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Protective role of Sepia ink against doxorubicin-induced myocardial infarction in rats

Papel protetor da tinta de sépia contra o infarto do miocárdio induzido por doxorrubicina em ratos

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ABSTRACT

Introduction: Myocardial infarction (MI) is caused by a reduction or total cessation of blood supply to a section of the myocardium. Despite its potential efficacy in the treatment of various malignancies, doxorubicin's (DOX) capabilities are limited due to its extensive cardiotoxic effect. Sepia ink (SI) is an alternative medication with numerous therapeutic applications. It possesses anti-oxidant, anti-radiation, and anti-bacterial characteristics. The current study aims to evaluate the protective effect of SI against DOX -induced myocardial infarction in rats. **Methods:** A total cumulative dose of DOX (18 mg/kg body weight, i.p.) was administrated once daily on days 2, 4, 6, 8, 10, and 12 (i.p.) to induce MI in rats. 18 rats were divided into 3 groups; control, MI, and MI treated with SI (200 mg/kg, orally). **Results**: Treatment with SI restored the ST-segment to near-normal levels. SI decreased levels of cardiac troponin T, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, alanine amino transferase, malondialdehyde, While it increased nitic oxide, catalase, and reduced glutathione levels. The histological studies found that the SI-treated group demonstrated significant improvement in the heart and liver tissues with reduction in collagen deposition. **Discussion:** SI demonstrated cardioprotective properties in the myocardial infarcted rats by boosting the antioxidant system and stimulating nitric oxide production. **Conclusion**: This study showed that SI serve as a promising therapeutic agent for mitigating doxorubicin-induced cardiotoxicity mediated through its anti-inflammatory and antioxidant properties.

Key words: Myocardial infarction, Doxorubicin, Sepia ink, Oxidative stress, Cardioprotective effect.

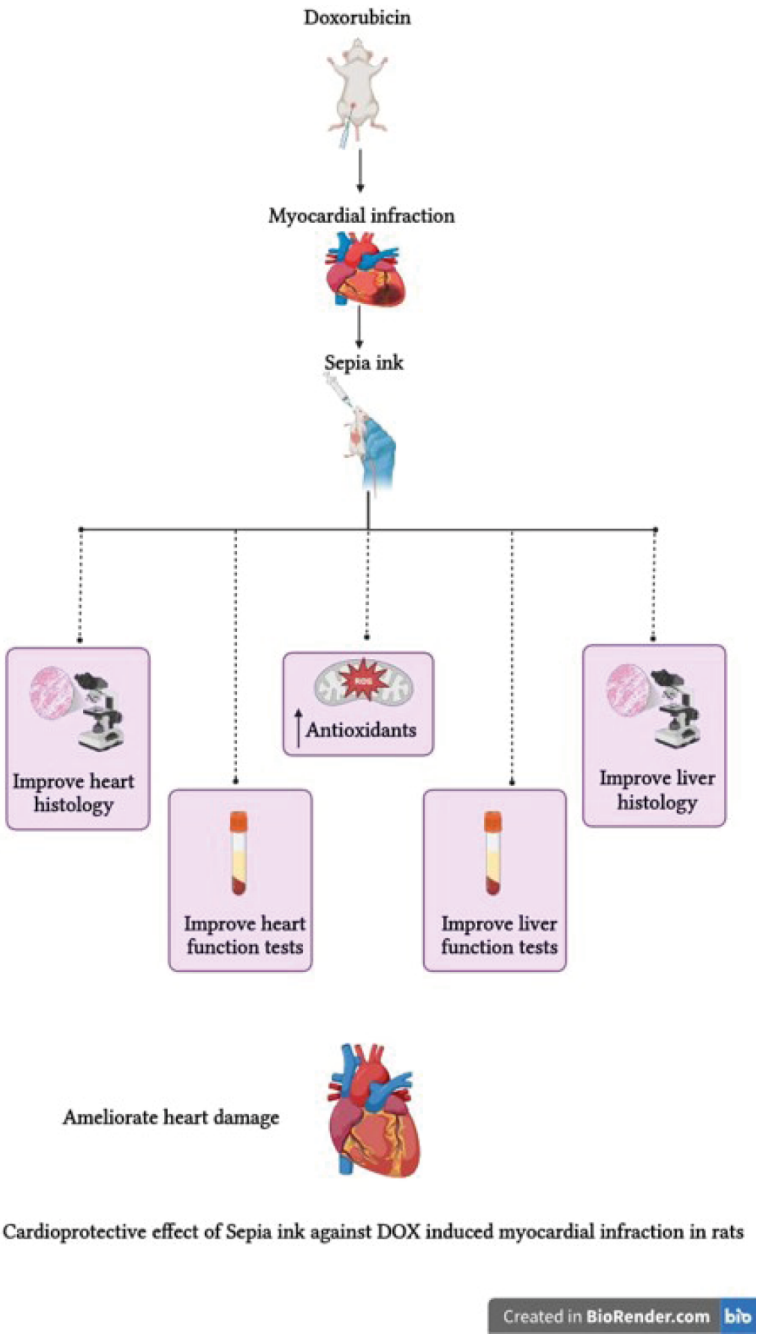
RESUMO

Introdução: O infarto do miocárdio (IM) é causado pela redução ou interrupção total do suprimento sanguíneo para uma região do miocárdio. Apesar de sua potencial eficácia no tratamento de diversas neoplasias malignas, o uso da doxorrubicina (DOX) é limitado por seus efeitos cardiotóxicos acentuados. A tinta de sépia (TS) é uma alternativa terapêutica com diversas aplicações, apresentando propriedades antioxidantes, antirradiação e antibacterianas. O presente estudo teve como objetivo avaliar o efeito protetor da TS contra o infarto do miocárdio induzido pela DOX em ratos. **Métodos:** Para induzir o IM nos ratos, foi administrada doxorrubicina por via intraperitoneal (i.p.), na dose cumulativa total de 18 mg/kg de peso corporal, uma vez ao dia, nos dias 2, 4, 6, 8, 10 e 12. Os 18 ratos foram divididos em três grupos: controle, IM e IM tratado com TS (200 mg/kg, por via oral). **Resultados:** O tratamento com TS restaurou o segmento ST a níveis próximos da normalidade. A TS reduziu os níveis de troponina cardíaca T, creatina quinase, lactato desidrogenase, aspartato aminotransferase, fosfatase alcalina, alanina aminotransferase e malondialdeído,

enquanto aumentou os níveis de óxido nítrico, catalase e glutationa reduzida. Os estudos histológicos demonstraram que o grupo tratado com TS apresentou melhora significativa nos tecidos cardíaco e hepático, com redução da deposição de colágeno. **Discussão:** A TS demonstrou propriedades cardioprotetoras em ratos com infarto do miocárdio, por meio do fortalecimento do sistema antioxidante e do estímulo à produção de óxido nítrico. **Conclusão:** Este estudo demonstrou que a TS constitui um potencial agente terapêutico para atenuar a cardiotoxicidade induzida pela doxorrubicina, por meio de suas propriedades anti-inflamatórias e antioxidantes.

