Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 51, Number 4, p363-374 December 2023
DOI: 10.21608/zvjz.2023.246616.1225

RESEARCH ARTICLE

The Efficacy of Grape Seed extract and lycopene as Antioxidants on Experimentally Induced Heart Toxicity in Male Albino Rats

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

* Corresponding author: Reemghanem78@gmail.com

Abstract

Cardiovascular disorders are the leading cause of death worldwide. Overly high blood cholesterol and oxidative stress are two major risk factors for heart disease. The purpose of this study was to ascertain if grape seed extract (GSE) and lycopene (LCP), two effective antioxidants, could protect against salbutamol's detrimental effects on cardiac functions.In the current study, 42 male albino rats weighing between 150 and 200 grams were divided into six sets of seven rats apiece at random. The experimental groups consisted of the following: (I) control group; (II) salbutamol group, which administered salbutamol (65 mg/kg BW) for two consecutive days in order to induce myocardial toxicity; (III) LCP group, which received LCP (1 mg/kg BW) once daily for three weeks; (IV) GSE group, which received GSE (100 mg/kg BW) once daily for three weeks; (V) LCP preventive group received a three-week pretreatment with LCP once daily for three weeks, followed by two doses of salbutamol; (VI) GSE preventive group received GSE once daily for three weeks before receiving salbutamol for two consecutive days. Every medication was administered orally once a day via gastric tube after being dissolved in regular saline. All rats' groups were examined for serum activity of cardiac enzymes (AST, LDH, CPK, and CK-MB), and serum levels of troponin T (cTnT) and troponin I (cTnI), as well as antioxidant enzymes (CAT, SOD, GPx), and MDA levels in heart tissues. Salbutamol toxicity was found to significantly ($P<0.001$) raise MDA, troponins, and serum enzyme activity while lowering antioxidant levels. When compared to the salbutamol-induced group, the rats treated with LCP or GSE exhibited a considerable restoration in the activities of antioxidant enzymes and cardiac biomarkers. We conclude that there is significant cardioprotective potential for both LCP and GSE; however, the beneficial effects of GSE are moderately better than LCP.

Key words: Grape Seed Extract, Cardiac biomarkers, Lycopene, Antioxidant enzymes, phytotherapeutics

Introduction

Heart disease is the leading cause of death in wealthy and developed countries, and its occurrence is alarmingly increasing in developing and impoverished nations [1]. Three main risk factors for heart disease are oxidative stress, low density lipoprotein, and high blood cholesterol [2]. One major risk factor for heart disease is an increase in free radical generation [3].

Salbutamol, also referred to as Albuterol, is a synthetic short-acting drug that selectively binds to β2-adrenoceptors (βAR). A frequently drug used in many different clinical contexts is salbutamol [4]. Its therapeutic efficacy is based on its strong smooth muscle relaxant properties, which limit bronchial smooth muscle contraction and resultant bronchodilation. It is the first selective short-acting β2-agonist (SABA) utilized as an alternative

asthma medicine [5]. β-Adrenergic amines induce tachycardia, which raises the heart's oxygen requirement. This induces myocardial hypoxia, which leads to ischemic myocardial necrosis, associated with a decrease in oxygen delivery since coronary artery perfusion decreases during hypotension [6].

A chiral medication containing (R)- and (S)-isomers is salbutamol [7]. Its binding to the human β2-adrenoceptor links its pharmacological activity to the (R)-enantiomer. There is debate regarding the (S)-enantiomer's activity [4]. This isomer is thought to be innocuous in humans, an experimental study revealed that the (S)-isomer might have unfavorable consequences that are clinically important [5, 7]. Salbutamol is not advised as a monotherapy; instead, it should be used as an alternative treatment option in some circumstances or in conjunction with low-dose inhaled corticosteroids (ICSs). Regular administration of this medication may actually have a pro-inflammatory impact, which could account for the reported greater incidence of exacerbations. Although numerous examples of oral salbutamol toxicity linked to cardiac arrhythmias, lactic acidosis, hypokalemia, hyperglycemia, and tremors have been documented [8].

According to previous studies, excessive salbutamol dosage exposure is known to produce harmful and deadly side effects [9]. Sprague-Dawley rats, aged 10 and 20 days, were given oral doses of salbutamol or a single intraperitoneal dosage of 200 mg/kg, with LD50 values above 1000 mg/kg [10]. It has been shown that whether or not 8-week-old Sprague-Dawley rats were fed or fasted affected the acute oral toxicity of salbutamol [11]. Conversely, no patient exhibited either higher Troponin levels or cardiac arrhythmias according to clinical data [12]. Of the children receiving albuterol treatment, 25% had high troponin-T levels and 60% had raised lactate levels [13]. Abuse of salbutamol can lead to heart problems such ventricular fibrillation and supraventricular tachycardia [14]. Furthermore, evidence suggests that people who abuse salbutamol have to be monitored by a physician in order to address electrolyte imbalances and malignant arrhythmias [14].

