Original Article

Protective Role of Ferulic acid and/or Gallic acid Against Pulmonary Toxicity Induced by Amiodarone in Rats

Reem Alazragi

Department of Biochemistry, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabi.

Abstract

Amiodarone is an orally active antiarrhythmic drug generally used throughout the world, causing pulmonary toxicity as one of its most harmful side effects. The efficacy of two phenolic acids; ferulic acid FA or gallic acid GA or their combination on the lung injury in amiodarone-induced lung toxicity were clarified. Fifty albino rats were divided into five groups as follows; G1: Healthy control kept without treatment, G2: Amiodarone group, rats were administered by Amiodarone AD in a daily oral dose (30 mg/kg bw)by gastric gavage for 6 weeks to induce pulmonary toxicity.G3: AD+FA, after induction of pulmonary toxicity, rats received an oral daily dose of FA (100 mg/kg bw) for 6 consecutive weeks. G4: AD + GA, after induction of pulmonary toxicity, rats received an oral daily dose of GA (200 mg/kg.bw) for 6 consecutive weeks. G5: AD + FA+GA, rats have received Amiodarone and a mixture of GA (200 mg/kg bw) and FA (100 mg/kg.bw) for 6 consecutive weeks. Lung samples were assayed for oxidative stress and inflammatory biomarkers along with lung histology and DNA comet assay. The study found that AD causes pulmonary toxicity manifested by a significant alteration in serum level of Lung Na^+-K^+ adenosine triphosphatase, lung tissue content of antioxidant enzymes, and inflammatory biomarkers. Also, lung DNA damage was indicated as a significant change in comet assay parameters, in addition to histological alterations. Results showed that oral administration of FA, GA, or a combination of them reversed these biochemical indices, such as histopathological alterations and DNA damage induced by AD. These findings designated that FA and GA have defensive effects against AD-induced pulmonary toxicity due to its anti-inflammatory and antioxidant properties. GA or a combination of both FA and GA has the most potent effect on inflammatory biomarkersand oxidative stress.Therefore, supplementingGA and FA combination as an adjuvant remedypossibly be a promising compound in lowering AD side effects.

Keywords: Ferulic acid -Gallic acid -Amiodarone- Pulmonary toxicity

Introduction

Medications were used for various treatments were linked to pulmonary complications including pulmonary oedema, bronchospasm, interstitial inflammation, and fibrosis [1]. The lung has a very high blood supply and large surface area and is extremely vulnerable to oxidative stress refereed injury. Furthermore, the mechanisms of inflammatory mediators and oxidative stress were responsible for the incidence of pulmonary distress. Acombinationofthese mechanisms regulate the production of the oxidants causing cellular stress and accordinglymechanicalinjury[2].

The useful therapeutic achievement of phenolic compounds is connected to their anti-inflammatory and antioxidant capacity [3]. Gallic acid GA (3,4,5-trihydroxy benzoic acid) is a potent anti-oxidant extract found ina large group of plant polyphenols identified as gallotannins. It can be found in tea leaves, grapes, blackberry, and gallnuts. Also, it can be found as a secondary metabolite in blueberries, apples, grapes, and tea [4]. Gallic acid is widely used as a dietary herbal supplement, as it has multiple biological properties as anti-allergic, anti-microbial, anti-cancer, anti-ulcer, anti-hyperglycemic, lipid homeostasis, and neuroprotective [5].

Ferulic acid (FA) (3-methoxy-4-hydroxycinnamic acid), first isolated from *Ferula foetida*, is a phenolic acid that exists in fruits. It was confirmed to be anti-inflammation, antioxidant, antidiabetic, and anticancer [6]. Also, it has a therapeutic effect on hypertension and insulin resistance compared to commercially available drugs [7]. Moreover, FA offers meaningful synergistic protection against skin cancer [7]. In diabetics administration of low doses, FA elevates

Address for correspondence: Reem Alazragi, Department of Biochemistry, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabi.
Email: rsalazragi @ uj.edu.sa

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 3.0 License, which allows others to remix, tweak, and build upon the work non commercially, as long as the author is credited and the new creations are licensed under the identical terms.

How to cite this article: Alazragi, R. Protective Role of Ferulic acid and/or Gallic acid Against Pulmonary Toxicity Induced by Amiodarone in Rats. Arch Pharma Pract 2020;11(4):83-90.

antioxidant enzyme activities, thus neutralizing free radicals which are the main cause of enhanced tissue damage [8].

Amiodarone (AD), a form of benzofuran, is a powerful mediator often used in treating cardiac arrhythmias. It has an inclusive variety of side effects, given its high effectiveness relative to other antiarrhythmics. Additionally, AD has many pharmacological characteristics that explain its toxicity, such as the existence of two iodine atoms, its lipophilic composition, a wide range of delivery, the long half-life, and strong tissue aggregation sensitivity. Consequently, AD builds up in fatty tissue, liver, lungs leading to leading to excessive organ toxicity [9].

Pulmonary toxicity, initially characterized by alveolitis and interstitial inflammation and eventually by pulmonary fibrosis, is the most severe adverse effect. These effects were due to the presence of N-desethylamiodarone, the main metabolite of AD, which possesses toxic activity thus causing lung tissue fibrosis [10]. The mechanism of AD to induce pulmonary injury includes alterations in membrane properties, phospholipidosisoxidant generation, inflammatory reactions, and immunological reactions. There are a few recognized risk factors for AD-induced pulmonary toxicity such as the extent of AD therapy and pre-existing lung disease [11]. This study examined the protective effect and therapeutic effectiveness of ferulic and gallic acid in rats with Amiodarone -induced pulmonary fibrosis by evaluating both oxidant-antioxidant and inflammatory status.

