radiation against DOX-induced nephropathy. Exposure to γ radiation 1 day prior to DOX administration afforded 81%, 77% and 91% protections against DOX-induced elevations in serum creatinine, urea and uric acid, respectively (Table 2). However, irradiation 3 days before DOX administration showed less protection. It produced 73%, 52% and 78% protections against DOX-induced elevations in these serum parameters, respectively (Table 2). On the contrary, no significant protection was observed when rats were irradiated 7 days before DOX injection. Accordingly, the subsequent set of experiment was designed to irradiate rats 1 day before DOX administration.

Daily treatment with curcumin, green tea, garlic or L-carnitine for 14 successive days prior to DOX injection provided 89%, 82%, 87% and 95% protections against DOX-induced elevation in serum creatinine, 84%, 80%, 81% and 88% protections against DOX-induced elevation in serum urea, and 97%, 92%, 89% and 90% protections against DOX-induced elevation in serum uric acid, respectively (Table 3).

It should be noted that the values of serum creatinine, urea and uric acid in normal, control (DOX; 5 mg/kg) and γ radiated (1 day before DOX) groups were determined in more than one set of experiments in the current study, however, no statistical differences were observed with respect to each group for the corresponding parameter in different sets of experiment.

DOX significantly decreased renal GSH content by 43% as compared to the normal value. γ-Radiation, 1 day prior to DOX administration, either alone or with prior treatment with any of the test agents completely protected against DOX-induced renal GSH depletion (Table 4). Moreover, curcumin, green tea or L-carnitine significantly elevated renal GSH content to 137%, 126% and 145% of the normal value, respectively. Compared to the irradiated value, only curcumin and L-carnitine increased GSH significantly.

DOX markedly increased renal lipid peroxidation as it elevated TBARS concentration to 219% of the normal value. γ-Radiation alone or with prior treatment with curcumin, green tea, garlic or L-carnitine provided 54%, 94%, 92%, 87% and 96% significant protections against DOX-induced increase in lipid peroxidation, respectively (Table 4). Treatment with any of the test natural products prior to irradiation significantly decreased renal TBARS concentration when compared to the irradiated value. Although afforded protection, γ radiation alone or with garlic treatment still showed significantly higher levels of renal TBARS as compared to the normal value.

Single DOX injection produced 53% increase in renal NO concentration as compared to the normal value. Prior irradiation alone or with preceding treatment with curcumin, green tea, garlic or L-carnitine produced significant protections against such increase recording 57%, 93%, 70%, 64% and 87%, respectively (Table 4). Only the curcumin and L-carnitine groups showed non-significant differences from the normal value in this aspect. In addition, these two groups exhibited significantly lower values than that of the irradiated group.

Renal Ca content was significantly elevated to 120% of the normal value by DOX administration. Neither γ radiation alone or with prior treatment with garlic provided any protection against DOX-induced renal Ca elevation. On the

Table 3 Effect of curcumin, green tea, garlic or L-carnitine on DOX-induced changes in serum levels of creatinine, urea, uric acid, total proteins (TP), albumin,% of albumin, LDL-C, HDL-C and HDL/LDL ratio of rats exposed to a single dose (0.3 Gy) of γ radiation, one day prior to DOX administration.

