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THE CARDIOPROTECTIVE EFFECT OF EXTRA VIRGIN OLIVE OIL AND VIRGIN COCONUT OIL ON ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN RATS

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Abstract

Routine consumption of extra virgin olive oil (EVOO) and virgin coconut oil (VCO) may improve cardiovascular function. This study examined the cardioprotective effect of the combination of EVOO and VCO on isoproterenol-induced myocardial infarction in rats. Rats (n = 30) were divided into five groups: a healthy group, an isoproterenol group without treatment, and three treatment groups that were given an EVOO–VCO combination at 1:1, 1:2 and 2:1 (2 mL/200 g) orally for 14 days before injecting isoproterenol (100 mg/kg) for two consecutive days. After 24 hours, blood samples were taken to analyse cardiac biomarkers, and the heart was examined for histopathological changes. Isoproterenol injection caused a substantial increase in the creatine kinase–myocardial band, lactate dehydrogenase and aspartate aminotransferase levels. Pretreatment with an EVOO–VCO combination in all ratios significantly reduced these biomarker levels ($p < 0.05$). Myocardial damage was significantly reduced, especially with the EVOO–VCO combination of 2:1. In conclusion, the EVOO–VCO combination significantly reduced cardiac injury biomarker levels and improved myocardial structure in isoproterenol-induced myocardial infarction in rats. The EVOO–VCO combination of 2:1 was superior in preserving myocardial structure compared to the other ratios.

Rezumat

Consumul uleiului de măsline extravirgin (EVOO) și a uleiului de cocos virgin (VCO) poate îmbunătăți funcția cardiovasculară. Acest studiu a examinat efectul cardioprotector al combinației de EVOO și VCO asupra infarctului miocardic indus de izoproterenol la șobolani. Șobolanii (n = 30) au fost împărțiți în cinci grupuri: un grup sănătos, un grup de izoproterenol fără tratament și trei grupuri de tratament cărora li s-a administrat o combinație de EVOO-VCO în proporție de 1:1, 1:2 și 2:1 (2 mL/200 g) pe cale orală timp de 14 zile înainte de injectarea de izoproterenol (100 mg/kg) timp de două zile consecutive. După 24 de ore, au fost prelevate probe de sânge pentru a analiza biomarkerii cardiaci, iar la nivelul inimii au fost examinate modificările histopatologice. Administrarea de izoproterenol a provocat o creștere substanțială a nivelurilor de creatin-kinază miocardică, lactat dehidrogenază și aspartat aminotransferază. Pretratarea cu o combinație EVOO-VCO, în toate proporțiile, a redus semnificativ aceste niveluri ale biomarkerilor ($p < 0,05$). Leziunile miocardice au fost diminuate, în special la administrarea combinației EVOO-VCO 2:1. În concluzie, asocierea EVOO-VCO a redus semnificativ nivelurile biomarkerilor cardiaci și a îmbunătățit structura miocardului în modelul experimental ales. Combinația EVOO-VCO de 2:1 a fost superioară în ceea ce privește conservarea structurii miocardice în comparație cu celelalte proporții.

Keywords: extra virgin olive oil, virgin coconut oil, cardioprotective, isoproterenol, myocardial infarction

Introduction

Isoproterenol (ISO) is a non-selective β-adrenergic (β-1 and β-2) agonist drug [1]. The injection of ISO in high doses has been used in experimental myocardial infarction (MI) in rat models [2, 3]. ISO can trigger an increase in calcium levels in the myocardium, resulting in overstimulation, increased contractile force, oxygen demand and excessive depletion of ATP, leading to myocardial injury [2, 4]. In addition, ISO can also cause an increase in oxidative stress, such as reactive oxygen species (ROS) and lipid peroxidase (LPO) [2, 5]. In most coronary artery diseases, a prolonged lack of oxygen in the myocardium leads to necrosis, which initiates the formation of MI [6].

Lifestyle changes and routine consumption of healthy foods such as vegetables, fruits and antioxidant-rich plants can help improve cardiovascular function [7]. Extra virgin olive oil (EVOO) and virgin coconut oil (VCO) have high fatty acids (FAs) and antioxidants [8]. VCO contains capric acid, caproic acid and lauric acid, which have potential as anti-thrombogenic, anti-arthritis, antihyperlipidemic, cardioprotective, anti-osteoporosis, anti-inflammatory, antimicrobial, hepato-protective and neuroprotective agents [8]. EVOO

contains FAs that are rich in oleic acid in the form of triacylglycerols and vitamin E [8]. It has potential antimicrobial, antioxidant and anti-inflammatory effects [9]. EVOO at a dose of 10 g/day can reduce the risk of cardiovascular disease by up to 10% [10]. A clinical trial reported that consuming 10 - 50 mL *per* day of EVOO could significantly reduce blood pressure [11]. Additionally, a preclinical study reported that a single administration of VCO had a cardioprotective effect against cardiac remodelling and blood pressure elevation [12]. The protective effects of EVOO and VCO in combination were shown to be superior against oxidative stress in multiple organs in doxorubicin-treated rats [13]. The study showed that the combination could prevent the development of myocardial injury after doxorubicin injection [14]. Accordingly, the present study aimed to examine the protective effect of an EVOO and VCO combination in a ratio of 1:1, 1:2 and 2:1 in ISO-treated rats.

Materials and Methods

Chemicals and drugs

EVOO and VCO were purchased from a local pharmacy in Makassar, Indonesia. The identification of the chemical compounds in the oils was by gas chromatography–mass spectrophotometry (GC-MS). Ethanol 70%, diethyl ether, 20% formalin and ISO (USP Cat. from Sigma Chemical Co.) were purchased from official chemical distributors in Jakarta, Indonesia. Creatine kinase–myocardial band (CK-MB), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kit reagents (Human, Germany) were obtained from the official distributor for Human World Diagnostic in Indonesia.

Chemical component analysis

EVOO and VCO components were analysed using a Trace 1310 gas chromatograph with a TSQ 8000 Evo mass spectrometer (Thermo Scientific; Mundelein, IL, USA). The column size used was 20 mm x 0.18 mm (TG-5MS), with helium as a carrier. The initial temperature of the oven was 50ºC, increasing to 330ºC at the end.

