Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 3, 432-438

Protective effect of lycopene and grape seed extract against salbutamol toxicity on myocardial tissue

Gamal E. Shams[1], Suhair A. Abd El-latief[1], Reem I. Abdelrahman[2*]

[1]*Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt.*
[2]*Pharmacist at Directorate of Health Affairs, Universal Health insurance Authority, Cairo, Egypt.*

ARTICLE INFO

Recieved: 03 November 2023

Accepted: 03 December 2023

***Correspondence:**

Corresponding author: Reem I. Abdelrahman
E-mail address: Reemghanem78@gmail.com

Keywords:

Grape seed extract
Lycopene
Salbutamol
Immunohistochemistry
COX-2

ABSTRACT

Cardiovascular toxicity includes damage to the heart resulting from inflammation, oxidative stress, and toxin-induced abnormalities in electrophysiology, and muscle damage. The current study set out to investigate the potential cardioprotective properties of lycopene (LCP) and grape seed extract (GSE) as potential novel therapies to counteract the cardiotoxicity caused by salbutamol. Histopathological and immunohistochemical analysis were used to achieve this. Male albino rats weighing between (150_180g b.wt) were randomly divided into six sets, each containing seven rats. Group I received saline and kept as a control group. Group II was given oral administration of salbutamol (65 mg/kg b.wt.) for 2 consecutive days. Group III received oral dose of LCP (1 mg/kg b.wt.) for three weeks. Group IV was given oral dose of GSE (100 mg/kg b.wt.) for three weeks. Group V was pretreated orally with LCP for three weeks followed by salbutamol oral administration (65 mg/kg b.wt.) for two consecutive doses. Group VI was pretreated orally with GSE (100 mg/kg b.wt.) for three weeks followed by salbutamol oral administration (65 mg/kg b.wt.) for two consecutive doses. All treatments were administered once daily by oral route using gastric tube. Results revealed that salbutamol induced cardiac damage manifested by congestion of myocardial blood vessels, intermuscular hemorrhages, endocardial degenerative changes, microcalcification and subendocardial congestion and hemorrhage, focal cardiomyocytes hyalinization with nuclear peripheralization and pyknosis and individual cellular apoptosis. In comparison with salbutamol group, pre-treatment of rats with GSE demonstrated a moderate ameliorative effect comparable to that of LCP pretreated group; however the residual tissue changes are little bit worse. Immunohistochemistry results supported the histopathological investigations. In conclusion, pre-administration of GSE and LCP provide potential cardioprotective effects by reducing salbutamol-induced cardiac damage linked to alterations in histopathology and immunohistochemistry.

Introduction

Myocardial diseases, which are caused by an abrupt blockage of the coronary arteries, include rheumatic heart disease, peripheral artery disease, ischemic heart disease, congestive heart failure, cardiomyopathy, coronary heart disease, and congenital heart diseases. These are the most serious fatal diseases in the world (Jahan *et al*., 2012; Amran *et al*., 2015; Liaqat *et al*., 2023). Myocardial infarction is defined as cardiomyocyte necrosis due to prolonged myocardial ischemia as a result of an imbalance between coronary blood supply and myocardial demand (Daoud *et al*., 2017; Nguelefack-Mbuyo *et al*., 2022).

The mechanism of drug- induced cardiotoxicity is very difficult and varies depending on the medications, but it generally involves the production of free radical oxygen species and oxidative stress throughout different stages of drug metabolism (Kushwah *et al*., 2022). Drug-induced cardiotoxicity initially causes cardiac muscle dysfunction that progressively leads to myocardial infarction and heart failure (Loh *et al*., 2007). Salbutamol, which consists of an R-isomer and S-isomer, is an effective and widely used β2 adrenoreceptor agonist that may possess anti-inflammatory properties in addition to its bronchodilator activity (Beng *et al*., 2023). Numerous illustrations of oral salbutamol toxicity linked to tremors, hyperglycemia, hypokalemia, and cardiac arrhythmias have been documented (Zheng and Yadav, 2021). Subcutaneous administration of albuterol for 3 days (0.5-50 mg/kg) to Sprague-Dawley rats resulted in a slight myocardial necrosis in the apex and papillary muscle characterized by edema, hemorrhage, and mononuclear cell infiltration in the necrotic areas (Magnusson and Hansson, 1973). A decrease in oxygen supply is linked to salbutamol toxicity because hypotension reduces coronary artery perfusion, which in turn results in myocardial hypoxia; ischemic myocardial necrosis and other cardiac complications include supraventricular tachycardia and ventricular fibrillation (Greaves, 1990; Petruska *et al*., 1997; Uysal *et al*., 2016). Also, Libretto (1994) reported that salbutamol toxicity increases the heart weight linked to fibrosis, localized myocardial necrosis, muscle fiber hypertrophy, and inflammation in rats. Furthermore, Liaqat *et al*. (2023) showed congestion and necrosis in the heart of rabbits treated with salbutamol (65 mg/kg b.wt.) once a day for two days to induce myocardial injury.

The number of cardiac patients is increasing rather than decreasing as a result of the ongoing efforts of pharmaceutical and medical scientists to treat heart diseases (Ojha *et al*., 2011). Besides, the synthetic cardioprotective medications that are already on the market have a lot of side effects and are quite expensive (Kchaou *et al*., 2014). Therefore, the easy availability, comparatively less side effects, and low cost of medicinal plants make them more attractive therapeutic agents which are potential agents for preventing and treating oxidative stress and heart diseases (Liu *et al*., 2014).

Lycopene (LCP) is one of over 600 carotenoids naturally found in tomatoes, watermelon, pink grapefruit, papaya, apricots, and guava which impart a characteristic red pigmentation (Carvalho *et al*., 2020). The main sources of LCP in the diet are tomatoes and tomato-based products (Colle *et al*., 2010). Due to the bioactive dietary compounds in LCP, it may be important in counteracting oxidative stress and diminish excess ROS contributes to initiating arterial hypertension and cardiovascular disease. Additionally, LCP is crucial in preventing cardiovascular complications, such as blood clots resulting from atherosclerotic alterations (Przybylska and Tokarczyk, 2022). This could help to explain LCP's potential as a cardio-

ISSN: 2090-6277/2090-6269/

protective agent as it has good antioxidant properties include scavenging singlet oxygen and free radical (Bansal *et al*., 2006).

Grape seed extract (GSE) is a natural powerful antioxidant that is safe without any toxicity (Safaei *et al*., 2017). It possesses several other biologic and pharmacologic actions including cardioprotective, anti-inflammatory, antitumor, antibacterial, and antiviral activities. Potential therapeutic values of GSE are associated with its antioxidant and anti-inflammatory properties (Zhang *et al*., 2011a). Grape seed proanthocyanidin extract (GSPE) is an active a biological polyphenolic compound extracted from grape seeds with various pharmacologic effects, has antioxidant, free radical scavenging, anti-inflammatory, and other biological activities (Hao *et al*., 2018; Tu *et al*., 2019). Several studies have suggested that GSE may beneficially affect endothelium injury, platelet aggregation, and inflammation involved in the pathogenesis of systemic diseases (Sano *et al*., 2005; Zhang *et al*., 2011b). Furthermore, GSE showed a protective activity against cardiovascular diseases and myocardial infarction, in addition to antithrombotic properties in different animal studies (Sato *et al*., 2001; Shao *et al*., 2003; Ammar *et al*., 2013;Nour *et al*., 2017).

Therefore, the main objective of this study was to evaluate the cardioprotective potential effects of LCP and GSE against myocardial toxicity induced by salbutamol in rats, by assessing histopathological and immunohistochemical analysis.

Materials and methods

Animal ethical statement

Animal handling, medications, and euthanization were carried out following the guidelines for the care of experimental animals and approved by the Research Ethical Committee, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt according to the Animal Research Ethics Committee Guide for care and use of laboratory animals (Approval number: ZU-IACUC/2/F/156/2023).

