IJBPAS, March, 2016, 5(3): 736-52

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE PROPHYLACTIC EFFICIENCY OF LYCOPENE AGAINST GAMMA IRRADAITION-INDIUCED CARDIAC OXIDATIVE DAMAGE, PATHOLOGY AND APOPTOSIS IN RATS

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ABSTRACT

The present study was designed to clarify the protective effect of lycopene against irradiation induced cardio-toxicity.Rats were divided into four equal groups. Gp1, control rats.Gp2, rats administered Lyc(5mg/kg, 5times / week) for 30days. Gp3, rats exposed to gamma irradiation (5Gy) on the days 24th, 26th, 28th, and 30th of the experimental duration.Gp4, rats administrated Lyc as in Gp2 then irradiated as Gp3. The results demonstrated that lycopene pre-treatment significantly reduced the gamma irradiation induced cardiac tissue injury as evidenced by decrement in heart enzymes LDH, AST and CK, ameliorated the augmented levels of AOPP, TNF-α, IL-6 and TGF-β and oxidative stress markers. This was coincided with decrement of DNA fragmentation damage, caspase 3 andBax protein levels, with increment of Bcl-2 and Bax/ Bcl-2 ratio.Lycopene also protected the heart against the vascular changes, degeneration and necrosis of myocardial muscle fibres and pericarditis caused by irradiation exposure.

Keywords: Gamma rays, cardiotoxicity, oxidative stress, apoptosis, pathology, Lycopene.

INTRODUCTION

Radiation therapy has been linked to the development of many cardiovascular diseases. There is evidence that the heart or at least part of the heart was irradiated during radiation therapy for breast cancer [1] and thoracic chest wall [2].During specific radiation therapy procedures, heart is exposed to high doses of irradiation inducing tissue damage and resulting in increased risk of circulatory diseases with micro-vascular damage and pro-inflammatory effects[3].

Ionizing radiation is known to generate reactive oxygen species (ROS) in irradiated tissue resulting in cardiomyopathy. On the other hand, ROS have also been demonstrated to perform certain functions in the early stages of apoptosis. Apoptosis can be induced through two distinct pathways; one involves the ligation of the TNF/Fas-receptor with its ligand, which then followed by caspase-8 activation. The other pathway is the mitochondria mediated caspase-9 activation pathway. Both pathways converge in caspase-3, culminating in cell death[4].

Inflammation was reported as one of the major consequences of radiation with the subsequent induced tissue injury. In addition, acute inflammatory responses triggered by over expression of inflammatory mediators could be the reason for radiation-induced tissue injury[5].

Natural antioxidants present in fruits and vegetables shared with the body enzymes in combating the effect of free radicals.Lycopene (Lyc) is a natural pigment synthesized by plants and microorganisms but not by animal cells. It is most commonly located within cell membranes and other lipid components as it is highly lipophilic. Therefore, it is expected that in the lipophilic environment, lycopene will have maximum ROS scavenging effects. Many epidemiological studies and supplementation human trials mentioned that lycopene could reduce cardiovascular risks due to its antioxidant characteristics.Long term administration of lycopene can protect myocardium against ischemia reperfusion injury[6].Because of its high number of conjugated double bonds, lycopene have been shown to exhibit higher singlet oxygen quenching ability acting as potent antioxidant so has the ability to prevent the oxidative damage of cellular macromolecules including lipids, proteins and DNA [7]. Lycopene also demonstrated as a potent anticancer, anti-inflammatory, anti-proliferative and hypo-cholesterolemic agent[8].

Keeping this in view, this study was designed for evaluating the cardio protective potential of lycopene in experimentally gamma irradiation–induced oxidative stress, myocardial injuries, inflammation and apoptosis in rats.

MATERIALS AND METHODS

Chemicals

Lycopene was purchased from Sigma (St. Louis, MO, USA). It was dissolved in olive oil and given to the animals (*i.p*) at a dose of 5mg/kg (0.2ml / animal).

All chemicals used for measurement of antioxidants markers were purchased from Sigma Chemical Co. (St. Louis, MO).

ELISA Kits (R&D Quantikine USA). Catalog Number RTA00, R6000B and MB100B were used for estimation of TNF-α, IL6 and TGF-β1 respectively.

ELISA kit (Uscn Life Science E90626Ra, E91824Ra and E90778Ra, China) were used for analysis of caspase, Bax and Bcl-2 respectively.

Animals

A total number of 36 male Sprague-Dawley rats with an average weight of 160-170g were obtained from the animal house of Research institute of Ophthalmology, Giza, Egypt. Rats were housed in regular designed cages and maintained in good ventilation, at a temperature of 25 ±5^{o}C, 60% humidity, and suitable illumination conditions (light/dark cycle). They were allowed standard pellet diet and fresh water *ad libitum*. Animals were allowed to acclimatize to the laboratory environment for 1 week prior to starting the onset experiment.