Preparation of animals

Male albino rats (*Rattus norvegicus*) were used as the animal model. Thirty rats weighing 180 - 300 g were cared for in plastic cages and given standard feed and drinking water ad libitum. The research was conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, and the experimental protocol was approved by the research ethics committee of the Faculty of Medicine at Hasanuddin University, Indonesia, (number 671 /UN4.6.4.5.31/PP36/22).

Preparation of isoproterenol and EVOO–VCO combination

The dose of isoproterenol used was 100 mg/kg body weight (20 mg/200 g body weight of rats) dissolved in 1 mL of 0.9% NaCl. The combination of EVOO and VCO was made in ratios of 1:1, 1:2 and 2:1, with an oral administration volume of 10 mL/kg rat body weight.

Experimental protocol

The rats were divided into five groups of six: (1) a healthy control group, (2) an ISO group (without any pre-treatment) and (3 - 5) the treatment groups, which received pre-treatment with EVOO and VCO in ratios of 1:1, 1:2 and 2:1, respectively. The treatments were given for 14 days, followed by ISO 100 mg/kg sub-cutaneous (s.c.) injection for 2 days in a row. After 24 hours following the last ISO injection, blood samples were withdrawn to analyse the levels of serum biomarkers for MI: CK-MB, LDH, AST and ALT. The blood samples were centrifuged at 3,000 rpm for 10 minutes to obtain serum. The obtained serum was prepared according to the instructions from the reagent kit to analyse the levels of CK-MB, LDH, AST and ALT using a spectrophotometry instrument (Humalyzer 3500). Surgical procedures were performed to remove the heart. Once removed, the hearts were rinsed in 0.9% NaCl, drained and weighed. The ratio of the organ weight to the body weight of rats was calculated to obtain the relative organ weight.

Histopathological examination

The rats' hearts were fixed in 10% formalin. After 48 hours, the tissue was vertically cut, processed in a tissue processor for 12 hours, and prepared into paraffin blocks. The tissue was sliced 4 - 5 μm thick using a microtome and floated in a water bath. The specimens were placed on glass slides and dried. After 2 hours, staining was performed using haematoxylin and eosin. Histopathological examination was carried out using a light microscope (Olympus®), and photo-micrographs were taken with a magnification of 200x. The histopathology score was used to obtain semi-quantitative data for the intensity of myocardial injury. The scores were determined by an anatomical pathologist who was blinded to the treatments given to the rats. A score of 0 indicated no damage in the observed tissue, a score of 1 indicated mild injury (< 25%), a score of 2 indicated moderate damage (26 - 50%), a score of 3 indicated severe damage (51 - 75%) and a score of 4 indicated massive damage (> 75%) [15].

Statistical analysis

The normality of the data was determined using Shapiro–Wilk analysis. The biomarker levels were compared using one-way ANOVA followed by Tukey's HSD test. The histopathological data were compared using the Kruskal-Wallis and Mann-Whitney U tests to determine significant differences between groups. The data are presented as mean ± standard deviation

(SD). The level of statistical significance was set at $p < 0.05$.

Results and Discussion

Chemical components

The GC-MS analysis of the EVOO used in this study revealed 55 peaks of chemical compounds (Table I). FAs, squalene and a-tocopherol were also found. The VCO had 49 peaks (Table II). Like EVOO, VCO is high in FAs. Based on the GC-MS analysis, the FA content of the EVOO used in this study consisted of long-chain fatty acids (LCFAs) (Table III). Of these, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were higher in EVOO than in VCO. In VCO, no SCFA content was found, but the MCFA content was much higher than that of EVOO. The LCFA content was lower in VCO than in EVOO. Different from EVOO, the VCO had a high SFA content compared to MUFAs and PUFAs.

Table I

The chemical compounds of the extra virgin olive oil used based on GC-MS analysis

No.	RT.	RM.	Component	Synonim	Rel. Area (%)
1.	3.772 - 4.068	$C_3H_8O_3$	Gliserin	Gliserin	0.06190
2.	3.955	$C_8H_{14}O_2$	Pentanoic acid, 2-propenyl ester	Velaric acid	0.01697
3	4.241	$C_5H_8O_2S$	4-Oxopentanethioic acid	4-Oxopentanethioic acid	0.00108
4.	4.891	$C_9H_{18}O_2$	Octanoic acid, methyl easter	Caprilic acid, methyl ester	0.00999
5.	6.173	$C_{11}H_{22}O_2$	Decanoic acid, methyl ester	Capric acid methyl ester	0.00979
6.	7.452	$C_{15}H_{24}$	α-farnesene	Trans,trans-.alpha.-Farnesene	0.00258
7.	7.54 - 7.615	$C_{13}H_{26}O_2$	Dodecanoic acid, methyl ester	Lauric acid methyl ester	0.08249
8.	8.802 - 8.86	$C_{15}H_{28}O_2$	Methyl myristoleate	Cis-9-tetradecanoic acid methyl ester	0.01155
9.	8.914 - 9.026	$C_{15}H_{30}O_2$	Methyl tetradecanoate	Tetradecanoic acid, methyl ester	0.09688
10.	9.424 - 9.591	$C_{19}H_{36}O_2$	9-Octadecanoic acid (Z)-, methyl ester	Oleic acid, methy ester, cis-	0.07185
11.	9.747	$C_{16}H_{32}O_2$	Pentadecanoic acid, methyl ester	Lauric acid	0.01121
12.	10.159 - 10.213	$C_{17}H_{32}O_2$	9-Hexadecanoic acid, methyl ester,(Z)-	Palmitoleic acid methyl ester	1.05613
13.	10.295 - 10.608	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	28.1459
14.	10.832 - 11.064	$C_{18}H_{34}O_2$	Cis-10-Heptadecenoic acid, methyl ester	Cis-10-Heptadecenoic acid, methyl ester	0.72335
15.	11.247 - 11.287	$C_{18}H_{36}O_2$	Heptadecanoic acid, methyl ester	Margaric acid, methyl ester	0.40585
16.	11.206 - 11.353	$C_{18}H_{36}O_2$	Hexadecanoaic acid, 14-methyl-, methyl ester	14-methylhexadecanoic acid methyl ester	0.04777
17.	11.414	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid methyl ester	0.14758
18.	11.462 - 11.523	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	trans-Vaccenic acid methyl ester	5.08255
19.	11.56 - 12.067	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-	Methyl trans-oleate	13.79887
20.	11.679 - 12.839	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, Methyl ester, Cis-	37.19101
21.	12.308	$C_{19}H_{38}O_2$	Methyl stearate	Stearic acid, methyl ester	3.14399
22.	12.455	$C_{20}H_{38}O_2$	Cis-10-Nonadecanoic acid, methyl ester	Cis-10-Nonadecanoic acid, methyl ester	0.29706
23.	12.54	$C_{20}H_{40}O_2$	Nonadecanoic acid, methyl ester	Methyl nonadecanoate	0.08822
24.	12.921	$C_{19}H_{36}O_3$	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	cis-9,10-Ethoxystearic Acid, methyl ester	0.04413
25.	12.995	$C_{21}H_{40}O_2$	Cis-11-Eicosenoic acid, methyl ester	Eicosenoic acid methyl ester, 11-(Z)-	1.70871
26.	13.118 - 13.149	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester	Methyl cis-11-eicosenoate	2.26083

