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ORIGINAL ARTICLE

Cardioprotective Effect of Oregano Oil against Doxorubicin-Induced Myocardial Infarction in Rats

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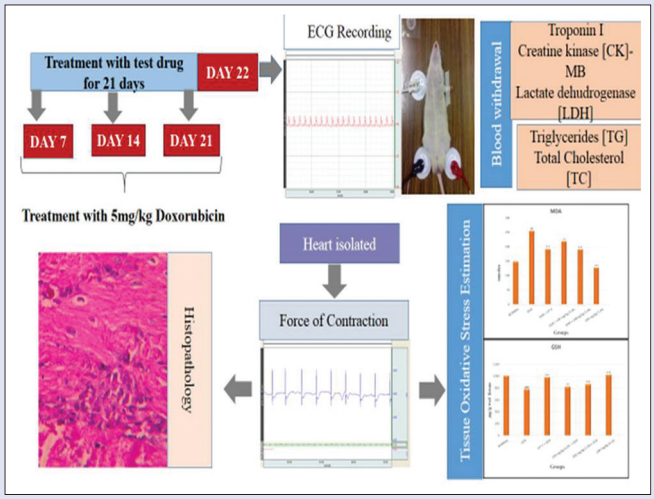
ABSTRACT

Aim: The objective of the study was to evaluate the cardioprotective effect of oregano oil (O.OIL) in doxorubicin (DOX)-induced myocardial infarction (MI) in rats. **Materials and Methods:** O.OIL (100 mg/kg and 200 mg/kg, p.o) was administered for 21 days in rats. MI was induced by DOX (5 mg/kg, i.p.) administered on the 7th, 14th, and 21st day of the study to obtain a cumulative dose of 15 mg/kg. On day 22, changes in electrocardiogram (ECG); force of contraction; serum markers: lactate dehydrogenase (LDH), creatine kinase (CK) MB isoenzyme, troponin I; lipid profile: total cholesterol and triglycerides; antioxidant enzymes level: malondialdehyde (MDA) and glutathione (GSH); heart and body weight; and histopathology of heart were determined. **Results:** Pretreatment with O.OIL significantly protected the myocardium from the toxic effects of DOX by reducing the elevated level of CK-MB, LDH, and troponin I to the normal levels. O.OIL increased the GSH levels and decreased the MDA levels in cardiac tissue. It also restored the changes in ECG and force of contraction and showed significant recovery of heart tissue in histopathological studies. **Statistical analysis:** All results are expressed as mean ± standard error of the mean. The results were analyzed for statistical significance by one-way ANOVA followed by Dunnet's Multiple Comparison Test using GraphPad version 5.01, $P < 0.01$ was considered statistically significant. **Conclusion:** O.OIL (100 and 200 mg/kg p.o.) reduced cardiac complications in DOX-induced MI in rats.

Key words: Cardioprotective, doxorubicin, myocardial infarction, oregano oil

SUMMARY

- Oregano oil (O.OIL) treatment could be considered as a potentially useful drug in combination with DOX to limit free radical-mediated organ injury. Pretreatment with O.OIL indicated dose-dependent cardioprotection against experimentally induced myocardial infarction. The cardioprotective effect of O.OIL could be owing to its antioxidant, anti-inflammatory, anti-lipid peroxidation, and free radical scavenging potential.



Abbreviations used: ECG: Electrocardiogram; O.OIL: Oregano oil; DOX: Doxorubicin; MI: Myocardial infarction.

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INTRODUCTION

Myocardial infarction (MI) is an acute condition of myocardial necrosis, where prolonged interruption of blood supply to an area of the heart causes oxygen deprivation to the heart muscle.[1] It is a leading cause of mortality and disability globally.[2]

Doxorubicin (DOX) is an anthracycline antibiotic. Anthracyclines are a class of antitumor drugs with a vast spectrum of activity in human cancers, and only a few cancers (e.g., colon cancer) do not respond to them.[3] The clinical use of DOX is limited, despite its broad therapeutic effectiveness, by a dose-dependent and cumulative cardiotoxicity.[4] Several mechanisms have suggested, that an increase in oxidative stress, along with reductions in the level of antioxidants play role in the pathogenesis of DOX-induced cardiomyopathy. Therefore, the use of natural or synthetic antioxidants might protect against the oxidative stress caused by DOX and other cytotoxic drugs.[5]

