ORIGINAL ARTICLE

Cardioprotective Effects of Nigella Sativa and Enalapril in Doxorubicin-induced Cardiotoxicity

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ABSTRACT

Objective: To determine the combined cardioprotective effects of *Nigella Sativa* and enalapril in doxorubicin-induced cardiotoxicity in rats.

Study Design: Experimental randomized control trials.

Place and Duration of Study: This research was carried out from September 2020 to August 2021, in the department of pharmacology, in collaboration with the National Institute of Health (NIH) Islamabad, Pakistan.

Materials and Methods: For this experiment, 4 groups of adult male rats were taken, each containing 10 rats. Group 1 rats acquired a normal diet without any medication throughout the experiment. On day 8 (after acclimatization) cardiotoxicity was induced in groups 2, 3, and 4 rats by administering doxorubicin 5mg/kg intraperitoneally for 3 consecutive days. After the confirmation of cardiotoxicity, Group 3 rats were administered only angiotensin-converting enzyme inhibitors (ACEI) enalapril 2mg/kg, while group 4 rats were given a combination of *Nigella sativa* 100mg/kg and Enalapril 2mg/kg orally for 14 days. Baseline blood samples were taken on day 0 to obtain normal values of Cardiac Troponin T (cTnT), Cardiac Troponin I (cTnT), and CK-MB enzyme. To confirm cardiotoxicity 2nd sampling was done on day 11, and the final sampling was done through cardiac puncture on day 26. Serum biochemical estimation was done and data were analyzed through SPSS 22 by using one-way ANOVA and paired t-test. A P-value < 0.05 was believed statistically considerable.

Results: Enalapril alone produced significant cardioprotective effects as shown by the marked reduction in cTnT, cTnI, and CKMB levels in group 3 ($p<0.05$), but combined administration of *Nigella sativa* and enalapril in group 4 mice produced a more significant reduction in Trop T, Trop I, and CK-MB levels ($P <0.05$).

Conclusion: *Nigella sativa* and enalapril in combination significantly lower cardiac enzyme in Doxorubicin-induced cardiotoxicity in rats.

Key Words: *Cardiotoxicity, Doxorubicin, Enalapril, Nigella Sativa.*

Introduction

Doxorubicin is an extensively used anthracycline for the treatment of several solid tumors and childhood malignancies for years. The main concern about the use of this drug is its cardiac adverse effects because about twenty percent of patients who receive doxorubicin can develop adverse cardiac effects.[1] Cardiomyocytes have a lesser capability to regenerate therefore, they are potentially more vulnerable to the long-term adverse effects of doxorubicin.[2] Its cardiotoxicity occurs because of its quinone group-based metabolites that produce reactive oxygen species and free radicals.[3] Cardiomyocytes are intrinsically more susceptible to oxidative stress, so these free radicals & reactive oxygen species impose mitochondrial and nuclear DNA lesions in cardiomyocytes. [4,5] Doxorubicin-mediated reactive oxygen species also stimulate mitogen-activated protein kinases, which induce the expression of several proapoptotic proteins. These processes lead to cardiomyocyte death either by necrosis or apoptosis and cause the release of

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cardio-specific contractile proteins; cardiac troponins I (cTnI), cardiac troponin T (cTnT), and creatinine kinase MB into the circulation.[6] Cardiotoxicity is more specifically expressed by cTnI and cTnT because they are exclusively expressed in the myocardium and are more abundant than CK-MB in the myocardium. Therefore, the quantitative approximation of these sensitive biomarkers is used for the presence of cardiotoxicity at the subclinical as well as clinical stages.[7,8] Several strategies have been recommended for the prevention of doxorubicin-induced cardiotoxicity. Still, the protection deliberated is not always effective and is expensive as well.[9] Several clinical studies and meta-analyses assessing the use of angiotensin-converting enzyme inhibitors (ACEIs) in anthracycline-induced cardiotoxicity have recently been revealed, and these studies suggested that ACEIs are effective as cardioprotective agents because of their antioxidant and anti-inflammatory properties.[10,11]

Nigella sativa has been used medicinally for decades because it is a marvel herb with a wide range of pharmacological properties and astonishing religious background.[12] This herb has proven antioxidant, anti-inflammatory, cardioprotective, hepatoprotective, antidiabetic, neuroprotective, and anticancer, effects among others.[13] The cardioprotective effects of both *Nigella sativa* and enalapril have been studied and proved separately, but according to the best of our knowledge, their combined effects are not yet explored. So, this study aimed to evaluate the extent of the protective role exerted by *Nigella sativa* in the prevention of cardiotoxic effects of doxorubicin in combination with an ACEI enalapril.