Chemical and plants

Salbutamol sulfate (VENTOLIN SYRUP) was purchased from GlaxoSmithKline Australia EGYPT. Bottle contains salbutamol as the sulfate 2.0 mg/5 mL in an orange flavoured sugar free and dye free formulation. Lycopene and Grape seed extract were purchased from 21st Century HealthCare Company, Inc. 443 West Alameda Dr. Tempe, AZ 85282.

Experimental animals

In this study Forty two adult male albino rats were chosen in a good clinical health (150-180g b.wt.) provided from the Animal House of the Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. The animals were housed in metal cages and kept under standard laboratory conditions of aeration and room temperature at about 25ºC. The animals were fed with a standard feed based on pellets, and purified water was given ad libitum throughout the experimental period.

Experimental design

The forty two rats were divided randomly into six experimental groups with seven rats each. Group I received standard diet only and served as normal control group. Group II received salbutamol (65 mg/kg b.wt.) orally once a day for 2 consecutive days to induce myocardial injury (Liaqat *et al*., 2023). Group lll was given LCP with a daily oral dose (1mg/kg b.wt.) for three weeks (Bansal *et al*., 2006). Group lV was given a daily oral dose of GSE (100 mg/kg b.wt.) once a day for three weeks (Razmaraii *et al*., 2016). Groups Vand VI were pretreated with LCP or GSE respectively for three weeks followed by salbutamol administration for 2 consecutive days.

All medicaments were dissolved in normal saline and given once daily through oral route using gastric tube. Drug solutions were freshly prepared before administration.

Histopathological analysis

At the end of experiment, the animals were euthanized and the hearts were excised, weighted, then washed with normal saline and fixed immediately in 10% neutral buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. The samples were then dehydrated using a gradient of ethanol concentrations (70–100%), and then followed by clearing in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, embedded and blocked out. Paraffin sections (4–5 µm) were stained with hematoxylin and eosin (Suvarna *et al*., 2013). Under a light microscope, the pathologic structural alterations were examined by a veterinary pathologist.

Immunohistochemistry analysis

The tissue sections were microwave treated. The presence of antigens in the heart tissues was identified by immunostaining, using a two-step process. The primary antibody was first bound to the related antigen, and then the reaction was visualized using a biotin– streptavidin (BSA) system (Hsu *et al*., 1981). 3,30 -Diaminobenzidine (DAB) was used for the permanent preparation, and hematoxylin was used for counterstaining. Five-micron-thick paraffin sections were mounted on positively charged glass slides (Biogenex, Freemont, CA, USA). Paraffin sections were soaked in xylene overnight and then passed through ethanol in concentrations of 100%, 95%, 75%, and 50%. The excess buffer was blotted off, and the slides were dried. One drop of supersensitive primary monoclonal antibodies (COX-2 and P53) was placed on the sections. After incubation for 60 min, the slides were rinsed for 5 min in phosphate-buffered saline (PBS). Two drops of DAKO EnVision were applied for 20 min, followed by rinsing with PBS. DAB chromogen was applied for 10–20 min until the desired brown color was obtained, and then the slides were washed in the buffer to remove the DAB. Mayer'shematoxylin (Hx) was used to counterstain the nuclei in the sections. In accordance with the degree of nuclear staining, sections were placed in Hx solution for 3–5 min, then washed in tap water and differentiated in acid–alcohol before being washed again in tap water. Air-dried slides were mounted with Canada balsam.

Morphometric analysis

Image analysis slides were digitized using an Olympus digital camera (Olympus LC20- Tokyo, Japan) installed on an Olympus microscope (Olympus BX-50, Tokyo, Japan) with a 1/2× photo adaptor, using a 40× objective. The resulting images were analyzed on an Intel® Core I3®-ased computer using Video Test Morphology 5.2 software (Mosco, Russia) with a specific built-in routine for immunohistostaining analysis and stain quantification. The system measured the area percentage of caspase-3 positive expression. Images from five slices per tissue were taken 200 µm apart. Five visions per slice were randomly chosen for assessing positive cells using image analysis software (JID801D). The average grayscale of the positive cells was calculated automatically (Hashish and Kamal, 2015).

Statistical analysis

The results are expressed as mean values ± SEM (standard error of the mean). To assess the influence of the treatment groups on the different biochemical parameters, a one-way analysis of variance (ANOVA) was used. All analyses and charts were performed using the Statistical Package for Social Sciences version 28.0 (SPSS, IBM Corp., Armonk, NY, USA).

Results

The histopathological changes in rats' myocardium of all the study groups are shown in Figures 1-6. Control negative rats' group (G1) showed apparently normal cardiomyocytes with a single centrally located nuclei, sarcolemma cells, interstitial tissue space, intermuscular blood vessels, endocardium, aorta and aortic valves (Fig. 1). However, the salbutamol treated rat's group (G2, control positive) displayed characteristic histopathological changes when matched with the control group. There were a variety of changes represented by pale acidophilic cytoplasm of many cardiac myocytes with gradual disappearance of the sarcoplasm and pyknosis of sarcolemma cells (early cardio-malacia), focal cardiomyocytes hyalinization with nuclear peripheralization and pyknosis and individual cellular apoptosis. There were also congestion of myocardial blood vessels, moderate interstitial edema with occasional intermuscular hemorrhages, beside endocardial degenerative changes, microcalcification and subendocardial congestion and hemorrhage (Fig. 2). The LCP treated rat's group (G3) revealed a micromorphological characterizations comparable to that of the control negative group with keeping features of the cardiomyocytes morphology, epicardial and endocardial structures, intercalated discs, interstitial tissue, sarcolemma cells, coroner's, and aortic structure (Fig. 3). Also, the GSE treated rat's group (G4) revealed histological features comparable to that of the control negative group with a preserved morphology of cardiomyocytes, epicardial and endocardial structures, intercalated discs, interstitial tissue, sarcolemma cells, coroner's, and aortic structure (Fig. 4).

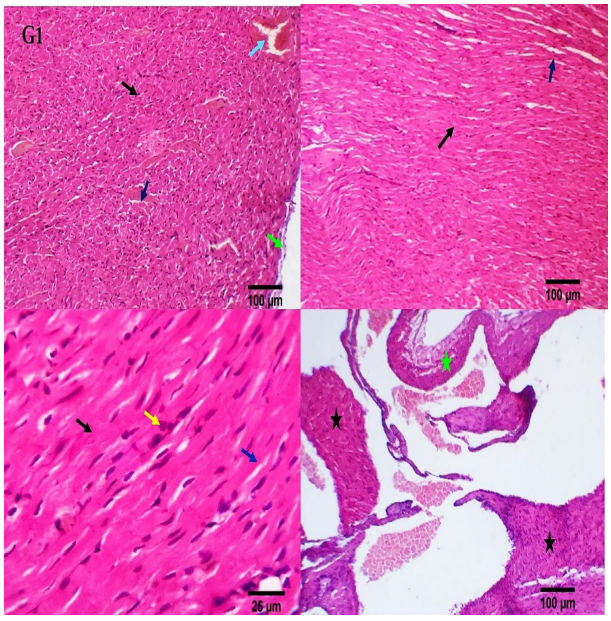

Fig.1. Photomicrograph from rat's heart of control negative group showing apparently normal cardiomyocytes with a single centrally located nuclei (black arrow), sarcolemma cells (yellow arrow), interstitial tissue space (dark blue arrow), intermuscular blood vessels (light blue arrow), endocardium (light green arrow), aorta (black and green asterisks) and aortic valves (light blue asterisk). H&E Scale bars 25um, 100um.

The LCP- pretreated rat's group followed by salbutamol administration (G5, preventive control) declared a moderate ameliorative effect of the used extract. Residual tissue changes were still recorded and were represented by focal myocardial degeneration (hyaline), apoptosis, congested coronary, intermuscular and occasionally subendocardial blood vessels. Mild interstitial edema and mild focal cardio malacic foci were seen. The aorta and other large blood vessels were apparently normal (Fig. 5).

