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Possible curative role of curcumin and silymarin against nephrotoxicity induced by gamma-rays in rats

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ABSTRACT

Curcumin (CUR) and silymarin (SLM) are powerful antioxidant and anti-inflammatory compounds with beneficial protective effects against renal diseases. The purpose of this study was to evaluate the efficacy of CUR and SLM alone or in combination on radiation (IR) induced kidney injury. The results showed that CUR and SLM alone or in combination attenuated the oxidative stress denoted by a reduction in the level of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and advanced oxidation protein products (AOPP) along with a marked increase of glutathione GSH content and total antioxidant capacity (TAC). Additionally, a significant decrease in the level of blood urea nitrogen (BUN), creatinine, Cystatin-C (CYT-C), neutrophil gelatinase-associated lipocalin (N-GAL) and Kidney Injury Molecule-1 (Kim-1) was recorded. Moreover, the treatment resulted in a remarkable decline in the serum levels of interleukin-18(IL-18), tumor necrosis factor- alpha (TNF-α), C reactive protein (CRP), BCL2 associated X protein (Bax), Factor-related Apoptosis (FAS) and the activity of Caspase-3 associated by an increase of B-cell CLL/lymphoma 2 (Bcl2) level. The results were confirmed with the histopathological examination. Kidney of irradiated showed glomerular atrophy, massive necrotic changes of expanded tubules with hyaline cast inside some tubules and apoptotic changes were recorded in some renal tubules. While irradiated rats treated with CUR and SLM exhibited marked preservation of the cellular structure of their kidney tissue. In conclusion, the combination of CUR and SLM could be more potent than a single agent on the biochemical and histological changes of the irradiated rat renal tissue.

1. Introduction

Ionizing radiation (IR) can induce different cellular reactions depending upon rate of exposure and dose (Park et al., 2015). The toxic effects of high-level IR on biological system are largely produced by the excessive production of reactive oxygen species (ROS) that overwhelm the levels of antioxidants. ROS considers as the chief mechanism for radiation–induced nephrotoxicity through lipid peroxidation, protein oxidation, and depletion of anti-oxidant elements (Einor et al., 2016). The kidney is an important organ necessary for the body to do several essential functions such as detoxification, and excretion of toxic metabolites and drugs. Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Kidney is a radiosensitive organ. Histopathological surveys of radiation-induced nephropathy have shown the occurrence of damage to glomeruli, blood vessels, tubular epithelium, and interstitium (Cohen and Robbins, 2003).

It is well known that compounds with antioxidant and anti-inflammatory properties exhibited radioprotective effects to maintain the integrity of cells against oxidative stress-induced tissue damage (Shedid et al., 2019). Combinations of phytochemicals may offer additive or synergistic effects, which would enhance their efficacy at low doses making them potent treatment modalities to inhibit or eliminate tissue damage due to oxidative stress initiation.

Many medicinal plants have been used with no side effects and phytochemicals derived from them. Phytochemicals have been classified into six major categories based on their chemical structures and characteristics. These categories include carbohydrate, lipids, phenolics, terpenoids and alkaloids, and other nitrogen-containing compounds. Phenolics are the largest and most structurally varied group of phytochemicals, and this group includes the flavonoids and phenolic acids. Flavonoids include the largest and most versatile class (Saxena et al., 2013). The phenolic acids are types of aromatic acid compound

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containing hydroxybenzoic acids and hydroxycinnamic acids that originate from non-phenolic molecules of benzoic and cinnamic acid, respectively (Machu et al., 2015). Other important phytochemicals such as lectins, glucosinolates, terpenes, polysaccharides, carotenoids, and others have been documented (Campos-Vega and Oomah, 2013).

Phytochemicals possess many therapeutic properties such as anticarcinogenic (Thomas et al., 2015), antimutagenic (Batista et al., 2016), anti-inflammatory (Oliviero et al., 2018), and antioxidant (Choi et al., 2012) effects. Some phytochemicals are known to block the action of enzymes and other substances that promote the growth of cancer cells (Athar et al., 2009). Additionally, phytochemicals such as thymoquinone, olive phenolics are known to have antimicrobial through inhibition of the action of ATP synthase, where the enzyme synthase could be used as a molecular target for these phytochemicals (Ahmad et al., 2015; Liu et al., 2017) Moreover, phytochemicals have the ability to protect against nephrotoxicity (Hassan et al., 2017a; Hassan et al., 2019), and hepatic oxidative injury (Hassan et al., 2018). Also, it is well documented that phytochemicals such as silybin, curcumin (CUR), genistein, ellagic acid, silymarin (SLM), and isothiocynates appear to protect against oxidative stress hazards (Hassan et al., 2017b; Prasad et al., 2016).

Curcumin, (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a yellow crystalline powder from turmeric (*Curcuma longa L.*). It possesses a wide range of pharmacological properties against multiple chronic diseases (Kunnumakkara et al., 2017). Curcumin has shown to inhibit inflammatory processes and considers as a powerful scavenger of the ROS and reactive nitrogen species (RNS) as well as being able to preserve the renal mitochondrial redox balance during acute and chronic nephrotoxicity (Trujillo et al., 2013). Further, Kim et al. (2016); Hashish and Elgaml (2016); Ghoniem et al. (2012) recorded the protective effects of CUR on renal function and oxidative stress.

Silymarin (SLM) is used for hepatic tissues in traditional medicine. It is a flavonoid extracted from *Silybum marianum* (milk thistle) plant, useful as a protective agent in various clinical and both in-vivo and in-vitro experimental models of hepatotoxicity (Gad, 2017), cardiotoxicity (Avci et al., 2017) and nephrotoxicity (Nouri and Heidarian, 2019). SLM is safe in animal models and no significant adverse reactions are reported in human studies (Hogan et al., 2007; Ahmed et al., 2019). SLM in combination with CUR has shown inhibitory effects against liver diseases and colon cancer cells (Gad, 2017; Montgomery et al., 2016).

