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The Comparison of The Effects of Novel Antioxidant Compounds Ebselen, Isorhamnetin and Genistein on Cardiovascular System

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ABSTRACT

Objective: This study evaluated the effects of antioxidants Ebselen (EBS), Isorhamnetin (ISO), and Genistein (GEN) on oxidative stress (OS), hemodynamic parameters, and cardiovascular histopathology in rats.

Methods: Thirty two adult *Wistar albino* rats were randomized into four groups (n=8/group): control (vehicle), EBS (15 mg/kg/day), ISO (5 mg/kg/day), and GEN (10 mg/kg/day) for 7 days. Hemodynamic parameters, biochemical markers, and histopathological changes in cardiac and aortic tissues were analyzed. Caspase-3 immunoreactivity assessed apoptosis.

Results: EBS significantly increased diastolic and mean blood pressure versus control (p<.05). All treatments reduced cardiac malondialdehyde, indicating OS attenuation. GEN exhibited the strongest antioxidant effect, with improved glutathione and catalase activity. EBS paradoxically reduced aortic glutathione and increased malondialdehyde, suggesting pro-oxidant potential. Histopathology revealed myocardial degeneration in EBS and ISO groups, while GEN preserved cardiac structure. ISO and GEN enhanced aortic glutathione, but EBS showed mixed effects. Serum alanine aminotransferase elevation in ISO and GEN indicated mild hepatotoxicity. Caspase-3 immunoreactivity was elevated in EBS-treated hearts (p<.001), implying pro-apoptotic effects.

Conclusion: GEN demonstrated robust antioxidant and cardioprotective effects, making it a promising therapeutic candidate. ISO showed vascular benefits but requires further safety evaluation. EBS, despite reducing OS, may induce adverse cardiac effects, warranting caution. These findings underscore the need for targeted antioxidant therapies in cardiovascular diseases, balancing efficacy and safety.

Keywords: Antioxidant, Ebselen, Isorhamnetin, Genistein, Cardiovascular

Introduction

Oxidative stress (OS) is intricately linked to the cardiovascular system, playing a pivotal role in the onset and progression of various cardiovascular (CV) diseases. Understanding and managing OS through lifestyle changes and therapeutic interventions could be crucial in preventing and treating these conditions. Under normal conditions, reactive oxygen species (ROS) are involved in essential cellular signaling and function. However, excessive ROS production can lead to OS, contributing to cellular damage and death, particularly in CV tissues (Dubois-Deruy et al., 2020; Wang & Kang, 2020; Sack et al., 2017). OS is implicated in the development and progression of several CV conditions, including atherosclerosis, heart failure, myocardial infarction, ischemia-reperfusion injury, and cardiac arrhythmias. It affects the CV system by promoting endothelial dysfunction, inflammation, and tissue damage (Dubois-Deruy et al., 2020; Wang & Kang, 2020; Kibel et al., 2020; Farías et al., 2017; Dhalla et al., 2000). Conditions such as diabetes, obesity, smoking, and pollution exacerbate OS, increasing the risk of CV diseases. These factors contribute to increased ROS production, which can lead to cardiac dysfunction and other CV issues (Niemann et al., 2017; Rotariu et al., 2022). Antioxidant therapies are being explored to mitigate OS in CV diseases.

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These include traditional antioxidants, novel therapeutic strategies like miRNA and nanomedicine, and specific pharmacological treatments targeting ROS-related pathways (Wang & Kang, 2020; Farías et al., 2017; Dhalla et al., 2000).

Ebselen (EBS) exhibits significant protective effects on the CV system by enhancing antioxidant defenses, reducing OS, and preventing cellular apoptosis. These properties make it a promising candidate for treating and preventing CV conditions associated with oxidative damage. EBS mimics the activity of glutathione peroxidase, catalyzing the reduction of hydrogen peroxide and organic peroxides, which helps in protecting cellular components from oxidative damage (Hermenegildo et al., 1990; Azad & Tomar, 2014). It effectively inhibits OS in endothelial cells, which is crucial for preventing endothelial dysfunction, a precursor to CV diseases (Ahwach et al., 2015).

Isorhamnetin (ISO) exhibits a wide range of protective effects on the CV system, including reducing cardiac hypertrophy, protecting against myocardial injury, and preventing thrombosis. Its mechanisms involve antioxidant activity, inhibition of apoptosis, and modulation of key signaling pathways, making it a promising candidate for CV disease prevention and treatment. It enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, reducing OS in CV tissues. ISO modulates apoptosis-related proteins, decreasing pro-apoptotic markers and increasing anti-apoptotic markers, thereby protecting heart cells from damage (Xu et al., 2020; Zhao et al., 2018; Abudalo et al., 2024).

Genistein (GEN) exhibits several CV benefits, including improvements in lipid profiles, blood pressure (BP), and glycemic control, along with protective effects against cardiac dysfunction. The antioxidant properties of GEN help in reducing OS and inflammation, which are key contributors to CV diseases (Jafari et al., 2023; Farruggio et al., 2019). GEN has been shown to improve lipid profiles by reducing total cholesterol and low-density lipoprotein cholesterol, and it may also lower systolic BP in certain populations, particularly postmenopausal women with metabolic syndrome (Jafari et al., 2023; Amerizadeh et al., 2022; Squadrito et al., 2013).

The aim of this study was to investigate the effects of antioxidant EBS, ISO and GEN on hemodynamic parameters and OS markers in cardiovascular tissue.

Methods

Inonu University Experimental Animal Ethics Committee approval (Date: 28.07.2016 Protocol no: 2016/A-103) was obtained for this experimental study. This study was performed in accordance with the principles of "Guide for the Care and Use of the Laboratory Animals" and animal rights were protected (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Adult and healthy *Wistar albino* rats were used for the study. The rats were obtained from Inonu University Experimental Animal Research Center. They were housed in temperature (21±2 °C) and humidity (60±5%) controlled rooms under 12:12 hours light/dark cycle. The rats were divided into four groups by simple randomized and double blind method.

-Control group (n=8): 0.5 mL volume of dimethyl sulfoxide:ethanol (DMSO:EtOH 2:1) used as vehicle solvent was administered intraperitoneally (i.p.) for 7 days.

-EBS group (n=8): 15 mg/kg EBS was administered as a single dose i.p. daily for 7 days.

-ISO group (n=8): 5 mg/kg ISO was administered as a single dose i.p. daily for 7 days.

-GEN group (n=8): 10 mg/kg GEN was administered as a single dose i.p. daily for 7 days.