Animals

This study comprised 50 male albino rats (150-180 g body weight) divided into five groups. Rats were kept under normal conditions and were allowed free access to a standard requirement diet and water *ad Libitum*. Rats were housed under controlled conditions at King Fahd Medical Research Center. They were acclimatized in the environment of the experiment for 4 days before dosing initiation. Experiments were approved by the Ethical Committee of King Fahd Medical Research Center, Jeddah, KSA. Approval number (163-19).

Rats were randomized into 5 groups (10 / group) as follow:

Group 1: Healthy control kept without treatment

Group 2: Amiodarone group, experimental rats were administered with amiodarone in a daily oral dose (30 mg/kg bw), via gastric gavage for 6 weeks to induce pulmonary toxicity [12].

Group 3: Amiodarone + FA, rats were administered amiodarone in a daily dose of 30 mg/kg body weight, orally via gastric gavage, and FA in a daily dose of 100 mg/kg body weight via gastric gavage (given 2 hours preceding amiodarone) for 6 consecutive weeks according to Dhaliwal, J., et al., [13].

Group 4: Amiodarone + GA, rats administered Amiodarone in a daily dose of 30 mg/kg body weight, orally by gastric gavage, and GA in a daily oral dose of 200 mg/kg bw, by gastric gavage (2 hours preceding amiodarone) for 6 consecutive weeks according to Sen, S., et al., [14].

Group 5: Amiodarone + FA+GA, rats administered amiodarone(30 mg/kg bw) orally by gastric gavage and a mixture of GA (200 mg/kg bw) and FA (100 mg/kg bw) for 6 consecutive weeks.

At the end of the experimental period, rats were dissected under ether anesthesia and blood samples were collected and centrifuged and kept in a deep-freezer at 20ºC till for biochemical analysis.

Preparation lung homogenate

Lung homogenate was obtained from the right lung. Lungs were removed and washed in cold saline. The tissue was homogenized with Tris hydrochloride (Tris-HCl) buffer (pH 7.4). The homogenate was centrifuged (9000 ×g, 30min), the supernatant was used for measurement of oxidative stress and inflammation indices.

Biochemical measurements:

Lung Na^+-K^+ adenosine triphosphatase (ATPase) activities and serum levels of sodium Na and potassium K were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain). Antioxidant status in lung homogenate was assessed by determination of malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD), and advanced oxidation protein products (AOPP) spectrophotometrically using (Biovision Kit, CA. USA). Also, Serum Transforming Growth Factor-beta (TGF-β) was performed by Enzyme-Linked Immunosorbent Assays (ELISA) technique (Cayman Chemical, Ann Arbor, MI, USA). Serum Transforming Growth Factor-beta (TGF-β), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6) activities were performed by Enzyme-Linked Immunosorbent Assays (ELISA) technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. While, advanced oxidation protein products (AOPP)was detected calorimetrically according to the assay protocol of (Abcam, USA).

Assessment of DNA damage by comet assay

Comet assay was performed as described according to de Souza, M.F., et al., [15]. Lungs tissues from control and treated groups were removed and used for the assessment of DNA damage. The evaluation of the length of the DNA migration (i.e., the diameter of the nucleus plus migrated DNA) was measured using image analysis Axiovision 3.1 software (Carl Zeiss, Canada) [16].

Histology

The left lungs of rats from each group were kept in formalin at 4°C for 24 h, then embedded in paraffin, lungs were cut

into slices then stained with hematoxylin and eosin (H&E) and evaluated using a light microscope (Olympus BX61, Hamburg, Germany) connected to a high-resolution digital camera (Olympus, E330, Imaging Corp) at ×200, as described previously [17].

Statistical analysis

Data were statistically analyzed using the statistical software, SPSS. Results were presented as mean ± SE. The differences between mean values were determined by analysis of variance (ANOVA test), $p \leq 0.05$ was considered significant.

RESULTS:

Table 1: Effect of different treatments on serum level of Lung Na+-K+ ATPase and the levels of MDA, CAT, SOD, and AOPP in lung tissue homogenates of all studied groups

Parameters Groups	Lung Na^+-K^+ ATPase (µmol/pi/h/ml)	MDA (nmol/min /mg protein)	CAT (nmol/min /mg protein)	SOD (U/mg protein)	Advanced Oxidation Protein Products (AOPP) (nmol /gm tissue)
G1(Control)	13.68±0.83	1.32±0.19	0.98±0.09	4.78±0.89	248±12.45
G2 (AD)	6.15±0.15[a]	2.35±0.16[a]	0.55±0.06[a]	1.35±0.76a	315±14.2$_9$[a]
G3 (AD+FA)	9.75±0.83[ab]	1.92±0.12[ab]	0.71±0.08[ab]	2.61±0.83[ba]	278±12.83[ba]
G4 (AD+GA)	11.75±0.99[abc]	1.45±0.18[abc]	0.82±0.04[abc]	3.22±0.55[abc]	252±12.55[abc]
G5 (AD+FA+GA)	11.64±0.93[abc]	1.65±0.13[abd]	0.87±0.06[abc]	3.57±0.51[abc]	260±11.51[abc]

Values are expressed as means ±S.D, n=10.
[a]: significant with G1, [b]: significant with G2,[c]: significant with G3, at ($p \leq 0.05$)
AD; Amiodarone, FA: Ferulic acid, GA: Gallic acid

Results obtained in the table (1) revealed that AmiodaroneAD supplementation causes pulmonary toxicity manifested by a significant reduction($p \leq 0.05$) in serum value of Lung Na^+-K^+ ATPase as compared to the control group. Moreover, AD induced oxidative stress in lung tissue homogenate observed as significant ($p \leq 0.05$) elevation in MDA and AOPP with a parallel significant ($p \leq 0.05$) decrease on CAT and SOD level. Thus, using either FA or GA or a combination of them claimed to decrease the harmful effect of AD. Comparing results showed that gallic acid treatment was more efficient if compared to FA treatment, no significant ($p \leq 0.05$) change was recognized between G4 (AD+GA) and G5(AD+FA+GA).