Materials and Methods

This experimental randomized controlled study was performed from September 2020 to August 2021 in the Department of Pharmacology, after getting approval from the institutional review committee. This study was initiated on a total of 40 healthy male albino rats weighing 200-250 g, 8 weeks of age, and with normal cTnI, cTnT, and CKMB levels. Rats were placed in well-aerated cages for acclimatization, a room temperature of 22 ± 2 ° C, and a 12-hour light-dark cycle was maintained.[14] Rats were randomly divided into 4 groups of 10 rats each. Group 1, the control group, consumed a normal diet and tap water throughout the experiment while group 2, 3, and 4 rats were given three intraperitoneal injections of doxorubicin at a dose of 5mg/kg for three consecutive days (cumulative dose 15 mg/kg).[15] After the confirmation of cardiotoxicity by measuring and analyzing cTnI, cTnT, and CK-MB levels; group 3 rats were given enalapril 2mg/kg/day[16] alone while group 4 rats were given *Nigella sativa* seed powder 100mg/kg/day[17] and enalapril 2mg/kg/day dissolved in distal water for 14 days.[16] Nigella sativa and enalapril powders were prepared at the Riphah Pharmaceutical Science Institute (RIPS) in Islamabad. On day 0, blood samples were taken for a baseline evaluation, and on day 11, a second sampling was done for the confirmation of cardiotoxicity in groups 2, 3, and 4. The final sampling happened on the 26th day of the experiment, all samplings were accomplished by cardiac puncture. The sample was centrifuged at 3000 rpm for 5 minutes, and the serum was separated and stored at -8 ºC in eppendorf tubes for further biochemical analysis. cTnT, cTnI, and CK-MB were assessed using the chemistry Analyzer, Micro lab 200 (Merck). These parametric data were statistically analyzed by using SPSS 22. The mean and standard error of the mean was calculated for all four groups. One Way ANOVAs were done for comparison among different groups while comparison between two groups was done by using the paired t-test. Results were considered significant at a p-value less than 0.05.

Results

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) were measured in ng/l ±SD while CK-MB activity was measured in U/l ±SD. On day 11 the confirmation of cardiotoxicity in groups 2, 3, & 4 was

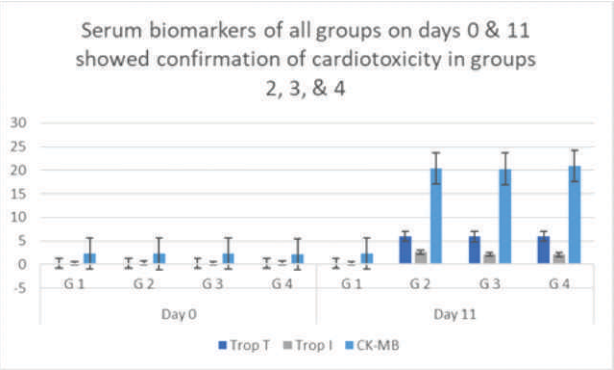

Figure 1: Comparison of Biomarkers Among Groups on Days 0 & 11.

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done by the results shown in figure 1. The p-values for cTnI, cTnT, and CKMB were 0.001, 0.003, and 0.000 respectively.

When we analyzed the 26th-day results of all groups, remarkably significant improvement was found in the cardiotoxic profiles of groups 3 & 4, their parameters were almost equal to normal control values and their day 0 levels (table 1).

Table I: Comparison Among Serum Biomarkers of all Groups on days 0 and 26.

Serum Tests	Day 0				Day 26				*p*-value
	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	
Trop T (ng/l) ±SD	0.17± 0.01	0.18± 0.03	0.17± 0.01	0.17± 0.05	0.17± 0.30	5.96± 0.37	0.22± 0.37	0.18± 0.37	0.001*
Trop I (ng/l) ±SD	0.26± 0.17	0.21± 0.33	0.22± 0.14	0.25± 0.08	0.25± 0.10	2.60± 0.21	0.32± 0.11	0.26± 0.17	0.000*
CK-MB (U/L) ±SD	2.35 ± 0.08	2.25 ± 0.07	2.32 ± 0.05	2.29 ± 0.07	2.35± 0.07	20.4 ± 1.31	2.79 ± 1.31	2.30 ± 1.31	0.000*

*Significant *p*-value

To confirm our hypothesis, we further compared the serum markers of group 3, & 4 and we observed *p*-values of 0.000, 0.023, and 0.000 respectively for trop T, trop I, & CKMB levels. These results showed that *Nigella sativa* increased the efficacy of enalapril.

