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

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ABSTRACT

The present study was aimed to investigate the protective effect of grape seed oil (GSO) on cardiac toxicity induced by carbon tetrachloride (CCl_4) in γ-irradiated rats (7 Gy). A significant elevation in the serum levels of LDH, CK-MB and Troponin T were exerted in CCl_4-intoxicated, γ-irradiated or γ-irradiated+CCl_4-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 CCl_4, 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 CCl_4, 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.

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INTRODUCTION

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin, besides it causes disorders in different tissues, including heart via free radicals generation [1]. The CCl_4 toxicity arises from the formation of reactive intermediates such as trichloromethyl ($^{\bullet}CCl_3$ and/or $^{\bullet}CCl_3OO$) and oxygen centered lipid radicals ($LO^{\bullet}$ and/or $LOO^{\bullet}$) [2,3,4,5]. Frequently, CCl_4 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 CCl_4-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 (CCl_4) 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.

CCl_4 treated group (CCl_4): Rats were orally administered water, then they were intraperitoneally (i.p) injected with acute single dose (2 ml/kg body weight) of CCl_4 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+CCl_4 treated group (R+CCl_4): 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 CCl_4.

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+CCl_4 treated group (GSO+CCl_4): Rats were orally administered with GSO for seven days. Then they were administered with acute single dose of CCl_4 (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+CCl_4 treated group (R+GSO+CCl_4): 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 CCl_4 (2 ml/kg body weight, ip).

The rats were kept overnight fasting. At the end of the experiment, after 16 h of CCl_4 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^{o}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 (H_2O_2) in the presence of horseradish peroxidase, remaining H_2O_2 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 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^{TM} first strand cDNA synthesis kit (Ferments life science, Thermo Scientific, USA) by incubating at 37^{o}C for l 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 CTT GTC 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 GCT AAG TGT AGA CAC G-3′ [32]**,** iNOS Forward: 5′-GTG GTG ACA AGC ACA TTT GG-3′, Reverse: 5′-GGC 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 TCA 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 CCl_4- and R-intoxicated rats as compared to the control animals (Fig. 1). However, these values were augmented in the combined treatment (R+CCl_4 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 CCl_4- and R-intoxicated rats as compared to the control group (Fig. 1). However, this value was amplified in the combined

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treatment (R+CCl_4 treated animals). Oral administration of GSO showed a significant decrement in the level of serum troponin T as compared to the intoxicated rats.

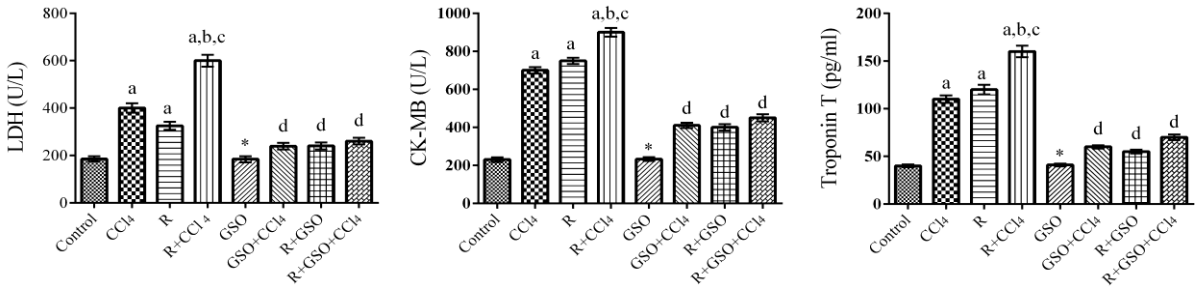

Figure 1. Effect of GSO on the cardiac markers in the blood serum; lactate dehydrogenase (LDH), creatine phosphokinase (CK-MB) and troponin T (TNT).

Control: untreated group, CCl_4: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl_4: 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 CCl_4- and R-intoxicated rats as compared to the normal animals. The levels of MDA and NO were enhanced in the combined treatment (R+CCl_4 treated animals). However, GSO treatment significantly decreased the levels of MDA and No as compared to the corresponding intoxicated groups (Fig. 2).