Table 2: The effect of different treatments on lung tissue homogenate level of inflammatory biomarkers (TGF-β, IL-1β, IL-6, and TNF-α) of all studied groups

Parameters Groups	Serum Transforming Growth Factor-beta (TGF-β) Ng/l	Interleukin-1β (IL-1β) (pg/ml)	Interleukin-6 (IL-6) (pg/ml)	Serum Tumor Necrosis Factor-alpha (TNF-α) (pg/ml)
G1(Control)	94.6±3.76	0.75±0.16	2.03±0.65	1.43±0.95
G2 (AD)	220.6±11.84[a]	1.96±0.74[a]	5.14±0.43[a]	4.13±1.05[a]
G3 (AD+FA)	178.7±5.87[ab]	1.26±0.94[ab]	3.55±0.96[ab]	3.15±1.02[ab]
G4 (AD+GA)	143.9±7.53[abc]	1.11±0.21[abc]	2.69±0.44[abc]	2.21±0.95[abc]
G5 (AD+FA+GA)	149.2±9.83[abc]	1.13±0.23[abc]	2.75±0.69[abc]	2.42±0.75[abcd]

Values are expressed as means ±S.D, n=10.
[a]: significant with G1, [b]: significant with G2,[c]: significant with G3, [d]: significant with G4, at ($p \leq 0.05$)
AD; Amiodarone, FA: Ferulic acid, GA: Gallic acid

Results obtained in the table (2) detected the effect of Amiodarone AD supplementation on increasing inflammations in the pulmonary tissues, established by a significant ($p \leq 0.05$) elevation in inflammatory biomarkers (TGF-β, IL-1β, IL-6, and TNF-α)of all studied group as compared to control rats. Meanwhile, the administration of either FA or GA or a combination of them attenuated the destructive effect of AD. Similarly, comparing results showed that Gallic acid treatment was more efficient when compared to FA treatment, no significant ($p \leq 0.05$) change was recognized between G4 (AD+GA) and G5(AD+FA+GA).

Histopathological observation:

The histological examination of control lung sections showed normal architecture of lung parenchyma (Fig.1A).

Histological examination of lung tissues for the amiodarone group (Fig.1B) showed lung fibrosis and interstitial inflammatory infiltration (yellow arrows). On the other hand, an improvement was observed in G3 (AD+FA)and G4 (AD+GA) (Fig. 1C and 1D respectively) as compared to G2. A lower number of inflammatory cells were observed in G5(Fig. 1D) in which a combination of ferulic and gallic acid was used for treatment.