The GSE- pre-treated group (G6, preventive control) demonstrated a moderate ameliorative effect comparable to that of LCP pretreated group; however the residual tissue changes are little bit worse. There were focal myocardial degeneration, macro-steatosis, and apoptosis. Moderate congestion of the coronary blood vessels, intestinal edema with associated cardiomyocytic atrophy together with mild focal cardio-malacic foci were recorded. The aorta and other large blood vessels appear normal (Fig. 6).

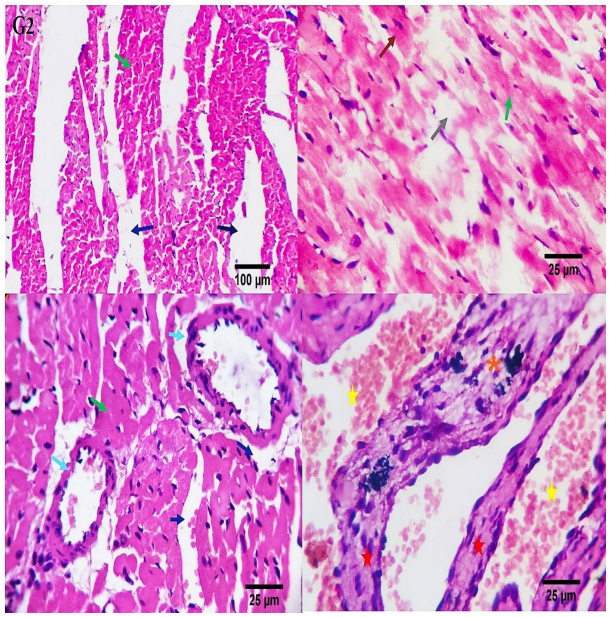

Fig. 2. Photomicrograph from rat's heart of control positive, salbutamol treated group showing pale acidophilic cytoplasm of many cardiac myocytes with gradual disappearance of the sarcoplasm and pyknosis of sarcolemma cells (early cardio-malacia) (gray arrow), focal cardiomyocytes hyalinization with nuclear peripheralization and pyknosis (dark green arrows) in addition to individual cellular apoptosis (brown arrow). There is also congestion of myocardial blood vessels (light blue arrows), moderate interstitial edema with occasional intermuscular hemorrhages (dark blue arrows) beside endocardial degenerative changes (red asterisks), microcalcification (orange asterisk) and subendocardial congestion and hemorrhage (yellow asterisks). H&E .Scale bars 25um, 100um.

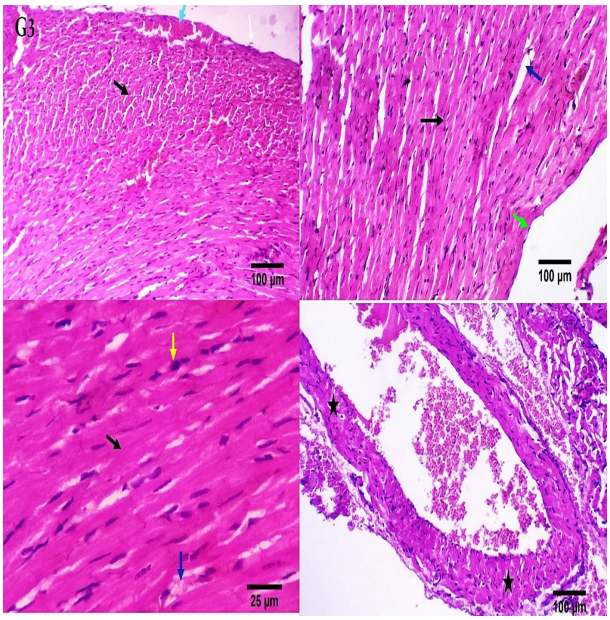

Fig.3. Photomicrograph from rat's heart of LCP treated group showing keeping features of the cardiomyocyte's morphology (black arrows), epicardial (white arrow) and endocardial structures (light green arrow), interstitial tissue (dark blue arrow), sarcolemma cells (yellow arrow), coroner's, and aortic structure (light blue arrow and black asterisks). H&E .Scale bars 25um, 100um

Examined COX- 2- immune-stained tissue sections from different experimental groups denoted negative expression of the inflammatory marker COX- 2 in the cardiomyocytes, vascular endothelium, interstitial fibrocytes and the normally detected fixed tissue histiocytes in groups 1, 3, 4, 5 and 6. Very few vascular endothelial cells were weakly reactive in groups 1and 3. Salbutamol treated rats (G2) revealed moderate expression of the used marker in the vascular endothelial cells and in a few to moderate number of degenerated cardiomyocytes. A few numbers of the above mentioned cells were recorded in the pretreated LCP and GSE groups, G5and G6 (Fig. 7).

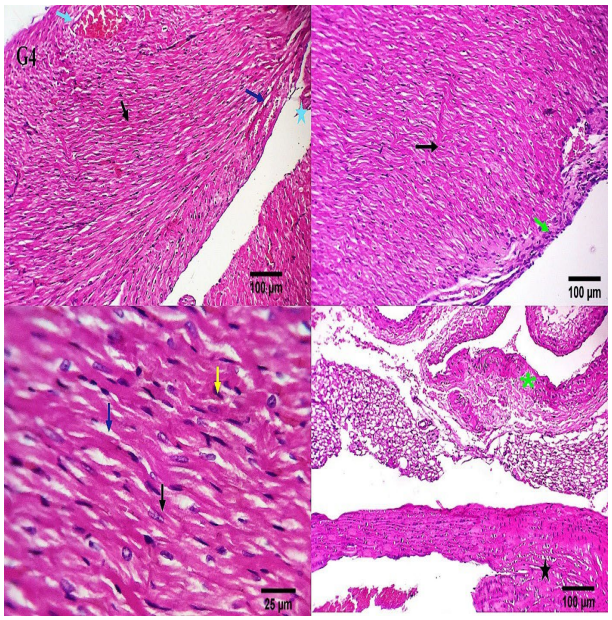

Fig. 4. Photomicrograph from rat's heart of GSE treated rats (G4) showing keeping features of the cardiomyocyte's morphology (black arrows), epicardial (white arrow) and endocardial structures (light green arrow), interstitial tissue (dark blue arrow), sarcolemma cells (yellow arrow), coroner's, and aortic structure (light blue arrow, green and black asterisks). H&E .Scale bars 25um, 100um.

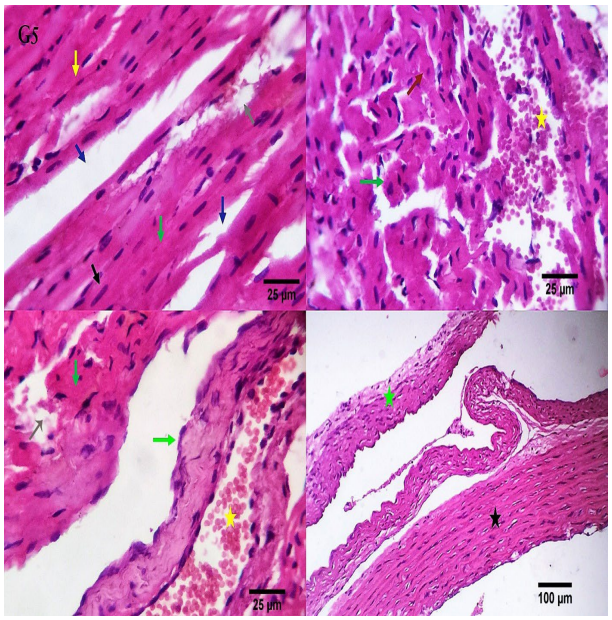

Fig. 5. Photomicrograph from rat's heart of LCP- pretreated group (G5) showing residual tissue changes represented by focal myocardial degeneration (hyaline) (dark green arrows), apoptosis (brown arrows) , congested intermuscular and subendocardial blood vessels(yellow asterisks) . Mild interstitial edema is also seen (dark blue arrow). Mild focal cardio malacic foci are observable (gray arrows). Aorta and large blood vessels appear normal (black and green asterisks). H&E Scale bars. 25um, 100um..