Essential oils are natural complex multicomponent systems composed mainly of terpenes and nonterpene components.[6] Oregano oil (O.OIL) is extracted from the species *Origanum vulgare* (Labiatae) by the process of steam distillation.[7] Its chemical constituents include benzyl alcohol, eugenol, 2-phenylethanol, thymol, 3-hexen-1-ol, and carvacrol.[8]

Oregano has been used traditionally to improve circulation as an emmenagogue, for infections of the oral cavity, as a carminative for

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digestive health, and for the treatment of inflammatory disorders such as arthritis.[9] The oil is effective against intestinal parasites,[10] upper respiratory tract infections,[11] and antiproliferative effect against tumor cell lines.[12] Carvacrol which is one of the constituents of O.OIL is reported to have cardioprotective activity in experimental rats.[13]

Since there is no reported activity of O.OIL possessing cardioprotective activity, this study was planned to evaluate its potential.

MATERIALS AND METHODS

Reagents

DOX (United Biotech, Pvt. Ltd. India, DXIH4B2) and Vitamin E (VIT E) (Merck Serono India., 21714) were procured from the pharmacy at Dr. Prabhakar Kore Hospital and Medical Research Center, Belagavi, India. Troponin I Kit (CTN4060003) was procured from Abon Biopharm, Hangzhou, China. Other diagnostic kits: Lactate dehydrogenase (LDH) (FBCEROO30), creatine kinase (CK)-MB (FBCER0069), total cholesterol (TC) (FBCER0009), and triglycerides (TG) (FBCER0031) were purchased from Erba Diagnostics Mannheim GmbH, Germany. All other reagents used in experiments were of analytical grade.

Test material: Oregano oil

An essential oil: O.OIL (AOS/ORG/250814) was purchased from AOS products Pvt. Ltd. Ghaziabad, India (An ISO 9001–2008 and GMP Accredited Company). It was isolated by steam distillation of flowering herb from botanical source: *O. vulgare*. Its main components are carvacrol, thymol, *P*-cymene, α-terpineol, cis-ocimene, caryophyllene, and linalool.[14] Its quantitative analysis: organoleptic and physicochemical properties were performed by the industry and certificate of analysis was provided. Therefore, we focused toward a pharmacological aspect of the O.OIL for cardioprotective effect.

Experimental animals

Male Wistar Albino rats weighing about 150–200 g were used for the experiment. The animals were acclimatized for 1 week to laboratory conditions and fed with standard pellet diet and water *ad libitum*. The protocol of animal experiments was approved by the Institutional Animal Ethics Committee (IAEC) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (KLECOP/IAEC/Res. 20-09/08/2014).

Experimental design

After 1 week of acclimatization, the animals (n = 6) were randomly divided into six groups: Normal, DOX, VIT E + DOX, 100 mg/kg O.OIL + DOX, 200 mg/kg O.OIL + DOX, AND 200 mg/kg O.OIL. Except the normal group, MI was induced in rats by intraperitoneal injection (IP) of DOX hydrochloride at a dose of 5 mg/kg on 7th, 14th, and 21st day. Each rat received a total cumulative dose of 15 mg/kg.[15]

Electrocardiogram measurement

Twenty-four hour after the last DOX injection, the animals were anesthetized using IP administration of ketamine and xylazine combination at a dose of 70 and 10 mg/kg, respectively. The Lead II electrocardiograms (ECGs) of all animals were recorded using Biopac Student Lab PRO 3.7 software (Model No. MP35) make BIOPAC Systems, Inc. 42 Aero Camino, Goleta, CA 93117, USA. The ECG parameters such as heart rate (BPM), QRS complex (in seconds), and QT interval (in seconds) were measured.

Force of contraction measurement

After anesthesia overdose, the heart was isolated and mounted on Radnoti Langendorff constant pressure nonrecirculating (Radnoti 130105EZ) system. The force of contraction was estimated.

Cardiac marker and lipid profile estimation

For estimation of cardiac marker profiles, blood samples were centrifuged at 2500 rpm/min for 15 min to separate plasma, which was used as qualitative, membrane-based immunoassay for the detection of cardiac Troponin I.[16] It was also used for quantitative estimation of CK-MB,[17] LDH,[18] TG,[19] and TC.[20]

Tissue antioxidants analysis

The isolated heart was washed with ice-chilled physiological saline. A known weight (200 mg) of the heart tissue was homogenized in 5 ml of 0.1 M Tris-HCL (pH 7.4) buffer solution. The homogenate was centrifuged at 2000 rpm for 10 min at 4°C. The supernatant thus obtained was used for the estimation of thiobarbituric acid reactive substances[21] and reduced glutathione (GSH).[22]

Histopathological studies

At the end of the experiment, myocardial tissues from all groups were subjected to histopathological studies. The tissues were fixed in formalin (10%), processed and embedded in paraffin wax. Paraffin sections were cut in glass slides and stained with hematoxylin and eosin after dewaxing and examined under a light microscope.