There is a growing trend in the treatment of cardiovascular illnesses with herbal medications, according to several research [15–17]. Using phytotherapeutics is thought to be a natural way to manage myocardial infarction (MI). Prophylactic therapy using different bioactive nutrients and antioxidant-rich substances may also have a good impact on apoptosis, inflammation, antioxidant capacity, and the treatment of disorders linked to oxidative stress [2, 18].

A naturally occurring carotenoid pigment that is fat soluble and found in many red foods, including tomatoes, papayas, pink grapefruits, pink guavas, carrots, and watermelon, is called lycopene [19]. Fresh tomatoes are not as good a source of LCP as processed tomato products, which also have higher bioavailability. Despite not being a pro-vitamin A carotenoid, LCP has shown signs of possible antioxidant action. Research has demonstrated that antioxidant properties of LCP were effectively reduced cardiac toxicity and apoptosis induced by methotrexate [20].

One active ingredient that is derived from grape seeds is called grape seed proanthocyanidin extract (GSPE) [21]. Flavane-3-ol molecules, such as epicatechin and catechin monomers and their corresponding oligomers, are derived from it [22]. A class of naturally occurring polyphenolic bioflavonoids called proanthocyanidins can be found in a wide range of foods, including fruits, vegetables, nuts, seeds, flowers, wood, and grape seeds in particular.

Procyanidins and proanthocyanidins are two polyphenols found in grape seed extract (GSE) that are effective at scavenging free radicals [23]. Among the phytochemicals that have been thoroughly studied recently is GSE. It is a rich source of condensed tannins, a naturally occurring family of oligomeric proanthocyanidins that are found in a variety of fruits and vegetables and are known as polyphenolic antioxidants Proanthocyanidins have been found to have advantageous benefits because of their ability to scavenge free radicals, which is 20 times more effective than other well-known antioxidants like vitamin C, vitamin E, or β-carotene [24].

Thus, the primary objective of this work was to compare between lycopene (LCP) and grape seed extract (GSE) effects to protect rats' hearts from the damaging effects of salbutamol by evaluating cardiac biomarkers, and antioxidant activity.

Materials and Methods

Experimental animals and experimental design

Forty-two mature adult male albino rats in good clinical health, weighing between 150 and 200 grams, were acquired from the Animal House of the Zagazig University's Faculty of Veterinary Medicine in Zagazig, Egypt. The animals were kept in metal cages with normal laboratory setups, which included aeration and a room temperature of roughly 25ºC. The animals had unrestricted access to water and conventional feed. The ZU-IACUC Committee approved the experimental protocol and assigned it an approval number (ZU-IACUC/2/F/156/2023).

The forty-two rats were divided into six groups of seven rats each, at random, as follows:

Group I (Control group): Rats served as the standard control group and administered only saline.

Group II (salbutamol control group): In accordance with a recent work [2], rats were given salbutamol (65 mg/kg BW) orally once a day for two days in a row to cause myocardial damage. Salbutamol sulfate (VENTOLIN SYRUP) was purchased from GlaxoSmithKline Egypt. Bottle contains salbutamol as the sulfate 2.0 mg / 5 mL in an orange flavored sugar free and dye free formulation.

Group III (Base line LCP group): For three weeks, rats received a daily dose of LCP (1 mg/kg BW) [19]. Lycopene (LCP) was purchased from 21st Century HealthCare company, Inc. 443 West Alameda Dr.Tempe, AZ 85282.

Group IV (Base line GSE group): For three weeks, rats received GES (100 mg/kg BW) [26]. Grape seed extract (GSE) was purchased from 21st Century HealthCare company, Inc. 443 West Alameda Dr.Tempe, AZ 85282.

Group V (LCP preventative group): Rats in this group received salbutamol (65 mg/kg BW) for two days in a row after receiving a three-week pretreatment of LCP (1 mg/kg BW).

Rats in Group VI (GSE preventative group) received salbutamol (65 mg/kg BW) on two days in a row after receiving a three-week pretreatment with grape seed extract (100 mg/kg BW).

Every medication was administered orally once a day via gastric tube after being dissolved in regular saline.

Blood testing

After completion of the experiment, blood samples were taken from the supra-orbital venous plexus under anesthesia by thiopental sodium (45mg/kg BW, IP.) according to Paget and Barnes[25]. The blood samples were placed in n glass

tubes, let to clot & then centrifuged at 3000 rpm for 15 minutes to separate the serum to be used for biochemical tests.

Biochemical evaluation

Calculating Heart Biomarker Levels

Using commercially available kits and a chemistry analyzer (Semar S 1000-elite), the cardiac biomarkers aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), creatine kinase MB (CK-MB), cardiac troponin T (cTnT), and troponin I (cTnI) were measured.

