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Cardioprotective effect of aqueous *Commiphora myrrha* extract against alcoholic cardiomyopathy in rats

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This study aimed to evaluate the cardioprotective efficacy of *Commiphora myrrha* resin in alcoholic cardiomyopathy (ACM) through integrated biochemical, histopathological, and NF-κB-related immunohistochemically assessments. Although ACM is a well-recognized consequence of chronic alcohol consumption, characterized by progressive cardio myocyte degeneration and necrosis, current effective and safe therapeutic strategies remain limited. Moreover, despite accumulating evidence implicating inflammatory signaling pathways—particularly NF-κB—in ACM pathogenesis, the potential modulatory role of *C. myrrha* resin in this context has not yet been systematically investigated, thereby representing a critical gap in the existing studies. For inducing the ACM rat model, rats were given ethanol for 30 days. Other rats were given distilled water as a negative control, *C. myrrha* as a positive control, and ethanol plus *C. myrrha*. The MDA, 4-HNE, MDA, NO, TNF-α, and HSP70 were measured. The histopathological alterations as well as the NF-κβ expression in cardiac tissue were investigated. The data were analyzed using ANOVA followed by Tukey's test. Alcohol increased the level of MDA, 4-HNE, TNF-α, NO, and HSP70, reduced NF-kB expression of the heart, and caused blood vessel congestion, cardiomyocyte necrosis, and myocardial fibrosis. While *C. myrrha* restored heart integrity and improved cardiac tissue since these compounds can overexpress HSP70 and alter cell cytokine expression. Our findings uniquely suggest a new mechanism by which furano-sesquiterpenoids and triterpenes from *C. myrrha* possess antioxidant and free radical-neutralizing properties, making them key mediators of protection against myocardial injury and providing scientific support for developing *C. myrrha* as a nutritional supplement.

Keywords: Antioxidants, TNF-α, HSP70, lipid peroxidation, NF-kB

Alcoholic cardiomyopathy (ACM) is a progressive myocardial disease induced by chronic and excessive alcohol consumption and characterized by ventricular dilation, impaired systolic function, and cardiac remodeling[1]. Prolonged ethanol exposure exerts a reduction in the efficiency of synthesis of the structural and non-structural proteins in the myocardial tissue, leading to the deterioration of cardiac myocytes and stimulating cell necrosis, in addition to the emergence of cases of hypertrophy and interstitial fibrosis in the myocardial tissue, causing heart failure[2]. Alcohol also was thought to have a negative impact on the mitochondrial energy production system via altering oxidative regulatory processes[3].

Despite advancements in understanding ACM pathogenesis, effective and safe therapeutic interventions remain limited. Plant-based natural compounds have been the subject of extensive studies due to their potential as environmentally friendly natural resources, attracting the attention of scientists and researchers around the world as a highly promising step towards producing effective medicines[4]. Several plant materials are major sources of natural antioxidants due to their rich content of phytochemical constituents like phenolics, flavonoids, terpenoids, and alkaloids[5]. One of the naturally occurring materials is myrrh gum resin, which is extracted from the bark of specific species of trees belonging to the genus *Commiphora* (family Burseraceae). *Commiphora* species are prevalent in Saudi Arabia, especially in rocky environments and mountainous areas, particularly in the Tihama, constituting a unique component of Saudi flora[6]. In Arab communities, myrrh is commonly used as a traditional treatment for stomach disorders, fevers,

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colds, and skin infections. Additionally, it acts as an antiseptic and is effective in wound healing[7]. Modern pharmacological studies have demonstrated that the myrrh resin extract and its isolated metabolites have possessed several therapeutic efficacies, such as antidiabetic, antioxidant, anti-inflammatory, and anticancer effects, by various mechanisms of action, including stimulating the endogenous antioxidant system, decreasing oxidative stress, and scavenging reactive oxygen species[8]. All of this resin's biological actions are related to its phytochemical content, which includes sesquiterpenoids, diterpenes, and triterpenes, which are accountable for antioxidant capacity; lignans, which have a cytotoxic effect on malignancies; and steroids, which have anti-inflammatory and antidiabetic properties[9,10].