Irradiation

Irradiation was performed through the use of a Canadian Gamma Cell-40 (^{137}Cs) at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate was 0.675 Gy/minute. Animals were exposed to 20Gy of whole body gamma irradiation (4x5Gy) on days 24th, 26th, 28th, and 30th of the treatment period. The protocol of animal treatment was approved by the Animal Care Committee of the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Study Design

Rats were divided into four groups. Gp1 (vehicle controlGp);each rat received 0.2ml olive oil *i.p*for thirty days (5 times / week).Gp2; rats were administered Lycopene*i.p*(5mg/kg b wt., 5 times/week) for thirty days. Gp3; animals subjected to four irradiation doses each of 5Gy on 24th, 26th, 28th, and 30th days of the experimental duration. Gp4; animals treated with Lyc as in Gp2 then exposed to irradiation as in Gp3. All animals were monitored for behavioural

changes, morbidity and mortality. At the end of the experiment, rats of all groups were euthanized under gentle diethyl ether anaesthesia, prior to which blood samples were collected for plasma separation. Hearts were immediately removed from the body; part of each heart was kept in 10% buffered neutral formalin forhistopathological examination. The other part was stored at -20^0c for estimation of various biochemical parameters.

Biochemical Analysis:

Biochemical parameters estimated in plasma:

Cardiac injury enzyme markers

The activities of lactate dehydrogenase (LDH), Creatine kinase (CK)[9]and aspartate aminotransferase (AST)[10]were evaluated.

Inflammatory mediators:

Advanced oxidation protein products (AOPP)[11], tumour necrosis factor- alpha TNF-α, interleukin IL-6 [12] and the transforming growth factor-beta (TGF-β1)[13] were estimated using ELISA Kits (R&D Quantikine USA) according to manufacturer's instructions.

Biochemical parameters estimated in heart tissue homogenate:

Cardiac oxidative stress markers

Lipid peroxidation end product; malondialdehyde (MDA)[14], nitric oxide (NO)[15], glutathione (GSH) content[16], glutathione peroxidase (GPx) activity[17], superoxide dismutase **(**SOD)[18]and catalase activity (CAT)[19] were assayed in heart tissue homogenates.

Apoptotic markers:

Apoptotic DNA Fragmentation Assay

DNA fragmentation was estimated in heart tissue using ladder assay[20]and DNA damages (fragmentation %)was evaluatedby DPA assay[21]. In brief, hearts were rapidly removed, washed, and homogenized. The homogenized tissue was transferred to centrifuge tube with extraction buffer (10 mMTris-HCl, pH 8.0, 25 mM EDTA, and 0.25% Triton X-100) on ice for 30 min. After centrifugation, the supernatants were incubated with 100 μg/ml RNase at 37°C for 30 min then incubated with 200 μg/ml proteinase K at 56°C overnight. The mixture was extracted with phenol-chloroform and precipitated with ethanol. The pellets were resuspended in Tris-EDTA buffer and subjected to agarose gel electrophoresis.

Apoptotic protein levels

The activity of Caspase-3, the expression of the pro apoptotic protein Bax and anti-apoptotic protein Bcl-2wereevaluated in heart tissue homogenatesusing ELISA kit (Uscn Life Science E90626Ra, China) according to manufacturer's instructions.

Histopathological examination

Formalin fixed heart specimens were routinely dehydrated in graded series of alcohol, cleared in xylol and finally embedded in paraffin. Paraffin blocks were serially sectioned at 4-5 µm thickness and stained with H&E[22].

Statistical Analysis:

The obtained data were expressed as mean ± standard error of the mean (M±SE). The significant differences among groups were determined by one-way analysis of variances using SPSS package program, version 20. The results were considered significant if the values of p were <0.05.

RESULTS

Rats of all groups survived the whole duration of the experiment.Both of control and lycopene treated rats neither showed any significant change in any of the investigated biochemical parameters nor their cardiac histology.