Evaluation of Malondialdehyde and Antioxidant Enzymes in Heart Tissues

After the completion of the experiment, the animals were euthanized. Then the heart tissues were excised, cleaned with isotonic saline, and then homogenizing in 10% ice-cold phosphate buffer (pH=7) according to Hameed *et al.* [27]. This homogenate was then centrifuged, and the supernatant was separated out for ELISA analysis of antioxidants, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA), which is a marker of lipid peroxidation.

Statistical analysis

The one-way ANOVA- F test was used to statistically assess the data generated from this experimental work using SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). The outcomes are shown as means ± standard error (SE). Statistical significance is shown when p is less than 0.05.

Results

In relation to the assessment of cardiac function in this investigation, the salbutamol-intoxicated group (G.II) had significantly ($p < 0.01$) higher serum activity of AST, LDH, and CPK enzymes than the control group. In contrast to group II, the treatment with LCP (G. V) or GSE (G.VI) three weeks prior to Ventolin significantly decreased the elevated level of cardiac biomarkers generated by salbutamol. Except for CPK activity, all cardiac enzymes (AST, LDH, and CPK) recovered fully to their control levels. The beneficial effects of GSE are moderately better than LCP. When compared to control rats, treatments with LCP (G.III) or GSE (G.IV) showed no discernible alterations (Table 1).

When comparing the rats treated with salbutamol to the control group, there was a significant ($p < 0.001$) rise in the cardiac levels of CK-MB, cTnT, and cTnI (Table 2). When rats were pretreated with LCP or GSE prior to salbutamol-induced cardiac toxicity, the aforementioned parameters significantly decreased in comparison to group II's mean values of control (G I). The favorable effects of GSE are moderately better than LCP, but as compared to the control group, non-statistically significant differences were only seen in the rats who received LCP or GSE (Table 2).

When comparing the salbutamol intoxication group (II) to the control group, it was discovered that the activity of the antioxidant enzymes CAT, SOD, and GPx in the heart tissue were significantly lower ($p < 0.01$). While there was a substantial rise ($p < 0.001$) in the salbutamol-intoxicated group compared to the control group in the level of cardiac MDA, a marker of lipid peroxidation. On the other hand, by raising the levels of CAT, SOD, and GPxin close proximity to the control group, three weeks of pretreatment of rats with LCP or GSE prior to salbutamol administration restored the antioxidant status (Table 3). By comparing the effects of both treatments, GSE effects are ascetically more favorable than LCP. Table 3 shows that treatment with LCP or GSE led to lower levels of oxidative stress as indicated by lower MDA levels. These

findings suggest that increased oxidative stress and decreased antioxidant status occurred in the heart tissue because of the myocardial damage caused by salbutamol. Furthermore, the rats that were intoxicated with salbutamol showed a significant reduction in oxidative stress and an increase in antioxidant status after receiving treatment with LCP or GSE. For three weeks, rats treated separately with LCP (G.III) or GSE (G.IV) did not statistically differ from the control group (G. I).

Table 1. The serum cardiac enzymes' activity in different groups (n= 6 groups, 7 rats each).

Parameters Groups	AST u/l	LDH u/l	CPK u/l
I (Control)	31.00 ^{b} ±2.06	241.20 ^{b} ±1.92	4890.0 ^{c} ±1.76
II (Salbutamol)	98.75 ^{a} ±2.13	550.00 ^{a} ±2.44	8548.0 ^{a} ±3.83
III (Lycopene)	33.00 ^{b} ±3.11	254.40 ^{b} ±1.88	5110.0 ^{c} ±2.70
IV (Grape seed extract)	29.55 ^{b} ±3.00	252.80 ^{b} ±2.97	4756.0 ^{c} ±3.70
V (Salbutamol +Lycopene)	35.00 ^{b} ±1.16	262.40 ^{b} ±3.78	6562.0 ^{b} ±2.55
VI (Salbutamol + Grape seed extract)	34.00 ^{b} ±2.00	275.80 ^{b} ±3.95	6550.0 ^{b} ±3.51

Means within the same column with different a, b, & c letters are significantly different at $p<0.05$.
AST= Aspartate aminotransferase, LDH= lactate dehydrogenase, CPK= creatinine phosphokinase.

Table 2. The serum CK-MB, cTnT and cTnI levels in different groups (n= 6 groups, 7 rats each).

Parameters Groups	CK-MB pg/ml	cTnTpg/ ml	cTnI pg/ml
I (Control)	162.00 ^{c} ±2.05	76.40 ^{c} ±0.95	102.00 ^{d} ±1.15
II (Salbutamol)	337.80 ^{a} ±3.32	490.00 ^{a} ±1.05	597.00 ^{a} ±2.28
III (Lycopene)	157.00 ^{c} ±2.11	80.80 ^{c} ±1.02	135.40 ^{cd} ±1.13
IV (Grape seed extract)	160.80 ^{c} ±2.42	79.60 ^{c} ±1.04	142.00 ^{cd} ±1.25
V (Salbutamol +Lycopene)	189.59 ^{b} ±2.95	160.00 ^{b} ±1.11	415.20 ^{b} ±3.12
VI (Salbutamol + Grape seed extract)	185.8 ^{b} ±2.25	136.80 ^{b} ±1.00	474.60 ^{b} ±2.25

Means within the same column with different a, b, c & cd letters are significantly different at $p<0.05$.
CK-MB= creatine kinase MB, cTnT= cardiac troponin T, cTnI= cardiac troponin I.

