Elettaria cardamomum extract counteracts cardio-hematological and immunological alterations of Doxorubicin-induced cardiotoxicity in rats

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Background and objective
As *Elettaria cardamomum* possesses wide phytochemical and biological activities; therefore, this study aimed to evaluate the ameliorating efficiency of *Elettaria cardamomum* ethanolic extract (ECEE) in Doxorubicin-induced cardiotoxicity.
Materials and methods
Male rats (150–180 g) were arranged into four groups (8 rats/group) as: (1) normal rats act as control, (2) normal rats daily ingested with ECEE (100 mg/kg, dissolved in water) four 28 days, (3) rats cardio-intoxicated intraperitoneally with Doxorubicin (DOX) at a dose 2.5 mg/kg on alternate days for 14 days and (4) cardio-intoxicated rats treated orally with ECEE for 28 days.
Results and conclusion
After 28 days of treatment, the results revealed that ECEE restored the cardiological, hematological, and immunological deteriorations induced by Doxorubicin; this was evidenced by the significant reduction of serum aminotransaminases (ALAT and ASAT), lactate dehydrogenase, creatin kinase MB, total cholesterol, triglycerides, tumor necrosis factor-alpha, interleukin-1β, caspase-3 and nuclear factor kappa-light-chain-enhancer of activated B cells as well as cardiac malondialdehyde, nitric oxide levels and cardiac DNA-fragmentation coupled with marked improvement in cardiac reduced glutathione, superoxide dismutase and glutathione peroxidase. Hematological indices and hemoglobin derivatives were markedly restored. Moreover, the ECEE induced prominent cardio-histological regeneration. In conclusion, ECEE possessed anti-cardio-hematotoxicities exhibition that could be performed through the radical scavenging and antioxidant characteristics of its active constituent's especially high phenolic content, reflecting the promising potency of ECEE as cardio protective supplement.

Keywords:
cardiotoxicity, Doxorubicin, *Elettaria cardamomum*, immunomodulatory, rat

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Introduction

The WHO reports that cancer and cardiovascular disease (CVD) account for most deaths globally, accounting for 17.9 million and around 10 million deaths annually, respectively [1,2]. The prognosis for cancer has greatly improved over the past few decades because of advancements in treatment and early identification [3]. Doxorubicin (DOX), an anthracycline medication, is a well-known treatment for lymphoma, sarcoma, leukemia, and breast cancer. It is an extremely successful cancer medication. However, because of its dose-dependent cardiotoxicity, which is manifested as a fall in left ventricle ejection fraction (LVEF), weaker heart muscle, and enlarged left ventricle (LV), clinical use is restricted [4]. About 40% of cancer patients treated with anthracyclines have mild, moderate, or severe cardiotoxicity, and 11% of breast cancer patients die from cardiovascular disease (CVD) [5,6]. Multiple factors contribute to the pathophysiology of DOX-induced cardiotoxicity, including mitochondrial damage in cardiomyocytes, oxidative stress caused by a disrupted redox balance, and remodeling of fibrotic and inflammatory tissues [7]. An increasing amount of data suggests that DOX also interferes with mitochondrial and cellular iron metabolism, resulting in iron overload in cardiomyocytes and iron-dependent cell death known as ferroptosis [8,9]. Furthermore, DOX inhibits DNA topoisomerase II beta (TOP2β) activity, which prevents DNA transcription and replication and leads to double-stranded breaks in DNA and cardiomyocyte mortality [10].

In addition to causing severe inflammation in the heart, DOX treatment can cause severe inflammation in the liver, gut, kidney, and blood vessels. Pro-inflammatory cytokines like TNFα, interleukin 1 beta (IL-1β), and Il-6 are strongly increased when DOX is administered; however, preventing this impact can significantly reduce the tissue damage it causes [11,12].

Globally, medicinal plants are extremely important to health. Antibacterial, antifungal, antiviral, anti-helminthic, anti-allergic, anticarcinogenic, and larvicidal are only a few of their numerous properties [13]. The existence of chemicals with therapeutic activity, including tannins, oils, and gums, as well as our growing understanding of the chemical makeup of plants, are responsible for their medicinal qualities. Scientists studying natural products, microbiologists, botanists, and pharmacologists are searching the planet for phytochemicals that may one day be used to treat a wide range of illnesses. Numerous contemporary medications are derived from plants [14].

Fruit that has been dried from the perennial herbaceous *Zingiberaceae* plant is called *E. Cardamomum* [15]. The most expensive spice is cardamom, followed by saffron and vanilla. It smells and tastes remarkably rich [16]. It is also known as 'real cardamom' and 'green cardamom.' [17]. Two varieties of cardamom are available: small-green and large-black. The biological source of little green cardamom is *E. Cardamomum*, the most common species of cardamom. At the same time, India, the world's largest cardamom grower, is where black cardamom is mainly farmed [18]. Worldwide, high latitudes with humid conditions are ideal for growing *E. cardamomum* plants. Still, the evergreen rainforests of Sri Lanka and southern India (Kerala, Karnataka, and Tamil Nadu) are home to numerous of this plant's ancestors. Kerala is the largest producer of cardamom in India, accounting for 70% of the total production. Karnataka (20%) and Tamil Nadu (5%) come next [19].