Cardiac injury enzyme markers

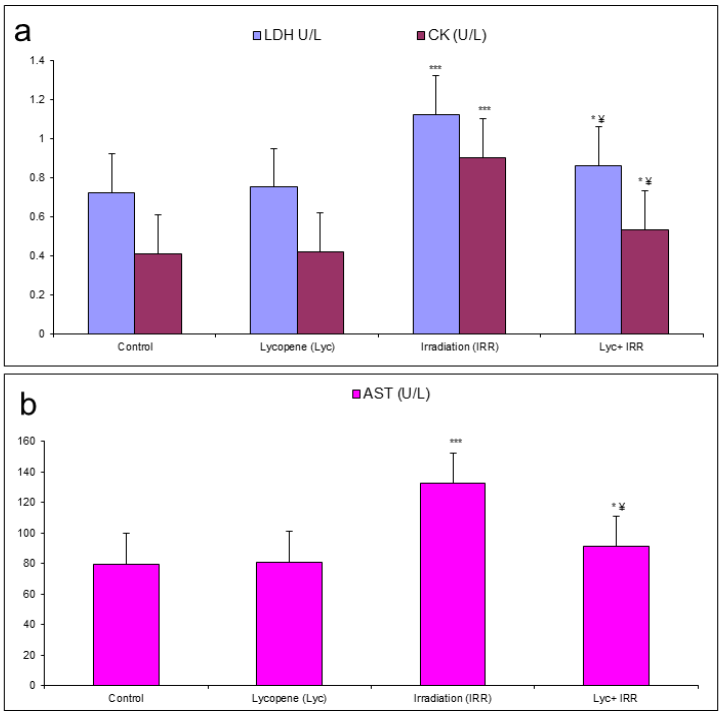
Rats exposed to gamma raysshowed highly significant elevation ($p \leq 0.001$) in their plasma activities of LDH, AST and CK compared to the control levels. However, pre-irradiationexposureto lycopeneameliorated the drastic effect of irradiation as evidenced by highly significant ($p \leq 0.001$) decrement in these enzyme activities compared with the values of irradiated group**(Fig. 1).**

Inflammatory mediators:

The present study showed significant increase ($p \leq 0.001$) in AOPPs, TNF-α, IL-6 and TGF-β levelsin irradiated ratscompared to control values. On the contrary, administration of lycopene pre-irradiation exposure significantly brought down that elevated mediatorslevels($p \leq 0.001$) compared to irradiated rats **(Fig. 2).**

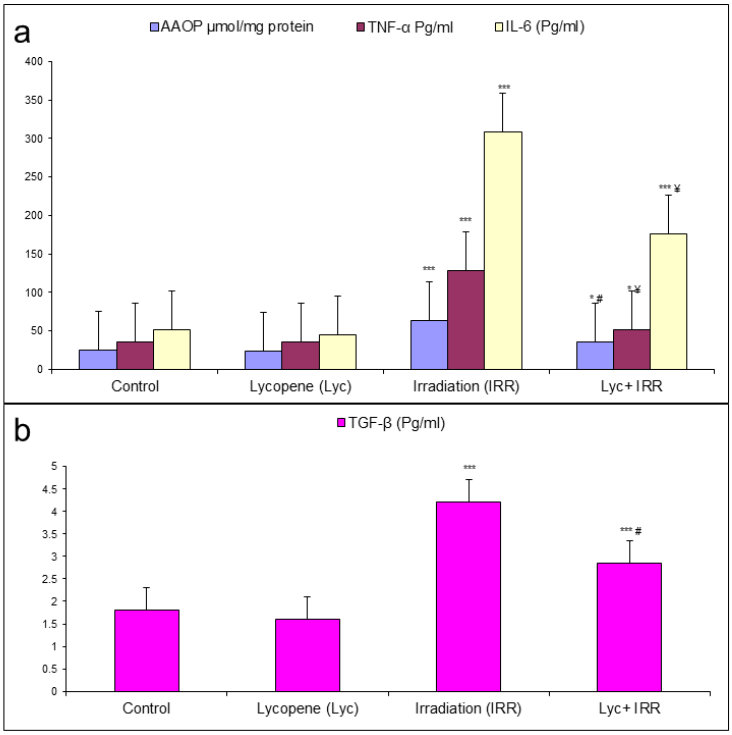
Cardiac oxidative stress markers

There was significant increase ($p \leq 0.001$) in MDA and NO levels accompanied with significant decrease in GSH content ($p \leq 0.001$), GPx ($p \leq 0.01$), SOD ($p \leq 0.01$), and CAT ($p \leq 0.001$) activities in cardiac tissue homogenates of irradiated rats compared to control set. However, pre-irradiation exposure to lycopene rendered protection against irradiation-induced oxidative stress and caused significant ($p \leq 0.001$) reduction in MDA and NO levels coincided with significant rise in GSH and CAT ($p \leq 0.05$), SOD ($p \leq 0.01$) andGPx ($p > 0.05$) levels compared to irradiated group **(Fig. 3).**