Palavras-chave: Infarto do miocárdio; Doxorrubicina; Tinta de sépia; Estresse oxidativo; Efeito cardioprotetor.

GRAPHICAL ABSTRACT



Cardioprotective effect of Sepia ink against DOX induced myocardial infraction in rats

1. INTRODUCTION

Myocardial infarction (MI) is a widely recognized manifestation of cardiovascular illness, primarily associated with an imbalance between coronary blood supply and myocardial demand (1). One of the leading causes of death worldwide and a significant contributor to the overall illness burden is MI (2). MI can also result in notable symptoms, including myocardial fibrosis and heart enlargement (3).The precise process involving MI has been demonstrated to be linked with oxidative stress, apoptosis, and inflammation (4). Annually, over 3 million individuals experience MI, while more than 4 million exhibit ST elevation pathology, characterized by the flat, isoelectric segment of the electrocardiograph (ECG) between the conclusion of the S wave and the onset of the T wave (5).

Doxorubicin (DOX), an anthracycline antibiotic utilized in the treatment of several malignancies, has been identified as a cause of cardiotoxicity in instances of overdose (6). DOX's cardiac effects can be observed in a variety of ways, such as alterations in ECG, pericarditis, myocarditis, arrhythmias, myocardial infarction, heart failure, cardiomyopathy, and congestive heart failure (7). These complications may manifest during the course of treatment, immediately following its conclusion, or even weeks later (8). The molecular pathways via which this antineoplastic drug causes cardiac damage are thought to differ from its therapeutic mode of action. While the precise mechanisms are yet to be completely clarified, alterations in calcium and iron homeostasis, mitochondrial dysfunction, inhibition of DNA replication through DNA modification, and direct membrane damage resulting from lipid peroxidation have all been recognized as significant causes (9).

The marine environment is a valuable source of both food and unique natural compounds (10). The marine environment contains several bioactive components with significant nutritional and functional benefits (11). Marine-derived bioactive compounds are used in beverages as functional and therapeutic supplements (12). Sepia ink (SI), a natural defense system, has been shown to have numerous therapeutic applications (13). It includes various bioactive chemicals, including oligopeptides, melanin, flavonoids, fatty acids, angiotensin-converting enzyme, alkaloids, tyrosinase, DOPA, melanin synthase, catecholamines, minerals, polyphenols, and polysaccharides (14). Numerous pharmacological and therapeutic properties of SI have been documented, including anti-cancer, antibacterial, antioxidant, antiviral, anti-ulcerative, antihypertensive, and hepatoprotective activities (15) . Hence the current study aimed to evaluate the effect of SI against doxorubicin-induced myocardial infarction in rats.

2. MATERIALS AND METHODS

2.1. CHEMICALS AND REAGENTS

DOX was purchased from Misr Company for pharmaceuticals. While all the other chemicals used were obtained from Sigma-Aldrich (St. Louis, MO, USA). Biochemical parameters were determined using kits obtained from Spectrum Diagnostics Company (Obour City, EGY). While the oxidative stress kits were bought from the Biodiagnostic Company (Giza, Egypt) and (Life Diagnostics, West Chester, AS).

2.2 PREPARATION OF SEPIA INK EXTRACT (SI)

SI were prepared using the method stated by Saleh et al. (2015) (16). Fresh cuttlefish (*Sepia officinalis*) were acquired immediately from a fishmonger. They were swiftly transported to the laboratory, where they were dissected, and their ink was promptly collected and diluted with an equal volume of purified water, followed by complete mixing. The mixture was promptly collected, concentrated, and lyophilized to yield a black residue utilizing a lyophilizer (LABCONCO, shell freeze system, USA).

2.3. GC-MS ANALYSIS

SI underwent GC-MS analysis utilizing a split injection ratio of 1:20 in hexane on a Shimadzu GC--MS QP 2010 gas chromatograph (Japan), featuring a capillary column measuring 30 m in length, 0.25 mm in diameter, and 0.25 µm in film thickness. The GC-MS operating settings were injector temperature at 280°C, column oven temperature at 45°C, flow control mode set to linear velocity, and helium (99.9% purity) column flow at 1.40 mL/min. The oven temperature was set at 45°C for 2 minutes and

300°C for 10 minutes, with a total holding duration of 36.5 minutes. The mass spectrometry conditions employed were electron impact at 40 eV, ion source temperature at 200°C, and interface temperature at 240°C. Individual components were discovered through matching with the Wiley 139.LIB and NISTO.5 LIB databases. The percentage composition was established by area normalization.

2.4. ANIMALS

In the current investigation, adult male Wistar rats (*Rattus norvegicus*) weighed 150–170 g was employed. Animals were supplied with a standard laboratory diet and water *ad libitum* and were grouped and confined in polypropylene cages (three animal/cage) at a temperature of (22–25) ˚C throughout a natural day/night cycle. The animals were obtained from the National Research Center (NRC), Egypt.

2.5. ETHICAL CONSIDERATION

The Cairo University Institutional Animal Care and Use Committee (IACUC) (Egypt) (CU/I/F/22/24) granted approval for the experimental protocol and procedures utilized in this study. The experimental procedures were implemented in accordance with international standards for the care and management of laboratory animals. Sample size was calculated statistically G*Power 3.1.9.7 and approved by the IACUC committee.

2.6. INDUCTION OF MYOCARDIAL INFARCTION

A cumulative dose of DOX (18 mg/kg body weight) was provided via intraperitoneal injection once daily on days 2, 4, 6, 8, 10, and 12 to induce myocardial infarction (MI) in rats (17).

2.7. EXPERIMENTAL DESIGN AND ANIMAL TREATMENT

Eighteen-rats were separated into three groups, each with six rats as follows:

Group 1 (control): rats received distilled water orally daily for two weeks, in conjunction with 0.9% saline administered intraperitoneally on days 2, 4, 6, 8, 10, and 12.

Group 2 (MI): rats were administered Dox (3 mg/kg body weight, intraperitoneally) on days 2, 4, 6, 8, 10, and 12, and received distilled water orally daily.

Group 3 (MI + SI): rats were administered Dox (3 mg/kg body weight, intraperitoneally) on days 2, 4, 6, 8, 10, and 12, and received SI (200 mg/kg, orally) (Saleh et al., 2015) every day for 2 weeks.