E. cardamomum has demonstrated therapeutic advantages about lipid homeostasis and glycemic management [20]. Alkaloids, anthocyanins, flavonoids, phenolic acids, monoterpene contents including 1, 8-cineole, α-pinene, α-terpineol, linalool, and nerolidol, as well as ester constituents like α-terpinyl acetate, are the main ingredients of *E. cardamomum*, a functional food. *E. cardamomum* contains phytochemicals that support its anti-inflammatory, antiviral, antifungal, antioxidant, chemotherapeutic, and anti-ulcer properties [16].

The purpose of this work is to examine the potential protective effects of *Elettaria cardamomum* ethanolic extract (ECEE) against immunological and cardio-hematological changes brought on by DOX.

Materials and methods

Chemicals and plant seeds

DOX (Doxorubicin hydrochloride) vials (10 mg/5 ml) were purchased from Pfizer, Cairo, Egypt. The seeds of Cardamom (*E. cardmomum*) were obtained from a local supplier for medicinal plants and herbal products, Cairo, Egypt; then the plant was authenticated and found belonging to *Zingiberaceae* family and carrying a taxonomic ID number 105181.

Seed ethanolic extraction

The dry seeds of Cardamom were left at 60°C for two hours before electrically grinded; then the ethanolic (70%) extract of the seeds powder was carried out as described by Gomaa *et al.* [14]. The powder was immersed in ethanol 70% (1 : 10 w/v) with stirring; after two days, the mixture was filtered; then the solvent was evaporated, and the moisture was removed. The solid extract was preserved at −20°C till the biological application.

In vitro assessment

The extract yield was calculated as previously stated [21]. Total phenolic content of the ECEE was determined as catechin equivalent using Folin-Ciocalteu reagent The radical scavenging activity (RSA) or triplicates was determined as previously described [22]; however, the percentage of RSA was calculated using the equation $[\text{RSA}\ (\%) = (\frac{A_{\text{control sample}} - A_{\text{sample extract}}}{A_{\text{control sample}}})]$; reducing power (RP) was tested method described by Sethiya *et al.* [23], however, the RP was calculated as equivalent to ascorbic acid.

Experimental design

Adult male Wistar rats (150–180 g) were purchased from Animal Colony, National Research Centre, Egypt. The rats received human care in compliance with the standard institutional criteria for the care and use of experimental animals, where the study proposal was approved by the ethical committee of Faculty of Science, Al-Azhar University, Assiut (approval number AZHAR 14/2023).

Study animals' groups

One week post acclimatization, the rats were divided into four groups (8 rats each); (1) control group

included normal rats received water; (2) normal rats daily received 100 mg/kg of ECEE dissolved in water [24] for 28 days; (3) rats cardio-intoxicated intraperitoneally with DOX at a dose 2.5 mg/kg each 2 days for 14 days [25]; (4) DOX-cardio-intoxicated rats treated orally with same dose of ECEE for 28 days.

Blood and tissue sampling

Post-treatment period, all rats were fasted and weighed, and then specimens of blood were collected. Each blood specimen was separated into two portions; the first was heparinized for hematological measurements, while the second for sera separation where the sera were divided into aliquots and kept at −80°C for biochemical measurements. Immediately post blood-collection, the animals were sacrificed, and the hearts of all rats were dissected out: five hearts of each group rolled in a piece of aluminum foil and preserved at −80°C for assessment of oxidative stress markers and DNA fragmentation; the other three hearts (of each group) were immersed in formaldehyde-saline (10% v/v) buffer for histopathological examination.

Hematological measurements

Cell blood counter (Japan) was used for measuring of red blood corpuscles (RBCs) count, hemoglobin (Hb) concentration. Hb derivatives were measured as percentage of the total Hb concentration; methemoglobin (met-Hb) was determined as described by Evelyn and Malloy [26]; sulf-Hb (Hb-S) was determined as described by Van Kampen and Zulstra [27]; while carboxy-Hb (Hb-CO) was evaluated using the method of Van Assendelft [28]. The percentage of oxy-Hb ($Hb\text{-}O_2$) was calculated from the formula $Hb\text{-}O_2$ (%)= 100−[met-Hb + S-Hb + Hb-Co].

Biochemical and immunological measurements

The activity of serum ASAT and ALAT was assayed using kits of Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany; total cholesterol and triglycerides levels were tested using kits purchased of DiaSys Diagnostic Germany; tumor necrosis factor-alpha (TNF-α), and caspase-3 levels were measured using rat ELISA-kits of SinoGeneClon, China. Serum levels of interleukin 1 beta (IL-1β) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) were measured using rats' ELISA-kit of CUSABIO, USA. Serum activity of lactate dehydrogenase (LDH) and creatine kinase MB (CK-MB) was determined kinetically using a reagent kit purchased from ATLAS MEDICAL, Cambridge, UK.