No.	RT.	RM.	Component	Synonim	Rel. Area (%)
27.	13.213	$C_{25}H_{42}O_2$	Cyclopropanebutanoic acid, 2-[[2-[(2-pentylcyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]-, methyl ester	Butanoic acid	0.0109
28.	13.298	$C_{20}H_{38}O_2$	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	Methyl dihidrosterculate	0.049981
29.	13.39	$C_{20}H_{36}O_2$	Linoleic acid ethyl ester	Linoleic acid ethyl ester	0.09103
30.	13.444	$C_{20}H_{36}O_2$	Methyl 2-octylcyclopropene-1-octanoate	Sterculic acid methyl ester	0.0725
31.	13.567	$C_{19}H_{36}O_2$	10-Octadecenoic acid, methyl ester	10-Octadecenoic acid, methyl ester	0.00591
32.	13.676	$C_{19}H_{36}O_2$	16-Octadecenoic acid, methyl ester	16-Octadecenoic acid, methyl ester	0.00484
	14.873				
	15.305				
33.	13.73	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	0.09611
34.	14.084	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2-Glyceryl monooleate	0.10305
35.	14.135	$C_{23}H_{40}O_3$	11-(3,4-Dimethyl-5-pentyl-2-furyl)-dodecanoic acid, methyl ester	11-(3,4-Dimethyl-5-pentyl-2-furyl)-dodecanoic acid, methyl ester	0.00577
36.	14.169	$C_{19}H_{36}O_3$	Methyl 5-oxo-octadecanoate	5-Oxostearic acid methyl ester	0.02064
37.	14.257	$C_{23}H_{44}O_2$	13-Docosenoic acid, methyl ester, (Z)-	Erucic acid methyl	0.01851
38.	14.308	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2-Palmitoylglycerol	0.01476
39.	14.434	$C_{23}H_{46}O_2$	Docosanoic acid, methyl ester	Behenic acid methyl ester	0.73223
40.	14.614	$C_{19}H_{36}O_2$	14-Octadecenoic acid, methyl ester	14-Octadecenoic acid, methyl ester	0.00862
41.	15.101	$C_{24}H_{48}O_2$	Tricosanoic acid, methyl ester	Methyl tricosanoate	0.15563
42.	15.795	$C_{25}H_{50}O_2$	Tetracosanoic acid, methyl ester	Lignoceric acid methyl ester	0.35066
43.	16.254	$C_{27}H_{52}O_4Si_2$	9,12,15-Octadecatrienoic, 2,3-bis-[(trimethylsilyl)oxy] propyl ester, [Z,Z,Z]-	Monolinolenin TMS	0.00233
44.	16.51 - 16.57	$C_{30}H_{50}$	Squalene	Squalene	3.46622
45.	16.91	$C_{35}H_{70}$	17-Pentatriacontene	17-Pentatriacontene	0.01202
46.	17.121	$C_{27}H_{54}O_2$	Hexacosanoic acid, methyl ester	Cerotic acid methyl ester	0.04682
47.	17.189 - 17.689	$C_{26}H_{44}O_5$	Ethyl iso-allocholate	Cholic acid ethyl ester	0.01695
48.	17.322 - 21,066	$C_{27}H_{56}O_4Si_2$	1-Monolinoleylglycerol trimethylsilyl ether	Monoolein TMS	0.03142
49.	18.689	$C_{29}H_{50}O_2$	(+)-α-Tocopherol	(+)-α-Tocopherol	0.05603
50.	19.658	$C_{28}H_{48}O$	Campesterol	Campesterol	0.00841
51.	20.593	$C_{29}H_{50}O$	Sitosterol	Angelicin (Steroid)	0.19757
52.	20.764	$C_{30}H_{50}O$	Cholest-5-en-3-0l, 24-propulidene-, (3β)-	(E)-24-propylidenecholesterol	0.01523
53.	21.002	$C_{30}H_{50}O_2$	Betulin	Betulin	0.00586
54.	21.417	$C_{32}H_{52}O_3$	9,19-Cyclolanost-24-en-3-ol, acetat, (3β)-	9,19-Cyclolanost-24-en-3-ol, acetat, (3β)-	0.00854
55.	22.24	$C_{30}H_{48}O_5$	9,19-Cyclolanost-24-en-3-ol, methylene, (3β)-	Cimigol	0.06169
Total					100

Table II

The chemical compound of the virgin coconut oil used is based on GC-MS analysis

No.	RT.	RM.	Component	Synonim	Rel. Area (%)
1.	3.456 - 3.806	$C_7H_{14}O_2$	Hexanoic acid, methyl ester	Caproic acid methyl ester	1.54681
2.	3.942 - 4.262	$C_6H_{12}O_2$	Hexanoic acid	Caproic acid	0.25573
3.	4.911 - 5.02	$C_9H_{18}O_2$	Octanoic acid, methyl ester	Caprilic acid methyl ester	3.55331
4.	5.211 - 5.84	$C_8H_{16}O_2$	Octanoic acid	Caprilic acid	5.55028
5.	6.221 - 6.292	$C_{11}H_{22}O_2$	Decanoic acid, methyl ester	Capric acid methyl ester	4.31038