Treatments		Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	TP (mg/dl)	Albumin (mg/dl)	% of albumin	LDL-C (mg/dl)	HDL-C (mg/dl)	HDL/LDL ratio
Group 1:	Normal (saline)	0.59 ± 0.03	20.30 ± 0.94	1.82 ± 0.07	5.73 ± 0.22	3.23 ± 0.10	56.71 ± 2.13	71.83 ± 2.66	49.66 ± 2.65	0.696 ± 0.044
Group 2:	Control (DOX)	1.21* ± 0.09	37.50* ± 1.20	4.42* ± 0.25	3.64* ± 0.24	1.69* ± 0.09	47.38 ± 4.25	102.72* ± 4.89	64.70* ± 2.27	0.639 ± 0.042
Group 3:	γ radiation + DOX	0.78$^{@}$ ± 0.08	22.06$^{@}$ ± 2.00	1.88$^{@}$ ± 0.32	5.22$^{@}$ ± 0.29	2.36$^{*@}$ ± 0.16	46.50 ± 5.01	98.41* ± 3.22	60.03 ± 2.63	0.617 ± 0.045
Group 4:	Curcumin (50 mg/kg, i.p.) + γ radiation + DOX	0.66$^{@}$ ± 0.04	23.10$^{@}$ ± 1.46	1.90$^{@}$ ± 0.11	5.53$^{@}$ ± 0.30	3.02$^{@\#}$ ± 0.11	55.03 ± 2.44	87.56$^{*@}$ ± 2.44	53.22$^{@}$ ± 2.65	0.614 ± 0.045
Group 5:	Green tea (300 mg/kg, p.o.) + γ radiation + DOX	0.70$^{@}$ ± 0.05	23.71$^{@}$ ± 1.19	2.02$^{@}$ ± 0.11	5.36$^{@}$ ± 0.27	2.65$^{@}$ ± 0.15	50.55 ± 4.64	84.76$^{@}$ ± 3.18	52.65$^{@}$ ± 2.41	0.630 ± 0.049
Group 6:	Garlic (100 mg/kg, p.o.) + γ radiation + DOX	0.67$^{@}$ ± 0.05	23.49$^{@}$ ± 1.52	2.11$^{@}$ ± 0.15	5.42$^{@}$ ± 0.35	2.77$^{@}$ ± 0.19	52.11 ± 4.81	74.24$^{@,\#}$ ± 3.64	50.81$^{@}$ ± 2.44	0.690 ± 0.038
Group 7:	L-carnitine (40 mg/kg, i.p.) + γ radiation + DOX	0.62$^{@}$ ± 0.05	22.32$^{@}$ ± 1.80	2.07$^{@}$ ± 0.15	5.30$^{@}$ ± 0.22	2.53$^{*,@}$ ± 0.11	48.15 ± 2.84	78.29$^{@}$ ± 3.15	51.33$^{@}$ ± 2.54	0.663 ± 0.050

Curcumin, green tea, garlic and L-carnitine were administered daily for 14 successive days and then rats were injected with a single dose of DOX (5 mg/kg, i.p.). Irradiated groups were subjected to a single dose (0.3 Gy) of whole body γ radiation, 1 day before DOX administration. Blood samples were collected from the animals 15 days following DOX administration.
Each value represents mean ($n = 6$) ± S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test.
* $p < 0.05$*vs. Group 1.*
$^{@}$ $p < 0.05$ *vs. Group 2.*
$^{\#}$ $p < 0.05$ *vs. Group 3.*

Table 4 Effect of curcumin, green tea, garlic or L-carnitine on DOX-induced changes in renal GSH, TBARS, NO and Ca contents of rats exposed to a single dose (0.3 Gy) of γ radiation, one day prior to DOX administration.

Treatments		GSH (mg/g protein)	TBARS (nmol/g protein)	NO (μmol/g protein)	Ca (mmol/g protein)
Group 1	Normal (saline)	61.83 ± 2.24	256.47 ± 5.16	151.38 ± 3.11	102.73 ± 2.50
Group 2	Control (DOX)	35.23* ± 2.30	561.37* ± 6.05	232.04* ± 3.47	123.30* ± 2.55
Group 3	γ radiation + DOX	67.94$^{@}$ ± 2.69	397.45$^{*,@}$ ± 8.40	186.17$^{*,@}$ ± 3.90	118.63* ± 2.54
Group 4	Curcumin (50 mg/kg, i.p.) + γ radiation + DOX	84.60$^{*,@,\#}$ ± 2.67	274.31$^{@,\#}$ ± 6.58	157.26$^{@,\#}$ ± 3.12	108.94$^{@}$ ± 3.75
Group 5	Green tea (300 mg/kg, p.o.) + γ radiatio + DOX	77.81$^{*,@}$ ± 2.81	282.15$^{@,\#}$ ± 5.59	175.57$^{*,@}$ ± 3.50	112.10 ± 3.06
Group 6	Garlic (100 mg/kg, p.o.) + γ radiation + DOX	72.86$^{@}$ ± 2.94	294.70$^{*,@,\#}$ ± 8.75	180.12$^{*,@}$ ± 3.59	120.29* ± 3.28
Group 7	L-carnitine (40 mg/kg, i.p.) + γ radiation + DOX	89.54$^{*,@,\#}$ ± 2.66	268.43$^{@,\#}$ ± 7.66	161.94$^{@,\#}$ ± 3.66	103.98$^{@,\#}$ ± 3.21

Curcumin, green tea, garlic and L-carnitine were administered daily for 14 successive days and then rats were injected with a single dose of DOX (5 mg/kg, i.p.). Irradiated groups were subjected to a single dose (0.3 Gy) of whole body γ radiation, one day before DOX administration. Kidney tissues were isolated from the animals 15 days following DOX administration.
Each value represents mean ($n = 6$) ± S.E of the mean.
Statistical analysis was carried out by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test.
* $p < 0.05$ *vs. Group 1.*
$^{@}$ $p < 0.05$ *vs. Group 2.*
$^{\#}$ $p < 0.05$ *vs. Group 3.*

contrary, treatment with curcumin or L-carnitine produced 70% and 94% protections, respectively. However, when compared to the irradiated value, only L-carnitine significantly reduced the renal Ca content. Renal Ca content of green tea-treated group was non-significantly varied from either normal or control groups (Table 4).

