

Ameliorative effect of Silybum marianum extract " Milk thistle " against lithium-induced cardiac toxicity in adult male albino rats.

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ABSTRACT

KEYWORDS

Cardiotoxicity,
Lithium carbonate,
Histological stains,
Cardiac muscle,
Ki-67.

Lithium carbonate is the first line of treatment for bipolar disorder. With its narrow therapeutic index and prolonged therapeutic doses, it causes multiple toxic effects. Cardiotoxicity is one of the serious toxicities induced by lithium treatment. Silybum marianum; an Asteraceae plant; with Silymarin as its major active constituent, has antioxidant, anti-inflammatory, and immunomodulatory properties. To assess the ameliorative effect of Silybum marianum extract "Milk thistle" on the histopathological changes of the cardiac muscle caused by lithium in adult male albino rats, using histological and immunohistochemical techniques. Twenty-Four adult male albino rats were equally randomized into 4 groups and received all treatments by oral gavage for 4 weeks. Group I (control) received distilled water. Group II received Silybum marianum extract (200 mg/kg/day). Group III received Lithium carbonate (50 mg/Kg). Group IV, Lithium carbonate concomitant with Silybum marianum extract was given. All animals were sacrificed after 4 weeks by decapitation. Prepared sections were subjected to hematoxylin and eosin stain, Mallory's trichrome stain, iron hematoxylin, and Ki-67 immunohistochemistry. lithium-induced widely disorganized and separated cardiac muscle fibers with pyknotic nuclei, without visible striations, increased collagen deposition and showed negative Ki-67 nuclear immune reaction. The use of Silybum marianum extract "Milk thistle" reversed cardiac muscle fibers alterations, decreased collagen amount, and restored Ki-67 nuclear immune reaction. Silybum marianum extract "Milk thistle" protects against lithium-induced cardiotoxicity in rats.

Introduction

Lithium has been used for a long time in the treatment of psychiatric disorders (Thakur et al., 2003). Lithium carbonate (a highly ionizable form of inorganic lithium) is considered now the first line of treatment for bipolar disorder associated with mania and depression (Ge and Jakobsson, 2019). Additionally, Alkarim et al. (2008) mentioned

the importance of lithium's role in the management of suicidal behavior. Moreover, abundant research work confirms that lithium is also effective in the management of neurodegenerative diseases herpes virus infection, and seborrheic dermatitis (Saad et al., 2017; Ge and Jakobsson, 2019). Some studies also investigated the potential effect of lithium on tumor growth suppression (Bgatova et al., 2014).

Lithium is easily absorbed from the gastrointestinal tract reaching its peak therapeutic level at 2-4 hours (Abdel Hamid et al., 2020). Lithium has a volume of distribution that ranges between 0.6 and 0.9 L/kg, it is water soluble and distributes freely to total body water with much higher distribution to the thyroid, kidney, and bone. Lithium poorly binds to plasma proteins and is mainly eliminated through the kidneys where

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over 60% of its dose is reabsorbed by the proximal tubules. Lithium has a narrow therapeutic index as the recognized therapeutic plasma concentration level is 0.6–1.2 mEq/L (Greller, 2019), so most lithium intoxications are linked to its therapeutic uses (Ufelle and Barchowsky, 2019).

Although lithium is efficient in treating psychiatric and neurological disorders, its prolonged therapeutic doses cause multiple toxic effects (Thakur et al., 2003; Saad et al., 2017). Lithium's toxic effects include neurotoxicity, nephrotoxicity and urethral cell damage, dermatologic complications, goiter, and hypothyroidism (Zarnescu and Zamfirescu, 2006). Teratogenic effects and diabetes insipidus were also reported (Thakur et al., 2003). Besides reduced fertility (Nciri et al., 2008), toxic effects of lithium on the reproductive system were also reported after prolonged administration in experimental animals in the form of testicular structural and morphological defects in both mature and immature testes (Alkarim et al., 2008).

Cardiotoxicity is one of the serious toxicities induced by lithium treatment (Aichhorn et al., 2006). Reported cardiac toxic manifestations include sinus bradycardia, junctional arrhythmias (Narayanan et al., 2015), atrioventricular block, premature atrial and ventricular beats (Aichhorn et al., 2006), and non-specific changes in the electrocardiogram (Goto et al., 2018). Congenital heart diseases were also reported (Young, 2009). Some histological studies were conducted to demonstrate lithium-induced cardiotoxicity and showed cardiomyocyte degeneration, subendocardial hemorrhages caused by chronic inflammation, and many tiny hemorrhages in myocardial muscle bundles (Shah et al., 2015).

It was suggested that lithium's toxic effects are mediated by different cellular functions via oxidative stress (Abdel Hamid et al., 2020) due to reduced mitochondrial respiratory functions resulting in free radicals production. Therefore, supplementation with safe and natural products with antioxidant

effects is needed to counteract lithium oxidative damage (Saad et al., 2017).

Silybum marianum, sometimes referred to as milk thistle (MT), is a plant belonging to the Asteraceae family (Fanoudi et al., 2020). Silymarin is the major active constituent of *Silybum marianum*. Silymarin is a blend of flavonolignans that include silibinin A and B, isosilybin, silydianin, and silychristin (Post-White et al., 2007).

Silymarin has different medicinal uses especially its known hepatoprotective role in liver diseases (Soleimani et al., 2019). Other pharmacological properties include antioxidant properties (Abenavoli et al., 2018), anti-inflammatory effects, anticancer activities, immunomodulatory properties, antibacterial effects, and neuroprotective properties. Additionally, some studies recorded the remarkable role of silymarin in diabetic patients in reducing plasma glucose levels and diminishing diabetes complications (Soleimani et al., 2019; Fanoudi et al., 2020).

