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Effects of Gamma-Irradiated Aqueous Extract of Turmeric on Doxorubicin Induced Cardio-Nephrotoxicity in Male Rats

A.M. Abdul Azeem[1], A.N. El shahat[1], Mohamed H.M. Abd El Megid[2] and Amal A. Mansour[1]*

[1]*Food Irradiation Research Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt*
[2]*Natural Products Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt*

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AAM and AMAA performed animal experiments. ANE and MHMAM performed the biological study and collected blood samples. The four authors wrote the manuscript.

Key words
Gamma-irradiation, Doxorubicin, Turmeric, Curcumin, Nephrotoxicity

ABSTRACT

This study was designed to investigate the effectiveness of gamma irradiation process (5 kGy) on the total phenolic contents, antioxidant activity and curcumin content of turmeric powder. Also, this study was aimed to investigate the influences of aqueous extract gamma-irradiated turmeric on doxorubicin (DOX)-induced cardio-nephrotoxicity in male rats. The result indicates that the total phenolic content, antioxidant activity and curcumin content of turmeric powder were significantly increased by γ-irradiation. The results of biological study revealed that inoculation of DOX (3 mg/kg b.w./week/6 weeks) to rats induced cardiac and renal dysfunction, inflammation, and oxidative stress with significant reduction of cardiac and renal antioxidant parameters compared to control group. Co-administration of DOX with either raw (RTAE) or γ-irradiated turmeric aqueous extract (GTAE) (100 mg/kg b.w/ day/6 weeks) significantly reduced side effects of DOX which evidenced by significant reduction in the oxidative parameter (malondialdehyde), cardiac markers, kidney function and inflammatory factors (TNF-α and IL-6) associated with elevation in the antioxidant parameters (glutathione content, superoxide dismutase and catalase) in kidney and heart tissues compared to DOX-group. So, the results concluded that gamma irradiation (5 kGy) can be effective in increasing the *in vivo* and *in vitro* antioxidant potential as well as enhancing the medicinal value of turmeric against tissues damage.

INTRODUCTION

Doxorubicin (DOX), also known as adriamycin (ADR), is an effective anticancer agent that related to anthracycline family and used with a wide range of action in human neoplasms such as breast cancer, acute lymphoblastic leukemia, and testicular carcinoma. However, it's harmful perspective i.e., cardiotoxicity, hepatic damages, and nephrotoxicity have reticent its clinical practice (Alia *et al*., 2021). The toxicity of DOX are due to mitochondrial dysfunction, the production of reactive oxygen species (ROS), the stimulation of apoptosis and inflection of nitric oxide (NO) (Hashisha *et al*., 2021). DOX-prompted harmfulness to liver tissues and possibly will alter blood supply to the kidney and change the xenobiotic reclamation, hence forth periphrastically executing nephropathy. So, the heart and kidney can be protected from DOX-induced cardiotoxicity by qualifying the ROS generated by DOX (Hashisha *et al*., 2021). A lot of antioxidant combinations have been recommended as chemo-deterrent for DOX persuaded toxicity (Afsar *et al*., 2020).

Herbal medicines are commonly used and considered as a convenient treatment because of their safety, efficacy, and cost effectiveness as well as better compatibility. Turmeric (*Curcuma longa* Linn.) is a rhizomatous perennial herb that is known as the golden spice as well as the spice of life. It is widely used as a spice, food preservative and colouring material (Kumari and Paul, 2020). The most important chemical components of *Curcuma longa* are curcuminoids (natural antioxidants), which includes curcumin and represent the majority compound within the rhizome (3-8%) (Islas-Ortiz *et al*., 2020). As a medicine, due its bioactive ingredients, turmeric has long been used

* Corresponding author: mansouramal350@gmail.com
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to treat many health problems such as cardiac dysfunction, liver diseases, inflammation, digestive disorders, and skin diseases (Hasimun *et al*., 2021). Till now, *Curcuma longa* is used in a wide range as a natural antioxidant medicinal herb without side any effects (Nahar *et al*., 2018).

