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Potential Cardioprotective effect of Scopoletin on Dox-triggered Cardiotoxic effect in Rodents

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Abstract

Doxorubicin is a chemotherapeutic drug commonly employed in cancer treatment., renowned for its efficacy in destroying cancer cells. However, it is also associated with cardiotoxicity, which leads to damage in heart muscle cells (cardiomyocytes). A total of fifty-five adult rats were utilized and categorized divided into four separate groups for the study: To evaluate the overall health and potential cardiac impacts, we measured BW, heart weight, and Electrocardiogram (ECG) measurements. Serum levels of cardiac troponin-I were assessed to gauge heart muscle damage and cardiotoxicity. Additionally, serum malondialdehyde (MDA) levels were analyzed to determine lipid peroxidation, a sign of oxidative stress. Total serum antioxidant capacity (TAC) was also measured to assess the body's overall antioxidant defense. Furthermore, histopathological examination of heart tissue samples was conducted under a microscope to identify any structural or cellular damage resulting from the treatments. In the DOX group, notable alterations in ECG parameters were observed, signaling cardiac impairment. Additionally, there was a noteworthy rise in serum cardiac troponin-I level ($P < 0.001$), indicating damage to heart muscle. Serum malondialdehyde (MDA) levels, a marker of oxidative stress, also increased markedly ($P < 0.001$). In contrast, TAC showed a noteworthy decline ($P < 0.001$), suggesting diminished antioxidant defenses. Histopathological examination with haematoxylin and eosin staining revealed signs of cardiomyopathy, including structural abnormalities in the heart tissue. Furthermore, the apoptotic index, assessed through caspase-3 staining, was significantly higher ($P < 0.001$), reflecting increased cell death in the heart comparingwith the control group. Conversely, the animal group pre-treatment with scopoletin (SPT) exhibited noteworthy improvements. ECG parameters showed reduced signs of cardiac dysfunction, suggesting enhanced cardiac health. Levels of serum trponin ($P < 0.001$) and malondialdehyde ($P < 0.001$) were notably lower, indicating decreased heart muscle damage and oxidative stress. The TAC levels increased significantly ($P < 0.001$), reflecting improved antioxidant defenses. Histopathological analysis revealed better preservation of heart tissue structure with fewer abnormalities. Additionally, the apoptotic index was significantly lower ($P < 0.001$), suggesting reduced cell death in cardiac tissue comparing to doxorubicin group.

Keywords: Doxorubicin, free radicals, cardiotoxicity, apoptosis, scopoletin

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Introduction

Doxorubicin, an anthracycline antibiotic, is commonly utilized to treat a range of cancers, is limited by severe cardiotoxicity. This adverse effect is dose-dependent, affecting over 4% of patients at 500–550 mg/m² and more than 36% at 601 mg/m². DOX damages cardiomyocytes throughROS production, mitochondrial dysfunctionand cardiac apoptosis. Cardiomyocytes, with short levels of anti-oxidant enzyme, are particularly susceptible to oxidative stressinjury. Quercetin (SPT), a dietary flavonoid with anti-cancer, anti-inflammatory and antioxidant properties, scavenges ROS and inhibits kinase activation. This research explored the potential shielding effect of SPT pretreatment on doxorubicin-induced cardiotoxicity in animals[1,2,3,4,5,6]

Materials and Methods

Fifty-five male SD rat (150-200 gms), were housed in enclosures containing five rats eachat standard room temperature, with access to food and water. Duringstudy, there were casualties: four rats from the DOX group, two from the SPT + DOX group, and two from the SPT group. The control group experienced no fatalities. Each experimental group ultimately comprised 10 surviving rats.

Rats were separated into four different treatment groups: Control (Here's a rephrased version:
administered saline by mouth for a duration of 4 weeks.), DOX (given 2.5 mg/kg via intraperitoneal injection every two days for a period of 2 weeks), SPT + DOX (given SPT orally for 4 weeks, followed by a combination of SPT and DOX for the final 2 weeks), and SPT (received SPT orally for 4 weeks). BW was recorded at the commencement of research, after 2 weeks, and at conclusion. Upon euthanasia, HW was measured, and thenH:BW ratio was measured. Additionally, electrocardiograms (ECGs) were taken 24 hours after the final DOX dose to assess heart rate and QT interval.