Limited research was found to explore *Silybum marianum*'s potential role to protect against lithium-induced cardiotoxicity. Most of these studies pursued different methodologies. So, this work was conducted to evaluate the preventive role of *Silybum marianum* extract (MT) versus lithium-induced cardiotoxicity using light & immuno-histochemical study.

Materials and Methods

The research was conducted at the Suez Canal University, Faculty of Medicine, Department of Histology and Cell Biology in Ismailia, Egypt. Animal care was performed per institutional guidelines animal care ethics committee at the Faculty of Medicine, Suez Canal University (FOM. SCU) (Reference number: 5236).

Experimental animals

Twenty-four adult male albino rats of similar body weight (150–180 g) and age (90 days) were used in the experiment. Animals for this work were purchased from the animal

house, Center of Excellence (FOM. SCU). The animals were kept in the animal house with optimal husbandry conditions for one week to acclimate before the beginning of the experiment. They had free access to water and a regular diet of animal pellets and were kept in plastic cages. The animal environment was kept at a controlled room temperature (22–24 °C), with 12 hr of daylight and 12 hr of darkness.

Drug preparation

- Lithium carbonate was purchased as "Priamil C-R" tablets (400 mg each) from El-Nile Company for Pharmaceuticals and Chemical Industries in Cairo, A.R.E. R.C.C. 115668.
- Silybum marianum extract (MT) was obtained from Shana (Brand name) in the form of jars of "Shana Milk Thistle", each containing 30 g (MT) powder.

Experimental design

- At the beginning of the experiment, rats were assigned randomly into four groups; each of which included six rats. For 4 weeks, all treatment regimens were delivered by oral gavage.
- Group I (Control group): animals were given distilled water orally for 4 weeks.
- Group II (the Silybum marianum group) animals received Silybum marianum extract (MT) orally for 4 weeks at a dose of 200 mg/kg/day (Abed et al., 2022).
- Group III (Lithium carbonate group): animals received lithium carbonate, (50 mg/Kg) orally for 4 weeks (Bondok et al., 2018), which is approximately 1/10 of LD50 of 525 mg/kg (Badawy et al., 2022).
- Group IV (Lithium carbonate & Silybum marianum group): animals received lithium carbonate concomitant with Silybum marianum extract (MT), in the same doses and duration.

Tissue sample collection

- After 4 weeks, all the animals were chloroformed-anesthetized and sacrificed

by being decapitated. A midline incision was made in the chest, the sternocostal connections were cut, and the heart was removed and isolated. It was then embedded in paraffin sections for 24 hours, fixed in 10% (v/v) neutral formalin, and sliced at a thickness of 5 µm. The following approaches were used for the obtained sections:

- H&E stain: for the general architecture of the cardiac muscle (Bancroft and Layton, 2019).
- Mallory's trichrome stain: for connective tissue examination (Bancroft and Layton, 2019).
- Iron Hematoxylin (Hx) stain: for cardiac muscle striations (Bancroft and Layton, 2019).
- Ki-67 immunohistochemistry is a proliferating cell marker used in cardiac muscle nuclear reaction detection. Ki-67: (1:40, code No. M7187, Dako, Cambridge, UK) (1:40, code No. M7187, Dako, Cambridge, UK). On tissue sections, Mayer's hematoxylin was used as a counterstain. Controls that were both negative and positive were used. The endogenous peroxidase was blocked by deparaffinizing, rehydrating, and submerging poly-L-lysine-coated slides in 3% hydrogen peroxide. The antigen was retrieved using a microwave technique. After that, primary rabbit polyclonal anti-Ki-67 antibodies were incubated on the slides (ab833). purchased an antibody from (Abcam Co., Cambridge, UK) (Abd-Elhafiz et al., 2021).

Histopathological and immunohistochemical assessment of cardiac muscle

Qualitative and quantitative assessments were done for histological and immunohistochemical changes in the cardiac muscles.

- By examining at 5 high power fields (x 400) in ten serial sections from each animal of all tested groups, a qualitative assessment was carried out. For each

histopathological change in each of the examined groups, the frequency distribution was calculated. With a resolution of 10 MP (megapixels) (3656 2740 pixels per image), all images were taken using a calibrated standard digital microscope camera (Tucsen ISH1000 digital microscope camera) with an Olympus® CX21 microscope. For image capture and improvement, "IS Capture" was designed.

➤ Quantitative measurements using software Image J. The following measurements were assessed in different groups:

1. The mean collagen fiber area %.
2. Mean percentage area of the nuclear brownish reaction given by the Ki-67 antibody.

Statistical analysis

The Microsoft Excel of Microsoft Office 365 Software Package (from Microsoft Corporation, USA) was used for data entry. Following that, a statistical analysis was carried out utilizing IBM Corporation's SPSS version 25 (Statistical Program for Social Sciences), which was developed in Chicago, USA. The information was displayed as mean and standard deviation (SD). One-way analysis of variance (ANOVA) and the posthoc Tukey test were used to examine the data for the differences in histopathological alterations

between groups. To evaluate statistical significance, a P-value of under 0.05 was applied.

Results

Regarding the H&E stain of cardiac muscle sections, the control group shows a normal architecture of the cardiac muscle with centrally placed vesicular nuclei and acidophilic sarcoplasm (Photo 1A). Sections of the Silybum marianum group are almost the same as the control group (Photo 1B). The lithium-treated group shows widely separated cardiomyocytes with pyknotic nuclei surrounded by a halo and a markedly dilated blood vessel is also obvious (Photo 1C). These changes are statistically significant compared to the control group ($P < 0.05$) (Table 1). Sections of Lithium carbonate & Silybum marianum group rats show apparently normal architecture of the cardiac muscle with vesicular nuclei. However, a few spaces between muscle fibers and a few pyknotic nuclei are still present (Photo 1D). These changes are statistically significant compared to the Lithium carbonate group ($P < 0.05$) (Table 1). The histopathological changes of the cardiac muscle in the different studied groups are presented in (Table 1).