The literature studies survey indicated that myrrh resins exhibit cytoprotective characteristics. However, there are not enough experimental studies on the effect of this resin against ACM. Therefore, the aim of this research was to investigate the protective and antioxidant efficiency of *C. myrrha* resin on myocardial tissue from biochemical, histopathological, and immunohistochemical aspects for the development of a novel therapeutic strategy to attenuate the adverse consequences of alcohol.

Materials and Methods

Chemicals and Kits

Malondialdehyde MDA (Item No. ab238537), 4-hydroxynonenal 4-HNE (Item No. ab238538), Nitric Oxide NO (Item No. ab65328), Heat shock protein 70 HSP70 (Item No. ab133060), and Tumor Necrosis Factor-α TNF-α (Item No. ab236712) assay kits were purchased from Abcam (Boston, USA). Anti-NF-kβ p65 Antibody (Item No. A93736) was purchased from Antibodies company (Cambridge, UK). Other chemicals were obtained from the central lab at the biology department, science college, King Khalid University.

Plant material preparation and its phytochemical screening

The *C. myrrha* was purchased from Elhekma Market in Jeddah, Kingdom of Saudi Arabia, in the form of gum resin. After washing and drying, *C. myrrha* was ground and then dissolved in distilled water (1:20) under the evaporation temperature and kept for 6 hours. The solution was filtered, and the supernatant was stored at 4°C until use[11].

The phytochemical composition of aqueous *C. myrrha* extract was performed using a Trace GC-TSQ mass spectrometer GC/MS (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The components were identified by comparison of their mass spectra with those of the WILEY 09 and NIST 14 mass spectral databases.

Animals

Twenty male Sprague Dawley rats (200–250 gm) were supplied from the Animal House of Science College at King Khalid University (Abha, Saudi Arabia). All rats were acclimatized for 10 days in ventilated propylene cages in normal temperature (22 ± 2°C) and humidity under a light/dark cycle of 12 h with unlimited access to rodent food and water. All procedures were performed according to the ARRIVE guidelines and approved by the Research Ethics Committee at King Khalid University (ECM# 2022-2125).

Experimental design

After acclimatization, rats were randomly distributed into four groups (n=5) as follows: the *negative normal control (-NC)*, in which animals were given distilled water; the *positive normal control (+NC)*, in which animals were treated with *C. myrrha* resin extract (500 mg/kg); the *alcoholic Cardiomyopathy (ACM)*, in which animals were administered ethanol (40%; 3 g/kg); and the *alcohol + C. myrrha group (ACMC)*, in which animals received ethanol and then *C. myrrha* after two hours as previous doses. The treatment was given orally by gastric tube for 30 days. The doses of *C. myrrha* and ethanol were carefully selected according to previous studies[12,13].

On day 31 of the study, all rats were anesthetized with diethyl ether and euthanized by cervical dislocation, and their hearts were taken out. The hearts were rinsed with physiological saline and then were divided into two parts. The first part was homogenized in 10% cold PBS and centrifuged at 3000 rpm (4°C)[14]. The supernatants were kept at −20°C for further biochemical analysis. The second part was fixed in 10% formaldehyde for histopathological and immunohistochemical investigations.

Biochemical analysis

Assay for lipid peroxidation (LPO)

To determine the LPO in the cardiac tissues, MDA (nmol/g tissue) and 4-HNE (nM/g tissue) quantities

were measured as major products in LPO by a colorimetric assay according to the instructions of the kits. The absorbance of the resultant products was measured at 450 nm.