Further, the CCl_4- 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.

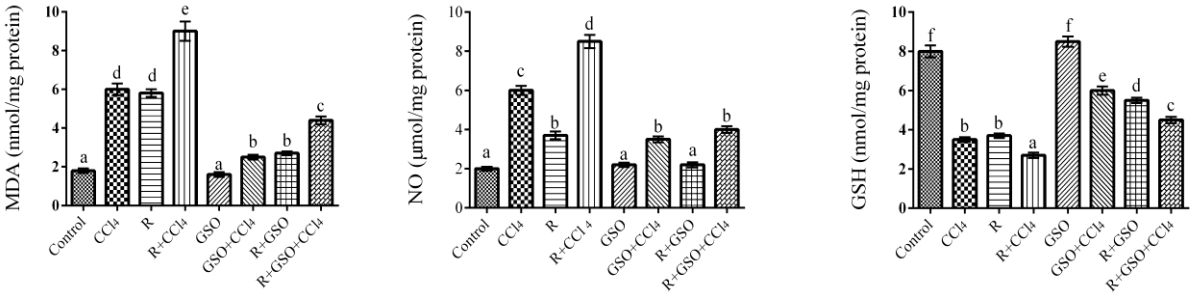

Figure 2. Effect of GSO on the oxidative stress markers in heart tissues.

MDA: malondialdehyde, NO: nitric oxide and GSH: reduced glutathione. Control: untreated group, CCl_4: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl_4: 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).

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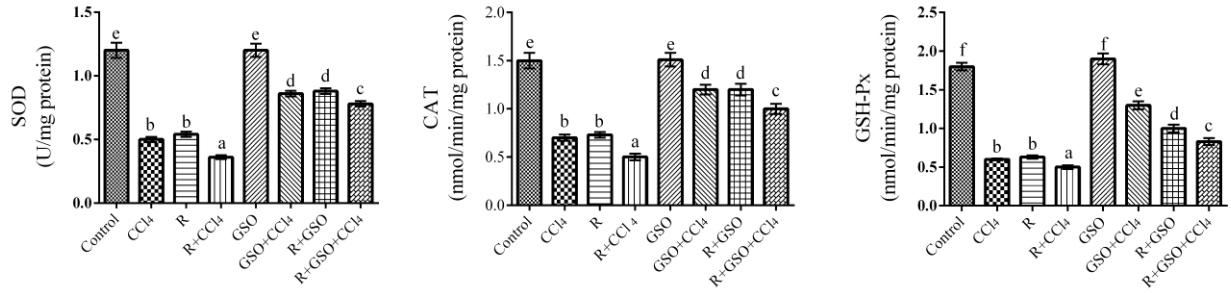

Figure (3): Effect of GSO on the antioxidant enzymes of the cardiac tissues.

SOD: superoxide dismutase, CAT: catalase and GSH-Px: glutathione peroxidase enzymes. Control: untreated group, CCl_4: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl_4: 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).