Statistical analysis

The results were expressed as the mean ± standard error of the mean and analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data were computed for statistical analysis using Prism 5 for windows, version 5.01, GraphPad Prism software, INC.

RESULTS

Body weight and heart weight

In DOX-treated group, there was highly significant ($P < 0.001$) decrease in body weight and heart weight (149.4 ± 1.435, 0.558 ± 0.008) as compared to that of normal rats (205.6 ± 1.691, 0.727 ± 0.007). In groups pretreated with 100 mg/kg, the increase in body weight and heart weight (161.6 ± 3.586, 0.606 ± 0.006) observed was statistically significant ($P < 0.01$) and highly significant ($P < 0.001$) with 200 mg/kg of O.OIL (169 ± 1.643, 0.693 ± 0.014) when compared with DOX-treated rats. The rats pretreated with VIT E-100 mg/kg followed by DOX administration showed a highly significant ($P < 0.001$) increase in body weight and heart weight (200.8 ± 2.709, 0.745 ± 0.001) compared to DOX-treated rats. The rats that received only O.OIL (200 mg/kg) showed a highly significant ($P < 0.001$) increase in body weight and heart weight (196.6 ± 3.750, 0.717 ± 0.003) compared to the DOX-treated group [Table 1].

Serum biomarker enzymes

Administration of DOX significantly ($P < 0.001$) increased the level of serum biomarker enzymes, namely, LDH (1899 ± 10.96) and CK-MB (1545 ± 9.317) compared to normal rats (16.87 ± 0.9930, 14.19 ± 0.988, 1008 ± 12.70, 1023 ± 5.896). Pretreatment with 100 mg/kg O.OIL before DOX administration showed significant decrease ($P < 0.05$) in the levels LDH (1764 ± 45.52) and CK-MB (1446 ± 23.46) compared to the DOX-treated group. Pretreatment with 200 mg/kg O.OIL before DOX administration showed highly significant ($P < 0.001$) decrease in the levels of LDH (1657 ± 61.94) and a significant ($P < 0.01$) decrease

was seen in the level of CK-MB (1413 ± 21.25) as compared to the DOX-treated group. The group pretreated with VIT E before DOX administration and the group which received only 200 mg/kg O.OIL showed highly significant ($P < 0.001$) decrease in the levels of LDH (1121 ± 24.46, 1041 ± 7.632) and CK-MB (1167 ± 41.49, 1069 ± 3.955) as compared to the DOX-treated group [Figure 1a].

In the DOX-treated group, all the rats showed the presence of troponin I, while the normal group all the rats showed the presence of troponin I in whole blood. In VIT E-pretreated group followed by DOX administration, among the six animals only one animal showed the presence of troponin I. In the group that received lower dose of oil followed by DOX administration three animals among six showed the presence of troponin I. In the group that received higher dose of oil followed by DOX administration, only one rat showed the presence of troponin I. The group that received only higher dose of oil showed the absence of troponin in all the six rats [Table 2].

Table 1: Effect of O.OIL on the body weight and heart weight of rats in DOX induced MI in rats.

Treatment	Body weight (g)	Heart weight (g)
NORMAL	205.6±1.691	0.727±0.007
DOX	149.4±1.435$^{\#\#\#}$	0.558±0.008$^{\#\#\#}$
VIT. E+DOX	200.8±2.709***	0.745±0.001***
100 mg/kg O.OIL + DOX	161.6±3.586**	0.606±0.006**
200 mg/kg O.OIL + DOX	169±1.643***	0.693±0.014***
200 mg/kg O.OIL	196.6±3.750***	0.717±0.003***

All values are expressed as mean±SEM for (n=6) in each group using one-way analysis of variance (ANOVA) followed by Dunnets multiple comparison test. Compared with Normal control: $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001. Compared with Disease control: *P<0.05, **P<0.01, ***P<0.001.