Assessment of oxidative stress markers

In ice-cold phosphate buffer (50 mM, pH 7.4), heart tissue was homogenized to give 10% homogenate (w/v); after centrifugation, the supernatant was subjected to determination of glutathione (GSH) and NO, superoxide dismutase (SOD), and glutathione peroxidase (GPx) using kits of Biodiagnostic, Egypt. Cardiac malondialdehyde (MDA) was evaluated according to Ruiz-Larnea *et al.* [29].

Cardiac DNA fragmentation percentage

The degree of DNA fragmentation (%) was determined as explained previously [30].

Histopathological examination and scoring

Samples of the heart from all animals were fixed in isotonic formalin (10%), washed in running water, dehydrated with ethanol, subjected to clearing with xylene, and then embedded in paraffin. Sections (5 μm thickness) were then stained with hematoxylin and eosin (H and E) for the histological examination [31].

The scoring method used to evaluate the cardiac pathology in this study involved the categorization of changes in degeneration, inflammation, and hemorrhage across several fields within each group was carried out with the validated histopathological scoring principles advocated by Gibson-Corley *et al.* [32].

Statistical analysis

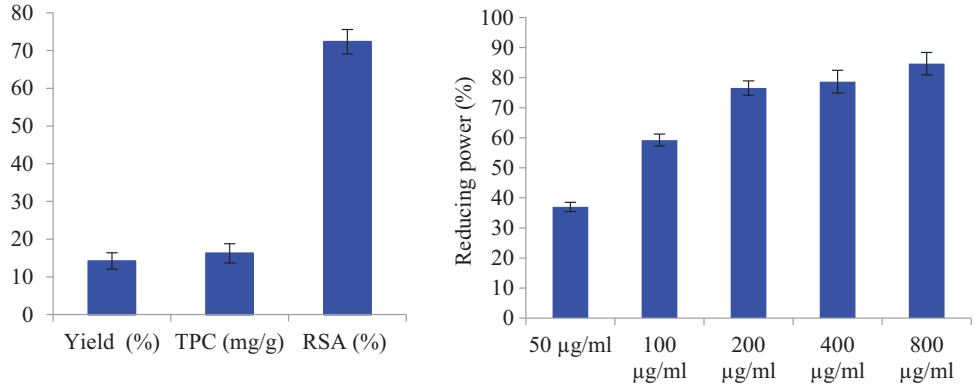
One-way analysis of variance followed by Duncan post hoc test at levels of P less than or equal to 0.05 was used to analyze the obtained results using a statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results

The *in vitro* results showed that ECEE has a considerable number of phenolic compounds and exhibited higher RSA and RP (Fig. 1).

Intoxication of animals with DOX resulted in detectable hematological disorders which monitored from the drop in RBCs count (−12.5) and Hb concentration (−49.7%). Regarding the Hb-derivatives, the nonfunctional forms (met-Hb and Co-Hb) were significantly elevated, while the functional derivative (oxy-Hb) was reduced significantly; however, S-Hb was not affected. Favorably, Treatment of DOX-intoxicated rats with

Figure 1



In vitro results (Yield, total phenolic compounds (TPC), radical scavenging activity (RSA), and reducing power (RP) of the *Elettaria cardamomum* ethanolic extract (ECEE).

ECEE markedly improved these hematological measurements (Table 1).

DOX-intoxication resulted in marked elevations in serum LDH, CK-MB, ALAT, and ASAT activities as well as cholesterol and triglycerides levels; however, administration of healthy rats with ECEE did not disturb these measurements in compared with the corresponding values of normal control. Post-treatment of DOX-intoxicated rats with ECEE led to a marked restoration in the abovementioned biochemical markers (Table 2).

Similarly, the current study revealed that DOX-intoxication led to significant upregulation in the level of serum level of immunological markers (TNF-α, IL-1β, and NF-κB) and apoptotic one (Caspase-3); while administration of healthy rats with ECEE neither deteriorate the level of inflammatory nor apoptotic markers in compared with the those of normal control. Favorably, treatment of DOX-intoxicated rats with ECEE recorded detectable downregulation in levels of the mentioned immunological markers (Fig. 2).

Regarding the oxidative stress markers of cardiac tissue (Table 3), the results recorded that DOX induced a detectable increase in the oxidative stress that monitored from the clear rise in the cardiac level of MDA and NO associated with the sharp drop in the

Table 1 Hematological parameters and hemoglobin derivatives of normal, doxorubicin, and doxorubicin ~ *Elettaria cardamomum* ethanolic extract treated rats

