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The beneficial role of milk thistle (*Silybum marianum*) oil against CCl4-induced cardiotoxicity in mice

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ABSTRACT:

The present study is arranged to investigate the role of milk thistle (*Silybum marianum*) oil to improve the histological alterations as well as the deposition of collagen fibres, and the expression of the desmin by IHC against cardiac muscle induced experimentally in mice (*Mus musculus*) by CCl4. Sixty adult male albino mice were used and divided into six groups (n=10/each) ; GI: normal group received standard diet and free access to water without any treatment for one week; GII: control group, each mouse administered with M.T.O daily at a dose 1 ml/kg/bw/d orally for 4 weeks; GIII & GIV: mice injected intraperitoneally (i.p.) with 1 :1 (v/v) mixture of CCl4 and olive oil (1 ml/kg/bw) twice weekly for 4 & 6 weeks. GV & GVI: mice treated with CCl4 as in GIII & GIV then administered with M.T.O as in GII. Histological results of the cardiac muscle sections of normal and control mice groups (GI & GII) showed typical myocardial architecture with exposed normal distribution of delicate collagen fibers in the endomysium between the muscle fibers and around the blood vessels (by using Masson's trichrome dye). Cardiac muscles of CCl4-intoxicated mice groups (GIII& GIV) revealed a loss of cardiac muscle architecture and expressed excess deposition of collagen fibers in the endomysium of cardiomyofibers and around the blood vessels that increase with time. Mice treated with CCl4 then administered with M.T.O in GV& GVI. showed an obvious improvement in myocardial cells, where most of the muscle fibers looked normal with intact oval nuclei and reduction of the inflammatory leukocytes as well as thE reduction in the distribution of collagen fibers which appeared similar to the control ones. Immunostain against desmin expressed the cardiac muscle fibers of the ordinary mice groups (GI&GII) as a normal moderate to strong immunopositive reaction in the intercalated discs and Z lines. The intensity of the immunoreactivity to desmin was almost diminished in cardiomyocytes of CCl4 mice group for 4 & 6 weeks (GIII and GIV due to the disappearance of intercalated discs and Z lines in most cardiomyocytes. The mice groups took M.T.O (GIV and GVI) illustrated a marked recovery of desmin in the intercalated discs and Z lines and expressed approximately similar to the normal one. In conclusion, M.T.O has a beneficial role in the amelioration of mice's cardiac muscle disorders induced by CCl4 The current results promise to overcome cardiac disorders in human by M.T.O as a natural plant product.

KEY WORDS: Cardiac muscle, Milk thistle (*Silybum marianum*), CCl4, Histology, collagen fibres, IHC, desmin, mice.

INTRODUCTION:

Cardiovascular diseases (CVDs) are the main cause of death and morbidity worldwide. CVDs are including heart disease, stroke, rheumatic heart disease, and other conditions. Five CVDs deaths are caused by heart attacks and strokes, and one-third of these deaths occur prematurely in people under 70 years of age. Significant evidence suggests that raised oxidative stress plays a major role in the pathogenesis of cardiovascular disease, including atherosclerosis, hypertension, vascular endothelial dysfunction, and ischemic heart disease. Cellular oxidative stress results in endothelial cells and vascular smooth muscle cells, releasing toxic free radicals that interfere with cell components such as proteins, DNA, or lipids, contributing to cardiovascular pathology **(Zalat *et al*., 2021).**

Myocardial fibrosis is a common final pathway in chronic myocardial disease and is the structural correlate of heart failure. It has usually been divided into interstitial fibrosis and replacement fibrosis, although more recent histology data have suggested significant overlap between these two states. Diffuse interstitial fibrosis occurs earlier in the course of disease and represents collagen synthesis and deposition by differentiated myofibroblasts in response to a variety of stimuli. Significantly, reactive interstitial fibrosis is reversible **(Chang *et al*., 2014).** A subgroup of interstitial fibrosis encapsulates infiltrative pathologies that deposit proteins in the interstitium (e.g. cardiac amyloidosis). Replacement fibrosis represents collagen deposition that occurs following myocytes apoptosis or necrosis. It is an irreversible and is of prognostic relevance across a broad spectrum of myocardial diseases **(Bing and Dweck, 2019)**.