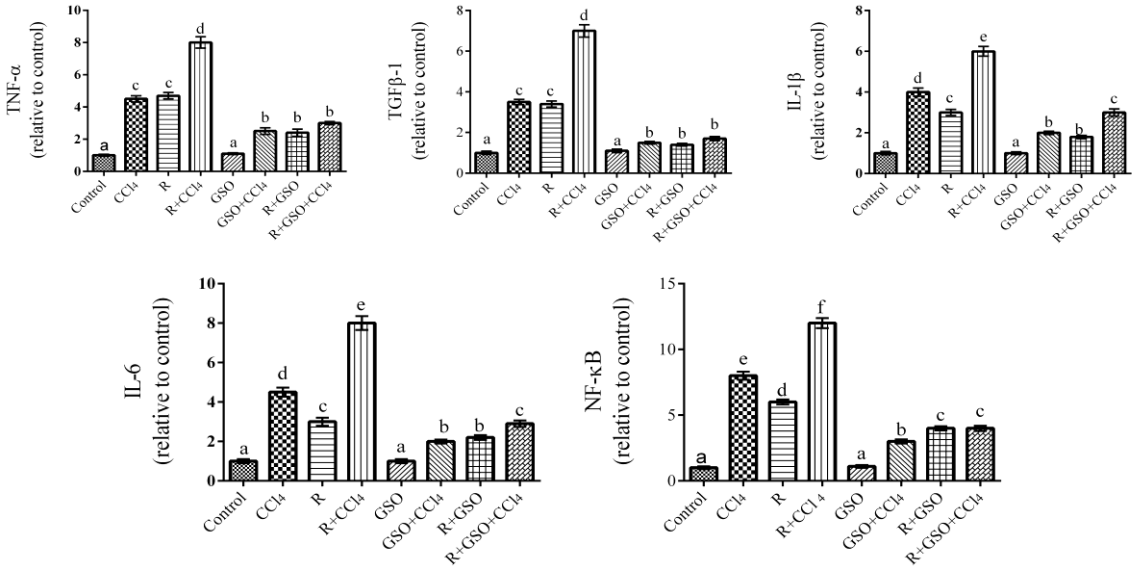

Figure 4. Effect of GSO on the gene expression of the cardiac proinflammatory markers.

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), intrleukin-1β (IL-1β, interleukin-6 (IL-6), Transforming growth factor beta 1 (TGF-β1) and nuclear factor kappa B (NF-κB). Control: untreated group, CCl_4: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl_4: 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).

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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 CCl_4- and R-intoxicated animals, which was enhanced due to the combined effect (R+CCl_4 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).

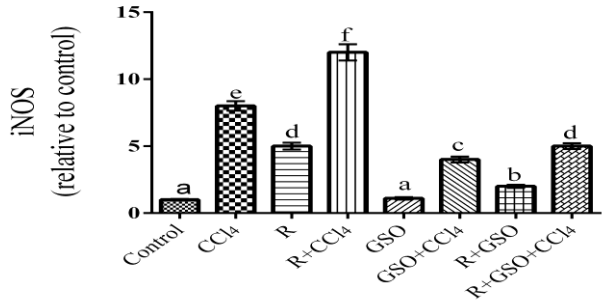

Figure 5. Effect of GSO on the gene expression of the cardiac inducible nitric oxide synthase (iNOS).

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, CCl_4: carbon tetrachloride treated group, R: γ-irradiated group (7 Gy), GSO: grape seed oil treated group, GSO+CCl_4: 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 CCl_4. The data indicate that the treatment of rats with CCl_4 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 CCl_4. Several studies proved that generation of ROS and oxidative stress were observed due to γ-radiation exposure and CCl_4 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 CCl_4 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 CCl_4 toxicity. The oxidative stress resulting from increased free radical production due to CCl_4 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 CCl_4 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 CCl_4 intoxicated-γ-irradiated rats as seen in this study could indicate the acute cardiac tissue damages. It was established that intra-peritoneal administration of CCl_4 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 ($O2^{\bullet-}$) is converted to H_2O_2 and molecular oxygen (O_2) by the SODs systems (Cu/Zn-SOD and Mn-SOD), the decrease in cardiac SOD activities remarked in CCl_4-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 H_2O_2 through its reduction to water and O_2 [43].

The observed decline in catalase and GSH-Px activities in the cardiac tissues of CCl_4-intoxicated and/or -γ-irradiation treated rats is evident that their ability to detoxify H_2O_2, however, the accumulation of H_2O_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_4-, R- and R+CCl_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 H_2O_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 ($HO^{\bullet}$), 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_4-induced free radical damage in the cardiac tissues. Furthermore, NO appears to increase in heart due to CCl_4 intoxicated-γ-irradiated rats in the present study. Reactive NO may combine with $O_2^{\bullet-}$ 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_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_4 intoxicated-γ-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_4-intoxicated-γ-irradiated rats. Consequently, GSO significantly reduced the NO production by attenuated iNOS expression and enhanced the antioxidant status of the heart tissues in CCl_4-injected-γ-irradiated rats.