Measurement of cardiac NO level

Nitric oxide production was measured in cardiac tissue using the colorimetric assay at optical density 540 nm, following the manufacturer's protocol of the kit. NO is rapidly converted to nitrite and nitrate, which are utilized to determine the quantity of NO production through transformation of nitrate to nitrite using nitrate reductase, followed by Griess reagents to convert nitrite to a deep purple azo compound[15].

Measurement of TNF-α level

The quantitative measurement of TNF-α (pg/mg) in cardiac tissue was determined, as a primary cytokine that can be produced during acute inflammation, using Enzyme-Linked Immunosorbent Assay (ELISA) at 450 nm according to the manufacturer's instructions.

Measurement of HSP70 level

To assess the relationship between cardiac injury and production of HSP70, inducible HSP70 (nm/g) from cardiac tissue of all rats was measured via ELISA technique at 450 nm following the protocol of the kit.

Histopathological examination

The fixed part of the heart of each rat was embedded in paraffin wax, sectioned at 4 μm thickness, then mounted on a glass slide to stain with hematoxylin and eosin (H&E) in order to investigate the histopathological alterations in cardiac muscles. Other slides were stained with Masson trichrome to detect collagen fiber production in cardiac tissues. All slides were examined using a digital microscope (Omax-M837ZL, China).

Determination of cardiac NF-κβ expression via immunohistochemistry stain

Detection of endogenous NF-κβ p65 expression in cardiac tissue was performed immunohistochemically using rabbit polyclonal antibody to NF-κβ p65 when phosphorylated at Ser276. Briefly, paraffin-embedded cardiac sections undergo dewaxing hydration, antigen retrieval, blocking via H_2O_2 closed endogenous peroxidase, adding primary antibody (NF-κβ p65), adding secondary antibody, and finally adding substrate for color development. The coated slides were investigated using a digital microscope (Omax-M837ZL, China).

Data analysis

Results were presented as means ± SEM. The data were statistically analyzed by the one-way analysis of variance (ANOVA) test using SPSS software (version 25). Tukey's multiple range test was used to evaluate the significance between multiple groups at ($P \leq 0.05$).

Results

The phytochemical screening of aqueous *C. myrrha* extract

The GC/MS analysis detected 26 components in the aqueous *C. myrrha* extract during retention time (RT) from 12.59 to 44.60 min, which were classified as sesquiterpenoids, triterpenes, fatty acid esters, steroids, phenols, and retinoids (Table 1). The most abundant major components identified belonged to the group of furano-sesquiterpenoids (44.22%), including furanoeudesma-1,3-diene (24.55%) and curzerene (19.67%). Other major components are triterpenes such as à-Amyrin (16.73%).

Biochemical analysis

Ethanol toxicity attenuation by C. myrrha on the products of LPO

In this study, we noticed that there was a significant increase ($P \leq 0.0001$) in myocardial contents of both MDA and 4-HNE in rats after ethanol was administered compared to the -NC rats. On the other hand, we found that the treatment with ethanol and *C. myrrha* has attenuated MDA and 4-HNE levels significantly as compared to the rats that were treated with ethanol only ($P \leq 0.05$ and $P \leq 0.0001$, respectively). Further, no significant alterations were recorded in the contents of MDA and 4-HNE in +NC as compared to -NC ($P > 0.05$) (Table 2).

Ineffectiveness of C. myrrha in protecting against ethanol toxicity on NO content

Compared to the -NC group, our results indicated a significant increase in NO level after treatment with ethanol only ($P \leq 0.0001$), while no significant change was observed after treatment with *C. myrrha* only ($P > 0.05$). Moreover, combined treatment with ethanol and *C. myrrha* did not show a significant change in NO level ($P > 0.05$) compared to rats treated with ethanol alone (Table 2).