Carbon tetrachloride (CCl4) is a xenobiotic hazardous hepato-toxin (Xiao et al., 2012). It also causes disorders in tissues other than liver including heart by generating free radicals **(Botsoglou *et al*., 2009).** Severe conditions may develop through multiple organ dysfunctions by these free radicles. CCl 4 leads to cellular damage by alteration of cellular structure through lipid peroxidation, and the cardiac lipid peroxidation was significantly increased. Chronic toxicity caused by treatment of CCl_4 to the rats significantly changed the cardiac function test, decreased the activities of antioxidant enzymes. CCl_4 is also caused DNA fragmentation in rats and histopathalogical abnormalities (Sahreen *et al*., 2014).

According to **WHO (2013),** herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients**.** Herbs have recently attracted attention as health beneficial food and as source materials for drugs development. Herbal medicines derived from plant extracts are being progressively used to treat a wide variety of diseases **(Shaker *et al*., 2010; El-Desouki *et al*., 2017- 2019, El Rabey *et al*., 2021).** Milk thistle "*Silybum marianum*" (MT) is an annual to biannual plant of the botany family of Asteraceae growing to 1.5 m **(Rambaldi *et al*., 2005).** MT is widely used as therapeutic herb in many diseases. MT provides cardioprotection and limits adverse

remodeling post-myocardial infarction by mitigating oxidative stress and reactive fibrosis **(Vilahur *et al.,* 2018).** It is also used as protecting liver protectant **(Gillessen and Schmidt, 2020),** and anticancer **(Elyai, 2021).**

Silymarin, an extract from milk thistle seeds, has been used for centuries to treat many diseases. Conventionally the seeds have been used in Europe as galactagogic in nursing mothers, bitter tonic, and treating depression (antidepressant), gallstones, dyspepsia, splenic congestions, varicose veins, diabetes, amenorrhea, uterine hemorrhage and menstrual problems. Preclinical data indicate that silymarin can reduce oxidative stress and consequent cytotoxicity. Silymarin has antioxidant activities against CVDs and offers protection against oxidative stress-induced hypension, atherosclerosis and cardiac toxicity **(Taleb *et al*., 2018).** Silymarin can protect the heart, brain, liver and kidneys against ischemia reperfusion injury, probably by preconditioning, the mechanism by which silymarin protects the heart from ischemia remains largely unknown **(Zholobenko and Modriansky, 2014).** However, **Khazaei *et al.* (2022)** recorded that the silymarin is a free radical scavenger, and a strong antioxidants.

The cytoskeleton is a complex and dynamic network of interlinking protein filaments present in the cytoplasm of all cells, and spreads from the cell nucleus to the cell membrane and is composed of similar proteins in the various organisms. There are three major known components, microfilaments, intermediate filaments (IF) and microtubules **(McKinley *et al*., 2015).** Desmin is the IF protein of muscle and endothelial cells with 53.5 kDa protein composed of 470 amino acids and a crucial subunit of the intermediate filament in cardiac, skeletal and smooth muscles **(Zong *et al*., 2013; Tsikitis *et al.,* 2018).**

The name of desmin (desmos = link) is for its alleged function of linking sarcomeres. Desmin filaments are mainly located at the periphery of Z-disk of striated muscles, and they play a critical role in the maintenance of structural and mechanical integrity of the contractile apparatus in muscle tissues. Desmin can be a useful marker in muscle derived tumors **(Leader *et al*., 1987; Costa *et al*., 2004).**

The present work is aimed to study the ameliorative effect of M.T.O on the histological and IHC changes of the cardiac muscle induced by CCl4 for two different durations in mice.

