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Original Article

The Influence of Melatonin in Doxorubicin-Induced Cardiotoxicity: In Vivo study

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ABSTRACT

Doxorubicin (DOX) is one of the most effective chemotherapeutic agents; yet, its clinical application is limited due to dose dependent cardiotoxicity, which worsens by increasing cumulative dose and is largely associated with oxidative stress and damage to cardiomyocyte. Despite numerous studies, proper methods to protect the heart during DOX therapy remain a critical challenge. Melatonin (MLT), a naturally endogenous hormone with antioxidant and cardio-protection properties, identified as a possible treatment to reduce DOX-induced cardiac damage. This study aimed to evaluate the protective role of MLT against DOX-induced cardiotoxicity using experimental rat models. Thirty male albino rats were separated into three groups: control group, DOX-treated group (3mg/kg/week, intraperitoneally, for 6 weeks), and DOX+MLT co-administration group (MLT;30 mg /kg/day, orally). After 42 days, serum biomarkers of oxidative stress and cardiac injury, as well as histological examination of cardiomyocyte tissue, were assessed. The administration of DOX resulted in considerable cardiac stress, as demonstrated by a significant increase in serum brain natriuretic peptide (BNP) ($p<0.0001$) and elevated malondialdehyde (MDA) levels($p<0.05$), indicating oxidative lipid damage. Co-treatment with MLT greatly reduced the BNP ($p<0.0001$), and MDA levels. Other serum markers, including cardiac troponin-I (cTnT-I), lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), nitric oxide (NO), showed non-significant changes. Collectively, the analysis revealed a significant structural advantage, MLT treatment markedly improved DOX-induced myocardial damage, significant reduction in cardiomyocyte hypertrophy, coronary artery wall thickening, and necrotic areas, thereby reducing the injury from moderate or severe to mild changes. These findings indicate that MLT exerts cardio-protection effects, likely through reducing oxidative stress and maintaining myocardial structure.

Keywords: Doxorubicin, Melatonin, cardiotoxicity, Oxidative stress, Reactive oxygen species.

1 Introduction

Cancer is one of the most serious health and economic burdens in the 21st century. It is responsible for about one in six deaths globally (16.8%) and one in four deaths (22.8%) linked to noncommunicable diseases around the world [1]. Advances in cancer screening program, diagnostic imaging and therapeutic techniques are significantly improved cancer survival rates; and the link between side effects from treatment and higher cancer survival rates could have an enormous impact on patients health status and quality of life [2] .Conventional chemotherapy continues to be a widely used treatment despite the availability of other therapeutic approaches[3].In recent years, cancer -related mortality has recorded a significant decline; despite continuing progress in chemotherapeutic agents, doxorubicin continue to be one of the most widely used anticancer agent in clinical practice[4] .The world health organization (WHO) reports that cardiovascular disease (CVD) caused approximately 17.9 million deaths worldwide in 2019, representing 32% of all deaths in the world [5] . Heart disease consisted of several CVDs and recognized as the main cause of worldwide [6]. CVD includes many disease conditions that affect the heart and vascular system, such as coronary artery disease, myocardial infarction, angina, hypertensive heart disease, heart failure (HF), cardiomyopathy, arrhythmia, congenital heart disease, valvulopathy, aortic aneurysm, endocarditis, rheumatic heart disease, venous thrombosis, thromboembolic disease, and peripheral vascular disease [7]. Anthracyclines were the first group of chemotherapeutic agents recognized to affect cardiac function, and they are still the most commonly used drugs to treat solid tumors and hematological malignancies [8]. DOX is an anthracycline glycoside antibiotic and anticancer agent which induces DNA intercalation and inhibits DNA replication. It exhibits a broad spectrum of antineoplastic activity, including efficacy against breast cancer, leukemia, and sarcoma. A significant side effect of DOX is cardiotoxicity, which may clinically appear as congestive heart failure [9]. This may