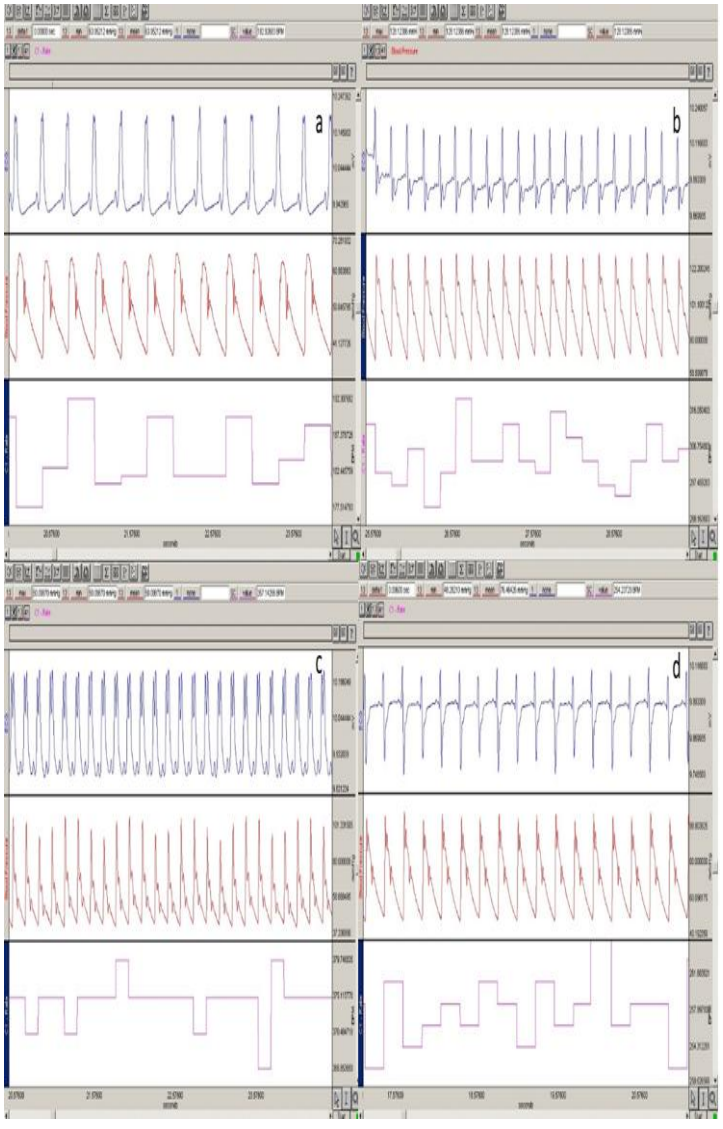
EBS (Sigma-Aldrich USA; CAS number: 60940-34-3), ISO (Selleck Biotechnology GmbH USA; CAS number: 480-19-3) and GEN (Selleck Biotechnology GmbH USA; CAS number: 446-72-0) doses, routes of administration and dose intervals were determined with reference to previous studies (Kalayci et al., 2005; Sun et al., 2013; Tan et al., 2022).

Under 1.2 g/kg ethyl carbamate (Urethane®, Sigma-Aldrich USA; CAS number: 51-79-6) anesthesia carotid arteries of rats were cannulated to determine CV function and electrocardiography (ECG) recordings were obtained by attaching ECG electrodes to the chest. Heart rate (HR) and BP were also determined. After the recordings were completed, histopathologic and biochemical analyses (oxidant and antioxidant parameters) were performed on heart and aortic tissue samples. Lactate dehydrogenase (LDH), creatine kinase (CK), myoglobin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in blood samples.

Measurement of Hemodynamic Parameters

In the Cardiovascular Research Laboratory in the Department of Medical Pharmacology, hemodynamic parameters of the rats were recorded with the Biopac MP-100 Data Acquisition system shown in Figure 1. Left carotid arteries were cannulated and systolic, diastolic and mean BP, HR and ECG changes were recorded using 3-lead ECG electrodes. During this period, the body temperature of the rats was kept in the range of 36-37° C. After the completion of the experimental protocol, blood samples were obtained from the inferior vena cava and heart and thoracic aorta tissues were obtained by autopsy. Both arrhythmia variety and PR, QRS and QT durations were calculated from the computer recordings according to Lambeth Convention criteria (Walker et al., 1988).

Figure 1.
Branch block in control group (a), T negativity in EBS group (b), branch block in ISO group (c), ST depression in GEN group (d).



Biochemical Analysis

Malondialdehyde Measurement

Malondialdehyde (MDA) was measured spectrophotometrically by the Uchiyama and Mihara method (Mihara & Uchiyama, 1978). This method is based on the treatment of lipid peroxidation product MDA with thiobarbituric acid at 95°C. After the treatments applied to the samples, the n-butanol phase on the top of the tubes was read at 535 and 520 nm wavelength using a spectrophotometer. The values obtained from the samples were expressed in nmol/mg protein.

Glutathione Measurement

Glutathione (GSH) concentration in the homogenate was measured spectrophotometrically by the Ellman method (Ellman, 2022). Homogenate samples were mixed with 10 mM 5,5-dithiobis (2-nitrobenzoic acid) in tubes containing a mixture of 17.5 M ethylene diamine tetraacetic acid and 100 mM potassium phosphate buffer (pH 7.5). 0.4 mM β-Nicotinamide adenine dinucleotide 2'-phosphate (NADPH) and 0.5 units of glutathione reductase (GR) were added to initiate the reaction. The absorbance of the samples was measured at 410 nm wavelength with a spectrophotometer 5 minutes after the reaction started. A standard curve for GSH concentration was constructed using the measured absorbances. The values obtained from the samples were expressed in nmol/mg protein.

Protein Amount Measurement

In order to calculate the data for the other methods studied, protein content analysis was performed by the modified Lowry method (Waterborg & Matthews, 1994). Folin reagent was added to the alkaline copper-protein solution containing the samples and the mixture was vortexed. With these procedures, the folin reagent was reduced before disintegration. The absorbances of the standards and samples were read at a wavelength of 750 nm. A standard curve was constructed using the measured absorbances. The values obtained from the samples were expressed in µg/mL.

Superoxide Dismutase Activity Measurement

SOD activity was measured spectrophotometrically by the method of Sun et al. (1988). The SOD activity assay is based on the principle of inhibition of Nitroblue Tetrazolium Chloride reduction. The absorbance value of formazone is inversely proportional to SOD activity when measured at 560 nm wavelength. The values obtained from

the samples were expressed in U/mg protein.

Catalase Activity Measurement

CAT activity was measured spectrophotometrically by Aebi's method (Aebi, 1984). The assay is based on the determination of hydrogen peroxide (H_2O_2) decomposition rate or rate constant (k, s-1) at a wavelength of 240 nm. Decomposition starts instantaneously by adding sample supernatants to the fresh H_2O_2 solution present in the test tube. The change caused by this decomposition is monitored in the spectrophotometer. These changes are monitored for a specified period of time and the rate constant is found with the activity calculated by the absorbance change. The values obtained from the samples were expressed in U/mg protein.

Total Antioxidant Status and Total Oxidant Status

Total antioxidant status (TAS) and Total oxidant status (TOS) measurements were performed with Rel Assay Diagnostics (MEGA TIP San. Tic. Ltd. Sti.) brand kits specially produced for TAS and TOS measurements in accordance with the steps in the manual.