Table (1): The frequency distribution of the different histopathological changes of the cardiac muscle of animals in the different groups (n= 24 as the total number of animals).

Histopathological changes	Groups (n= 6 animals in each group)			
	Control group** %	Silybum marianum group ** %	Lithium carbonate group* %	Lithium carbonate & Silybum marianum group ** %
• Separation & Disorganization of cardiac muscle fibers	1	2	93	15
• Nuclear changes (Pyknosis)	0	0	83	7
• Dilatation of blood vessels	0	0	87	0

n: number, *Statistically significant compared to the control group ($P < 0.05$), **Statistically significant compared to the Lithium carbonate group ($P < 0.05$).

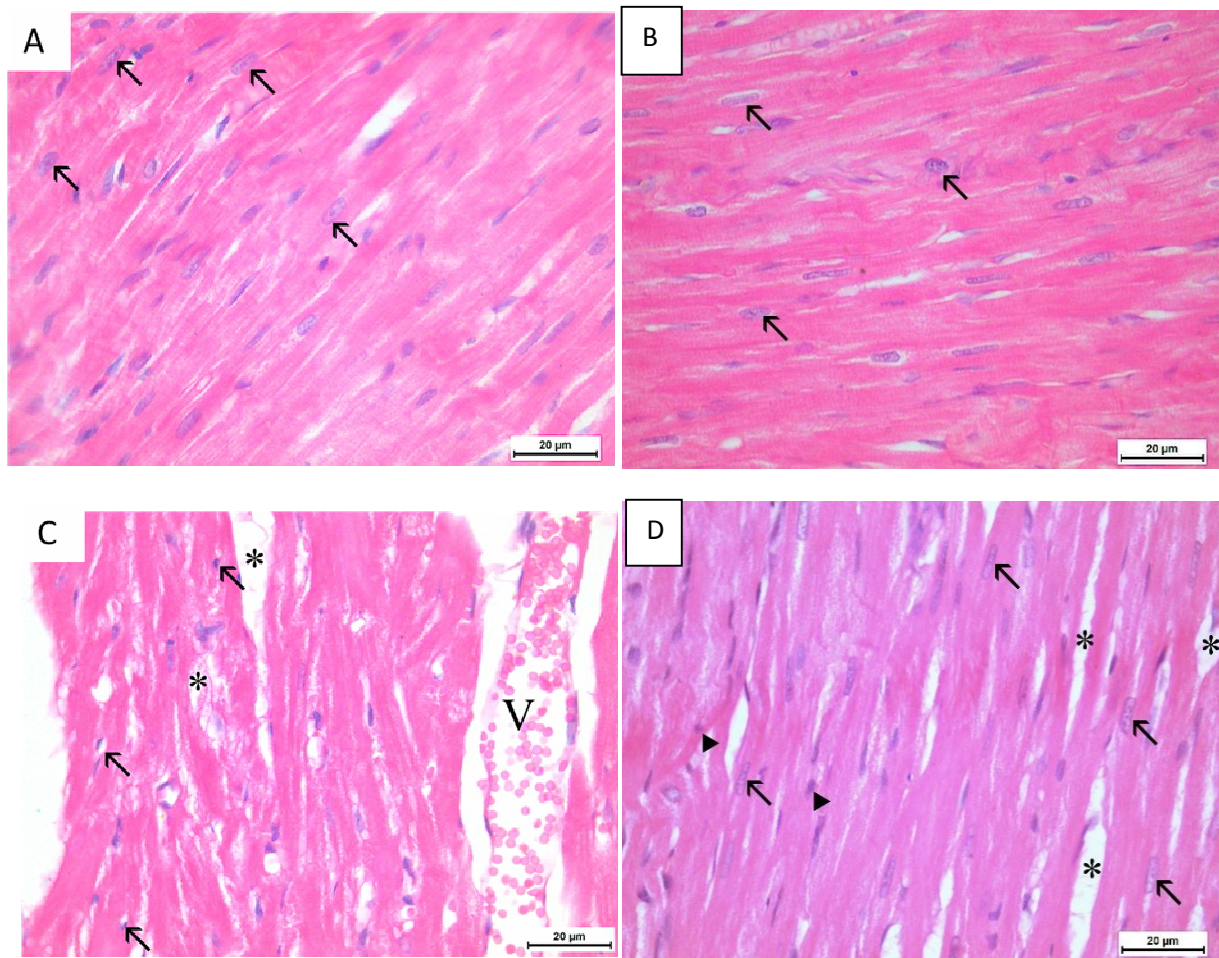


Photo (1): Photomicrographs of cardiac muscle sections stained with H&E x 630 from different groups. A. Control group shows a normal architecture of cardiac muscle that exhibits acidophilic sarcoplasm and centrally located vesicular nuclei (↖). B. Silybum marianum group (group II) is nearly similar to the control group. It shows organized cardiomyocytes and centrally positioned vesicular nuclei (↖). C. Lithium carbonate group (group III) reveals widely separated cardiomyocytes (*), pyknotic nuclei surrounded by halo (↖) and markedly dilated blood vessel (V). D. Combined Lithium carbonate & Silybum marianum group (group IV) demonstrate apparently normal architecture of the cardiac muscle with vesicular nuclei (↖). Few spaces between muscle fibers (*) and few pyknotic nuclei are still shown.

Mallory's trichrome stained sections from both the control group and the Silybum marianum group reveal minimal collagen fibers between cardiomyocytes. The increased amount of collagen fibers deposition between the disorganized cardiomyocytes, in lithium-treated rats, is statistically significant

compared to the control group ($P < 0.05$). In the Lithium carbonate & Silybum marianum group, little collagen deposits between cardiomyocytes are visible, which is statistically significant compared to lithium-treated rats ($P < 0.05$) (Photo 2 & Table 2).