2.8. ECG MEASUREMENT

At the end of the study period, the rats underwent an overnight fast followed by anesthesia with 3% sodium pentobarbital. Rats were positioned supinely and leads from the conventional electrocardiograph (ECG 100G single-channel Electrocardiograph-China) were attached to the dermal layer of both forepaws and rear limbs for ECG measurement.

2.9. ANIMAL HANDLING AND SPECIMEN ASSEMBLY

Blood samples were promptly obtained in sterile centrifuge tubes via the exsanguination procedure. The obtained serum was kept at -20˚C until utilized for biochemical tests. The heart and liver were excised and placed on filter paper to eliminate blood residues. Parts of the liver and heart were preserved at -80˚C for biochemical investigation. Additional parts of the heart and liver were immersed in 10% formal saline for fixation in preparation for histological analysis.

2.10. DETERMINATION OF SERUM BIOCHEMICAL PARAMETERS

The accessible kits were utilized for the quantification of creatinine kinase (CK), alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), albumin (ALB), and total protein (TP) employing Spectrum (Obour City Industrial Area, Cairo, Egypt), while the available ELISA kit from CUSABIO (Houston, Texas, 77054, US) was employed for troponin T quantification.

2.11. DETERMINATION OF OXIDATIVE STRESS PARAMETERS

The heart and liver tissues were homogenized at a concentration of 10% w/v in ice-cold 50 mM

phosphate buffer at pH 7.4. The homogenate underwent centrifugation for 15 minutes at 4 ˚C and 4000 rpm. Markers of oxidative stress were evaluated in the supernatant. The concentrations of malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), and catalase (CAT) were quantified.

2.12. HISTOPATHOLOGICAL EXAMINATION

Heart and liver tissues were preserved in 10% neutral buffered formalin. The specimens were fixed, cleaned, dehydrated, and embedded in paraffin wax. The tissues were sliced to a thickness of 4-5 µm and stained with hematoxylin and eosin (H&E). picrosirius red stain (PSR) was used for normal bright field and polarized light examinations to assess the degree of fibroplasia, which was quantified as an area percentage.

2.13. STATISTICAL ANALYSIS:

Means and ±SE were used to express values. Data normality was evaluated using the Shapiro–Wilk test, while homogeneity of variances was examined using Levene's test. Values were reported as means ± SEM. One-way ANOVA with Duncan post hoc test was used to compare group means, with p-values < 0.05 indicating statistical significance. Duncan post hoc test was chosen because it provides greater sensitivity for detecting differences among treatment means in biological experiments involving multiple groups, while maintaining an acceptable balance between Type I and Type II error rates." The statistical analysis was performed using SPSS for Windows (version 15.0).

3. RESULTS

3.1. GC- MASS ANALYSIS

Figure 1 illustrates that the GC-MS analysis of SI revealed a prominent peak for cis-13-eicosenoic acid (20.37 %), trans-13-octadecenoic acid (15.0%), ethanol, 2-(9-octadecenyloxy) (13.87%), and E,E,Z-1,3,12-nonadecatriene-5,14-diol (10.65 %).

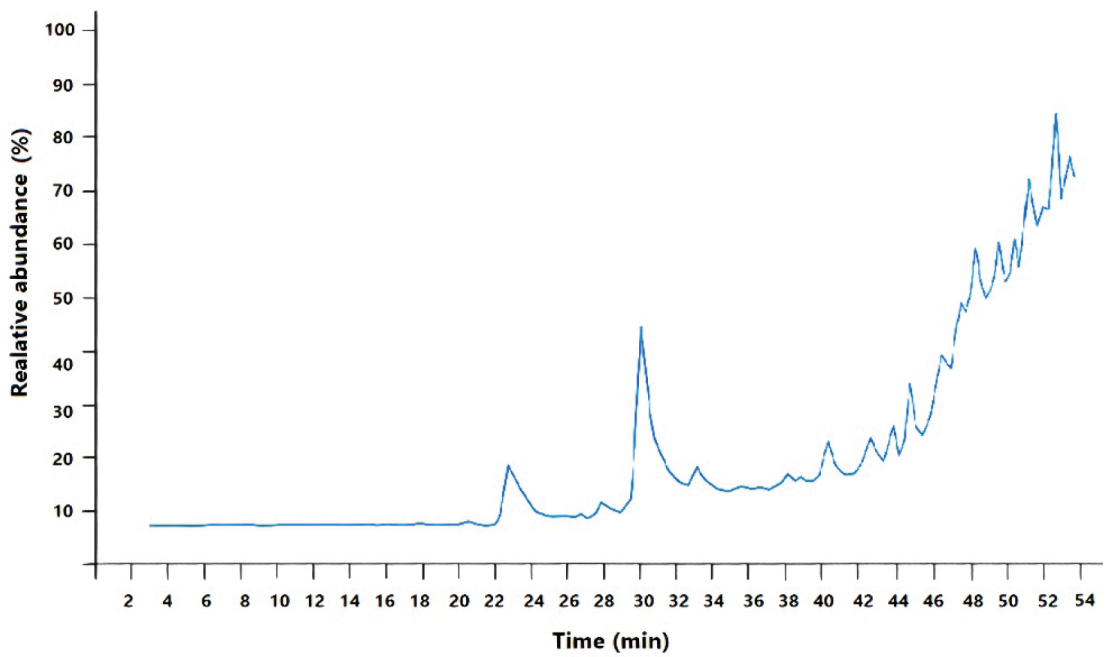
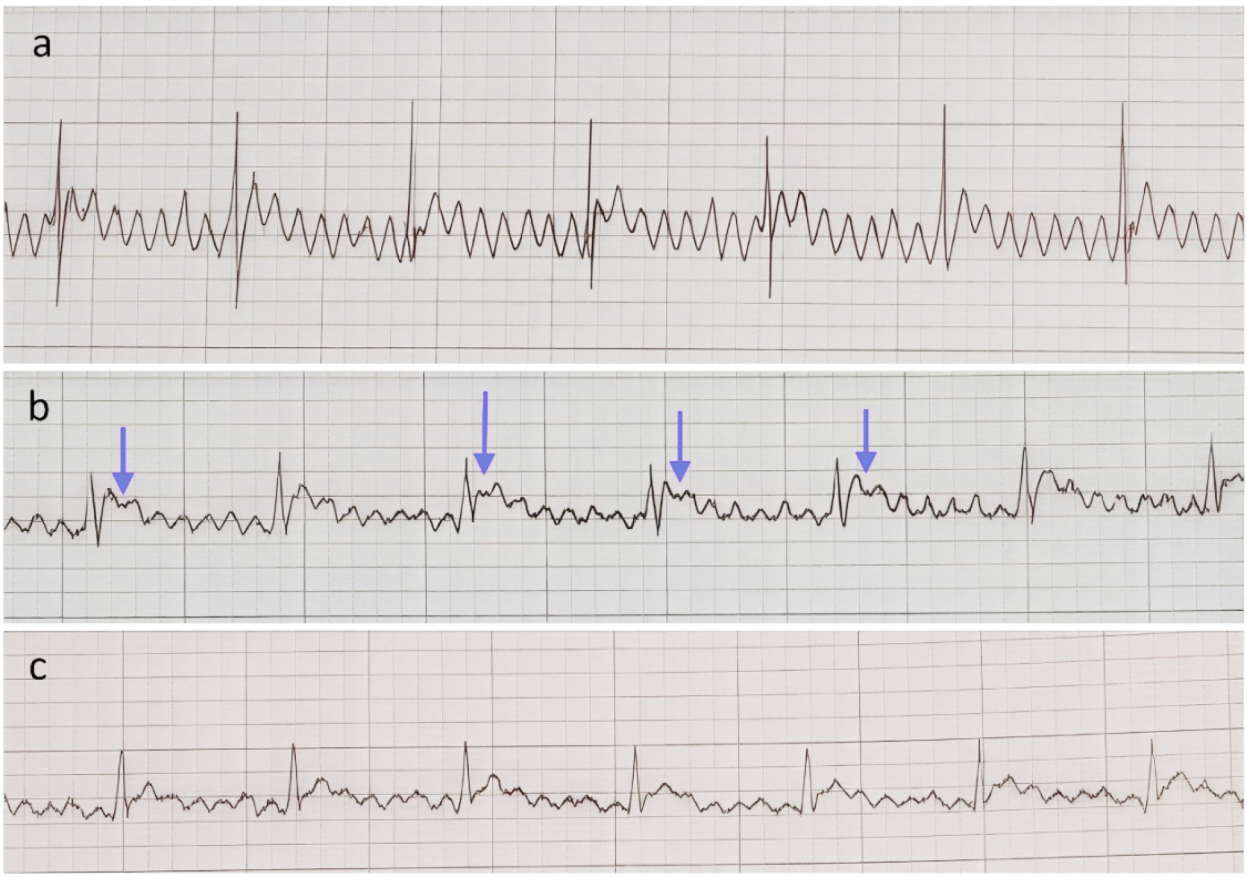