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ultimately result in early or late onset irreversible degenerative cardiomyopathy, with the loss of terminally differentiated cardiomyocytes being the primary pathogenic part of HF. Various mechanisms influencing DOX-induced cardiotoxicity have been examined, including apoptosis, dysregulated autophagy, and necrosis [10]. The toxicity of DOX minimizes its application in chemotherapy, the adverse effects of DOX on cardiomyocytes are a major issue. Various pathways contribute to the cardiotoxicity associated with DOX; thus, numerous studies have demonstrated that oxidative stress, inflammation, and apoptosis are involved in this pathological process [11-13]. Oxidative stress occurs when generation of reactive oxygen species (ROS) exceeds the capacity of the body's antioxidant defense system. DOX markedly diminishes endogenous antioxidant levels, resulting in disrupting redox imbalances and increased oxidative stress. oxidative complications in the myocardium, in contrast to other tissues, are likely associated with decreased levels of antioxidant enzymes [14]. Oxidative stress contributes to cardiotoxicity due to the heart's lack of adequate antioxidant mechanisms [15] . MLT (N-acetyl-5-methoxytryptamine) is an indolamine formed from the amino acid tryptophan though serotonin which serves as an intermediate step. Its main production site is the brain's pineal gland. MLT participates in numerous physiological functions, such as circadian rhythms, reproduction, mood and behavior, cancer, and inflammation. It demonstrates anticancer and anti-apoptotic properties, MLT act as a potent antioxidant, combating oxidative stress both by directly scavenging ROS and enhancing the antioxidant defense system through a rise in antioxidant enzyme activities [16]. Significant scientific evidence demonstrates that MLT and its metabolites exert a beneficial impact on DNA, lipids, and protein as a result of both endogenous and exogenous free radical production processes [17]. Moreover, MLT enhances cardioprotective benefits against several cardiovascular disorders [18] . The current investigation aims to evaluate the protective therapeutic effectiveness of MLT against DOX-induced cardiotoxicity through an in vivo experimental approach, by assessing serum biomarkers of cardiac damage, oxidative stress indicators along with histopathological changes in myocardial tissue.

2 Materials and methods

2. 1 Chemicals

DOX was sourced from Stadapharm (Germany). Melatonin was obtained from GNB Pharma (Turkey). for the experiment, MLT was solubilized in 1% ethanol to a final working concentration of 5 mg/ml.

2. 2 Animals

The research was conducted on male Albino rats (Rattus norvegicus) with weights 250 to 300 grams. Animals were housed in the animal house of the Department of Biology, College of Science, University of Sulaimani, Sulaimaniyah, Iraq. The animals were kept in sterilized cages at 22 ± 2 °C, maintained under a 12-hour light/dark cycle, and provided with standard hygienic conditions, along with unrestricted access to standard rat feed and water. Ethics Committee of the College of Science sanctioned the research project No. (1) on August 24,2025 at the University of Sulaimani, Sulaymaniyah, Iraq.

2. 3 Doxorubicin Cardiotoxicity Induction

DOX solution was injected intraperitoneally into experimental rats at a dosage of 3 mg/kg body weight until the completion of the 42-day experiment, with injections occurring once per week.

2. 4 Melatonin solution preparation

MLT tablets (10 mg) were solubilized in 1% ethanol to prepare the working MLT solution. Rats received liquid MLT (30 mg/kg body weight) orally through a feeding tube (gavage) on a daily basis.

2. 5 Experimental Design

The current investigation is conducted to assess the influence of MLT on DOX-induced cardiotoxicity in albino rats. Experimental rats were randomly divided into three groups (n=6), each group was subjected to the following treatments:

1. Control group served as control rats: Administered intraperitoneal injection of sodium chloride once weekly for a duration of six weeks.

2. Doxorubicin group served as DOX group: Administered weekly intraperitoneal injection of DOX at 3 mg/kg over six weeks.

3. DOX+ MLT Group served as treated group: MLT was provided via oral daily gavage (10 mg/kg, six days per week) one hour after the administration of the 3 mg/kg DOX dose. Body weights were continuously measured weekly before the drug was administered throughout the research period.

2. 6 Blood Collection and Serum Preparation

Upon completion of the experimental period, following 42 days of treatment, all rats were subjected to overnight fasting. Animals were anesthetized using a ketamine–xylazine combination via intraperitoneal injection. Blood samples were obtained by means of cardiac puncture and immediately transferred into serum gel tubes. Following clot formation, samples were centrifuged at 3000 rpm for 30 minutes to separate the serum, The harvested serum was stored at −80 °C for biochemical analysis of both cardiac biomarkers such as BNP**,** CK-MB, LDH, cTnT-I levels**,** using enzyme-linked immunosorbent assay (ELISA) technique, and oxidative stress biomarkers, involve serum MDA and NO levels, conducted spectrophotometrically. Biochemical assessments were performed using ELISA kits in accordance with the manufacturer's instructions.

2. 7 Histological Analysis

Heart samples were excised from anesthetized rats and fixed in 10% formal saline; the selected sections were identified by a

histopathologist. The tissues received dehydration through ascending concentrations of ethanol (70%, 95%, and 100%), were cleaned with xylene, and later infiltrated and embedded in paraffin wax. Paraffin sections, four micrometers (4 µm) thick, were produced, and histological analysis was performed by examining hematoxylin and eosin (H&E)-stained slides under a light microscope. A microscope camera connected to the microscope and Amscope software (USA) enabled the analysis of histopathological images.

Statistical Analysis

The quantitative results are expressed as mean± standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way ANOVA, followed by Tukey's post-hoc test for significant results; A-p < 0.05 was considered statistically significant. GraphPad prism (Version 9.0.0) was used to make bar charts of the results. Histological examinations were statistically evaluated using SPSS software (version 23), with group means compared to determine significant differences between the experimental groups.

