Egypt. J. Rad. Sci. Applic. **Vol. 36,** No.1-2, pp.99 -109 (2023)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

Amlodipine Repurposing in Amending Liver Toxicity Induced by Gamma Irradiation

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LIVER is a highly sensitive organ towards irradiation.The present study aims to determine the potential repurposing effect of amlodipine (AML) (antihypertensive medicine)against acute hepatotoxicity induced by gamma radiation, using Silymarin (Sily) as a standardized hepatoprotective supplement in experimental rats. Thirty-two male rats were distributed into four groups: Control, Radiation (Rad),Rad+Sily, and Rad+AML. Following exposure to a single dose of gamma radiation (6Gy), rats received daily AML or Sily for a week. Results showed that administration of AML or Sily post gamma irradiation causes amelioration in the liver enzymes (AST, ALT) and the levels of total proteins and albumin,oxidative stress markers (NOx, MDA, GSH, ROS, XOand GST) and inflammatory markers (TNF-α, IL-6, and NF-κβ p65) also showed significant improvement, when compared to the irradiated group. Additionally, AML ameliorates gamma irradiation-induced histological changes and alleviates the severity of hepatic injury thus histopathological finding supports the biochemical outcomes. It may be concluded that AML produces a hepatoprotective response as well as Silymarin (a standardized hepatoprotective supplement) against gamma irradiation -induced acute liver injury in rats.

Keywords: Amlodipine, Calcium channel blocker, Gamma irradiation, Liver, Inflammatory markers, Oxidative stress

Introduction

Drug repurposing is also named as drug repositioning or therapeutic switching. Repurposing process is applied to identify new therapeutic use from the present FDA approved clinically used drug molecules. It is an effective approach to develop drug candidates with new pharmacological activities or therapeutic uses. As the drug discovery is over-priced, time-consuming, difficult, and extremely risky, the novel approach of drug repositioning is used to boost the success rate of medication development(Sahoo et al. 2021).The study demonstrated that chloroquine and hydroxychloroquine might be effective weapons against COVID-19 is an instructive and contemporary example of helpful repositioning(Jourdan et al. 2020).For the development of drugs, numerous target protein and the characteristics of ligands and receptors have been utilized to find novel targets for medicines that already exist(Jang et al. 2016).Drug repositioning frequently use the docking technique, which is a powerful tool for drug repositioning and drug rescue. It involves docking a small-molecule drug in the potential binding cavities of a set of clinically significant macromolecular targets (Kharkar et al. 2014).

Amlodipine is an oral dihydropyridine third generation calcium channel blocker, which selectively acts on vascular smooth muscle and is used to treat hypertension and stable angina(Li et al. 2023).AML was shown to exhibit anti-inflammatory activity (Qasim et al. 2020). Amlodipine was well established before, that it has an antioxidant activity (Mason 2002). Studies verified that calcium ions played a vital role in the synthesis and release of mediators that trigger inflammation process (Abdollahi et al. 2001). Research data also displayed that a reduction in calcium ions in tissue reduced pain

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Received / / ; Accepted / /
DOI:

and inflammation(Carnevale and Cathcart 2001). Radiation damage, including DNA double-strand breaks and lipid peroxidation, has been seen at the molecular level in investigations utilizing cells or experimental animals. Such damage is thought to trigger a variety of metabolic processes that ultimately result in disease states. Following, exposure to high doses of radiation during cancer radiotherapies, liver fibrosis as a type of acute radiation damage has been documented (Kim and Jung 2017).Hepatocyte's lipids, proteins, and nucleic acids are among the cellular structures affected primarily by ROS. Lipid peroxidation disrupts the normal membrane structure, while protein oxidation affects signal transduction and DNA repair enzymes. However, the effects of ROS are counteracted by endogenous antioxidants, e.g., glutathione (GSH), the most important antioxidant molecule, superoxide dismutase (SOD) and GSH peroxidase (Kim and Jung 2017).

A series of cytokines and chemokines are released shortly after irradiation, which prolongs and exaggerates the inflammatory response and causes tissue damage and chronic inflammation(Gawish et al. 2020). Additionally, due to the formation of ROS and increased chemokine gene expression after radiation exposure, liver cells exhibit inflammatory responses (Malik et al. 2010).

According to the literature, Amlodipine showed a high binding relationship with TNF-, IL-6, and IL-1 using molecular docking studies (Sumera Qasim 2020). AML has a positive impact on the balance between inflammatory cytokines and oxidant/antioxidant levels(Lee et al. 2011). Thus, AML can be used not only in antihypertensive therapy, but alsopotentially in the treatment of many diseases experimentally. Hence, the aim of the current work is to investigate the potential biological and pharmacological effects of AML on gamma irradiation-induced acute hepatotoxicity in experimental animals. In addition, the probable mechanisms through assessment of oxidative stress and inflammatory biomarkers as well as by histological techniques were clarified.