Blood sample were gathered for the separation of serum and subsequent analysis of cardiac MDA, troponin-I andTAC. Histopathological examination of heart tissues was carried out using hematoxylin and eosin (H&E) staining to evaluate structural changs, and immunohistochemical (IHC) staining was employed to identify apoptotic cells.

Serum levels of cTn-I were measured with an ELISA kit, MDA levels were determined using spectrophotometry, and TAC was evaluated using a hydrogen peroxide assay kit. Heart tissues were fixed in formalin, and H&E staining was utilized for qualitative assessment of tissue morphology. Apoptotic cells were identified through IHC staining with a caspase-3 antibody, and the apoptotic index was calculated by counting cells in 10 high-power fields.

Statistical analysis was conducted using SPSS software, with results articulated as mean ± SEM. One-way ANOVA test were integrated as appropriate, followed by Tukey or Mann-Whitney post-hoc tests, with significance considered at $P < 0.05$. [7,8,9,10].

Results

Initially, all rat groups showed comparable weight gain during the first two weeks of the study. However, from that point onward, significant weight loss in both the SPT + DOX and DOX group. The weight loss was more pronounced in the DOX group compared to the SPT + DOX group, with the DOX group showing a substantial reduction in body weight relative to the control group (-23.70 ± 0.29 vs. 30.10 ± 0.25; $P < 0.001$). On the other hand, SPT treatment notably alleviated the body weight loss caused by DOX (-7.90 ± 0.23 vs. -23.70 ± 0.29; $P < 0.001$) when done comparison to the DOX group (see Table 1 for comprehensive data).

In terms of cardiac parameters, the DOX group demonstrated a significant decrease in heart weight (0.50 ± 0.009 g) and the heart-to-body weight ratio (3.92 ± 0.05 g/kg) compared to the control group (0.89 ± 0.01 g and 4.98 ± 0.04 g/kg, respectively; $P < 0.001$). Conversely, SPT treatment led to a significant increase in both heart weight (0.65 ± 0.007 g) and the heart-to-body weight ratio (4.46 ± 0.02 g/kg) compared to the DOX group (0.50 ± 0.009 g and 3.92 ± 0.05 g/kg, respectively; $P < 0.001$) (see Table 1 for detailed results).

Table 1: The effect of scopoletin on changes in BW, HW, the H:BW ratio, andECG parameter in rat with dox-induced cardiac injury

Parameters	Groups			
	Control (n=10)	DOX (n=10)	SPT+DOX(n=10)	SPT(n=10)
Initial body weight (g)	150.10±0.48	153.0±0.89	155.10±1.82	148.20±0.77
Body weight after 2 weeks (g)	165.40±0.54	164.60±0.33	166.70±1.25	165.0±0.29
Final body weight (g)	180.20±0.71	129.30±0.61*	147.20±0.71◊	180.60±0.58
Body weight change (g)	30.10±0.25	−23.70±0.29*	−7.90±0.23◊	32.40±0.24
Heart weight (g)	0.89±0.01	0.50±0.009*	0.65±0.007◊	0.90±0.006
Heart/body weight index (g/kg)	4.98±0.04	3.92±0.05*	4.46±0.02 ◊	5.01±0.02
HR (beats/min)	428.40±0.76	375.50±1.05*	398.80±0.93◊	429.30±0.73
QT interval (ms)	71.80±0.76	95.10±0.89*	81.20±0.71◊	72.60±0.70

All measurements are presented as mean ± SEM. The study included a total of 40 rats. Changes in body weight (in grams) were measured by subtracting the IBW from the FBW. The heart-to-body weight index (in grams per kilogram) was computed by dividing heart weight (in grams) by the final body weight (in kilograms). In this context, DOX stands for doxorubicin, HR denotes heart rate, and SPT represents scopoletin. Statistical significance was indicated as *P ≤ 0.05 for differences between the DOX group and the control group, and ◊P ≤ 0.05 for differences between the SPT + DOX group and the Doxorubicin group.

Doxorubicin (DOX) causes various changes in ECG parameters, including bradycardia, increased T wave amplitude, elevated ST segment, and prolonged QT interval. ECG recordings taken 24 hours after the final DOX dose showed significant bradycardia and a prolonged QT interval in DOX group comparing to the control group (375.50 ± 1.05 vs. 428.40 ± 0.76 for heart rate, and 95.10 ± 0.89 vs. 71.80 ± 0.76 for QT interval; P < 0.001). Scopoletin (SPT) theraphycaused a notable rise in heart rate and a reduction in QT interval comparing to DOX group (398.80 ± 0.93 vs. 375.50 ± 1.05 for heart rate, and 81.20 ± 0.71 vs. 95.10 ± 0.89 for QT interval; P < 0.001) (see Table 1).