Administration of DOX significantly reduced serum total proteins (TP) by 36% and albumin by 48% as compared to the corresponding normal values. γ-Radiation alone or with previous treatment with curcumin, green tea, garlic or L-carnitine exhibited significant protections against DOX-induced serum TP depletion. These protections recorded 76%, 90%, 82%, 85% and 79%, respectively (Table 3). Similarly, these groups recorded 44%, 86%, 62%, 70% and 55% significant protections against DOX-induced hypoalbuminemia, respectively. Nevertheless, γ radiation alone or with L-carnitine treatment showed significantly lower levels of serum albumin as compared to the normal value. On the other hand, curcumin significantly elevated serum albumin when compared to the irradiated value. However, the percentage of albumin in TP did not show any significant changes between all groups (Table 3).

DOX injection resulted in 43% increase in serum LDL-C and 30% increase in serum HDL-C as compared to the corresponding normal values. γ-Radiation before DOX administration did not produce any significant protection against DOX-induced increases in such parameters. On the other hand, pretreatment with curcumin, green tea, garlic or L-carnitine afforded degrees of protections recording 49%, 58%, 92% and 79% against the increase in serum LDL-C level and 76% 80%, 92% and 89% against the increase in serum HDL-C level, respectively (Table 3). Furthermore, pretreatment with garlic significantly reduced serum LDL-C level as compared to the irradiated value. Despite these results, no significant differences were observed between groups with respect to HDL/LDL ratio.

4. Discussion

Results of the present study indicated that single i.p. injection of DOX in the three tested dose levels; 5, 10 and 15 mg/kg induced nephropathy in rats manifested by increased serum levels of creatinine, urea and uric acid. The onset of nephropathy was dose-dependent. DOX, in the highest dose (15 mg/kg) elevated all these three parameters 3 days following administration, while DOX in the doses of 5 and 10 mg/kg elevated such parameters after 15 and 10 days of administration, respectively. The observed high serum creatinine, urea and uric acid might be attributed to impaired glomerular filtration function so that the rate of filtration fails to balance that of production. Furthermore, the possible increase in tissue breakdown that might be caused by DOX[20] might aggravate these elevations.

The present data showed that DOX (5 mg/kg) elevated serum urea firstly after 3 days of administration, followed by elevation of creatinine after 10 days of administration, and finally uric acid after 15 days of administration. This is in accordance with the proposed impaired glomerular filtration rate (GFR). If renal dysfunction is caused by a reduction in GFR, serum urea concentration tends to rise faster than that of creatinine. In addition, serum creatinine concentration may not exceed the upper limit of the reference range until the GFR, and therefore the creatinine clearance has been reduced by approximately 60%.[21]

The observed delay in the appearance of hyperuricemia might indicate the possible presence of tubular dysfunction accompanied by the glomerular one. When GFR falls, substances which are little affected by tubular action, such as urea and creatinine are retained so their serum levels elevate. However, serum urate level depends on the balance between the degree of retention due to reduced glomerular function and the degree of loss due to reduced proximal tubular reabsorptive capacity. Accordingly, the net effect on serum uric acid level depends on which dysfunction predominates. Thus, predominance of glomerular dysfunction elevates serum urate and predominance of proximal tubular dysfunction reduces serum urate.[21] Based on this principle, DOX might induce balanced glomerular and proximal tubular dysfunctions early after administration so that no apparent changes in serum uric acid level appeared. This was followed by a progression and predominance of glomerular dysfunction lately.

The current investigation found that DOX induced hypoalbuminemia. This finding might be ascribed to DOX-induced nephrotic syndrome, in which the glomerular basement membrane permeability increased leading to albumin loss. A probable fluid retention secondary to reduced GFR might also reduce serum albumin concentration leading to dilutional hypoalbuminemia.[21] However, this dilutional hypoalbuminemia was not the case in the present study since no significant differences in the percentage of albumin in total proteins (TP) between all groups were observed. Also, data showed a reduction in serum TP concentration. This observation could be a result of DOX-induced hypoalbuminemia. Most individual plasma proteins, except albumin, contribute little to TP concentration so that quite or large percentage change in the concentration of one may not cause a detectable change in TP concentration. On the other hand, marked changes of a major constituent of plasma protein, such as albumin are likely to alter TP concentration significantly.[21] The observed reduction in serum albumin and TP levels were in accordance with earlier observations.[22]