Materials and Methods

Animals

Male albino rats weighing 180-210 g were obtained from the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The rats were kept in a controlled environment with a constant light cycle (12 h light/dark) and temperature (25 °C), and unrestricted access to food, water, and a regular rodent chow diet. The Ethics Committee for Animal Experimentation approved this work As well as The Animal Care Committee of the NCRRT - Egyptian Atomic Energy Authority, Cairo, Egypt (Permit Number: 2 A/ 23).

Drugs and Chemicals

Amlodipine (Norvasc 10 mg Tb) was brought from the Pfizer Pharmaceutical Company, Egypt. Silymarin capsules (140mg/cap) were brought from Sedico Company. All other chemicals and reagents, unless otherwise specified, were obtained from Sigma-Aldrich Company, USA.

Exposure of rats to γ radiation

Irradiation of rats was accomplished at the NCRRT using a Gamma cell-40 biological irradiator supplied with a Caesium-137 irradiation source (Atomic Energy of Canada Ltd). Rats were exposed to a single radiation dose level of 6 Gy which is the threshold dose for the development of radiation-induced hepatotoxicity in rats (Abd-Al-Haleem, 2019) at a dose rate of 0.36Gy/min.

Experimental design

Four groups of eight rats each were classifies as; Group I: control group; rats received saline (P.O),Group II: Radiation (Rad);rats were exposed to a single dose of gamma radiation at 6 Gy, Group III: Radiation+ Silymarin (Rad+ Sily);rats were exposed to a single dose of gamma radiation (6Gy) then silymarin(200mg/kgb.wt) was administered orally(Zhang et al. 2013), one hour after irradiation, every day for one week. GroupIV: Radiation+ Amlodipine (Rad+ AML), rats were irradiated by gamma radiation (6Gy) then AML was administered (10 mg/kgb.wt) orally (Puzyrenko et al. 2013), one hour after irradiation, daily for one week.

Twenty-four hours after the last dose of medication, the rats were anaesthetized with urethane (1.2 g/kg, i.p.)(Flecknell 1993). Blood was obtained from the retro-orbital sinus under anaesthesia using non-heparinized capillary tubes for serum separation. The rats were dissected and livers detached, washed with saline, dried and weighed. Two sets of experiments were conducted for each group; one for biochemical measurements and the other for histological examination.

Tissue collection and processing

Sera were obtained after centrifugation at 3000rpm for 15 min and kept frozen at − 80 °C. Hepatic tissue samples were homogenized at 1:5 (weight: volume) (w: v) in phosphate

buffer (pH 7.4) using Homogenizer (Glas-Col, USA.), afterward, the supernatant separation was completed by centrifugation at 10,000rpm for 15 min (Cooling centrifuge, Hettich, MIKRO 22R, Germany) then kept at − 80 °C for hepatic oxidative stress and inflammatory biomarkers analysis.

Biochemical assessment

*In vitro antioxidant activity assay by 1***,** *diphenyl-2-picryl hydrazyl(DPPH) radical scavenging method*

The antioxidant activity of AML was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH according to Brand-Williams et al.(1995) with some modifications. The antioxidant compounds neutralized the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH, thereby changing the colour from purple to the yellow-colored stable diamagnetic molecule diphenylpicrylhydrazine). In brief, 0.1 mM solution of DPPH in ethanol was prepared. Sample in ethanol (1mg/ml) was prepared. 1ml of DPPH solution was added to 3ml sample, then shacked, incubated at room temperature for 30 minutes. The absorbance was read at 517 nm using spectrophotometer. Ascorbic acid was used as a standard. The DPPH scavenging effect percentage was determined as follows: DPPH % = A0-A1/A0 x 100 (where A0 = control reaction and A1= OD of sample).

Assessment of serum enzyme markers of liver damage

To evaluate acute liver injury, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and albumin levels were measured according to (Gornall et al. 1949) and(Doumas et al. 1971),respectively using enzymatic colorimetric kits(Biodiagnostics, Egypt).