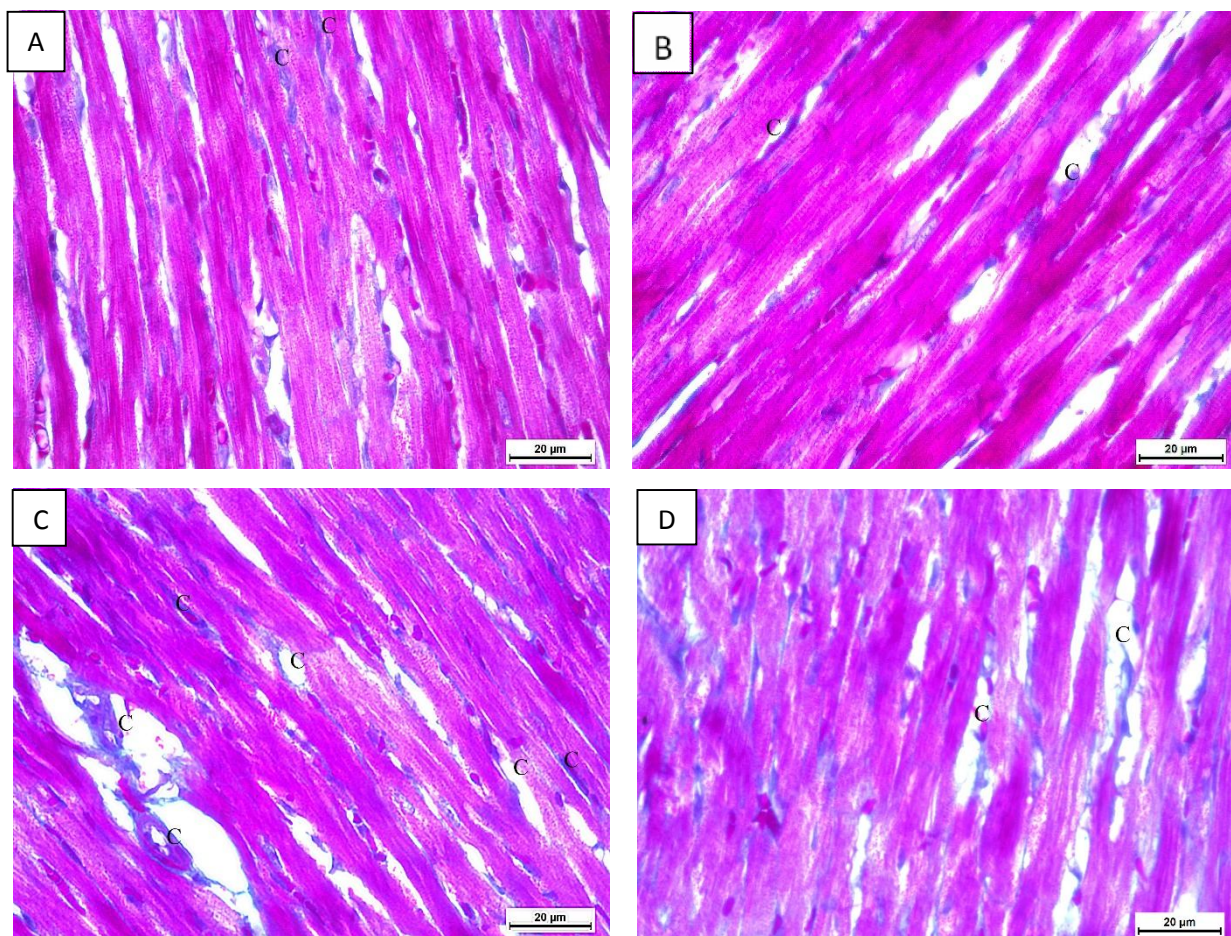


Photo (2): Photomicrographs of cardiac muscle sections stained with Mallory's trichrome x 630 from different groups. A. The control group shows minimal collagen fibers interspersed between cardiomyocytes (C). B. Silybum marianum group rat's cardiac muscle section is almost as the control group (C). C. Lithium carbonate group (group III) demonstrates a notable increased amount of collagen fibers deposition between the disorganized cardiomyocytes (C). D. Lithium carbonate & Silybum marianum group shows little collagen deposits between cardiomyocytes (C).

Table (2): The mean and standard deviation (SD) of the area percentage of collagen (Mallory's trichrome stain) in the different experimental groups (n= 24 as the total number of animals).

Groups (n= 6 animals in each group)	Mean ± Standard deviation (%)
1. Control group	14.16 ± 0.48
2. Silybum marianum group	14.02 ± 1.9
3. Lithium carbonate group	32.09 ± 1.4 *
4. Lithium carbonate & Silybum marianum group	24.25 ± 1.61 **

n: number, * Statistically significant when compared to the control group (P<0.05), ** Statistically significant when compared to the lithium carbonate group (P<0.05).

Iron Hx examined sections from both the control group and the Silybum marianum group show organized cardiomyocytes with their vesicular nuclei and dark black cross striations. The lithium-treated group shows widely separated cardiomyocytes with no

obvious striations. Sections of the Lithium carbonate & Silybum marianum group are more or less similar to that of the control group with organized cardiomyocytes with vesicular nuclei and striations (Photo 3).