TAS was measured spectrophotometrically according to the Erel method using the supernatant obtained (Erel, 2004). The method is based on the oxidation of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) molecule to $ABTS^+$ molecule in the presence of H2O2. In this method, ABTS radical is used. The ABTS radical loses its green-blue color according to the antioxidant capacity and the amount of antioxidant in the environment. Data are obtained by measuring the green-blue color change at 660 nm wavelength. There is an inverse correlation between the amount of antioxidants in the samples and the green-blue color change. In the method, the reaction rate is adjusted with Trolox. The unit is nmol/mg protein.

TOS was measured colorimetrically according to the Erel method using the supernatant obtained (Erel, 2005). The method is based on the oxidation of the ferrous ion-o-dianisidine complex of oxidants to ferric ion. The presence of glycerol molecule in the reaction medium is necessary for oxidation to occur. In acidic medium, ferric ions form a colored complex with xylenol orange chromogen. At a wavelength of 530 nm, the intensity of this complex is measured with a spectrophotometer. The unit is nmol/mg protein.

Histopathological Analysis

The heart and vascular tissues taken at the end of the experiment were fixed in 10% formaldehyde. After tissue tracing, 4–5 µm thick sections were taken from the paraffin blocks prepared. The sections were stained with hematoxylin-eosin staining method to determine the general morphologic structure.

Heart sections were evaluated for congestion-hemorrhage and cardiomyocyte degeneration (dense eosinophilic cytoplasm, pyknotic nuclei). Ten randomly selected areas were examined and the areas were scored according to the degree of histologic changes; 0: no change, 1: mild, 2: moderate, 3: severe change (Tan et al., 2022).

In the evaluation for the aorta, the whole area was examined on 2 separate sections for each sample and tunica intima-media thickness was measured in 10 randomly selected areas from each section.

Analyses were performed using a Leica DFC-280 research microscope and Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Immunohistochemical Analysis

For immunohistochemical analysis, deparaffinized and rehydrated sections were placed in a pressure cooker and boiled in 0.01 M citrate (pH 6.0) for 15–20 min. The sections were treated with 3% hydrogen peroxide for 12 min to block endogenous peroxidase enzyme activity. Protein block (ultra V block) was applied to the sections washed with Phosphate buffered saline (PBS) for 5 min. The sections were then incubated with primary antibody (Caspase-3, Thermo Scientific) at 37 ˚C for 60 min. The tissues were washed with PBS and treated with biotinylated secondary antibody at 37 ˚C for 10 min. After this process, the sections were incubated with streptavadin peroxidase at 37 ˚C for 10 min. The chromogen-treated sections were then stained with hematoxylin and covered with water-based sealer.

Staining was scored semiquantitatively based on the prevalence (0: no staining, 1: 1%–25%, 2: 26%–50%, 3: 51%–75%, 4: 76%–100%) and severity (0: none, +1: mild, +2: moderate, +3: severe) of immunoreactivity. Total staining score was obtained by calculating prevalence × severity (Ozbek et al., 2013).

Statistical Analysis

Statistical analyses were performed with SPSS statistical software program (SPSS for Windows version 26). Normally distributed data were analyzed by one-way ANOVA test followed by posthoc Tukey test. For non-normally distributed data, Kruskal-Wallis analysis of variance, a nonparametric test, was used for the overall comparison of groups in terms of all variables, and comparisons between paired groups were made by Mann-Whitney U test with Bonferroni correction. Data were expressed as median (minimum - maximum), median (quartiles) and arithmetic mean ± standard deviation. p<.05 was considered significant.

Results

Hemodynamic Findings

Hemodynamic findings are presented in table 1. When hemodynamic parameters were compared, there was no statistically significant difference in HR, systolic BP, PR, QRS and QT intervals between the groups, whereas there was a statistically significant difference in diastolic and mean BP. In the EBS group, diastolic and mean BP were significantly increased compared to the control and ISO groups (p<.05).

Table 1.
Distribution of hemodynamic parameters according to groups

Parameters	Control Group	EBS Group	ISO Group	GEN Group	*p*
Heart rate (beat/min)	280 (185-309)	296.5 (215-384)	250 (230-375)	220 (165-261)	.07
Sistolic BP (mm-Hg)	94 (66-137)	118.5 (97-132)	107 (84-127)	124 (74-141)	.1088
Diastolic BP (mm-Hg)	42 (29-65)[a]	67 (45-75)[b]	45 (35-56)	53 (42-66)	**.0146**
Mean BP (mm-Hg)	60 (43-88)[a]	86.5 (64-95)[b]	62 (51-83)	73 (56-93)	**.0143**
PR interval (ms)	36 (30-46)	31 (30-48)	36 (30-44)	38 (34-44)	.6327
QRS interval (ms)	78 (74-104)	82 (56-96)	86 (72-100)	88 (64-120)	.6306
QT interval (ms)	122 (108-150)	126 (98-138)	118 (102-130)	134 (96-154)	.3046

a: Different compared to EBS group (p < .05); b: Different by ISO group (p < .05); c: Different according to GEN group (p < .05).
* BP: blood pressure, EBS: ebselen, ISO: isorhamnetin, GEN: genistein.

Arrhythmia variability is presented in table 2. Two of the eight rats in the control group had branch block, but no arrhythmia like extrasistol, ST depression or T negativity. Two of the eight rats in the EBS group had ST depression, one had T negativity, but no arrhythmia or branch block. One of the eight rats in the ISO group had branch block, but no arrhythmia, ST depression or T negativity were observed. One of the eight rats in the GEN group had ST depression, one had branch block, while arrhythmia and T negativity were not observed.

Table 2.
Changes in heart rhythm

	Arrhythmia	ST depression	T negativity	Block
Control Group (n:8)	0	0	0	2
EBS Group (n:8)	0	2	1	0
ISO Group (n:8)	0	0	0	1
GEN Group (n:8)	0	1	0	1

***** EBS: ebselen, ISO: isorhamnetin, GEN: genistein

Biochemical results

Parameters associated with oxidative stress in cardiac tissue are presented in Table 3. When SOD, TAS and TOS parameters were compared between the groups, there was no statistically significant difference, whereas there was a significant difference between the groups in MDA, GSH and CAT parameters. When MDA values were compared, there was a statistically significant decrease in EBS, ISO and GEN groups compared to the control group (p<.05). When MDA values are compared, there is a statistically significant decrease in ISO and GEN groups compared to EBS group (p<.05). When MDA values are compared, there is a statistically significant decrease in GEN group compared to ISO group (p<.05). When GSH values are compared, there is a statistically significant decrease in EBS, ISO and GEN groups compared to the control group (p<.05). When GSH values are compared, there is a statistically significant increase in ISO and GEN groups compared to EBS group (p<.05). When CAT values are compared, there is a statistically significant decrease in EBS, ISO and GEN groups compared to the control group (p<.05). When CAT values are compared, there is a statistically significant decrease in

ISO and GEN groups compared to EBS group (p<.05). When CAT values are compared, there is a statistically significant decrease in GEN group compared to ISO group (p<.05).

