Physiology and Pharmacology 29 (2025) 315-322
Experimental Research Article

# S-Adenosylmethionine protected 5-Fluorouracil-induced cardiotoxicity in male albino rats

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## ABSTRACT

**Introduction:** 5-fluorouracil is commonly used for solid cancers, with cardiotoxicity being the main side effect. S-adenosylmethionine (SAMe) is known to have therapeutic benefits in several human diseases, including cancer, osteoarthritis, Alzheimer's disease, depression, and chronic liver diseases. SAMe also possesses cytoprotective and antioxidant properties. The present investigation aims to evaluate the effects of SAMe on 5-Fluorouracil-induced cardiotoxicity in rats by comparing it with a typical medication, Silymarin (SIL).

**Methods:** Forty male albino rats were divided into five groups as follows: Control (D.W. only), 5-Fluorouracil (100 mg/kg), SAMe (100 mg/kg), 5-Fluorouracil 100 mg/kg+SAMe100 mg/kg, and 5-Fluorouracil 100 mg/kg+200 mg/kg SIL. Cardiotoxicity was induced with an intraperitoneal injection of a single dose of 5-Fluorouracil (100 mg/kg). Serum was collected, and histological analysis of the heart was performed to evaluate the rat model-5-FU's toxicity alone and in combination with SAMe and SIL. In addition to a heart histology examination, serum cardiac enzyme testing, pro-oxidant/antioxidant status, and cyclooxygenase-2 expression in cardiac tissue were examined.

**Results:** 5-Fluorouracil induced severe cardiotoxicity, as evidenced by increased histological deterioration, cardiac enzymes, cyclooxygenase-2 expression, and malondialdehyde concentrations. The overall antioxidant capacity was likewise reduced by 5-FU treatment. While oxidative stress, cardiac enzymes, histological degenerations, and cyclooxygenase-2 expression in cardiac tissue were reduced, total antioxidant capacity was improved by SAMe or SIL treatment.

**Conclusion:** The cardiotoxicity induced by 5-fluorouracil in rats was ameliorated by SAMe or SIL therapy confirmed by reserved histological and biochemical analysis.

**Keywords:**
Antioxidant effect
Cardiotoxicity
Chemotherapy

## Introduction

Chemotherapy has been increasingly reported to be associated with cardiotoxicity, limiting the dose of anticancer used and thereby the response to the therapy (Sara et al., 2018a). The cardiotoxicity is represented as cardiomyopathy, mild arrhythmias, and irregular blood pressure. The etiology of cardiotoxicity is multifactorial, including the production of free oxygen radicals, which

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Received 17 July 2024; Revised from 26 September 2024; Accepted 28 October 2024

Citation: Alrawi D.I., Khalaf M.M., Alnori M.K. S-Adenosylmethionine protected 5-Fluorouracil- induced cardiotoxicity in male albino rats. Physiology and Pharmacology 2025; 29: 315-322. http://dx.doi.org/10.61882/phypha.29.3.315

can damage the cells, and the heart's antigen-presenting cells, which can trigger immune reactions. Furthermore, the effects of chemotherapy on certain phospholipids, e.g., cardiolipin, could potentiate cardiac damage (Koleini et al., 2019).

5-fluorouracil (5-FU) is an antimetabolite anticancer agent that has been commonly applied for the treatment of head, neck, breast, and gastrointestinal cancers. It affects the S phase of the cell cycle via suppression of thymidylate synthase, hindering DNA synthesis and ultimately ending with cell death (Iqbal et al., 2019). Its application is associated with side effects including bone marrow suppression, gastrointestinal upset, leucopenia, and thrombocytopenia. These adverse effects, along with a variety of others, include cardiotoxicity, hepatotoxicity, and nephrotoxicity, limiting its clinical applications (Rashid et al., 2014).