On the other hand, the developed ROS could induce several intracellular pathways, including transcription factors e.g. nuclear factor kappa B (NF-κB) [65]. When NF-κB activated by oxidative stress, it induces the over expression of the proinflammatory cytokines such as TNF-α, IL-1β and IL-6 [66]. These proinflammatory cytokines TNF-α, IL-1β and IL-6 as well as TGF-β 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_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-κB deactivation [70]. Our results showed that the relative ratio of the gene expression of TNF-α, IL-6 and TGF-1β were elevated in CCl_4-, γ-irradiated or combined treatment of CCl_4-intoxicated-γ-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 antiinflammatory action of GSO could assign to its antioxidant activity. In addition, NF-κB 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-κB deactivation and inhibition of the proinflammatory cytokines; TNF-α and IL-6 and inhibition of the TGF-β1 release of the intoxicated groups. Besides, the decrease in NO levels in GSO treated CCl_4-intoxicated-γ-irradiated rats could be attributed to the inhibition of TNF-α and ROS production, which evoke NF-κB deactivation and the subsequent iNOS gene expression [74]. Accordingly, the ameliorating effect of GSO on CCl_4 and γ-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 CCl_4 and γ-irradiation-induced cardiotoxicity, oxidative stress and inflammation in rat heart. Protective effect of grape sees oil against CCl_4 and γ-irradiation might be due to the recovery of the redox system and inhibition of iNOS and proinflammatory cytokines gene expression.

REFERENCES

[1] Sahreen S, Khan MR, Khan RA and Alkreathy HM. BMC Research Notes 2014; 7: 224-233.
[2] Xiao J, Liong EC, Ching YP, Chang RC, So KF, Fung ML and Tipoe GL. J Ethnopharmacol 2012; 139(2): 462-470.
[3] Ozturk F, Ucar M, Ozturk IC, Vardi N and Batcioglu K. Urology 2003; 62: 353-356.
[4] Jayakumara T, Sakthivel M, Thomasb PA and Geraldinea P. Chem Biol Interact 2008; 176(2-3): 108-120.
[5] Botsoglou NA, Taitzoglou IA Botsoglou E Lavrentiadou S et al. J Agric Food Chem 2008; 56: 6287-6293.
[6] Karakus E, Karadeniz A, Simsek N, Can I, Kara A, Yildirim S, Kalkan Y and Kisa F. J Hazard Mater 2011; 195: 208-213.
[7] Maurya DK, Devasagayam TP, Krishnan C and Nair K. Indian Journal of Experimental Biology 2006; 44: 93-114.
[8] de Freitas RB, Boligon AA, Rovani BT, Piana M, de Brum TF, Jesus R, Rother FC, Alves NM, da Rocha JB, Athayde ML, Barrio JP, de Andrade ER and Bauerman L. Molecules 2013; 18: 12154-12167.
[9] Al-Rasheed NM, Al-Rasheed NM, Faddah LM, Mohamed AM, Mohammad RA and Al-Amin M. Saudi Journal of Biological Sciences 2014; 21: 265-274.
[10] Donder E, Baydas G, Ozkan Y, Ercel E and Yalniz M. Biomed Res 1999; 10: 141-145.
[11] Turkdogan MK, Agaoglu Z, Yener Z, Sekeroglu R, Akkan HA and Avci ME. Deutschetierarztliche Wochenschrift 2001; 108: 71-73.
[12] Shaker ME, Zalata KR, Mehal WZ, Shiha GE and Ibrahim TM. Toxicol Appl Pharmacol 2011; 252: 165-175.
[13] Bail S, Stübiger G, Krist S, Unterweger H and Buchbauer G. Food Chemistry 2008; 108: 1122-1132.
[14] Ismail AFM, Essawy MMT and Salem AAM. J Agric Chem Biotechn. Mansoura University 2014; 5: 287-303.
[15] Ismail AFM, Moawed FSM and Mohamed MA. J Photochem and Photobiol B: Biol 2015; 153: 317-323.
[16] Maier T, Schieber A Kammerer DR and Carle R. Food Chem 2009; 112: 551-559.
[17] Poudel PR, Tamura H, Kataoka I and Mochioka R. J Food Comp Anal 2008; 21: 622-625.
[18] Tangolar SG, Zoğul Y, Tangolar S and Orun A. Int J Food Sci Nutri 2009; 60: 32-39.
[19] Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG and Vinson JA. Mutat Res 2003; 523-524: 87-97.
[20] Uma Maheswari M and Rao PGM. Indian J Pharmacol 2005; 37: 179-182.
[21] Cho BO, Ryu HW, So Y, Jin CH, Baek JY, Park KH, Byun EH and Jeong IY. Food Chem 2013; 138: 107-115.
[22] Begum N, Rajendra PN and Thayalan K. Int J Nut Pharmacol Neurol Diseases 2012; 2: 145-152.
[23] Buhl SN and Jackson KY. Clin Chem 1978; 24: 828-831.
[24] Szasz G. Proceedings of the Second International Symposium on Clinical Enzymology. Chicago, IL, USA 1975.
[25] Satoh K. Clinica Chimica Acta 1978; 90: 37-43.
[26] Montgomery HA and Dymock JF. Analyst 1961; 86: 414-416.
[27] Beutler E, Duron O, Kelly BM. J Lab Clin Med 1963; 61: 882-890.
[28] Nishikimi M, Roa NA and Yogi K. Biochem Biophys Res Commun 1972; 46: 849-854.
[29] Aebi H.; Catalase. In: Methods in Enzymatic Analysis. Bergmeyer HU (ed); Chemic. Academic Press; Inc. Verlag. 1984; 2: 673-678.
[30] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Science 1973; 179: 588-590.