Spices and herbal medicines are subject to contamination by microorganisms from soil, air, and water. It can be influenced by environmental factors, handling practices and the storage conditions (Almeida *et al*., 2018). Products like turmeric powder need an efficient decontamination method, preferably one that do not leave chemical residues (Almeida *et al*., 2018). Irradiation techniques compared to other decontamination techniques are faster, safer, more convenient, and eco-friendlier (Rahayu *et al*., 2016). This method appears to be more useful than electro-beams to improve food safety and can be realized after packaging and provides minimal changes in fresh, perishable and "ready to eat" products (Almeida *et al*., 2018). Doses up to 10 kGy were applied and no changes in curcuminoids content were founded (Almeida *et al*., 2018).

Therefore, the aim of the present study was to evaluate the effectiveness of gamma irradiation process (5 kGy) on turmeric, related to quantification of phenolic compounds, antioxidant activity and quantification of curcumin. Also, this study was aimed to investigate the protective effect of gamma-irradiated turmeric on DOX-induced cardio-nephrotoxicity in rats.

MATERIALS AND METHODS

All experiments were carried out during 2021 at the Egyptian atomic energy authority, food irradiation department. The dried turmeric rhizomes were purchased from the store of spices, grains, and oils (Cairo, Egypt). Turmeric rhizomes were powdered by using domestic grinder and then sieved (mesh 20) to obtain powder of uniform particle size. Then, the samples were kept in airtight container and protected from light until further use.

Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Gamma irradiation treatment

The turmeric powder was air packaged in polyethylene packets and treated with gamma rays at the dose of 5 kGy, using Indian Gamma Cell (Ge 4000 A) ^{60}Co source at a dose rate of 0.8053 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Determination of total phenolic contents and antioxidant activity

The total phenolic content of raw and gamma-irradiated turmeric powder was determined by the method described by Singleton and Rossi (1965). The antioxidant activity of the raw and gamma-irradiated samples was determined by the method described by Brand-Williams *et al*. (1995) that based on the reduction of 2,2-diphenyl-1-picril-hydrazyl free radical (DPPH).

Quantification of curcumin

The percentage of curcumin in raw and gamma-irradiated turmeric powder was quantified by measuring the absorbance at λ max 425 nm by using double beam UV spectrophotometer (Pawar *et al*., 2014). About 2 gm from each turmeric powder was dissolved in 100 mL of methanol separately in a round bottom flask. The mixture was refluxed for 1 and 2 h on a heating mantle at an optimum temperature of 50-60 °C. The extract was filtered to get the clear filtrate and then the volume was made up to 100 mL with methanol. An aliquot of 0.5 mL was taken and further diluted with methanol to 50 mL in a volumetric flask. Then, the percentage of curcumin at was quantified λ max 425 nm.

Total curcumin (%) = [Absorbance/ (1680 x concentration of sample)] X 100

Animals

Sprague Dawley Male rats (200-230g body weight (B.WT)) 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 stock rodent diet and tap water that were allowed *ad libitum*. All animals' procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH guidelines).

Preparation of samples for dosage

Doxorubicin (DOX) injection was diluted in saline to set quantity for inoculation. A total dose of 18 mg/kg b.w (single dose (3 mg/ kg b. w.)/ week /6 weeks) was injected into rats during the experiment (Zhao *et al*., 2012).

Preparation of turmeric powder aqueous extract

To prepare raw or gamma-irradiated turmeric aqueous extract (RTAE or GTAE). 100 g of either raw or gamma-irradiated powder was dissolved in 1000 mL distilled sterilized water and boiled for 60 minutes (Kim *et al*., 2005). This solution was filtered and stored at 4°C.

The aqueous extract of RTAE or GTAE was administered orally (100 mg/kg b.w/day) the dose was

detected after the LD50 estimation (700 mg/Kg b. w.) as described by Kumari and Paul (2020).