The purpose of this study was to investigate the mitigating effects of CUR and SLM alone or in combination against IR-induced nephrotoxicity by assessment biochemical indices and the histological changes in the kidney tissue of male rats.

2. Material and methods

2.1. Animals

Male Wistar albino rats (180–200 g) obtained from the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, housed in specially designed cages and kept under standard conditions of temperature (25C° ± 5C°), humidity and light controlled room-(12:12 h light: dark cycle) kept on a standard laboratory diet and water ad libitum. The study was approved by Research Ethics Committee (REC) for experimental studies (Human and Animal subjects) at the National Centre for Radiation Research and Technology (NCRRT)-Egyptian atomic Energy Authority, Cairo. Serial No. A7/19.

2.2. Radiation facility

Animals were located in specially designed, well-ventilated, acrylic cages. The whole body of the animals was exposed to 8 Gy gamma radiation from a biological irradiator with Cs-137 (Gamma cell-40) source (manufactured at the atomic energy agency, Canada) belonging to NCRRT, Cairo. The dose was calculated according to the Dosimetry Department in NCRRT, with a dose rate: 0.649 Gy/min.

2.3. Reagents and chemicals

CUR and SLM were purchased from Sigma-Aldrich, St Louis, MO, USA product No. C1386 and S 0292, respectively. The dose of CUR was 100 mg/Kg/day and SLM was100 mg/Kg/day (for 14 consecutive days (Avci et al., 2017)) post-irradiation. Chemicals and reagents were purchased from Sigma-Aldrich, St Louis, MO, USA unless otherwise mentioned. Measurement of absorbance was performed using a T60 UV/VIS spectrophotometer, PG instruments, London, UK. All chemicals used were of the highest purity grade available.

2.4. Experimental design

Rats were divided into eight equal groups ($n = 8$) as following (I) Control; rats received orally phosphate buffer solution (PBS) PH 8 1 ml for each rat (as a vehicle) for 14 days. (II) CUR; rats received curcumin in a dose of 100 mg/kg daily dissolved in PBS by gastric tube for14 days. (III) SLM; rats received silymarin in a dose of 100 mg/kg daily dissolved in PBS by gastric tube for 14 days. (IV) CUR + SLM; rats received as in group II and III for 14 days. (V) IR; rats were exposed to an acute single dose of 8 Gy gamma rays; (VI) IR + CUR; rats were exposed to an acute single dose of 8 Gy, then followed by CUR for 14 days after one hour from irradiation; (VII) IR + SLM; rats were exposed to an acute single dose of 8 Gy, then followed by SLM for 14 days after one hour from irradiation; (VIII) IR + CUR + SLM; rats were exposed to an acute single dose of 8 Gy, then followed by CUR and SLM for 14 days after one hour from irradiation.

2.5. Preparation of the samples

At the end of the experiment, rats were sacrificed 15th day after a fasting period of 12 h next day following the last dose of the treatment. Blood samples were obtained via heart puncture by sterilized syringe, kidney tissues were rapidly excised. The blood was left to coagulate to obtain serum after centrifugation at 3000*g* for 15 min. Homogenized kidney tissues were prepared according to the method of the kit's instructions.

2.6. Measurement of oxidant and antioxidant markers

Total Antioxidant Capacity (TAC) was determined using TAC Assay Kit Cat. No. MAK187. GSH was determined according to Beutler et al. (1963), malondialdehyde (MDA) was assayed according to Ohkawa et al. (1979) and hydrogen peroxide (H_2O_2) was determined according to Aebi (1984) using commercial kits (Biodiagnostic, Egypt). Advanced oxidation protein products (AOPP) was determined according to the method of Witko-Sarsat et al. (1996) using Rat AOPP ELISA Assay Kit Cat. No. CSB-EQ027429RA from CUSABIO. The tissues were prepared according to the instructions of the method.

2.7. Measurement of serum kidney injury markers

BUN and creatinine were determined according the method of Fawcett and Scott (1960); Schirmeister et al. (1964), respectively using commercial kits (Biodiagnostic, Egypt). Cystatin-C (CYS-C), serum Neutrophil Gelatinase Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (Kim-1) were determined using Rat CYS-C ELISA Kit Cat. No. MBS042119, Rat NGAL Elisa kit Cat. No. MBS724401 and Rat Kim-1 ELISA Kit Cat. No. MBS355395, respectively from MyBioSource.com.

2.8. Measurement of inflammatory and apoptosis markers in the serum

B-cell CLL/lymphoma 2 (Bcl2), Bax, Factor-related Apoptosis (FAS),

Interleukin 18(IL-18), tumor necrosis factor alpha (TNF-α), C-Reactive Protein (CRP) and Caspase-3 were determined using Rat Bcl2 ELISA Kit Cat.No. CSB-E08854r, Rat Bax ELISA Kit Cat No. CSB-EL002573RA, Rat FAS ELISA Kit Cat. No. CSB-E07324r, Rat IL-18 Elisa Kit Cat.No. CSB-E04610r, Rat TNF-α ELISA kit Cat. No. CSB-E11987r, Rat CRP ELISA Kit Cat. No. CSB-E07922r and Rat Casp-3 ELISA Kit Cat. No. CSB-E08857r, respectively from CUSABIO.

2.9. Determination of TNFα, IL-18, Bax, Bcl2 and caspase-3 gene expression

The mRNA gene expression of TNFα, IL-18, Bax, Bcl2 and caspase-3 was determined by Real time-Polymerase Chain Reaction (Real time-PCR). The method is based on the RNA extraction and the reverse transcription into cDNA. Total RNA was extracted from the kidney tissue using SV Total RNA Isolation system (Promega, Madison, WI, USA). The isolation of RNA needs effective disruption of cells, denaturation of nucleoproteins complexes, inactivation of endogenous ribonuclease (RNase) activity and elimination of contaminating DNA and proteins. The reverse transcription into cDNA was carried out in thermal cycler AB, Applied Biosystem 2720, USA using RT-PCR Kit (Stratagene, USA.). The primer pairs used for the genes are illustrated in Table 1. Relative expression the genes were calculated by means of the comparative threshold cycle method. All values were normalized to the β- Actin gene.