DOX-treated group showed significant increase ($P < 0.001$) in the levels of TC (149.5 ± 2.473) and TG (186 ± 2.269) when compared to normal group (95.86 ± 4.346, 89.50 ± 3.667). Pretreatment with 100 mg/kg O.OIL showed statistically significant ($P < 0.01$) reduction and pretreatment with 200 mg/kg O.OIL and VIT E showed highly significant ($P < 0.001$) reduction in the levels of TC and TG in toxic rats. The rats treated with 200 mg/kg O.OIL alone showed a highly statistically significant ($P < 0.001$) decrease in the levels of TC (86.9 ± 2.177) and TG (83.67 ± 2.076) as compared to the group that received DOX [Figure 1b].

Antioxidant status

Myocardial malondialdehyde (MDA), a marker of lipid peroxidation, was found to be significantly higher ($P < 0.001$) in DOX-treated group (247 ± 8.331) when compared with normal (141.0 ± 4.266). The decrease in MDA levels in rats pretreated with 100 mg/kg O.OIL was statistically significant ($P < 0.01$) and highly statistically significant ($P < 0.001$) with 200 mg/kg O.OIL- and VIT E-pretreated rats compared to DOX-treated rats (210.8 ± 5.093, 183.4 ± 4.996, and 184.6 ± 5.105). The rats that received only O.OIL (200 mg/kg) showed a highly statistically significant ($P < 0.001$) decrease in MDA levels (119.8 ± 4.903) as compared to the DOX-treated group [Table 3].

A statistical significant ($P < 0.001$) decline in the activity of endogenous antioxidant enzyme GSH was observed in the heart tissues of DOX-treated rats (743.1 ± 9.270) as compared with normal

Table 2: Effects of O.OIL on Troponin I in DOX induced MI in rats

No. of Animals	NORMAL	DOX	VIT E + DOX	100 mg/kg O.OIL + DOX	200 mg/kg O.OIL + DOX	200 mg/kg O.OIL
1	–	+	–	–	–	–
2	–	+	–	–	–	–
3	–	+	–	+	–	–
4	–	+	–	+	+	–
5	–	+	+	–	–	–
6	–	+	–	+	–	–

+ = Presence and - = Absence of Troponin I in serum, (n=6)

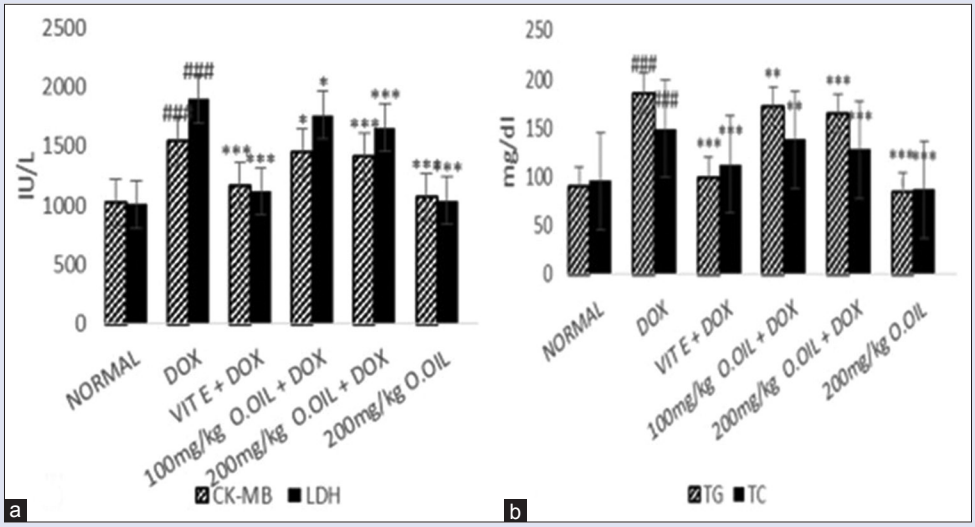

Figure 1: Effects of oregano oil on serum biomarker enzymes: a = CK-MB (Creatine Kinase MB); LDH (Lactate Dehydrogenase) and lipid profile: b = TG (Triglycerides); TC (Total Cholesterol) in doxorubicin induced myocardial infarction in rats. All values are expressed as mean ± standard error of the mean for (n = 6) in each group using one way analysis of variance followed by Dunnet's multiple comparison test. Compared with normal control: # $P < 0.05$, # # $P < 0.01$, # # # $P < 0.001$. Compared with disease control: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

group (968.9 ± 4.128). Pretreatment with O.OIL (100 and 200 mg/kg) and VIT E also significantly ($P < 0.01$) increased the depleted GSH enzyme level (784.2 ± 7.39O, 828.8 ± 11.31) when compared to DOX-treated rats. The rats that received only 200 mg/kg O.OIL showed highly statistically significant ($P < 0.001$) increase in the levels of antioxidant enzyme (984.7 ± 2.298) when compared to the DOX-treated group [Table 3].