3 Results

3. 1 Role of MLT on DOX-Induced Alterations in BNP Levels

Figure (1) shows a significant increase in serum BNP levels in the DOX-treated group relative to the control group. The co-treatment of DOX+MLT led to a significant reduction in BNP levels compared to the DOX group. The BNP levels in the DOX+MLT group were slightly higher than in the control group, but the difference was not significant.

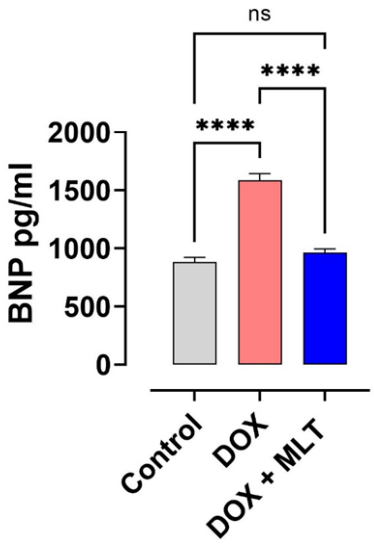

Figure 1: Serum brain natriuretic peptide (BNP) levels. BNP concentrations (pg/ml) in control, DOX-treated, DOX+MLT treated group. Compared to the control group DOX administration resulted in significant increase in BNP levels (**** p < 0.0001), whereas treatment with MLT along with DOX significantly lowered BNP levels compared with DOX group (*** p < 0.0001). Data are presented as mean ± SEM; ns = not significant.

3. 2 Role of MLT on DOX-Induced Alterations in Cardiac Troponin-I (cTnT-I) Levels

Figure (2) shows a significant rise in serum (cTnT-I) after DOX exposure in contrast with control group. The co-administration of DOX + MLT resulted in a minor reduction in cTnT-I levels compared with the DOX group; however, the observed reduction was not significant. The DOX+MLT group showed no significant difference from the control group.

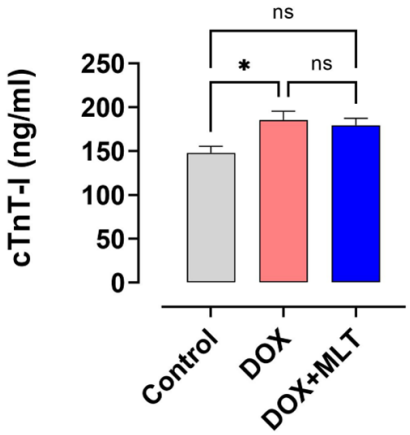

Figure 2: Serum Troponin-I levels; cTnT concentrations in control, DOX-treated, and DOX + MLT-treated rats. DOX significantly elevated troponin-I relative to control (* p < 0.05), while MLT co-treatment showed a non-significant decrease compared with DOX alone (ns, p > 0.05). Data presented as mean ± SEM.

3. 3 Role of MLT on DOX-Induced Alterations in LDH Activity

Figure (3) demonstrates that serum LDH levels exhibited no significant variations among the control, DOX + MLT groups. DOX injection caused a minor, non-significant elevation in LDH activity compared to the control. The co-administration of MLT did not significantly affect LDH activity compared with the DOX or control groups.

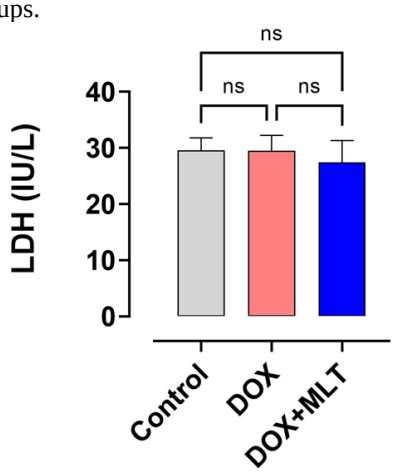

Figure 3: Serum LDH; activity in control, DOX-treated, and DOX + MLT -treated groups. Statistical analysis revealed no significant variation among the groups (ns, p > 0.05). Values are expressed as mean ± SEM.

3. 4 Role of MLT On DOX-Induced Alterations in CK-MB Levels

Figure (4) shows that serum CK-MB concentrations did not differ among the experimental groups. DOX therapy produced a minor, insignificant elevation in CK-MB relative to the control. The co-treatment of DOX + MLT resulted in a slight decrease in CK-MB levels than the DOX group, but the difference was not significant.