Oxidative stress markers

Lipid peroxidation was assessed as MDA using the Yoshioka et al., method (1979), while the hepatic NOx concentration was estimated according to Miranda et al., method (2001). Reduced glutathione (GSH) was determined in the hepatic tissue according to Ellman's method(1959).ROS generation was measured by the assay for intracellular conversion of nitro blue tetrazolium (NBT) to formazan by superoxide anion(Vrablic et al. 2001).Glutathione S-transferase (GST) activity was detected in hepatic tissue (Mannervik et al. 1985).The activity of the enzyme XO was measured according to the method described by (Putter and Becker 1974).

Assessment of hepatic inflammatory markers

Tumor necrosis factor-α (TNF-α) level was assessed in liver homogenates using commercially existing rat-specific ELISA kit (RayBiotech, Inc., Norcross, Georgia). In addition, the content of interleukin-6 (IL-6) was measured in hepatic tissues according to the manufacturer's instructions utilizing rat IL-6 ELISA kit (RayBiotech, Inc.).

Histopathological examinations

Liver tissue specimens were fixed in 10% formalin, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, fixed in paraffin blocks and sectioned at 4-6 µm thick. Tissue sections were deparaffinized using xylol and stained using Hematoxylin and Eosin (H&E) for histopathological examination through the electric light microscope(Layton and Suvarna 2013).The frequency and severity of lesions in the liver were semi-quantitatively assessed as previously reported byPlaa et al. (2007)using a scale where, grade 0: No apparent injury, grade I: Swelling of hepatocytes, grade II: Ballooning of hepatocytes, grade III: Lipid droplets in hepatocytes and grade IV: Apoptosis and /or Necrosis of hepatocytes.

Immuno-histochemical analysis

The immunohistochemistry method was used to evaluate the expression of cleaved NFκβ p65 in liver tissues. Paraffinized liver sections of 4 mm thickness were deparaffinized with xylene then hydrated in graded ethanol solution and heated in citrate buffer (pH 6.0) for 5 min. Thereafter, sections were blocked with 5% bovine serum albumin in Tris buffered saline (TBS) for 2 h. Then slides were incubated with anti- NFκβ p65 antibody (SC-32251, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4ºC. After rinsing with TBS the slides were incubated in a solution of 0.02% diaminobenzidine containing 0.01% H2O2 for 10 min. The fraction of NFκβ p65 immunoreactive area, in 6 fields/section, was measured (X400).

Statistical analysis

The whole data were checked for normality in addition to homogeneity of variance by means of the Kolmogorov-Smirnov test and Bartlett's test, respectively. The data that agreed with the assumptions for parametric analysis were examined using one-way ANOVA followed by Tukey's multiple comparisons test. The results are presented as the

mean ± SEM. Statistical analysis was performed by means of Graph Pad Prism software.

Results

In vitro DPPH Radical Scavenging Activity Assay

Amlodipine showed efficiency as scavenger of DPPH *in vitro*. The free radical scavenging activity DPPH of AML was 60.98**%**at concentration of 1000µg/ml, while was 96.38% for ascorbic acid at the same concentration of AML (1000µg/ml).

Effects of Amlodipine or Silymarin administration on serum liver markers in irradiated rats

The irradiated group displayed a significant increase in ALT and AST activities by 32.42% &36.66%, respectively as equated to the control group, while albumin and total protein concentrations decreased significantly post irradiation by -41.23%& -29.29%, respectively from the control. Treatment of the irradiated rats with Amlodipine revealed no significant amelioration in ALT& AST activities, significant amelioration in albumin, and total proteins concentrations, compared to their respective levels in the irradiated group, the recorded percentage changes from the control were 29.85%, 24.06%, -20.72, -10.11%, respectively. Silymarin administration post irradiation reverses the effect of irradiation on ALT, AST, albumin, and total proteinsto be closer to the normal level (Table 1).

Data presented as mean ± S.E of the mean, (n=5). Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. a: significantly different from normal control group, b: significantly different from irradiated group. $p < 0.05$. Rad, Radiation; Sily, Silymarin; AML, Amlodipine.

Effects of Amlodipine or Silymarin administration on oxidative stress markers in irradiated rats

Exposure to gamma radiation triggered a state of oxidative stress, as demonstrated by significant increases in hepatic NOx and MDA levels in comparison to the control group by 122%and 41.57%, respectively. Conversely, hepatic GSH was significantly reduced post irradiation as compared to the normal level(-24.54%). Compared to the irradiated group, Silymarin or AML administration after irradiation demonstrated significant decreases in NOx and MDA levels by -30.83%;-24.88% and -11.98%;-35%, respectively. AML significantly increased hepatic GSH content by 16.5%,as compared with the irradiated group (Fig.1).Amlodipine administration recorded better results than that of Silymarin in MDA level and GSH content.