Grouping of animals

The animals were randomly divided into 4 groups, each consisted of 7 rats; Group C: rats fed on balanced diet and served as control, Group DOX: drug control was inoculated with DOX (3 mg/kg b. w./ week) for 6 weeks (Zhao *et al*., 2012). Group DOX & RTAE: rats treated with an oral dose of raw turmeric aqueous extract (RTAE; 100 mg/kg b.w) daily for 6 weeks along with DOX inoculation once per week (Kumari and Paul, 2020). Group DOX & GTAE: rats treated with an oral dose of gamma-irradiated turmeric aqueous extract (GTAE; 100 mg/kg b.w) daily for 6 weeks along with DOX inoculation once per week (Kumari and Paul, 2020).

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, kidney 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 tumour 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. Serum urea was measured by enzymatic colourimetric method as described by Coulomb and Farreau (1963), serum creatinine was measured by the method of Husdan and Rapoport (1968) and serum uric acid was measured by the method of Caraway (1995).

Renal 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) was according to Yoshioka *et al*. (1979), GSH content by Beutler *et al*. (1963), superoxide dismutase (SOD) activity 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 analysed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Data were statistically analysed by 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 quantification of phenolic compounds of raw and γ-irradiated samples revealed that the total phenolic content and antioxidative activity of γ-irradiated turmeric powder were significantly increased by percent change 3.96% and 5.5%, respectively comparing with raw sample (Table I). Also, the results obtained that the percentage content of curcumin was increased in γ-irradiated turmeric compared to raw samples by percent change 16.6 %.

Table I. Total phenolic compounds and antioxidant activity of raw and γ-irradiated turmeric.

Radiation dose (kGy)	Total phenolic (mg GAE/g)	Antioxidative activity (mg/g dry sample)	Total curcumin %
0.0	572.9 ± 18.5	10.8 ± 0.9	2.77
5.0	595.6 ± 21.6	11.4 ± 0.7	3.23

Values are means of three replicates (± SD)

The results showed that DOX administration markedly increased the levels of cardiac markers (LDH, CPK, CK-MB and cTnI), inflammatory factors (TNF-α and IL-6), kidney function (urea, uric acid, and creatinine), and the level of renal and cardiac MDA accompanied by reduction in the level of renal and cardiac GSH and the activity of SOD and CAT compared to control group (Tables II, III).

Treatment of rats with DOX along with RTAE and GTAE induced significant reduction in the levels of cardiac markers, inflammatory factors, kidney function and the level of renal and cardiac MDA with a significant elevation in the concentration of renal and cardiac and the activity of SOD and CAT compared to DOX-group.

DISCUSSION

Turmeric powder samples were exposed to gamma radiation at dose level of 5kGy. The effect of irradiation on total phenolic content, DPPH antioxidant activity and curcumin content was studied for all the irradiated and raw samples. In this study, the total phenolic content and antioxidant activity of raw (0 kGy) turmeric powder were increased under the effect of gamma-irradiation (at 5 kGy).

Table II. Effect of raw and γ-irradiated turmeric on cardiac function, TNF-α and IL-6 levels and kidney function in DOX-treated rats.

Parameters	C	DOX	DOX&RTE	DOX& GTE
CPK (U/L)	255.75±6.42^{d}	461.32±9.12^{a}	358.42±8.65^{b}	312.81±9.52^{c}
CK-MB(ng/mL)	3.23 ±0.79^{d}	7.33±0.82^{a}	5.85 ±0.94^{b}	4.67±0.86^{c}
CTnI (ng/mL)	25.43±1.04^{d}	60.77±1.16^{a}	46.37±1.03^{b}	38.48±1.02^{c}
LDH (U/ml)	226.31±14.11^{d}	535.43±15.31^{a}	395.31±17.34^{b}	304.61±16.45^{c}
TNF-α (pg/mL)	673.31 ± 43.12^{d}	895.96±56.43^{a}	763.16 ± 51.26^{b}	719.57 ± 54.32^{c}
IL-6 (pg/mL)	325.75 ± 24.16^{d}	511.34±32.13^{a}	418.94 ± 31.47^{b}	363.42 ± 32.12^{c}
Urea (mg/dl)	25.48±0.82^{d}	53.86±1.13^{a}	40.53±1.14^{b}	33.78±1.16^{c}
Uric acid(mg/dl)	3.67± 0.41^{d}	7.46±0.53^{a}	5.52±0.42^{b}	4.63±0.37^{c}
Creatinine(mg/dl)	0.91± 0.04^{d}	2.27±0.06^{a}	1.62±0.05^{b}	1.30±0.04^{c}