[31] Xiao J, Emily C, Lionga L, Chinga YP, Chuen R, Changa C, Soa KF and Yokel RA. Neurotoxicology 2000; 21: 813-28.
[32] Abdel Azim SA, Darwish HA, Rizk MZ, Ali SA and Kadry O. Experimental and Toxicologic Pathology 2015; 67: 305-314.
[33] Livak KJ and Schmittgen TD. Methods 2001; 25: 402-408.
[34] Schmidt-Ullrich RK, Dent P, Grant S, Mikkelsen RB, et al. Radiat Res 2000; 153: 245-247.
[35] Joshi MS, Ponthier JL and Lancaster R. Free Radic Biol Med 1999; 27: 1357-1-366.
[36] Boje KM. Front Biosci 2004; 9: 763-776.
[37] Karadeniz A, Yildirim A, Çelebi F. Atatürk Üniversitesi Vet Bil Derg 2007; 2: 117-121.
[38] Bayraktar N, Devay SD, Taslipinar MY, Ucankus NL, Omeroglu S, Gumuslu S, Kavutcu M and Canbolat O. Turk J Biol 2011; 36: 283-289.
[39] Szymonik LS, Czechowska G, Stryjecka ZM, Slomka M, et al. J Hepato Biliary Pancreat Surg 2003; 10: 309-315.
[40] Yao JK and Keshavan MS. Antioxid Redox Signal 2011; 15: 2011-2035
[41] Recknagel RO, Glende EA, Dolak JA and Waller RL. Pharmacol Ther 1989; 43: 139-154.
[42] Lavrentiadou SN, Tsantarliotou MP, Zervos IA, Nikolaidis E, et al. Food Chem Toxicol 2013; 61:196-202.
[43] Halliwell B. Drugs Aging 2001; 18: 685-716.
[44] Usoh FI, Akpan EJ, Etim EO and Farombi EO. Pak J Nutr 2005; 4: 135-141.
[45] Sies H. Am J Med 1991; 91: 31-38.
[46] Reiter R and Wendel A. Biol Interact 1982; 40: 365-374.
[47] Navarro J, Obrador E, Carretero J, Petschen I, et al. Free Radic Biol Med 1999; 26: 410-418.
[48] Sies H. Angew Chem 1986; 25: 1058-1071.
[49] Cui J, Holmes EH and Liu PK. J Neurochem 1999; 73: 1164-1174.
[50] Epe B, Ballmaier D, Roussyn I, Brivibaand K, et al. Nucleic Acids Res 1996; 24: 4105-4110.
[51] Halliwell B. Pathol Biol 1996; 44: 6-13.
[52] Huang D, Shenoy A, Cui JK, Huang W, et al. J Fed Am Soc Exp Biol 2009; 14: 407-417.
[53] Pacheret P, Beckman JS and Liaudet L. Physiol Rev 2007; 87: 315-424.
[54] Lee SY, Lee SJ, Han C, Patkar AA, et al. Prog Neuro Psychopharmacol Biol Psychiatry 2012; 46: 224-235.