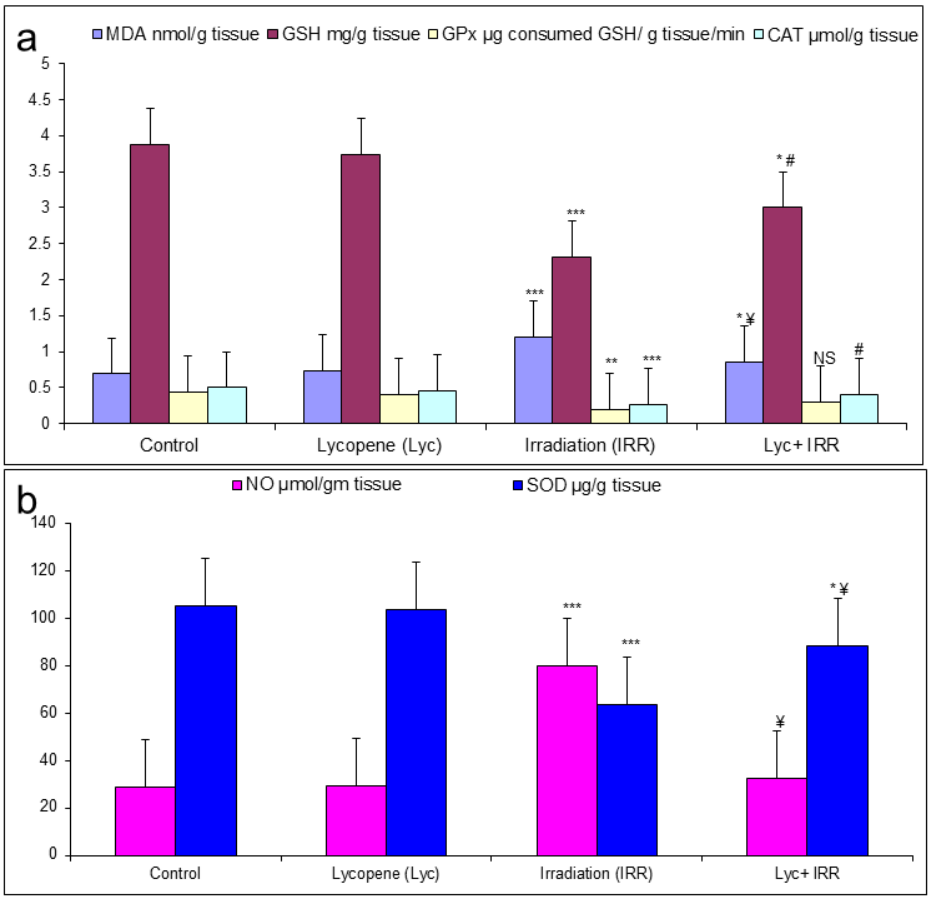
Data expressed as mean ± SE. The significant difference from the control values at *p≤0.05, **p≤0.01, ***p≤0.01. The significant difference from the irradiated values at ¥p≤0.01, #p≤0.05

Figure1: Effect of lycopene on cardiac enzymes of irradiated rats; (a) LDH and CK and (b) AST.



Data expressed as mean± SE, The significant difference from the control values at, ***p≤0.01. The significant difference from the irradiated values at ¥p≤0.001, #p≤0.01

Figure 2.Effect of lycopene on cardiac inflammatory mediators of irradiated rats; (a) AOPPs, TNF-α and IL-6 and (b)TGF-β.



Data expressed as mean± SE, The significant difference from the control values at, ***p≤0.01. The significant difference from the irradiated values at ¥p≤0.001, #p≤0.01

Figure 3.Effect of lycopene onantioxidant markers in heart tissue of irradiated rats; (a) MDA, GSH, GPx and CTA (b) NO and SOD.

Apoptotic markers in cardiac tissue

Apoptotic DNA fragmentation

Lycopene, in its own didn't cause significant change in the percentage of DNA (2.32±0.44) fragmentation compared with control rats (2.55±0.48). However, exposure of rats to gamma irradiation resulted in an obvious type of DNA fragmentation pattern that was observed in DNA ladder assay **(Fig. 4)** and caused significant increase (14.61±0.96) in the percentage of DNA damage compared to control rats**(Fig. 5a).** Both of DNA fragmentation percentageand DNA ladder assay were intimately correlated with each other. Lyc treatment prior to irradiationexposure could significantly reduce the levels of DNA damage ($P < 0.05$) in comparison tothe irradiated group.

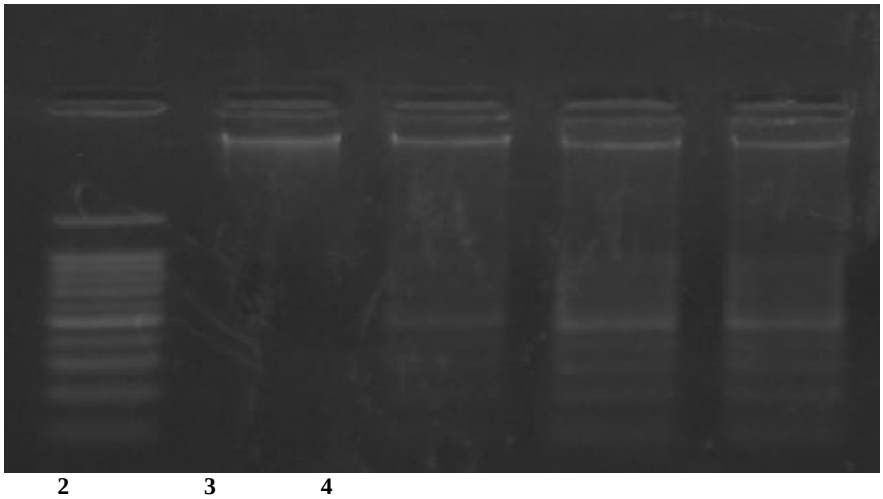
Apoptotic protein levels

The sole lycopene treatment didn't cause any significant change (P>0.05) in the investigated apoptotic markers levels compared to control set. However, irradiation exposure significantly (P<0.001) increased caspase 3