Table 3. The antioxidants enzymes and Malondialdehyde (MDA) levels in cardiac tissues of different groups (n= 6 groups, 7 rats each).

Parameters Groups	CAT u/mg protein	SOD u/mg protein	GPx mu/mg protein	MDA nmol/mg protein
I (Control)	6.00 ^{ab} ±0.40	124.00 ^{a} ±4.93	9.06 ^{a} ±0.38	2.21 ^{c} ±0.17
II (Salbutamol)	2.46 d^{d} ±0.26	60.66 ^{d} ±4.40	4.19 ^{c} ±0.18	6.14 ^{a} ±0.19
III (Lycopene)	6.36 ^{a} ±0.38	127.00 ^{a} ±4.93	9.03 ^{a} ±0.33	1.76 ^{c} ±0.12
IV (Grape seed extract)	6.26 ^{a} ±0.38	125.00 ^{a} ±4.04	8.96 ^{a} ±0.26	1.73 ^{c} ±0.18
V (Salbutamol +Lycopene)	4.13 ^{c} ±0.20	87.33 ^{c} ±2.72	6.10 ^{b} ±0.55	4.33 ^{b} ±0.18
VI (Salbutamol + Grape seed extract)	4.90^{bc} ±0.05	104.00 ^{b} ±2.64	8.03 ^{a} ±0.12	3.63 ^{b} ±0.21

Means within the same column with different a, b, c, ab, bc& d letters are significantly different at $p<0.05$.
CAT= Catalase, SOD= Superoxide dismutase, GPx= Glutathione peroxidase, MDA= malondialdehyde.

Discussion

While a large body of research has been done on the effects of isoproterenol-induced cardiotoxicity, relatively less has been done on the effects of myocardial infarction caused by salbutamol [28]. It is believed that salbutamol's mode of action may be comparable to isoproterenol due to their structural similarities [2]. Prior research has demonstrated a correlation between oxidative stress and salbutamol toxicity [29].

When compared to the normal control group, salbutamol-induced toxicity group showed a substantial rise in the levels of blood cardiac marker enzymes such as AST, LDH, CPK, CK-MB, cTnT, and cTnI, indicating a myocardial infarction in rats. This could be because salbutamol induces cardiomyocytes to release enzymes into the bloodstream. These enzymes are discharged into the bloodstream from the heart when myocardial damage occurs as a result of oxygen or glucose shortage, the collapse of cellular and subcellular compartments, a ruptured or permeable cell membrane, or other factors that reflect pathological changes in the myocardium [30–32]. The degree of cardiac marker leakage served as a sensitive indicator of myocyte injury and signaled the beginning of a myocardial infarction [15]. According to other observations, salbutamol induced oxidative stress and cardiac cell necrosis, which in turn boosted the activity of the enzymes [33- 34]. Comparably, a high dose of isoproterenol (85 mg/kg) can destroy the heart and result in cardiotoxicity because of cytosolic Ca2+ overload. The destruction of the heart also causes the secretion of cytosolic enzymes, such as CK-MB, LDH, AST, and ALT, into the blood, which can be used as diagnostic indicators of cardiotoxicity [1]. Additionally, earlier studies revealed that the administration of salbutamol caused cardiotoxicity, which improved the lipid profile, elevated cardiac markers, and reduced antioxidant enzymes [34]. It was noted in a different animal experiment that myocardial infarction results from the histological analysis of the heart

following salbutamol administration [35]. Isoproterenol (85 mg/kg BW) injections for two days in a row significantly increased the activities of cardiac marker enzymes (CK-MB, AST, ALT, and LDH) in rabbits [1].

Moreover, higher Ca^{+2} concentration in blood from salbutamol may be the cause of the rise in serum enzyme activity and Troponins (cTnT&cTnI) levels. This would lead to an increase in enzyme secretion. The severity of the myocardial cell necrosis caused by salbutamol is indicated by the high levels of these measures. An increase in lipid peroxidation may be the cause of the myocardial cell necrosis [2]. Additionally, it was said that administering the optimal dosage of salbutamol (80 mg/kg) for two days in a row could confirm the commencement of myocardial infarction [31]. According to the latter reporters, the development of myocardial infarction was confirmed by the positive indication of Troponin I. The protein known as troponin, which is composed of the three subunits cTnT, cTnI, and cTnC, is located in the thin filament of striated muscles and is present in cardiac tissue. The two biochemical indicators for the detection of myocardial damage among the three troponins are cTnT and cTnI [36]. The substantial rise in Trop I levels may be the consequence of Trop I seeping into the bloodstream from the injured cardiac tissues as a result of the rats' optimal dosage of salbutamol-induced necrosis.