MATERIAL AND METHODS:

Animals:

Sixty male albino mice (*Mus musculus*) weighing 25±3g, with the age of approximately three months were obtained from Vacsera 51 Wezaret EL Zeraa ST. Agouza, Giza, Egypt. All animals were housed in plastic cages with stainless steel wire-bar covers (10 per cage) for one-week acclimatization under the same condition of temperature and natural dark- light cycle, using a wooden dust free litter as a bedding material. The National Research Centre's Ethics Committee and the National Institutes of Health's Guide for Care and Use of Laboratory Animals were followed

when performing animal procedures. The experimentation and animal care were performed and handled in compliance with the ethical guidelines approved by the animal care and use committee, Faculty of Science, Tanta University, Egypt (IACUC-SCI-TU-).

Chemicals:

CCl4 and olive oil were obtained from Vacsera 51 Wezaret EL Zeria ST. Agouza, Giza, Egypt. CCl4 is a colorless non-flammable liquid, of molecular weight 153:84. CCl4 is one of the most used in the experimental studies to induce cardiotoxins **(Botsoglou *et al.*, 2009).**

Induction of cardiotoxin by CCl4 and treatment:

Cardiac injury was induced by intraperitoneal (i.p.) injections of CCl4 (1 ml/kg/bw) twice a week for 4 & 6 weeks using a concentration of 50% (v/v) of CCl4 suspended in olive oil at a ratio of (1:1) starting from the first week **(Sahreen *et al.*, 2014) .** M.T.O was orally administered by a gastric tube daily to each mouse at a dose1 ml/kg/bw/d for 4 weeks **(Vilahur *et al.*, 2018)**. M.T.O was purchased from a local market of agricultural seeds, and medicinal plants with affair degree of quality assurance (Alexandria, Egypt).

Experimental design:

Sixty male albino mice were divided into 6 groups (n=10/each). Group (GI): normal group, animals received free access to standard laboratory feed and water for one week (n = 10). Group (GII): control group, received orally a dose of M.T.O (1 ml/kg/bw/d/ mouse) for 4 weeks. Groups (GIII & GIV): two different durations, (n = 20 for each group); each mouse was injected i.p. by CCl4 at a dose 1 ml/kg/bw that added to olive oil (1:1 v/v) twice a week for 4 & 6 weeks. Groups (GV & GVI): M.T.O administered orally as in GII. At the end of each experimental period, the mice of all groups were euthanized and sacrificed Left ventricles of cardiac tissue specimens from all groups were taken and cut into small pieces to process for histological and immunohistochemical (IHC) study **(Hsu *et al.*, 1981; Bancroft and Stevens, 1982),** then examined by light microscope.

Histological study:

The specimens were fixed in 10% neutral buffered formalin, dehydrated in accending ethanol, cleared in xylene, embedded in paraffin wax and sectioned at 5 µ thickness using microtome. Paraffin sections were further processed by H&E to demonstrate the histological changes, and with Masson's trichrome to display the collagen fibres.

IHC study:

IHC technique was used to express the desmin in the cardiac muscle tissues by the avidin-biotin complex (ABC) method in which a biotinylated secondary antibody reacts with peroxidase conjugated streptavidin molecules **(Hsu *et al.*, 1981).** . Endogenous peroxidase activity was inhibited by incubation with 3% H2O2 for 5 min. The sections were blocked with normal goat serum for 1h to prevent non-specific binding followed by incubation with the primary monoclonal antibody against desmin (RD30) was used. The sections were incubated with the secondary antibody (anti-rabbit peroxidase) for 30 min. The staining was visualized by using 3,

3'diaminobenzidine (DAB) chromogen solution for colouring the reaction into brown colour. Then, the slides were washed and counterstained with haematoxylin.

Image analysis:

Digital images were analysed by a semi quantitative system (Fiji-Image J software, Java-based application for analysing images). The brown colour of desmin positive cells was immunohistochemically expressed in cardiomyocyte sections; the percentage-coloured stained area (area fraction) per field area was determined by measuring five randomly photographed high-power fields; X 400 magnifications **(Schindelin *et al.*, 2012).**

Statistical analysis:

Statistical analysis data was expressed as mean and standard error and carried out by one-way analysis of variance (ANOVA). Significant differences in means were set at $P \leq 0.05$.