2.10. Histopathological study

Tissue specimens from the kidney were collected and fixed in 10% buffered formalin solution followed by dehydration, clearing and embedding in paraffin. Paraffin sections of 5 μm thickness were prepared and stained routinely with hematoxylin and eosin according to Bancroft et al. (1996) and examined microscopically.

2.11. Statistical analysis

Statistical comparisons of data were analyzed using (SPSS/PC) computer program. The results were analyzed using ANOVA, and multiple comparisons were done with Tukey's post-hoc test using SPSS/PC software program. The data were expressed as mean standard deviation. The significance levels were set at $P < .05$, $P < .01$ and $P < .001$ (Snedecor and Cochran, 1989).

Table 1
The primer sequence of the studied gene expression.

Target gene	Primer sequence
TNF-α	Forward: 5′ - ACT GAA CTT CGG GGT GAT-3′ Reverse: 5′ - GCT TGG TGG TTT GCT ACG - 3′
IL-18	Forward: 5′ - CAGACCACTTTGGCAGACTTCA-3′ Reverse: 5′ -ACACAGGCGGGTTTCTTTTGT-3′
Caspase-3	Forward: 5′-GCAGCAGCCTCAAATTGTTGAC-3′ Reverse: 5′-TGCTCCGGCTCAAACCATC-3′
Bax	Forward: 5′-AGACACCTGACCTTGGA-3′ Reverse: 5′-TTGAAGTTGCCATCAGCAAACA-3′
Fas	Forward: 5′-GAATGCAAGGGACTGATAGC-3′ Reverse: 5′-TGGTTCGTGTGCAAGGCTC-3′
Bcl2	Forward: 5′ - ATCGCTCTGTGGATGACTGAGTAC- 3′ Reverse: 5′ - AGAGACAGCCAGGAGAAATCAAAC- 3′
β-actin	Forward: 5′-GAGATTACTGCCCTGGCTCCTA-3′ Reverse: 5′-CATCGTACTCCTGCTTGCTGAT-3′

3. Results

3.1. Effect of CUR or SLM alone or in combination on the kidney redox state markers

Table 2 showed that administration of CUR or SLM alone or in combination to normal rats had no significant change ($p > .05$) in the levels of GSH content, MDA, H_2O_2 and AOPP, whereas a significant increase ($p \leq .05$) in the value of TAC in rats that received CUR alone or CUR in combination with SLM by 14%, 18% respectively, compared to their values of control group. Irradiated rats induced a significant increase in the level of MDA, H_2O_2 and AOPP by 89%, 102%, 76% respectively associated with a significant decrease of GSH content and TAC by 68%, 64% respectively, compared to control rats. On the other hand, irradiated rats that received CUR alone revealed a significant decrease ($p \leq .001$) of MDA, H_2O_2 and AOPP by 41%, 34%, 34%, respectively associated with a significant increase of GSH content and TAC by 144%, 125%, respectively, compared to irradiated group. Also, irradiated rats that received SLM alone showed a significant decrease ($p \leq .001$) of MDA, H_2O_2 and AOPP by 36%, 34%, 34%, respectively associated with a marked increase ($p \leq .001$) of GSH and TAC by 135%, 100%. While irradiated rats that received CUR in combination with SLM revealed the additive effect manifested by a significant decrease of MDA, H_2O_2 and AOPP by 43%, 42% and 37% respectively accompanied with a significant increase of GSH content and TAC by 169%, 150%, respectively, compared to irradiated rats (Table 2).

3.2. Effect of CUR or SLM alone or in combination kidney injury markers

Normal rats that received CUR or SLM alone had no significant change ($p > .05$) in the serum level of BUN, creatinine, Cystatin-C, N-GAL and Kim-1, compared to their values in the control group. In group 5, exposure of rats to irradiation led to a noticeable elevation ($p \leq .001$) in the serum levels of BUN, creatinine, Cystatin-C, N-GAL and Kim-1 by 90%, 85%, 97%, 128%, and 114%, respectively relative to the normal group (Table 3). While, in irradiated rats received CUR alone resulted in a remarkable decline ($p \leq .001$) in serum levels of BUN, creatinine, Cystatin-C, N-GAL and Kim-1 by 35%, 31%, 36%, 45%, 45%, respectively relative to the irradiated group. Similarly, irradiated rats that received SLM alone resulted in a remarkable decline ($p \leq .001$) in serum levels of BUN, creatinine, Cystatin-C, N-GAL and Kim-1 by 27%, 28%, 35%, 42%, 43%, respectively. Moreover, CUR in combination with SLM displayed the additive effects on the studied parameters that decreased by 44%, 37%, 45%, 49%, 49% respectively, compared to irradiated rats (Table 3).

3.3. Affect of CUR or SLM alone or in combination on serum inflammatory and apoptotic markers

Normal rats that received CUR or SLM alone had no significant change ($p > .05$) in the gene expression and serum level of IL-18, TNF-α,CRP, Bax, Bcl2, FAS, and the activity of Casp-3, compared to their values in the control group. Rats exposed to irradiation showed a noticeable elevation ($p \leq .001$) in the gene expression and serum levels of IL-18,TNF-α,CRP, Bax, FAS, and the activity of Casp-3 by 163%, 175%, 109%, 108%, 223%, 54%, respectively associated with a significant decrease of Bcl2 by 63% relative to the normal group While, in irradiated rats that received CUR alone resulted in a remarkable decline ($p \leq .001$) in the gene expression and serum levels of the of IL-18,TNF-α, CRP, Bax, FAS, and the activity of Casp-3 by 51%, 52%, 34%, 33%, 59%, 23%, respectively associated with an increase of Bcl2 level by 80% relative to the irradiated group. Similarly, irradiated rats that received SLM alone resulted in a remarkable decline ($p < .001$) in the gene expression and serum levels of IL-18,TNF-α,CRP, Bax, FAS, and the activity of Casp-3 by 49%, 47%, 33%, 26%, 57%, 21%, respectively associated by an increase of Bcl2 level by109%, respectively (Table 4,

Table 2
Effect of CUR and SLM on total antioxidant capacity (TAC), glutathione (GSH) content, malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and advanced oxidation protein products (AOPP) in the kidney tissue of different animal groups.