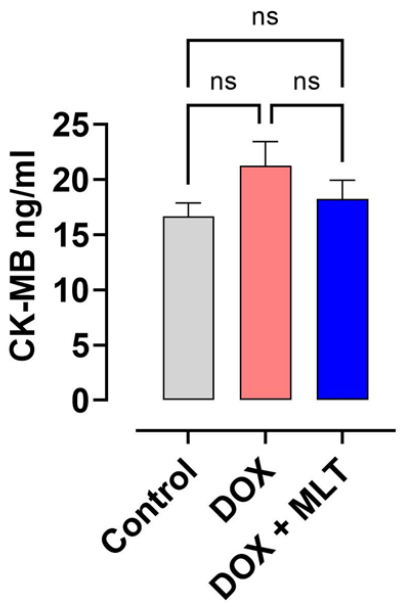

Figure 4: Serum creatine kinase–MB levels; (CK-MB) concentrations (ng/ml) in control, DOX-treated, and DOX + MLT-treated rats. Results (mean ± SEM) showed no statistically significant differences between experimental groups (ns, $p > 0.05$).

3.5. Role of MLT on DOX-Mediated Lipid Peroxidation

As Figure (5) presented, MDA concentration was considerably increased in rats receiving DOX injection compared to the control group. The co-treatment of DOX + MLT caused a minor decrease in MDA levels relative to the DOX group; however, this reduction did not reach significant. Moreover, no significant distinction was found among the control and DOX + MLT groups.

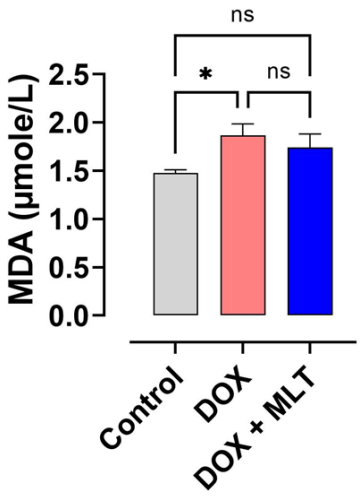

Figure 5: Serum malondialdehyde (MDA) levels; MDA levels (µmol/L) in control, DOX-treated, DOX+MLT treatment. The injection of DOX caused a significant increase in MDA than control group ($p < 0.05$), which means that lipid peroxidation was enhanced. Co-treatment with MLT exhibited a non-significant reduction in comparison to DOX-treated group ($p > 0.05$).

3. 6 Role of MLT on DOX-Mediated Alterations In NO Level

As shown in Figure (6), serum NO concentrations revealed no significant changes among the experimental groups. The injection of DOX caused slight, non-significant elevation in NO concentration relative to the control group. Similarly, the co-administration of DOX + MLT did not significantly alter NO levels compared with either the DOX-treated or the control groups.

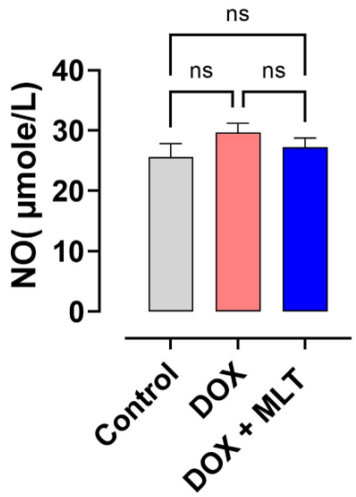

Figure 6: Serum nitric oxide (NO) levels; NO concentrations (µmol/L) in control, DOX-treated, and DOX + MLT co-treated rats. The analysis revealed no significant differences between the experimental groups (ns, $p > 0.05$). Data exhibited as mean ± SEM.

Table 1: Effect of MLT in DOX-induced cardiotoxicity on serum cardiac injury biomarkers (BNP, cTnT, LDH, CK-MB) and serum oxidative stress biomarkers (NO, MDA) level (Mean ± SEM).

	Serum Parameters	Groups		
		Control	DOX	DOX + MLT
Cardiac Markers	BNP (pg.ml)	883.9 ± 39.27	1587±57.23****	963.7±31.58
	LDH (IU/L)	29.59± 2.224	29.51± 2.735	27.37± 3.955
	CK-MB (ng/ml)	16.68± 1.197	21.26± 2.180	18.22± 1.729
	cTnT (ng/ml)	148.0±7.375	185.3± 9.994*	179.3± 8.009
Oxidative Stress Markers	MDA (µmole/L)	1.477±0.03451	1.864± 0.1197*	1.741± 0.1394
	NO (µmole/L)	25.60± 2.220	29.69± 1.511	27.21± 1.544

3.7. Histological Examination

Effect of MLT On Histological Alterations in DOX-Induced Cardiotoxicity

To assess cardiomyopathy at 400 x magnification, three distinct parameters in cardiac tissue were analyzed. Myocardial hypertrophy was evaluated by measuring the myocyte width in each group across 20 regions of the left ventricular cardiac wall. The width of myocytes was measured in micrometers, and the mean value was calculated. The coronary artery in the left ventricular cardiac wall was assessed in several regions around the circular circumference of the artery wall for each group, and the mean values were determined. Additionally, the necrotic area in the left ventricular wall was measured, and the means were calculated. Grading and scoring were performed as outlined in Table (2).