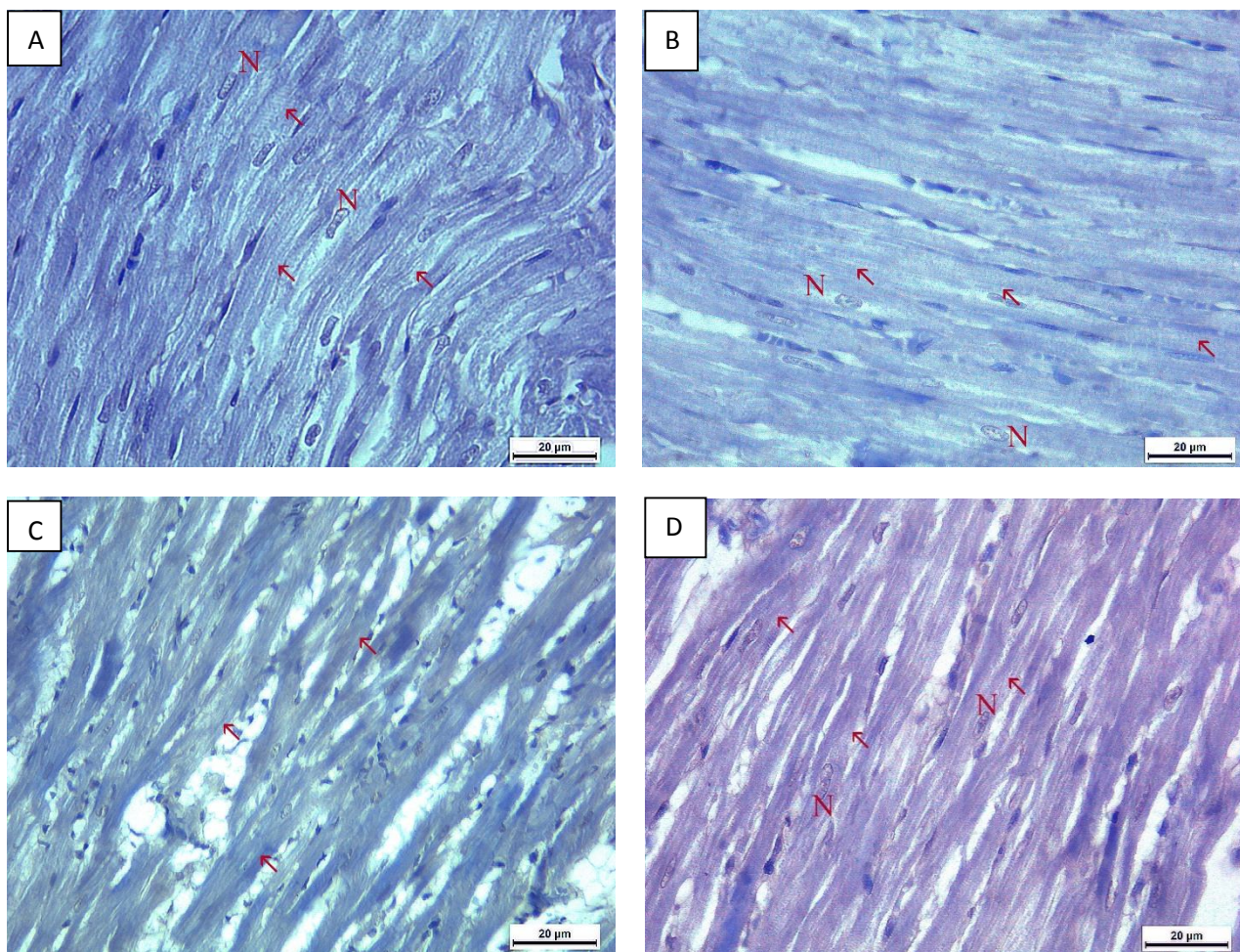


Photo (3): Photomicrographs of cardiomyocytes stained with Iron hematoxylin x 630 from different groups. A. The control group shows striated and organized cardiomyocytes with their vesicular nuclei (N), and darkly black cross-striations (Λ). B. Silybum marianum group (group II), which is almost similar to the control group. C. Lithium carbonate group shows widely separated cardiomyocytes with no obvious striations (Λ). D. Lithium carbonate & Silybum marianum group rat showing more or less organized cardiomyocytes with vesicular nucleus (N) and striations (Λ).

The KI-67 nuclear immune reaction of the control group is positive. The Silybum marianum group displays a nuclear immune reaction that is similar to that of the control

group. In comparison to the control group, the lithium carbonate group displays a negative immune reaction that is statistically significant ($P < 0.05$). In the nucleus of

cardiomyocytes of the Lithium carbonate & Silybum marianum group, a positive immunoreaction is observed and is

statistically significant to that of the lithium carbonate group ($P < 0.05$) (Photo 4 & Table 3).