Parameters groups	TAC (mg/g)	GSH (mg/g)	MDA (nmol/g protein)	H_2O_2 (U/mg protein)	AOPP (μmol/L)
Control	22 ± 2.2	1.67 ± 0.21	380 ± 36	90 ± 11	125 ± 20
CUR	25 ± 2.0[a]	1.74 ± 0.22	374 ± 33	88 ± 12	122 ± 18
SLM	23 ± 1.1	1.72 ± 0.23	376 ± 36	87 ± 11	123 ± 17
CUR + SLM	26 ± 2.2[a]	1.77 ± 0.11	374 ± 32	88 ± 10	123 ± 18
IR	8 ± 1.2[a]	0.54 ± 0.03[a]	719 ± 55[a]	182 ± 37[a]	220 ± 39[a]
IR + CUR	18 ± 2[a2b3]	1.32 ± 0.02[a2b3]	423 ± 50[a1b3]	120 ± 23[a3b3]	147 ± 28[a1b3]
IR + SLM	16 ± 2.2[a2b3]	1.27 ± 0.01[a2b3]	458 ± 48[a2b3]	121 ± 22[a3b3]	145 ± 22[a1b3]
IR + CUR + SLM	20 ± 2.0[b]	1.45 ± 0.41[a1b3]	410 ± 48[b3]	106 ± 21[a1b3]	139 ± 28[a1b3]

Values are expressed as Means ± standard deviation (SD) ($n = 8$). Differences between means were considered significant (a1b1) at $p \leq .05$, highly significant (a2b2) at $p \leq .01$ and very highly significant (a3b3) at $p \leq .001$.
[a] vs Normal control group.
[b] vs Irradiated group (IR).

Fig. 1). Moreover, CUR in combination with SLM displayed the additive effects on the level of IL-18, TNF-α, CRP, Bax, FAS, and the activity of Casp-3 by 58%, 58%, 41%, 47%, 64%, 31%, respectively associated byan increase of Bcl2 level by 122%, respectively compared to irradiated rats (Table 4), as well as positive modulation in their gene expression was detected (Fig. 1).

3.4. Histopathological finding

The renal corpuscle of control rat consists of glomerulus and Bowman's capsule. At the urinary pole, the incomplete layer of the Bowman's capsule contains simple squamous epithelium altered to cuboidal epithelium in the proximal convoluted tubules, (Fig. 2A). The microscopic picture of kidney of CUR and SLM treated groups showed normal histological structures as control one (Fig. 2B&C). In the irradiated group, renal cortex showed atrophy of glomeruli due to shrunken and fibrosis of the rest of glomerular tuff, besides an increase in the capsular space (Fig. 3A). Some cases, showed massive necrotic changes of expanded tubules with hyaline cast inside some tubules and loss of their brush borders, in other cases the necrotic tubules were replaced by leukocytes (Fig. 3B&C). Moreover the lesions in some tubules were progressive to hyalinization and massive necrosis, which led to loss of its details (Fig. 3D). In other cases apoptotic changes were recorded in the renal tubules (Fig. 3E). Renal cortex in the kidney of irradiated rats treated with CUR showed prevention of irradiation damage in the renal cortex without pathological renal lesions changes in most cases with or without dilated inter-tubular blood vessels (Fig. 3F). Kidney of irradiated rats treated with SLM showed an increase in the tubular leukocytes (Fig. 3G) and brush borders loss in some expanded renal tubules. While in the kidney of irradiated rats treated with CUR in combination with SLM showed normal glomeruli and normal renal tubules with or without congested blood vessels (Fig. 3H&I).

4. Discussion

Using medicinal plants in the control of various human illnesses is an early idea. The renal tissue is highly vulnerable to damage caused by ROS, might due to the abundance of polyunsaturated fatty acids in the renal tubular cells (Singh et al., 2006). Thus, the administration of antioxidants that counteract oxidative stress may protect the kidneys from resultant injury. Nephrotoxicity studies have confirmed the real roles for antioxidant medicinal plants against kidney toxicity (Hassan et al., 2019; Hassan et al., 2017a; Changizi-Ashtiyani et al., 2017). In the current study, it was noticed that IR (8Gy) induced oxidative stress that revealed negative effects on the renal function associated with kidney tissue damage and the present results are in concordance with previously published findings by Schüler et al. (2015); Abozaid et al. (2017) Shao et al., (2018); Talebpour Amiri et al., (2018); Sayed et al., (2019). Oxidative stress indicated by an elevation in the level of MDA and AOPP due to the reaction of OH· that is the most reactive among all ROS with lipids and proteins molecules of the cells, respectively. Moreover, the elevation in the level of oxidative stress markers was along with a decrease of TAC and GSH content and this might be attributed to increased utilization of antioxidants to neutralize the excess of ROS. The obtained results are in agreement with Azzam et al. (2012), Shedid et al. (2019).

Curcumin (CUR) is a polyphenolic diketone natural product isolated from the rhizome of the plant *Curcuma longa*, commonly known as turmeric, with a variety of pharmacologic properties. It possesses pleiotropic molecular effects, where it interacts with its numerous molecular targets. CUR may directly bind and modulate their activity, or indirectly regulate their functions. > 30 different proteins have been found to interact with curcumin directly such as DNA polymerase (Takeuchi et al., 2006), focal adhesion kinase (FAK) (Leu et al., 2003), thioredoxin reductase (Fang et al., 2005), lipoxygenase (LOX)

Table 3
Effect of CUR and SLM on kidney injury markers [blood urea nitrogen (BUN), creatinine, Cystatin-C, neutrophil gelatinase-associated lipocalin (N-GAL) and Kidney Injury Molecule-1 (Kim-1)] in the serum of different group of rats.