No.	RT.	RM.	Component	Synonim	Rel. Area (%)
6.	6.615 - 7.326	$C_{10}H_{20}O_2$	n-Decanoic acid	Capric acid	4.16892
7.	7.605 - 8.016	$C_{13}H_{26}O_2$	Dodecanoic acid, methyl ester	Lauric acid methyl ester	12.06083
8.	8.054 - 8.918	$C_{12}H_{24}O_2$	Dodecanoic acid	Lauric acid	18.47322
9.	8.993 - 9.319	$C_{15}H_{30}O_2$	Methyl tetradecanoate	Miristic acid methyl ester	9.49689
10.	9.435 - 10.101	$C_{14}H_{28}O_2$	Tetradecanoic acid	Miristic acid	8.79295
11.	10.224	$C_{18}H_{34}O_2$	Oleic acid	Eladoic acid	0.02006
12.	10.346 - 10.618	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	Palmitic acid methyl ester	7.48533
13.	10.642 - 10.982	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Palmitic acid	3.14541
14.	11.02 12.105 12.717	$C_{18}H_{36}O_2$	Octadecanoic acid	Stearic acid	0.531309
15.	11.482	$C_{19}H_{36}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid methyl ester	0.02808
16.	11.547 - 11.564 11.72 - 11.741	$C_{19}H_{36}O_2$	9-Octadecanoic acid (Z)-, methyl ester	Oleic acid methyl ester	3.58107
17.	11.618 11.669	$C_{19}H_{36}O_2$	9-Octadecanoic acid, methyl ester, (E)-	Elaidic acid	3.11044
18.	11.632	$C_{19}H_{36}O_2$	11-Octadecanoic acid, methyl ester	Trans veccanic acid methyl ester	1.30384
19.	11.771	$C_{15}H_{30}O_4$	Dodecanoic acid, 2,3-dihidroxypropyl ester	Laurin 1-mono	1.12757
20.	11.822 - 11.907	$C_{19}H_{38}O_2$	Methyl stearate	Stearic acid methyl ester	4.34627
21.	11.921	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	0.72384
22.	11.975	$C_{18}H_{34}O_2$	9-Octadecanoic acid, (E)-	Trans-oleic acid	0.42989
23.	11.996 - 12.383 13.155 13.431	$C_{18}H_{34}O_2$	Oleic acid	Oleic acid	1.01164
24.	12.785	$C_{19}H_{36}O_3$	Methyl 5 oxo-octadecanoate	Methyl 5 oxo-octadecanoate	0.00843
25.	12.843	$C_{19}H_{36}O_3$	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	Ricinoleic acid methyl ester	0.01373
26.	12.887	$C_{21}H_{40}O_2$	Cis-11-Eicosenoic acid, methyl ester	Cis-11-Eicosenoic acid, methyl ester	0.132
27.	12.945 - 13.019	$C_{17}H_{34}O_4$	Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Miristic acid beta monoglyseride	0.69048
28.	13.37	$C_{13}H_{26}O_6$	Xylitol, 1-O-octanoyl	Octanoil-o-xylitol	0.04892
29.	13.06	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester	Arracidic acid methyl ester	0.23068
30.	14.067	$C_{22}H_{46}O_3Si$	Hexadecanoic acid, 3-[(trimethylsilyl)oxy) propyl ester	3-[trimethylcilyn)oxy) prophyl palmitat	0.00585
31.	14.247	$C_{19}H_{38}O_4$	Octadecanoic acid, 9,10-dihidroxy-, methyl ester	Methyl 9,10 dihidroxystearate	0.01784
32.	14.325	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Palmiric acid, beta monogliseride	0.2536
33.	14.407	$C_{19}H_{38}O_4$	Docosanoic acid, methyl ester	Behenic acid methyl ester	0.05816
34.	14.601 14.73	$C_{13}H_{26}O_4$	Decanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Decanoic acid 2 monogliseride	0.13775
35.	14.665 14.764	$C_{15}H_{30}O_4$	Docosanoic acid, 2,3-dihydroxypropyl ester	Laurin acid alpha monogliseride	0.18336
36.	14.863 15.448	$C_{35}H_{68}O_5$	Hexadecanoic, 1-(hydroxymethyl)-1,2-ethenediyl ester	Dipalmitin	0.00583
37.	15.087	$C_{24}H_{48}O_2$	Methyl 21-methyldodosanoat	Methyl 21-methyldodosanoat	0.00514
38.	15.298	$C_{14}H_{26}F_2O_2$	2,2-Difluoroheptacosanoic acid	2,2-Difluoroheptacosanoic acid	0.00425
39.	15.591	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Olein 2-mono	0.13879
40.	15.74	$C_{21}H_{42}O_4$	Octadecanoic acid, 2,3-dihidroxypropyl ester	Stearin 1-mono	0.02981
41.	15.778	$C_{25}H_{50}O_2$	Tetracosanoic acid, methyl ester	Lignoceric acid methyl ester	0.05691

No.	RT.	RM.	Component	Synonim	Rel. Area (%)
42.	15.968 - 20.781	$C_{27}H_{52}O_5$	Dodecanoic acid, 1-(hydoxymethyl)-1,2-ethanediyl ester	Ethyl laurat	2.77783
43.	16.485	$C_{39}H_{50}$	Squalene	Squalene	0.01603
44.	17.118	$C_{18}H_{36}O_2$	Hexadecanoic acid, 14-methyl-, methyl ester	Montanic acid methyl ester	0.00466
45.	18.57	$C_{29}H_{54}O_6$	Dinonanoin monocaprylin	Dinonanoin monocaprylin	0.00421
46.	19.682	$C_{28}H_{48}O$	Campesterol	Campesterol	0.00237
47.	19.975	$C_{29}H_{48}O$	Stigmasterol	Stigmasterol	0.0031
48.	20.339	$C_{27}H_{50}O_6$	Glycerol tricaprylate	Caprilic acid trigliseride	0.06873
49.	21.478	$C_{23}H_{52}O_3$	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)-	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)-	000417
Total					100

Table III

The types of fatty acid content of EVOO and VCO

Types of Fatty Acid				
EVOO	SCFA	0.11%	SFA	35.99%
	MCFA	0.10%	MUFA	60.23%
	LCFA	99.77%	PUFA	3.71%
VCO	SCFA	-	SFA	89.49%
	MCFA	49.92%	MUFA	9.74%
	LCFA	50.07%	PUFA	0.77%

SCFA**:** short-chain fatty acids; MCFA: medium chain fatty acids: LCFA: long-chain fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Relative organ weights

The relative organ weight in the healthy control group was 4.15 ± 0.19 mg/g. The relative heart weight significantly increased in the ISO group, with an average of 6.03 ± 0.51 mg/g ($p < 0.0001$). Pre-treatment with a combination of EVOO and VCO in all ratios maintained the relative organ weights of the rats; hence, the weights were similar to those in the healthy control group (Figure 1).