Immune stained tissue sections for apoptotic protein P53 from different experimental groups denoted negative expression of the used marker in the cardiomyocytes, vascular endothelium, interstitial fibrocytes and tissue histiocytes in groups 1, 3, 4, 5 and 6. Very few vascular endothelial cells were weakly reactive in groups 1, 3. On the other hand Salbutamol treated rats (G2) revealed mild to moderate expression of the used marker in a few to moderate numbers of cardiomyocytes and vascular endothelial cells (Fig. 8).

Morphometric analysis of COX-2 immune-stained heart tissue section from different experimental groups revealed an estimated average expression of positive cells as 1.86, 12.13, 0.45, 0.31, 2.57 and 1.88 in control free (G1), salbutamol intoxicated (G2), lycopene (G3), grape seed extract (G4), lycopene preventive (G5) and grape seed preventive (G6) groups respectively (Fig. 9) . Morphometric analysis of P53 immune-stained heart tissue section from different experimental groups revealed an estimated average expression of positive cells as 2.46 , 10.12 , 1.96, 0.37, 0.40 and 0.98 in control free (G1), salbutamol intoxicated (G2), lycopene (G3), grape seed extract (G4), lycopene preventive (G5) and grape seed preventive (G6) groups respectively (Fig. 9).

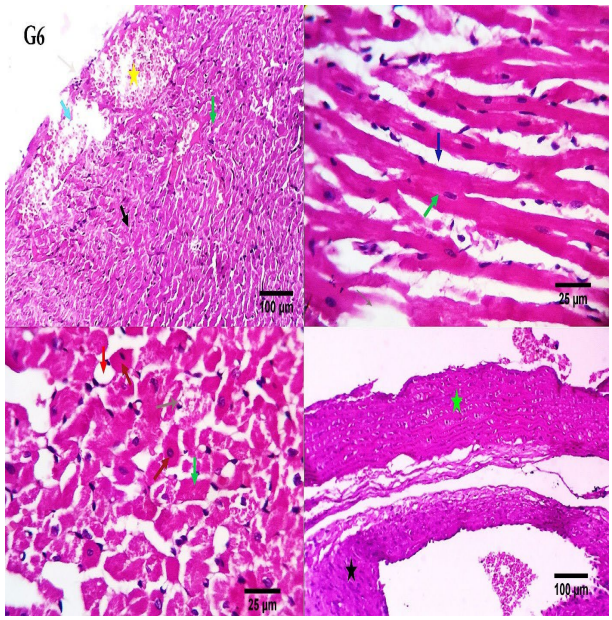

Fig. 6. Photomicrograph from rat's heart of GSE pre-treated group (G6) showing focal myocardial degeneration (dark w associated cardiomyocytic atrophy (dark blue arrow) together with mild focal cardio-malacic foci (gray arrows) can be seen. The aorta and other large blood vessels appear normal (black and green asterisks). H&E. Scale bars. 25um, 100um.

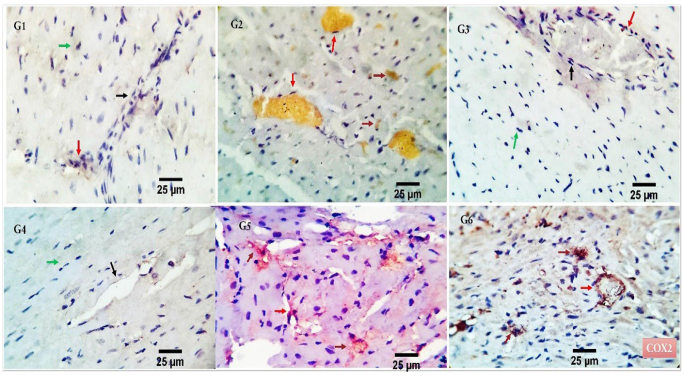

Fig. 7. Photomicrograph from COX-2-immune stained tissue sections showing negative expression (green arrows) of the inflammatory marker COX 2 in the cardiomyocytes, vascular endothelium, interstitial fibrocytes and tissue histiocytes in groups 1, 3 &4. Very few vascular endothelial cells appears weakly reactive in groups 1&3 (red arrows). Salbutamol treated rats (G2) shows moderate expression of the used marker in the vascular endothelial cells (red arrows) and in a few to moderate number of degenerated cardiomyocytes (brown arrows) .A few numbers of the above mentioned cells are seen in the preventive groups (5&6). COX- 2 .Scale bar 25um.

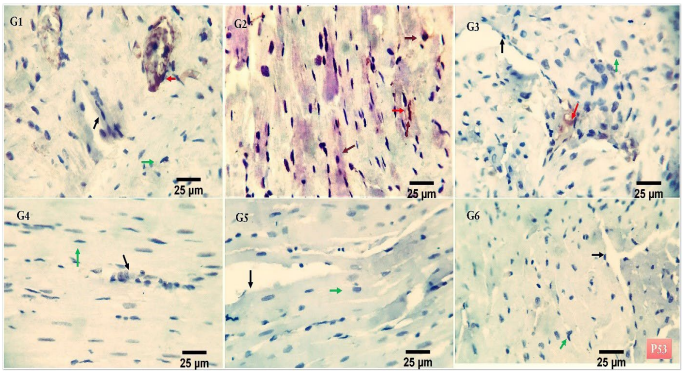

Fig. 8. Photomicrograph from P53-immune stained tissue sections showing negative expression of the used marker in the cardiomyocytes (green arrows), vascular endothelium (black arrows) beside the interstitial fibrocytes and tissue histiocytes in groups 1,3,4, 5 and 6. Very few vascular endothelial cells appear weakly reactive in groups 1and 3 (red arrows). Salbutamol treated rats (G2) showing mild to moderate expression of the used marker in a few to moderate numbers of cardiomyocytes (brown arrows) and vascular endothelial cells (red arrows). P53 .Scale bar 25um.

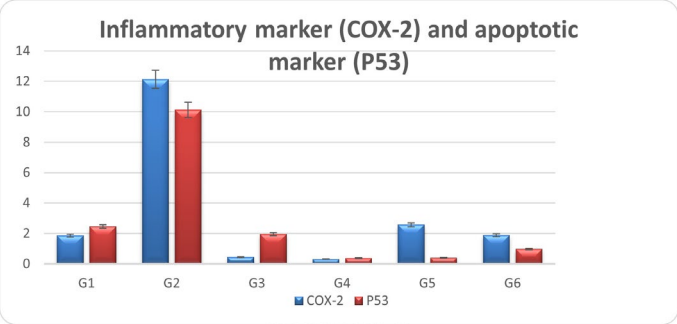

Fig. 9. demonstrating the estimated average expression of positive cells for COX- 2 and P53 in the heart muscles of different experimental groups.

Discussion

Because oxidative stress plays a key role in the pathogenesis of cardiac toxicity, avoiding this stress is the fundamental need of preventing cardiac injury. This can be achieved either by enhancement of endogenous antioxidant enzyme or reducing the production of reactive oxygen species (Jain *et al*., 2018).

Cardiomyopathy is the most important toxic outcome in patients or experimental animals receiving salbutamol. Former studies according to Libretto (1994) findings, rats with salbutamol toxicity exhibited fibrosis, myocardial necrosis, hypertrophy of muscular fibres, and inflammation. In addition, rabbits given salbutamol (65 mg/kg b.wt.) once daily for two days in order to cause myocardial damage had congestion and necrosis in their hearts (Liaqat *et al*., 2023). New studies have associated with an increased in the morbidity and mortality in asthmatic patients with underlying heart disease currently being treated with salbutamol (Nagra *et al*., 2011).