Values are expressed as means ± S.E. (n=7). Values in the same row with different superscript are significantly different at P<0.05.
DOX, Doxorubicin; RTAE, raw turmeric aqueous extract; GTAE, gamma-irradiated turmeric aqueous extract. CPK, creatine phosphokinase; CK-MB, creatinine kinase-MB; cTnI, Troponin I; LDH, lactate dehydrogenase; TNF-α, tumour necrotic factor- α; IL-6, interleukin-6.

Table III. Effect of raw and γ-irradiated turmeric on renal and cardiac antioxidant status system and lipid peroxidation in DOX-treated rats.

Parameters		C	DOX	DOX&RTE	DOX&GTE
MDA (n mol/g tissue)	Kidney	223.31±3.85^{d}	486.52±4.23^{a}	361.37±4.94^{b}	292.25±4.12^{c}
	Heart	133.65±3.41^{d}	307.62±4.31^{a}	239.53±3.38^{b}	173.42±3.42^{c}
GSH (mg/g tissue)	Kidney	34.68±0.52^{a}	18.98±0.82^{d}	23.92±0.67^{c}	28.79±0.81^{b}
	Heart	5.42 ± 0.26^{a}	2.85 ± 0.25^{d}	3.71 ± 0.21^{c}	4.68 ± 0.24^{b}
SOD (U/mg protein)	Kidney	45.58±0.95^{a}	27.35±0.91^{d}	34.35±0.97^{c}	39.82±0.89^{b}
	Heart	28.84±0.94^{a}	16.93±0.88^{d}	19.74±0.86^{c}	22.96±0.87^{b}
CAT (U/mg protein)	Kidney	49.78±0.69^{a}	31.87±0.80^{d}	38.76±0.72^{c}	42.65±0.75^{b}
	Heart	43.15±0.85^{a}	21.48±0.79^{d}	31.52±0.93^{c}	37.29±0.92^{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.
C, control; DOX, Doxorubicin; RTAE, raw turmeric aqueous extract; GTAE, gamma-irradiated turmeric aqueous extract; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, Catalase activity. For other details, see Table II.

Irradiation may break the large phenolic compounds to facilitate release of active ingredients, which were contributed to increase the total phenolic content (Alothman *et al*., 2009). Furthermore, gamma-radiation processing might increase the total phenolic contents and enhance the antioxidant activity of turmeric powder by increasing the activity of phenylalanine ammonia-lyase responsible for the synthesis of phenolic compounds or by increasing the extractability from the tissues by depolymerization and dissolution of cell wall polysaccharides (Alothman *et al*., 2009; Bhatt *et al*., 2007).

Curcumin is of great importance among the biological activities of turmeric and possess high antioxidant activity (Almeida *et al*., 2018). So that, it's important in this work to investigate the effect of gamma-irradiation on the percentage of curcumin content in turmeric powder. The results in this study indicated that gamma-irradiation significantly increase the percentage of curcumin content which could be due to the damaging of turmeric's cell membrane by gamma-radiation thus increased the extractability of curcumin and other medicinal compounds (Dhanya *et al*., 2011). The same result was observed by the study of Sagirroglu *et al*. (2014) and Khalil *et al*. (2012).