Serum AST, LDH, CPK, CK-MB, cTnT, and cTnI levels clearly show that LCP and GSE therapy protected against acute salbutamol toxicity. Three weeks prior to salbutamol, pre-administration of GSE and LCP once daily resulted with a substantial decrease in cardiac biomarkers relative to the normal control. The potential of plants to repair and protect the membrane due to antioxidant polyphenols, thereby limiting the secretion of enzymes, may be the cause of the decrease in enzyme activity and troponins (cTnTandcTnI) levels [17, 37].

Salbutamol significantly ($p<0.01$) reduced the activity of antioxidant enzymes (CAT, SOD, and GPx) and increased the amount of MAD in the heart tissue as compared to the normal control group in this study's examination of antioxidants and lipid peroxidation markers. Following salbutamol treatment, CAT, SOD, and GPx levels decrease, indicating an overabundance of free radicals that damage the heart through oxidative stress. Since excessive lipid peroxidation increases the consumption of antioxidant enzymes, these enzymes offer protection against peroxidative damage in oxidative stress. Antioxidant enzyme activity was hindered by the production of highly reactive free radical species [17, 38]. Because salbutamol and isoproterenol have similar structures and mechanisms of action, they are likewise synthetic catecholamines that cause severe myocardial stress and necrosis [35]. Additionally, the release of inflammatory mediators from mast cells and eosinophils is inhibited by the high amount of cAMP [39]. Excessive salbutamol dosage caused tachycardia, which could result in myocardial infarction [16, 31]. Previous authors suggested that β2-adrenergic receptors be added to the list of medications that may cause myopathy, and they suggested that salbutamol may be the cause of the harmful muscular effects brought on by the release of free radicals, which causes myocardiopathy [40]. Numerous investigations document the concurrent use of salbutamol and cardiovascular problems (such as hypertension, cardiac arrhythmias, and coronary insufficiency) [41- 42].

The heart concentrations of antioxidants (CAT, SOD, and GPx) increased in the rat groups V and VI after receiving herbal therapies, indicating that these treatments had a positive impact on

the heart toxicity caused by salbutamol. The increased production of these enzymes due to a cellular adaptation mechanism may be the cause of the rise in endogenous antioxidant enzyme levels, which has been linked to the antioxidant polyphenols' capacity to scavenge free radicals in medicinal plants [38]. Numerous investigations shown that the administration of LCP or GSE would alleviate the cardiotoxicity caused by salbutamol because they would stop the lipid chain reaction, suppress lipid peroxidation, and stop the drop of CAT, SOD, and GPx in the myocardium [20-23, 38, 43]. Also, the decreased serum CPK and CPK-MB activity suggests that LCP or GSE may have a membrane stabilizing impact. Our results agree with references [2- 9- 44]. In the heart of rats with isoproterenol-induced myocardial infarction, further research revealed that LCP also dramatically suppressed lipid peroxidation and MDA production, and prevented the depletion of antioxidants (SOD, CAT, GPx, and GSH), myocyte injury marker enzymes (CK-MB and LDH), and both [17].

It has previously been observed that supplementing with LCP or GSE reduced MDA levels in the heart tissue and, as a result, lipid peroxidation, indicating a lower risk of coronary heart disease [44-45]. Previous research has indicated that LCP is the most effective biological carotenoid singlet oxygen quencher and that it contributes to the initial line of defense that is upheld by SOD and CAT [46]. LCP proved to have a preventive impact against myocardial damage, confirming the heart's health benefits [47].

According to recent research, GSE may be able to stop cardiomyopathy and apoptosis by lowering inflammatory factors, oxidative stress, xanthine oxidase activity, and activating the cardiomyocytes' built-in antioxidant system [48- 49]. Numerous of the previously stated investigations had findings and interpretations that were similar [18- 45].The antioxidant, anti-inflammatory, anti-cytotoxic and anti-microbial effects of GSE because it was rich in polyphenol compounds like proanthocyanidins, phenolic acids like gallic and gallic acids, catechin and epicatechin [50].

In conclusion, the results show that grape seed extract and lycopene guard against the cardiotoxicity caused by salbutamol, and they could be a good option to employ in conjunction with salbutamol medication.However, the effect of grape seed extract is more evident than lycopene.

Conflict of interest

None of the authors have any conflictof interest to declare**.**

References

[1] Jahan, N.; Rahman, K.U.; Ali, S.; Asi M.R. and Akhtar, A. (2012); Cardioprotective potential of gemmomodified extract of Terminalia arjuna against chemically induced myocardial injury in rabbits. Pak Vet J, 32(2): 255-259.