Table 1 — GC-SM analysis of aqueous *C. myrrha* extract

No.	M. F.	Chemical Name	Area (%)	RT	Nature of Compound
1	$C_{15}H_{24}$	δ-EIemene	0.62	12.59	fatty acid ester
2	$C_{15}H_{24}$	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	3.97	13.88	fatty acid ester
3	$C_{15}H_{20}O$	Curzerene	9.47	16.17	Furano-sesquiterpenoid
4	$C_{15}H_{24}$	ç-Muurolene	0.93	16.68	sesquiterpene
5	$C_{15}H_{24}$	ç-Elemene	2.48	17.63	fatty acid ester
6	$C_{15}H_{18}O$	Furanoeudesma-1,3-diene	17.09	19.02	Furano-sesquiterpenoid
7	$C_{15}H_{18}O$	Furanoeudesm-1,3-diene	7.46	19.16	Furano-sesquiterpenoid
8	$C_{15}H_{24}$	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimeth yl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-	2.83	19.40	sesquiterpene
9	$C_{15}H_{24}$	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dime thyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aà)]-	1.47	20.28	sesquiterpene
10	$C_{15}H_{20}O$	Curzerene	10.20	20.41	Furano-sesquiterpenoid
11	$C_{16}H_{22}O_2$	(R,5E,9E)-8-Methoxy-3,6,10-trimethyl-4,7,8,11-tetrahydrocyclodeca[b]furan	4.16	21.04	sesquiterpene lactone
12	$C_{15}H_{18}O_2$	Furanoeudesma-1,4-diene	1.96	22.51	Furano-sesquiterpenoid
13	$C_{16}H_{22}O_2$	(R,5E,9E)-8-Methoxy-3,6,10-trimethyl-4,7,8,11-tetrahydrocyclodeca[b]furan	1.87	23.41	sesquiterpene lactone
14	$C_{15}H_{20}O_2$	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-met hylene-7-(1-methylethenyl)-, [3aS-(3aà,6à,7á,7aá)]-	1.31	24.31	sesquiterpene
15	$C_{15}H_{20}O_2$	Furosardonin A	1.24	24.54	Fatty acid ester
16	$C_{23}H_{34}O_2$	cis-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	0.86	25.32	Fatty acid ester
17	$C_{18}H_{24}O_2$	5,8,11-Heptadecatriynoic acid, methyl ester	3.35	25.95	Fatty acid ester
18	$C_{15}H_{18}O$	1-Naphthalenol, 4,7-dimethyl-2-(1-methyleth yl)-	3.65	26.49	phenol
19	$C_{28}H_{48}O$	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.92	28.76	steroid
20	$C_{15}H_{24}O$	cis-Z-à-Bisabolene epoxide	1.95	29.36	sesquiterpene
21	$C_{17}H_{28}O_4$	Nerolidol-epoxy acetate	1.36	29.92	sesquiterpene
22	$C_{21}H_{30}O_2$	Retinoic acid, methyl ester	0.69	41.50	retinoid
23	$C_{30}H_{50}O$	à-Amyrin	4.60	41.67	triterpene
24	$C_{30}H_{50}O$	à-Amyrin	12.13	42.10	triterpene
25	$C_{24}H_{32}O_4$	Resibufogenin	0.87	44.31	steroid
26	$C_{21}H_{30}O_2$	Retinoic acid, methyl ester	2.56	44.60	retinoid

Table 2 — Impacts of *C. myrrha* extract and/or ethanol treatment on the biochemical markers in myocardial tissue. -NC, no treatment; +NC, *C. myrrha* extract (500 mg/kg); ACM, 40% ethanol (3 g/kg); and ACMC, 40% ethanol followed by *C. myrrha* extract.