Figure 1: GC- mass analysis of Sepia ink extract.

3.2. MEASUREMENT OF ELECTROCARDIOGRAM (ECG):

The control rats exhibited normal ECG patterns **(Figure 2a)**, but the rats administered DOX displayed a significant elevation of the ST segment (blue arrows) **(Figure 2b)**. SI therapy normalized the ST-segment **(Figure2c)**.

Figure 2: Electrocardiogram (ECG) of control group (a), MI group (b), and SI group (c).



3.3. SERUM BIOCHEMICAL PARAMETERS

As presented in Table 1, the myocardial infarction (MI) group exhibited significant elevations ($p < 0.05$) in serum cardiac injury biomarkers, including cardiac troponin T, creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), as well as liver function markers, namely alanine aminotransferase (ALT) and alkaline phosphatase (ALP), compared with the control group. Administration of sepia ink (SI; 200 mg/kg b.w., orally) significantly reduced the levels of these biomarkers relative to the untreated MI group ($p < 0.05$), suggesting a protective effect against doxorubicin-induced myocardial injury and associated hepatic dysfunction. In contrast, serum total protein and albumin concentrations did not differ significantly among the experimental groups ($p > 0.05$).

Table 1: The curative potency of SI on serum biochemical parameters of myocardial infarcted rats.

Parameter	Control	MI	MI+SI
cTnT (ng/ml)	0.74 ± 0.02^{a}	1.24 ± 0.01^{c}	0.94 ± 0.05^{b}
CK-MB (U/L)	196.06 ± 5.12^{a}	341.46 ± 5.17^{c}	244.76 ± 5.12^{b}
LDH (U/L)	1221.47 ± 36.46^{a}	1998.64 ± 76.88^{c}	1365.57 ± 48.54^{b}
AST (U/L)	119.51 ± 1.31^{a}	166.47 ± 1.34^{c}	121.01 ± 2.58^{b}
ALT (U/L)	47.64 ± 1.21^{a}	79.31 ± 1.07^{b}	50.8 ± 2.95^{a}
ALP (U/L)	342.88 ± 3.26^{a}	408.44 ± 2.31^{c}	367.88 ± 3.2^{b}
Total protein (g/dl)	6.61 ± 0.21^{a}	6.15 ± 0.04^{b}	6.5 ± 0.12^{b}
Albumin (g/dl)	2.97 ± 0.08^{a}	2.62 ± 0.06^{b}	2.82 ± 0.04^{b}

- The values are shown as means ± SEM for six rats in each group.
- Values are designated by different superscript letters according to their statistical ranking, where 'a' indicates the lowest mean value, followed by 'b' for higher values, and 'c' for the highest values. Means sharing the same letter are not significantly different, whereas means with different letters differ significantly.

3.4. OXIDATIVE STRESS MARKERS

As shown in **Table 2**, the MI group exhibited a significant increase in malondialdehyde (MDA) levels in both cardiac and hepatic tissues compared with the control group ($p < 0.05$), indicating enhanced lipid peroxidation and oxidative damage induced by doxorubicin. Concurrently, myocardial infarction was associated with a marked depletion of endogenous antioxidant defenses, as evidenced by significant reductions in reduced glutathione (GSH), catalase (CAT), and nitric oxide (NO) levels in both tissues ($p < 0.05$). These alterations suggest a disruption of redox homeostasis and impaired antioxidant capacity. Treatment with sepia ink (SI; 200 mg/kg b.w., orally) significantly attenuated MDA accumulation while restoring GSH, CAT, and NO levels toward normal values compared with the untreated MI group ($p < 0.05$).

Table 2: The curative potency of SI on liver and cardiac oxidative stress markers of myocardial infarcted rats.