[2] Liaqat, N.; Jahan, N.; Rahman, K.U.; Tahseen, I.; Anwar, T. and Qureshi, H. (2023): Investigation of phytotherapeutic potential of herbal mixtures and their effects on salbutamol induced cardiotoxicity and hyperlipidemia in rabbits. Bot Stud.; 64(1):23.

[3] Oguntibeju, O.; Esterhuyse, A.J. and Truter, E.J. (2009): Cardiovascular disease and the potential protective role of antioxidants. Afr J Biotechnol 8(14): 3107–3117.

[4] Kumar, A.; Prajapati, P.; Singh, G.; Kumar, D.; Mishra, V.; Kim, S.-C.; Raorane, C.J.; Raj, V. and Kushwaha, S. (2023): Salbutamol Attenuates Diabetic Skeletal Muscle Atrophy by Reducing Oxidative Stress, Myostatin/GDF-8, and Pro-Inflammatory Cytokines in Rats. Pharmaceutics, 15(8): 2101.

[5] Marques L. and Vale N., (2022): Salbutamol in the Management of Asthma: A Review. Int J Mol Sci.; 23(22): 14207.

[6] Greaves, P. (1991): Histopathology of preclinical toxicity studies. Interpretation and prelevance in drug safety evaluation. Else' Oxford, 1st ed., pp 229-277.

[7] Patel, M. and Thomson, N.C. (2011): (R)-Salbutamol in the Treatment of Asthma and Chronic Obstructive Airways Disease. Expert Opin. Pharmacother.; 12: 1133–1141.

[8] Zheng, B. and Yadav, K. (2021): Acute salbutamol toxicity in the emergency department: A case report. World J Emerg Med.; 12(1):73-75.

[9] Libretto, S.E. (1994): A review of the toxicology of salbutamol (albuterol). Arch Toxicol.; 68(4):213-6.

[10] Nishioeda, R., Tamagawa, M., Minami, T., Hatori, M., Tanaka, N. (1981). Acute toxicity of procaterol hydrochloride in rat offspring. 1: macometrics 22:191 – 197.

[11] Kast, A. and Nishikawa, J. (1981): The effect of fasting on oral acute toxicity of drugs in rats and mice. Lab Anim.; 15(4):359-64.

[12] Gunathilaka, P.K.G.; Punchihewa, P.M.G.; Kitulwatte, N.C.; Athukorala, T.J.; Kankananarachchi, I.; Fernando, C.M.P. and Athugalpura, D.M.A.A. (2016): Does intravenous salbutamol therapy cause cardiac toxicity in children with acute severe asthma?. Sri Lanka Journal of Child Health; 45(4): 250-255.

[13] Carroll, C.L.; Coro, M.; Cowl, A.; Sala, K.A. and Schramm, C.M. (2014): Transient occult cardiotoxicity in children receiving continuous beta-agonist therapy. World J Pediatr.; 10(4): 324-9.

[14] Uysal, E.; Solak, S.; Carus, M.; Uzun, N. and Cevik, E. (2016): Salbutamol Abuse is Associated with Ventricular Fibrillation. Turk J Emerg Med.; 15(2):87-9.

[15] Nandave, M.; Ojha, S.K.; Joshi, S.; Kumari, S. and Arya, D.S.(2007): cardioprotective effect of Bacopamonneira against isoproterenol inducedmyocardial necrosis in rats. Int. J. Pharmacol. 3:358-392.

[16] Hina, S., Rehman, K. and Dogar, Z.H. (2010): Cardioprotective effect of gemmotherapeutically treated Withaniasomnifera against chemically induced myocardial injury. Pakistan J. Bot.; 42(3): 1487–1499.

[17] Ojha, S.; Bharti, S.; Sharma, A.K.; Rani, N.; Bhatia, J.; Kumari, S. and Arya, D.S. (2011): Cardioprotective effects of Commiphoramukul against isoprenaline-induced cardiotoxicity: A biochemical and histopathological evaluation. J Environ Biol., 32(6):731–738.

[18] Zarei, S.; Taghian, F.; Sharifi, G. and 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(3):833-845.

[19] Bansal, P.; Gupta, S.K.; Ojha, S.K.; Nandave, M.; Mittal, R.; Kumari, S. and Arya, D.S. (2006): Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury. Mol Cell Biochem.; 289(1-2):1-9.

[20] Aboubakr, M.; Farag, A.; Elfadadny, A.; Alkafafy, M.; Soliman, A. and Elbadawy, M. (2023): Antioxidant and anti-apoptotic potency of allicin and lycopene against methotrexate-induced cardiac injury in rats. Environ Sci Pollut Res Int.; 30(38):88724-88733.

[21] Tu, X.; Wang, M.; Liu, Y.; Zhao, W.; Ren, X. and 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 (7): 953-961.

[22] Bladé, C.; Arola L. and Salvadó, M.J. (2010): Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular

mechanisms. Mol. Nutr. Food Res.; 54 (1): 37–59.