	-NC	+NC	ACM	ACMC
MDA (nmol/g)	31.48 ± 4.11	30.31 ± 5.49	56.43 ± 7.99****	46.06 ± 8.90$^{\#}$
4-HNE (nM/g)	89.85 ± 11.96	86.46 ± 11.47	162.50 ± 18.96****	125.46 ± 16.82$^{\#\#\#}$
NO (µmol /mg)	82.03 ± 9.11	92.20 ± 10.43	149.58 ± 21.93****	139.12 ± 16.80
TNF-α (pg/mg)	139.88 ± 7.99	184.01 ± 8.94	380.81 ± 11****	248.41 ± 12.13$^{\#\#\#\#}$
HSP70 (nM/g)	103.90 ± 12.42	92.08 ± 12.52	132.98 ± 9.16***	156.98 ± 21.44$^{\#}$

All values are expressed as mean + standard deviation (SD) of n=5. ***$P \leq 0.001$ and ****$P \leq 0.0001$ *vs.* -NC; $^{\#}P \leq 0.05$, $^{\#\#\#}P \leq 0.001$, and $^{\#\#\#\#}P \leq 0.0001$ *vs.* ACM

Ethanol toxicity reduction by C. myrrha on the TNF-α production

It is noted in Table 2 that the ethanol administration led to high elevation in the production of TNF-α in myocardial tissue compared to the control ($P \leq 0.0001$), while *C. myrrha* when given together with ethanol, has shown noticeable protection on TNF-α production ($P \leq 0.0001$). Additionally, there was no significant alteration between -NC and +NC ($P > 0.05$).

HSP70 level elevation by ethanol plus C. myrrha treatment

As seen in Table 2, HSP70 production was notably increased ($P < 0.001$) after ethanol intake compared to the -NC. In contrast, there is no significant alteration in its level between both -NC and +NC groups

($P > 0.05$). While it was found that the ethanol plus *C. myrrha* combination highly elevated the levels of HSP70 compared to its level after treatment with ethanol only ($P < 0.05$).

Histopathological examination

The cardiac sections obtained from -NC and +NC rats illustrated normal endomysium distribution and structure of cardiomyocytes (Fig. 1A and B) respectively, except slight degeneration of the cardiac muscle with intermuscular edema was noticed in few areas of the +NC group. In contrast, the ethanol intake alone caused intense congestion of the cardiac blood vessels with degeneration of their walls, loss of myofibrils, cardiomyocyte necrosis, and loss of striation (Fig. 1C and D), whereas its combination with *C. myrrha* resulted in high improvement in myocardial tissue with a decrease in the degree of congestion and necrosis, and the myocardium cells appear to be near normality (Fig. 1E and F).

Masson's trichrome staining for cardiac tissues obtained from both -NC and +NC showed normal distribution of collagen fibers in the extracellular matrix surrounding cardiomyocytes (Fig. 2A and B, respectively). In ACM groups (Fig. 2C and D), we found an excessive deposition of collagen fibers around congested vessels (perivascular fibrosis), in an extracellular matrix surrounding cells (interstitial fibrosis), and in the myocardium (replacement fibrosis). On the other hand, ACMC showed improvement in the distribution of collagen fibers as revealed by remarkably reduced interstitial fibrosis (Fig. 2E).

Cardiac NF-κβ expression

In both the -NC and +NC groups, we noticed intense expression of NF-κβ p65 distributed throughout the myocardial fibers, with more prominent expression in the blood vessels (Fig. 3A and B, respectively). After treatment with ethanol alone, weak expression of NF-κβ p65 was noticed in cardiac blood vessels (Fig. 3C). While its expression was illustrated to be moderate after treatment with a combination of *C. myrrha* and ethanol (Fig. 3D).