In the current study, salbutamol exposed animals revealed diversity of histopathological lesions represented by pale acidophilic cytoplasm of many cardiac myocytes with gradual disappearance of the sarcoplasm and pyknosis of sarcolemma cells, focal cardiomyocytes hyalinization with nuclear peripheralization and pyknosis and individual cellular apoptosis, beside congestion of myocardial blood vessels, moderate interstitial edema with occasional intermuscular hemorrhages, endocardial degenerative changes, microcalcification and subendocardial congestion and hemorrhage. These alterations may be due to oxidative stress with production of free radical oxygen species causing cardiac injury (Kushwah *et al*., 2022). Farías *et al*. (2017) reported that oxidative stress referred to the remarkable imbalance in normal oxidant scavenging enzyme systems leading to toxic intermediates accumulation and circulation. It is believed that salbutamol's mode of action may be comparable to isoproterenol due to their structural similarities (Liaqat *et al*., 2023). The histopathological findings in salbutamol induced cardiotoxicity may be due to oxidative stress which is a key pathomechanism that led to progress in autophagia, apoptosis, and irreversible injury in cardiomyocytes (Xiang *et al*., 2021). The obtained findings were in agreement with other investigators showing that salbutamol significantly increased apoptosis/necrosis compared to non-treated cardiomyocytes subjected to hypoxia/reoxygenation (Nagra *et al*., 2011). Also, Nour *et al*. (2017) stated that rats received two intra-peritoneal injections of Isoproterenol (ISO) (85 mg/kg) showed focal areas of myocardial infarction, mononuclear cell infiltration, a significantly increased percentage area of fibrosis and degeneration of cardiomyocytes which viewing loss of striation and hypereosinophilic vacuolated cytoplasm with condensed nuclei. Furthermore, evidently discernible myocyte necrosis was encountered in the heart of rats after clenbuterol (0.1 mg /kg b.wt.) administration (Burniston *et al*., 2002). Also, significant cardiac damage in the form of myocardial necrosis, nuclear pyknosis, edema, vacuolization, inflammatory infiltration and muscle fibrosis was recorded in ISO induced group (Jain *et al*., 2018). Our results of histopathological analysis are in line with many previous studies (Hina *et al*., 2010; Fathiazad *et al*., 2012). Similarly, the hearts of doxorubicin (DOX)- treated animals showed moderate to severe focal myocardial degeneration accompanied by mononuclear cell infiltration (Karimi *et al*., 2005). Additionally, when compared with the myocardium of the control rats, signs of necrosis with the infiltration of numerous inflammatory cells as well as muscle fibers separation were seen in ISO-induced myocardial infarction (MI) in rats (Amran *et al*., 2015).

On the other hand, treatment of experimental animals with LCP or GSE decreased salbutamol- induced heart lesions. LCP treated rats showed a moderate ameliorative effect of the used extract. Residual tissue changes were still noticed and were exemplified by focal myocardial degeneration, apoptosis, congested coronary, intermuscular and occasionally subendocardial blood vessels, with mild interstitial edema and mild focal cardio malacic foci were seen. The aorta and other large blood vessels were apparently normal. These results may be endorsed to the protective and antioxidant effects of LCP as previously reported that LCP is characterized by a high antioxidant potential, the highest among carotenoid pigments. For this reason, epidemiological studies show a number of favorable properties between the consumption of lycopene in the diet and a reduced risk of cardiovascular disease (Cheng *et al*., 2019; Przybylska and Tokarczyk, 2022). In recent years, oxidative stress and its counter strategies have attracted biomedical research that LCP has been described to directly interact with ROS, which can help to avert chronic diseases, including diabetes, cardiovascular and neurodegenerative diseases (Bin-Jumah *et al*., 2022). Similar histopathological outcomes were attained by Karimi *et al*. (2005) who observed slight myocardial inflammation and mild cell injury in animals treated with 2.4 g/kg of extract or 3.5 mg/kg of LCP. Also, Abdel-Daim *et al*. (2018) reports marked amelioration and regression of histopathological lesions was established in the tulathromycin–lycopene- and diclofenac sodium (DFS)–lycopene-treated groups, with mild vacuolar degeneration of cardiomyocytes and restoration of cardiac myocytes that appeared relatively similar to the control group.

GSE- pre-treated group (G6) demonstrated a moderate ameliorative effect comparable to that of LCP pretreated group; however the residual tissue changes are little bit worse. There were focal myocardial degeneration, moderate coronary blood vessels congestion, intestinal edema and mild focal cardio-malacic foci were recorded. The aorta and other large blood vessels appear normal. Such mild findings in rats treated with GSE may be attributed to antioxidant activity of GSE which significantly protected the heart tissue; this leading to conclusion that GSE acts as a potent antioxidant to alleviate heart damage (Yalçin *et al*., 2010; Boghdady *et al*., 2013). Also, recent experimental studies stated that consumption of the GSE prevented excessive ROS accumulation in heart tissue and might increase antioxidant capacity (Shao *et al*., 2003). Moreover, GSE had scavenging properties that reduced the free radicals (Aldubayan, 2020; Zarei *et al*., 2022). Others reported that GSE treatment markedly attenuated DOX-induced toxicity, structural changes in myocardium and improved ventricular function in Wistar Rats (Razmaraii *et al*., 2016). This was in line with that pretreatment of rats with Cassia fistula methanolic extract in ISO-induced myocardial infarction exhibited moderate necrosis and kept normal morphology of cardiac muscles significantly by reducing infarct size as compared to the other groups (Kushwah *et al*., 2022). In harmony with this evidence, Karthikeyan *et al*., (2009) have confirmed the efficacy of GSPE as a cardioprotective agent in alleviating isoproterenol-induced myocardial injury in rats. Moreover, in the diabetic rats, GSPE suppressed the hypertrophy of cardiomyocytes, decreased interstitial fibrosis, and led to light microscopic findings similar to those of the control rats (Cheng *et al*., 2007).

Regarding immunohistochemistry in the current work, immune-stained heart tissue sections from different experimental groups denoted negative expression of the inflammatory marker COX- 2 in the cardiomyocytes, vascular endothelium, interstitial fibrocytes and normally detected in fixed tissue histiocytes (G1, and 3- 6). Very few vascular endothelial cells were weakly reactive in groups 1&3. However, salbutamol administration (G2) exposed moderate expression of COX- 2 marker in the vascular endothelial cells and in a few to moderate number of degenerated cardiomyocytes. A few numbers of the above mentioned cells were expressed COX- 2 marker in the pretreated LCP and GSE groups (G5and G6). These results indicate that inflammatory reaction in myocardial infarction (MI) caused salbutamol is probably mediated COX-2. Prostaglandins are produced as a result of COX-2 activation and are likely implicated in the inflammatory response that follows MI. This response is crucial for healing MI, but inflammation may also have negative effects (Heymans *et al*., 1999; Zidar *et al*., 2005). In rats, COX-2 expression increase was associated with elevations in reactive oxygen species (Kim *et al*., 2001) causing myocardial toxicity induced by salbutamol. Experimental research have highlighted the significance of COX-2 in MI by demonstrating its participation in the pathophysiology of persistent heart failure resulting from MI (LaPointe *et al*., 2004; Saito *et al*., 2004). In the present study, COX-2 would not be present in the normal rat's heart, and experimental groups (3-6) although weak focal expression of COX-2 were observed in few numbers of cardiomyocytes, vascular endothelium, interstitial fibrocytes in the pretreated LCP and GSE groups. Similar findings have been described in some other studies, both in humans (Yajosima *et al*., 1999; Wong *et al*., 1998) and experimental animals (Kim *et al*., 2001). Some authors, on the contrary, did not find any COX-2 positivity in the normal human (Abbate *et al*., 2004) and animal hearts (LaPointe *et al*., 2004). The significance of COX-2 in the normal heart has not been elucidated. It has been suggested that COX-2 is the source of the reactive oxygen species which are well known to be responsible for oxidative stress characteristic for aging processes (Kim *et al*., 2001). A recent study by Zidar *et al*. (2007) reported that COX-2 was either not presents or it was present in occasional myocytes in the normal hearts. In myocardial infarction, its expres-

sion was induced in cardiomyocytes as well as in interstitial inflammatory cells, and in capillaries and myofibroblasts in granulation tissue. This was due to COX-2 probably mediates inflammatory reaction in myocardial infarction caused as a result of salbutamol toxicity.

