Grape seed oil ameliorates carbon tetrachloride-mediated cardiac oxidative stress and inflammation in γ-irradiated rats

Amel F. M. Ismail¹, Mahgoub M. Ahmed²*, and Mamdouh M. T. Eassawy³

¹Drug Radiation Research Department, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt.
²Molecular Drug Evaluation Department, National Organization for Drug Control and Research (NODCAR), Giza 12553, Egypt
³Regional Center for Food and Feed (RCFF), Agriculture Research Center, Giza, Egypt.

ABSTRACT

The present study was aimed to investigate the protective effect of grape seed oil (GSO) on cardiac toxicity induced by carbon tetrachloride (CCl₄) in γ-irradiated rats (7 Gy). A significant elevation in the serum levels of LDH, CK-MB and Troponin T were exerted in CCl₄-intoxicated, γ-irradiated or γ-irradiated+CCl₄-intoxicated rats. Furthermore, the malondialdehyde (MDA), nitric oxide (NO) levels were significantly increased accompanied with a significant decrease of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities and glutathione (GSH) content in the cardiac tissues after exposure to CCl₄, γ-irradiation or their combinations. Moreover, up-regulation gene expression of the proinflammatory markers; tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), nuclear factor-kappa B (NF-κB) and inducible nitric oxide synthase (iNOS) in the cardiac tissues was observed. However, the oral administration of GSO (3.7 g/kg body weight) ameliorated the studied parameters by decreasing elevated serum markers. In addition, significantly suppressed the cardiac lipid peroxidation nitric oxide and gene expression of the proinflammatory markers as well as recovered the activities of antioxidant enzymes and GSH levels in the heart. The cardioprotective effects of GSO on CCl₄ γ-irradiation or their combinations could be attributed to its potent antioxidant and anti-inflammatory activities.

Keywords: Grape seed oil, carbon tetrachloride, γ-irradiation, cardiotoxicity, proinflammatory cytokines oxidative stress, gene expression.

*Corresponding author
INTRODUCTION

Carbon tetrachloride (CCl₄) is a well-known hepatotoxin, besides it causes disorders in different tissues, including heart via free radicals generation [1]. The CCl₄ toxicity arises from the formation of reactive intermediates such as trichloromethyl (CCl₃) and/or carbon centered lipid radicals (LO• and/or LOO•) [2,3,4,5]. Frequently, CCl₄ at a high dose quickly causes an acute tissue injury, whereas it displayed a cellular necrosis, oxidative stress and inflammation, leading to apoptotic organ failure [6]. On the other hand, radiation being progressively used for medical and occupational purposes, however, the destructive effect of radiation destroys the tumor cells as well as the healthy normal tissues. The exposure to ionizing radiation increases the production of the reactive oxygen species (ROS) and this can lead the irradiated cells into a state of oxidative stress, or imbalance between ROS production and the detoxification process by the biological systems [7].

The heart is a bio-vital organ and generates intense oxidative imbalances because of its intense activity, thus it presents a less potent antioxidant system as compared to other organs [8]. Generally, maintaining the antioxidant status and inhibiting the pro-inflammatory markers could function as major mechanisms in preventing damage burden induced by toxic agents. The association of oxidative stress and inflammation in the etiology and progression of several acute and chronic clinical disorders could be resisted by the agents with antioxidant and anti-inflammatory properties [9]. Several antioxidant agents have been described to reduce CCl₄-induced toxicity [10,11,12].

Various reports focused on the health promoting and antioxidant effects of grapes. Interest in the health benefits of grapes has been increased due to their high phenolics contents, most of these phenolics were detected in the seeds. The Grape seed oil (GSO) contains high amounts of phenolic compounds; including resveratrol, gallic acid, catechin, epicatechin and procyanidins, as well as a high level of the vitamin E (60-120 mg/100 g) in addition, to a high amount of essential-polyunsaturated fatty acids such as linoleic acid (69-78%), palmitic acid (5-11%), oleic acid (15-20%) and stearic acid (3-6%) as well as considerable amount of macro and micro elements such as phosphorus, potassium, calcium, magnesium, iron, zinc, copper and manganese [13,14,15,16,17,18]. The potent antioxidant property and the biological activity are claimed to be the protective mechanism of GSO [19,20].

Therefore, the objective of this study was carried out to investigate the protective effect of GSO on acute dose of carbon tetrachloride induced cardiac toxicity in γ-radiated rats.