Table 3.
Distribution of cardiac parameters according to groups

Parameters	Control Group	EBS Group	ISO Group	GEN Group	*p*
MDA (nmol/mg protein)	0.19 (0.02)[a,b,c]	0.15 (0.01)[b,c]	0.08 (0.07)[c]	0.05 (0.02)	**<.001**
GSH (nmol/mg protein)	7.59 (1.11)[a,b,c]	5.34 (0.73)[b,c]	6.03 (0.56)	6.2 (1.03)	**<.001**
SOD (U/mg protein)	71.29 (17.71)	75.45 (13.66)	67.26 (21.1)	68.3 (9.64)	.666
CAT (U/mg protein)	33.64 (2.99)[a,b,c]	31.75 (5.31)[b,c]	26.89 (1.58)[c]	27.88 (2.13)	<.001
TAS (nmol/mg protein)	0.14 (0.04)	0.13 (0.03)	0.13 (0.06)	0.2 (0.11)	.1371
TOS (nmol/mg protein)	0.58 (0.43)	0.44 (0.2)	0.3 (0.14)	0.45 (0.18)	.1411

a: There is a statistically significant difference according to the EBS group (p<.05). b: There is a statistically significant difference according to ISO group (p<.05). c: There is a statistically significant difference according to GEN group (p<.05).

* MDA: malondialdehyde, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, TAS: total antioxidant status, TOS: total oxidant stastus, EBS: ebselen, ISO: isorhamnetin, GEN: genistein.

The findings of oxidative parameters in aortic tissue are presented in Table 4. When oxidative stress parameters in aortic tissue were compared between the groups, MDA, GSH, SOD, TAS and TOS parameters were statistically significant, while CAT parameter was not statistically significant. When MDA parameters in aortic tissue were compared, there was a significant increase in EBS and GEN groups compared to the control group (p<0.05). When MDA parameters in aortic tissue were compared, there was a significant increase in EBS group compared to ISO and GEN groups (p<.05). When GSH parameters in aortic tissue were compared, there was a statistically significant increase in EBS, ISO and GEN groups compared to the control group (p<.05). When GSH parameters in aortic tissue were compared, there was a statistically significant increase in ISO and GEN groups compared to EBS group (p<.05). When SOD parameters in aortic tissue were compared, there was a statistically significant increase in EBS and ISO groups compared to the control group (p<.05). When SOD parameters in aortic tissue were compared, there was a statistically significant increase in EBS and ISO groups compared to GEN group (p<0.05). When TAS parameters in aortic tissue were compared, there was a statistically significant decrease in EBS group compared to ISO and GEN groups (p<.05). When TOS parameters in aortic tissue were compared, there was a statistically significant increase in the ISO group compared to the EBS and GEN groups (p<.05).

Table 4.
Distribution of aortic parameters according to groups

Parameters	Control Group	EBS Group	ISO Group	GEN Group	*p*
MDA (nmol/mg protein)	1.07 (1.62)[a,c]	0.36 (0.78)[b,c]	2.66 (0.58)	2.9 (0.74)	**<.001**
GSH (nmol/mg protein)	19.92 (2.79)[a,b,c]	26.02 (5.61)[b,c]	72.31 (3.94)	58.99 (18.69)	**<.001**
SOD (U/mg protein)	10.33 (4.4)[a,b]	15.46 (2.5)[c]	23.79 (17.59)[c]	11.37 (3.23)	**.0197**
CAT (U/mg protein)	19.65 (4.31)	20.65 (7.55)	21.72 (11.01)	22.02 (3.59)	.6128
TAS (nmol/mg protein)	0.47 (0.64)	0.34 (0.02)[b,c]	0.59 (0.16)	0.53 (0.14)	**.0111**
TOS (nmol/mg protein)	25.14 (10.85)	15.93 (1.43)[b]	27.06 (10.41)	16.41 (5.49)[b]	**.0239**

a: There is a statistically significant difference according to the EBS group (p<.05). b: There is a statistically significant difference according to ISO group (p<.05). c: There is a statistically significant difference according to GEN group (p<.05).

* MDA: malondialdehyde, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, TAS: total antioxidant status, TOS: total oxidant stastus, EBS: ebselen, ISO: isorhamnetin, GEN: genistein.

The results of biochemical analysis of serum are presented in Table 5. When LDH, CK, myoglobin and AST parameters were compared, there was no significant difference between the groups, while there was a statistically significant difference between the groups in ALT parameter. When ALT prameter in serum was compared between the groups, there was a statistically significant increase in ISO and GEN groups compared to the control group (p<.05). The increase in the Gene group was statistically significantly higher than in the EBS group

(p<.05).

Table 5.

Distribution of serum biochemistry variables according to groups

Parameters	Control Group	EBS Group	ISO Group	GEN Group	*p*
LDH (U/L)	474 (151-1368)	604.5 (130-997)	958 (273-2396)	653.5 (277-1285)	.4113
CK (U/L)	912.5 (329-1945)	522.5 (167-6304)	1033 (549-2283)	1365 (486-2343)	.5497
Myoglobin (ng/mL)	337.8 (95.3-967.5)	236.2 (42.2-1022)	496.5 (117.7-1200)	273.2 (101.9-1156.5)	.4799
AST (U/L)	126 (61-198)	113.5 (50-234)	129 (93-435)	120.5 (104-319)	.7751
ALT (U/L)	7.5 (6-14)[b,c]	11.5 (6-21)[c]	13.5 (7-16)	16 (9-27)	.0115

a: There is a statistically significant difference according to the EBS group (p<.05). b: There is a statistically significant difference according to ISO group (p<.05). c: There is a statistically significant difference according to GEN group (p<.05).

* LDH: lactate dehydrogenase, CK: creatine kinase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, EBS: ebselen, ISO: isorhamnetin, GEN: genistein.

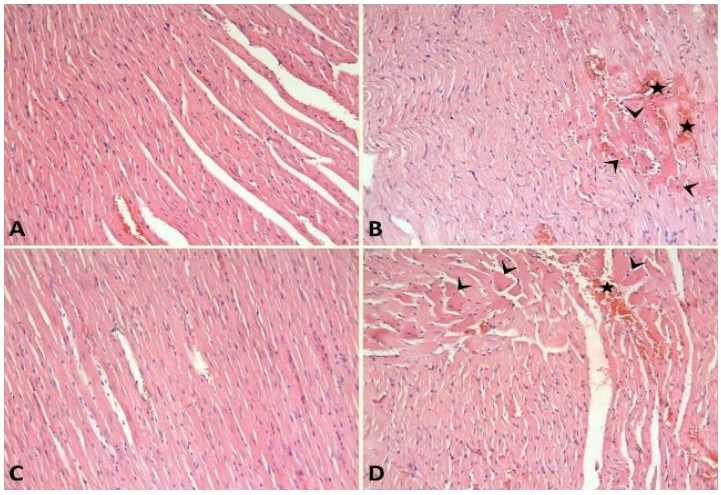
Histopatohological and Immunohistochemical Results

Heart

In general morphologic evaluation, the myocardium was evaluated for congestion-hemorrhage and degenerated cardiomyocytes. In the control group, except for mild changes, the heart tissue had a normal histologic appearance (Figure 2 A). It was observed that the heart tissue of the GEN group had a similar appearance to the control group (Figure 2 A and c). On the other hand, there was a significant increase in the density of degenerated cardiomyocytes in the EBS and ISO groups compared to the control group (p=.014) (Figure 2 B and D). A slight increase in congestion-hemorrhage was also observed in these groups.