	Control	ECEE	DOX	DOX~ECEE
RBCs (10^6/ccm)	7.2 ± 0.22^{A}	7.3 ± 0.24^{A}	6.3 ± 0.17^{B}	6.8 ± 0.23^{B}
Hb (g/dl)	17.3 ± 0.91^{A}	17.6 ± 0.93^{A}	8.7 ± 0.62^{C}	13.9 ± 0.8^{B}
O_2-Hb (%)	98.1 ± 0.95^{A}	98.16 ± 0.88^{A}	80.5 ± 1.22^{C}	91.3 ± 1.18^{B}
Met-Hb (%)	0.84 ± 0.19^{C}	0.78 ± 0.16^{C}	10.40 ± 0.63^{A}	5.2 ± 0.37^{B}
Co-Hb (%)	0.58 ± 0.11^{C}	0.57 ± 0.13^{C}	8.61 ± 0.44^{A}	3.2 ± 0.04^{B}
S-Hb (%)	0.48 ± 0.09^{A}	0.49 ± 0.11^{A}	0.49 ± 0.16^{A}	0.5 ± 0.0^{A}

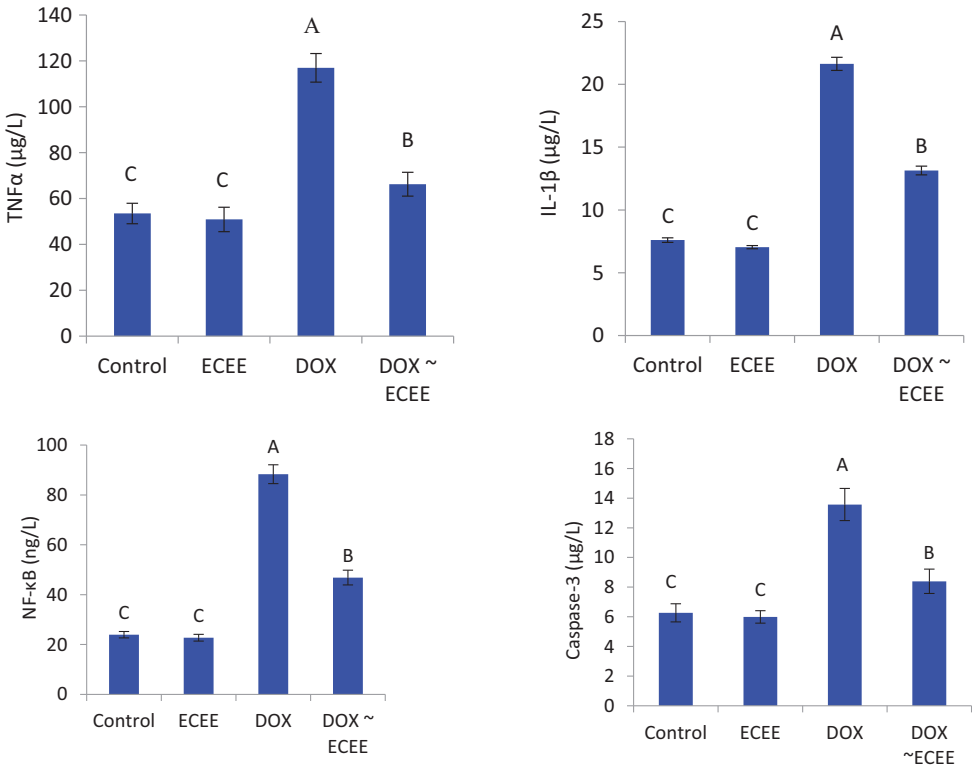
Data are presented as mean±standard error; within the same raw, means with superscript different letters are significantly different at *P* less than or equal to 0.05.

Table 2 Serum biochemical markers of normal, doxorubicin, and doxorubicin ~ *Elettaria cardamomum* ethanolic extract treated rats

	Control	ECEE	DOX	DOX~ECEE
ALAT (U/l)	89.5 ± 1.38^{C}	86.2 ± 1.11^{C}	187.3 ± 3.1^{A}	98.5 ± 1.88^{B}
ASAT (U/l)	112.4 ± 2.18^{C}	107.3 ± 2.06^{C}	255.2 ± 3.7^{A}	123.5 ± 2.01^{B}
Cholesterol (mg/dl)	113.5 ± 3.55^{C}	105.5 ± 2.44^{C}	243.1 ± 4.1^{A}	133.4 ± 2.37^{B}
Triglycerides (mg/dl)	67.7 ± 1.91^{C}	64.6 ± 1.77^{C}	174.7 ± 2.55^{A}	87.6 ± 2.31^{B}
CK-MB (U/l)	13.8 ± 0.95^{C}	13.2 ± 0.83^{C}	22.7 ± 1.01^{A}	16.2 ± 1.0^{B}
LDH (U/l)	7.43 ± 0.33^{C}	7.12 ± 0.27^{C}	20.8 ± 0.95^{A}	10.7 ± 0.71^{B}

Data are presented as mean±standard error; within the same raw, means with superscript different letters are significantly different at *P* less than or equal to 0.05.

Figure 2



Serum immunological markers of normal, doxorubicin, and doxorubicin ~ *Elettaria cardamomum* ethanolic extract treated rats.