Compared to the control, gamma irradiation caused a significant elevation (120.59%) in the concentration of ROS in the liver homogenate, while treatment of the irradiated rats with Sily or AML induced a significant reduction in hepatic ROS level as compared to the irradiated group and the levels were higher than the control by 27.65, and 35.62%, respectively. Furthermore, hepatic xanthine oxidase activity post irradiation was significantly higher than the control level by 39.66%. Sily or AML reduced significantly the activity of XO *in vivo* as compared to the irradiated group and the levels were higher than the control by 16.99%, and 25.77%, respectively. On the other hand, hepatic activity of GST post irradiation was significantly lower than the control level by -23.15%. Administration of Sily or AML to irradiated rats ameliorated the GST activity in the hepatic homogenate to be close to the control level (Fig. 2).

TABLE 1. Effects of Amlodipine or Silymarin administration on liver function tests in rats with gamma radiation-induced acute hepatotoxicity.

Parameters / Groups	ALT (U/ml)	AST (U/ml)	Albumin (g/dl)	Total proteins (g/dl)
Control	42.31± 2.09	103.9± 2.10	4.39± 0.330	7.51± 0.337
Rad	56.03± 0.961^{a}	142.0± 4.18^{a}	2.58± 0.151^{a}	5.31± 0.331^{a}
Rad+ Sily	46.44± 2.67^{b}	109.3± 5.38^{b}	3.63± 0.050^{b}	6.68± 0.286 ^{b}
Rad+ AML	54.94± 0.980^{a}	128.9± 5.95^{a}	3.48± 0.037^{a,b}	6.75± 0.437 ^{b}

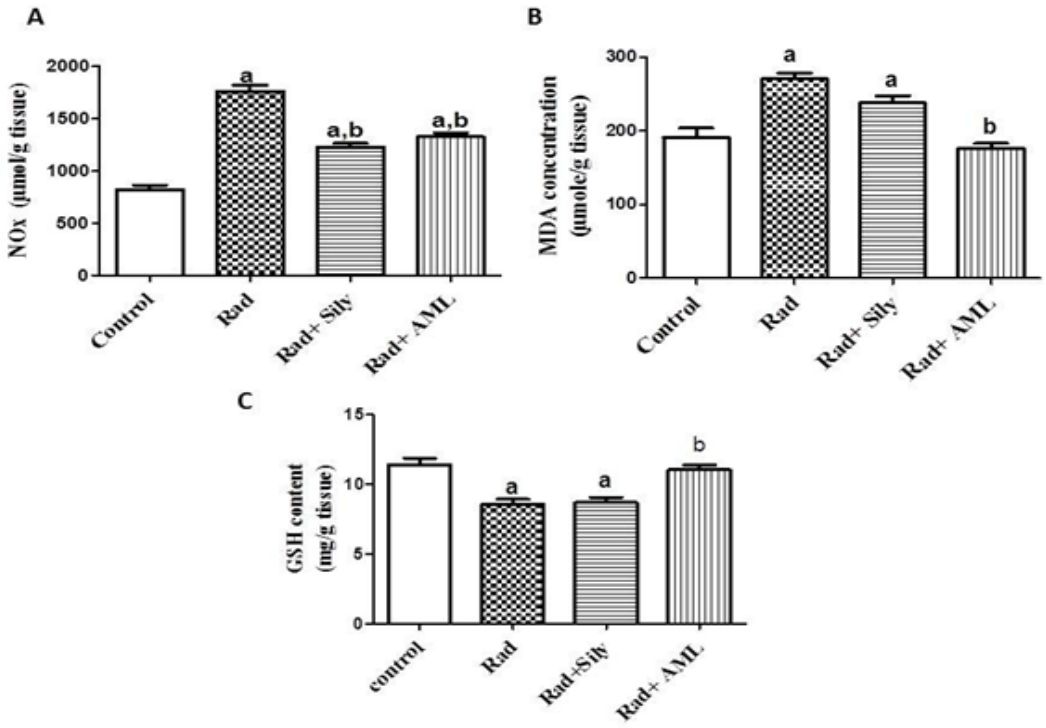

Fig.1. Effect of Amlodipineon hepatic NOx, MDA, and GSH levels in irradiated rats.

(A) NOx, (B) MDA, and (C)GSH levels in irradiated rats.Data presented as mean ± S.E of the mean(n=5). Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. a: significantly different from control group, b: significantly different from irradiated group. $p < 0.05$.Rad, Radiation; Sily, Silymarin; AML, Amlodipine.