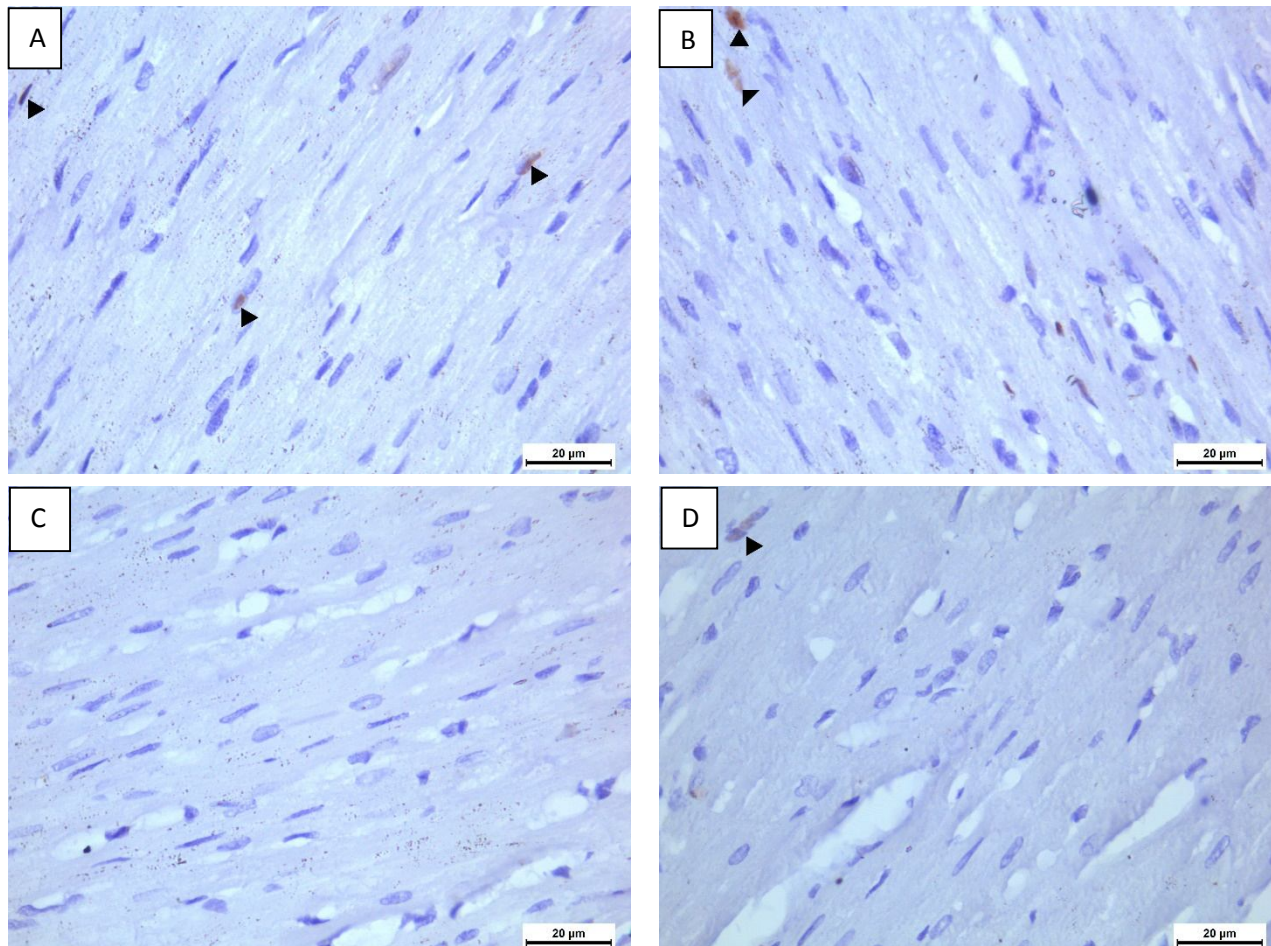


Photo (4): Photomicrographs of cardiomyocytes stained with Ki-67 x 630 from different groups. A. The control group shows positive immunoreaction in the nucleus of cardiomyocytes (▶). B. Nuclear immune reaction of the Silybum marianum group (group II) is similar to the control group (▶). C. Lithium carbonate group (group III) demonstrates a negative immune reaction. D. The Lithium carbonate & Silybum marianum group (group IV) demonstrates a positive nuclear immune reaction (▶).

Table (3): The mean and standard deviation (SD) of the area percentage of Ki-67 immunostaining in the different experimental groups (n= 24 as the total number of animals).

Groups (n= 6 animals in each group)	Mean ± Standard deviation (%)
1. Control group	0.57 ± 0.08
2. Silybum marianum group	0.55 ± 0.1
3. Lithium carbonate group	0 *
4. Lithium carbonate & Silybum marianum group	0.336 ± 0.04 **

n: number, * Statistically significant when compared to the control group ($P < 0.05$), ** Statistically significant when compared to the lithium carbonate group ($P < 0.05$).

Discussion

Lithium is the primary medication used in the treatment of bipolar disease, but because of its tight therapeutic index, it can generate various toxic effects (Abdel Hamid et al., 2020). Even though Lithium cardiotoxicity is becoming more well-recognized, no effective preventative or therapeutic medication is now available (Mezni et al., 2017). The current study sought to determine the preventive role of *Silybum marianum* extract (MT) on cardiotoxicity induced by lithium in male rats.

In the present study, H & E and Iron Hematoxylin-stained cardiac muscle sections of lithium-treated animals displayed many histopathological alterations in the form of widely separated cardiomyocytes with pyknotic nuclei and no obvious cross striations. These findings line up with other studies (Mezni et al., 2017; Saad et al., 2017). Similar histopathological alterations were reported by Abdel Hamid et al. (2020) who documented these changes with prolonged use of lithium for 12 weeks. Another study revealed that degenerative myocardial changes induced by lithium are duration-dependent (Shah et al., 2015).

Lithium's toxicity is linked to the increased genesis of reactive oxygen species (ROS), which culminates in oxidative stress in cells (Musik et al., 2015). A group of nitric oxide synthases (NOS), including endothelial and neuronal NOS, produces nitric oxide (NO) from its precursor L-arginine. The heart's myocytes, conductive tissue, and endothelium all express eNOS. In the heart, eNOS controls the physiological effects of NO, including control of cardiac contractility, vascular tone, platelet aggregation, and endothelial function (Lee et al., 2016).

The cardiac muscle section of the lithium-treated group in this study also showed a markedly dilated blood vessel. Similar findings were made by Afsharimani et al. (2007) who discovered that continuous lithium treatment increased Ach-induced endothelium-derived vasodilation in rat mesenteric bed. This impact might be explained by an increase in nitric oxide synthase (NOS) activity or by suppressing the cyclo-oxygenase (COX) pathway and activation of the endothelium-derived hyperpolarizing factor (EDHF) pathway (Afsharimani et al., 2007).