Table 3 Cardiac oxidative stress markers of normal, doxorubicin, and doxorubicin ~ *Elettaria cardamomum* ethanolic extract treated rats

	Control	ECEE	DOX	DOX~ECEE
MDA (nmol/g tissue)	72.4 ± 1.87^{C}	69.3 ± 1.77^{C}	214 ± 4.12^{A}	97.9 ± 1.87^{B}
NO (µmol/g tissue)	265.7 ± 6.66^{C}	268.5 ± 5.22^{C}	465.3 ± 4.22^{A}	315.5 ± 2.1^{B}
GSH (mg/g tissue)	5.87 ± 0.21^{A}	6.08 ± 0.25^{A}	2.62 ± 0.13^{C}	4.55 ± 0.19^{B}
GPx (U/g tissue)	7228 ± 168^{A}	7333 ± 175^{A}	3654 ± 95^{C}	6666 ± 132^{B}
SOD (U/g tissue)	6682 ± 107^{A}	6729 ± 111^{A}	3111 ± 78^{C}	5442 ± 113^{B}

Data are presented as mean±standard error; within the same raw, means with superscript different letters are significantly different at *P* less than or equal to 0.05.

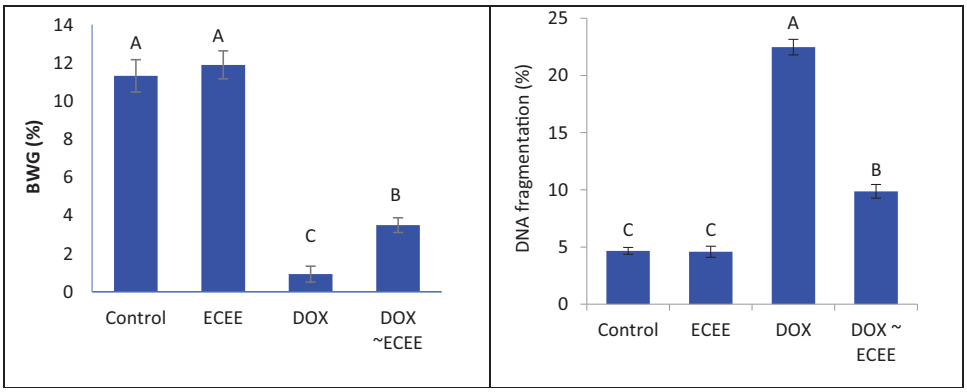
values of GSH, GPx, and SOD in compared with the values of normal control group; however, healthy rats ingested with ECEE showed values close to those control group. In a promising manner, treatment of DOX-intoxicated rats with ECEE showed antioxidant performance that was achieved from the clear reduction in MDA and NO levels coupled with a detectable increase in the level of GSH and activities of GPx and SOD in compared with the matched values of DOX group (Table 3).

Moreover, DOX-intoxicated rats showed a significant decrease in the percentage of body weight gain matched with an increase in the percentage of DNA-fragmentation; while ECEE-administration to healthy rats did not influence both in compared with normal control group. Favorably, treatment of DOX-intoxicated rats with ECEE performed a notable body weight gain increase, and DNA-fragmentation decreased (Fig. 3).

Histopathological findings

The morphological assessment of hearts across various experimental groups revealed a spectrum of alterations, ranging from absence of injury (Control and ECEE groups) to mild lesions (DOX+ECEE group) to severe damage (DOX). The control group showed normal histological architecture characterized by branched striated cardiac myocytes with acidophilic cytoplasm and centrally located, vesicular, and oval nuclei; similarly, ECEE group exhibited intact and densely packed striated myocardial fibers, indicating

Figure 3



Shows the percentage of body weight gain and DNA fragmentation of normal, doxorubicin, and doxorubicin ~ *Elettaria cardamomum* ethanolic extract treated rats' groups. means with superscript different letters are significantly different at *P* less than or equal to 0.05.

preservation of normal myocardial architecture. In contrast, DOX-intoxicated group displayed diverse myocardial alterations, including cytoplasmic degeneration, distorted striations of cardiac muscle, irregular spacing within interstitial regions, focal necrosis in small clusters of myocardial fibers, focal cellular infiltration, noticeable expansion in interstitial spaces, and extra-vasated RBCs, suggesting cardiotoxicity. Favorably, posttreatment of DOX intoxicated rats with ECEE predominantly displayed nearly normal myocardial architecture with mild degenerative alterations, including intracellular edema and mild cytoplasmic vacuolization, without any inflammatory response compared with control groups, implying a discernible ameliorative effect of the toxin (Fig. 4a–f).

Cardiac pathology scoring

Depending on the severity of the degenerative changes observed, the scoring analysis across the study groups revealed varying degrees of degeneration, inflammation, and hemorrhage. Control and ECEE groups exhibited detectable signs of inflammation or hemorrhage, while the doxorubicin group displayed severe degeneration alongside varying levels of inflammation and hemorrhage. Interestingly, treatment of doxorubicin-intoxicated rats with ECEE resulted in a moderate degeneration with mild inflammation and hemorrhage (Table 4).