Electrocardiogram changes

DOX-treated group showed significant changes in the repolarization phase of the ECG, significant prolongation of the QT interval, elevation of the ST segment, and alteration in the QRS complex as compared to the normal group. In addition, a statistical significant ($P < 0.001$) increase in the heart rate of DOX-treated rats was observed as compared to normal group. Pretreatment with O.OIL (100 and 200 mg/kg) and VIT E significantly reduced the ECG alterations when compared to the DOX-treated group. There was no change observed in the ECG of the rats treated with 200 mg/kg O.OIL when compared to that of the normal rats [Figure 2].

Table 3: Effect of O.OIL on MDA and GSH in DOX induced MI in rats

GROUPS	MDA (nmol of MDA/min)	GSH (µg/g of heart tissue)
NORMAL	141.0±4.266	968.9±4.128
DOX	247±8.331$^{\#\#\#}$	743.1±9.270$^{\#\#\#}$
VIT. E+DOX	184.6±5.105***	944.3±2.682***
100 mg/kg O.OIL + DOX	210.8±5.093**	784.2±7.39O**
200 mg/kg O.OIL + DOX	183.4±4.996***	828.8±11.31***
200 mg/kg O.OIL	119.8±4.903***	984.7±2.298***

All values are expressed as mean±SEM for (n=6) in each group using one-way analysis of variance (ANOVA) followed by Dunnets multiple comparison test. Compared with Normal control: $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. Compared with Disease control: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Force of contraction

The height of response in force of contraction of DOX-treated rats heart was significantly decreased ($P < 0.001$) in comparison to normal rats. The increase in force of contraction observed in rats pretreated with 100 mg/kg was statistically significant ($P < 0.05$) and highly statistically significant ($P < 0.001$) with 200 mg/kg of O.OIL when compared with DOX-treated rats. The rats pretreated with VITE 100 mg/kg followed by DOX administration showed a highly significant ($P < 0.001$) increase in force of contraction, compared to DOX-treated rats. The rats that received 200 mg/kg O.OIL alone resulted in a highly significant ($P < 0.001$) increase in the force of contraction as compared to the DOX-treated group [Table 4].

Histopathological studies

Rats that received 100 mg/kg and 200 mg/kg of O.OIL followed by DOX administration showed mild vascular congestion when compared to DOX group. Sporadic early necrosis of fiber, moderate vascular congestion and interstitial edema, degenerative changes, and cytoplasmic vacuolation was observed in DOX-treated animal which indicates moderate damage. Pretreatment with VIT E indicated mild vascular congestion, while animals received O.OIL alone indicated normal architecture [Figure 3].

DISCUSSION

The study results suggested that O.OIL prevents DOX-induced MI in rats. The following lines of evidence can be highlighted from the present study.

The existence and degree of myocyte injury can be assessed by cardiac enzymes CK-MB, LDH, and troponin I found primarily in