Additionally, plasma level of cTn-I was significantly higher in the doxorubicin group comparing to control group (0.254 ± 0.013 ng/ml vs. 0.014 ± 0.001 ng/ml; P < 0.001). Scopoletin treatment significantly reduced serum cTn-I levels comparing to DOX group (0.130 ± 0.011 ng/ml vs. 0.254 ± 0.013 ng/ml; P < 0.001) (see Table 2).

Table 2: Effect of scopoletin on biochemical markers and the apoptotic index, evaluated via caspase-3 immunohistochemistry, in rats with cardiotoxicity induced by doxorubicin

Parameters	Groups			
	Control (n=10)	**DOX (n=10)**	**SPT+DOX(n=10)**	**SPT(n=10)**
Troponin (ng/ml)	0.014±0.001	0.254±0.013*	0.130±0.011◊	0.010±0.000
MDA (nmol/ml)	10.10±0.95	58.80±1.06*	29.80±0.97◊	9.80±0.81
TAC (mM/l)	0.085±0.005	0.011±0.001*	0.044±0.002◊	0.103±0.005
Apoptotic index %	1.20±0.29	14.60±1.0*	4.50±0.34◊	1.10±0.23

Results are presented as mean ± SEM, with "n" indicating the amount of rat per group. In this study, DOX stands for doxorubicin, MDA refers to malondialdehyde, SPT denotes scopoletin, and TAC represents total antioxidant capacity. The apoptotis index was determined by together with the number of apoptotic cell per 100 normal cells. Statistical significance was indicated by *P ≤ 0.05 for differences between the DOX group and the control group, and ◊P ≤ 0.05 for differences between the SPT + DOX group and the DOX group.

The average serum MDA level (in nmol/ml) was notably higher in the DOX group comparingto control group (58.80 ± 1.06 vs. 10.10 ± 0.95; P < 0.001). Scopoletin treatment significantly lowered serum MDA levels in

comparing to DOX group (29.80 ± 0.97 vs. 58.80 ± 1.06; P < 0.001) (see Table 2).
The average serum TAC level (in mM/l) was pointedly reduced in the doxorubicin group comparing to control group (0.011 ± 0.001 vs. 0.085 ± 0.005; P < 0.001). Scopoletin treatment notably increased serum TAC levels relative to DOX group (0.044 ± 0.002 vs. 0.011 ± 0.001; P < 0.001) (refer to Table 2). Histopathological investigation of cardiac tissues, done using H and E and caspase-3 staining, showed that control rat hasregular cardiac tissue morphology and histology, with none caspase-3 staining detected (see Table 3 and Figs. 1a, 2a).