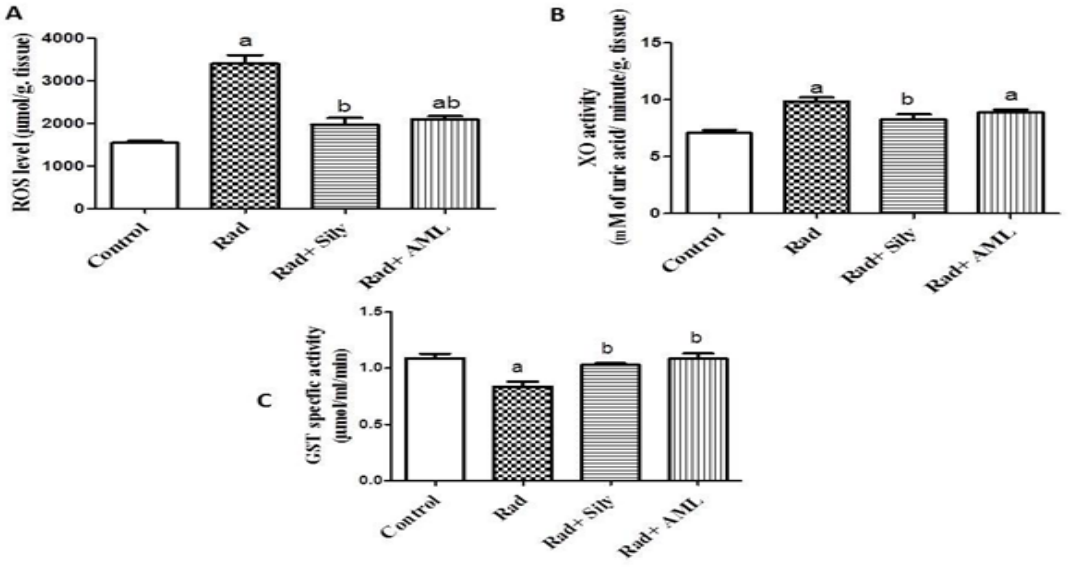

Fig. 2. Effect of Amlodipineon hepatic ROS, XO, and GST levels in irradiated rats.

(A) ROS, (B) XO, and (C) GST levels in irradiated rats.Data presented as mean ± S.E of the mean(n=5). Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. a: significantly different from control group, b: significantly different from irradiated group. $p < 0.05$.Rad, Radiation; Sily, Silymarin; AML, Amlodipine.

Effects of Amlodipine or Silymarin administration on inflammatory markers in irradiated rats

The state of oxidative stress was accompanied by an inflammatory status, as demonstrated by the significant elevation in the levels of hepatic IL-6 and TNF-α as compared to the control group(67.94%; 137.91%, respectively). Silymarin or Amlodipine administration after irradiation, has significantly diminished the levels of hepatic IL-6and TNF- α as compared to the irradiated group to the same extent (IL-6: -43.63%;-44%and TNF-α:-52.88%;-51.12%,respectively)(Fig. 3).

Effects of Amlodipine or Silymarin administration on histopathological changes in irradiated rats

The control group showed a normal histological structure of the hepatic lobules and organization of hepatic cords with prominent central hepatic vein. Polygonal hepatic cells were joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes (grade 0). The hepatic parenchyma of animals exposed to radiation showed disorganization of hepatic cords and apoptosis of hepatocytes which appeared as deeply eosinophilic bodies scatter all over the hepatic lobules. Hyperplasia of Kupffer cells with dilatation of hepatic sinusoids were noticed (grade IV). Hepatic tissue section of animals exposed to radiation and treated by Silymarin showed a mild improvement in comparison with untreated group. Few numbers of apoptotic bodies with hyperplasia of Kupffer cells were seen (grade II). On the other side, liver tissue section of animals exposed to radiation and treated by Amlodipine showed much improvement in comparison with Silymarin treatment. Hepatic lobules showed a mild swelling of hepatocytes and granularity of its cytoplasm. Narrowing of hepatic sinusoids and hyperplasia of Kupffer cells were detected (grade I) (Fig. 4).

Immunohistochemistry results

The positive result of NF-κβ p65 was considered as cytoplasmic staining. The interpretation of the results considered both the staining intensity and the percentage of positive cells. The reactivity was classified as: negative (0), weak (+), moderate (++), marked (+++). The control rats showed a negative cytoplasmic reactivity (0) for NF-κβ p65 in hepatocytes of peri-portal and peri-venular areas; the irradiated rats showed a marked cytoplasmic reactivity (+++); rats in the groups Rad+Silyand Rad+ AML showed a weak cytoplasmic reactivity (+) for NF-κβ p65 in hepatocytes of peri-portal and peri-venular areas (Fig. 5).