Figure 2.

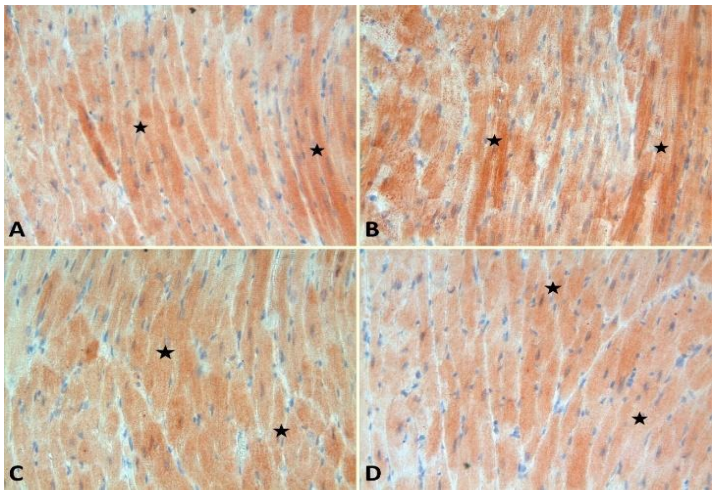
*Appearance of myocardium in control (A), EBS (B), GEN (C) and ISO (D) groups. Degenerated cardiomyocytes (arrowheads) and hemorrhagic areas (stars) in the myocardium of EBS and ISO groups. H-Ex20. * EBS: ebselen, ISO: isorhamnetin, GEN: genistein.*



In the evaluation of caspase-3 immunoreactivity in cardiomyocytes, GEN and ISO groups were found to be similar to the control group (p>.05), while the severity of immunoreactivity was significantly higher in the EBS group compared to the control group (p<.001) ((Figure 3).

Figure 3.

Caspase-3 immunoreactivity (brownish staining stars) in cardiomyocytes of control (A), EBS (B), GEN (C) and ISO (D) groups. It is noteworthy that the severity of immunoreactivity is higher in the Ebselen group compared to the other groups. Caspase-3 immunostaining x40.



Histologic evaluation results and caspase-3 immunoreactivity are given in Table 6.

Aorta

The vessel wall had a normal histologic appearance in all experimental groups. Tunica intima-media thicknesses were 122.68±20.19 µm in the control group, 106.44±20.05 µm in the EBS group, 123.08±20.20 µm in the GEN group

and 93.01±21.19 µm in the ISO group. In terms of tunica intima-media thickness, GEN group was similar to the control group, whereas tunica intima-media thickness was found to be decreased in EBS and ISO groups compared to the control group (p<.05) (Figure 4).

In the evaluation of caspase-3 immunoreactivity in vascular smooth muscle cells, the severity of immunoreactivity was significantly lower in EBS and ISO groups compared to the control group (p=.005) (Figure 5).

Tunica intima-media thickness and caspase-3 immunoreactivity are given in Table 7.

Figure 4.

Appearance of the vessel wall in control (A), EBS (B), GEN (C) and ISO (D) groups. Decreased tunica intima-media thickness is observed in the EBS and ISO groups. Double-headed arrows indicate tunica intima-media thickness. H-E x20.

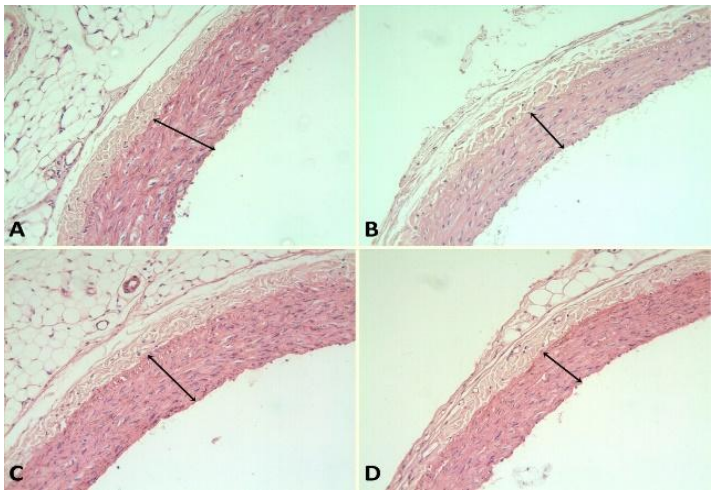
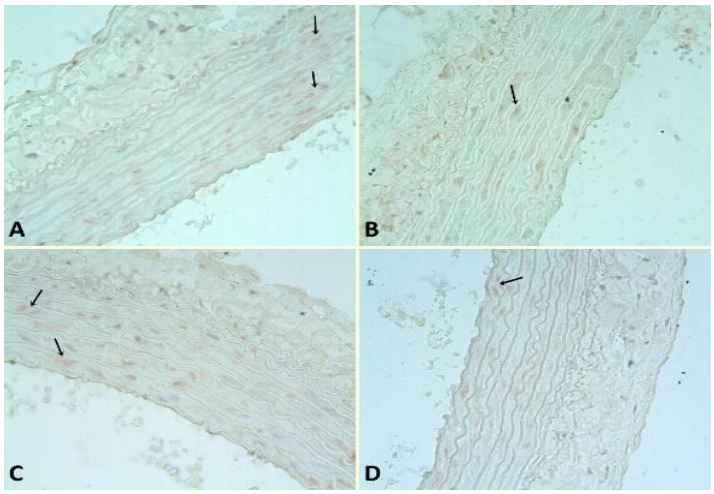


Figure 5.

Caspase-3 immunoreactivity in vascular smooth muscle cells (arrows). Control (A), EBS (B), GEN (C), ISO (D). Caspase-3 immunostaining x40



. **Table 6.**

Hemorrhage, cardiomyocyte degeneration and caspase-3 immunoreactivity

Groups	Hemorrhage	Cardiomyocyte degeneration	Caspase-3 immunoreactivity
Control	0.0 (0.0-2.0)	0.0 (0.0-1.0)	3.0 (1.0-9.0)
EBS	0.0 (0.0-2.0)	0.0 (0.0-2.0)[a]	6.0 (3.0-9.0)[b]
ISO	0.0 (0.0-3.0)	0.0 (0.0-2.0)[a]	3.0 (1.0-8.0)
GEN	0.0 (0.0-2.0)	0.0 (0.0-1.0)	2.0 (1.0-8.0)

[a] Statistical increase compared to the control group (p=.014).