Discussion

Our research was focused on the potential protective role of *C. myrrha* on cardiotoxicity of ethanol in rats. Lipid peroxidation products such as MDA and 4-HNE are critical indicators of oxidative stress[16]. We showed the highest levels of both markers in cardiac tissues obtained from ACM rats compared to other groups, suggesting that the alcohol-treated rats are under oxidative stress, which is like

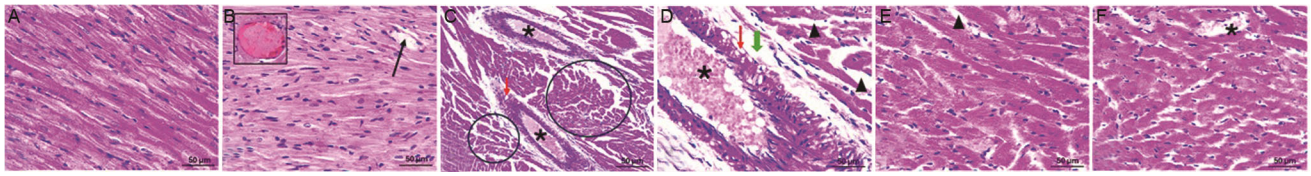

Fig. 1 — Cardiac longitudinal sections stained with H&E showing the histological changes after treatment with ethanol and/or *C. myrrha*. (A) -NC group showed a normal pattern of cardiomyocytes and normal endomysium distribution. (B) +NC group showing normal structure of cardiomyocyte with slight degeneration of the cardiac muscle (arrow) and intermuscular edema (square). (C & D) ACM group showed congested blood vessels (star) with degeneration of their walls (red arrow), loss of myofibrils (green arrow), cardiomyocyte necrosis (green arrow), and loss of striation (circle). (E & F) ACMC group showed high improvement in myocardium with decreased degrees of necrosis (arrowhead) and congestion (star). -NC, no treatment; +NC, *C. myrrha* extract (500 mg/kg); ACM, 40% ethanol (3 g/kg); and ACMC, 40% ethanol followed by *C. myrrha* extract. 200x.

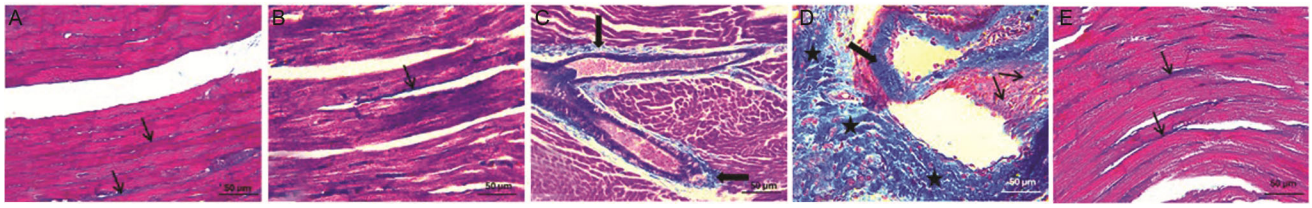

Fig. 2 — Masson's trichrome staining sections for cardiac tissues of experimental groups to visualize cardiomyocytes (red) and collagen fibers (blue). (A & B): -NC and +NC groups, respectively, showed a normal distribution of collagen fibers between cardiomyocytes. (C & D): The cardiac tissues of ACM rats showed an excessive deposition of collagen fibers around congested vessel (bold arrow), in an extracellular matrix surrounding cells (*), and in the myocardium (arrow). (E): The cardiac tissue of ACMC rat showed remarkable reduction in collagen fibers (blue) between cardiomyocytes. -NC, no treatment; +NC, *C. myrrha* extract (500 mg/kg); ACM, 40% ethanol (3 g/kg); and ACMC, 40% ethanol followed by *C. myrrha* extract. 200x.