activity, Bax leveland Bax/ Bcl2 ratio (P<0.001) concomitant with marked decrease (P<0.001)in Bcl2 compared to control rats**(Figs 5a and b).**The pre-irradiation administration of Lyc brought down the increase in caspase 3 (p<0.01)Bax (P<0.001), and the Bax/ Bcl2 ratio (P<0.001) as well as increased the level of Bcl2 (P<0.001)compared to irradiation set.

Histopathological examination

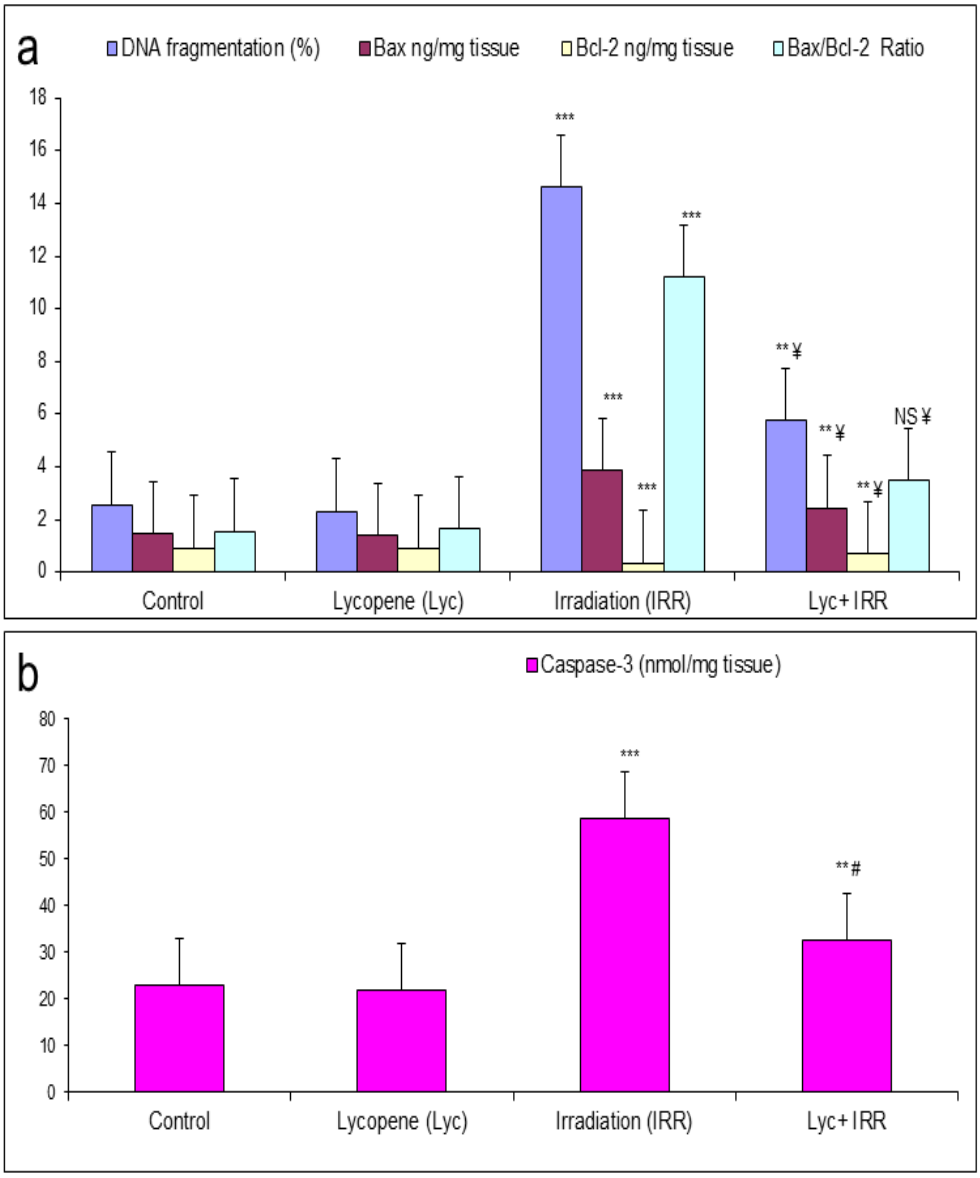
Microscopical examination of different heart sections of control and the sole lycopene treated rats' revealed normal cardiac muscular and vascular histology. Whole body exposure to gamma radiation resulted in deleterious histological alterations in the cardiac tissue represented by marked congestion of the myocardial blood vessels (Fig.6A), variable degrees of intermuscular hemorrhages (Fig. 6B), apparent thickening and hyalinization of the myocardial vascular walls (Fig.6C) and vasculitis (Fig.6D). The myocardial muscle fibers appeared swollen with variable degrees of granular (Figure 6E) and vacuolar degeneration (Fig. 6F).Intermuscularedema (Fig.7A), hyalinization of some muscle fibers (Fig. 7B and C) and fragmentation of others (Fig. 7D) were also noticed. Focal mononuclear inflammatory cells infiltrations were observed among the degenerated muscle fibers (Fig. 7E) with an obvious pericarditis (Fig.7F). On contrary, lycopene administration prior to irradiation exposure safeguarded the cardiac muscles against the harmful influence of radiation to a great extent, only mild to moderate degrees of vascular congestion (Fig. 8A and B) intermuscular edema and rare myocardial muscle granular degeneration were noticed.