MATERIALS AND METHODS

Chemicals

The grape seed oil was obtained from Sigma Chemical Co., Nasr City, Cairo, Egypt. Carbon tetrachloride (CCl₄) was obtained from Merck. All other chemicals and reagents used in this study were of analytical grade.

Irradiation of Animals

Whole-body gamma-irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using Canadian Gamma Cell-40 biological irradiator (137Cesium), manufactured by the Atomic Energy of Canada Limited, Ontario, Canada. The radiation dose rate was 0.456 Gy/min at the time of exposure. The total radiation dose was 7 Gy as a single dose of the whole body. Animals were not anesthetized before irradiation.

Experimental animals

Female Wistar rats (weighing 100-120 g) were obtained from the Nile Pharmaceutical Co., Cairo, Egypt. They were housed at the animal facility at the National Center for Radiation Research and Technology. Upon arrival, the animals were allowed to acclimatize for one week before starting the experiment. The animals were kept under standard laboratory conditions of light/dark cycle (12/12 h) a temperature of 25±2°C and humidity of 60±5%. The rats were housed in cages with free access to food and drinking water ad libitum. They were provided with a nutritionally adequate standard laboratory (pellet) diet. The study was conducted
in accordance with international guidelines for animal experiments and approved by the Ethical Committee of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

EXPERIMENTAL DESIGN

The rats were divided into the following groups (6 rats each)

Control group (C): rats administered water orally by gastric intubation for 7 days.

CCl₄ treated group (CCl₄): Rats were orally administered water, then they were intraperitoneally (i.p) injected with acute single dose (2 ml/kg body weight) of CCl₄ after 7 days from the beginning of the experiment [21].

Irradiated group (R): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation [22] then they were administered water orally.

Irradiated+CCl₄ treated group (R+CCl₄): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation. Irradiated rats were orally administered water by gastric intubation for seven days, then they were intraperitoneally (i.p) administered with an acute single dose (2 ml /kg body weight) of CCl₄.

Grape seed oil treated group (GSO): Rats were orally administered 3.7 g/kg body weight (4 ml/kg body weight) of GSO [20] till the end of the experimental period.

GSO+CCl₄ treated group (GSO+CCl₄): Rats were orally administered with GSO for seven days. Then they were administered with acute single dose of CCl₄ (2 ml/kg body weight, ip).

Irradiated+GSO treated group (R+GSO): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation, 30 min later was orally administered GSO till the end of the experimental period.

Irradiated+GSO+CCl₄ treated group (R+GSO+CCl₄): Rats were exposed to a single dose of 7 Gy of the whole body γ-irradiation; 30 min later was orally administered with GSO for seven days. Then they were administered with acute single dose of CCl₄ (2 ml/kg body weight, ip).

The rats were kept overnight fasting. At the end of the experiment, after 16 h of CCl₄ administration [21], rats were sacrificed by cervical dislocation and blood was collected. The separated serum was used for estimation of lactate dehydrogenase [23] and creatine phosphokinase [24] activities using commercial enzymatic kits. The serum troponin T (TNT) was determined using an ELISA kit for rat (Glory Science Co., Ltd, USA). The measurements were performed according to the catalogue instruction guidelines. The TNT level was calculated after plotting the standard curves and expressed as pg/ml.

Determination of the oxidative stress parameters and the antioxidant enzymes in the heart homogenates

The heart of each animal was excised immediately, washed with physiological saline and stored at -80°C. Part of the heart was weighed and homogenized (10%) in chilled 50 mmol phosphate buffered saline (pH 7.4), centrifuged at 1200 g, at 4 °C for 15 min, using cooling centrifuge (Sigma 30K, Germany), then the supernatants were used for the determination of the following parameters;

Lipid peroxidation, in terms of malondialdehyde (MDA) was measured according to the method of Satoh [25], using 1, 1, 3, 3-tetraethoxypropane as a standard.

Nitric oxide (NO) was determined as nitrite concentration. The method used depends on Griess reaction, which converts nitrite into a deep purple azo-compound, which photometrically measured at 540 nm according to the method of Montgomery and Dymock [26].

GSH concentration was measured according to Beutler et al. [27] using 5, 5'-dithionitrobenzoic acid (DTNB).

Superoxide dismutase (SOD) activity was determined according to Nishikimi et al. [28]. The assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye, which was followed photometrically at 560 nm.
Catalase (CAT) activity was assessed according to Aebi [29]. Catalase reacts with a known quantity of hydrogen peroxide (H₂O₂) in the presence of horseradish peroxidase, remaining H₂O₂ reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity measured at 510 nm, which is inversely proportional to the amount of catalase in the original sample.