Immune stained tissue sections for apoptotic protein P53 from different experimental groups denoted negative expression of the used marker in the cardiomyocytes, vascular endothelium, interstitial fibrocytes and tissue histiocytes in groups 1, 3, 4, 5 and 6. Very few vascular endothelial cells were weakly reactive in groups 1, 3. On the other hand, salbutamol treated rats (G2) revealed mild to moderate expression of the used marker in a few to moderate numbers of cardiomyocytes and vascular endothelial cells. Freshly investigation revealed that P53 is a gene that regulates the expression of many genes. It broadly regulates the Transcriptome to maintain cardiac architecture and function. Elevated P53 levels correlate with cardiomyocytes (CM) apoptosis and hypertrophy in end-stage human heart failure. Therefore, the impairment of P53 will have deleterious consequences on the heart as a result of reactive oxygen species (ROS) induces DNA damage (Song *et al*., 1999). In the present study administration of salbutamol induced cardiac injury manifested by immunohistochemistry as mild to moderate expression of P53 marker in a few to moderate numbers of cardiomyocytes and vascular endothelial cells compared with the control or treated animals which showed negative expression. The same findings were obtained by previous studies (Gottlieb and Vousden, 2010). Earlier study showed a marked increase in the protein expression of key regulators of apoptosis, namely the caspase -3 and Bax-proteins in rats with experimental myocardial infarction (MI) (Vahtola *et al*., 2010). Also, Mak *et al*. (2017) demonstrate that deletion of P53 was sufficient to trigger the development of spontaneous pathological heart hypertrophy in older mice. Formerly, a study investigated the mechanism of P53-induced myocardial apoptosis and cardiac dysfunction by activating the mitochondrion apoptotic pathway following coronary microembolization (CME) -induced cardiac dysfunction (Sun *et al*., 2017). Also, the latter authors revealed that P53, Bbc3 (PUMA), and cleaved caspase-3 expressions were significantly increased, and BCL-2 expression was declined in myocardial tissues of the CME group compared to the sham group. Jiang *et al*. (2015) found that P53 expression in myocardial tissues was upregulated during myocardial ischemia-reperfusion (I/R) and was positively correlated with the myocardial apoptosis level. Some studies reported that the downregulation of P53 expression could reduce myocardial apoptosis during myocardial infarction (Naito *et al*., 2010; Zhang *et al*., 2011b). In addition, P53 is closely related to the apoptotic pathway; it can not only upregulate Bax expression, but may also directly interact with Bcl-2 in order to inhibit any anti-apoptotic effect (Chi, 2014).

On contrary, immune stained heart tissue for apoptotic protein P53 in treated groups with LCP or GSE denoted negative expression. Our results are in consistent with Roy *et al*. (2005); Pei *et al*. (2018) and Przybylska and Tokarczyk (2022). The authors before stated that the addition of Thymoquinone inhibited DOX-induced cardiac fibrosis (TGF-β, Smad3, collagen I, collagen III, and α-SMA) and apoptosis (P53, bcl-2, caspase-3, caspase-9, and BAX) in rats, indicating that thymoquinone may be a potential therapeutic target for cardiac damage caused by DOX-induced heart failure. Furthermore, immunohistochemistry revealed an increased Bax/Bcl-2 ratio in the myocardium of isoproterenol- induced oxidative stress and myocardial infarction, while Kaempferol restored hemodynamic, left ventricular functions, decreased cardiac injury markers, TNF-α level and apoptosis (Suchal *et al*., 2016). Previous research conducted by Hong *et al*. (2015), they found that lycopene (in the form of watermelon powder) reduced inflammation by reducing the activity of the pro-inflammatory mediator cyclooxygenase 2 (COX-2), disrupting the production of prostaglandins E2 and I2. In contrast, lycopene administration to hypercholesteremic rats reduced the levels of Bax and caspase-3 in both organs (heart and kidneys) noticeably and increased the levels of Bcl-2 in comparison to the control group (Albrahim, 2022). Concerning rats treated with GSE, our findings disagree with earlier report that showed a slight positive reaction for P53 expression in the heart sections from mice in the control and GSPE groups, while the heart sections from mice with Ehrlich solid tumor (EST) exhibited strong positive reactions for P53 expression. By contrast, mild-to-moderate positive reactions for P53 expression were observed in the heart sections from mice in the GSPE + EST group and EST + GSPE group (Aldubayan, 2020).

Conclusion

The present study revealed that LCP and GSE mitigate myocardial damage in salbutamol-induced cardiac injury by reducing inflammation owing to its anti-apoptotic, anti-inflammatory and antioxidant activities. It may be concluded that a diet containing LCP or GSE may be beneficial in those who are at the risk of myocardial injury.

Conflict of interest

None of the authors have any conflict of interest to declare.

References

Abbate, A., Santini, D., Biondi-Zoccai, G.G., Scarpa, S., Vasaturo, F., Liuzzo, G., Bussani, R., Silvestri, F., Baldi, F., Crea, F., Biasucci, L.M., Baldi, A., 2004. Cyclo-oxygenase-2 (COX-2) expression at the site of recent myocardial infarction: friend or foe? Heart 90, 440-443.

Abdel-Daim, M.M., Eltaysh, R., Hassan, A., Mousa, S.A., 2018. Lycopene Attenuates Tulathromycin and Diclofenac Sodium-Induced Cardiotoxicity in Mice. Int. J. Mol. Sci. 19, 344.

Albrahim, T., 2022. Lycopene Modulates Oxidative Stress and Inflammation in Hypercholesterolemic Rats. Pharmaceuticals 15, 1420.

Aldubayan, M.A., 2020. Evaluation of the Cardiac Protection Conferred by Proanthocyanidins in Grape Seeds against Development of Ehrlich Solid Tumors in Mice. Biomed. Res. Int. 2020, 3530296.

Ammar, E., Said, S.A., El-Damarawy, S.L., Suddek, G.M., 2013. Cardioprotective effect of grape-seed proanthocyanidins on doxorubicin-induced cardiac toxicity in rats. Pharm. Biol. 51, 339-44.

Amran, A.Z., Jantan, I., Dianita, R., Buang, F., 2015. Protective effects of the standardized extract of Zingiber officinale on myocardium against isoproterenol-induced biochemical and histopathological alterations in rats. Pharm. Biol. 53, 1795-802.

Bansal, P., Gupta, S.K., Ojha, S.K., Nandave, M., Mittal, R., Kumari, S., Arya, D.S., 2006. Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury. Mol. Cell. Biochem. 289, 1-9.

Beng, H., Hu, J., Wang, S., Liang, X., Qin, H., Tan, W., 2023. Effects of R-salbutamol on the inflammatory response and acute lung injury in endotoxemic mice. Int. Immunopharmacol. 121, 110482.

Bin-Jumah, M.N., Nadeem, M.S., Gilani, S.J., Mubeen, B., Ullah, I., Alzarea, S.I., Ghoneim, M.M., Alshehri, S., Al-Abbasi, F.A., Kazmi, I., 2022. Lycopene: A Natural Arsenal in the War against Oxidative Stress and Cardiovascular Diseases. Antioxidants 11, 232.

Boghdady, N.A., 2013. Antioxidant and antiapoptotic effects of proanthocyanidin and ginkgo biloba extract against doxorubicin-induced cardiac injury in rats. Cell Biochem. Funct.. 31,344–51.

Burniston, J.G., Ng Y., Clark W.A., Colyer J., Tan L.B., Goldspink D.F., 2002. Myotoxic effects of clenbuterol in the rat heart and soleus muscle. J. Appl. Physiol. 93,1824-32.

Carvalho, G.C., Sábio, R.M., Chorilli, M., 2020. An overview of properties and analytical methods for lycopene in organic nanocarriers. Crit. Rev. Anal. Chem. 51, 1–13.