[55] ZorrillaZubiletea MA, Rıosc H, Silbermanb DM, Guelmana LR, et al. Brain Res 2005; 1051: 8-16.
[56] Parlakpinar H, Ozer MK and Acet A. Mol. Cell Biochem 2005; 277: 137-142.
[57] Zaman J, Jeddi S, Daneshpour MS, Zarkesh M, Daneshian Z and Ghasemi A. Gene 2015; 570: 185-190.
[58] Fan Q, Yang XC, Liu Y, Wang LF, Liu SH, Ge, YG. Chen, ML, Wang W, Zhang LK, Irwin, MG and Xia Z. Clin Sci 2011; 120: 251-261.
[59] Donnini S and Ziche M. Antioxid Redox Signaling 2004; 4: 817-823.
[60] Shah AM and Wainwright CL. Pharmacol Therapeut 2000; 2000 (86): 49-86.
[61] Chen WL, Ouyang KX, Li HG, Huang ZQ, Li JS and Wang JG. J Craniofac Surg 2009; 20: 171-175.
[62] Kane AJ, Barker JE, Mitchell, GM, Theile D.B, Romero R, Messina A, Wagh M, Fraulin FOG, Morrison WA and Stewart AG. Br J Pharmacol 2001; 132: 1631-1638.
[63] Zhou L and Zhu DY. Nitric Oxide 2009; 20: 223-230.
[64] Busserolles J, Gueux E, Balasinska B, Piriou Y, et al. Int J Vitam Nutr Res 2006; 76: 22-27.
[65] Kvietys PR, Inauen W, Bacon Br and Grisham MB. Am J Physiol 1989; 257: 1640-1646.
[66] Umezawa K, Arigaand A and Matsumoto N. Anticancer Drug Des 2000; 15: 239-244.
[67] Euler G. Frontiers in Physiology 2015; 6: 66.
[68] Shukla SK, Sharma SB and Singh UR. Redox Report 2015; 20 (2): 49-59.
[69] Zhou DY, Du Q, Li RR, Huang M. et al. Planta Med 2011; 77: 1575-1581.
[70] Gessner DK, Ringseis R, Siebers M, Keller J, et al. J Anim Physiol Anim Nutr 2012; 96: 1074-1083.
[71] Flohé L, Brigelius-Flohé R, Saliou C, Traber MG, et al. Free Radic Biol Med 1997; 6: 1115-1126.
[72] Mohan N and Meltz ML. Radiat Res 1994; 140: 97-104.
[73] BaeumlBehrends H, Peter U, Mueller RU, Kammerbauer S, et al. Free Radic Res 1997; 27: 127-142.
[74] Kwon KB, KimEK, Jeong ES, Lee YH., et al. World J Gastroenterol 2006; 12: 4331-4337.
[75] Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG and Vinson JA. Mutat Res 2003; 523-524: 87-97.
[76] Bagchi D, CA Kuszynski J Balmoori M Bagchi et al. Phytother Res 1998; 12: 568-571.
[77] Morin B, Narbonne JF, Ribera D, Badouard C et al. Food Chem Toxicol 2008; 46: 787-796.