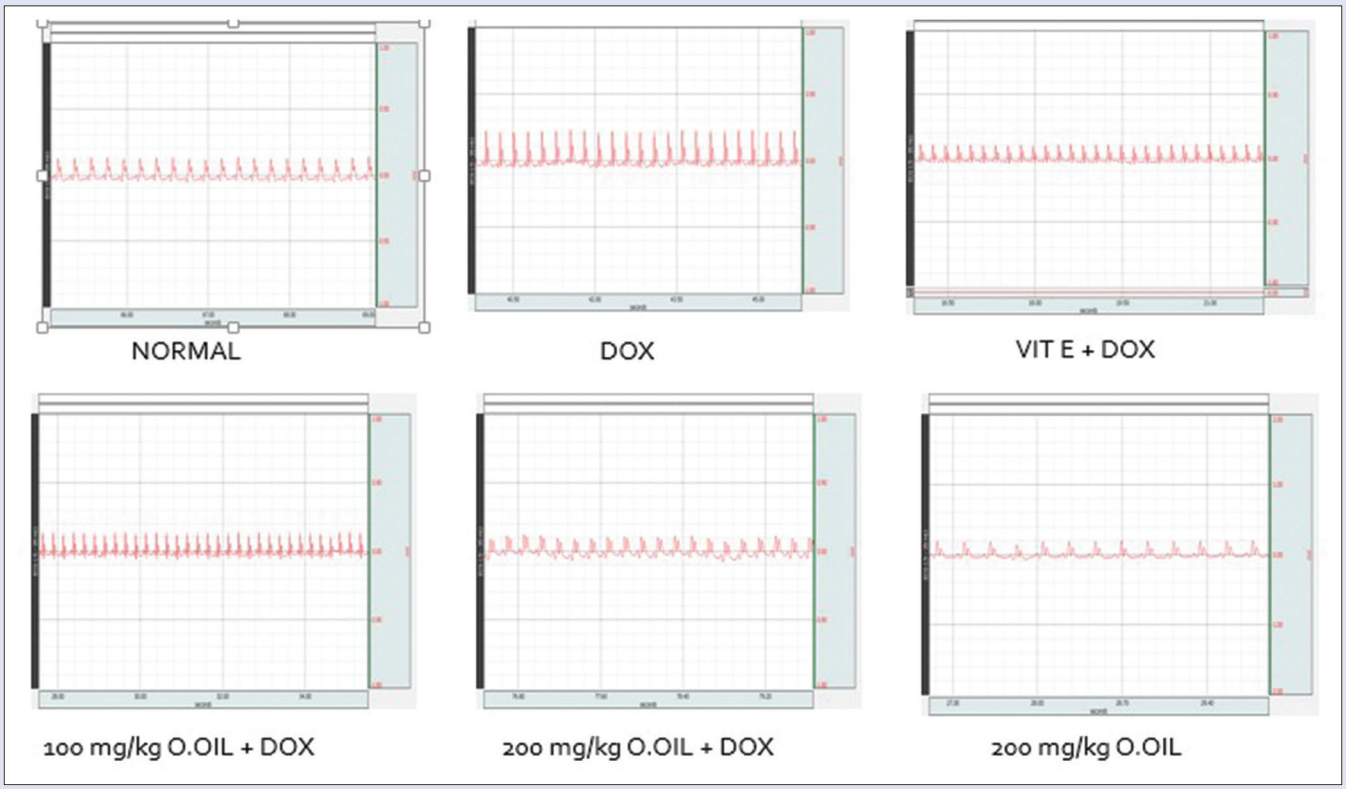

Figure 2: Electrocardiogram changes on various treated groups

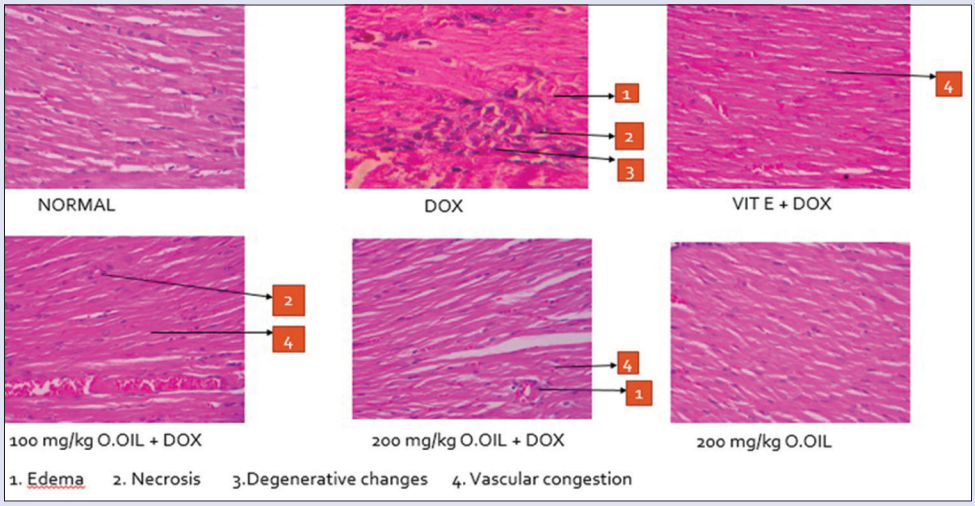

Figure 3: Histopathological studies on various treated groups

Table 4: Effect of O.OIL on DOX induced changes in the force of contraction

GROUPS	HEIGHT OF RESPONSE (cm)	% RESPONSE
NORMAL	1.933±0.033	100
DOX	1.133±0.033$^{\#\#\#}$	58.61
VIT E+DOX	1.800±0.057***	93.11
100 mg/kg O.OIL+DOX	1.333±0.033*	68.96
200 mg/kg O.OIL+DOX	1.600±0.057***	82.77
200 mg/kg O.OIL	1.867±0.033***	96.58