Silymarin (*Silybum marianum*) or milk thistle is a herbal product used for the treatment of gallbladder, liver disorders, liver protection from snake bites and bug stings, alcoholism, and mushroom poisoning, due to their effectiveness in hepatocyte regeneration, immune modulation, antifibrotic, anti-inflammatory, anti-lipid peroxidative, and antioxidant effects (Abed et al., 2022). In addition, silymarin restores the endogenous antioxidant enzymes, inhibiting neutrophil infiltration, and lowering serum malondialdehyde- the final byproduct of cardiac lipid peroxide (Okiljević et al., 2024). Furthermore, many small-scale, observational, or randomized clinical investigations have proved the positive effects of silymarin extract and its primary component, silibinin, on the cardiovascular system (Islam et al., 2021).

S-adenosyl-L-methionine (SAMe) is an endogenous biomolecule in humans that functions as a trans-sulfur, transmethyl, and transaminopropyl (Chen et al., 2016). SAMe can efficiently improve degenerative joint disease, cholestasis, spinal cord deformity, fibromyalgia, and abnormal liver functions in clinical settings (Pascale et al., 2022). Moreover, it has been discovered that SAMe utilizes polyamines to stimulate cell regeneration and mitigate oxidative stress-induced cell damage (Madeo et al., 2018). There are only limited or no studies performed on SAMe effects on myocardial damage and myocardial tissue angiogenesis following myocardial infarction. Therefore, the present work aimed to determine the potential cardioprotective effect of S-adenosylmethionine (SAMe) against cardiotoxicity induced by 5-FU in male albino rats.

## Materials and Methods

*Drugs and Chemicals*

SAMe capsules 200 mg from NOW (USA) purchased from Amazon store, dried extract of milk thistle (Legalon forte) capsule 140 mg from Meda (Germany), and 5-Fluorouracil 50 mg/ml solution for injection or infusion from Accord (UK) were bought from local pharmacies in Mosul and Erbil (Iraq), respectively. Other chemicals, including assay kits for heart markers: Rat TNNI3 (Troponin I Type 3, Cardiac) ELISA Kit was obtained from Elk Biotechnology (China), and Lactate dehydrogenase colorimetric kit was obtained from Giesse Diagnostics srl (Italy). The total antioxidant capacity (T-AOC) Assay Kit and Malondialdehyde (MDA) content assay Kit were obtained from Solarbio Life Science (China). The COX2 Polyclonal Antibody Kit was obtained from Elabsciences (USA). The remaining chemical compounds were used at the analytical level. All of these parameters were measured using the manufacturer's manuals included with the kits. The contents of the SAMe 200mg capsule were suspended in distilled water at a concentration of 25mg/ml, then animals were administered a SAMe dose of 100 mg/kg body weight (Jeon and Lee 2001). The contents of the SIL 140 mg capsule were dissolved in distilled water at a concentration of 50 mg/ml, then animals were administered a SIL dose of 200 mg/kg body weight (Abed et al., 2022). These drugs were freshly prepared and orally administered by tip-balled gavage needle**.**

*Animal experimental design*

This is a randomized experimental study performed on 40 male albino rats (10-12 weeks, 188-286 g), which were brought from Jehan University (Erbil, Iraq). They have been kept in the Animal House of the College of Veterinary Medicine at the University of Mosul for two weeks before the start of the trial. The study was registered and ethically approved by the Institutional Animal Care and Use Committee in the College of Veterinary Medicine/University of Mosul (Approval letter UM.VET.2023.022). The animals were kept in a 12-hour light-dark cycle and housed in a controlled environment with specified temperatures (23±2°C) and humidity (50%). The Animals were kept in sterile metallic cages and had free access to food and tap water during