Table 7.

Tunica intima-media thickness and caspase-3 immunoreactivity

Groups	TIM thickness	Caspase-3 immunoreactivity
Control	122.68 ± 20.19	0.0 (0.0-2.0)
EBS	106.44 ± 20.05[a]	0.0 (0.0-1.0)[b]
ISO	123.08 ± 20.20	0.0 (0.0-2.0)
GEN	93.01 ± 21.19[a]	0.0 (0.0-1.0)[b]

[a] Statistical decrease compared to the control group (p<.001).
[b] Statistical decrease compared to the control group (p=.005).
* TIM: tunica intima-media, EBS: ebselen, ISO: isorhamnetin, GEN: genistein.

Discussion

This study investigated the effects of three antioxidant compounds -EBS, ISO, and GEN- on OS markers, hemodynamic parameters, and histopathological changes in CV tissues. The results provide valuable insights into the potential cardioprotective roles of these compounds while also highlighting their varying degrees of efficacy and safety.

In a study involving rats exposed to chronic intermittent hypoxia, EBS treatment resulted in a significant decrease in mean BP, suggesting its role in reducing hypertension induced by OS. EBS has been noted to increase nitric oxide levels in certain contexts, which can lead to vasodilation and reduced BP (Moya et al., 2016). In our study EBS administration significantly increased diastolic and mean BP compared to control and ISO groups, while ISO and GEN had no such effect. The studies reviewed do not support the notion that ebselen increases mean and diastolic blood pressure in rats. Instead, ebselen appears to have a protective role against hypertension, primarily through its antioxidant properties and modulation of nitric oxide levels.

This aligns with prior reports suggesting EBS may modulate vascular tone, though the mechanism remains unclear. Increased BP could reflect enhanced vascular resistance or altered nitric oxide bioavailability, as EBS's GSH peroxidase-like activity might influence redox-sensitive pathways regulating vasomotor function. Conversely, ISO and GEN, which improved cardiac antioxidant status, did not affect BP, indicating distinct mechanisms of action. While previous research suggests that EBS enhances antioxidant defenses and reduces OS, its impact on BP warrants further investigation. Unlike EBS, neither ISO nor GEN exhibited significant hemodynamic changes, suggesting they may not adversely affect cardiovascular function in this regard (Hermenegildo et al., 1990; Azad & Tomar, 2014).

The analysis of OS markers revealed that all three antioxidants reduced MDA levels in cardiac tissue compared to the control group, confirming their ability to mitigate lipid peroxidation. However, the GEN group exhibited the greatest reduction in MDA levels, suggesting superior antioxidant activity in cardiac tissue. The reduction in MDA levels by GEN is often associated with its antioxidant properties. GEN enhances the activity of antioxidant enzymes such as SOD and glutathione peroxidase, which help mitigate OS. It also modulates pathways like the Nrf2/HO-1 pathway, which plays a crucial role in cellular defense against oxidative damage (Moya et al., 2016; Jia et al., 2019). In aortic tissue, EBS and GEN were associated with increased MDA levels, raising concerns about potential pro-oxidative effects under certain conditions. GEN exhibited the strongest effect, followed by ISO and EBS, consistent with their known roles in scavenging ROS and upregulating antioxidant enzymes (Xu et al., 2020; Zhao et al., 2018; Abudalo et al., 2024; Farruggio et al., 2019; Amerizadeh et al., 2022; Squadrito et al., 2013). However, EBS paradoxically decreased GSH and CAT levels, suggesting a potential pro-oxidant effect or accelerated GSH utilization. In a study on radiocontrast media-induced hepatotoxicity, EBS decreased CAT levels when combined with the media, suggesting a protective mechanism against oxidative damage (Basarslan et al., 2013). EBS can both increase and decrease CAT activity in rats, depending on the dose and the specific OS context. Lower doses may enhance CAT activity, while higher doses or certain stress conditions may lead to a decrease. This highlights the importance of dosage and context in the use of EBS for modulating OS responses. This contrasts with ISO and GEN, which preserved GSH and CAT activity, indicating more sustainable antioxidant support. The reduction in CAT activity across all groups may reflect compensatory downregulation due to decreased ROS burden, though this requires further validation.

GSH levels were significantly lower in the EBS group compared to ISO and GEN, suggesting that EBS might deplete cellular antioxidant reserves. Conversely, ISO and GEN increased GSH levels, reinforcing their potential as protective agents. Similarly, CAT activity was significantly reduced in the EBS group, which may indicate an insufficient adaptive response to OS.

Serum ALT levels were elevated in ISO and GEN groups, signaling potential hepatotoxicity, particularly with GEN. This aligns with prior studies noting GEN's dose-dependent liver effects (Amerizadeh et al., 2022; Squadrito et al., 2013). However, cardiac injury markers (LDH, CK, myoglobin) remained unchanged, suggesting that the observed myocardial degeneration did not progress to necrosis within the 7-day study period.

EBS and ISO induced cardiomyocyte degeneration and congestion, despite reducing oxidative stress. This discrepancy may reflect off-target effects, such as mitochondrial dysfunction or altered calcium homeostasis. Conversely, GEN preserved myocardial architecture, consistent with its protective role in ischemia-reperfusion injury (Farruggio et al., 2019). GEN has been shown to protect against doxorubicin-induced cardiotoxicity in rats. It reduces cardiomyocyte autophagy and apoptosis through the ERK/STAT3/c-Myc signaling pathway, thereby preventing cardiac dysfunction and pathological remodeling (Wu et al., 2024). In a model of hypertension induced by NG-Nitroarginine methyl ester (L-NAME), GEN prevented cardiac remodeling and dysfunction. It reduced OS and inflammation, which are key contributors to cardiomyocyte degeneration (Poasakate et al., 2021). GEN has demonstrated protective effects against cardiac inflammation and OS in diabetic rats. It improved cardiac function and reduced structural damage in the myocardium, indicating its potential to mitigate cardiomyocyte degeneration (Gupta et al., 2015). Caspase-3 immunoreactivity was elevated in EBS-treated hearts, suggesting pro-apoptotic effects, whereas ISO and GEN had no significant impact. The studies reviewed do not support the notion that EBS increases caspase-3 activity in rats. Instead, EBS appears to have protective effects against apoptosis by inhibiting caspase-3 activation in various models of toxicity and injury. In a study on rat hippocampal astrocytes, EBS did not activate caspase-3, suggesting that its effects on cell viability and mitochondrial function do not involve caspase-3 activation (Santofimia-Castaño et al., 2013). The studies provided do not report any instances where genistein did not alter caspase 3 activity in rats. Instead, they highlight that genistein can either reduce or increase caspase 3 activity depending on the context, such

as the dosage and the specific physiological conditions being studied. GEN has been shown to reduce caspase 3 activity in various contexts. For instance, genistein-3'-sodium sulfonate, a derivative of genistein, decreased caspase 3 activity in rat models of cerebral ischemia, suggesting a protective effect against neuronal injury (Li et al., 2017). Similarly, GEN reduced caspase 3 activity in the pancreas of diabetic rats, indicating its role in decreasing apoptosis (Yousefi et al., 2017). In contrast, high doses of GEN increased the levels of cleaved caspase 3 in rat brain tissue, indicating an induction of apoptosis (Choi & Lee, 2004). This suggests that the effect of genistein on caspase 3 may depend on the dosage and specific conditions of the study. In aortic smooth muscle, EBS and ISO reduced caspase-3 activity, indicating vascular protection. In human brain microvascular endothelial cells, ISO reduced the expression and activity of caspase-3 and caspase-8, indicating its role in inhibiting apoptosis through both intrinsic and extrinsic pathways (Li et al., 2016). While ISO has demonstrated the ability to reduce caspase-3 activity in various cell types, including macrophages, endothelial cells, and cardiomyocytes, there is no direct evidence from the provided studies regarding its effect on caspase-3 in vascular smooth muscle cells.