Despite the widespread use of DOX as an antineoplastic drug used in the cancer treatment, it has severe adverse effects such as cardiotoxicity and nephrotoxicity (Hashish *et al*., 2021). The results in this work revealed that DOX induced cardiac and renal dysfunction which evidenced by a significant increasing in the levels of cardiac markers (LDH, CPK, CK-MB and cTnI) and elevation of kidney function (urea, uric acid, and creatinine). Also, injection of DOX to rats induced significant reduction in the antioxidant status along with significant increase in the MDA and inflammatory factors

compared to control group. These results were in covenant with the study of Abdel-Moneim *et al*. (2014) and El-Sheikh *et al*. (2012) which demonstrated an increase in lipid peroxidation and subsidence of antioxidant defence system in the kidney after DOX treatment.

In addition, Kalyanaraman *et al*. (2002) reported that DOX reduced the endogenous levels of the antioxidant; these effects can induce cardiomyocyte apoptosis. DOX could induced renal and cardiac tissues injury and inflammation by increasing the production of ROS, oxidative stress, apoptosis, and decreasing in antioxidant enzymes (Abdelmeguid *et al*., 2010). The over generation of ROS could be due to reduction of DOX into the semiquinone form by the effect of mitochondrial enzymes that enhance the increase in the concentration of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) (Carvalho *et al*., 2016). Lee and Harris (2011) suggested that DOX may induce nephrotoxicity directly through its accumulation in the kidney and induction of renal damaging or indirectly by causing damaging to other organs as the heart and the liver may modulate blood supply to the kidney and alter xenobiotic detoxification processes.

In comparison to DOX intoxicated group, the results showed that co-administration of DOX with either RTAE or GTAE can ameliorate DOX toxicity. However, GTAE has high significant ameliorating effect against DOX than that of RTAE which could attributed to the increasing effect of gamma-irradiation on the total phenolic, antioxidant activity and curcumin content. The anti-toxic effect of RTAE or GTAE in this study was observed in the form of reduction in the oxidative parameter (MDA), cardiac markers, kidney function and inflammatory factors (TNF-α and IL-6) associated with elevation in the antioxidant parameters (GSH, SOD and CAT) in kidney and heart tissues. Bami *et al*. (2017) and Tvrdá *et al*. (2016) reported that the antioxidant activity of turmeric could be attributed to its active ingredient like curcumin which have dual antioxidant activity and acts through the scavenging reactive oxygen species (ROS) including superoxide and hydroxyl radicals due to its phenolic structure and induces the upregulation of several endogenous cytoprotective and antioxidant proteins.

The antioxidant effect of turmeric could be due to increasing nuclear factor erythroid like-2 (NrF2) which enhances the transcription through antioxidant response elements (ARE) resulting in an increasing antioxidant activity (He *et al*., 2012). Hussein *et al*. (2018) found that curcumin treatment was able to protect rat myocardium against isoproternol induced myocardial ischemic damage and the protective effect was attributed to its antioxidant properties by inhibiting free radical generation. The study of Toldo *et al*. (2015) indicated curcumin exerts its anti-inflammatory effects via several mechanisms, i.e., curcumin down regulates the nuclear factor-κB (NF-κB), resulting in a decrease in the expression of TNF-α, interleukin-1B (IL-1B) and interleukin-6 (IL-6).

CONCLUSION

From the obtained results it could be concluded that, turmeric (RTAE or GTAE) administration significantly curtails the effects of cardio-nephrotoxicity induced by DOX that verified by remarkable protection effect of kidney and heart function, enhance the antioxidant status, and reduce MDA concentration with potent anti-inflammatory influence.

Also, the results concluded that gamma-irradiated turmeric (5 kg) had higher level of total phenolic and curcumin content, higher in vivo and in vitro antioxidant activity and high significant cardio-nephroprotective effect against DOX than the raw one. So, the results suggest that gamma irradiation (5 kg) can be applicable as a food processing technique and can enhance the medicinal value of turmeric against tissues damage.

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IRB approval and ethical statement

All procedures related to animals were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH guidelines).

Statement of conflict of interest

The authors have declared no conflict of interest.

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