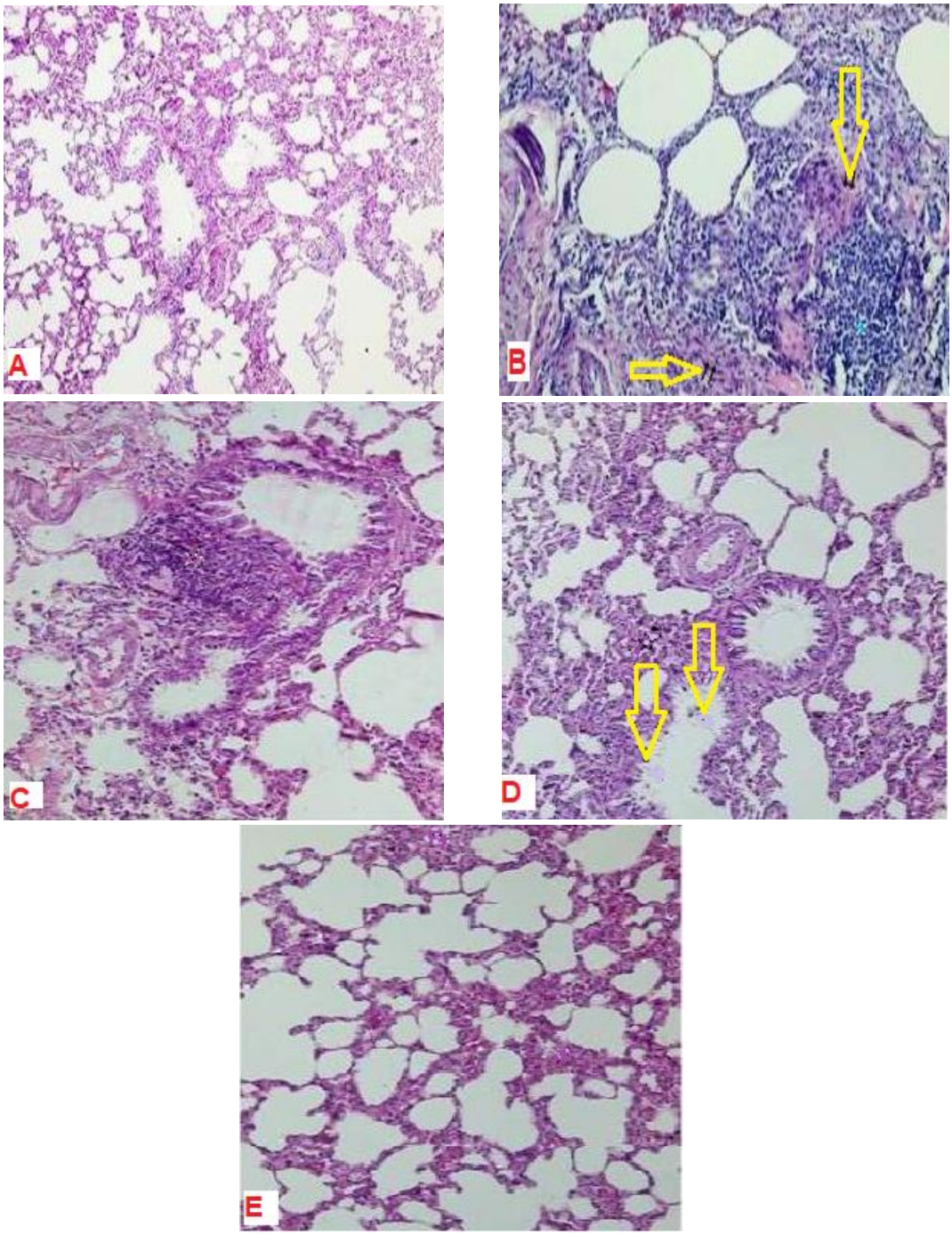

Figure (1): Micrographs of lung histopathology. (A)G1 control group; No abnormalities were seen; lung tissues show normal architectureAlveolar spaces are almost normal in size (B) G2 (AD); Amiodarone rat group showing lung fibrosis and interstitial inflammatory infiltration (yellow arrows)and thickening of the alveolar septa (C) G3 (AD+FA); Amiodarone rat group treated with ferulic acid, showed moderate interstitial inflammatory infiltration (D) G4 (AD+GA); Amiodarone rat group treated with Gallic acid, inflammation was lessened and the number of inflammatory cells decreased (5) (AD+FA+GA); Amiodarone rat group treated with Ferulic acid and Gallic acid, inflammation was lessened and the number of inflammatory cells decreased, appears near the normal architecture

Comet assay result:

Comet assay was applied to observe DNA damage in lung tissues in rats (Fig 2). Compared with the control group (Fig. 2A), DNA damage was observed in the tissues of the group treated with Amiodarone (Fig. 2B). The essay revealed the reduced DNA damage in rat groups treated with (AD+FA) and the group treated with (G4 (AD+GA) (Fig. 2C and Fig. 2D). The assay also revealed a significant decrease in DNA damage in rats treated with (AD+FA+GA) (Fig. 2E).

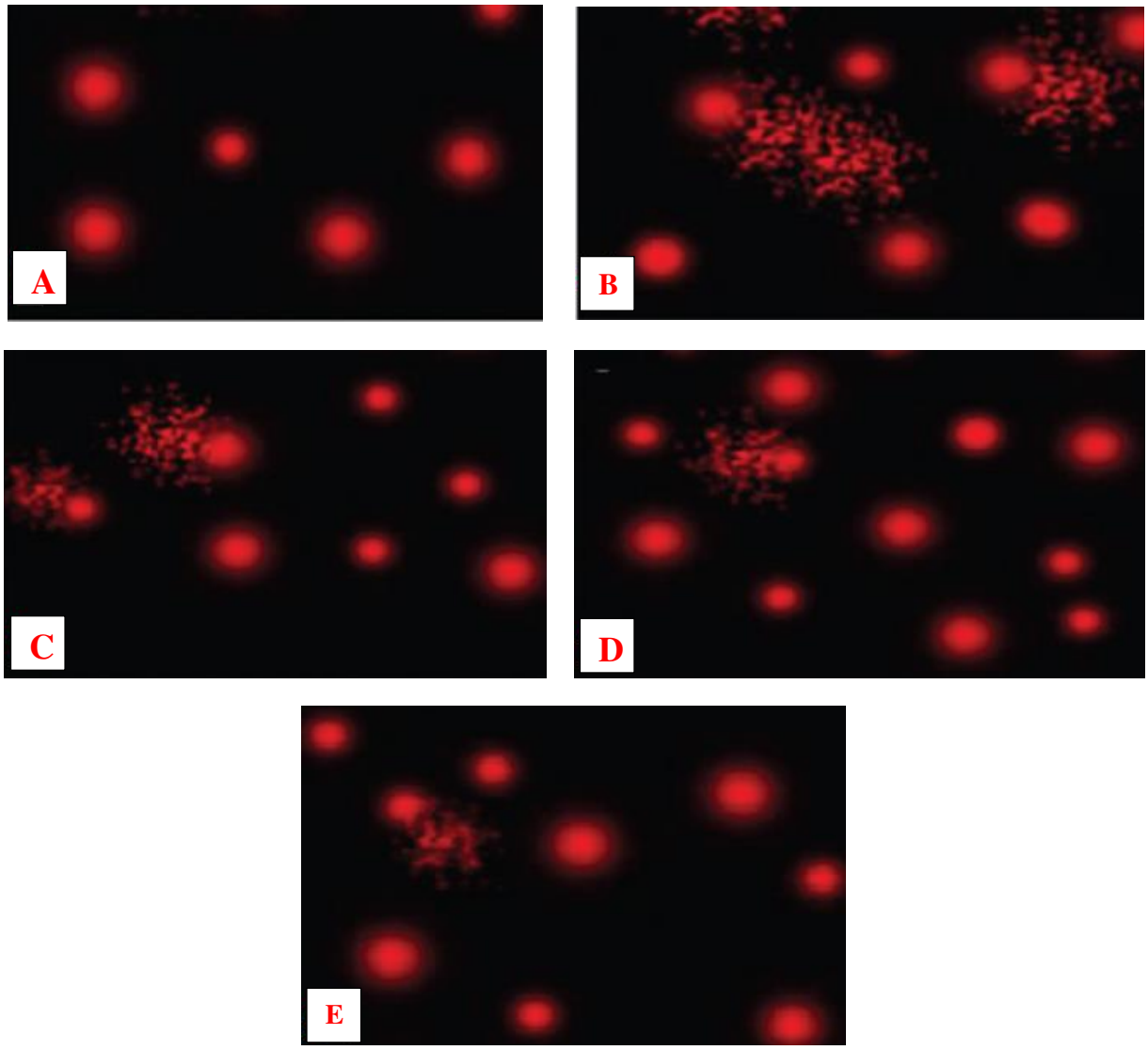

Figure 2. COMET assay showing the degree of DNA damage in the lung tissue in rats subjected to different treatments. (A) Control; normal control group, (B) (AD); Amiodarone rat group showed DNA damage (C) G3 (AD+FA); Amiodarone rat group treated with ferulic acid(D) G4 (AD+GA); Amiodarone rat group treated with Gallic acid (E) (AD+FA+GA); Amiodarone rat group treated with Ferulic acid and Gallic acid.