Parameters groups	BUN (mg/dl)	Creatinine (mg/dl)	Cystatin-C (mg/L)	N-GAL (ng/ml)	Kim-1 (ng/ml)
Control	10.5 ± 2.1	0.60 ± 0.09	1.10 ± 0.07	40 ± 6	0.51 ± 0.01
CUR	10.3 ± 1.9	0.58 ± 0.08	1.09 ± 0.10	39.8 ± 5	0.50 ± 0.02
SLM	10.6 ± 1.8	0.59 ± 0.09	1.11 ± 0.09	39.7 ± 4	0.50 ± 0.01
CUR + SLM	10.3 ± 0.9	0.60 ± 0.08	1.11 ± 0.07	39.6 ± 4	0.51 ± 0.02
IR	20 ± 3.2[a3]	1.11 ± 0.10[3]	2.17 ± 0.15[a3]	91 ± 15[a3]	1.09 ± 0.09[a3]
IR + CUR	13.0 ± 2.1[a2b3]	0.77 ± 0.09[a2b3]	1.39 ± 0.19[a2b3]	50 ± 12[a2b3]	0.60 ± 0.08[a1b3]
IR + SLM	14.6 ± 2.0[a3b3]	0.80 ± 0.07[a2b3]	1.42 ± 0.11[a2b3]	53 ± 13[a3b3]	0.62 ± 0.09[a2b3]
IR + CUR + SLM	11.2 ± 1.9[b3]	0.70 ± 0.08[a1b3]	1.20 ± 0.10[b3]	46 ± 7 [a1b3]	0.56 ± 0.1[b3]

Values are expressed as Means ± standard deviation (SD) ($n = 8$). Differences between means were considered significant (a1b1) at $p \leq .05$, highly significant (a2b2) at $p \leq .01$ and very highly significant (a3b3) at $p \leq .001$.
[a]vs Normal control group.
[b]vs Irradiated group (IR).

Table 4
Effect of CUR and SLM on serum interlukin-18(IL-18), tumor necrosis factor alpha (TNF-α), C reactive protein (CRP), Factor-related Apoptosis (FAS) B-cell CLL/lymphoma 2 (Bcl2) levels and the Caspase-3 activity of different animal groups.

Parameters groups	IL-18 ng/ml	TNF-α (pg/ml)	CRP (ng/ml)	Bax (ng/ml)	Fas (ng/ml)	Caspase- 3 (ng/ml)	Bcl2 (ng/ml)
Control	2.23 ± 0.5	40 ± 4	400 ± 40	1.44 ± 0.02	2.10 ± 0.4	3.89 ± 0.6	15 ± 2.2
CUR	2.21 ± 0.4	39.7 ± 5	401 ± 38	1.42 ± 0.02	2.15 ± 0.5	3.88 ± 0.5	14.7 ± 1.9
SLM	2.22 ± 0.4	39.8 ± 4	399 ± 37	1.41 ± 0.01	2.11 ± 0.4	3.90 ± 0.7	14.5 ± 1.8
CUR + SLM	2.23 ± 0.5	39.7 ± 5	398 ± 36	1.38 ± 0. 02	2.13 ± 0.5	3.81 ± 0.6	14.9 ± 1.9
IR	5.87 ± 1.1^{a3}	110 ± 12^{a3}	834 ± 67^{a3}	2.99 ± 0.18^{a3}	6.78 ± 2.3^{a3}	5.99 ± 1.0^{a3}	5.5 ± 0.8^{a3}
IR + CUR	2.90 ± 0.8^{a1b3}	53 ± 5^{a3b3}	550 ± 0.8^{a2b3}	2.00 ± 0.19^{a3b3}	2.80 ± 0.33^{a3b3}	4.61 ± 0.4^{a2b3}	9.90 ± 1.0^{a3b3}
IR + SLM	2.99 ± 0.7^{a2b3}	58 ± 4^{a3b3}	562 ± 1.3^{a3b3}	2.22 ± 0.18^{a3b3}	2.90 ± 0.6^{a3b3}	4.71 ± 1.09^{a3b3}	11.51 ± 1.4^{a2b3}
IR + CUR + SLM	2.44 ± 0.5^{b}	46 ± 4^{a1b3}	490 ± 1.2^{a2b3}	1.59 ± 0.2^{b3}	2.42 ± 0.5^{a1b3}	4.11 ± 0.5^{b3}	12.2 ± 1.4^{a2b3}

Values are expressed as Means ± standard deviation (SD) ($n = 8$). Differences between means were considered significant (a1b1) at $p \leq .05$, highly significant (a2b2) at $p \leq .01$ and very highly significant (a3b3) at $p \leq .001$.
[a]vs Irradiated group (IR).
[b]vs Normal control group.

(Skrzypczak-Jankun et al., 2003). Besides, CUR can also bind to certain divalent metal ions such as Fe, Cu, Mn and Zn (Baum and Ng, 2004). Similarly, silymarin (SLM) is a standardized extract of the milk thistle seeds, containing a mixture of flavonolignans consisting of silibinin, isosilibinin, silicristin, silidianin, and others. Previous studies recorded that SLM has molecular targets for cancer prevention. It interferes with the expression of the cell cycle regulators and proteins involved in apoptosis. Thus, it can modulate the balance between cell survival and apoptosis (Lee et al. 2013).