These findings highlight the dual nature of EBS, which may protect against oxidative damage while promoting apoptosis in cardiomyocytes—a critical consideration for therapeutic applications. The histopathological analysis showed that while GEN and ISO preserved myocardial structure, EBS was associated with increased cardiomyocyte degeneration and caspase-3 immunoreactivity. This suggests that, despite its antioxidant properties, EBS may trigger apoptotic pathways in cardiac tissue. On the other hand, GEN and ISO did not show significant adverse effects on cardiomyocyte integrity, aligning with their known cardioprotective roles in reducing apoptosis (Xu et al., 2020; Zhao et al., 2018; Abudalo et al., 2024; Farruggio et al., 2019).

A limitation of this study is that it was conducted in an animal model, and further research is necessary to determine whether these findings translate to human subjects. Additionally, longer treatment durations and different dosages should be explored to fully understand the potential benefits and risks of these compounds.

Conclusion

These findings suggest that while all three compounds exhibit antioxidant effects, their impact on CV health varies. GEN appears to be the most beneficial, with strong antioxidant properties and minimal adverse effects (Jafari et al., 2023; Gupta et al., 2015; Poasakate et al., 2021). ISO also shows promise, but its impact on tunica intima-media thickness requires further study (Xu et al., 2020; Abudalo et al., 2024). EBS, despite its potent antioxidant capacity, may have adverse effects on cardiomyocyte integrity, indicating that its use in cardiovascular therapy should be approached cautiously (Azad & Tomar, 2014; Santofimia-Castaño et al., 2013).

Overall, the results highlight the importance of selective antioxidant therapy in CV disease (Farías et al., 2017; Dhalla et al., 2000). The fact that the effects of EBS, ISO and GEN on the CV system are being studied comparatively for the first time emphasizes the originality of the study. While GEN and ISO show potential for clinical application (Farruggio et al., 2019; Zhao et al., 2018), further research is needed to assess the safety profile of EBS (Ahwach et al., 2015; Hermenegildo et al., 1990). Future studies should explore molecular mechanisms underlying these effects to optimize antioxidant-based therapeutic strategies for CV diseases (Sack et al., 2017; Rotariu et al., 2022).

Availability of data and material: All data generated or analysed during this study are included in this published article (and its Supplementary Information files).
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References

Abudalo, R., Gammoh, O., Altaber, S., Bseiso, Y., Qnais, E., Wedyan, M., Oqal, M., & Alqudah, A.. (2024). Mitigation of cisplatin-induced cardiotoxicity by isorhamnetin: Mechanistic insights into oxidative stress, inflammation, and apoptosis modulation. *Toxicology Reports*, *12*, 564–573.

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105*, 121–126.

Ahwach, S. M., Thomas, M., Onstead-Haas, L., Mooradian, A. D., & Haas, M. J. (2015). The glutathione mimic ebselen inhibits

oxidative stress but not endoplasmic reticulum stress in endothelial cells. *Life Sciences*, *134*, 9–15.

Amerizadeh, A., Asgary, S., Vaseghi, G., & Farajzadegan, Z. (2022). Effect of genistein intake on some cardiovascular risk factors: An updated systematic review and meta-analysis. *Current Problems in Cardiology*, *47*(9), 100902.

Azad, G. K., & Tomar, R. S. (2014). Ebselen, a promising antioxidant drug: Mechanisms of action and targets of biological pathways. *Molecular Biology Reports*, *41*(8), 4865–4879.

Basarslan, F., Yilmaz, N., Davarci, I., Akin, M., Ozgur, M., Yilmaz, C., & Ulutas Turker, K. (2013). Effects of ebselen on radiocontrast media-induced hepatotoxicity in rats. *Toxicology and Industrial Health*, *29*(8), 746–752.

Choi, E. J., & Lee, B. H. (2004). Evidence for genistein-mediated cytotoxicity and apoptosis in rat brain. *Life Sciences*, *75*(4), 499–509.

Dhalla, N. S., Temsah, R. M., & Netticadan, T. (2000). Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension*, *18*(6), 655–673.

Dubois-Deruy, E., Peugnet, V., Turkieh, A., & Pinet, F. (2020). Oxidative stress in cardiovascular diseases. *Antioxidants*, *9*(9).

Ellman, G. L. (2022). Reprint of: Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *726*, 109245.

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, *37*(4), 277–285.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, *38*(12), 1103–1111.

Farías, J. G., Molina, V. M., Carrasco, R. A., Zepeda, A. B., Figueroa, E., Letelier, P., & Castillo, R.L. (2017). Antioxidant therapeutic strategies for cardiovascular conditions associated with oxidative stress. *Nutrients*, *9*(9).

Farruggio, S., Raina, G., Cocomazzi, G., Librasi, C., Mary, D., Gentilli, S., et al. (2019). Genistein improves viability, proliferation and mitochondrial function of cardiomyoblasts cultured in physiologic and peroxidative conditions. *International Journal of Molecular Medicine*, *44*(6), 2298–2310.

Gupta, S. K., Dongare, S., Mathur, R., Mohanty, I. R., Srivastava, S., Mathur, S., & Grossini, E. (2015). Genistein ameliorates cardiac inflammation and oxidative stress in streptozotocin-induced diabetic cardiomyopathy in rats. *Molecular and Cellular Biochemistry*, *408*(1–2), 63–72.

Hermenegildo, C., Nies, E., Monsalve, E., Puertas, F. J., Higueras, V., & Romero, F. J. (1990). Some aspects of cardiac antioxidant defence: Ebselen (PZ 51) treatment increases glutathione peroxidase activity in the rat heart. *Biochemical Society Transactions*, *18*(6), 1193–1194.

Jafari, S., Shoghi, M., & Khazdair, M. R. (2023). Pharmacological effects of genistein on cardiovascular diseases. *Evidence-Based Complementary and Alternative Medicine*, *2023*, 8250219.