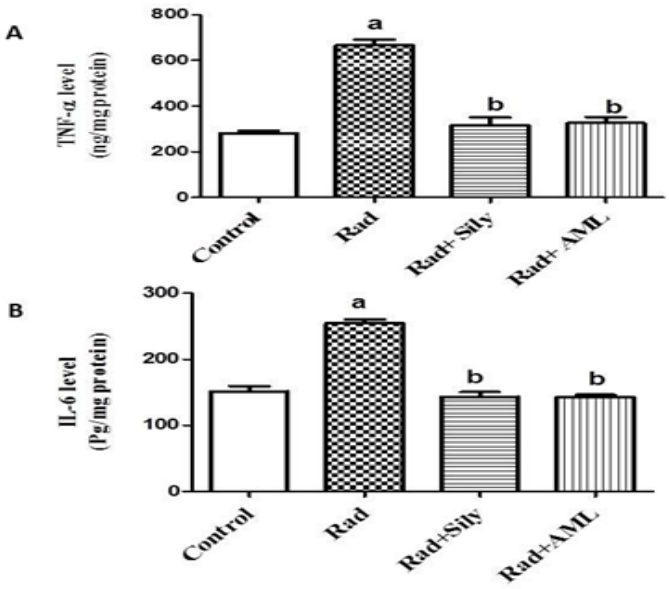

Fig. 3. Effect of Amlodipine on hepatic IL-6 and TNF-α levels in irradiated rats.
(A) TNF-α and (B) IL-6levels in irradiated rats.Data presented as mean ± S.E of the mean(n=5). Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. a: significantly different from control group, b: significantly different from irradiated group. $p < 0.05$. Rad, Radiation; Sily, Silymarin; AML, Amlodipine.

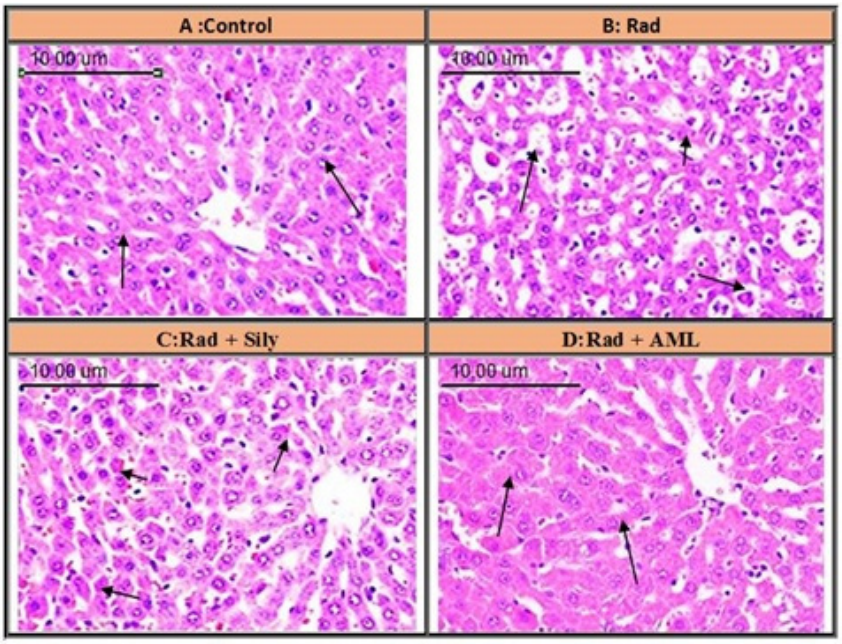

Fig. 4. Effects of Amlodipine or Silymarin administration on histopathologicalchanges in irradiated rats. Photomicrograph of liver tissue sections (A–D) were stained with H&E, 400× magnification.
(A): normal histological structure of hepatic lobules and organization of hepatic cords (**black arrow**). (B): apoptosis of hepatocytes and dilatation of hepatic sinusoids (**black arrow**). (D): few numbers of apoptotic bodies (**black arrow**). (C): swelling of hepatocytes and narrowing of hepatic sinusoids (**black arrow**). Rad, Radiation; Sily, Silymarin; AML, Amlodipine.