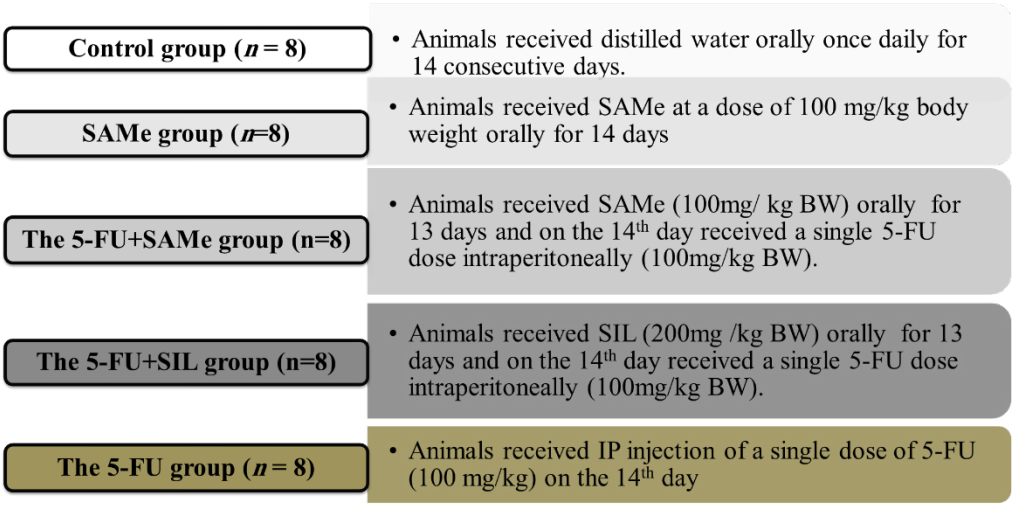


**FIGURE 1.** A Schematic representation of experimental design.

the whole experimental period. Treatment and grouping are listed in Figure 1.

*Induction of acute cardiotoxicity*

The induction of cardiotoxicity is conducted by a single dose of 5-Fluorouracil solution at a dose of 100mg/kg body weight given intraperitoneally (Safarpour et al., 2022).

*Serum and tissue sample collection*

On day 15 of the experiment, blood samples were collected from each rat using a capillary tube puncture in its retro-orbital plexus. Blood samples were taken into sterile gel separator plain tubes, which were centrifuged for 15 minutes at 3000 rpm to separate the serum after clotting. The clear serum was transferred and divided using clean 1.5 ml Eppendorf tubes. These fractions were kept at -20°C for biochemical analysis. Animals were sacrificed by cervical dislocation as soon as blood was drawn, and the heart tissues were quickly taken out and cleaned with cold saline. Heart tissue specimens were immersed in 10% formalin (formaldehyde solution) for COX-2 immunohistochemistry expression and histological evaluation (Parsaei et al., 2024). The COX-2 expression was evaluated based on scoring the intensity of expression, where score 0 represents no expression, score 1 represents weak positive expression, score 2 represents clear expression, and score 3 denotes intensely expressed COX-2 samples.

*Histopathological assessment*

The heart tissue fixation procedure was started by immersing the samples in 10% neutral buffered formalin after tissue collection for at least 72 hours. After several steps, a Hematoxylin-eosin stain was used to demonstrate the general histological structure (Abdullah et al., 2022).

*Statistical analysis*

The data were represented as mean±standard deviation (SD). One-way Analysis of Variance (ANOVA) tests were used to compare differences amongst all groups, and these were followed by post-hoc Tukey's multiple comparison analyses. The SPSS (V. 23) software was used to analyze the data. P-values $< 0.05$ are regarded as significant.

## Results

Compared to the control group, 5-FU and SAMe+5-FU induced a significant ($p<0.5$) increase in cardiac troponin levels, while the SAMe and SIL+5-FU group produced a significant decrease in the level of Troponin ($p=0.03$) in comparison to the 5-FU group. Statistically, there is a significant ($p=0.01$) difference between SAMe+5-FU and SIL+5-FU (Figure 2A). The level of LDH rises significantly ($p=0.007$) with 5-FU in comparison to the control group, and the LDH level was significantly (0.01) reduced by SAMe. On the other hand, SAMe+5-FU and SIL+5-FU groups showed no significant changes when compared with the 5-FU group (p=