Glutathione-peroxidase (GPx) activity was measured according to Rotruck et al. [30] that based on indirect determination of GPx, whereas GPx reacted with known amount of glutathione (GSH), then the residual GSH reacted with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB). The color developed was read at 412 nm.

Detection of relative gene expression ratio by Quantitative Real Time PCR (qRT-PCR)

**RNA Isolation and Reverse Transcription**

Briefly, the heart tissues (100 mg) were homogenized in 1 ml ice-cold TRIzol reagent following the manufacturer’s instruction. The RNA quality was verified using spectrophotometric and agarose gel electrophoresis. cDNA was synthesized from 2 µg total RNA using RevertAid™ first strand cDNA synthesis kit (Ferments life science, Thermo Scientific, USA) by incubating at 37°C for 1 h with reverse transcriptase with random hexanucleotides according to the manufacturer’s instructions.

**Quantitative Real Time PCR**

qRT-PCR was performed using the Real-Time PCR Systems (Step One instrument, Applied Biosystems, Foster City, CA, USA). Each 10 µl reaction contained 5 µl SYBR Green Master Mix (Applied Biosystems), 0.3 µl gene-specific forward and reverse primers (10 µM), 2.5 µl cDNA and 1.9 µl nuclease-free water. The sequences of PCR primer pairs used for each gene as follows, TNF-α, Forward: 5′-ATG AGC ACA GAA AGC ATG ATC-3′, Reverse: 5′-TAC AGG CTG ACT CGA ATT-3′ [31], IL-1β Forward: 5′-CAG GCA GGC AGT ATC ACT CA-3′, Reverse: 5′-AGG CCA CAG GTA TTT TGT CG-3′ [31], IL-6 Forward: 5′-CCG GAG AGG AGA CTT CAC AG-3′, Reverse: 5′-GGA AAT TGG GGT AGG AAG GA-3′ [31], TGF-β1 Forward: 5′-CTT CAG CTC CAC AGA GAA GAA CTG C-3′, Reverse: 5′-CAC GAT CAT GTT GGA CAA CTG CTC C-3′ [31], NF-κB Forward: 5′-CAT GAA GAG AAG ACA CTG ACC ATG GAA A-3′, Reverse: 5′-TGG ATA GAG CCT AAG TGT AGA CAC G-3′ [32], iNOS Forward: 5′-GTG GTG ACA AGC ACA TTT GG-3′, Reverse: 5′-GTC TGG ACT TTT CAC TCT GC-3′ [31] and GAPDH Forward: 5′-CCT TCA TTG ACC TCA ACT ACA TGG T-3′, Reverse: 5′-TCA TTG TAC CAG GAA ATG AGC T-3′ [31]. The relative expression of the studied genes was calculated using the comparative threshold cycle method. All values were normalized to the GAPDH genes [33].

**Statistical Analysis**

All statistical analyses were conducted by using the statistical package for Windows Version 15.0 (SPSS Software, Chicago, IL). The results for continuous variables were expressed as mean±standard error. Values were compared by one-way analysis of variance (ANOVA). Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD) test. A value corresponding to P<0.05 was considered statistically significant.

**RESULTS**

Assessment of serum cardiac enzymes

In this study, there was a remarkable elevation (P<0.05) in the serum CK-MB and LDH activities of CCl₄- and R-intoxicated rats as compared to the control animals (Fig. 1). However, these values were augmented in the combined treatment (R+CCl₄ treated animals). Meanwhile, oral treatment of GSO resulted in a significant reduction (P<0.05) in the levels of these enzymes towards normal as compared to the intoxicated rats.

Assessment of serum cardiac troponin-T (TNT) of different treated rats

The serum troponin T level showed a significant statistical increase (P<0.05) in CCl₄- and R-intoxicated rats as compared to the control group (Fig. 1). However, this value was amplified in the combined
treatment (R+CCl₄ treated animals). Oral administration of GSO showed a significant decrement in the level of serum troponin T as compared to the intoxicated rats.

Figure 1. Effect of GSO on the cardiac markers in the blood serum; lactate dehydrogenase (LDH), creatine phosphokinase (CK-MB) and troponin T (TNT).