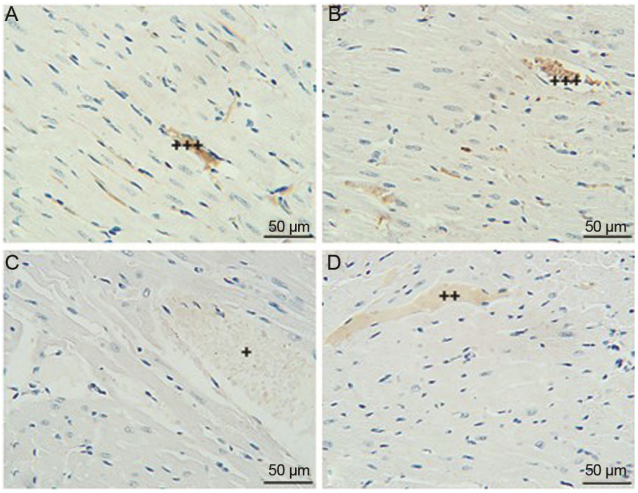

Fig. 3 — Immunohistochemical staining for NF-κB p65 expression (brown) in heart tissues of experimental groups. (A & B) -NC and +NC groups, respectively, showed intense expression of NF-κβ p65 (+++) throughout the myocardial fibers and blood vessels. (C) The ACM group showed weak expression (+) in cardiac blood vessels. (D) ACMC showed moderate expression of NF-κβ p65 (++) throughout the cardiac tissue. -NC, no treatment; +NC, *C. myrrha* extract (500 mg/kg); ACM, 40% ethanol (3 g/kg); and ACMC, 40% ethanol followed by *C. myrrha* extract. 200x.

that reported by previous research[17]. These effects were confirmed by histopathological signs in cardiac tissues such as blood vessel congestion, cardiomyocyte necrosis, and myocardial fibrosis. After cardiomyocyte necrosis, the heart attempts to regenerate and repair the injured tissue[18]. At the same time, its ability to repair is reduced due to severe ethanol-induced injury, leading to the promotion of ineffective repair processes such as fibrosis[19]. After alcohol intake, the active metabolite of alcohol inside the body is acetaldehyde, which elicits lipid peroxidation, leading to permanent myocardial injury as a result of increasing the permeability of the myocardial membrane and causing the presence of excessive amounts of intracellular calcium[20]. Furthermore, acetaldehyde and lipid peroxidation products can interact with myocardial proteins to produce protein-adduct compounds, which directly stimulate the inflammatory response as observed in our study through elevated levels of TNF-α and NO in the cardiac tissue of ethanol-treated rats[21]. The presence of protein-adduct compounds in myocardium promotes inflammatory responses by modulating NF-κβ signaling. NF-κβ regulates the expression of pro-inflammatory genes, thus leading to the release of cytokines and inflammatory mediators (COX-2 and iNOS), which contribute to inflammation[22]. NF-κβ is a crucial transcription factor in control of inflammation via regulating the pro-inflammatory gene expression, which is responsible for releasing chemokines and cytokines[23]. Additionally, the NF-κβ signaling pathway contributes to the production of C-reactive protein, cyclooxygenase 2 (COX-2), and nitric oxide synthase (iNOS), which leads to multi-coordination and an improvement in the inflammatory innate response[24]. In our findings, among the alcohol-caused toxicity biomarkers is the elevation of HSP70 in cardiac tissue, which is consistent with the previous study on testicular tissue[8]. It is a conserved protein that acts to shield cells from stress[25,26]. When cells are exposed to harmful products, they rapidly respond via stimulating regulatory genes; among those is the HSP70 as a physiological mechanism of cellular stability. HSP70 production may regulate cytokine and chemokine expression through the modulation of the NF-κβ pathway[27,13].

In our study, we have shown a marked drop in the cardiac MDA and 4-HNE levels when given *C. myrrha* together with alcohol, demonstrating the antioxidant role of *C. myrrha* in mitigating oxidative stress in cardiac tissue. These results were consistent with those of Lebda *et al.*[28]. Furthermore, *C. myrrha* extract significantly decreased TNF-α level as well as increased the level of HSP70, proposing its fundamental impact in attenuating cardiac damage through anti-inflammatory activities.