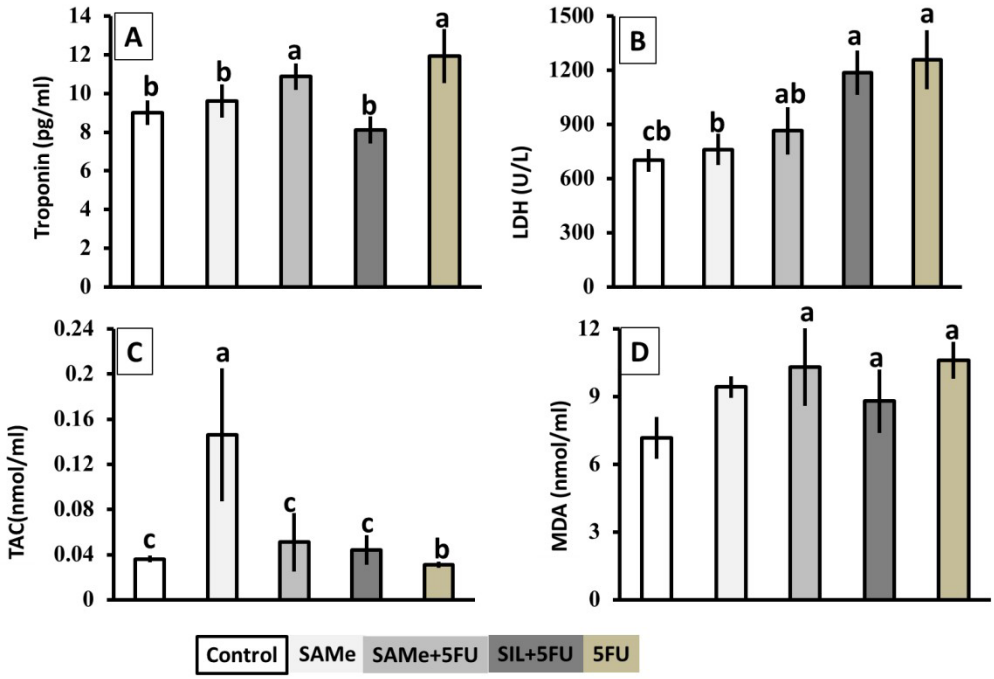


**FIGURE 2.** Effects of S-adenosylmethionine (100mg/kg) and Silymarin (200mg/kg) on the serum levels of measured biochemical parameters in albino male rats with 5-FU. Analysis was obtained by a One-way ANOVA test followed by Tukey's multiple comparison test. Data expressed as mean±SD (n=8 each). Similar letters indicate non-significant differences between tested groups, different letters indicate significant differences between tested groups, and $p<0.05$ is considered a significant value. LDH=lactate dehydrogenase, TAC=total antioxidant capacity, MDA=Malondialdehyde, SAMe=S-adenosylmethionine, 5-FU= 5-fluorouracil, SIL=Silymarin.

0.08 and 0.7, respectively), no appreciable (p= 0.8) differences in LDH measurement between SAMe+5-FU and SIL+5-FU (Figure 2B). In contrast to the control group, the levels of TAC were significantly ($p<0.05$) reduced by 5-FU. Compared to the 5-FU group, the TAC level is non-significantly increased in the SAMe+5-FU and SIL+5-FU groups (p=0.4 and 0.3, respectively). There were no appreciable differences (p=0.8) in TAC levels between SAMe+5-FU and SIL+5-FU. SAMe seems to significantly induce TAC levels compared to other groups (Figure 2C). 5-FU causes the level of MDA to rise significantly (p = 0.01) in comparison to the control group. Compared to the 5-FU group, the SAMe+5-FU and SIL+5-FU groups result in a non-significant decrease in MDA levels (p=0.8 and p=0.2, respectively) (Figure 2D).

A cardiac slice of a rat from the 5-FU group demonstrates necrosis, inflammatory cell infiltration, and hyaline degeneration of the myocardial muscle cells. While the cardiac muscle fibers and blood vessels in the control, SAMe, and SIL+5-FU groups were normal. Furthermore, normal cardiac muscle fibres and minor blood vessel congestion with haemorrhage were present in the SAMe + 5FU group (Figure 3). The 5-FU group showed the highest COX-2 expression (scoring 3+), whereas the Control, SAMe, and SIL+5-FU groups showed the lowest (score 0), while the SAM+5-FU group displayed a weak positive (score of 1+) (Figure 4).

## Discussion

Chemotherapy is currently one of the mainstays of cancer treatment. However, a primary issue with this treatment is the related adverse reactions, which include intestinal inflammation and destruction of the liver, spleen, and heart tissues (Cove-Smith et al., 2017). Several studies have been performed to lessen these medications' related adverse effects. Among these, herbal treatments may be able to protect patients from the harmful effects of chemotherapy medications (Lin et al., 2020).