[23] Hassan, H.A., Edrees, G.M.; El-Gamel, E.M. and El-Sayed E.A. (2014): Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage. Cytotechnology, 66 (3): 419-429.

[24] Hajati, H.; Hassanabadi, A.; Golian, A.; Nassiri-Moghaddam, H. and Reza Nassiri, M. (2015): The effect of grape seed extract and vitamin C feed supplementation on some blood parameters and HSP70 gene expression of broiler chickens suffering from chronic heat stress. Ital. J. Anim. Sci., 14: 1-9.

[25] Paget, G.E. and Barnes, J.M. (1964) Chapter 6—Toxicity Test. In: Laurence, D.R. and Bacharach, A.L., Eds., Evaluation of Drug Activities, Academic Press, Massachusetts, 135-166.

[26] Razmaraii, N.; Babaei, H.; Nayebi, A.M.; Assadnassab, G.; Ashrafi Helan, J. and Azarmi, Y. (2016): Cardioprotective Effect of Grape Seed Extract on Chronic Doxorubicin-Induced Cardiac Toxicity in Wistar Rats. Adv Pharm Bull.; 6(3):423-433.

[27] Hameed, A.; Shah, T.M.; Atta, B.M.; Haq, M.A. and Sayed, H. (2008): Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. Pakistan Journal of Botany, 40 (3): 1033-1041.

[28] Kousar, F.; Jahan, N.; Rehman, K. and Nosheen, S. (2012): Cardioprotective potential of Coriandrum sativum. Plant Sci J.; 1:01–06.

[29] Uzkeser, H.; Cadirci, E.; Halici, Z.; Odabasoglu, F.; Polat, B.; Yuksel, T.N.; Ozaltin, S. and Atalay, F. (2012): Anti-inflammatory and antinociceptive effects of salbutamol on acute and chronic models of inflammation in rats: involvement of an antioxidant mechanism. Mediators Inflamm.; 2012:438912.

[30] Jaffe, A.S.; Babuin, L. and Apple, F.S. (2006): Biomarkers in acute cardiac disease: the present and the future. J. Am. Coll. Cardiol. 48: 1-11.

[31] Afsheen, N.; Khalil-ur-Rehman, Jahan, N.; Khan, K.M. and Zia, M.A. (2017): Salbutamol: a substituent of isoproterenol to establish an experimental animal model to induce myocardial infarction. The J. Anim. Plant Sci. 27(4): 1208-1202

[32] Shalaby, N.M.M. and Soliman, W.I. (2017): Comparative study on the effects of lycopene and saffron on doxorubicin-induced cardiotoxicity in adult male albino rats: a histological and biochemical assessment. Egypt J. Forensic Sci. Appli. Toxicol.; 17 (1): 237 – 259.

[33] Dianita, R.; Jantan, I.; Amran, A.Z. and Jalil J. (2015): Protective effects of Labisia pumila on biochemical and histopathological alterations of cardiac muscle cells in isoproterenol-induced myocardial infarction rats. Molecules. 20: 4746-4763.

[34] Zafar, F.; Jahan, N.; Rahman, K.U.; Khan, A. and Akram, W. (2015): Cardioprotective potential of polyphenolic rich green combination in catecholamine induced myocardial necrosis in rabbits. Evid. Based Complement. Alternat. Med. 1-9

[35] Aslam, S.; Jahan, N. and Khan, K.M. (2015): Efficacy of herbal mixture for the treatment of salbutamol induced myocardial necrosis in rabbits. Pakistan Vet. J. 35(3): 355- 359.

[36] Subashini, R. and M. Rajadura (2011). Evaluation of cardioprotective efficacy of Nelumbo nucifera leaf extract on isoproterenol-induced myocardial infarction in wistar rats. Int. J. Pharm. Bio. Sci.; 2: 285-294.

[37] Qureshi, H.; Asif, S.; Ahmed, H.; Al-Kahtani, H.A. and Hayat, K. (2016): Chemical composition and medicinal

significance of Fagoniacretica: a review. Nat Prod Res 30(6):625–639.

[38] Karthikeyan, K.; Bai, B.R. and Devaraj S.N. (2007): Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. Int J Cardiol, 115(3): 326-333.

[39] Rahman, A.; Khanum, S. and Rahman, S.T. (2012). Levo salbutamol versus salbutamol for treatment of acute exacerbation of asthma in Bangladesh children. J. Aller. Ther. 3 (3): 1131-5.

[40] Hellier, J.; Baudrimont, M.; Dussaule, J.and Berenbaum, F. (2002): Reversible Selective B2-Adrenoceptor Agonist-Induced Myopathy. Rheumatology (Oxford); 41(1):111-113.

[41] Neville, E.; Corris, P.; Vivian, J.; Nariman, S. and Gibson, G. (1982): Nebulised salbutamol and angina. Br Med J (Clin Res Ed); 285(6344):796-7.