Attenuation of lipid peroxidation and inflammation is a mechanism we assume to arbitrate the protective action of *C. myrrha* against myocardial injury induced by ethanol. Interestingly, the *C. myrrha* extract exhibited a radical scavenging capacity, as revealed by the significantly diminished lipid peroxidation in cardiac homogenate. Likewise, earlier investigations illustrated that myrrh supplementation significantly decreased lipid peroxidation in rats and hepatocarcinogenesis through scavenging free radicals and promoting antioxidant activities[29]. Pharmacological activities of *C. myrrha* may be attributed to its bioactive phytochemicals, including furano-sesquiterpenoids such as furanoeudesma-1,3-diene and curzerene, as well as containing triterpenes such as à-Amyrin. Several studies have demonstrated that furano-sesquiterpenoids and triterpenes may decrease TNF-α release in cells, indicating that they may be useful as anti-inflammatory medications[30,31]. Furthermore, low furano-sesquiterpenoid concentrations can prevent macrophages from releasing NO, which

in turn may influence cell signaling activation[30,32]. Our results suggested that overexpression of HSP70 modified cytokine expression in cells in a way that was comparable to heat conditioning. This action was linked to HSP70's capacity to suppress NF-κβ p65, indicating that HSP70 downregulates the NF-κβ pathway, which makes cells resistant to TNF-α and apoptosis[13]. These physiological events explain the reason for the elevation of the HSP70 level and the reduction of TNF-α in heart samples after being treated with alcohol plus *C. myrrha*.

Furano-sesquiterpenoids and triterpenes also have been shown to have antioxidant and free radical-neutralizing qualities, as well as to reduce levels of ROS within cells[10,33]. These many beneficial findings suggest that both components are safe and effective medications to enhance cardiac tissue and restore heart integrity, which increases the likelihood that they will be used to treat deadly illnesses. On the other hand, the antioxidant and anti-inflammatory properties of *C. myrrha* shouldn't be evaluated just in relation to its major components. Also, the synergistic effect of other minor ingredients such as fatty acid esters, steroids, phenols, and retinoids may contribute to enhancing the pharmaceutical activity[34].

Our immunohistochemical examination showed intense brown staining of NF-κβ in certain areas of myocardial tissues of both -NC and +NC groups as well as moderate staining in the ACMC group. These results could be indicative of its critical function in preserving cellular function and heart homeostasis[35]. These findings are consistent with previous study showing that NF-κβ in cardiac tissue is important for promoting the expression of anti-apoptotic genes, which are necessary for preserving the integrity of myocardial cells, even in the absence of pathogenic stimuli[36]. Furthermore, as part of a natural adaptive process, NF-κβ may be activated in response to mechanical stress since the heart is continuously exposed to hemodynamic pressures[37].

Meanwhile, we observed weak NF-κβ staining in cardiac tissue of the ACM group. This unexpected result might be attributed to the dose-dependent impacts of ethanol, where chronic ethanol administration triggers adaptive cellular tolerance, which could prevent or delay the activation of the signaling pathways like NF-κβ[38]. Furthermore, it may also be possible that this result was due to the timing of tissue collection, which might have missed the peak NF-κβ activation[39]. Also, the observed staining patterns may potentially be the result of technical factors and adaptive biological mechanisms[40].

Conclusion

Based on the results and discussion of this study, we have concluded that furano-sesquiterpenoids and triterpenes, which are isolated from *C. myrrha*, uniquely have antioxidant and free radical-neutralizing features through overexpressing HSP70 and altering cell cytokine expression, making cells resistant to TNF-α and apoptosis. Therefore, this study supports the potential of *C. myrrha* as a biologically safe and effective agent for restoring heart integrity and improving cardiac tissue, providing scientific evidence for developing *C. myrrha* as a novel therapeutic and nutritional supplement. More *in vitro* and *in vivo* investigations supported by isolation and specific structural identification of each constituent molecule are advised to be applied as a promising therapeutic agent against other disorders.

Ethical statement

The experimental protocols for animals were approved by the Research Ethics Committee at King Khalid University (ECM# 2022-2125).

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Conflict of interest

The authors declare no competing interests.

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