Parameter		Control	MI	MI+SI
MDA (nmol/g.tissue)	liver	0.69 ± 0.02^{a}	1.35 ± 0.01^{c}	0.87 ± 0.03^{b}
	cardiac	0.51 ± 0.03^{a}	0.74 ± 0.01^{b}	0.55 ± 0.01^{a}
GSH (mmol/g.tissue)	liver	0.33 ± 0^{c}	0.14 ± 0.02^{a}	0.25 ± 0.01^{b}
	cardiac	1.13 ± 0.03^{c}	0.61 ± 0.03^{a}	0.88 ± 0.01^{b}
Catalase (U/g.tissue)	liver	373.62 ± 2.45^{c}	305.46 ± 5.15^{a}	356.55 ± 1.9^{b}
	cardiac	64.87 ± 2.17^{c}	6.94 ± 0.74^{a}	41.11 ± 3.75^{b}
NO (µmol/g.tissue)	liver	187.18 ± 2.88^{c}	78.32 ± 1.72^{a}	162.86 ± 2.74^{b}
	cardiac	96.31 ± 0.84^{c}	41.98 ± 2.69^{a}	84.84 ± 1.99^{b}

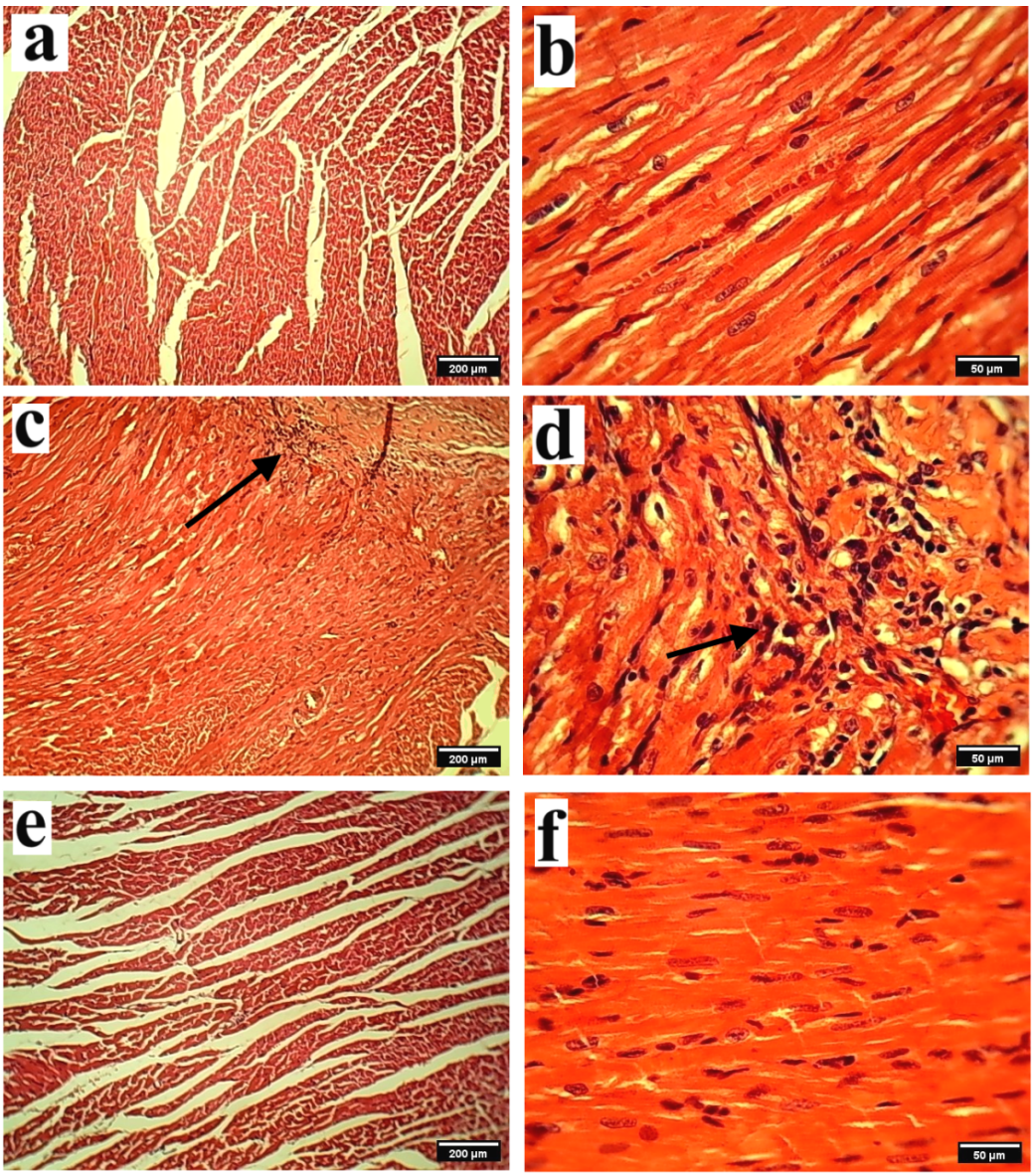
- The values are shown as means ± SEM for six rats in each group.
- Values are designated by different superscript letters according to their statistical ranking, where 'a' indicates the lowest mean value, followed by 'b' for higher values, and 'c' for the highest values. Means sharing the same letter are not significantly different, whereas means with different letters differ significantly.

3.5. HISTOLOGICAL EXAMINATION

3.5.1. HISTOPATHOLOGY OF THE HEART

The control group exhibited a typical histological structure, as illustrated in **Figures 3a** and **3b**. The myocardium exhibited cylindrical, branching cardiomyocytes with centrally located oval nuclei and acidophilic sarcoplasm. The MI group exhibited myocarditis and fibrosis (solid arrow) as shown in **Figures 3c** and **3d**. While SI treated group **Figures 3e** and **3f** demonstrated enhancement in the histological architecture of the heart, which appears similar to the normal structure of the control rats.