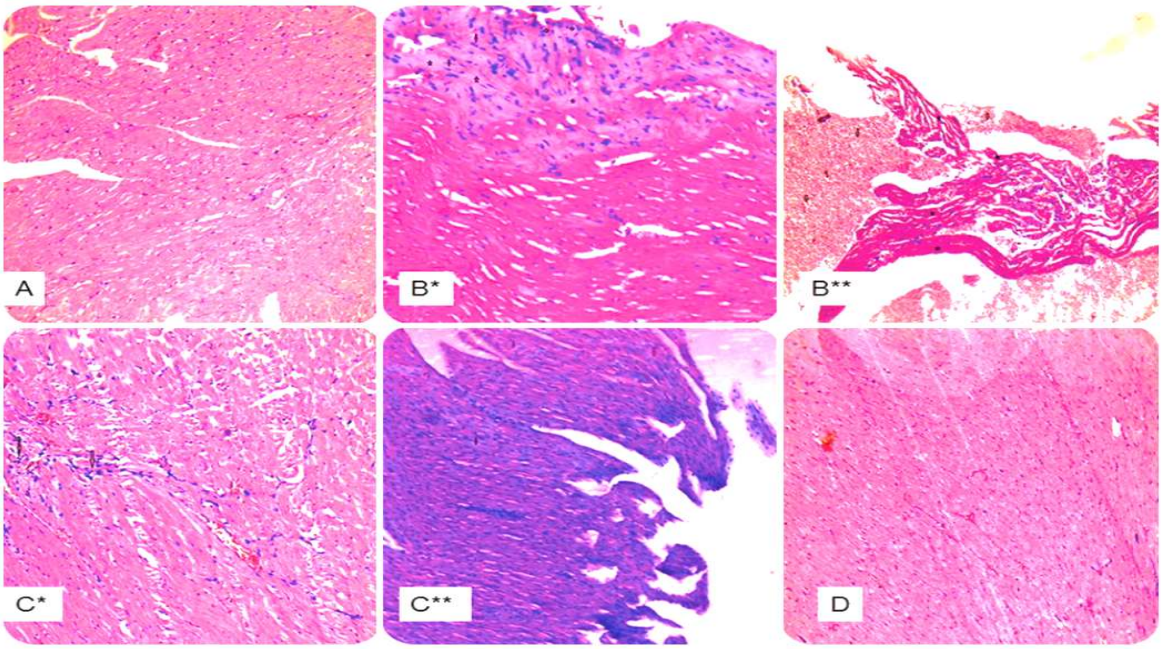

Figure. 1 Light microscopic analysis of heart sections stained with H&E revealed the following observations: (A) Sections from the control group displayed normal cardiac muscle cells with well-preserved structure; (B*) Sections from the DOX group showed signs of inflammatory cell infiltration, including plasma cells and lymphocytes, along with muscle degeneration; (B**) Some sections from the DOX group exhibited significant hemorrhage and further muscle degeneration; (C*) Sections from the SPT + DOX group revealed mild inflammatory infiltrates, predominantly lymphocytes; (C**) Other sections from the SPT + DOX group displayed minor degenerative changes; (D) Sections from the SPT group were similar in appearance to the cardiac muscle cells observed in the control group (H&E staining, ×100 magnification for A, B, C, and D).

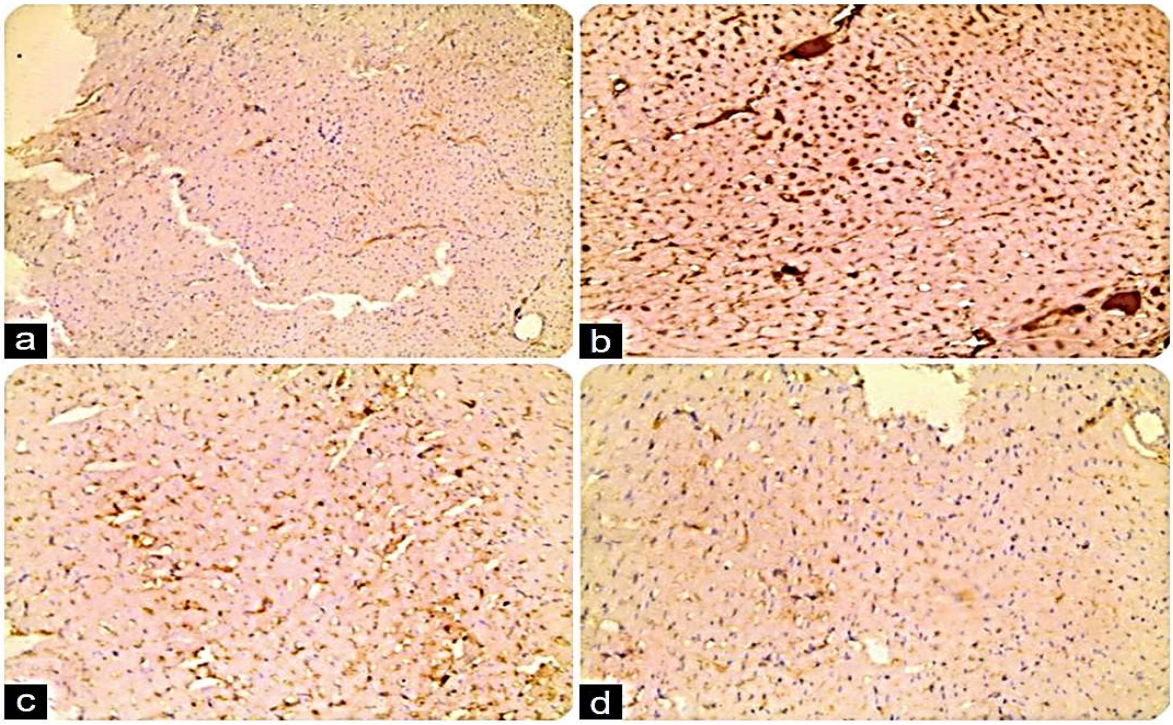

Figure. 2 Light microscopic examination of heart sections with IHC staining revealed the following: (a) Control group sections exhibited negative caspase-3 staining; (b) DOX group sections showed positive caspase-3 staining with a higher percentage of expression at ×200 magnification; (c) SPT + DOX group sections displayed positive caspase-3 staining with a lower percentage of expression at ×200 magnification; (d) SPT group sections showed negative caspase-3 staining at ×100 magnification (caspase-3, ×100 for a and d, caspase-3, ×200 for b and c).