Cheng, H.M., Koutsidis, G, Lodge., J.K., Ashor, A.W., Siervo, M., Lara, J., 2019. Lycopene and tomato and risk of cardiovascular diseases: A systematic review and meta-analysis of epidemiological evidence. Crit. Rev. Food Sci. Nutr. 59, 141-158.

Cheng, M., Gao, H.Q., Xu, L., Li, B.Y., Zhang, H., Li, X.H., 2007. Cardioprotective effects of grape seed proanthocyanidins extracts in streptozocin induced diabetic rats. J. Cardiovasc. Pharmacol. 50, 503-509.

Chi, S.W., 2014. Structural insights into the transcription-independent apoptotic pathway of p53, BMB Rep. 47, 167–172

Colle, I., Lemmens, L., Van Buggenhout, S., 2010. Effect of thermal processing on the degradation, isomerization and bioaccessibility of lycopene in tomato pulp. J. Food Sci. 75, 735–759.

Daoud, A., Ben Mefteh, F., Mnafgui, K., Turki, M., Jmal, S., Ben Amar, R., Ayadi, F., ElFeki, A., Abid, L., Rateb, M.E., Belbahri, L., Kadri, A., Gharsallah, N., 2017. Cardiopreventive effect of ethanolic extract of Date Palm Pollen against isoproterenol induced myocardial infarction in rats through the inhibition of the angiotensin-converting enzyme. Exp. Toxicol. Pathol. 69, 656-665.

Farías, J.G., Molina, V.M., Carrasco, R.A., Zepeda, A.B., Figueroa, E., Letelier, P., Castillo, R.L., 2017. Antioxidant therapeutic strategies for cardiovascular conditions associated with oxidative stress. Nutrients 9, 966.

Fathiazad, F., Matlobi, A., Khorrami, A., Hamedeyazdan, S., Soraya, H., Hammami, M., Maleki-Dizaji, N., Garjani, A., 2012. Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of Ocimum basilicum L. (basil) against isoproterenol induced myocardial infarction in rats. Daru. 20,87.

Gottlieb, E., Vousden, K.H., 2010. p53 regulation of metabolic pathways. Cold Spring Harb. Perspect. Biol. 2, a001040.

Greaves, P., 1990. Histopathology of preclinical toxicity studies. terpretation and relevance in drug safety evaluation. Else' Oxford, pp. 229-277.

Hao, J.P., Shi, H., Zhang, J., Zhang, C.M., Feng, Y.M., Qie, L.Y., Dong, M., Ji, X., 2018. Role of GSPE in improving early cerebral vascular damage by inhibition of Profilin-1 expression in a ouabain-induced hypertension model. Eur. Rev. Med. Pharmacol. Sci. 22, 6999–7012.

Hashish, H., Kamal, R., 2015. Effect of curcumin on the expression of Caspase-3 and Bcl-2 in the spleen of diabetic rats. J. Exp. Clin. Anat. 14, 18–23.

Heymans, S., Luttun, A., Nuyens, D., Theilmeier, G., Creemers, E., Moons, L., 1999. Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. Nat. Med. 5, 1135-1142.

Hina, S., Rehman K., Dogar, Z.H., 2010. Cardioprotective effect of gemmotherapeutically treated Withania somnifera against chemically induced myocardial injury. Pakistan J. Bot. 42, 1487–1499.

Hong, M.Y., Hartig, N., Kaufman, K., Hooshmand, S., Figueroa, A., Kern, M., 2015. Watermelon consumption improves inflammation and antioxidant capacity in rats fed an atherogenic diet. Nutr. Res. 5, 251–258

Hsu, S.-M., Raine, L., Fanger, H., 1981. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am. J. Clin. Pathol., 75, 734–738.

Jahan, N., Ali, S., Asi, M.R., Akhtar, A., 2012. Cardioprotective Potential of Gemmomodified Extract of Terminalia arjuna against Chemically Induced Myocardial Injury in Rabbits. Pak. Vet. J. 32, 255-259.

Jain, PG, Mahajan, U.B., Shinde, S.D., Surana, S.J., 2018. Cardioprotective role of FA against isoproterenol induced cardiac toxicity. Mol. Biol. Rep. 45, 1357-1365.

Jiang, X., Guo, C.X., Zeng, X.J., Li, H.H., Chen, B.X., Du, F.H., 2015. A soluble receptor for advanced glycation end-products inhibits myocardial apoptosis induced by ischemia/reperfusion via the JAK2/STAT3 pathway. Apoptosis. 20, 1033-1047.

Karimi, G., Ramezani, M., Abdi, A., 2005. Protective effects of lycopene and tomato extract against doxorubicin-induced cardiotoxicity. Phytother Res. 19, 912-4.

Karthikeyan, K., Bai, B.R., Devaraj, S.N., 2009. Efficacy of grape seed proanthocyanidins on cardioprotection during isoproterenol-induced myocardial injury in rats. J. Cardiovasc. Pharmacol. 53,109–15.

Kchaou, W., Abbès, F., Attia, H., Besbes, S., 2014. In vitro antioxidant activities of three selected dates from Tunisia (Phoenix dactylifera L.). J. Chem. 367681, 8.

Kim, J.W., Baek, B.S., Kim, Y.K., Herlihy, J.T., Ikeno, Y., Yu, BP, Chung, H.Y., 2001. Gene expression of cyclooxygenase in the aging heart. J. Gerontol. Biol. Sci. 56, 350–355.

Kushwah, A.S., Mittal, R., Kumar, M., Kaur, G., Goel, P., Sharma, R.K., Kabra, A., 2022. Nainwal, L.M. 2022. Cardioprotective Activity of Cassia fistula L. Bark Extract in Isoproterenol-Induced Myocardial Infarction Rat Model. Evid. Based Complement. Alternat. Med. 2022, 6874281.

LaPointe, M.C., Mendez, M., Leung, A., Tao, Z., Yang, X.P., 2004. Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. Am. J. Physiol. Heart Circ. Physiol. 286, H1416– 424.

Liaqat, N., Jahan, N., Rahman, K.U., Tahseen, I., Anwar, T., Qureshi, H., 2023. Investigation of phy-