Jia, Q., Wang, Y., Liu, X., Ma, S., & Yang, R. (2019). [Effects of genistein on Nrf2/HO-1 pathway in myocardial tissues of diabetic rats]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, *44*(8), 850–856.

Kalayci, M., Coskun, O., Cagavi, F., Kanter, M., Armutcu, F., Gul, S., & Acikgoz, B. (2005). Neuroprotective effects of ebselen on experimental spinal cord injury in rats. *Neurochemical Research*, *30*(3), 403–410.

Kibel, A., Lukinac, A. M., Dambic, V., Juric, I., & Selthofer-Relatic, K. (2020). Oxidative stress in ischemic heart disease. *Oxidative Medicine and Cellular Longevity*, *2020*, 6627144.

Li, L., Xue, J., Liu, R., Li, X., Lai, L., Xie, J., Huang, Z., & Huang, C. (2017). Neuroprotective effects of genistein-3'-sodium sulfonate on focal cerebral ischemia in rats. *Neuroscience Letters*, *646*, 43–48.

Li, W., Chen, Z., Yan, M., He, P., Chen, Z., & Dai, H. (2016). The protective role of isorhamnetin on human brain microvascular endothelial cells from cytotoxicity induced by methylglyoxal and oxygen-glucose deprivation. *Journal of Neurochemistry*, *136*(3), 651–659.

Mihara, M., & Uchiyama, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, *86*(1), 271–278.

Moya, E. A., Arias, P., Varela, C., Oyarce, M. P., Del Rio, R., & Iturriaga, R. (2016). Intermittent hypoxia-induced carotid body chemosensory potentiation and hypertension are critically dependent on peroxynitrite formation. *Oxidative Medicine and Cellular Longevity*, *2016*, 9802136.

National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press.

Niemann, B., Rohrbach, S., Miller, M. R., Newby, D. E., Fuster, V., & Kovacic, J. C. (2017). Oxidative stress and cardiovascular risk: Obesity, diabetes, smoking, and pollution: Part 3 of a 3-part series. *Journal of the American College of Cardiology*, *70*(2), 230–251.

Ozbek, E., Simsek, A., Ozbek, M., & Somay, A. (2013). Caloric restriction increases internal iliac artery and penil nitric oxide synthase expression in rat: Comparison of aged and adult rats. *Archivio Italiano di Urologia, Andrologia: Organo Ufficiale della Società Italiana di Ecografia Urologica e Nefrologica*, *85*(3), 113–117.

Poasakate, A., Maneesai, P., Rattanakanokchai, S., Bunbupha, S., Tong-Un, T., & Pakdeechote, P. (2021). Genistein prevents nitric oxide deficiency-induced cardiac dysfunction and remodeling in rats. *Antioxidants*, *10*(2).

Rotariu, D., Babes, E. E., Tit, D. M., Moisi, M., Bustea, C., Stoicescu, M., Radu, A.F., Vesa, C.M., Behl, T., Bungau, A.F., & Bungau, S.G. (2022). Oxidative stress – Complex pathological issues concerning the hallmark of cardiovascular and metabolic disorders. *Biomedicine & Pharmacotherapy*, *152*, 113238.

Sack, M. N., Fyhrquist, F. Y., Saijonmaa, O. J., Fuster, V., & Kovacic, J. C. (2017). Basic biology of oxidative stress and the cardiovascular system: Part 1 of a 3-part series. *Journal of the American College of Cardiology*, *70*(2), 196–211.

Santofimia-Castaño, P., Salido, G. M., & González, A. (2013). Ebselen alters mitochondrial physiology and reduces viability of rat hippocampal astrocytes. *DNA and Cell Biology*, *32*(4), 147–155.

Squadrito, F., Marini, H., Bitto, A., Altavilla, D., Polito, F., Adamo, E. B., D'Anna, R., Arcoraci, V., Burnett, B.P., Minotoli, L., Di Benedetto, A., Di Vieste, G., Cucinıtta, D., de Gregorio, C., Russo, S., Corrado, F., Saitta, A., Irace, C., Corrao, S., & Licata, G. (2013). Genistein in the metabolic syndrome: Results of a randomized clinical trial. *The Journal of Clinical Endocrinology & Metabolism*, *98*(8), 3366–3374.

Sun, J., Sun, G., Meng, X., Wang, H., Luo, Y., Qin, M., Ma, B., Wang, M., Cai, D., Guo, P., & Sun, X. (2013). Isorhamnetin protects against doxorubicin-induced cardiotoxicity in vivo and in vitro. *PLoS ONE*, *8*(5), e64526.

Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*, *34*(3), 497–500.

Tan, M., Toplu, Y., Varan, E., Sapmaz, E., Özhan, O., Parlakpınar, H., & Polat, A. (2022). The effect of genistein on cisplatin-induced ototoxicity and oxidative stress. *Brazilian Journal of Otorhinolaryngology*, *88*(1), 105–111.

Walker, M. J., Curtis, M. J., Hearse, D. J., Campbell, R. W., Janse, M. J., Yellon, D. M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W.G., Higgins, A.J., Julian, D.G., Lab, M.J., Manning, A.S., Northover, B.J., Parratt, J.R., Riemersma, R.A., Riva, E. Russell, D.C., …, Woodward, B. (1988). The Lambeth Conventions: Guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovascular Research*, *22*(7), 447–455.

Wang, W., & Kang, P. M. (2020). Oxidative stress and antioxidant treatments in cardiovascular diseases. *Antioxidants*, *9*(12).

Waterborg, J. H., & Matthews, H. R. (1994). The Lowry method for protein quantitation. *Methods in Molecular Biology (Clifton, NJ)*, *32*, 1–4.

Wu, J., Feng, A., Liu, C., Zhou, W., Li, K., Liu, Y., Shi, Y., Adu-Amankwaah, J., Yu, H., Pan, X., & Sun, H. (2024). Genistein alleviates doxorubicin-induced cardiomyocyte autophagy and apoptosis via ERK/STAT3/c-Myc signaling pathway in rat model. *Phytotherapy Research: PTR*, *38*(8), 3921–3934.

Xu, Y., Tang, C., Tan, S., Duan, J., Tian, H., & Yang, Y. (2020). Cardioprotective effect of isorhamnetin against myocardial ischemia reperfusion (I/R) injury in isolated rat heart through attenuation of apoptosis. *Journal of Cellular and Molecular Medicine*, *24*(11), 6253–6262.

Yousefi, H., Karimi, P., Alihemmati, A., Alipour, M. R., Habibi, P., & Ahmadiasl, N. (2017). Therapeutic potential of genistein in ovariectomy-induced pancreatic injury in diabetic rats: The regulation of MAPK pathway and apoptosis. *Iranian Journal of Basic Medical Sciences*, *20*(9), 1009–1015.

Zhao, T. T., Yang, T. L., Gong, L., & Wu, P. (2018). Isorhamnetin protects against hypoxia/reoxygenation-induced injury by attenuating apoptosis and oxidative stress in H9c2 cardiomyocytes. *Gene*, *666*, 92–99.