In the DOX group, heart sections revealed marked inflammation (40% of rat hearts), significant hemorrhage (20%), extensive degeneration (60%), congested blood vessels (10%), and focal necrosis (20%) as observed in H&E staining (Table 3 and Fig. 1b*, b**). The SPT + DOX group exhibited mild inflammation (70% of rat hearts) and mild degeneration (30%), without hemorrhage, congested blood vessels, or necrosis (Table 3 and Fig. 1c*, c**).

DOX administration significantly increased apoptotic cells (Fig. 2b) and the apoptotic index (14.60 ± 1.0 vs. 1.20 ± 0.29; $P < 0.001$) as indicated by caspase-3 staining (Table 2). Conversely, the SPT + DOX group showed fewer apoptotic cells (Fig. 2c) and a significantly lower apoptotic index (4.50 ± 0.34 vs. 14.60 ± 1.0; $P < 0.001$) comparing to the DOX group (Table 2).

In contrast, the SPT group showed no noteworthychanges in BW, HW, the HW:B ratio, heart rate, or QT interval when comparing to control group. Serum level of cTn-I, malondihyde, and TAC were all within normal limits. Histopathological evaluation of the cardiac tissues from this group revealed normal heart muscle structure, with no signs of inflammation, degeneration, hemorrhage, or necrosis, and no caspase-3 staining was observed (see Tables 1–3 and Figs. 1d, 2d).

Table 3: Scopoletin's effect on histopathological parameters, assessed via haematoxylin and eosin (H&E) staining,

Parameters	**Groups**			
	Control (n=10)	**DOX (n=10)**	**SPT+DOX(n=10)**	**SPT(n=10)**
Inflammation				
Absent	10 (100.0)	2 (20.0)	3 (30.0)	10 (100.0)
Mild		2 (20.0)	7 (70.0)	

Moderate		2 (20.0)		
Marked		4 (40.0)		
Haemorrhage				
Absent	10 (100.0)	5 (50.0)	10 (100.0)	10 (100.0)
Mild		3 (30.0)		
Marked		2 (20.0)		
Congested blood vessels				
No	10 (100.0)	9 (90.0)	10 (100.0)	10 (100.0)
Yes		1 (10.0)		
Necrosis				
No	10 (100.0)	6 (60.0)	10 (100.0)	10 (100.0)
Focal		2 (20.0)		
Marked		2 (20.0)		
Degeneration				
No	10 (100.0)	1 (10.0)	7 (70.0)	10 (100.0)
Mild		3 (30.0)	3 (30.0)	
Marked		6 (60.0)		

Each parameter was expressed as theamount of rats(n) and the % of rats affected.

Discussion

Doxorubicin is a chemotherapy drug, treats a range of cancer, including both solid tumors and blood cancers. While effective, DOX is known to have considerable and potentially severe side effect, with cardiac damage, the important serious concerns[11].Scopoletin (SPT) is a polyphenolic compound present in numerous plant species. It is well-known for its strong anti-oxidant capabilities, which allow it to neutralize damaging free radicals and protect cells from oxidative stress. Additionally, SPT has anti-inflammatory properties, helping to regulate inflammation and support tissue health. It also exhibits antiapoptotic effects, meaning it can prevent programmed cell death, especially in stressed or injured cells. These characteristics make SPT a potential candidate for therapeutic strategies aimed at reducing oxidative damage and inflammation-related conditions[5]. Past studies, including research by Chen et al., have highlighted this connection[12], Research has explored the potential benefits of combining scopoletin (SPT) with doxorubicin (DOX) to counteract the latter's cardiotoxic effects. SPT, a polyphenolic compound with known antioxidant properties, may help alleviate DOX-induced heart damage by neutralizing free radicals and reducing oxidative stress.