RESULTS:

I. Histological observations:

Ia. Haematoxylin and eosin stain (H & E):

The histological examination of the cardiac muscle sections of the normal and control mice groups (GI, GII) showed typical myocardial architecture. The myocardium is striated and organized in a linear array that branched and anastomosed in a specific shape giving the appearance of a sheet. The cardiac muscle fibers are connected by intercalated discs, representing that the fibril is dividing, combining, and then spreading again. The intercalated disc represents the intracellular junction between two cardiac muscle cells (cardiomyocytes). The myocytes possess centrally located oval nuclei and homogeneous acidophilic sarcoplasm.The rich capillaries supplied intercellular areas (Fig.1).

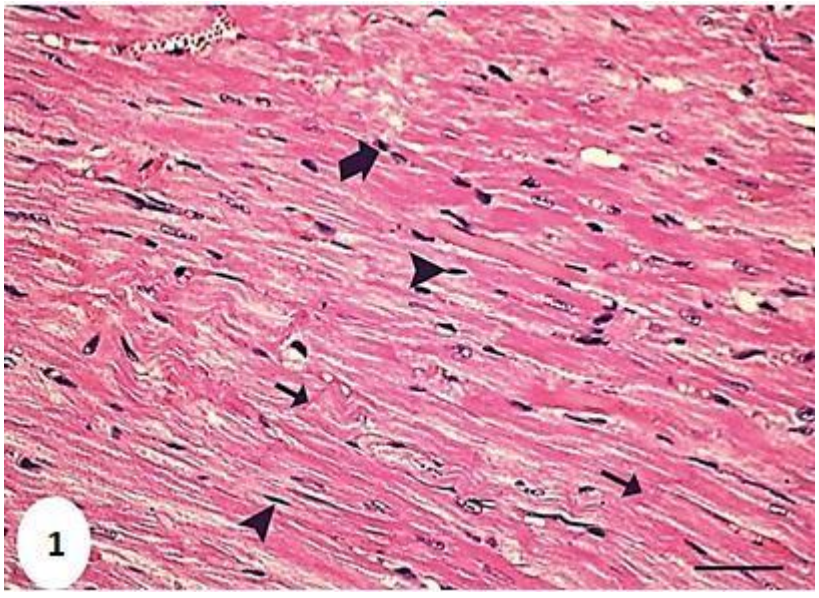


Fig.1. Longitudinal section of the cardiac muscle of a normal mouse showing normal architecture of branched striated cardiomyocytes with intact intercalated discs (arrows),

acidophilic homogeneous sarcoplasm and centrally located oval nuclei (arrowheads). Occasional muscle cells possess two nuclei (thick arrow). H&E, scale bar = 12.5 μm.

Cardiac muscles of CCl_4-intoxicated mice groups (GIII&GIV) revealed a loss of cardiac muscle architecture, demonstrating many cardiac myocytes with remarkable disorganization and fragmentation of myofibrils, necrosis appearance in myocardial cells, vacuolar degeneration, leucocytes recruitment, loose of cross striation of cardiac muscle and disappearance of intercalated discs in many cardiomyocytes with pyknotic nuclei. Marked congestion with dilatation of the myocardial blood vessels were also observed (Fig.2). Moreover, the cardiac muscle sections of cirrhotic mice group showed more disarrangement of cardiomyocytes with a marked sarcoplasmic vacuolation and pyknotic nuclei, necrosis of myocardial cells and congestion of blood vessels (Fig.3).