In addition, Mallory's trichrome-stained sections of rats treated with lithium in the current study showed an increase in collagen fibers deposition between cardiomyocytes that were statistically significant which could be attributed to fibrosis. Similarly, lithium-induced interstitial renal collagen deposition after short-term exposure in rat model for 14 and 28 days with more evident interstitial renal fibrosis after 28 days (Mehta et al., 2022). The fibrosis in the heart tissues of rats following prolonged lithium treatment was also noted by other researchers (Shah et al., 2015; Abdel Hamid et al., 2020). According to Moradi et al. (2019), lithium-induced atrial fibrosis was attributed to the increment in collagen I alpha 1 gene expression. However, in a study on cell culture taken from fibroblasts withdrawn from adult healthy myocardium, lithium could not increase the pro-fibrotic capacity of human myocardial fibroblasts at peak therapeutic levels (1.0 mM), while it boosts fibroblasts' pro-fibrotic abilities above therapeutic peak levels (>1.0 mM). Lithium may have a dose-dependent influence on the pro-fibrotic activity of cardiac fibroblasts (Chen et al., 2021).

Regarding the immunohistochemical results in the current study, the negative Ki-67

nuclear immune reaction, in lithium-treated animals, could be attributed to the degenerated cardiac muscle fibers as well as the loss of cross striations induced by lithium. According to Doi et al. (2010) lithium administration for 14 days was unable to restore the density of Ki-67-positive cells within the dentate gyrus of the hippocampus after continuous ACTH therapy had markedly reduced it. Furthermore, a recent study conducted in Shanghai on a water environment polluted with lithium batteries to demonstrate lithium toxicity to human cardiomyocytes reported that lithium inhibited cells growth, proliferation, and viability, and triggered cardiomyocytes apoptosis significantly (Shen et al., 2020). It was suggested that the apoptotic and antiproliferative activities of lithium were via inhibiting glycogen synthase kinase-3 β (GSK-3 β), an active regulator of cell proliferation and apoptosis (Wang et al., 2017; Shen et al., 2020).

Heart failure, cardiac hypertrophy, atherosclerosis, and other cardiovascular conditions are all significantly influenced by oxidative stress. Lithium was implicated in studies by Vijaimohan et al. (2010) and Mezni et al. (2017) for inducing oxidative stress and DNA damage. Lithium was also charged with causing oxidative injury to the rat's heart tissues. Based on the preceding findings, the cardiac toxicity of lithium in the current study might be related to oxidative stress.

Silymarin is one of the well-known antioxidants, and its antioxidant properties have been reviewed. Free radicals direct elimination, ion chelation of iron and copper in the intestine, inhibition of the enzymes that produce reactive oxygen species (ROS), facilitating the generation of protective elements like heat shock proteins, sirtuins,

and thioredoxin that protect from stressful stimuli, and antioxidative enzymes stimulation are just a few of the potential antioxidant mechanisms of silymarin (Surai, 2015).

In the present study, co-treatment of animals with *Silybum marianum* extract "Milk thistle" restored most of the cardiac muscle fibers organization, vesicular nuclei, and striations. These findings were confirmed ultrastructurally by Cecen et al. (2011) who demonstrated that silymarin greatly restored the ultrastructural damage to myofibrils and sarcoplasmic reticulum and reduced the doxorubicin-induced toxicity to the rat heart, kidneys, and liver and suggested the use of silymarin as a supportive agent in the course of doxorubicin anti-cancer regimen. Additionally, Kumaş et al. (2016) examined the histopathological and biochemical effects of silymarin on cardiac damage brought on by a high dose of isotretinoin (ISR). They discovered that silymarin protected against isotretinoin's high-dose-induced damage to the heart tissue by boosting the activities of antioxidant enzymes, particularly plasma GSH enzyme levels, and reducing lipid peroxidation by lowering E-MDA and P-MDA levels. Additionally, it was shown to have an apoptosis-lowering impact by partially reducing isotretinoin-induced apoptotic cell death. As a result, it was determined that silymarin has an antioxidant and antiapoptotic impact on heart tissue. In human investigations, silymarin's cardioprotective effects were also noted. An investigation into the effects of silymarin co-administration with anthracycline chemotherapy on certain cardiac blood parameters, such as LDH, CK-MB, cTnI, Anticardiolipin IgG, Fe, ferritin, and TIBC, concluded that silymarin provides a novel treatment strategy for improving the control of cardiotoxicity

brought on by anthracyclines (Zalat et al., 2020). In another study, silymarin was shown to possess a beneficial preventive role in the cardiotoxicity and hepatotoxicity caused by methotrexate and mercaptopurine in leukemic children. These effects were manifested by decreased ejection fraction and improved systolic function after three months in both the case and control groups, and after six months particularly in the control group (Ghaderian et al., 2017).

Mallory trichrome-stained sections from rats given both *Silybum marianum* extract (MT) and lithium carbonate in the current study revealed minimal collagen deposits between the cardiomyocytes compared to the lithium carbonate group. Vilahur et al. (2018) findings' revealed that prolonged silymarin treatment for three weeks after an induced myocardial infarction (MI) in pigs reduced collagen buildup and cardiac reactive fibrosis and may have limited scar growth and led to enhanced cardiac repair and heart contractility post-MI. According to a different study, silymarin dramatically reduced cardiac fibrosis and collagen buildup in diabetic rats. It was reported that silymarin inhibits gluconeogenesis and glycogenolysis through the inactivation of the intrahepatic NF- κ B signaling pathway and lowers hepatic fibrosis via TGF- β 1, while silymarin treatment was shown to inhibit collagen deposition and cardiac fibrosis through the TGF- β 1/Smad signaling pathway (Meng et al., 2019).