Our results show that SPT pre-treatments significantly decreases DOXorubicin-induced oxidative stress, reduces cardiac cell apoptosis, lessens the overall cardiotoxic impact of DOX. These findings underscore SPT's potential as a therapeutic agent to prevent DOX-related heart damage. This aligns with previous research by Kocahan et al., reinforcing the idea that SPT could provide significant benefits in reducing DOX's negative effects on the heart. [13] Razmaraii et al. found that scopoletin (SPT), a flavonoid, effectively reduced the hepatotoxic and nephrotoxic effects caused by oxidative stress from doxorubicin (DOX) and cyclophosphamide. Their study revealed that SPT increased antioxidant levels and decreased oxidant levels and lipid peroxidation, thereby reducing oxidative damage. This highlighted SPT's protective capabilities against toxicity caused by ROS in the kidney and liver.

In our research, same as of Razmaraii et al., DOX treatment resulted in reduced BW, HW, and the H:BW ratio in rats, reflecting the systemic impact of DOX-induced illness. These observations are reliable with those observed by Razmaraii et al., further emphasizing the destructive effect of Doxorubicin on these physiological parameters.[14] who relatedthe decrease in these parameters to DOX toxicityand also with Periyasamy et al. [15] Matouk et al. reported different findings, indicating that the reductions in BW and HW observed in rat given with doxorubicin might not be solely due to decreased food intake. Their research likely investigated other contributing factors, such as metabolic disturbances, oxidative stress, or specific cardiotoxic mechanisms associated with DOX that affect tissue structure and function.

In our study, consistent with the observations of Matouk et al., we noted significant decreases in both BW and

HW in rats given with Doxorubicin. These results underscore the complex nature of DOX-induced cardiotoxicity, suggesting that factors beyond mere changes in food intake contribute to alterations in body and cardiac weights.[9]Ma et al. expanded on the understanding of Doxorubicin-induced cardiac injury by linking the observed rise in HW and the H:BW ratio in treated animals to cardiac hypertrophy and remodeling. They attributed these changes to the release of proinflammatory cytokines triggered by doxorubicin, which are associated with reactive oxygen species (ROS) produced by the drug. These ROS contribute to oxidative stress and subsequent cellular damage in the heart. Their research highlights the intricate pathophysiological processes involved in DOX-induced cardiotoxicity, emphasizing the interplay between inflammatory responses and oxidative stress in causing structural changes in the heart[16] Ma et al. attributed the observed increase in heart weight following doxorubicin (DOX) treatment to an increase in myofibrillar thickness, indicating a hypertrophic response of the heart muscle. This change may represent a compensatory mechanism to counteract damage caused by DOX. Variations in study outcomes, including those investigated by Matouk et al. and Razmaraii et al., could be due to differences in experimental variables such as the rat strains used, DOX dosages, and timing of evaluations. These factors can affect the severity and characteristics of cardiotoxicity observed in preclinical models of DOX-induced cardiomyopathy[9,14–16]. Our study also found that pretreatment with scopoletin (SPT) alleviated weight loss and enhanced the H:BW ratio compared to the doxorubicin-treatment group. These observations are consistent with earlier research that has highlighted SPT's protective properties against oxidative stress and its potential therapeutic role in reducing doxorubicin-induced cardiotoxicity[15].

In our research, the occurrence of cardiac injury in the doxorubicin group was indicated by notable changes in ECG parameters, such as bradycardia, a prolonged QT interval, and an elevated ST segment. These results align with those observed by Ma et al., underscoring the cardiotoxic effects of doxorubicin, particularly through alterations in heart rate and QT interval prolongation [16]. However, scopoletin (SPT) demonstrated protective effects on the heart by enhancing heart rate and normalizing QT intervals. These findings are reliable with the result reported by Santos et al [17] who observed that SPT-elicited positive inotropism was dependent on β1-adrenoceptors and was associated with increased intracellular CAMP.Also, Santos et al. [17] demonstrated that the beneficial cardiac inotropic effect of scopoletin (SPT) is linked with rise in the amplitude wave of L-type channelcalcium currents.

Troponin I is particularly specific to cardiac muscle and is not present in skeletal muscle tissue. This specificity makes troponin I a highly effective biomarker for identifying myocardial damage[18].