Table 2: Scoring and grading assessment for myocardial pathology parameters.

Cardiomyopathy Parameters	Threshold	Score	Grade
Myocyte Hypertrophy (µm)	≤ 1.25 ٪	0	No pathology
	1.25 - 2.25	1	Mild
	2.25 - 3.25 ٪	2	Moderate
	> 3.25 ٪	3	Severe
Coronary Artery Wall Thickness in the Left Ventricle (µm)	≤ 1.50 ٪	0	No pathology
	1.50 - 2.50 ٪	1	Mild
	2.50 - 5.00 ٪	2	Moderate
	> 5.00 ٪	3	Severe
Necrotic Area (µm²)	0٪	0	No pathology
	≤ 10٪	1	Mild
	10 - 20٪	2	Moderate
	> 20٪	3	Severe

The mean myocyte width in the normal group was 1.24 µm, which means that there were no pathological changes. In the model group that received DOX, the myocyte width increased up considerably to 2.61 µm, resulting in a score of 2, which means moderate hypertrophy. In the MLT-treated group, myocyte width significantly decreased to 1.39 µm, corresponding to a score of 1, indicating mild hypertrophy. This grading was based on quantitative measurement of myocyte width, in accordance with previously published methods [19].

Relating to the thickness of the coronary artery in the left ventricular wall, the normal group had a mean thickness of 1.48 µm, which is score 0. There were no signs of pathological changes. In the DOX-treated group, the thickness significantly increased to 5.36 µm, which is score 3, indicating severe thickness. The MLT-treated group had a significant decrease in thickness to 1.95 µm, corresponding to 1, which means mild pathological changes. For necrotic area, in the control group, there was no necrosis, with an area of 0 µm², corresponding to score 0, indicating no pathology. In the DOX-treated group, the necrotic area significantly increased to 29.61 µm², which is a score of 3, indicating severe necrosis. In the MLT-treated group, the necrotic area was greatly reduced to 8.93 µm², corresponding to score1, indicating mild necrosis. All these results indicate significant improvement in the MLT treated group, with a $p < 0.005$.

Table 3: Reveals the mean ± SD for different groups, with length measured in micrometers (µm) and area in square micrometers (µm²). Capital letter "A" indicates a significant difference between treated groups, with a $p < 0.005$, using one-way ANOVA (N = 5).

Cardiomyopathy Parameters	Group	Mean ± SD %	Score	Grade
Myocyte Hypertrophy (µm)	Normal	1.24 ± 1.13	0	No
	Doxorubicin	2.61 ± 1.18^{A}	2	Moderate
	Melatonin	1.39 ± 0.32^{A}	1	Mild
Coronary Artery Wall Thickness in the Left Ventricle (µm)	Normal	1.48 ± 0.29	0	No
	Doxorubicin	5.36 ± 1.19^{A}	3	Sever
	Melatonin	1.95 ± 0.32^{A}	1	Mild
Necrotic Area (µm²)	Normal	0.00 ± 0.00	0	No
	Doxorubicin	29.61 ± 1.86^{A}	3	Sever
	Melatonin	$8.93^{A} \pm 3.1$	1	Mild