Biomarker analysis

Four cardiac serum biomarkers were used to confirm the presence of MI: CK-MB, LDH, AST and ALT. In the healthy control group, the mean CK-MB, LDH, AST and ALT levels were 29.59 ± 8.39 U/L, 108.50 ± 26.53 U/L, 105.20 ± 5.86 U/L and 59.55 ± 9.78 U/L, respectively. The levels of these biomarkers, except for ALT, were significantly increased with ISO injection without pretreatment ($p < 0.05$). In contrast, the CK-MB and LDH levels of rats treated with an EVOO–VCO combination at 1:1, 1:2 and 2:1 were not significantly elevated after ISO injection; the values were not significantly different from that of the healthy control. Furthermore, the EVOO–VCO combination in a 1:2 and 2:1 ratio, but not 1:1, significantly inhibited the increase in AST levels ($p < 0.05$) (Figure 2).

Histopathological analysis

Representative histopathological features of the heart tissue can be seen in Figure 3, which was scored based on the intensity of injury in Table IV. The healthy control group showed no sign of myocardial tissue damage. In contrast, rats in the ISO group without EVOO–VCO pretreatment had evident myocardial injury characterized by diffused necrotic areas, haemorrhage and inflammation. In the treatment groups, the administration of EVOO–VCO in any ratio before the injection of ISO reduced the damage to myocardial tissue, but only the ratio of 2:1 led to superior improvement of the myocardial tissue. With EVOO–VCO (2:1), the occurrence of necrosis was only scattered in small areas, with no excessive bleeding, and the infiltration of inflammatory cells was not prominent.

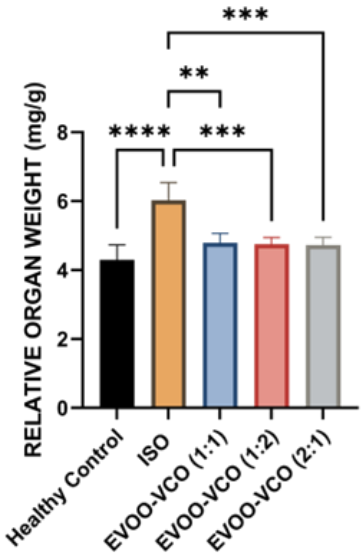

Figure 1.

Comparison of the relative weights of rats' heart among treatment groups

(**) indicates $p < 0.001$ (***) indicates $p < 0.001$, (****) indicates $p < 0.0001$

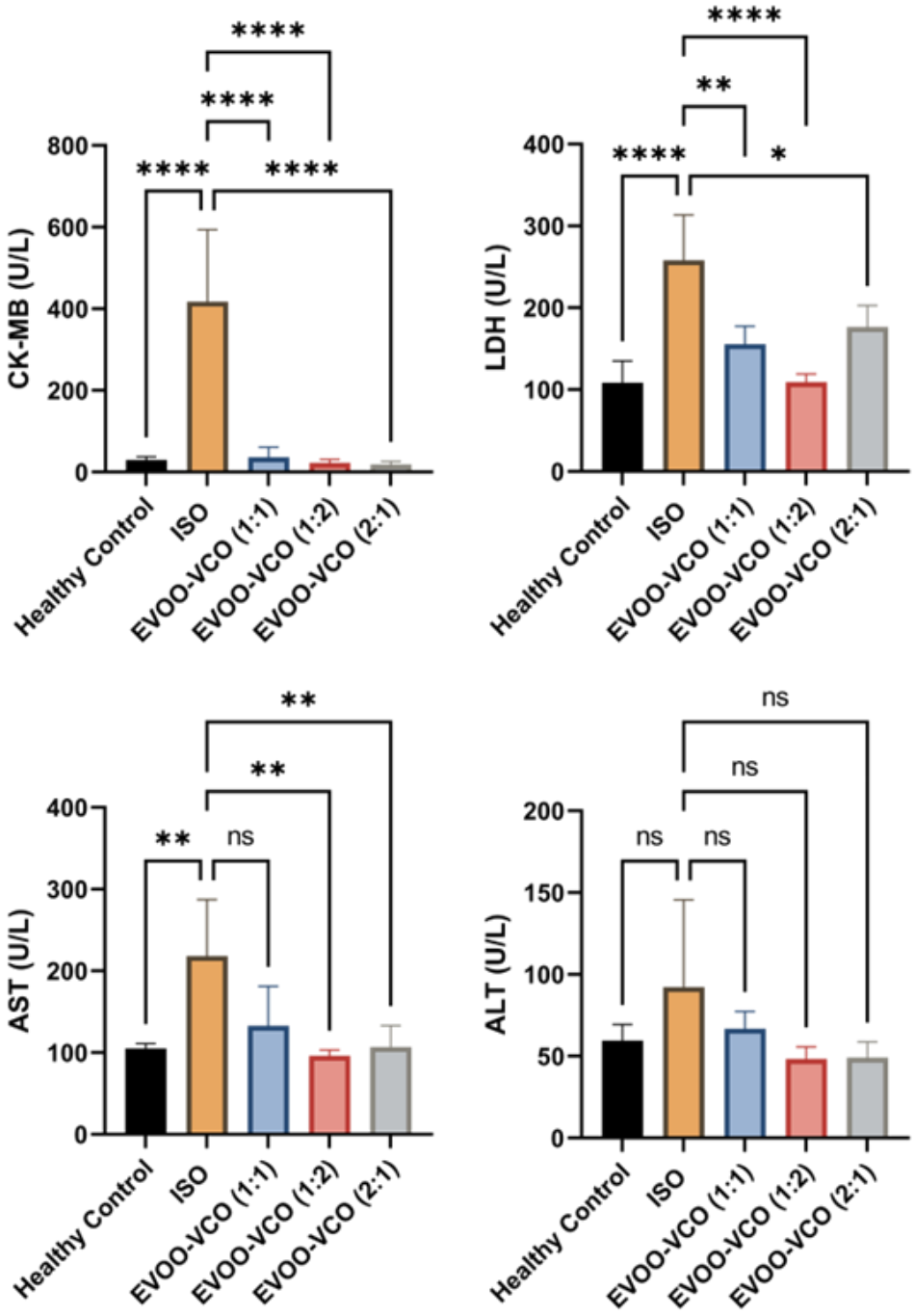

Figure 2.