Table 4 Shows the scoring analysis across the study groups

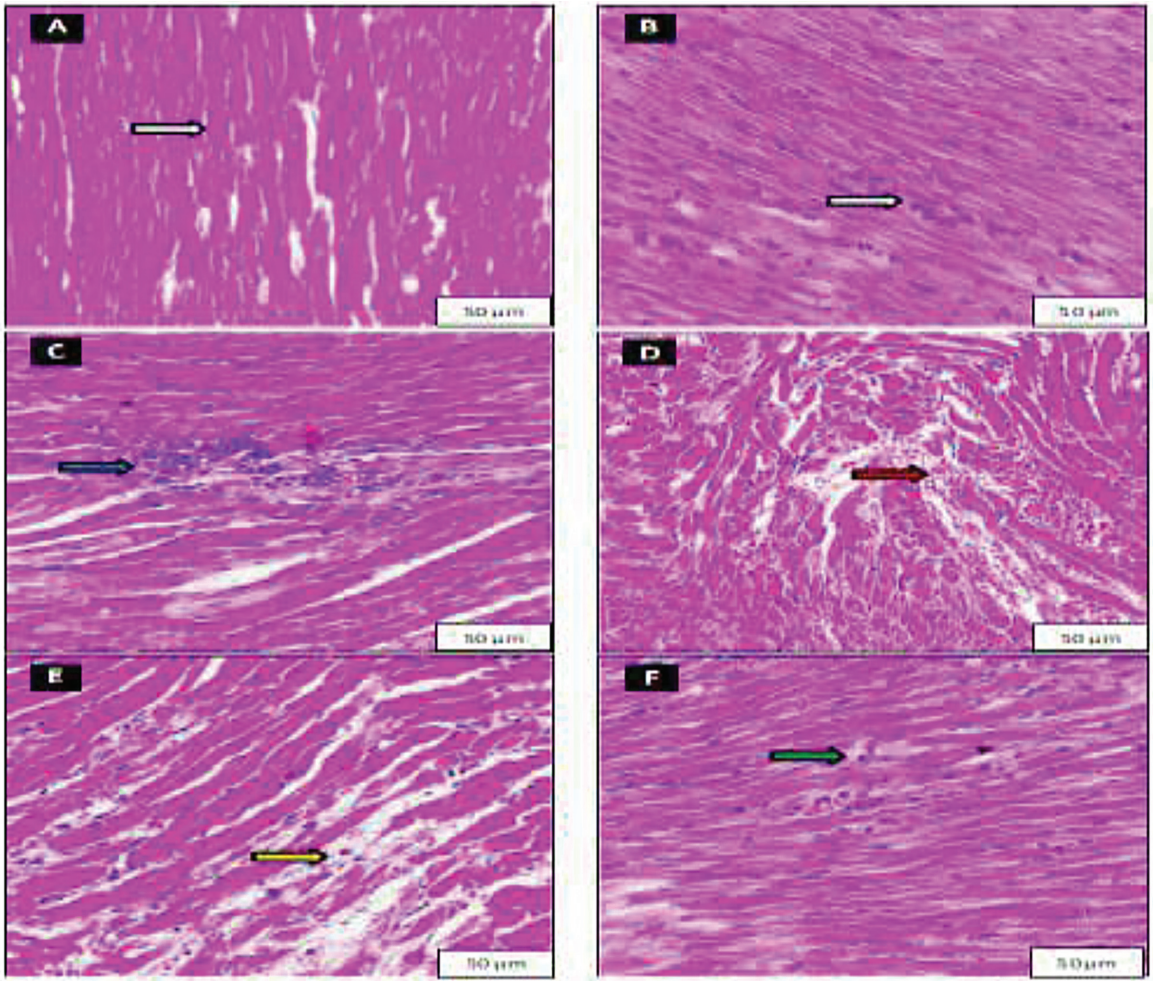
Group	Observation	Positive	Negative
Control	Degeneration	1	9
	Inflammation	0	10
	Hemorrhage	0	10
ECEE	Degeneration	3	7
	Inflammation	0	10
	Hemorrhage	0	10
DOX	Degeneration	8	2
	Inflammation	5	5
	Hemorrhage	5	5
DOX+ECEE	Degeneration	3	7
	Inflammation	2	8

Discussion

DOX is a double-edged sword used in the treatment of different types of cancer, mostly due to its propensity to cause cardiotoxicity as a side effect, which is contingent upon the dosage administered. The cytotoxic effects of DOX result in myocyte injury, ultimately contributing to the development of cardiomyopathy [33]. Studies show that several factors, such as the total dosage of DOX, the dosing regimen, and the patient's age, affect the likelihood of DOX-induced cardiotoxicity [34]. The cardiotoxicity generated by DOX might manifest as acute, subacute, or chronic. Acute cardiotoxicity generated by DOX typically manifests during a period of 2–3 days following DOX injection, with an estimated incidence rate of roughly 11%.