All values are expressed as mean±SEM for (n=6) in each group using one-way analysis of variance (ANOVA) followed by Dunnets multiple comparison test. Compared with Normal control: $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001. Compared with Disease control: *P<0.05, **P<0.01, ***P<0.001.

the myocardium.[23] In the present study, DOX-treated rats showed significant elevation in the levels of these diagnostic marker enzymes. Moreover, elevated levels of these enzymes are an indicator of the severity of DOX-induced myocardial damage.[24]

The prior administration of O.OIL showed significant reduction in DOX-induced elevated serum marker enzymes. This reduction of the marker enzymes may be due to the ability of the essential oil to maintain the normal structure and architectural integrity of cardiac myocytes, thereby restricting the leakage of these enzymes, which is evident from the near normal levels of CK-MB, LDH, and troponin I.

Excess lipids in blood are accelerating the development of atherosclerosis and the major risk factor in MI. High levels of circulating cholesterol and its circulation in heart tissue are well associated with cardiovascular damage. They also modify the composition, structure, and stability of cellular membranes which contribute to cardiovascular disease.[25]

Increase in the levels of serum TC and TG in the DOX-treated group indicate that DOX may be interfering with metabolism or biosynthesis of lipids. The present results are in good agreement with the earlier findings.[26] The groups pretreated with O.OIL showed significant reduction in levels of TC and TG. Earlier findings have shown that oregano supplement and carvacrol have shown to have antihyperlipidemic properties.[27,28]

The mechanism of cardiotoxicity induced by DOX is not clearly known, although evidence supports that DOX administration is associated with a decrease in endogenous antioxidants and increase in the oxygen free radicals resulting in increase in oxidative stress, which is followed by development of a variety of subcellular changes in the myocardium, typical of DOX-induced cardiac injury.[29]

DOX has high affinity to phospholipids leading to accumulation of DOX in heart tissue and since the heart tissue contains low levels of antioxidant enzymes, it is more prone to damage through oxidative stress.[30] Several antioxidants such as alpha-tocopherol, flavonoids, and resveratrol have been used to counteract DOX-induced cardiotoxicity.[31]

Pretreatment with O.OIL significantly increased the responses of force of contraction and showed cardiotonic property in cardiotoxic rats. In this study, DOX-treated rats showed an increase in the heart tissue MDA levels suggesting increased lipid peroxidation and decreased levels of GSH, which confirms the oxidative stress and cardiac damage. These results are in good agreement with the earlier findings where DOX treatment increased the levels of MDA and decreased the levels of GSH.[32,33]

O.OIL prevented the DOX-induced tissue damage by decreasing the oxidative stress and restoring the antioxidant status. ECG abnormalities are the main criteria generally used for the definite diagnosis of myocardial injury. Moreover, ECG changes are an indicator of the severity of DOX-induced MI.[34,35]

DOX-treated group showed significant prolongation of QT interval and elevation of ST segment, with significant increase in heart rate when compared to normal group. The increased heart rate of DOX-treated rats can lead to increase in oxygen consumption and can accelerate myocardial necrosis. Pretreatment with O.OIL significantly reduced the ECG alterations when compared to DOX-treated rats.

The protective effect of O.OIL was demonstrated by histopathological findings. Pretreatment with O.OIL showed recovery of the tissue close to normal structure and integrity.

CONCLUSION

The present study indicates that the pretreatment with O.OIL indicated dose-dependent cardioprotection against experimentally induced MI. The overall cardioprotective effect of O.OIL is probably due to its antioxidant, anti-inflammatory, anti-lipid peroxidation, and free radical scavenging activity. The study result suggests that O.OIL may be considered as a potentially useful drug in combination with DOX to limit free radical-mediated organ injury. However, molecular level of investigation needs to be done using different animal models and using different biochemical parameters to find out the possible mode of action of O.OIL as cardioprotective agent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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