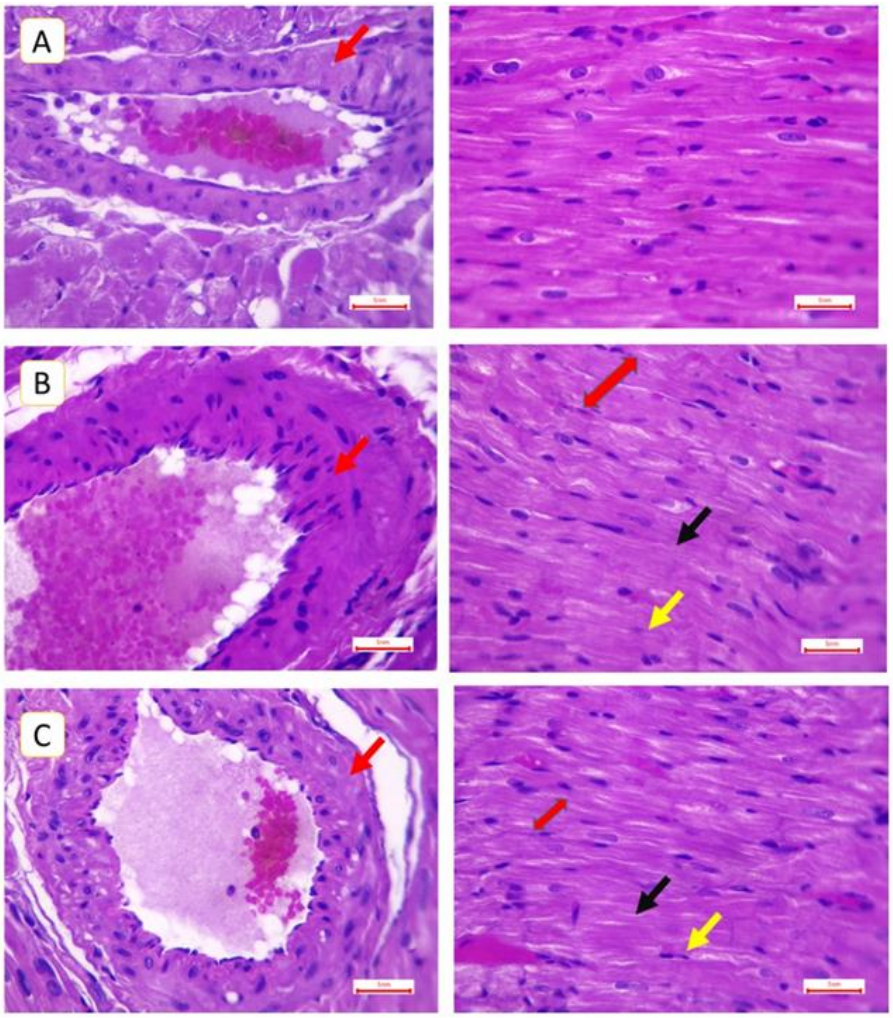

Figure 7: Histopathological micrographs in Panel A show preserved coronary artery wall thickness and organized cardiomyocytes in the left ventricle without significant pathological changes (score 0). In the DOX-treated group (Panel B), there is a marked increase in coronary artery wall thickness, extensive areas of necrosis (score 3), and moderate myocytic hypertrophy (score 2). In the MLT-treated group (Panel C), mild thickening of the artery wall, myocytic hypertrophy, and focal necrosis are observed (score 2). Red arrows indicate the artery wall, double-headed arrows denote cardiomyocyte width, black arrows highlight necrotic areas, and yellow arrows point to pyknotic nuclei within necrotic cardiomyocytes. Hematoxylin and Eosin (H&E) staining; original magnification ×400; scale bar = 5 µm.

4 Discussion

B-type natriuretic peptide is a hormone synthesized by the heart's ventricular myocardium when they are under ventricular stretching and wall stress. The level of serum BNP reflects the severity of HF and functionality of left ventricle; for this reason, serum BNP measurements are considered as essential indicators for both diagnosis and therapy [20]. Under the conditions of the present study, significant elevations in serum BNP levels were observed in the DOX-treated group in comparison to the control group, indicating the development of cardiac stress along with early ventricular dysfunction. these observations are consistent with ……. et al. [21], which shows BNP was greatly elevated in the DOX-treated rats compared to the control group. Increased BNP levels serve as clinical indicator for the beginning of acute HF, and its concentration correlated with the disease's severity [22]. The marked rise in BNP levels after DOX exposure is consistent with the known cardiotoxic effects of DOX, which may result in cardiomyopathy, arrhythmia, ischemia, systolic dysfunction, and HF. The primary findings leading to these disorders are caused by the death of cardiac cells and necrosis [23]. Moreover, a major rise in BNP levels after DOX exposure aligns with its recognized cardiotoxic effects, which include extensive cardiomyocyte damage, oxidative stress, mitochondrial dysfunction, and disrupted calcium homeostasis [24]. consistent with the prior research findings [25]. as ……. et al [26] explains, that natriuretic peptide hormones are released from cardiac atrial and ventricular myocardium when undergoing wall stress, and BNP concentration typically rise during chemotherapy treatment. MLT co-administration markedly diminished BNP concentrations, bringing them near to control levels, and did not differ significantly between the control and DOX+MLT groups. This discovery indicates that MLT alleviated DOX-induced cardiac stress, ……. et al. [27] explains that MLT, a significant natural antioxidant, could prevent DOX-induced oxidative damage. The study finding aligned with [28, 29], which revealed that MLT is a powerful free radical scavenger, shown to play an important role in preserving cell viability and cell membrane integrity under toxic conditions, primarily by diminishing oxidative damage from free radicals and suppressing inflammatory pathways. Moreover, MLT affects the cardiovascular system by mitigating the loss of essential cardiac tissue during ischemia/reperfusion injury [30].