In the current work, administration of CUR and SLM alone or in combination to irradiated rats attenuated the oxidative stress manifested by a decrease in the level of MDA, AOPP and H_2O_2 associated with an increase of GSH content and the capacity of antioxidant. This might result from the reduced levels of subunits of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase Nox4 and p67phox, which catalyzes the synthesis of O_2^- (Trujillo et al., 2013). Moreover, the current data revealed that the two agents are most effective in ameliorating these markers when given in combination and the results are in line with the previous study in which CUR had the ability to attenuate the renal oxidative stress through up-regulation haem oxygenase diabetic nephropathy rats (Kim et al., 2016). Similarly, silymarin (SLM) showed effectiveness in the balance between oxidative stress and antioxidant defense in the kidney that attributed to its stabilizing effects on the plasma membrane and proteins of the renal cells against ROS (Hashish and Elgaml, 2016). While, Nouri and Heidarian (2019) recorded that SLM had the ability to increase vitamin C, which could be considered as a reason for reducing ROS and the recovery of renal damage and morphological changes.

The immune system considers as the most important defense mechanisms against environmental agents. IR induces immune response disorders as well as deregulation of inflammatory cytokines production (Hekim et al., 2015). Numerous studies have recorded a strong link between renal impairment and different mediators and markers of inflammation including CRP, IL-18, TNF-α, and fibrinogen (Vanholder et al., 2003). Muslimovic et al. (2015) reported that the progression of kidney disease is significantly associated with inflammation. Therefore, oxidative stress and the overproduction of TNF-α and IL-18 are closely linked and contribute significantly to the renal cells damage post-irradiation. The result is similar to that in the previous findings by Baradaran-Ghahfarokhi (2012); Reuter et al. (2010).

Further, CRP is a sensitive indicator of inflammation and tissue damage; play an important role in radiation- induced damage (Koc

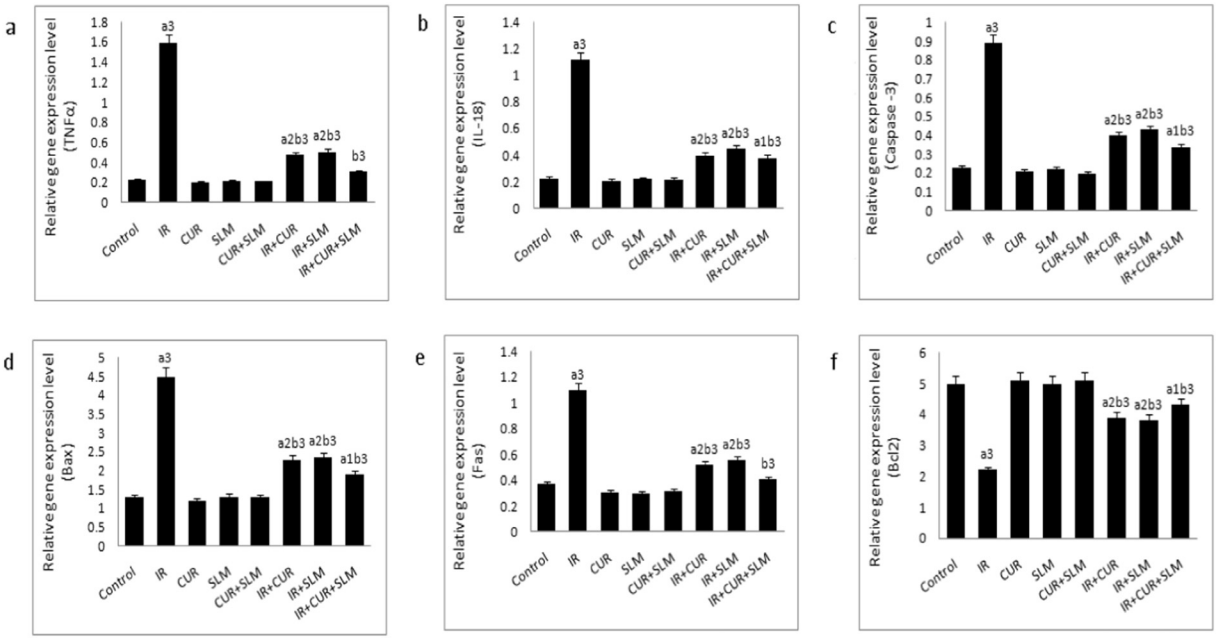

Fig. 1. Quantitative real -time polymerase chain reaction (RT-PCR) analysis mRNA of TNFα (a), IL-18 (b), caspase-3 (c), Bax (d), Fas (e) and Bcl2 (f) in the different animal groups. Values are expressed as Means ± standard deviation (SD) ($n = 8$). Differences between means were considered significant (a1b1) at $p \leq .05$, highly significant (a2b2) at $p \leq .01$ and very highly significant (a3b3) at $p \leq .001$. [a] vs normal control group. [b] vs irradiated group (IR).

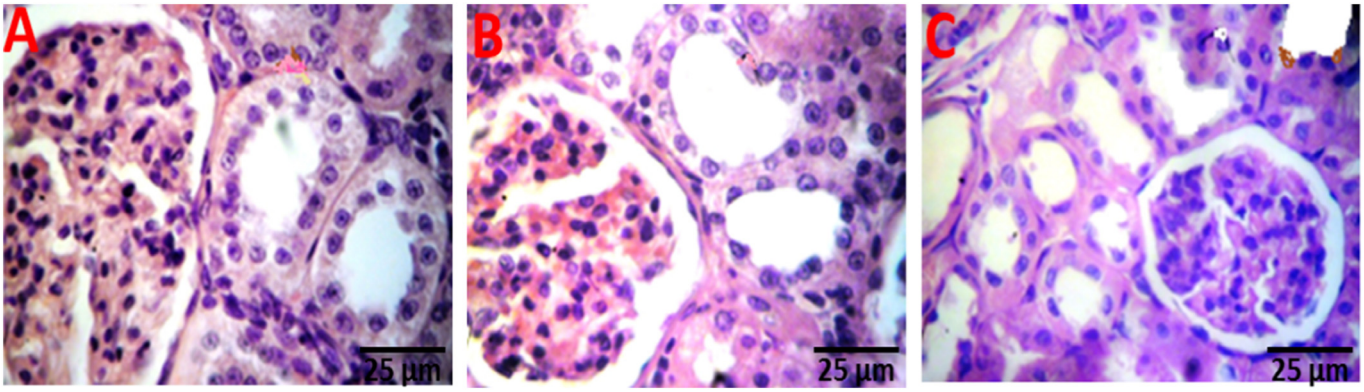

Fig. 2. A, B & C; kidneys of control, CUR and SLm rats showing normal structures (H&E 400×). H&E: hematoxylin and eosin.

et al., 2003) through the apoptosis, phagocytosis, nitric oxide release, and the production of cytokines. Further, a significant increase in the level of CRP following exposure of rats to γ-radiation, which might result in renal function abnormalities that promote acute kidney injury and this is in concordance with Lai et al. (2016).