Discussion

In our study, the administration of GA or FA or a combination of them could reduce the harmful effects of amiodarone. GA and FA prevented the elevation of inflammatory biomarkers, reduced the oxidative stress, and conserved the lung tissue integrity. These properties were moderately accredited to the commonly known antioxidant activities.

When taken by mouth, amiodarone is absorbed gradually and inaccurately, but if consumed with food, its bioavailability can be boosted. The high lipid solubility is an essential property of amiodarone. Amiodarone AD supplementation causes pulmonary toxicity manifested by a significant reduction in serum level of Lung Na^+-K^+ ATPase.This was partly related to its aggregation in adipose tissue and strongly perfumed organs such as the liver, lung, and spleen [3].

Na^+/K^+-ATPase (sodium-potassium adenosine triphosphatase is an enzyme present throughout the membrane of almost all animal cells. It plays a significant role in cell physiology. For each ATP molecule used by the pump, three sodium ions are extracted and two

potassium ions are imported; there is therefore a total export of a single positive charge per pump cycle [18]. Also, Na+-K+ ATPase activity loss may be due to the reduced levels of its substrate (ATP), and expanded membrane lipid peroxidation results in the modification of membranes by ROS [19].

A study bySchwaiblmair, M., et al., proposed that Amiodarone and its metabolites cause lung damage directly by a cytotoxic potency and indirectly by an immunological response. Its toxicity is complex and multifactorial, involving several mechanisms including direct toxicity, hypersensitivity, and elevated oxidative markers [20]. These results agreed with that of Sarova, G.A., et al., which showed that exposure of rats AD for 48 h decreased lung Na, K-ATPase hydrolytic activity [21].

The elevated activities of oxidative stress markers in lung tissue homogenate as MDA and AOPP along with reduced levels of CAT and SOD level was the observed result of AD supplementation.AD increases the production of free radicals and induces mitochondrial oxidation. Cumulative effects of reactive oxygen species (ROS) may result in significant damage to cell structures leading to oxidative DNA damage [22]. The increased lipid peroxidation indicates decreased enzymatic and nonenzymatic antioxidant defense mechanisms [23]. Catalase exists as a tetramer made up of four identical monomers, each comprises of active site heme group, Superoxide is the mainreasonforreleasing ROS, and its dismutation is a key factor in each cell [24]. AOPPs were first identified as a marker of protein oxidation throughout oxidative stress [25].

The effect of oxidative stress in developing pulmonary toxicity following amiodarone was suggested byAndrades, M., et al., who attributed these findings to the massive damage to the blood air barrier and increase in free radicals generation and mitochondrial hydrogen peroxide production [26]. Moreover, attributed these changes to direct toxicity of amiodarone, hypersensitivity, elevated oxidant markers.

Applying FA or GA or a combination of them claimed to decrease the harmful effect of AD on oxidative stress parameters. This study results agreed with Ramar, M., et al., that found that FA caused antioxidant mechanisms such as SOD, alcoholic violent CAT behaviors, and diabetic rats [27]. Also, in a previous study, FA was found to reduce lipid peroxidation by interacting with the inflammation pathways and by scavenging free radicals [28]. Similarly, FA scavenges ROS produced released during the liver damage induced by either D-galactosamine or CCl_4 [29]. Also, in nicotine-treated rats, FA lowered levels of MDA, DNA fragmentation, and regained antioxidant status with elevated rates of oxidative enzymes [6].

Several scientific studies have shown that GA's antioxidant activities demonstrate their pharmacological effects [30]. Remarkably, GA ameliorated the observed AD-induced alterations in lung tissues homogenates content of antioxidant stress markers in the rats. These findings correlate positively with the report of [31]. One study examining the protective capacity of specific phenols involving gallic acid, protocatechuic acid, chlorogenic acid, and vanillic acid revealed that GA demonstrated the highest potency for antioxidants [32]. A previous work byOmobowale, T.O., et al., Gallic acid has been shown to mitigate oxidative stress by decreasing the generation of ROS in the rat brain and enhancing antioxidant state [33]. Although, Cheng, Q., et al., stated that gallic acid-enhanced SOD activity and CAT-related enzymes in streptozotocin-induced diabetic brain damage and blocked free radicals production [34]. Also, Li, B., et al., examined the antioxidant substances that mostly inhibited the experimentally induced oxidative stress in ovarian tissue. They suggested that gallic acid worked as a potent antioxidant by decreasing the production of ROS and stimulating the enzyme antioxidant mechanism [35].

A few previous studies have indicated that GA behaves as an electrophilic mutagens scavenger [33]. In line with our findings Nikbakht, J., et al., was verified that GA strengthened the antioxidant-oxidant balance and recovered the fibrotic lung tissues to their normal state and thus could be an important natural substance for the management of bleomycin-induced pulmonary fibrosis of the lung [36].

Amiodarone AD supplementation increased inflammations in the pulmonary tissues, as recognized by a significant elevation in inflammatory biomarkers including. TGF-β, IL-1β, IL-6, and TNF-α Additional works have shown that amiodarone is metabolized to an aryl radical that can disrupt inflammatory cytokines. It was shown that both catalytic and scavenger antioxidants ameliorate lung damage and fibrosis in animals caused by amiodarone [37]. Reactive oxygen species cause endothelial dysfunction due to an increase in permeability, recruitment of leukocytes, adhesion and transmigration, thrombosis, and other inflammation-initiating and proliferating processes [38]. TNF-α and IL-1β play a role in starting the inflammatory responses. Up-regulation of TNF-α and IL-1β level is key in the regulation of host immune responses in the lung after exposure to γ-rays in the rat. IL-6, which is sharply and quickly produced in infections and tissue injury responses, contributes to the host defense through the stimulation of immune reactions [39].