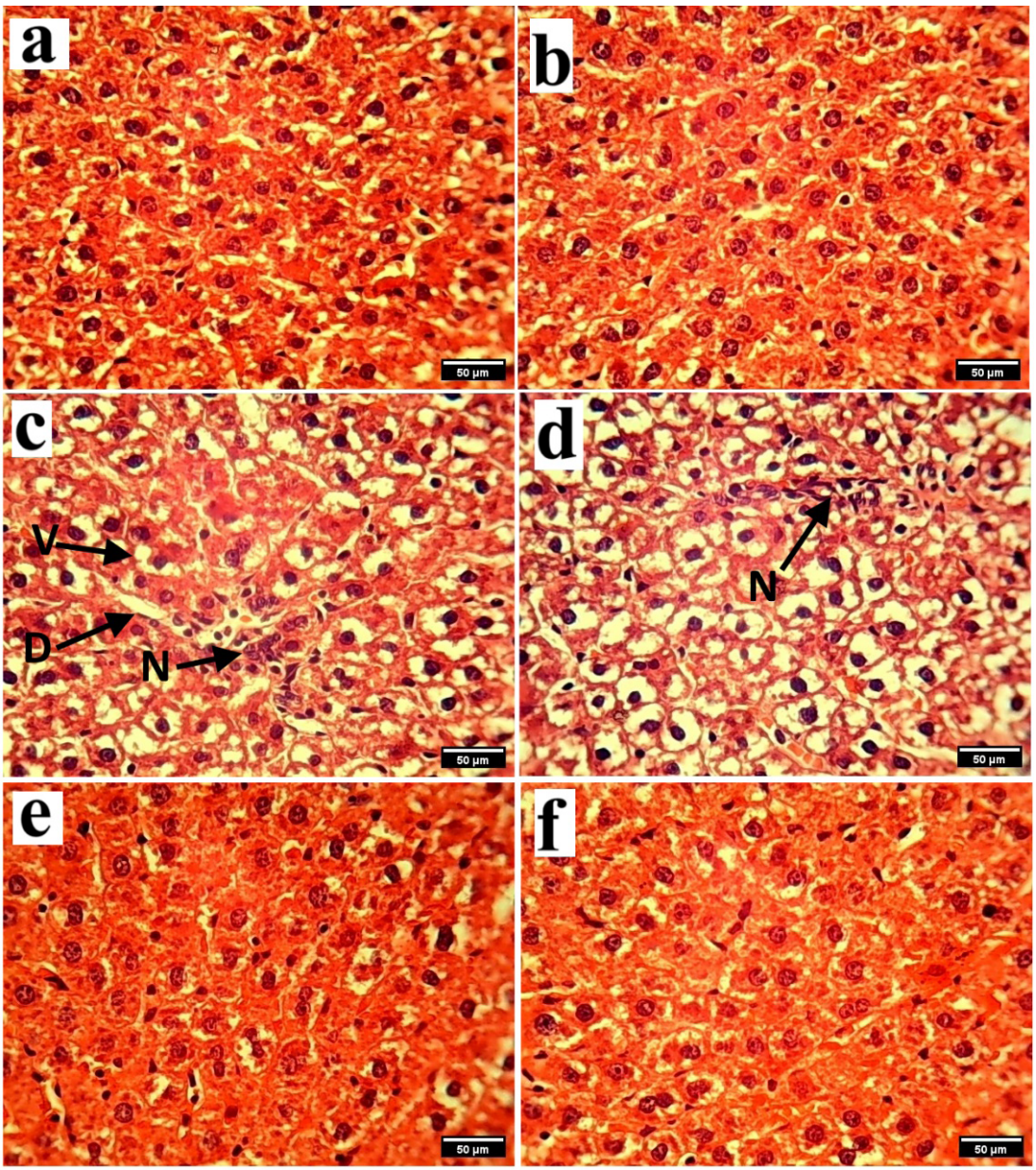
Figure 3: Photomicrograph of heart of control group (a and b), MI group (c and d), and SI treated group (e and f) (H&E).



3.5.2. HISTOPATHOLOGY OF THE LIVER

Figure 4 showed histopathological images of the liver tissues of all groups. No histopathological changes were observed in the liver of the control group **(Figures 4a and 4b)**. Meanwhile, severe histopathological alterations were noticed in the hepatic tissue of MI group which were represented by necrosis (N), dilation of sinusoidal space (D), and vacuolation of the cytoplasm (V) **(Figures 4c and 4d)**. SI treated group showed high improvement of the hepatic tissue like the typical histological structures of the liver **(Figure 4e and 4f)**.

Figure 4: Photomicrograph of liver of control group (a and b), MI group (c and d), and SI treated group (e and f) (H&E). V, vacuolation of the cytoplasm; D, dilation of sinusoidal space; N, necrosis.

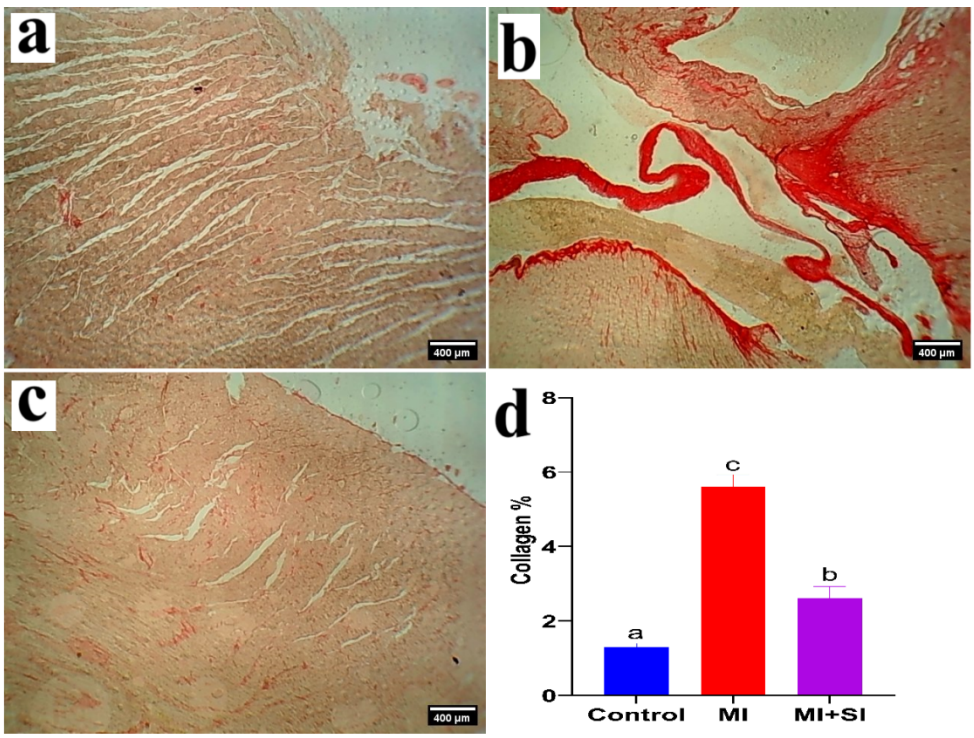


3.6. PICROSIRIUS RED STAINING

3.6.1. HEART

Control rats had a normal distribution of collagen in heart **(Figure 5a)**, whereas MI rats demonstrated signs of fibrosis (**Figure 5b)**. On other hand, treated group with SI showed less collagen deposition, as compared to MI groups demonstrating that the heart was repaired **(Figure 5c)**. A substantial reduction ($P > 0.05$) in picrosirius red was observed after the delivery of SI indicating the restoration of the normal collagen fiber distribution **(Figure 5d).**