Comparison of biomarker levels among rat treatment groups

a) CK-MB, b) LDH, c) AST and d) ALT; (*) indicate $p < 0.05$, (**) indicate $p < 0.01$, (****) indicates $p < 0.0001$ and (ns) indicates non-significant from the ISO group

Table IV

The score of myocardial damage based on the presence of necrosis, inflammation and haemorrhage

Group	Necrosis	Inflammation	Haemorrhage
Healthy group	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ISO group	2.33 ± 0.58	2.33 ± 0.58	1.00 ± 0.00
EVOO:VCO (1:1) + ISO	1.67 ± 0.58	1.33 ± 0.58	1.00 ± 0.00
EVOO:VCO (1:2) + ISO	1.33 ± 0.58	1.33 ± 0.58	0.67± 0.58
EVOO:VCO (2:1) + ISO	1.00 ± 0.00	1.00 ± 1.00	0.00 ± 0.00

Score 0: no damage; score 1: a mild injury (< 25%); score 2: moderate damage (26% - 50%); score 3: severe damage (51% - 75%); score 4: massive damage (> 75% - 100%)

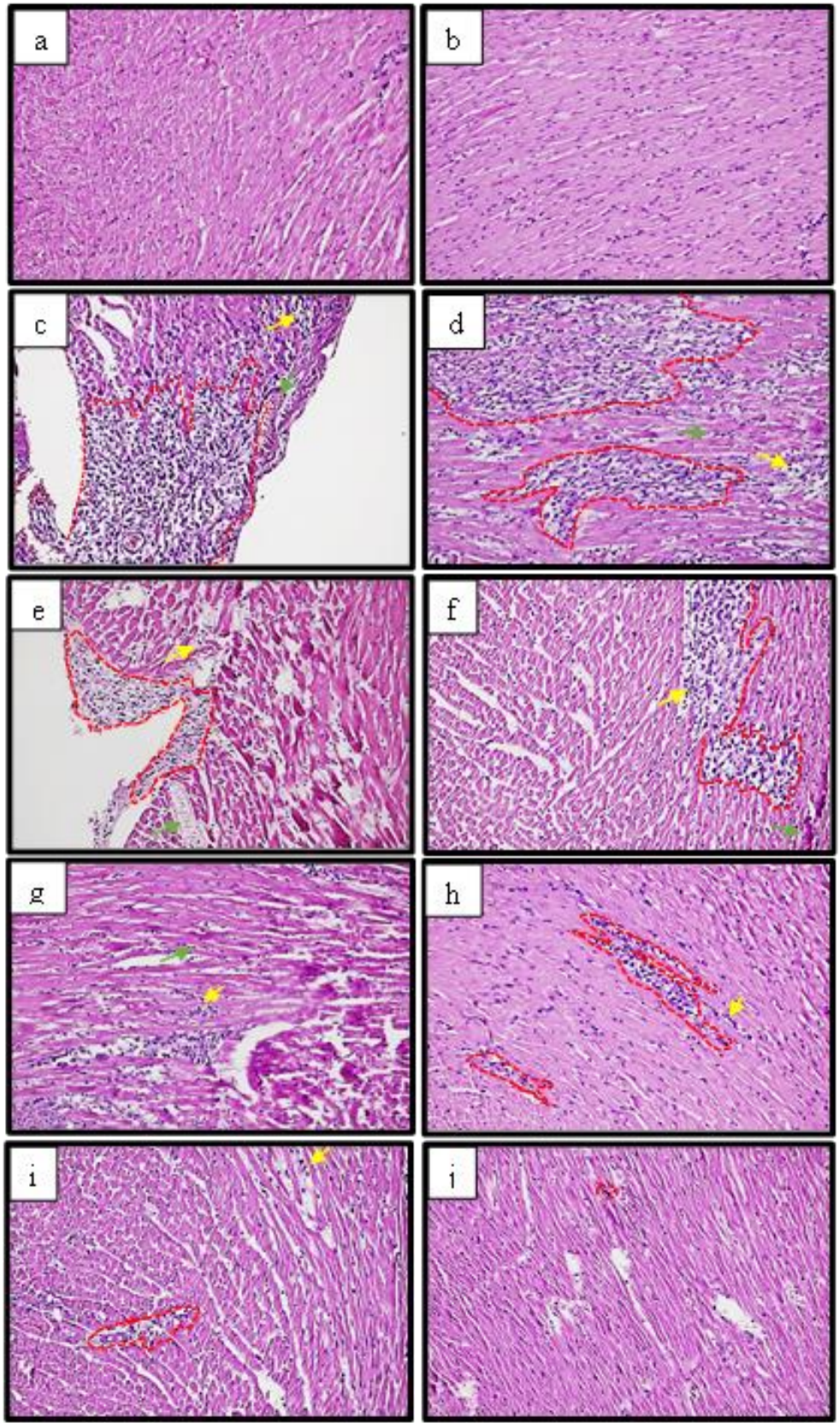

Figure 3.
Comparison of histopathological features of cardiac tissue
a-b: healthy group; c-d: ISO group 100 mg/kg BW; e-f: group EVOO:VCO ratio 1:1; g-h: group EVOO:VCO 1:2; i-j: group EVOO:VCO 2:1. Red dotted line (necrosis); yellow arrows (inflammation); and green arrows (haemorrhage)