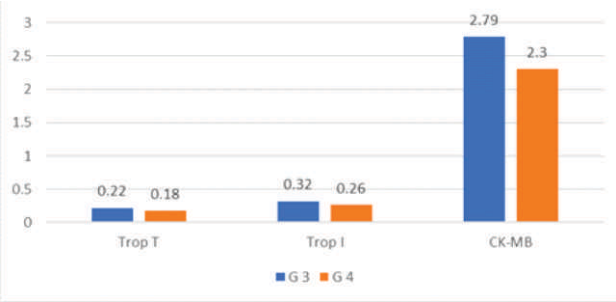

Figure 2: Comparison of Serum Markers of groups 3 & 4 on day 26.

Discussion

In the present study, we observed that *Nigella sativa* potentiates the cardioprotective effects of enalapril in doxorubicin-induced cardiotoxic rats as represented by the comparison of the cardiotoxicity indicating biomarkers. Enalapril alone also proved cardioprotective.

We artificially induced acute cardiotoxicity by a cumulative dose of doxorubicin 15 mg/kg/day in groups 2, 3, and 4 rats, this cardiotoxicity was confirmed by significant rises in serum biomarkers of cardiac injury including cTnI, cTnT, & CKMB. Doxorubicin's cardiotoxicity is a piece of evidence used by numerous researchers to induce cardiotoxicity in different animal models. Lin and his associates induce cardiotoxicity in rats with doxorubicin, they use an accumulative dose of 18mg/kg over 2 weeks.[18] Aziz and his colleagues observed similar changes in biomarkers following the use of doxorubicin as a single intraperitoneal injection in rats.[19] Sawyer and his colleagues observed such kinds of effects in their preclinical studies both in vitro and in vivo, while Georgakopoulos and coworkers observed the effects of doxorubicin in clinical studies on lymphoma patients and established that doxorubicin caused a significant and dose-dependent cardiomyocyte apoptosis and myocytes death.[20,21] Another study by Eisvand et al also supported the cardiac enzyme disruption after introducing a single dose of doxorubicin in rats.[22] In our study the rationale behind the measurement of CKMB was its historical value but our stress remained on cTnI & T as more specific and sensitive biomarkers, these markers are considered the gold standard biomarker of cardiac injury in all mammalian species.[23]

The second part of our study was a comparative analysis of the cardioprotection provided by enalapril alone and in combination with *Nigella Sativa*. The Group 3 animals were treated with enalapril for 14 days and they confer cardioprotection. The cardioprotective effects of enalapril and other ACEIs are well established. In a recent study, Ghasemi and his fellows comprehensively review the clinical trials performed on the prevention of doxorubicin-induced cardiotoxicity. They conclude that ACEIs plus doxorubicin are the best treatment for preventing cardiotoxicity in these patients.[24] Divergent effects of enalapril and eplerenone in primary prevention against doxorubicin-induced cardiotoxicity were studied by Hullin et al and they concluded that primary prevention with enalapril preserves left ventricular morphology and function in mice.[25] Georgakopoulos et al studied the cardioprotective effects of metoprolol and enalapril in lymphoma patients.[21]

Adıyaman used the same dose of *Nigella Sativa* seed powder for the prevention of doxorubicin-induced toxicity[17] while we use it for the treatment

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and in combination with enalapril to define their combined effects. A study conducted by El-Kerdasy also showed the beneficial effects of Nigella Sativa and ACEIs on myocardial fibrosis Induced by lipopolysaccharide.[26] Cardioprotective effects of the ethanolic extract of Nigella sativa (800mg/kg/day) were studied by Hassan and his colleagues, they observed improvement in different cardiac markers.[27]Extensive literature is present regarding the use of this herb in animals but very few studies have been done on humans and this is the need of time.

Conclusion

Nigella sativa and enalapril in combination significantly lower cardiac enzyme in Doxorubicin-induced cardiotoxicity in rats. So, Nigella sativa can be used as an adjunct with enalapril in the treatment of doxorubicin-induced cardiotoxicity.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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