Herein, administration of CUR and SLM alone or in combination to irradiated rats reduced the inflammation processes through decreasing NF-α, IL-18 and CRP. However, the combination is more effective than

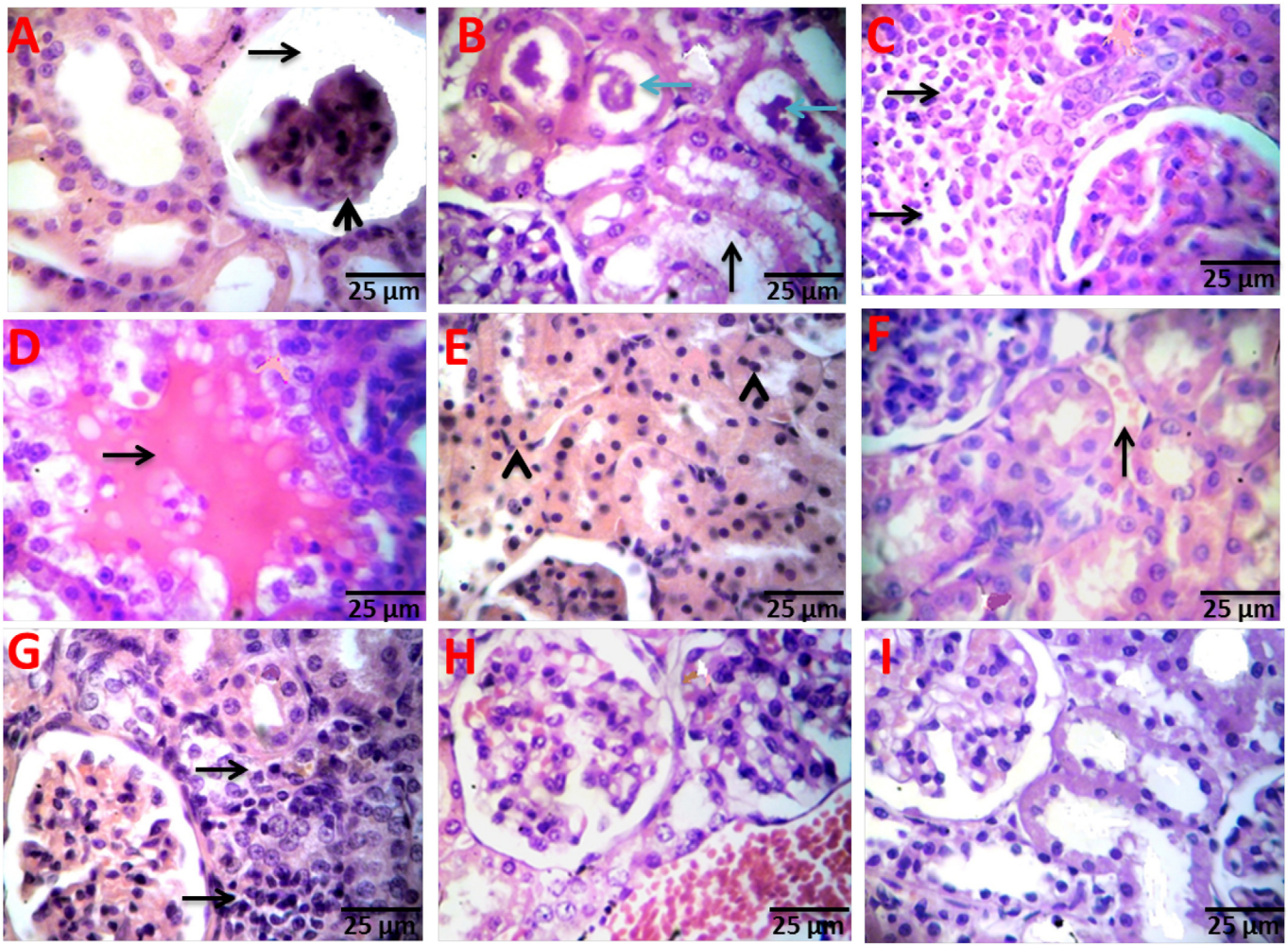

Fig. 3. Effects of CUR and SLM or in combination on renal tissues of irradiated rats (H&E 400 x). H&E: hematoxylin and eosin. A; Rat kidney of irradiated group showing atrophy of glomuli (↑) and increasing the capsular space (→). B; Kidney of irradiated group showing hyaline cast (←) inside expanded renal tubules (↑). C; Kidney of irradiated group showing necrotic tubules covered by leukocytes (→). D; Kidney of irradiated group showing massive necrosis of tubules with loss of its details (→). E; Kidney of irradiated group showing apoptosis changes of tubules (^). F; Kidney of Curcumin treated & irradiated group showing normal renal corpuscle with congested inter tubular blood vessels (↑). G; Kidney of Silymarin treated & irradiated group showing an increase in the leukocytes inside and around renal tubules (→). H; Kidney of Curcumin-Silymarin treated & irradiated group showing normal glomeruli with congested blood vessels (↑). I; Kidney of irradiated rats treated with curcumin in combination with silymarin showing slightly normal renal tissues.