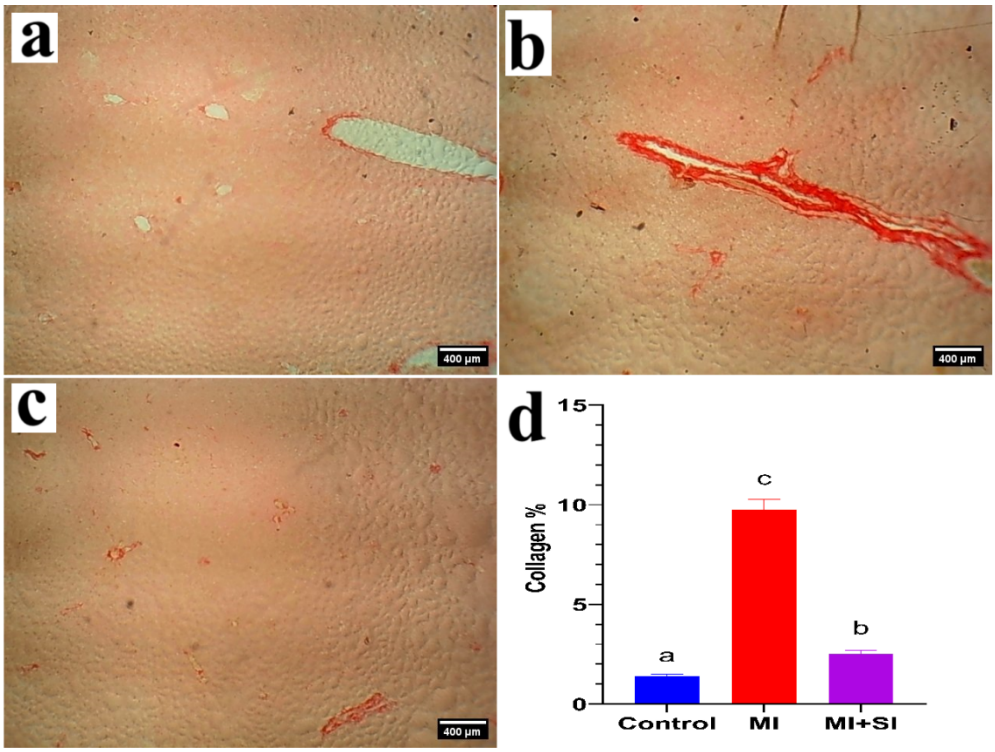
Figure 5: The photomicrographs of the hearts of the control group (a), the MI group (b), and SI treated group (c), and % of collagen (d) (Picrosirius red).



3.6.2. LIVER

Control group showed a normal collagen distribution in the liver (**Figure 6a)**, on the other hand MI group showed high collagen deposition indicating fibrosis (**Figure 6b)**. While SI treated group showed less collagen deposition, when compared to MI group demonstrating the repair of the liver (**Figure 6c)**. A substantial reduction ($P > 0.05$) in picrosirius red was observed after the delivery of SI signifying the restoration of the normal collagen fiber distribution (**Figure 6d).**

Figure 6: The photomicrographs of the livers of the control group (a), the MI group (b), and SI treated group (c), and % of collagen (d) (Picrosirius red).



4. DISCUSSION

MI is a disorder characterized by heart necrosis resulting from an imbalance between blood supply and demand (Liu et al., 2024). Evidence indicates that mechanisms such as cytosolic calcium excess, elevated superoxide anions and reactive oxygen species (ROS), cardiomyocyte apoptosis, and mitochondrial dysfunction contribute to MI (19).

The electrocardiogram (ECG) is a crucial measure of cardiac health, representing the biophysical electrical signal generated by the heart muscle (20). The most often employed imaging modality for diagnosing and managing persons presenting with symptoms indicative of acute coronary syndrome (ACS) is the ECG (21). The arrangement of ST segments, T waves, and QRS complexes is influenced by acute ischemia (22). The current investigation demonstrated DOX-induced myocardial infarction through ST-segment elevation in the ECG (23). Complete coronary artery occlusion is the primary cause of acute myocardial infarctions, and ST-segment elevation indicates transmural infarction (24). The reduction in cell membrane potential induced by oxidative stress in the infarcted heart may be responsible for these EEG abnormalities (25). In addition, Exposure to doxorubicin was demonstrated to induce mitochondrial fission, as indicated by elevated transcription of Drp1 and mitochondrial fragmentation, in cardiomyocytes *in vitro* (26). Indicating that mitochondrial fission plays a role in doxorubicin-induced cardiomyocyte apoptosis, siRNA-mediated Drp1 knockdown correlated with a reduction in caspase-3 expression (27). The effects of DOX may result in significant inflammatory responses, causing the invasion of mast cells, lymphocytes, neutrophils, and macrophages (28). The aggregation of these cells may lead to prolonged production of ROS, potentially resulting in alterations to the extracellular matrix (ECM) and fibrosis (29). Apoptotic cell death entails the activation of pro-apoptotic proteins, including caspase 3, and an elevated BAX/B-cell leukemia/lymphoma 2 (Bcl2) ratio (30). Prior research has indicat-

ed the participation of caspase 3 and BAX proteins in doxorubicin-induced cardiomyopathy in animal models (31,32).

Regarding biochemical indicators of cardiac function, DOX caused a notable increase in cardiac troponin T (cTnT), creatine kinase (CK), and lactate dehydrogenase (LDH). All skeletal and cardiac muscle cells possess troponins, which are protein complexes coupled to tropomyosin (33). Due to their exceptional sensitivities and specificities for myocardial injury, cTnI and cTnT are presently the standard biomarkers for the laboratory diagnosis of MI (34). Cytosolic enzymes, including CK-MB and LDH, are biomarkers that signal cardiac muscle injury or degradation (35). These enzymes are released into the bloodstream when the myocardium is compromised for whatever reason, resulting in elevated enzyme levels (36).

The liver is a vital organ for sustaining systemic hemostasis, and recent studies indicate that it also plays a crucial role in the body's defense against myocardial ischemia (37). Liver microsomal enzymes and cytoplasmic reductases facilitate the metabolism of doxorubicin, resulting in the accumulation of toxic and immunogenic intermediates associated with the onset of liver damage (38). The correlation between MI and the liver was demonstrated in the present study through the notable elevation of AST, ALT, and ALP. A histopathology examination indicated histological damage in the liver tissue of the MI group. The molecular pathways responsible for DOX-induced hepatotoxicity are thought to stem from oxidative stress caused by the production of ROS (39). This mechanism occurs during the metabolism of doxorubicin in the liver, ultimately resulting in inflammation and dysfunction of mitochondrial activity (40).