DOX intoxication was associated with a notable decrease in RBCs count, and total hemoglobin concentrations. Also, marked reduction was noticed in the level of the functional hemoglobin derivative ($Hb\text{-}O_2$) associated with a big rise in the nonfunctional hemoglobin derivatives (met-Hb, S-Hb, and Co-Hb), suggesting that DOX-induced oxidative stress on red blood cells that resulted in tissue hypoxia due to the resultant anemia; either physiological (reduction in RBCs and total Hb), or functional (decreased functional $Hb\text{-}O_2$ and increased non-functional met-Hb, S-Hb, and Co-Hb). Reactive oxygen species (ROS), such as the hydroxyl radical,

Figure 4



Illustrates histopathological findings of the heart of the study animals' groups. Control group (A) shows normal cardiac histological architecture with branched striated cardiac myocytes (white arrows); ~ *Elettaria cardamomum* ethanolic extract group (B) displays an intact myocardial structure with densely packed fibers; doxorubicin,-intoxicated group (C–E) reveals severe myocardial damage characterized by cytoplasmic degeneration (blue arrow), distorted striations, and focal necrosis (yellow arrows), cell infiltration (blue arrows), and red extra-vasated res blood cells evident (red arrows); doxorubicin ~ *Elettaria cardamomum* ethanolic extract group (F) shows only mild degenerative changes (green arrow) (H and E ×200).

superoxide anion O^{-2}, and H_2O_2, are produced when free radicals hit microsomal lipids and cause them to peroxide. They can also attach to microsomal lipids and proteins covalently [35]. The erythrocytes are particularly vulnerable to damage from superoxide anion and H_2O_2 molecules due to their contact with ROS, as well as their lack of the ability to synthesize new enzyme molecules throughout maturation. These reactive compounds are implicated in thiol-group oxidation of enzymes [36], lipid peroxidation [37], and Hb oxidation, conformational and/or configurational change. After that, met-Hb accumulates and forms Heinz bodies by covalently binding to the inside membrane of erythrocytes. This distorts the cell membrane and increases hemolysis and erythrocyte fragility [38]. Additionally, DOX has a considerable impact on the amount and functionality of the Hb molecule, whereas the level of met-Hb, Sulf-Hb, and Co-Hb increased concurrently with a significant drop in total Hb and Oxy-Hb. Thus, these modifications could be the result of an increase in oxidative stress brought on by free radicals produced during the metabolic breakdown of DOX; when erythrocytes are exposed to oxidative stress, their concentration of Met-Hb rises significantly. Met-Hb is known to be unable to bind oxygen reversibly [39]. When Hb oxidizes to the ferric form from the ferrous porphyrin complex, Met-Hb is created [40]. The major mechanism that reduces met-Hb *in vivo* is the NADH cytochrome b5-Met-Hb reductase system, with a secondary pathway being the NADPH-dependent met-Hb reductase [41]. It was proposed that limiting the production of met-Hb might need a high concentration of NADPH. Lipid peroxidation and met-Hb production are thought to occur at higher rates when NADPH and GSH are

depleted [42]. In addition to altering the Hb molecule's shape, free radicals can also cause it to attract unfavorable ligands other than oxygen, like sulfur (S) and carbon monoxide (CO). Because ECEE was able to decrease the levels of met-Hb, S-Hb and Co-Hb as well as increase the contents of total Hb and Oxy-Hb, hemoglobin function was enhanced both quantitatively and qualitatively. These findings support ECEE's potential as an antioxidant even further. This could enhance liver function, which would enhance the production of antioxidant enzymes and the reduced GSH needed to protect erythrocytes.

Rats intoxicated with DOX showed marked increases in LDH, CK-MB, ASAT, and ALAT activities; these results are consistent with earlier studies suggesting that DOX-induced oxidative stress can result in lipid peroxidation, which is followed by the release of these enzymes into serum [43,44]. Despite this, rats treated with ECEE demonstrated a considerable recovery of CK-MB and LDH enzymes, indicating that ECEE stabilized myocyte membranes and reduced leakiness in cardiomyocytes due to its suppression of lipid peroxidation and membrane disruption.

In comparison to the control, injection with DOX caused an obvious increase in cardiac NO and MDA as well as a decrease in cardiac GSH, SOD, and GPx. This exhibition consists with those of Gomaa *et al.* [45] and Basal *et al.* [46]; these findings might be the consequence of oxidative damage in the heart, which led to the corresponding formation of MDA and decrease in GSH levels. The capacity of DOX to promote the activation of nitric oxide synthase (NOS) and, consequently, NO release in the heart, explains the rise in NO level [47]. Previous research indicates that the eNOS-bound membrane is stimulated and dissociated upon endothelial cell stimulation with calcium mobilizing agents. Because intracellular H_2O_2 and calcium influx both contribute to DOX-induced toxicity, DOX treatment increases eNOS transcription and protein activity in aortic endothelial cells, which in turn increases NO production [48]. This decrease in GSH content was explained by the overproduction of ROS brought on by DOX injection, as these species are detoxified by endogenous antioxidants, primarily GSH, which causes their cellular stores to be depleted [44,46]. As demonstrated by the current investigation, increased activity in SOD and GSH metabolizing enzymes may also be the cause of the observed decrease in myocardial GSH concentration.