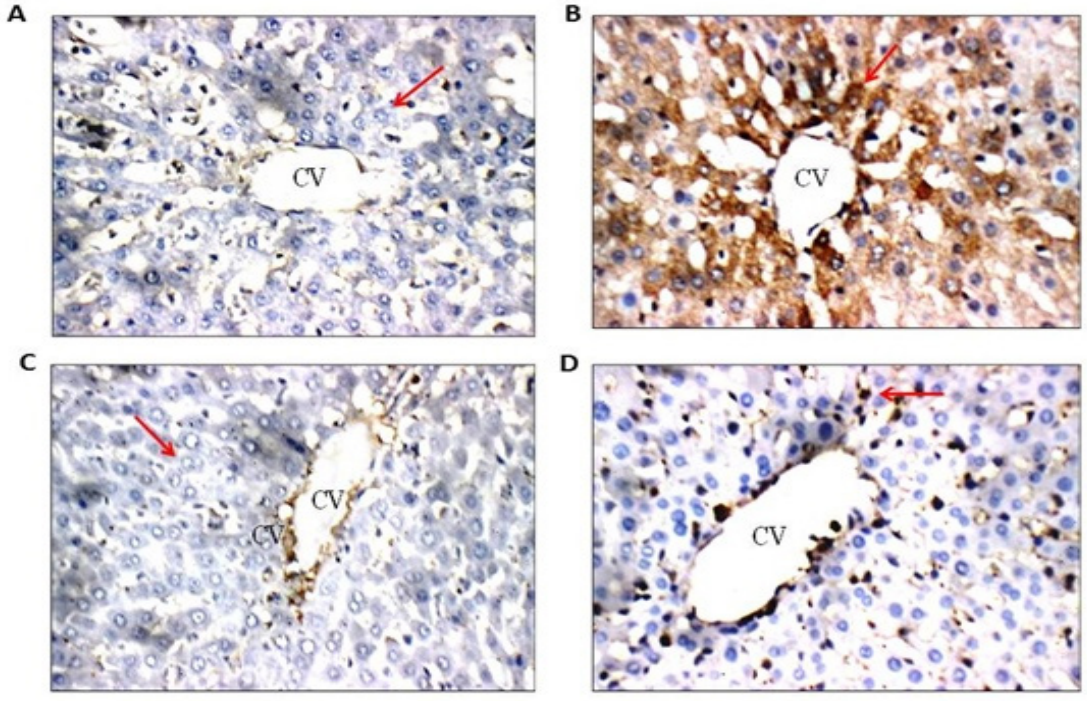

Fig. 5. Photomicrograph of NF-κβ p65 immune-stained liver tissue sections showing:
(A):Control showing a negative cytoplasmic reactivity(0) for NF-κβ p65 in hepatocytes of peri-portal area (**red arrow**) (B):Radiation: showing a marked cytoplasmic reactivity (+++)(**red arrow**) (C):Rad+Sily showing a weak cytoplasmic reactivity (+) (**red arrow**) (D):Rad+AML showing a weak cytoplasmic reactivity (+) (**red arrow**) (NF-κβ p65 immunostaining X 400).Rad, Radiation; Sily, Silymarin; AML, Amlodipine.

Discussion

Drug repurpose has the significant benefit of not requiring additional preclinical safety testing, because the candidate compound's safety has already been demonstrated (Ballard et al. 2020). To find new therapeutic targets for well-established medications, experimental and in silico methods are utilized. The computer methods are confirmed using biological and clinical studies (Wang et al. 2020). Based on prior research, Amlodipine exhibited a strong affinity for TNF-**α**, IL-6, and IL-1 utilizing molecular docking tests(Sumera Qasim 2020). Thus, the current study explores the commercially available calcium channel blocker AML, to repurpose against gamma radiation-induced liver injury in male adult rats using Silymarin as a reference standardized hepatoprotective supplement.

Radioprotectors are substances that shield healthy cells from the damaging effects of radiation(Szejk et al. 2016).A continuous effort is going on by researchers to develop clinically promising radioprotective agents and their mode of action while taking into account any potential therapeutic applications.