In this research, the presence of cardiac injury in the doxorubicin group was further confirmed by increased serum level of cTn-I, which is indicative of myocardial injury. This observation is consistent with earlier research by Atas et al., which established that elevated cTn-I levels are a reliable indicator of cardiac damage caused by doxorubicin[19].However, pretreatment with scopoletin (SPT) resulted in reduced serum level ofcTn-I. This outcome aligns with the results reported by Yaseen et al., which suggest that SPT offers protection against doxorubicin-induced cardiotoxicity by lowering cTn-I levels, thus indicating a reduction in myocardial damage[20].Scopoletin (SPT) appears to offer significant protective benefits against doxorubicin (DOX)-induced cardiotoxicity in rats, as demonstrated by improvements in ECG readings and decreased levels of serum cardiac troponin I (cTn-I). The involvement of reactive free radicals produced by DOX in causing oxidative damage to the heart is particularly critical due to the heart's high mitochondrial density and relatively lower antioxidant defenses compared to other organs, highlighting the serious nature of DOX-induced cardiotoxicity[21]. Upon entering cardiomyocytes, doxorubicin (DOX) is metabolized by mitochondrial enzymes into its semiquinone form. This process results in the production of increased level of ROS, including H_2O_2 and $O2^-$ [22].In accumulation to generating harmful radicals, doxorubicin also decline the level of natural antioxidants within cells. These combined effects promote apoptosis in cardiomyocytes, which is the programmed cell death of heart muscle cells [3].

Our findings showed an increase in serum malondialdehyde (MDA), indicating elevated ROS, and a decline in total TAC in DOX-treated group. These results align with the observations made by Sun et al. [23]. Additionally, scopoletin (SPT) has demonstrated protective effects in various conditions: it guards against myocardial infarction, offers neuroprotection in Alzheimer’s disease, protects the liver from non-alcoholic steatohepatitis, and provides chemoprotective benefits. These protective actions of SPT are largely attributed to

its antioxidant properties [24]. In this study, pretreatment with scopoletin (SPT) led to an increase in total antioxidant capacity (TAC), enhancing antioxidant activity, and a reduction inMDA, a biomarker of oxidative-stress. These findings are consistent with the results reported by Dong et al [25]. Scopoletin (SPT) pretreatment helps safeguard rat hearts from cardiotoxicity induced by doxorubicin (DOX) by mitigating oxidative stress and damage. After 2 weeks of DOX treatment, rats exhibited significant histopathological changes, including inflammation, bleeding, engorged blood vessels, tissue degeneration, and necrosis, in line with observations made by Iqbal et al[26].Furthermore, our study found that scopoletin (SPT) has anti-inflammatory properties, as evidenced by improvements in the histopathology of doxorubicin induced cardiotoxicity. SPT treatment led to reductions in degeneration, necrosis and inflammation, , necrosis within the cardiac muscles. These reports are steady with those investigated by Matouk et al[9].While the precise mechanisms behind doxorubicin (DOX)-induced cardiotoxicity are not fully elucidated, growing evidence indicates that cardiac apoptosis is a key factor. It is widely believed that oxidative stress caused by DOX plays a crucial role in activating apoptotic pathways, which subsequently leads to the death of cardiomyocytes[27].In our study, DOX-treated rats showed increased apoptosis, as showed by the induction of caspase-3 observed through immunohistochemical staining of cardiac tissue and a higher apoptotic index in this group. These results align with findings from earlier research conducted by Zhao and Zhang[28]. Additionally, our results demonstrate a reduction in apoptosis following pretreatment with scopoletin (SPT). This investigation is reliable with work of Chen et al., which suggests that SPT has a protective role in mitigating apoptosis caused by doxorubicin (DOX)[29].These investigation support the idea that scopoletin (SPT) may protection against doxorubicin induced cardiac injury, by reducing cardiac apoptosis. This aligns with earlier research, such as that conducted by Chen et al., which suggests that SPT helps to lessen apoptosis in cardiac tissues caused by DOX.

Conclusion

In summary, scopoletin (SPT) exhibits a protective effect on the heart against doxorubicin induced cardiac injury in rats by mitigating ROS. This reduction in ROS leads to declined apoptosis and helps maintain the structural integrity of myocardium. These finding suggests, SPT could be anvaluable candidate for managing DOX-related cardiotoxicity. However, additional research is necessary to clarify the exact mechanisms by which SPT counters oxidative stress.

Future studies should also explore whether administering SPT either prior to or alongside DOX affects the drug's antitumor effectiveness. It is essential to understand these interactions to develop therapeutic approaches that effectively balance heart protection with successful cancer treatment.

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