Control: untreated group, CCl₄: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl₄: carbon tetrachloride group pretreated with GSO, R+GSO: γ-irradiated group post treated with GSO. Values are expressed as means±SE (n=6). Values not sharing the same superscript letters were significantly different (P<0.05).

The oxidative stress and antioxidant status of the cardiac tissues

Fig. 2 and Fig. 3 showed the oxidative stress markers and the antioxidant enzyme activities determined in the cardiac tissues of different experimental groups. MDA and NO levels in the cardiac tissues showed significant elevation (P<0.05) in CCl₄- and R-intoxicated rats as compared to the normal animals. The levels of MDA and NO were enhanced in the combined treatment (R+CCl₄ treated animals). However, GSO treatment significantly decreased the levels of MDA and No as compared to the corresponding intoxicated groups (Fig. 2).

Further, the CCl₄- or R-treated animals showed a significant decrease (P<0.05) in the antioxidant enzymes; SOD, GSH-Px and CAT activities and GSH content in the cardiac tissues as compared to the normal animals (Fig. 2 and 3). However, the treatment of animals with GSO significantly (P<0.05) ameliorated the antioxidant system by enhancing the activities of SOD, GSH-Px and CAT and the GSH content as compared to the corresponding intoxicated groups.

Figure 2. Effect of GSO on the oxidative stress markers in heart tissues.

MDA: malondialdehyde, NO: nitric oxide and GSH: reduced glutathione. Control: untreated group, CCl₄: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl₄: carbon tetrachloride group pretreated with GSO, R+GSO: γ-irradiated group post treated with GSO. Values are expressed as means±SE (n=6). Values not sharing the same superscript letters were significantly different (P<0.05).
Figure (3): Effect of GSO on the antioxidant enzymes of the cardiac tissues.

SOD: superoxide dismutase, CAT: catalase and GSH-Px: glutathione peroxidase enzymes. Control: untreated group, CCl₄: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl₄: carbon tetrachloride group pretreated with GSO, R+GSO: γ-irradiated group post treated with GSO. Values are expressed as means±SE (n=6). Values not sharing the same superscript letters were significantly different (P<0.05).

The relative ratio of the gene expression of the proinflammatory cytokines; TNF-α, IL-1β, IL-6, TGF-β and the proinflammatory nuclear factor kappa B (NF-κB) showed a significant up regulation (P<0.05) in the intoxicated animals as compared to the control group. However, GSO treatment significantly down regulated the gene expression of these proinflammatory markers, as compared to the corresponding intoxicated groups (Fig. 4).

Figure 4. Effect of GSO on the gene expression of the cardiac proinflammatory markers.

Data are expressed as fold change (relative to control group). Using reverse transcriptase, cDNA was synthesized from 1 µg total RNA. Aliquots of cDNA were used as template for real-time PCR reactions containing primers for proinflammatory markers. Tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), Transforming growth factor beta 1 (TGF-β1) and nuclear factor kappa B (NF-κB). Control: untreated group, CCl₄: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl₄: carbon tetrachloride group pretreated with GSO, R+GSO: γ-irradiated group post treated with GSO. Values are expressed as means±SE (n=6). Values not sharing the same superscript letters were significantly different (P<0.05).
The relative ratio of the inducible nitric oxide synthase gene expression in the cardiac tissues

The relative ratio of the gene expression of the iNOS showed a significant up regulation (P<0.01) in the CCl₄- and R-intoxicated animals, which was enhanced due to the combined effect (R+CCl₄ treated animals) as compared to the control group. However, GSO treatment significantly down regulated the gene expression of the iNOS, as compared to the corresponding intoxicated groups (Fig. 5).

Data are expressed as fold change (relative to control group). Using reverse transcriptase, cDNA was synthesized from 1 µg total RNA. Aliquots of cDNA were used as template for real-time PCR reactions containing primer for iNOS. Control: untreated group, CCl₄: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl₄: carbon tetrachloride group pretreated with GSO, R+GSO: γ-irradiated group post treated with GSO. Values are expressed as means±SE (n=6). Values not sharing the same superscript letters were significantly different (P<0.05).