Troponins, particularly their cardiac isoforms, cTnI and cTnT, are commonly used in human medicine to identify cardiac injury. These proteins, which are vital for muscle contraction, are secreted from the intracellular environment of cardiomyocytes into the bloodstream in case of damaged heart muscle cells induced by factors such as ischemia or cardiotoxic agents [31]. In contrast, the current findings indicated a slight elevation in serum cTn-I concentration from DOX-treated rats relative to control group; although a significant variation was observed among the control and DOX groups, while MLT co-treatment caused a non-significant reduction. According to [32], cTn serves as a sensitive and specific marker of myocardial necrosis. Apoptosis or necrosis of cardiomyocytes leads to the secretion of key intracellular enzymes and biomarkers, such as CK or cTn into the bloodstream, with increased cTn levels correlating with the severity of cardiac injury. Still, there are limitations associated with the use of cTn, cTn is rapidly eliminated from rat plasma, requiring that blood collection begins before its clearance. A previous study indicated that cTnI and cTnT have significant ability to predict myocardial necrosis and structural heart damage rather than detecting early myocardial injury. However, the duration required for cardiotoxic drugs to reach their peak varies. In comparison, the serum level of cTnI exhibits a significant increase 72 hours after the administration of DOX (20 mg/kg), supporting its use as a specific and early marker for acute myocardial damage induced by DOX. These elevations appear to respond to certain drug treatments, including DOX [33]. The slight rise noted here may suggest minor cardiac stress or subclinical myofibrillar damage caused by DOX without significant necrosis. current findings align with ……et al. [34], which suggests that the gradual loss in cardiac function may not cause sufficient cardiomyocyte damage to produce consistent elevated of cTnI levels when measured by using assays. However, newer high – and ultra -high sensitivity assays can detect lower concentrations, potentially revealing more subtle, treatment related changes in cTnI that were previously undetectable. Importantly, evidence clearly shows that cTnI levels rise markedly with increasing cumulative dose of DOX [35]. The MLT-treated group did not show a significant increase, and this may indicate a partial reduction of oxidative or inflammatory damage due to its antioxidant abilities; however, the effect may not have been sufficiently strong to cause an important decrease in damage. This result indicates that although DOX caused slight cardiac damage, MLT's preventive effectiveness was limited within the current experimental model.

Lactate dehydrogenase (LDH) is a fundamental enzyme present in in the cells of various organisms; it plays a crucial role in carbohydrate metabolism by catalyzing the reversible conversion between lactate and pyruvate within the NAD+/NADH coenzyme system [36]. According to the current investigation, LDH activity exhibited no significant variations across experimental groups. This observation may be due to the relatively small cumulative dosage and short duration of DOX utilized in the present protocol. In line with another studies [37, 38], using various cumulative dosages of DOX indicated that the cardiotoxic effects of DOX are dose dependent, which ultimately give a rise to HF. However, there's considerable variability among protocols for the overall received cumulative amount of DOX, the intervals between doses, and the duration needed to produce cardiopathy, indicating that elevated cumulative DOX levels lead to more severe structural damage and systolic dysfunction. In addition, according to ……et al. [39], serum LDH activity generally increases above the normal range during 24 to 48 hours after an acute myocardial infarction, reaching maximal level of two- to tenfold within 3 to 6 days, and later returning to baseline values within 8 to 14 days. This temporal pattern indicates the enzyme's release kinetics from injured myocardial tissue. Despite their beneficial diagnostic performance of CK and LDH, they showed a negative correlation with myocardial

damage and had low specificity. In contrast to the present findings, several studies have reported elevated LDH levels following DOX administration [40, 41]. The lack of a detectable reduction following MLT co-treatment indicates that MLT did not produce a significant systemic cytoprotective effect identifiable by this general enzyme assay. MLT demonstrates antioxidant properties by directly eliminating ROS and by stimulating endogenous antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, thus mitigating oxidative damage [42].

Creatine kinase (CK) is an enzyme that catalyzes transformations of creatine phosphate into creatine and adenosine triphosphate, thereby supplying energy for muscle contraction [43]. CK-MB primarily exists in cardiac muscle, accounting for about 25–46% of overall CK activity within myocardium, resulting minimal concentrations present in skeletal muscle [44]. As revealed by the present analysis, serum CK-MB levels exhibited no significant differences across the control, DOX, and DOX + MLT groups. A minor, non-significant increase was detected after DOX treatment. This may suggest slight cardiomyocyte membrane damage rather than obvious necrosis. ……..et al. [32] indicates that CK is rapidly cleared from circulation and has a short half-life; blood samples must be obtained before clearance. This discrepancy might result from the reduced cumulative dose and limited exposure period, perhaps leading to only minor myocardial injury insufficient to produce a detectable rise in CK-MB activity, which in agreement with ……. et al. [45], which exhibited that DOX induced significant variations in the serum activity of the CK–MB biomarker, correlating with adverse effects identified in each group. It is important to mention that these alterations were correlated with both the cumulative DOX dosage and the duration of DOX withdrawal. According to ……. et al. [31], CK-MB serves as an alternative to cardiac troponins; yet it is not highly selective to the myocardium because it is also found in skeletal muscles. The slight improvement in the MLT-treated group may indicate a partial reduction in oxidative or inflammatory damage due to its antioxidant characteristics; however, the effect may not have been sufficiently strong to result in a significant reduction. This result indicates that although DOX caused slight cardiac damage, MLT's preventive effects were limited within the current experimental condition.