M 1 2 3 4

Figure 4.Agarose gel electrophoresis showing the DNA fragmentation pattern in the different studied groups.

Lanes from left;(M): high-molecular weight marker, (1): control (2): Lycopene, (3): Irradiation and (4) Lycopene and irradiation.



Data expressed as mean± SE, The significant difference from the control values at, ***p≤0.01. The significant difference from the irradiated values at ¥p≤0.001, #p≤0.01

Figure 5.Effect of Lycopene on apoptotic markers in cardiac tissue of irradiated rats; (a) DNA fragmentation, Bax, Bcl-2, Bax/ Bcl-2 ratio and (b) caspase-3 activity.

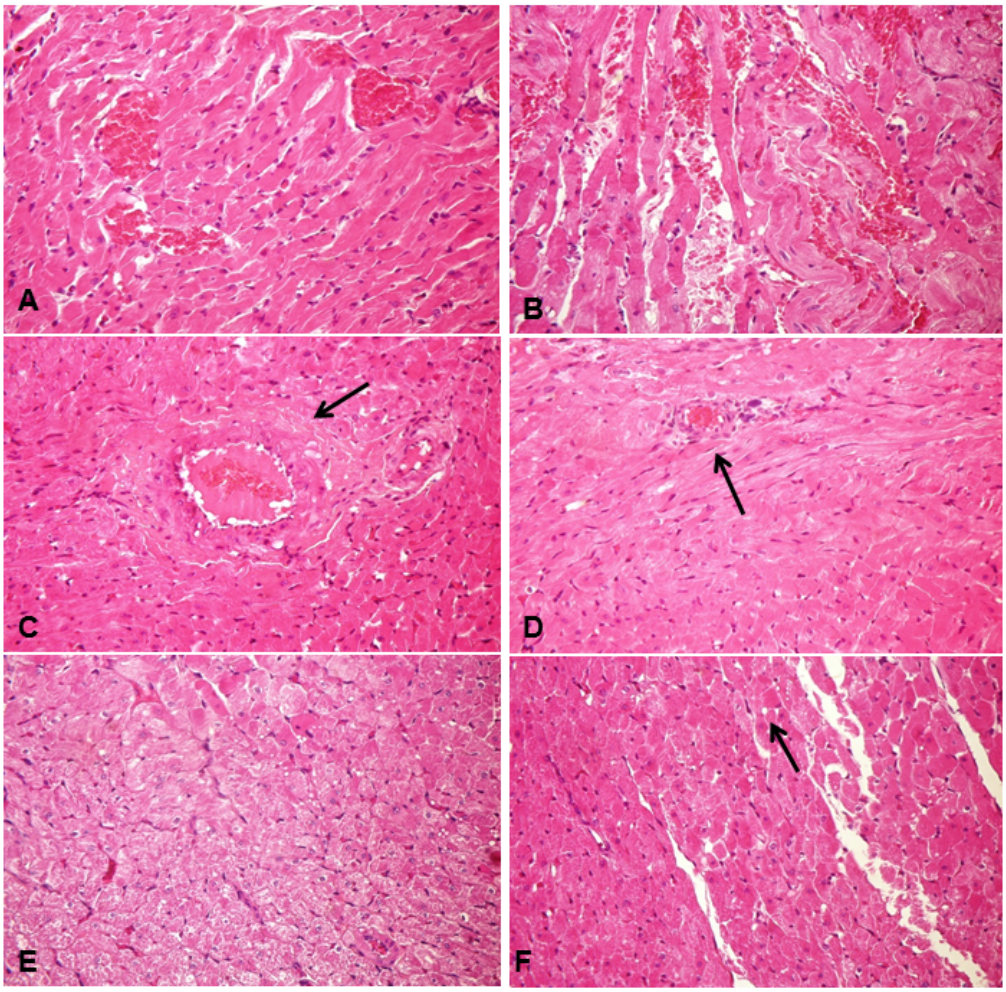


Figure 6.Heart of whole body irradiated rats showing; (A) marked congestion of the myocardial blood vessels. (B) Areas of intermuscularhemorrhages. (C) Thickening of the myocardial blood wall with apparent areas of hyalinization and mild edema. (D) Vasculitis, notice the inflammatory cells infiltration in the vascular wall (arrow). (E and F) Marked swelling of the myocardial muscle fibers with granular (E) and vacuolar (F) degeneration. (H&E X400)