DISCUSSION

In this study, we investigated the protective effect of GSO against oxidative stress in the cardiac tissues of an experimental rat model of γ-radiation and CCl₄. The data indicate that the treatment of rats with CCl₄ or γ-irradiation induced over production of ROS coupled with diminished cellular antioxidant activities as well as elevated MDA and NO levels in the cardiac tissues. Further, the gene expression of the proinflammatory cytokines, TNF-α, IL-1β, IL-6, TGF-β1 as well as the NF-κB was up regulated. Furthermore, the gene expression of iNOS was up regulated also in the cardiac tissues of these intoxicated rats. In addition, all these values were amplified due to the treatment of γ-irradiated animals with CCl₄. Several studies proved that generation of ROS and oxidative stress were observed due to γ-radiation exposure and CCl₄ administration [14,15,34,35,36,37,38,39]. The ionization radiation generates ROS/RNS, which activates several signal transduction pathways, causing the final radiation effects [34]. Various studies of carbon tetrachloride intoxication had established that CCl₄ generates free radicals in different tissues, including the heart [1,8,9], thus the activities of SOD, CAT, and GPx and level of GSH were diminished, accompanied by elevated levels of MDA and NO due to CCl₄ toxicity. The oxidative stress resulting from increased free radical production due to CCl₄ intoxication may play a critical role in the degenerative processes in the tissues [39]. Both oxidative and nitrosative stresses have been reported to alter lipids and proteins [40].

The mechanism of CCl₄ toxicity involves lipid peroxidation mediated by the free radicals that are generated during metabolism. Elevated lipid peroxidation can lead to oxidative stress when the antioxidant defense systems are inhibited. The peroxidation of membrane phospholipids eventually lead to loss of membrane integrity and finally to cell death. Further, the elevated lipid peroxidation contents that augmented in the cardiac tissues of CCl₄ intoxicated-γ-irradiated rats as seen in this study could indicate the acute cardiac tissue damages. It was established that intra-peritoneal administration of CCl₄ in rats induced lipid peroxidation and oxidative protein damage and increased the MDA level in the tissues [5,41]. These changes result in alteration of the enzymatic antioxidant defenses of the tissues [16,42]. SOD, CAT and GSH-Px are mainly the antioxidant enzymes that involved in the cell defense against oxygen cytotoxicity. The superoxide anion (O₂⁻) is converted to H₂O₂ and molecular oxygen (O₃) by the SODs systems (Cu/Zn-SOD and Mn-SOD), the decrease in cardiac SOD activities remarked in CCl₄-intoxicated-γ-irradiated rats could be attributed to the adaptive responses in protecting cells against oxidative stress damage. In addition, CAT and/or GSH-Px catalyze the removal of H₂O₂ through its reduction to water and O₃ [43].
The observed decline in catalase and GSH-Px activities in the cardiac tissues of CCl\textsubscript{4}-intoxicated and/or \(\gamma\)-irradiation treated rats is evident that their ability to detoxify \(\text{H}_2\text{O}_2\), however, the accumulation of \(\text{H}_2\text{O}_2\) inhibits their activities. Moreover, the decrease in the activities of the CAT noted in the present study could be attributed to the leakage of the enzymes from the injured cells that exposed to oxidative stress into the blood [44]. The significant decrease in the heart GSH-Px activity of CCl\textsubscript{4}, R-, and R+CCl\textsubscript{4}-intoxicated rats could be attributed to its inactivation by MDA by-products [45] or due to the decrease in GSH content [43,46]. GSH-Px acts in conjunction with GSH, the major non-enzymatic antioxidant to decompose \(\text{H}_2\text{O}_2\), or an organic peroxide (ROOH) to water/or alcohol while simultaneously oxidizing GSH. The resistance of many cells against oxidative stress is concomitant with high intracellular levels of GSH [47]. GSH acts directly as a free radical scavenger by neutralizing the hydroxyl radical (HO\(^{\cdot}\)), restores damaged molecules by hydrogen donation, reduces peroxides and maintains protein thiols in the reduced state [43,48]. The significant decline in the cardiac GSH content that was detected in this study could be attributed to the enhanced utilization of GSH in a large amount to combat with the ionizing radiation and CCl\textsubscript{4}-induced free radical damage in the cardiac tissues. Furthermore, NO appears to increase in heart due to CCl\textsubscript{4} intoxicated-\(\gamma\)-irradiated rats in the present study. Reactive NO may combine with \(\text{O}_2^{\cdot -}\) to form peroxynitrite that generates 3-nitrotyrosine in protein. Peroxynitrite is also could initiate MDA, which cause direct or indirect oxidative damage in the nucleic acids or promote apoptosis [49,50,51,52,53,54]. On the other hand, the relative ratio of iNOS gene expression was up regulated in the current study due to CCl\textsubscript{4} and R-induced cardiac intoxication, which could suggest the elevated level of NO. NO might be a part of the physiological response to injury. NO is synthesized from L-arginine by a family consisting of NO synthase (NOS) isoenzymes [55]. NO over production by iNOS could be contributed to ischemia–reperfusion injury [56,57,58]. Further, iNOS isoform has been reported to play an important role in reducing the infarct size during myocardial infarction, iNOS over expression could promote tumor angiogenesis and metastasis, where it was found to be increased and correlated with vascularization [59,60,61]. Furthermore, iNOS-derived NO was described to promote survival of ischemic tissue by stimulating angiogenesis [62]. Additionally, the iNOS is expressed by inflammatory cells induced by endotoxic or proinflammatory cytokines [63].