Heart and liver damage arises from an imbalance between ROS and endogenous antioxidants, leading to oxidative stress (41). DOX is induced oxidative stress in the hearts and livers of myocardial infarction rats, evidenced by a considerable increase in malondialdehyde (MDA) and a significant reduction in glutathione (GSH) and catalase (CAT) levels. DOX impairs redox homeostasis, resulting in elevated ROS and diminished antioxidant defenses (42). DOX-induced ROS can result in pathogenic sarcoplasmic reticulum calcium leakage, DNA damage, and interruption of autophagic flow, culminating in lipid peroxidation-dependent ferroptosis and other forms of regulated cell death (43). The redox cycle of DOX pertains to the presence of the quinone moiety, enabling DOX to function as an electron acceptor. A one-electron reduction of the quinone moiety by NADPH and cytochrome P450 reductase transforms Doxo into a semiquinone radical. The latter interacts with oxygen (O2), resulting in the production of the superoxide radical $O2^{\bullet-}$ and the concurrent regeneration of the quinone form (44).

Nitric oxide (NO) is considered a vital intracellular bioactive molecule within the cardiovascular system, playing several physiological and pathological roles, such as regulating cardiac contractility and the width of blood vessels (45). Reduced NO levels in ischaemic myocardium groups indicate dysregulation of cardiac activities, as nitric oxide regulates vascular tone, endothelial function, myocardial performance, haemostasis, and thrombosis(46). DOX has been demonstrated to diminish vascular smooth muscle cell contractility, potentially correlating with the noted reductions in NO production I pervious study (47).

The present investigation revealed many histological changes in heart and liver tissues following DOX administration. The MI group displayed myocarditis and fibrosis in cardiac tissue, while the hepatic tissue of the MI group exhibited necrosis, dilatation of sinusoidal spaces, and cytoplasmic vacuolation, indicating hepatic damage. These alterations may be attributed to ischemia/hypoxia, which leads to coagulative necrosis of cardiac myocytes due to prolonged oxygen deprivation, along with further damage from inflammatory and reperfusion reactions (48). Furthermore MI group demonstrated an increase in collagen deposition, likely due to the remodeling of the extracellular matrix (ECM) following a myocardial infarction, resulting in scar formation marked by elevated collagen levels (49). This scar, primarily composed of collagen type I, is essential for structural support to prevent rupture; nevertheless, excessive scarring may cause cardiac stiffness, reduced elasticity, and compromised cardiac function, potentially leading to heart failure (50).

The dose of sepia ink (200 mg/kg body weight) was selected based on previous preclinical studies demonstrating its biological efficacy and safety. At

this dose, sepia ink has been shown to exert significant antioxidant, anti-inflammatory, and cytoprotective activities without evidence of adverse effects (Saleh et al., 2015). Administering SI normalizes the ECG pattern, improves the functions tests and structure of the heart and liver, and reinstates the equilibrium of oxidants and antioxidants in rats with MI. This finding suggests that the cardioprotective benefit of SI may be associated with its antioxidant action against free radical species (51). The antioxidant properties of the SI may be ascribed to its potent free radical scavenging activity, facilitated by the presence of polysaccharides and melanin that function as free radical scavengers (52). Moreover, the melanin in SI functions as a potent superoxide dismutase (SOD) owing to the presence of dihydroxyindole (DHI), which catalyzes the disproportionation of superoxide anion (O_2•−) into hydrogen peroxide (H_2O_2) and molecular oxygen (O), hence inhibiting the free radical chain reaction initiated by superoxide (O^{-2}) (53). In addition to melanin and polysaccharides, SI comprises a significant quantity of taurine, which possesses antioxidant characteristics (54). Moreover (51)**)** stated that SI is essential in the treatment and prevention of liver and cardiac injury, as well as in the inhibition of enzyme leakage from the plasma membranes of cells (51). Additionally, SI possesses anti-inflammatory properties due to the presence of trans-13 octadecenoic acid which is a potent anti-inflammatory, and antiviral fatty acid (55). This fatty acid operate by reducing the production of pro-inflammatory cytokines like TNF-α and IL-1β in macrophages and also exhibits antioxidant properties that amplify its anti-inflammatory actions (56). Leading to a reduction in the inflammatory responses within cardiac and hepatic tissues (57). In addition E,E,Z-1,3,12-nonadecatriene-5,14-diol which is a constituent of SI has hypocholesterolemic and hepatoprotective actions as it works by anti-inflammatory, antioxidant, and antibacterial mechanisms due to the presence of the diol group and triene (three double bonds) structure (58). SI also contain cis-13-eicosenoic acid which has anti-inflammatory, analgesic, and antioxidant properties (59). The combination of these constituents together led to the cardio and hepatoprotective effects of SI as shown in the current study by restoring the cardiac and hepatic functions to the normal level and restoring the healthy structure of the cardiac and hepatic tissues. Moreover, SI caused a decline in collagen deposition this can be ascribed to the influence of SI on the levels of enzymes like MMP-1, which are involved in collagen remodeling during healing (60).

Moreover, SI administration led to increase in the production of NO which performs multiple essential functions in the heart, as vasodilation to regulate blood pressure and flow, avoiding thrombosis and atherogenesis by blocking platelet and leukocyte adhesion, and directly modulating cardiomyocyte contractility (61). It safeguards the heart from injury, fosters cellular growth and repair, and sustains vascular equilibrium (62). The reduction in NO production, referred to endothelial dysfunction, is a critical contributor to the onset of cardiovascular illnesses such as hypertension and atherosclerosis (63). Consequently, elevated NO levels after the injection of SI suggest enhanced cardiac function by suppressing vascular smooth muscle cell proliferation, platelet aggregation, monocyte adhesion, low-density lipoprotein oxidation, and vascular inflammation, while facilitating vasodilation (64).

5. CONCLUSION

Results showed that SI effectively protected rats from doxorubicin-inducing myocardial infarction by boosting their antioxidant system, reducing the inflammation and increasing their nitric oxide production. Based on these findings, SI may be a viable option for addressing cardiotoxicity caused by doxorubicin according to its anti-inflammatory, antioxidant properties, and stimulating nitric oxide production.

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Author contribution

The authors confirm contribution to the paper as follows:

Study conception and design: AMS. data collection: MFAS. Analysis and interpretation of results: ASM. Draft manuscript:ASM. All authors reviewed the results and approved the final version of the manuscript.

Limitations

The relatively small sample size may limit the statistical power and generalizability of the findings. Although the doxorubicin-induced cardiotoxicity model is widely used and provides valuable insights into myocardial injury and cardioprotection, animal models cannot fully reproduce the complexity of human myocardial infarction and cardiovascular pathology. Finally, only a single dose of sepia ink was evaluated, precluding assessment of dose-response relationships. Therefore, future studies with larger cohorts, multiple dosing regimens, and mechanistic analyses.

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