5-FU has been used to treat a variety of malignant diseases, but its therapeutic usefulness is limited because of the serious cardiac damage that is associated with its administration (Sara et al., 2018b). Hence, searching for prophylactic agents to inhibit or mitigate this car-

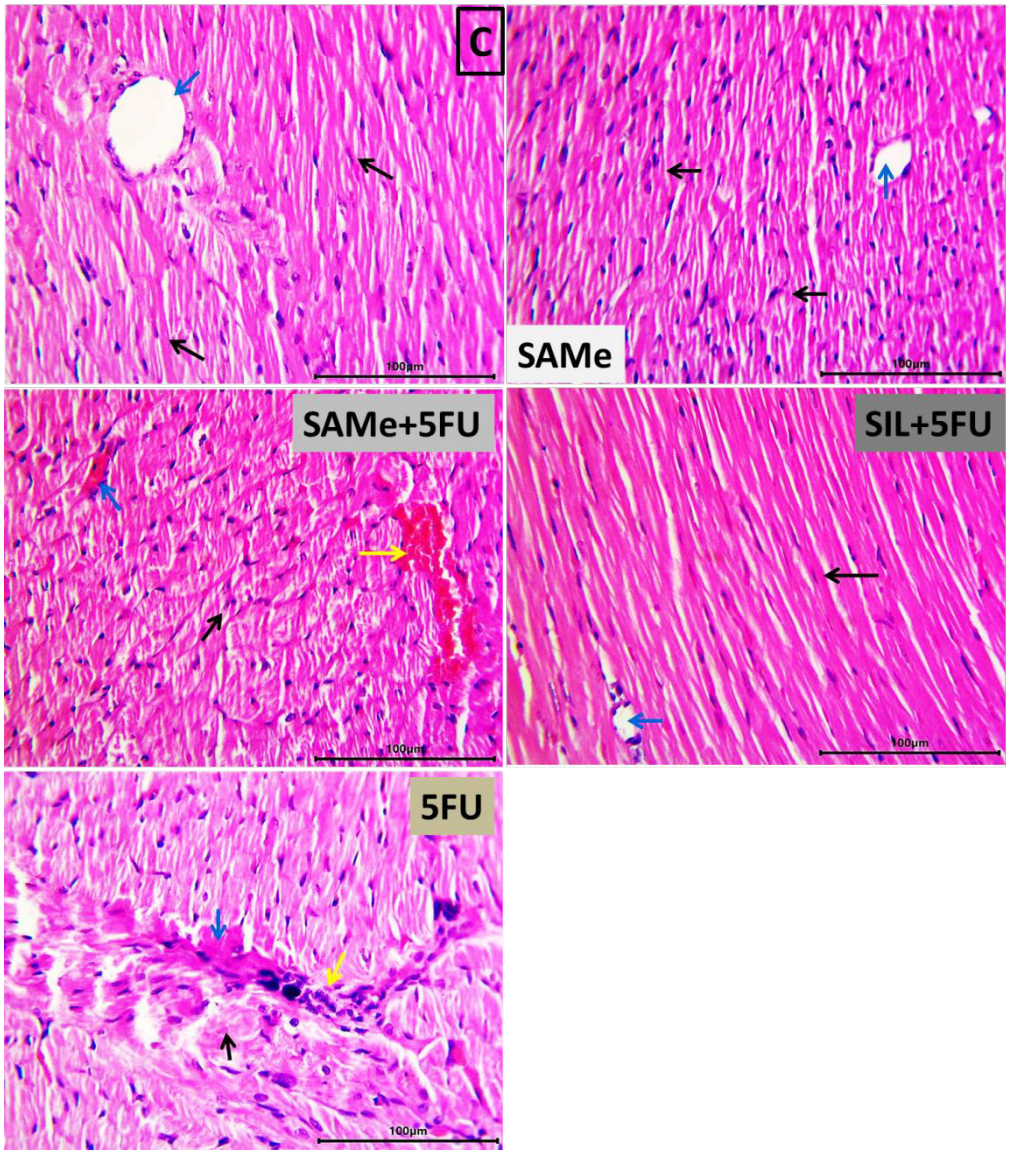


**FIGURE 3.** A representative image for heart histology of the studied groups. Heart slices stained with H&E, X400.

diotoxicity is crucial, with previous studies focused on inhibiting the oxidative damage and improving 5-FU toxicity (Polk et al., 2014), thereby protecting the cardiomyocytes from apoptosis (Li et al., 2021). SAMe has increasingly been reported to be a useful therapeutic tool due to its antioxidant and cytoprotective effects (Palipoch et al., 2016). The main therapeutic actions of SAMe include increased glutathione synthesis and endothelial nitric oxide synthase activation (Zhou et al., 2023).