No significant differences were observed across the experimental groups, suggesting that systemic NO bioavailability was not significantly changed or that compensating endothelium mechanisms preserved balance. The absence of significant change in this investigation may suggest that the endothelial dysfunction induced by the selected DOX dosage was insufficient to affect systemic NO levels. Owing to ……..et al. [46], administered high dose of DOX, leading to enhance NO generation via iNOS overexpression and higher serum nitrite levels; excessive NO production is linked to various cardiac disorders, including dilated cardiomyopathy and congestive heart failure. Prior research has indicated that elevated NO generation plays a role in DOX-induced increases in nitrosative stress. NO is endogenously produced through the enzymatic action of nitric oxide synthase (NOS), which catalyzes the conversion of arginine to citrulline [47] .as well as [48] explains that cardiotoxicity induced by DOX in mice is attributed with elevated serum NO concentration, oxidative and nitrosative stress, and myocardial inflammation. The co-administration of MLT did not significantly alter NO levels, aligning with its established free-radical scavenging characteristics. These findings suggest that DOX produced moderate oxidative stress without significant alterations in NO levels.

The assessment of oxidative stress parameters demonstrated a markedly elevated serum MDA levels in rats treated with DOX relative to control group, indicating that DOX exposure increased lipid peroxidation and oxidative stress. Current results are aligned with prior observations [13, 49], which explains that DOX treatment in rats resulted in an important rise in lipid peroxidation, revealed by major elevations in MDA levels. As DOX elevated ROS levels, ROS can promote and increase lipid peroxidation markers [50]. As a result, lipid peroxidation occurs, leading to oxidative damage to the mitochondria and plasma membrane of cardiomyocyte [51]. Since MDA serves as the primary biomarker for oxidative stress [52]. This is a final product of the peroxidation of polyunsaturated fatty acids [53]. The MLT co-treatment resulted in a minor reduction in MDA levels; however, this change did not achieve significance, indicating that the antioxidant protection may be partial and limited, possibly affected by the amount administered and treatment time. This finding is consistent with the ……. et al. [54], which display that MLT's antioxidant protection shows depends on both dose and time duration, indicating that low doses or shorter treatment durations could result in only partial antioxidant activity. Still, the decline in MDA indicates MLT's established free-radical scavenging properties and its capability to minimize lipid peroxidation to certain extents [55]. Research in nonuremic subjects has demonstrated that MLT treatment can decrease serum levels of MDA [56, 57].

Histopathological evaluation revealed that, in the DOX-treated group, it induced structural modifications in cardiac tissue, resulting in a significant increase in coronary artery wall thickness, large necrotic lesions, myocyte hypertrophy in the left ventricular myocardium, and mild myocytic hypertrophy. The modifications align with the widely known cardiotoxic potential of DOX. According to ……. et al. [58], cardiac hypertrophy and fibrosis, as well as cardiac cells, are recognized as the principal causes of DOX toxicity. In line with other studies [59, 60], which exhibits that Hearts treated with DOX exhibited an elevation in damaged cardiomyocytes, cellular swelling, localized necrosis, interstitial hypertrophy, and myocardial atrophy, demonstrating significant degenerative and structural damage of cardiac tissue. The MLT-treated group exhibits slight arterial wall thickening, myocytic hypertrophy, and localized necrosis. Recent data indicate that MLT significantly improved DOX-induced myocardial injury. MLT treatment markedly diminished myocyte width, coronary artery wall thickness, and necrotic area in contrast to the DOX-treated group, causing a reduction in

myocytic hypertrophy, vascular remodeling, and necrotic damage. Consistent with prior findings, the MLT+DOX group exhibited low myocyte disruption and slight inflammatory infiltration, with enhanced myocardial architecture and maintained cardiomyocyte morphology, suggesting that MLT successfully alleviated DOX-induced structural cardiac damage [61, 62]. The observed elevation may be linked to its cardio-protective properties, resulting from its strong antioxidant activity, as well as its ability to reduce oxidative stress and damage as a result of apoptosis of cardiomyocytes [63].

Conclusion

In conclusion, the current study demonstrates significant cardiotoxic effects in rats after DOX exposure, evidenced by elevated cardiac stress, enhanced lipid peroxidation, and marked structural damage to myocardial tissue. The increase in serum BNP and MDA levels, along with histopathological signs of cardiomyocytes hypertrophy, vascular wall thickening and necrosis, confirms the cardiotoxic effect of DOX. MLT co treatment markedly reduced these adverse effects, particularly by alleviating cardiac stress and maintaining myocardial architecture. Although circulating cardiac enzymes and systemic NO levels were not altered significantly, the substantial histological improvement suggests that MLT limits myocardial injury at the tissue level. Overall, these findings support MLT as a promising adjunctive agent for reducing cardiotoxicity induced by DOX- and warrant further investigation into its therapeutic application during chemotherapy.

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