An improvement was observed in inflammatory biomarkers after treatment with either FA or GA or a combination of them. In addition to the antioxidant activity of FA, we further observed a depleted inflammatory response in the lungs. The declining levels of TNF-ш, IL-1β, and IL-6 mentioned an immunomodulating influence of FA [40]. Cytokines including interleukin (IL)-1β and IL-6 [41]. Another study by Gerin, F., et al., suggested that FA can be used as a promising hepatoprotective agent against formaldehyde toxicity because of the obvious beneficial effects on inflammation parameters [42]. GA co-administration possibly regulated the lung expression of TNF-α, and IL-6, suggesting its important role in the attenuation of the amiodarone-induced inflammatory cascade and reflect its anti-inflammatory potential [43].

GA attenuates TNF- and IL-6 expression from human monocytes [44]. In a study, the effect of gallic acid on bleomycin-induced pulmonary fibrosis in rats was reviewed. The results indicate that intratracheal bleomycin administration substantially increased inflammatory or fibrotic alterations, collagen content, MDA level, and proinflammatory cytokines, such as TNF-5-007 and IL1β, in the lung [45]. In the present study, the amiodarone-treated group (G2) showed marked degenerative change, showing lung fibrosis and interstitial inflammatory infiltration. These findings were in agreement with Zickri, M.B., ET AL., who mentioned similar findings as to the thickening of the alveolar septa [46]. The results revealed ruptured interstitial inflammatory infiltration, which was similar to the findings of [47]. Moreover, Mahavadi, P., et al., confirmed the thickening of alveolar septa with patchy fibrosis and cellular infiltration [48]. In a study by Hosseini, S., et al., an oral administration of Gallic acid reversed the histopathological alterations induced by bleomycin [49].

The harmful effect of AD on DNA observed in this study agreed with da silvia [50]. While treatment with either FA or GA or a combination of them improved these results. Sudheer, A.R., et al., also reported a dramatic increase in comet parameter levels in nicotine-treated lymphocytes, which is the indication DNA strand breaks [51]. Also, using FA therapy has been reported in reducing DNA lymphocyte damage and sepsis-induced rats' liver and kidney cells, suggesting that FA improves the mechanisms of DNA repair mechanisms. Owing to its phenolic nucleus and prolonged side chain, FA is easily formed by a resonance stabilized phenoxy radical that contributes to its free radical-scavenging impacts. This allows FA to preserve the DNA and lipids via ROS against oxidation [28].

Our in vivo findings are line with prior in vitro reports indicating that the decline in protein expression of pro-apoptotic protein could be a cause for gallic acid's anti-apoptotic activity [52]. Previous studies have suggested that FA by its antioxidant activity is capable of neutralizing nitric oxide and hydroxy-radical groups which also cause DNA damage [53].

Conclusion

Results demonstrated that ferulic acid or gallic acid or their combination significantly recovered the effect of Amiodarone- induced pulmonary toxicity in rats probably throughout their anti-inflammatory and antioxidant properties, as evidenced by efficient modulation of the severity and degree of lung injury besides the histopathological and genetic lessening of the alterations caused by Amiodarone.

References

1. Kishida, K., Matsumoto, H. Urinary excretion rate and bioavailability of chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid in non-fasted rats maintained under physiological conditions. Heliyon, 2019. 5(10): e02708.
2. Elkady, A., Tawfik, s. Anti-inflammatory role of piperine against rat lung tissue damage induced by gamma-rays. International Journal of Radiation Research, 2018. 16(1): 75-84.
3. Eroglu C, Soyuer S, Saraymen R, Orhan O, Kaplan B. Protective effects of erdosteine against radiation-induced lung injury in rats. INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL MEDICINE. 2018 Jan 1;11(10):11155-60.
4. Zarandona I, Puertas AI, Dueñas MT, Guerrero P, de la Caba K. Assessment of active chitosan films incorporated with gallic acid. Food Hydrocolloids. 2020 Apr 1;101:105486.
5. Akbari G, Dianat M, Badavi M. Effect of gallic acid on electrophysiological properties and ventricular arrhythmia following chemical-induced arrhythmia in rat. Iranian Journal of Basic Medical Sciences. 2020 Feb;23(2):167.
6. Wang H, Sun X, Zhang N, Ji Z, Ma Z, Fu Q, Qu R, Ma S. Ferulic acid attenuates diabetes-induced cognitive impairment in rats via regulation of PTP1B and insulin signaling pathway. Physiology & behavior. 2017 Dec 1;182:93-100.
7. Hou T, Zhang L, Yang X. Ferulic acid, a natural polyphenol, protects against osteoporosis by activating SIRT1 and NF-κB in neonatal rats with glucocorticoid-induced osteoporosis. Biomedicine & Pharmacotherapy. 2019 Dec 1;120:109205.
8. Song Y, Wu T, Yang Q, Chen X, Wang M, Wang Y, Peng X, Ou S. Ferulic acid alleviates the symptoms of diabetes in obese rats. Journal of Functional Foods. 2014 Jul 1;9:141-7.
9. Moroi MK, Ruzieh M, Aboujamous NM, Ghahramani M, Naccarelli GV, Mandrola J, Foy AJ. Dataset for amiodarone adverse events compared to placebo using data from randomized controlled trials. Data in brief. 2020 Feb 1;28:104835.
10. van Erven L, Schalij MJ. Amiodarone: an effective antiarrhythmic drug with unusual side effects. Heart. 2010 Oct 1;96(19):1593-600.
11. Abdul-Hamid M, Galaly SR, Mahmoud H, Mostafa F. The protective effect of grape seed and Ginkgo biloba against hepatotoxicity induced by the antidysrhythmic drug “amiodarone” in male albino rats. Beni-Suef University Journal of Basic and Applied Sciences. 2018 Jun 1;7(2):223-30.
12. Gawad FA, Rizk AA, Jouakim MF, Abd El Halem MZ. Amiodarone-induced lung toxicity and the protective role of Vitamin E in adult male albino rat. Eur. j. anat. 2018 Jul 1;22(4):323-33.
13. Dhaliwal J, Dhaliwal N, Akhtar A, Kuhad A, Chopra K. Beneficial effects of ferulic acid alone and in combination with insulin in streptozotocin induced diabetic neuropathy in Sprague Dawley rats. Life Sciences. 2020 May 28:117856.
14. Sen S, Asokkumar K, Umamaheswari M, Sivashanmugam AT, Subhadradevi V. Antiulcerogenic effect of gallic acid in rats and its effect on oxidant and antioxidant parameters in stomach tissue. Indian journal of pharmaceutical sciences. 2013 Mar;75(2):149.
15. de Souza MF, Gonçales TA, Steinmetz A, Moura DJ, Saffi J, Gomez R, Barros HM. Cocaine induces DNA damage in distinct brain areas of female rats under different hormonal conditions. Clinical and Experimental Pharmacology and Physiology. 2014 Apr;41(4):265-9.
16. Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH. The acute pulmonary and thrombotic effects of cerium oxide nanoparticles after intratracheal instillation in mice. International journal of nanomedicine. 2017;12:2913.
17. Doi R, Tsuchiya T, Mitsutake N, Nishimura S, Matsuu-Matsuyama M, Nakazawa Y, Ogi T, Akita S, Yukawa H, Baba Y, Yamasaki N. Transplantation of bioengineered rat lungs recellularized with endothelial and adipose-derived stromal cells. Scientific reports. 2017 Aug 16;7(1):1-5.
18. Clausen MV, Hilbers F, Poulsen H. The structure and function of the Na, K-ATPase isoforms in health and disease. Frontiers in physiology. 2017 Jun 6;8:371.
19. Yan Y, Shapiro JI. The physiological and clinical importance of sodium potassium ATPase in cardiovascular diseases. Current opinion in pharmacology. 2016 Apr 1;27:43-9.
20. Schwaiblmair M, Berghaus T, Haeckel T, Wagner T, von Scheidt W. Amiodarone-induced pulmonary toxicity: an under-recognized and severe adverse effect?. Clinical Research in Cardiology. 2010 Nov 1;99(11):693-700.
21. Gusarova GA, Trejo HE, Dada LA, Briva A, Welch LC, Hamanaka RB, Mutlu GM, Chandel NS, Prakriya M, Sznajder JI. Hypoxia leads to Na, K-ATPase downregulation via Ca2+ release-activated Ca2+