CUR and SLM alone, which related to their anti-inflammatory and immunomodulatory beneficial properties due to their chemical structure and their ability to interact with numerous their molecular targets (Soetikno et al., 2011; Ghosh et al., 2012; Lu et al., 2017; Xu et al., 2018). The results are supported by previous studies (Al-Kuraishy et al., 2019; Montgomery et al., 2016; Nouri and Heidarian, 2019; Al-Rasheed et al., 2015, 2016; Kim et al., 2016; Ghosh et al., 2016).

The mitochondrial pathway is described by the liberation of apoptosis-stimulating factors, such as cytochrome *c* (Cyt c) and apoptosis-inducing factor (AIF) (Zhang et al., 2017). The liberation of Cyt c to cytosol can activate the caspases-dependent apoptosis (Huttemann et al., 2011). The Bcl-2 family proteins, including anti-apoptotic agents like Bcl-2 and pro-apoptotic like Bax members, play essential roles in the mitochondria-mediated pathway (Kushnareva et al., 2012). Accordingly, the current study investigated that irradiated rats displayed a significant increase in the caspase 3 activity and Bax level and their gene expressions associated with a depletion in the Bcl2 and Fas levels and their gene expressions. This might result from the overproduction of ROS resulting in a decrease of mitochondrial membrane potential, which then accelerated the liberation of Cyt c into the cytoplasm, leading to apoptosis by caspase 3 and 9 dependent pathway (Zhao et al., 2019). The results are similar to that in Wang et al., 2017; Shao et al. (2018).

On the other hand, irradiated rats received CUR and SLM alone or in combination effectively decreased the level and gene expression of TNF-α, IL-18, Bax, Fas and Caspase −3 along together an elevation of antiapoptotic markers Bcl2 level and its gene expression, compared to irradiated untreated animals. Moreover, the combination of the two agents was the most beneficial in modulating these markers near the normal level, suggesting their anti-apoptotic potential action and confirmed that these molecules could be considered as molecular targets for CUR and SLM to prevent radiation -induced oxidative damage and renal dysfunction. The results are in agreement with Tan et al. (2015); Wang et al., 2018; Zhang et al., 2018; Sandur et al. (2007); Okunieff et al. (2006).

In kidneys, urea is filtered out of the blood by glomeruli and is partially being reabsorbed with water (Corbett, 2008). Increased BUN is seen associated with kidney disease or failure (Mitchell and Kline, 2006). Creatinine is a breakdown result of creatine phosphate in muscle and is generally produced at a fairly constant rate by the body replying on muscle mass (Allen, 2012). Creatinine is commonly used as a measure of kidney function and is the first step in checking glomerular filtration rate (GFR). Cystatin C is a protease preventer and non-glycosylated, low molecular weight protein that results from nucleated cells at a constant rate. Cystatin C is proposed to be a biomarker of renal function and is freely filtered by the glomeruli and then completely reabsorbed and catabolized in the proximal tubular cells. Therefore, serum Cystatin C level has the potential to be an endogenous marker of GFR (Dharnidharka et al., 2002).

Herein, irradiated rats caused a marked increase in the levels of BUN, creatinine, Cystatin C, N-GAL and Kim-1 and this is might be due to the impairment of GFR (Sarumathy et al., 2011). Additionally, the rise in BUN level could be attributable to radiation-induced changes in the amino acid metabolism or excessive protein breakdown as a reflection of deteriorating renal performance (Badr El-Din, 2004). Moreover, the elevated level of inflammatory mediators such as TNF-α and, IL-18 being able to act as toxins participating in uremia complications and kidney injury. This is similar to that in Vanholder et al. (2003).

This study showed that the treatment of CUR and SLM alone or in combination to irradiated rats led to a partial recovery of renal function denoted by a reduction of BUN, creatinine and Cystatin C and this might be due to a reduction of renal damage and this is in concordance with Najafi et al., 2015, Trujillo et al. (2013) Guzel et al. (2019). Also, our result is in line with Hassan et al. (2017a, 2017b) who suggested a possible protective effect of silybin against polymyxin E-induced nephrotoxicity through reduction of the elevated serum renal biochemical markers.

Finally, the biochemical findings were confirmed by histopathological examination, which showed glomerular collapse, inflammatory cell infiltration, and loss of cellular architecture of kidney tissues of the irradiated rats. These data agree with that reported by Kokubo et al. (2010). Renal cortex showed glomerular atrophy, besides an increase in the capsular space, massive necrotic changes of expanded tubules with hyaline cast inside some tubules and loss their brush border. Other cases showed necrotic tubules that were replaced by leukocytes, lesions might be progressive to massive tubular necrosis or hyalinization and loss of tubular details. Apoptotic changes might be recorded in renal tubules of some cases (Abozaid et al., 2017). These histopathological observations supported our hypothesis that CUR and SLM alone or in combination represents a potential therapeutic approach in the treatment of γ-radiation induced nephrotoxicity as evidenced by the marked reduction of these pathologic changes with only congested blood vessels in some cases. This is in agreement with previous studies that showed the CUR and SLM has an ameliorative role against renal damage and oxidative stress (Nouri and Heidarian, 2019; Ali et al., 2018). The histopathogical observations showed that CUR in combination with SLM was more effective to reduce the kidney injure than CUR and SLM alone.

5. Conclusion

The obtained results showed that rats exposed to 8 Gy of γ-rays induced- severe nephrotoxicity, which greatly associated with many biochemical and histological changes. On the other hand, irradiated rats treated with CUR and SLM alone or in combination reduced the elevated levels of lipid peroxidation, attenuated the renal dysfunction, decreased the levels of inflammatory and apoptotic markers and normalized the altered renal morphology. Additionally, the combination of CUR and SLM revealed more effective and efficient on radiation induced kidney injury. Thereby, CUR in combination with SLM could be used as a medication for protection of patients during radiotherapy.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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