Data of the present study indicates that liver damage induced by gamma radiation was coupled with oxidative stress evident by significant elevation of tissue MDAassociated with significant reduction in tissue GSH levels. Moreover, there was a significant rise in the concentration of ROS, xanthine oxidase (XO) activity, coupled with a significant decrease in GST hepatic activity. Inflammatory progression was also evident, reported by significant elevations of tissue NOx production-**α**, IL-6 andNF-κB p65. Liver damage was substantiated by elevation of ALT, AST activities, and decreased albumin concentration. Injury to the hepatocytes may be due to increase in free radical production and/ or decrease free radical neutralization (Planjar-Prvan et al. 2013). Biochemical findings were strongly supported by the results of histopathological examination. The results corroborate previous studies (Fahmy et al. 2016; Mekkawy et al. 2020) reporting that oxidative stress brought on by radiation increases cell membrane permeability and permits the escape of enzymes from inside the cells. Elevation in the oxidative stress could be due to the generation of metabolites that increase the body burden of free radicals, as well as ROS generation from water radiolysis(Abd-Al-Haleem et al. 2019).Xanthine oxidase-derived reactive oxygen species triggered activation of the cascade of molecular events leading to oxidation, and eventually to liver inflammation. NF-κB is one of the major transcription factors involved in unleashing the cascade of events leading to inflammation(Romagnoli et al. 2010). ROS contribute to the expression of a variety of different inflammatory cytokines, chemokines, and adhesion molecules by activating redox-sensitive transcription factors such as NF-κB (Lin et al. 2009).As proven previously, activation of NF-κB inflammatory pathway, involved by ROS, could be used as the initiator of the inflammatory response. The established inflammatory response triggers hepatocyte damage and dysfunction by increasing IL-6 and TNF-α, thus eliciting inflammation and apoptosis that disturb lipid metabolism (Kim et al. 2020). Additionally, the elevation in IL-6 caused by gamma radiation reduces the production of albumin and transferrin. Therefore, it was proposed that after liver irradiation, a series of events occur that cause hepatocyte disruption, hepatic macrophage production of cytokines, and ultimately hepatocellular mortality. TNF-**α**, is a pro-inflammatory cytokine that stimulates the creation of acute phase protein (CRP, C-reactive protein) in hepatocytes and activates NF-kB. Over time, this causes inflammation, which in turn causes an increase in ROS generation, which makes TNF- α, an apoptotic agent. Anti-TNF- α medication may therefore provide protection from radiation-induced TNF-mediated cellular damage (Selim et al. 2020).

Rats that received AML treatment after being exposed to gamma radiation showed a significant amelioration in liver function as well as in the histopathological studies, this improvement may be due to the decrease in the downstream targets NF-κB65, TNF-**α**, and IL-6 which in turn decreased the oxidative stress state. The current data showed that AML may reduce the deleterious effects of gamma radiation-induced liver damage. This could be related to its hepatoprotective effect through preventing the oxidation of cell membranes and counteract the negative effects of free radicals. Several studies have demonstrated that amlodipine has antioxidant activity, relating this activity to its dihydropyridinereductant nature or hydrogen donor properties, respectively the ability of donating protons and electrons to the lipid peroxide molecules, thereby blocking the peroxidation process (Darvishi-Khezri et al. 2022; Vitolina et al. 2012). AML was more efficient in scavenging DPPH radical at a very low concentration of 50µg/mL(Rawal et al. 2020). The location of

the chlorophenyl ring of amlodipine is in close proximity to the acyl chain unsaturated bonds of membrane phospholipid, an important target for peroxidation. The conjugated phenyl ring moiety of amlodipine at this location, in turn, may be favorable for scavenging free radicals by electron donating and radical-resonating mechanisms. Calcium antagonists may effectively scavenge free radicals, thereby breaking the lipid peroxidation chain reaction in membrane and lipoprotein preparations (Godfraind 2005; Mason 2002).

Therefore, Amlodipine provided a strong anti-inflammatory response, which is consistent with the reduction of oxidative stress in Kupffer cells. These anti-inflammatory effects certainly reinforce the repurpose of AML in the management of radiation induced liver injury. The anti-inflammatory biomarker results were consistent with previous studies (Andrzejczak et al. 2006; Li et al. 2009).. The modulatory effect of AML treatment may be attributed to its positive impact on the oxidant/antioxidant balance and regulation of the expression of TNF-α and TGF-β (Kaya et al. 2018). Calcium channel blockers in general are reported to possess hepatoprotective activities in several *in vivo* and *in vitro*studies(Kamal 2013; Rajaraman et al. 2007). Amlodipine was reported to have hepatoprotective potential in other animal models of hepatotoxicity such as CCl4-induced hepatic damage(Abdel Salam et al. 2007).The tested AML is more or less equally active as that of the standard tested hepatoprotective Silymarin in most of tested parameters. Thus, it can be used as a good adjuvant in fighting the inflammatory and oxidation cascades upon radiation exposure without toxic effects. The present study may give a helpful starting point for additional trials on other drugs with similar mechanisms against comparable injury.

In conclusion, AML has a modulator and curative effect against hepatic damage induced by ionizing radiation through its anti-inflammatory and anti-oxidative molecular mechanisms. However, AML can be tested in the future to get profounder insight into the mechanism responsible for the hepatoprotective agent against the radiation-induced liver injury.

Conflicts of interest:

The authors report no conflict of interest.

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