The results of this study demonstrated that 5-FU treatment raised LDH and troponin; these effects were reversed by the use of SAMe. Moreover, MDA and TAC levels in the present study were assessed to establish the impact that oxidative stress plays on the cardiotoxicity caused by 5-FU. The current study demonstrated that 5-FU treatment raises MDA levels while lowering TAC levels. Conversely, the administration of SAMe or SIL resulted in a reduction in MDA levels and elevation in TAC, and these results were in line with earlier research as they demonstrated that SAMe was more efficient than GSH in scavenging radicals of hydroxyl (ȮH) and chelating iron ions to prevent the production of hydroxyl radical (Onaolapo et al., 2017). Furthermore, the current results of the biochemical analysis are confirmed by the assessment of the histological changes in heart tissue.

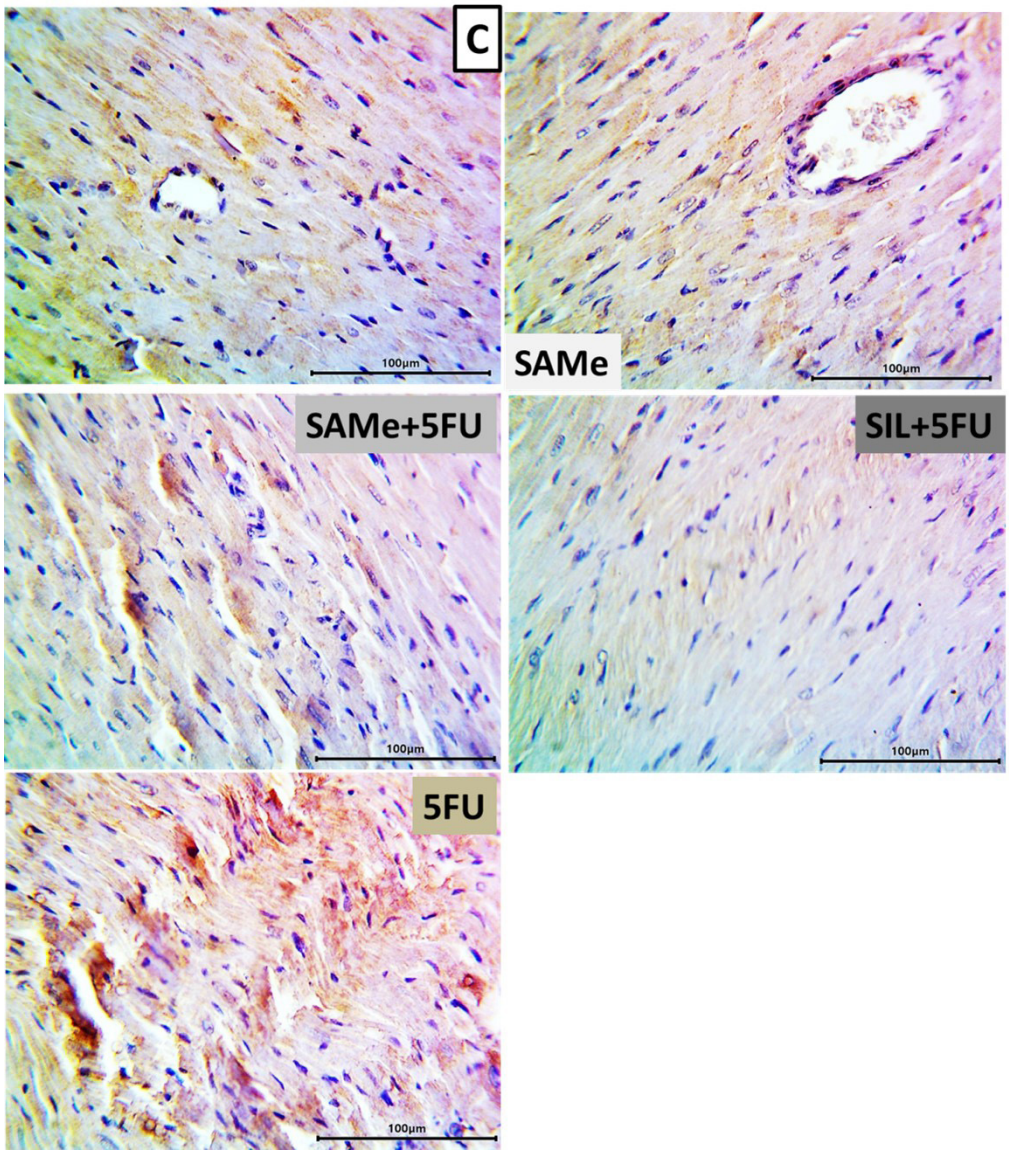


**FIGURE 4.** COX-2 expression in the hearts of the studied groups. Heart slices stained with H&E, X400. representative image for heart histology of the studied groups. Heart slices stained with H&E, X400.

The results of histological investigations in this study on albino rats showed that 5-FU treatment was followed by necrosis with hyaline degeneration of the cardiac muscle cells and inflammatory cell infiltration in the rat heart tissue. The preventive and anti-inflammatory properties of SAMe and SIL may have contributed to the reduction of cardiac tissue damage with unchanged myocardial muscle fibres and normal blood vessels after treatment. Our results are similar to the previous findings, which showed SAMe stimulates the Jagged1/Notch1 signaling pathway and inhibits fibrosis, promoting myocardial angiogenesis. SAMe prevents ventricular remodeling in rats after MI, enhancing their heart shape and function. The findings may create novel targets for the therapy of myocardial infarction (He et al., 2022).