[42] Price A.H., Clissold S.P. (1989): Salbutamol in the 1980: A Reappraisal of Its Clinical Efficacy. Drugs; 38(1):77–122.

[43] Yilmaz, S.; Atessahin, A.; Sahna, E.; Karahan, I. and Ozer S. (2006): Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology; 218(2-3): 164–171.

[44] Agarwal, S. and Rao, A.V. (1998): Tomato lycopene and low density lipoprotein oxidation. Lipids 33(10): 981–984.

[45] Saadh, M.J. (2023): Potential protective effects of red grape seed extract in a rat model of malathion-induced neurotoxicity. Veterinary World, 16(2): 380-385

[46] Albrahim, T. (2022): Lycopene Modulates Oxidative Stress and Inflammation in Hypercholesterolemic Rats. Pharmaceuticals, 15(11):1420.

[47] Kohlmeier, L.; Kark, J.D.; Martin, B.C.; Steck, S.E. and Riemersma, R. (1997):Lycopene and myocardial infarction risk in the EURAMICstudy. Am J Epidemiol 146(8): 618–626.

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

[49]Lian, Y.; Gao, L.; Guo, P.; Zhao, Y. and Lin, T. (2016): Grape seed proanthocyanidins extract prevents cisplatin-induced cardiotoxicity in rats. Food Science and Technology Research, 22(3): 403–408.

[50]Farid A.; Mohamed D.; Mostafa D.; Tarek R.; Sherif V. and Safwat, G. (2023): Novel grape seed extract nanoparticles attenuate amikacin-induced nephrotoxicity in rats. AMB Express.; 13(1):129.

الملخص العربى

افعالية مستخلص بذور العنب والليكوبين كمضادات للأكسدة على سمية القلب المستحثة تجريبيا في ذكور الفئران البيضاء

1جمال الدين شمس، 1سهير عبد اللطيف،2 ريم ابراهيم عبد الرحمن إبراهيم

1قسم الفارماكولوجيا- كلية الطب البيطري- جامعة الزقازيق
2صيدلي بمديرية الشؤون الصحية- الهيئة العامة للتأمين الصحي الشامل - القاهرة

على الصعيد العالمي، تشكل أمراض القلب والأوعية الدموية السبب الرئيسي للوفاة. الهدف من هذه الدراسة هو تحديد القدرة الوقائية المقارنة لليكوبين (LCP) ومستخلص بذور العنب (GSE) ضد تسمم القلب الناجم عن السالبوتامول. تم تقسيم اثنين وأربعين فأرًا (وزن الجسم 150-180 جم) بشكل عشوائي إلى ست مجموعات تحتوي كل منها على سبعة فئران. وتضمنت المجموعات التجريبية: (I) المجموعة الضابطة؛ (ثانيا) مجموعة السالبوتامول، أعطيت الفئران السالبوتامول (60 ملجم/كجم من وزن الجسم) لمدة يومين متتاليين لتحفيز سمية عضلة القلب؛ (III) مجموعة LCP التي تلقت (1ملجم/كجم من وزن الجسم) مرة واحدة يومياً لمدة ثلاثة أسابيع بواسطة أنبوب المعدة؛ (IV) مجموعة GSE التي أعطت جرعة من مستخلص بذور العنب (100ملجم / كجم من وزن الجسم) مرة واحدة يوميًا لمدة ثلاثة أسابيع؛ (V) المجموعة الوقائية LCP التي تمت معالجتها مسبقًا بـ LCP مرة واحدة يوميًا لمدة ثلاثة أسابيع ثم تم إعطاؤها السالبوتامول لجرعتين متتاليتين؛ (السادس) تمت معالجة المجموعة الوقائية من مستخلص بذور العنب مرة واحدة يوميًا لمدة ثلاثة أسابيع ثم تم إعطاؤها السالبوتامول لمدة يومين متتاليين. كل العلاجات أعطت عن طريق الفم.

تم تحليل النشاط المصلي للإنزيمات عضلة القلب (AST،LDH، CPK وCK-MB)، والتروبونين القلبي (cTnT) والتروبونين ((cTnI، والإنزيمات المضادة للأكسدة (CAT،SOD،CAT) وMDA في أنسجة القلب لفئران التجارب المختلفة ". أدت سمية السالبوتامول إلى زيادة معنوية في نشاط إنزيمات المصل والتروبونين وMDA، مع انخفاض مضادات الأكسدة. أظهرت الفئران المعالجة بـ LCP أو GSE استعادة كبيرة في أنشطة المؤشرات الحيوية للقلب والإنزيمات المضادة للأكسدة بالمقارنة مع المجموعة التي يسببها السالبوتامول. أكدت نتائج الكيمياء المناعية التحليل الكيميائي الحيوي. نستنتج من هذه الدراسة أن اليكوبين ومستخلص بذور العنبلديهما إمكانات قوية لحماية القلب.