The pretreatment of GSO to CCl\textsubscript{4} intoxicated-\(\gamma\)-irradiated rats eventually resulted in a reduction of the MDA level, which point out the antioxidant property of GSO that correlated with the direct scavenging activity towards peroxyl radicals both in the membrane and in the aqueous phase. Further, the antioxidant activity of the GSO is attributed to its high polyphenols contents including resveratrol, gallic acid, catechin and procyanidins as well as vitamin E [13,16,17,18,64]. In addition, GSO administration exhibited down regulation of the elevated iNOS gene expression relative ratio compared to CCl\textsubscript{4}-intoxicated-\(\gamma\)-irradiated rats. Consequently, GSO significantly reduced the NO production by attenuated iNOS expression and enhanced the antioxidant status of the heart tissues in CCl\textsubscript{4}-injected-\(\gamma\)-irradiated rats.

On the other hand, the developed ROS could induce several intracellular pathways, including transcription factors e.g. nuclear factor kappa B (NF-kB) [65]. When NF-kB activated by oxidative stress, it induces the over expression of the proinflammatory cytokines such as TNF-\(\alpha\), IL-1\(\beta\) and IL-6 [66]. These proinflammatory cytokines TNF-\(\alpha\), IL-1\(\beta\) and IL-6 as well as TGF-\(\beta\) were up regulated in ischemic heart diseases [67,68].

The data revealed that the pretreatment of GSO showed a statistical improvement of the harmful effects of ionizing radiation and CCl\textsubscript{4} on the antioxidant status in the cardiac tissues. Moreover, it was reported that GSO decreased the progress of inflammation by down regulating the iNOS expression [69]. The polyphenol-rich GSO is useful for the inhibition of the inflammatory processes via NF-kB deactivation [70]. Our results showed that the relative ratio of the gene expression of TNF-\(\alpha\), IL-6 and TGF-\(\beta\) were elevated in CCl\textsubscript{4}, \(\gamma\)-irradiated or combined treatment of CCl\textsubscript{4}-intoxicated-\(\gamma\)-irradiated rats than in controls. On the other hand, the amelioration occurs in inflammatory factors in GSO treated intoxicated groups could indicate that GSO ingredients have potent anti-inflammatory activities. The anti-inflammatory action of GSO could assign to its antioxidant activity. In addition, NF-kB activation could be facilitated by some oxidative stress responses [71]; however, a broad range of antioxidants inhibited it [72-73]. So, scavenging of ROS by GSO antioxidant components resulted in NF-kB deactivation and inhibition of the proinflammatory cytokines; TNF-\(\alpha\) and IL-6 and inhibition of the TGF-\(\beta\) release of the intoxicated groups. Besides, the decrease in NO levels in GSO treated CCl\textsubscript{4}-intoxicated-\(\gamma\)-irradiated rats could be attributed to the inhibition of TNF-\(\alpha\) and ROS production, which evoke NF-kB deactivation and the subsequent iNOS gene expression [74]. Accordingly, the ameliorating effect of GSO on CCl\textsubscript{4} and \(\gamma\)-irradiation induced inflammation involved the inhibition of a variety of
proinflammatory mediators via inhibiting the ROS/RNS, TNF-α and IL-6 production and the release of TGF-β. Furthermore, resveratrol, procyanidins, catechin and gallic acid were reported to be good cellular preventive agents against DNA oxidative damage and apoptosis via induction of endogenous antioxidant enzymes [75,76,77].

In Conclusion grape sees oil is effective in preventing CCl₄ and γ-irradiation-induced cardiotoxicity, oxidative stress and inflammation in rat heart. Protective effect of grape sees oil against CCl₄ and γ-irradiation might be due to the recovery of the redox system and inhibition of iNOS and proinflammatory cytokines gene expression.

REFERENCES