channels and AMPK activation. Molecular and cellular biology. 2011 Sep 1;31(17):3546-56.
22. Aggarwal D, Sharma M, Singla SK. The role of natural antioxidants as potential therapeutic agent in nephrolithiasis. Asian J Pharm Clin Res. 2013;6(3):48-53.
23. Fathy AH, Bashandy MA, Bashandy SA, Mansour AM, Azab KS. The beneficial effect of natural antioxidants from olive oil with fig and date palm fruit extracts on biochemical and hematological parameters in rats treated with doxorubicin and γ-radiation. FACETS. 2018 Jul 9;3(1):722-35.
24. Gazia MM, El-Kordy EA. Role of activated protein C and soya-bean in experimental lung toxicity in adult male albino rat: histological and Immunohistochemical studies. Life Sci J. 2012;9:73-85.
25. Ashry OM, Hasan HF. The Evolving Role of Erythropoietin in Lung of Irradiated Rats. Brazilian Archives of Biology and Technology. 2017;60.
26. Andrades M, Ritter C, de Oliveira MR, Streck EL, Moreira JC, Dal-Pizzol F. Antioxidant treatment reverses organ failure in rat model of sepsis: role of antioxidant enzymes imbalance, neutrophil infiltration, and oxidative stress. Journal of surgical research. 2011 May 15;167(2):e307-13.
27. Ramar M, Manikandan B, Raman T, Priyadarsini A, Palanisamy S, Velayudam M, Munusamy A, Prabhu NM, Vaseeharan B. Protective effect of ferulic acid and resveratrol against alloxan-induced diabetes in mice. European Journal of Pharmacology. 2012 Sep 5;690(1-3):226-35.
28. Trombino S, Cassano R, Ferrarelli T, Barone E, Picci N, Mancuso C. Trans-ferulic acid-based solid lipid nanoparticles and their antioxidant effect in rat brain microsomes. Colloids and Surfaces B: Biointerfaces. 2013 Sep 1;109:273-9.
29. El-Lakkany NM, El-Maadawy WH, el-Din SH, Saleh S, Safar MM, Ezzat SM, Mohamed SH, Botros SS, Demerdash Z, Hammam OA. Antifibrotic effects of gallic acid on hepatic stellate cells: In vitro and in vivo mechanistic study. Journal of Traditional and Complementary Medicine. 2019 Jan 1;9(1):45-53.
30. Nayeem N, Asdaq SM, Salem H, AHEl-Alfqy S. Gallic acid: a promising lead molecule for drug development. Journal of Applied Pharmacy. 2016;8(2):1-4.
31. Wang J, Tang L, White J, Fang J. Inhibitory effect of gallic acid on CCl 4-mediated liver fibrosis in mice. Cell biochemistry and biophysics. 2014 May 1;69(1):21-6.
32. Ola-Davies OE, Olukole SG. Gallic acid protects against bisphenol A-induced alterations in the cardio-renal system of Wistar rats through the antioxidant defense mechanism. Biomedicine & Pharmacotherapy. 2018 Nov 1;107:1786-94.
33. Omobowale TO, Oyagbemi AA, Ajufo UE, Adejumobi OA, Ola-Davies OE, Adedapo AA, Yakubu MA. Ameliorative effect of gallic acid in doxorubicin-induced hepatotoxicity in Wistar rats through antioxidant defense system. Journal of dietary supplements. 2018 Mar 4;15(2):183-96.
34. Cheng Q, Li C, Yang CF, Zhong YJ, Wu D, Shi L, Chen L, Li YW, Li L. Methyl ferulic acid attenuates liver fibrosis and hepatic stellate cell activation through the TGF-β1/Smad and NOX4/ROS pathways. Chemico-biological interactions. 2019 Feb 1;299:131-9.
35. Li B, Weng Q, Liu Z, Shen M, Zhang J, Wu W, Liu H. Selection of antioxidants against ovarian oxidative stress in mouse model. Journal of biochemical and molecular toxicology. 2017 Dec;31(12):e21997.
36. Nikbakht J, Hemmati AA, Arzi A, Mansouri MT, Rezaie A, Ghafourian M. Protective effect of gallic acid against bleomycin-induced pulmonary fibrosis in rats. Pharmacological Reports. 2015 Dec 1;67(6):1061-7.
37. Vellai RD, Chandiran S, Pillai SS. GTF-231, a mixture of gymnemic acid, trigonelline and ferulic acid significantly ameliorates oxidative stress in experimental type 2 diabetes in rats. Canadian journal of diabetes. 2018 Jun 1;42(3):237-44.
38. Al-Shammari B, Khalifa M, Bakheet SA, Yasser M. A mechanistic study on the amiodarone-induced pulmonary toxicity. Oxidative Medicine and Cellular Longevity. 2016 Jan 1;2016.
39. Salem AM, Mohammaden TF, Ali MA, Mohamed EA, Hasan HF. Ellagic and ferulic acids alleviate gamma radiation and aluminium chloride-induced oxidative damage. Life sciences. 2016 Sep 1;160:2-11.
40. Zhang S, Wang P, Zhao P, Wang D, Zhang Y, Wang J, Chen L, Guo W, Gao H, Jiao Y. Pretreatment of ferulic acid attenuates inflammation and oxidative stress in a rat model of lipopolysaccharide-induced acute respiratory distress syndrome. International journal of immunopathology and pharmacology. 2018 Jan 16;31:0394632017750518.
41. Sa Sadar SS, Vyawahare NS, Bodhankar SL. Ferulic acid ameliorates TNBS-induced ulcerative colitis through modulation of cytokines, oxidative stress, iNOs, COX-2, and apoptosis in laboratory rats. EXCLI journal. 2016;15:482.
42. Gerin F, Erman H, Erboga M, Sener U, Yilmaz A, Seyhan H, Gurel A. The effects of ferulic acid against oxidative stress and inflammation in formaldehyde-induced hepatotoxicity. Inflammation. 2016 Aug 1;39(4):1377-86.
43. Mojiri-Forushani H, Hemmati AA, Dehghani MA, Malayeri AR, Pour HH. Effects of herbal extracts and compounds and pharmacological agents on pulmonary fibrosis in animal models: a review. Journal of Integrative Medicine. 2017 Nov 1;15(6):433-41.
44. Kuppan G, Balasubramanyam J, Monickaraj F, Srinivasan G, Mohan V, Balasubramanyam M. Transcriptional regulation of cytokines and oxidative stress by gallic acid in human THP-1 monocytes. Cytokine. 2010 Feb 1;49(2):229-34.
45. Mirshekar MA, Sarkaki A, Farbood Y, Naseri MK, Badavi M, Mansouri MT, Haghparast A. Neuroprotective effects of gallic acid in a rat model of traumatic brain injury: behavioral, electrophysiological, and molecular studies. Iranian Journal of Basic Medical Sciences. 2018 Oct;21(10):1056.
46. Zickri MB, Fadl SG, Metwally HG. Comparative study between intravenous and intraperitoneal stem cell therapy in amiodarone induced lung injury in rat. International journal of stem cells. 2014 May;7(1):1.
47. Sarg NA, Kamal KM. Protection against lung toxicity induced by Amiodarone in Albino rats by fish oil. Journal of American Science. 2019;15(12).
48. Mahavadi P, Henneke I, Ruppert C, Knudsen L, Venkatesan S, Liebisch G, Chambers RC, Ochs M, Schmitz G, Vancheri C, Seeger W. Altered surfactant homeostasis and alveolar epithelial cell stress in amiodarone-induced lung fibrosis. Toxicological sciences. 2014 Nov 1;142(1):285-97.
49. Hosseini S, Imenshahidi M, Hosseinzadeh H, Karimi G. Effects of plant extracts and bioactive compounds on attenuation of bleomycin-induced pulmonary fibrosis. Biomedicine & Pharmacotherapy. 2018 Nov 1;107:1454-65.
50. da Silva CR, de Andrade Neto JB, Sidrim JJ, Ângelo MR, Magalhães HI, Cavalcanti BC, Brilhante RS, Macedo DS, de Moraes MO, Lobo MD, Grangeiro TB. Synergistic effects of amiodarone and fluconazole on Candida tropicalis resistant to fluconazole. Antimicrobial agents and chemotherapy. 2013 Apr 1;57(4):1691-700.
51. Sudheer AR, Muthukumaran S, Devipriya N, Devaraj H, Menon VP. Influence of ferulic acid on nicotine-induced lipid peroxidation, DNA damage and inflammation in experimental rats as compared to N-acetylcysteine. Toxicology. 2008 Jan 20;243(3):317-29.
52. Erol-Dayi Ö, Arda N, Erdem G. Protective effects of olive oil phenolics and gallic acid on hydrogen peroxide-induced apoptosis. European journal of nutrition. 2012 Dec 1;51(8):955-60.
53. Bami E, Ozakpınar OB, Ozdemir-Kumral ZN, Köroglu K, Ercan F, Cirakli Z, Sekerler T, Izzettin FV, Sancar M, Okuyan B. Protective effect of ferulic acid on cisplatin induced nephrotoxicity in rats. Environmental Toxicology and Pharmacology. 2017 Sep 1;54:105-11.