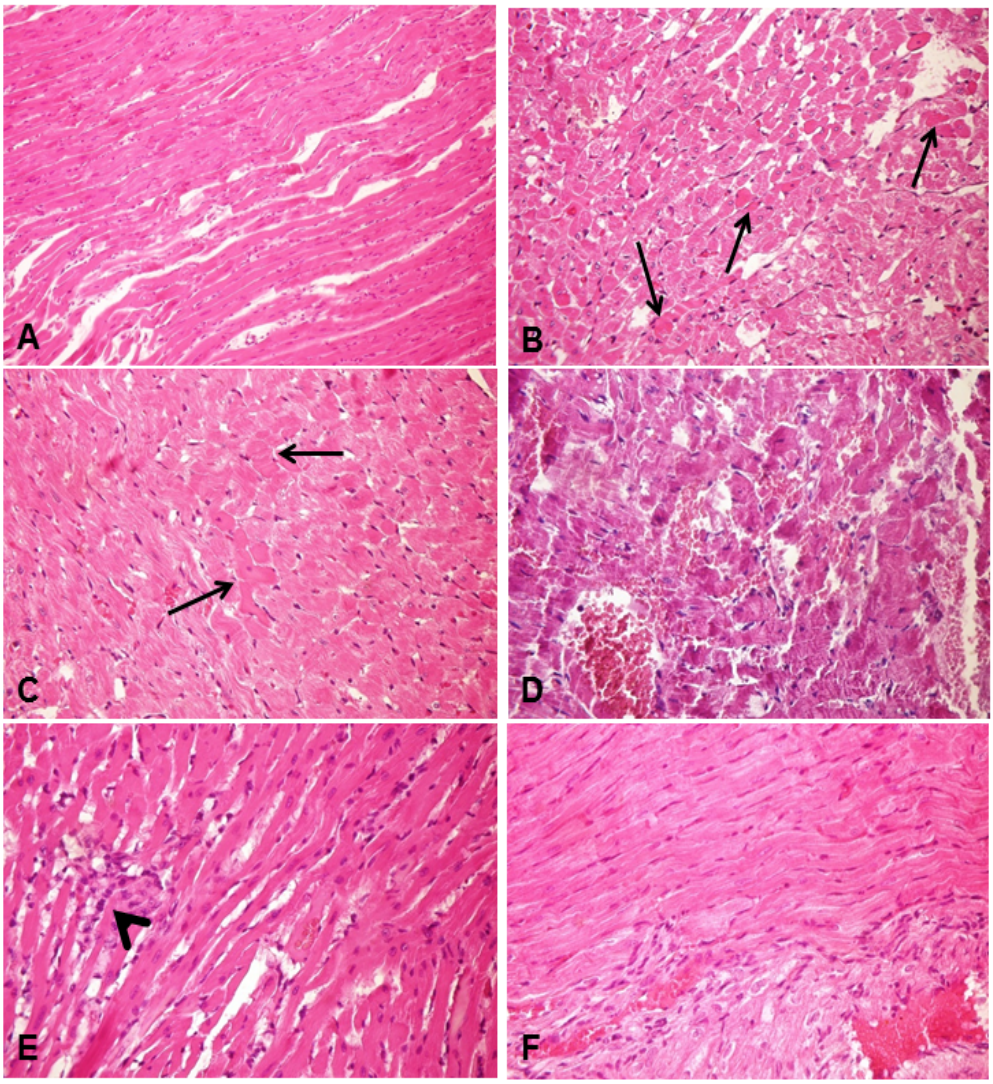


Figure 7. Heart of whole body ɣ- irradiated rats showing; (A) Intermuscularedema dispersing the muscle bundles apart. (B and C) Hyalinization of some muscle fibers (arrows) which appear homogemousesinophilic with pyknotic nuclei (B) or without nuclei (C). (D) Fragmentation of the muscle fibers with intermuscularhemorrhage. (E) Focal mononuclear inflammatory cells infiltrations in between the degenerated muscle fibers. (F) Pericarditis notice, the congested pericardial blood capillaries and mononuclear cell infiltration. (H&E X200 and 400).

DISCUSSION

Many phytochemicals have shown both anti-inflammatory and anti-oxidant activities in vitro and in vivo. They probably have multiple cellular mechanisms acting on multiple sites of cellular machinery[23]. The present study disclosed that whole body exposure to gamma radiation resulted in marked detrimental effects in the cardiac tissue that reflected by alteration of the various investigated parameters.