Furthermore, we saw a marked decline in the values of the GPx enzyme and GSH in the animals treated with DOX; in contrast, treatment with ECEE enhanced the actions of SOD and CAT and prevented the consumption of GSH in addition to restoring the activity of the GPx enzyme. These findings suggest that ECEE may have antioxidant and free radical scavenging properties. By maintaining enzymatic antioxidants, the first line of defense, ECEE has been reported in earlier studies as one of the effective antioxidants and free radical scavengers [49,50]. The antioxidant activity of ECEE was demonstrated in the current investigation, which supports its cardioprotective impact through its antioxidant action in the myocardium. Due to oxidative degradation of fatty acids in the cardiac membrane, DOX impairs antioxidant protection systems and increases the myocardium's susceptibility to lipid peroxidation, as seen by an increase in MDA levels. A measure of the extent of the cellular damage to the heart caused by DOX is the increased generation of MDA, a degradation product of lipid peroxidation [51]. This can be connected to changes in membrane structure and enzyme inactivation. Our findings suggest that increased antioxidant defense in the myocardium may be responsible for the lower MDA levels and concurrent increase in GSH that occurred after the cardamom administration.

DOX can cause cardiotoxicity, cardiomyopathy, and congestive heart failure by upregulating TNF-α and IL-1β, among other inflammatory pathways. Previous studies have shown that DOX treatment causes an increase in the levels of IL-1β, NF-κB, TNF-α, and caspase-3 in rats [52,53]. It has been demonstrated that the effects of DOX on adipose tissue cause NF-κB to become activated and inflammatory cytokines to be produced. Thus, blood levels of triglycerides and total cholesterol rise as results of Syukri *et al.* [54]. The phenomenon under observation could be explained by the downregulation of DOX-produced peroxisome proliferator-activated receptor gamma (PPARγ) in adipose tissue, which would lead to a decrease in the clearance of circulating free fatty acids, an increase in the number of macrophage cells, and the activation of inflammatory cytokines and NF-κB [12], this supported our findings; thus, a strong infiltration of inflammatory cells and a marked elevation of TNF-α and IL-1β were seen in the heart tissue of the DOX-treated group. The biochemical confirmation of DOX-induced apoptosis was demonstrated by elevated caspase 3 activity in rats administered with DOX. Our findings are in line with other research that found that exposure to DOX significantly increased

caspase 3 activity and protein expression while downregulating antiapoptotic proteins [55,56]. Furthermore, Bax translocation to the outer mitochondrial membrane caused by ROS triggered by DOX might result in the release of cytochrome C from the mitochondria into the cardiomyocyte cytoplasm, which in turn triggers caspase 3-dependent death [57]. In cardiac cells, DOX-induced apoptosis can also be stimulated by NF-κB [58]. Myocyte contractile function may be reduced because of Caspase 3's potential to cleave cardiac myofibrillar proteins, such as ventricular α actin, myosin light chain, troponin T, and α actinin [59]. Rats treated with ECEE both before and during DOX treatment showed a substantial decrease in caspase 3 immunostaining, in contrast to rats treated with DOX. Given that antioxidants prevent DNA fragmentation and apoptosis and that oxidative stress damages DNA [60], it is plausible that ECEE has both antioxidant and antiapoptotic properties. This is most likely due to its ability to modulate the caspase pathway in response to oxidative stress.

Treatment with ECEE stopped all the changes that DOX caused. We think that the most plausible mechanisms of action of cardamom are its antioxidant qualities [61], its capacity to inactivate NF-κB, and its ability to stop the generation of pro-inflammatory cytokines [62].

Furthermore, a histological analysis of the cardiac tissue in normal control animals showed a united, intact cell membrane free of inflammation, edema, and inflammatory cell infiltration; in contrast, a histological analysis of the rats' cardiotoxicity DOX revealed coagulative myonecrosis, inflammation, and inflammatory cell infiltration. Rats given ECEE, however, showed condensed myonecrosis, edema, and decreased inflammatory cell permeability. When combined with hemodynamic and biochemical recovery as well as histological salvage, ECEE appears nontoxic to cardiomyocytes, most likely due to the restoration of the endogenous antioxidant defense against DOX. It was reported that *E. cardamomum* contains many active ingredients with large medicinal activities; terpinyl acetate, terpineol, sabinene, nerol, α-pinene, geraniol, linalool, cineole, limonene, β-pinene, myrcene, and octanal represent the highest concentrations; this besides minute quantities of other constituents. These ingredients exhibit antioxidant, immune-modulatory, and other therapeutic activities that can treat life threatening diseases [63].

Conclusion

The current study concluded that ECEE exhibited successfully alleviating oxidative stress, amelioration, and retrieving efficiencies against the doxorubicin resultant cardiac deteriorations; therefore, post more validation via future research, this extract could be recommended as a supplement along doxorubicin therapy.

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Ethical considerations: This research was carried out in accordance with the standard institutional criteria for the care and use of experimental animals, and the proposal was approved by the ethical committee of Faculty of Science, Al-Azhar University, Assiut (approval number AZHAR 14/2023).

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Conflicts of interest

There are no conflicts of interest.

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