This study was conducted to determine the cardio-protective effect of various combinations (either 1:1, 1:2, or 2:1) of EVOO and VCO in MI rats induced by ISO. Different ratios of EVOO–VCO combinations may result in different cardioprotective effects. Previously, Utari *et al*. (2022) showed that an EVOO–VCO combination in a 1:1 ratio was superior in inhibiting myocardial injury in DOX-induced rats compared to either EVOO or VCO alone. The EVOO–VCO combination was believed to have a synergistic effect in inhibiting cellular damage and death [13]. EVOO and VCO are plant-based oils with very different chemical compounds and FA profiles. FAs can be classified based on the length of the carbon chains (SCFAs, MCFAs and LCFAs) [16], or the presence of double bonds in their carbon chains (SFAs, MUFAs and PUFAs) [17]. In this study, EVOO predominantly contained LCFAs, whereas the FA content of VCO primarily consisted of MCFAs and LCFAs. A high LCFA content in EVOO can function as an energy source and maintain heart contractile function [18]. The use of LCFA as the energy source of cardiac metabolism can result in better adenosine triphosphate (ATP) production when compared to glucose. A previous study demonstrated that the incidence of heart failure is more common when energy sources are derived from glucose [19, 20]. The rich MCFA content in VCO, apart from acting as an energy source, can molecularly increase and activate GPCR84 and play a role in the immune system and inflammatory pathways, which results in the inhibition of cAMP [21-23]. Therefore, the initial administration of the EVOO–VCO combination can inhibit the inflammation that occurs in ISO-induced MI rats. In addition, MCFAs such as caprylic acid may act as anti-inflammatory agents, inhibiting the toll-like receptor-4 (TRL4) activation and inhibiting the NF-κB pathway [24]. By combining EVOO and VCO, the FA content can optimally prevent heart damage and reduce mortality in patients with cardiovascular disease [25]. According to a meta-analysis study by Schwingshackl and Hoffmann in 2014, MUFAs may be beneficial to reduce cardiovascular events and death [26].

ISO can cause MI through several mechanisms [2, 27, 28]. The administration of ISO can cause an increase in Ca^{2+} levels in the myocardium, which results in an overstimulation of myocytes, with increased contractility and oxygen demand, causing ATP depletion [5]. The occurrence of excessive intracellular calcium affects the activation of phospholipase and protease enzymes that inhibit Na^{+}/K^{+}ATPase, which causes an increase in intracellular Na^{+} and Ca^{2+}, prompting cellular dysfunction and cardiotoxicity [29]. The alteration of myocardial metabolism also triggers an inflammatory reaction. Moreover, infarct-size reducing properties can be related to anti-inflammatory activity of a drug [30]. The neutrophil accumulation in the myocardium reaches its peak after 24 hours [31]. Monocytes and macrophages predominate in cellular infiltration. In this phase, an increase occurs in the production of several cytokines such as IL-1, IL-18, IL-6 and TNF [32]. Inflammatory cells release proteolytic enzymes and ROS, which damage the myocytes. MI is characterized by irreversible damage to cardiomyocytes and results in necrosis [18]. In this study, MI was noticeable after two injections of ISO at the dose of 100 mg/kg, characterized by moderate to severe necrosis in a large area of myocardium. The histological structures of cardiomyocytes were significantly different in the hearts of the rats receiving EVOO–VCO treatment at a ratio of 2:1. Although mild injury was still present, the areas of necrosis and inflammation were significantly reduced. The occurrence of myocardial injury triggers the release of cardiac biomarkers to the systemic circulation. Hence, CK-MB, LDH and AST were significantly elevated after ISO injection in the ISO group [27]. Based on the results of this study, the FA contained in the EVOO–VCO combination could inhibit cellular damage, presumably by providing an energy source that can be effectively metabolized to preserve myocardial energy when ISO induces overstimulation of beta-receptors. Another explanation may come from the anti-inflammatory effect of EVOO and VCO [20, 21], which helps reduce the pro-inflammatory response [24]. Interestingly, the 2:1 ratio elicited better protection against MI. This may indicate that the LCFA and MUFA content of EVOO was required in a higher proportion than the MCFAs contained in VCO. EVOO is also known to contain more antioxidants compared to VCO, which may also explain this result.

Conclusions

The combination of EVOO and VCO in ratios of 1:1, 1:2 and 2:1 inhibited the increase in the levels of CK-MB, LDH and AST, indicating a cardio-protective effect against ISO-induced MI. In addition, the combination of EVOO and VCO, especially in a ratio of 2:1, provided superior protection against myocardial necrosis and inflammation. Further studies are needed to measure the prooxidant and antioxidant levels in rats treated with ISO and EVOO–VCO to confirm whether this cardioprotective effect is related to its antioxidant activity.

Conflict of interest

The authors declare no conflict of interest.

References

1. Neumar RW, Otto CW, Link MS, Kronick SL, Shuster M, Callaway CW, Kudenchuk PJ, Ornato JP, McNally B, Silvers SM, Passman RS, White RD, Hess EP, Tang W, Davis D, Sinz E, Morrison LJ, Part 8: adult advanced cardiovascular life support:

2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation*, 2010; 122(18 Suppl 3): S729-S767.
2. Garg M, Khanna D, Exploration of pharmacological interventions to prevent isoproterenol-induced myocardial infarction in experimental models. *Ther Adv Cardiovasc Dis.*, 2014; 8(4): 155-169.
3. Shaik AH, Rasool SN, Kareem MA, Krushna GS, Akhtar PM, Devi KL, Maslinic acid protects against isoproterenol-induced cardiotoxicity in albino Wistar rats. *J Med Food*, 2012; 15(8): 741-746.
4. Szymanski MW, Singh DP, Isoproterenol. [Updated 2022 Sep 27], In: StatPearls, Treasure Island (FL): StatPearls Publishing, 2022; 1-15.
5. Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Yao L, Nagai Y, Fujisawa Y, Miyatake A, Abe Y, Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. *Cardiovasc Res.*, 2005; 65(1): 230-238.
6. Ojha S, Nandave M, Arora S, Arya DS, Effect of isoproterenol on tissue defense enzymes, hemodynamic and left ventricular contractile function in rats. *Ind J Clin Biochem.*, 2010; 25(4): 357-361.
7. Topliss JG, Clark AM, Ernst E, Hufford CD, Johnston GAR, Rimoldi JM, Weimann BJ, Natural and synthetic substances related to human health (IUPAC Technical Report). *Pure Appl Chem.*, 2002; 74(10): 1957-1985.
8. Kamri AM, Ningsih R, Wiyani L, Wiyani Lastri, Rahman S, Sistematic review: The effect of virgin coconut oil as a source of antioxidant. *IJRAR.*, 2021; 8(4): 213-219.
9. Yubero-Serrano EM, Lopez-Moreno J, Gomez-Delgado F, Lopez-Miranda J, Extra virgin olive oil: More than a healthy fat. *Eur J Clin Nutr.*, 2019; 72: 8-17.
10. Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, Ros E, Corella D, Recondo J, Gomez-Gracia E, Fiol M, Lapetra J, Serra-Majem, L, Muñoz MA, Pintó X, Lamuela-Raventós RM, Basora J, Buil-Cosiales P, Sorlí JV, Ruiz-Gutíerrez V, Martínez JA, Salas-Savadó J, Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Med.*, 2014; 12(78): 1-11.
11. Zamora-Zamora F, Martínez-Galiano JM, Gaforio JJ, Delgado-Rodríguez M, Effects of olive oil on blood pressure: A systematic review and meta-analysis. *Grasas y Aceites*, 2018; 69(4): 1-9.
12. Kamisah Y, Periyah V, Lee KT, Noor-Izwan N, Nurul-Hamizah A, Nurul-Iman BS, Subermaniam K, Jaarin K, Azam A, Faizah O, Qodriyah HMS, Cardioprotective effect of virgin coconut oil in heated palm oil diet-induced hypertensive rats. *Pharm Biol.*, 2015; 53(9): 1243-1249.
13. Utari AU, Djabir YY, Palinggi BP, A combination of virgin coconut oil and extra virgin olive oil elicits superior protection against doxorubicin cardiotoxicity in rats. *Turk J Pharm Sci.*, 2022; 19(2): 138-144.
14. Rus I, Tertiș M, Paşcalău V, Pavel C, Melean B, Suciu M, Moldovan C, Topală T, Popa C, Săndulescu R, Cristea C, Simple and fast analytical method for the evaluation of the encapsulation and release profile of doxorubicin from drug delivery systems. *Farmacia*, 2021; 69(4): 670-681.
15. Gibson-Corley KN, Olivier AK, Meyerholz DK, Principles for valid histopathologic scoring in research. *Vet Pathol.*, 2013; 50(6): 1007-1015.
16. Palm CL, Nijholt KT, Bakker BM, Westenbrink BD, Short-Chain fatty acids in the Metabolism of heart failure – rethinking the fat stigma. *Front Cardiovasc Med.*, 2022; 9: 1-9.
17. Savchenko L, Ivanauskas L, Jarukas L, Georgiyants V, Determination and comparison of fatty acids composition of apricot and peach oils. *Farmacia*, 2021; 69(5): 941-947.
18. Labarthe F, Gélinas R, Rosiers CD, Medium-chain fatty acids as metabolic therapy in cardiac disease. *Cardiovasc Drugs Ther.*, 2008; 22: 97-106.
19. Angelini A, Saha PK, Jain A, Jung SY, Mynatt RL, Pi X, Xie L, PHDs/CPT1B/VDAC1 axis regulates long-chain fatty acid oxidation in cardiomyocytes. *Cell Rep.*, 2021; 37(1): 1-14.
20. Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS, Stanley WC, Myocardial fatty acid metabolism in health and disease. *Physiol Rev.*, 2010; 90(1): 207-258.
21. Roopashree PG, Shetty SS, Kumari SN, Effect of medium chain fatty acid in human health and disease. *J Funct Foods*, 2021; 87: 1-11.
22. Toit ED, Browne L, Irving-Rodgers H, Massa HM, Fozzard N, Jennings MP, Peak IR, Effect of GPR84 deletion on obesity and diabetes development in mice fed long chain or medium chain fatty acid rich diets. *Eur J Nutr.*, 2018; 57: 1737-1746.
23. Wang J, Wu X, Simonavicius N, Tian H, Ling L, Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. *JBC.*, 2006; 281(84): 34457-34464.
24. Zhang X, Xue C, Xu Q, Zhang Y, Li H, Li F, Liu Y, Guo C, Caprylic acid suppresses inflammation *via* TLR4/NF-κB signaling and improves atherosclerosis in ApoE-deficient mice. *Nutr Metab (Lond.)*, 2019; 16(40): 1-16.
25. Balta I, Stef L, Pet I, Iancu T, Stef D, Corcionivoschi N, Essential Fatty Acids as Biomedicines in Cardiac Health. *Biomedicines*, 2021; 9(10): 1-24.
26. Schwingshackl L, Hoffmann G, Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids Health Dis.*, 2014; 13: 1-15.
27. Afroz R, Tanvir EM, Karim N, Hossain MS, Alam N, Gan SH, Khalil MI, Sundarban Honey Confers Protection against Isoproterenol-Induced Myocardial Infarction in Wistar Rats. *Biomed Res Int.*, 2016; 2016: 1-10.
28. Mohamed ME, Abduldaium MS, Younis NS, Cardioprotective effect of linalool against isoproterenol-induced myocardial infarction. *Life*, 2021; 11(2): 1-17.
29. Djabir Y, Dobson GP, Hemodynamic rescue and ECG stability during chest compressions using adenosine and lidocaine after 8-minute asphyxial hypoxia in the rat. *Am J Emerg Med.*, 2013; 31(11): 1539-1545.
30. Ramos K, Combs AB, Acosta D, Role of calcium in isoproterenol cytotoxicity to cultured myocardial cells. *Biochem Pharmacol.*, 1984; 33(12): 1989-1992.

31. Kain V, Halade GV, Role of neutrophils in ischemic heart failure. *Pharmacol Ther.*, 2020; 205: 1-22.
32. Turillazzi E, Pomara C, Bello S, Neri M, Riezzo I, Fineschi V, Meaning of different forms of structural myocardial injury, immune response and timing of infarct necrosis and cardiac repair. *Curr Vasc Pharmacol.*, 2015; 13(1): 6-19.