totherapeutic potential of herbal mixtures and their effects on salbutamol induced cardiotoxicity and hyperlipidemia in rabbits. Bot. Stud. 64, 23.
Libretto, S.E., 1994. A review of the toxicology of salbutamol (albuterol). Arch. Toxicol. 68, 213-216.
Liu, J, Peter, K, Shi, D, Zhang L, Dong G, Zhang D, Breiteneder H, Bauer R, Jakowitsch J, Ma Y., 2014. Anti-inflammatory effects of the chinese herbal formula sini tang in myocardial infarction rats. Evid. Based Complement. Alternat. Med. 2014, 309378.
Loh, H.K., Sahoo, K.C., Kishore, K., Ray, R., Nag, T.C., Kumari, S., Arya, D.S., 2007. Effects of thalidomide on isoprenaline-induced acute myocardial injury: a haemodynamic, histopathological and ultrastructural study. Basic Clin. Pharmacol. Toxicol. 100, 233-239.
Magnusson, G., Hansson, E., 1973. Myocardial necrosis in the rat: A comparison between isoprenaline, orciprenaline, salbutamol and terbutahne. Cardiology 58, 174-180.
Mak, T.W., Hauck, L., Grothe, D., Billia, F., 2017. p53 regulates the cardiac transcriptome. Proc Natl Acad Sci U S A. 114, 2331-2336.
Nagra, A., Maddock, H., Hussain, A., 2011. The bronchodilator salbutamol is associated with increased myocardial injury in a pre-clinical heart attack model. Heart 97, 1-2.
Naito, A.T., Okada, S., Minamino, T., Iwanaga, K., Liu, M.L., Sumida, T., 2010. Promotion of CHIP-mediated p53 degradation protects the heart from ischemic injury. Circ. Res. 106, 1692–1702.
Nguelefack-Mbuyo, E.P., Nokam, F., Tchinda, N.L., Goumtsa, A.F., Tsabang, N., Nguelefack, T.B., 2022. Vasorelaxant and Antioxidant Effects of Aframomum pruinosum Gagnep. (*Zingiberaceae*) Seed Extracts May Mediate Their Cardioprotective Activity against Isoproterenol-Induced Myocardial Infarction. Evid. Based. Complement. Alternat. Med. 2022, 7257448.
Nour, M.S., Sarhan, N.R., Mazroa, S.A., Gawish, S.A., 2017. Histological and immunohistochemical study of cardiac telocytes in a rat model of isoproterenol-induced myocardial infarction with a reference to the effect of grape seed extract. Acta Histochem. 119, 747-758.
Ojha, S., Bharti, S., Sharma, A.K., Rani, N., Bhatia, J., Kumari, S., Arya, D.S., 2011. Cardioprotective effects of Commiphora mukul against isoprenaline-induced cardiotoxicity: a biochemical and histopathological evaluation. J. Environ. Biol. 32, 731–738.
Pei, Z., Hu, J., Bai, Q., Liu, B., Cheng, D., Liu, H., Na, R., Yu, Q., 2018. Thymoquinone protects against cardiac damage from doxorubicin-induced heart failure in Sprague-Dawley rats. RSC Adv. 8, 14633-14639.
Petruska, J.M., Beattie, J.G., Stuart, B.O., Pai, S., Walters, K.M., Banks, C.M., Lulham, G.W., Mirro, E.J., 1997. Cardiovascular effects after inhalation of large doses of albuterol dry powder in rats, monkeys, and dogs: a species comparison. Fundam. Appl. Toxicol. 40, 52-62.
Przybylska, S., Tokarczyk, G., 2022. Lycopene in the Prevention of Cardiovascular Diseases. Int. J. Mol. Sci. 23, 1957.
Razmaraii, N., Babaei, H., Mohajjel Nayebi, A., Assadnassab, G., Ashrafi Helan, J., Azarmi, Y., 2016. Cardioprotective Effect of Grape Seed Extract on Chronic Doxorubicin-Induced Cardiac Toxicity in Wistar Rats. Adv Pharm Bull. 6, 423-433.
Roy, A.M., Baliga, M.S., Elmets, C.A., Katiyar, S.K., 2005. Grape seed proanthocyanidins induce apoptosis through p53, Bax, and caspase 3 pathways. Neoplasia. 7, 24-36.
Safaei, N., Babaei, H., Azarfarin, R., Jodati, A.R., Yaghoubi, A., Sheikhalizadeh, M.A., 2017. Comparative effect of grape seed extract (Vitis vinifera) and ascorbic acid in oxidative stress induced by on-pump coronary artery bypass surgery. Ann. Card. Anaesth. 20, 45-51.
Saito, T., Rodger, I.W., Hu, F., Robinson, R., Huyn, T., Giaid, A., 2004. Inhibition of COX pathway in experimental myocardial infarction. J. Mol. Cell. Cardiol. 37, 71–77.
Sano, T., Oda, E., Yamashita, T., Naemura, A., Ijiri, Y., Yamakoshi, J., Yamamoto, J., 2005. Anti-thrombotic effect of proanthocyanidin, a purified ingredient of grape seed. Thromb. Res. 115, 115-121.
Sato, M., Bagchi, D., Tosaki, A., Das, D.K., 2001. Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia/reperfusion-induced activation of JNK-1 and C-JUN. Free Radic. Biol. Med. 31, 729–737.
Shao, Z.H., Becker, L.B., Vanden Hoek, T.L., Schumacker, P.T., Li, C.Q., Zhao, D., 2003. Grape seed proanthocyanidin extract attenuates oxidant injury in cardiomyocytes. Pharmacol. Res. 47, 463-469.
Song, H., Conte, J.V.Jr, Foster AH, McLaughlin, J.S., Wei, C., 1999. Increased p53 protein expression in human failing myocardium. J. Heart Lung Transplant. 18, 744–749.
Suchal, K., Malik, S., Gamad, N., Malhotra, R.K., Goyal, S.N., Bhatia, J., Arya, D.S., 2016. Kampeferol protects against oxidative stress and apoptotic damage in experimental model of isoproterenol-induced cardiac toxicity in rats. Phytomedicine. 23, 1401-1408.
Sun, Y.H., Su, Q., Li, L., Wang, X.T., Lu, Y.X., Liang, J.B., 2017. Expression of p53 in myocardium following coronary microembolization in rats and its significance. J. Geriatr. Cardiol. 14, 292-300.
Suvarna K.S., Christopher L., Bancroft J.D., 2013. Bancroft's Theory and Practice of Histological Techniques, 7th Edition.
Tu, X., Wang, M., Liu, Y., Zhao, W., Ren, X., Li, Y., 2019. Pretreatment of grape seed proanthocyanidin extract exerts neuroprotective effect in murine model of neonatal hypoxic-ischemic brain injury by its antiapoptotic property. Cell Mol. Neurobiol., 39, 953-961.
Uysal, E., Solak, S., Carus, M., Uzun, N., Cevik, E., 2016. Salbutamol Abuse is Associated with Ventricular Fibrillation. Turk. J. Emerg. Med. 15, 87-89.
Vahtola, E., Louhelainen, M., Forstén, H., Merasto, S., Raivio, J., Kaheinen, P., Kytö, V., Tikkanen, I., Levijoki, J., Mervaala, E., 2010. Sirtuin1-p53, forkhead box O3a, p38 and post-infarct cardiac remodeling in the spontaneously diabetic Goto-Kakizaki rat. Cardiovasc. Diabetol. 9, 5.
Wong, S.C.Y., Fukuchi, M., Melnyk, P., Rodger, I., Giaid, A., 1998. Induction of cyclooxygenase-2 and activation of nuclear factor-nB in myocardium of patients with congestive heart failure. Circulation 98, 100– 103.
Xiang, M., Lu, Y., Xin, L., Gao, J., Shang, C., Jiang, Z., Lin, H., Fang, X., Qu, Y., Wang, Y., Shen, Z., Zhao, M., Cui, X., 2021. Role of oxidative stress in reperfusion following myocardial ischemia and its treatments. Oxid. Med.Cell. Long. 2021, 1–23.
Yajosima, K., Schwab, C., McGeer, E.G., McGeer, P.L., 1999. Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. Brain Res. 830, 226–236.
Yalçin, E., Oruç, E., Çavuşoğlu, K., Yapar, K., 2010. Protective role of grape seed extract against doxorubicin-induced cardiotoxicity and genotoxicity in albino mice. J. Med. Food 13, 917–25.
Zarei, S., Taghian, F., Sharifi, G., Abedi, H., 2022. Novel prevention insights into depletion of oxidative stress status through regular exercise and grape seed effective substance in heart ischemia rat model. Food Sci. Nutr. 10, 833-845.
Zhang, Y., Kohler, K., Xu, J., Lu, D., Braun, T., Schlitt, A., Buerke, M., 2011a. Inhibition of p53 after acute myocardial infarction: reduction of apoptosis is counteracted by disturbed scar formation and cardiac rupture. J. Mol. Cell Cardiol. 50, 471–478.
Zhang, Y., Shi, H., Wang, W., Ke, Z., Xu, P., Zhong, Z., Li, X., Wang, S., 2011b. Antithrombotic effect of grape seed proanthocyanidins extract in a rat model of deep vein thrombosis. J. Vasc. Surg. 53, 743-753.
Zheng, B., Yadav, K., 2021. Acute salbutamol toxicity in the emergency department: A case report. World J. Emerg. Med. 12, 73-75.
Zidar, N., Dolenc-Strazar, Z., Jeruc, J., Jerse, M., Balazic, J., Gartner, U., Jermol, U., Zupanc, T., Stajer, D., 2007. Expression of cyclooxygenase-1 and cyclooxygenase-2 in the normal human heart and in myocardial infarction. Cardiovasc. Pathol. 16, 300-304.
Zidar, N., Jeruc, J., Balazic, J., Stajer, D., 2005. Neutrophils in human myocardial infarction with rupture of the free wall. Cardiovasc. Pathol. 14, 247-250.