In the molecular part of this study, the COX-2 levels in the SAMe+5-FU and SIL+5-FU treatment groups decreased in comparison to the 5-FU group, suggesting that SAMe and SIL have anti-inflammatory qualities and the mentioned results were congruent with another study performed on S-adenosyl-L-methionine (SAMe), which has analgesic and anti-inflammatory activities in the experiment animals without causing harm to the mucosa of the gastrointestinal tract (Jalgaonkar et al., 2023), which provide evidence that the activity of

SAMe in controlling the acute inflammation reaction is connected with the ability of this drug to interact with the eicosanoid system. The pro-inflammatory enzyme COX-2 is activated by a variety of stimuli, including the presence of free radicals and hypoxia (Yagami et al., 2016). It is controversial how COX-2 contributes to heart damage. In heart tissue, 5-FU led to increased expression of COX-2, which in turn increased ROS generation (Polk et al., 2014). A study has shown that cardiac tissue expresses COX-2 in response to doxorubicin (DOX), which may indicate a role for COX-2 in DOX-induced cardiac injury (Yagami et al., 2016). The current findings were consistent with those of previous studies (Delgado III et al., 2004), as they found COX-2 inhibition reduced the cardiotoxicity and heart failure caused by DOX, respectively. Conversely, other research showed that DOX-induced elevation in COX-2 expression in cardiac tissues prevented cardiac cells from death and that inhibiting COX-2 was related to exacerbating heart damage (Yagami et al., 2016).

## Conclusion

The treatment with SAMe has to some extent cardiac protection effects in a similar way to that of the proposed cardiac defender (SIL). The results of this study may help further investigate the advantages of SAMe as a novel therapeutic approach for protecting the heart from chemotherapy medications.

## Acknowledgements

The authors are grateful to the University of Mosul, College of Pharmacy, for continuous support.

## Conflict of interest

All authors declare that they have no conflict of interest.

## Ethics approval

All study procedures were conducted following the approval by the Institutional Animal Care and Use Committee of the Veterinary Medicine Department at the University of Mosul in Iraq. Date: 1/9/2023, Ref: UM.VET.2023.022.

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