In the current investigation, compared to the lithium carbonate group, rats treated with milk thistle plus lithium demonstrated a positive nuclear Ki-67 immunoreactivity. Even though silymarin, a component of milk thistle seeds, has been shown to have anti-proliferative benefits against several malignancies, including prostate, colon, lung, bladder, and breast cancer cells, through cell cycle interruption, cyclin-dependent kinase

inhibition, and activation of apoptosis, it is also claimed to have the best regenerative effect on the liver (Meddeb et al., 2018). In rats with partial hepatectomy, milk thistle compounds, silymarin, and active substances like silybin were presented to promote liver regeneration (Yormaz et al., 2012; Cetinkunar et al., 2015; Yavuz et al., 2022). It was observed that silybin injection intraperitoneally significantly enhances the production of ribosomal RNA and polymerase I, resulting in more rapidly formed ribosomes, which ultimately hasten protein formation (Abenavoli et al., 2018). Furthermore, another study investigated milk thistle extract's influence on the development and longevity of brain cells and found that it can support neuronal survival and differentiation while shielding the neurons in the rat hippocampus from oxidative stress-related cell death (Kittur et al., 2002).

This study concluded that the *Silybum marianum* extract (MT) protects against lithium-induced cardiotoxicity in rats based on histopathological and immunohistochemical results. Accordingly, the authors recommend the administration of *Silybum marianum* extract (MT) as a cardioprotective agent for bipolar patients and patients with neurodegenerative diseases during lithium therapy to prevent or at least minimize symptoms of lithium-induced cardiotoxicity.

Declaration of interest

No conflicts of interest were reported by the authors.

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الدور المحسن لمستخلص سيليبوم ماريانوم "شوك الحليب" ضد سمية القلب المستحثة بالليثيوم في ذكور الجرذان البالغة البيضاء

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يستخدم الليثيوم منذ فترة طويلة في علاج الاضطرابات النفسية، ويعتبر كربونات الليثيوم الخط الأول في علاج الاضطراب ثنائي القطب المرتبط بالهوس والاكتئاب. على الرغم من أن الليثيوم فعال في علاج الاضطرابات النفسية والعصبية، إلا أن جرعاته العلاجية الطويلة تسبب آثاراً سامة متعددة، وتعتبر السمية القلبية واحدة من السميات الخطيرة التي يسببها علاج الليثيوم. سيليمارين هو المكون النشط الرئيسي لسيليبوم ماريانوم. سيليمارين له خصائص دوائية مختلفة والتي تشمل الخصائص المضادة للأكسدة، والتأثيرات المضادة للالتهابات، والأنشطة المضادة للسرطان، والخصائص المناعية، والتأثيرات المضادة للبكتيريا، وخصائص الحماية العصبية. توجد دراسات محدودة توضح الدور الوقائي لسيليبوم ماريانوم ضد السمية القلبية التي يسببها الليثيوم. لذا فقد أجريت هذه الدراسة لتقييم التأثير الوقائي لمستخلص سيليبوم ماريانوم "شوك الحليب" ضد السمية القلبية التي يسببها الليثيوم. ولقد أجريت هذه الدراسة على أربعة وعشرين من ذكور الجرذان البيضاء بعد تقسيمهم إلى أربع مجموعات بشكل عشوائي (٦ حيوانات لكل مجموعة). وقد تلقت حيوانات البحث جميع العلاجات تزقيمية عن طريق الفم. تلقت المجموعة الأولى (المجموعة الضابطة) الماء المقطر. تلقت المجموعة الثانية المستخلص المائي لشوك الحليب بجرعة (٢٠٠ مجم / كجم من وزن الجسم / يوميا). المجموعة الثالثة تلقت كربونات الليثيوم (٥٠ مجم / كجم). تلقت المجموعة الرابعة كربونات الليثيوم مصاحباً للمستخلص المائي لشوك الحليب. ثم بعد ٤ أسابيع ، تم ذبح الحيوانات بقطع رأسهم. ثم تم إعداد شرائح من القلب وصباغته بالهيماتوكسيلين والإيوسين ، وصبغة المألوري ، وصبغة هيماتوكسيلين الحديد ، والصبغة الهستوكيميائية المناعية ل Ki-67 لدراسة ألياف عضلة القلب من حيث التنظيم والتباعد وشكل نواتها وأوعيتها الدموية، ثم أجريت بعض القياسات باستخدام جهاز Image J وهي النسبة المئوية لألياف الكولاجين وللخلايا المتفاعلة مع الصبغة الهستوكيميائية المناعية ل Ki-67 ثم تم تحليل البيانات الناتجة إحصائياً. وقد أوضحت نتائج الدراسة أن الليثيوم قد تسبب بتغيرات هستوباثولوجية واضحة في أنسجة عضلة القلب حيث تسبب بألياف عضلية غير منظمة ومفصولة على نطاق واسع مع نوى متدنية، وألغى الشكل النمطي لعضلات القلب، وزاد من ترسب الكولاجين، وأظهر رد فعل مناعي نووي سلبي ل Ki-67. وقد أدى استخدام شوك الحليب إلى استعادة تغيرات ألياف عضلة القلب، وتقليل كمية الكولاجين، وتعزيز رد الفعل المناعي النووي Ki-67. ونستخلص من هذه الدراسة أن مستخلص شوك الحليب يحمي من السمية القلبية التي يسببها الليثيوم في ذكور الجرذان البالغة البيضاء، وهذا ويوصي الباحثون بإعطاء مستخلص "شوك الحليب" كعامل وقائي للقلب لمرضى الاضطراب ثنائي القطب والمرضى الذين يعانون من أمراض التنكس العصبي، أثناء العلاج بالليثيوم، لمنع أو على الأقل تقليل أعراض السمية القلبية التي يسببها الليثيوم.