The findings of the present study indicated significant elevation of LDH, AST and CPK activities in plasma of irradiated ratswhich were considered common characteristics of cardiotoxicity. It is well known that the

myocardium contains an abundant concentration of the marker enzymes such as LDH, AST and CPK and once metabolically damaged, it releases its contents into the extracellular fluid [24]. That elevated enzymes levels might be due to the excessive production of free radicals and lipid peroxides which induced leakage of the cytosolic enzymes[25]. It was observed that lycopene pretreatment alleviated the activities of these enzymes which could be attributed to its free radical scavenging properties that maintain the membrane integrity of cardiac muscle cells.

Our results detected elevated levels of the inflammatory mediators; AOPPs, TNF-α, IL-6 and TGF- β in irradiated rats which are in agreement with[26]who mentioned that; inflammation and persistent increases in pro-inflammatory cytokines behold as one of the mechanisms responsible for cardiovascular damage after whole body exposure to high doses of gamma rays. That elevated levels of inflammatory mediators may be attributed to radiation-induced oxidative damage and increase in inflammatory activity as well as pointing out the role of these cytokines in irradiation-induced cardiac toxicity[27]. The levels of these cytokines were brought down after pre-irradiation exposure to Lycsuggestingits role in diminishing radiation-induced oxidative injury and its anti-inflammatory effect[28]. Moreover, lycopene has been found to stimulate the production of anti-inflammatory cytokine IL-10, which controls inflammation and inhibits the production of pro inflammatory cytokines including TNF-β, IL- 6, and IL-8[29].

The present work showed that gamma irradiation exposure resulted in significant oxidative damage indicated by significant elevation of MDA level and NO production in cardiac tissueaccompanied with depletion in GSH, GP_X, SOD and CAT which are in agreement with de Freitas et al[30]. Oxidative stress is the major etiopathological factor in radiation-induced heart toxicity. Heart tissues are particularly vulnerable to ROS due to a relatively low content of endogenous antioxidant[31].The high MDA level could be indicative to oxidative damage to mitochondria and cardiac cell membranes caused by irradiation exposure which could also promote cell death processes such as radiation-induced apoptosis[32].While, the significant decline in the other antioxidants levels in irradiated rats could be due to their exhaustion as an attempt to detoxify the free radicals generated by radiation. The pre-irradiation exposure to lycopene resulted in depletion of lipid peroxidation and NO productionas well as improvedthe antioxidant

statuswhich may be a result of its potent-free radical scavenger[32]thus preventing the oxidative damage of critical biomolecules including lipids, proteins and DNA [7].

Radiation exposure in our work resulted in increased percentage of DNA damage and DNA fragmentation andraised caspase-3 activity (a common critical event for apoptosis)in cardiac tissue which pointed to irradiation induced cardiac apoptosis and necrosis due toROS generated by irradiation which can attack DNA resulting in its damage[33].In the same line with others, the present study demonstrated also an increase in Bax level and Bax/Bcl-2 ratio with a decrease in bcl-2 level which could enhance the induction of cell death and apoptosis in response to ROS induced by irradiation exposure.

The pre-irradiation exposure to Lycrevealed decrement in both of DNA fragmentation and damage, caspase-3 activity, Bax protein level and Bax/ Bcl-2 as well, meanwhile Bcl-2 level was increased. This indicates that Lyc displayed anti-apoptotic function and protected cardiac tissue against irradiation induced apoptosis. The later beneficial effect might be due to the potent antioxidant activity of Lyc which scavenge the highly toxic free radicals and prevents the DNA damage and thereby inhibiting caspase-3 activity.

Our histopathological evaluation revealed that whole body exposure to gamma radiation had cardiotox effect that represented by thickening of the myocardial vascular walls, vasculitis, and variable degrees of myocardial degenerative and necrotic changes as well as pericarditis. These changes were in accordance with those mentioned by Azab et al[34].Damage to the myocardium due to radiation is primarily caused by damage to the microvasculature, leading to inflammatory and thrombotic changes, focal ischemia and interstitial fibrosis after high doses[35].The radiation induced-cardiomyopathy may be related to increased formation of free radicals which damaged the integrity of the myofibers thus affecting cardiac performance resulting in contraction failure and structural damage of the myocardium. However, the pre-irradiation exposure to lycopene could ameliorate the hazardous effect of radiation as evidenced by absence of inflammatory reaction and decreased other pathological alterations. These data further confirmed the cardioprotective action of lycopene which could be related to itsantioxidant activity that makes Lyccapable of neutralizing the free radicals molecules making them less

reactiveso protecting the cardiac muscles against the deleterious effect of radiation.

CONCLUSION

The present study displayed that lycopene could confer a marked cardiac protection against gamma irradiation induced cardiac damage during whole body irradiation. The underlying mechanisms involved in this protection were inhibition of lipid peroxidation as well as anti-inflammatory and anti-apoptoticeffects.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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