The present work revealed that DOX resulted in elevation of LDL-C and HDL-C levels. These results were in agreement with previous reports.[22,23] However, no significant alteration of HDL/LDL ratio was observed. The increased level of these lipoproteins might be due to compensatory overproduction induced by hypoalbuminemia, which might be associated with DOX nephrotoxicity.[24]

The exact mechanisms underlying DOX-induced nephrotoxicity remain unknown. However, the present data revealed a marked increase in lipid peroxidation and GSH depletion in kidney tissue following DOX administration. Thus, oxidative damage should be concerned. These results correlate well with many previous studies.[25,26] The relation between oxidant and antioxidant states of the tissue is vicious. The increase in free radical production and oxidative stresses could consume and hence deplete the antioxidant defense mechanisms and, on the other hand, the impairment of the antioxidant defense mechanisms could enhance free radical production and oxidative damage. Two different pathways of free radical formation by DOX have been described. The first implicates the one-electron reduction of DOX to the corresponding DOX semiquinone. In the presence of oxygen, redox cycling of

DOX–derived quinine–semiquinone yields superoxide radicals ($O_2^{\cdot -}$). In the second pathway, DOX free radicals are produced by a non-enzymatic mechanism that involves reactions with iron. Iron–DOX complex can reduce oxygen to H_2O_2 and other active oxygen species, which cause oxidative damage of a variety of tissues including the kidneys.[27,28] In addition, DOX induced a significant increase in the renal NO content. This present finding was in agreement with those previously reported.[29,30] High NO production might be a consequence of oxidative stress-induced inducible nitric oxide synthase (iNOS) activation. On the other hand, the produced NO could aggravate the oxidative stresses. The reaction between the produced NO and superoxide anion could result in the formation of peroxynitrite radical, a potent and aggressive cellular oxidant,[31] that could participate in renal GSH depletion and increased lipid peroxidation.

DOX-induced dysfunction of glomerular filtration might perhaps be a consequence of renal circulatory insufficiency. It has been suggested that oxidative stress promotes the formation of a variety of vasoconstrictors that can affect renal function directly or by decreasing the glomerular capillary ultrafiltration coefficient, and thus reduce GFR.[32,33]

The present study demonstrated that whole body exposure to a single low dose (0.3 Gy) of γ radiation, 1 or 3 days before DOX injection exhibited significant protection toward elevated serum levels of creatinine, urea and uric acid. Such protections were more pronounced when irradiation was carried out 1 day before DOX injection. However, these protections ceased if DOX was administered after 7 days of irradiation. Accordingly, irradiation 1 day prior to DOX administration was selected to complete the study.

The current results verified that γ radiation prevented also the DOX-induced changes in serum levels of TP and albumin and in renal contents of GSH, TBARS and NO. However, irradiation did not modify DOX-induced changes in serum LDL-C and HDL-C levels as well as renal Ca content. These findings were consistent with previous reports.[34,35] The protection by low-dose irradiation could be a result of increased antioxidant pools secondary to radiation. In such a way, the low dose of radiation might enhance free radical formation with the concomitant enhancement of the antioxidant mechanisms that overwhelmed the formed free radicals and finally predominated. Thus, irradiation preconditioned the body to withstand the subsequent oxidative injury. This explanation is in accordance with the present results that showed a radiation-induced protection against DOX-induced renal GSH depletion and a consequent reduction in renal lipid peroxidation. Also, the observed termination of the protective effect of radiation after 7 days could be attributed to the return of the antioxidant pools to basal pre-irradiation level.

The current study was also planned to investigate the possible additive protective effect of certain natural products with antioxidant potentials on the prophylactic low dose of γ radiation. The results showed that the most additive protection was provided by L-carnitine, which enhanced the protection effect toward DOX-induced changes in renal GSH, TBARS, NO and Ca concentrations. Curcumin enhanced the protection toward the first three parameters only, while treatment with green tea or garlic exhibited only additive protection against DOX-induced increase in lipid peroxidation. Although percentage of albumin in TP as well as the HDL/LDL ratio was not affected by DOX, curcumin and garlic showed an additive protection against the changes in serum levels of albumin and LDL-C, respectively. These additive protections might indicate that these natural products added adaptive protective mechanisms rather than provided by low-dose radiation. Further more detailed investigations should be performed to clarify this hypothesis.

5. Conclusion

In conclusion, the role of oxidative stress on renal damage induced by DOX has been supported by the results of this experiment. Low-dose γ radiation could provide prophylactic potentials that could be attributed to a decrease in renal lipid peroxidation and enrichment of GSH stores which might be augmented by the use of certain natural antioxidants.

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