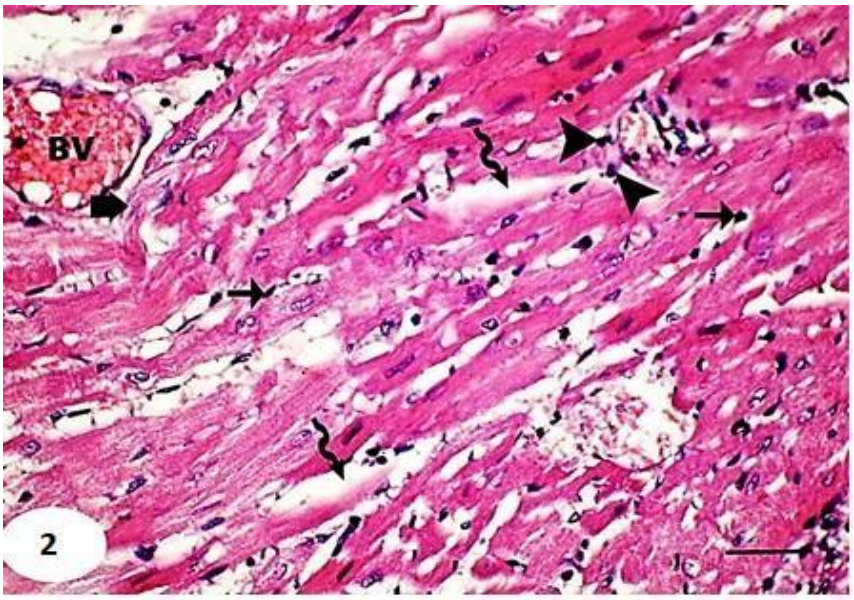


Fig.2. L.S. of the cardiac muscle of a mouse injected i.p. with CCl_4 at a dose of 1 ml/kg/bw twice weekly for 4 weeks (GIII) showing marked disarrangement of muscle fibers (thick arrow), loose of cross striation of cardiomyocytes, disappearance of intercalated discs in many cardiac fibers, vacuolation of the sarcoplasm, appearance of pyknotic nuclei (arrows), degeneration of muscle fibers (zigzag arrows), congestion of blood vessel (BV), and abundant of inflammatory leucocytes (arrowheads). H&E, scale bar = 12.5 μm.

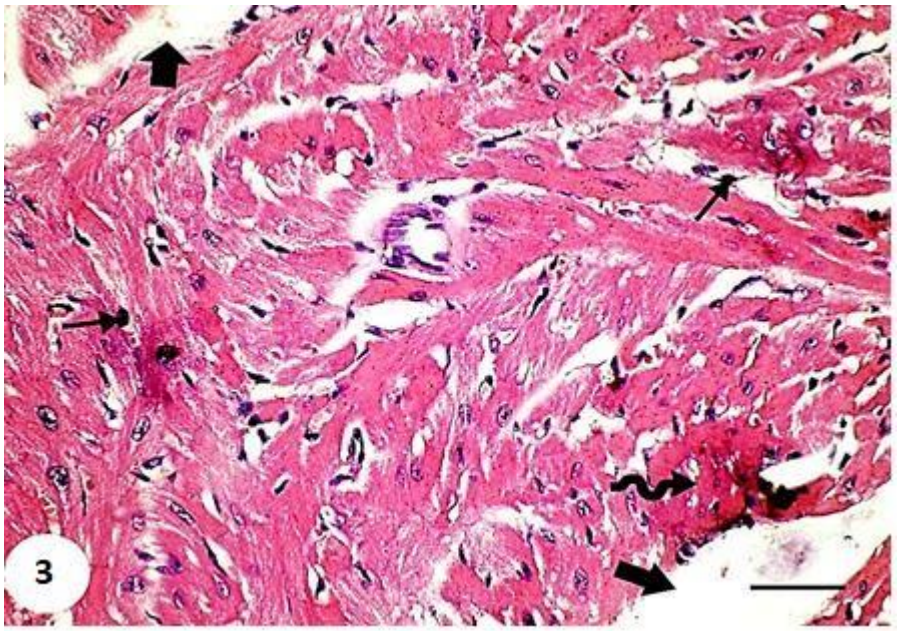
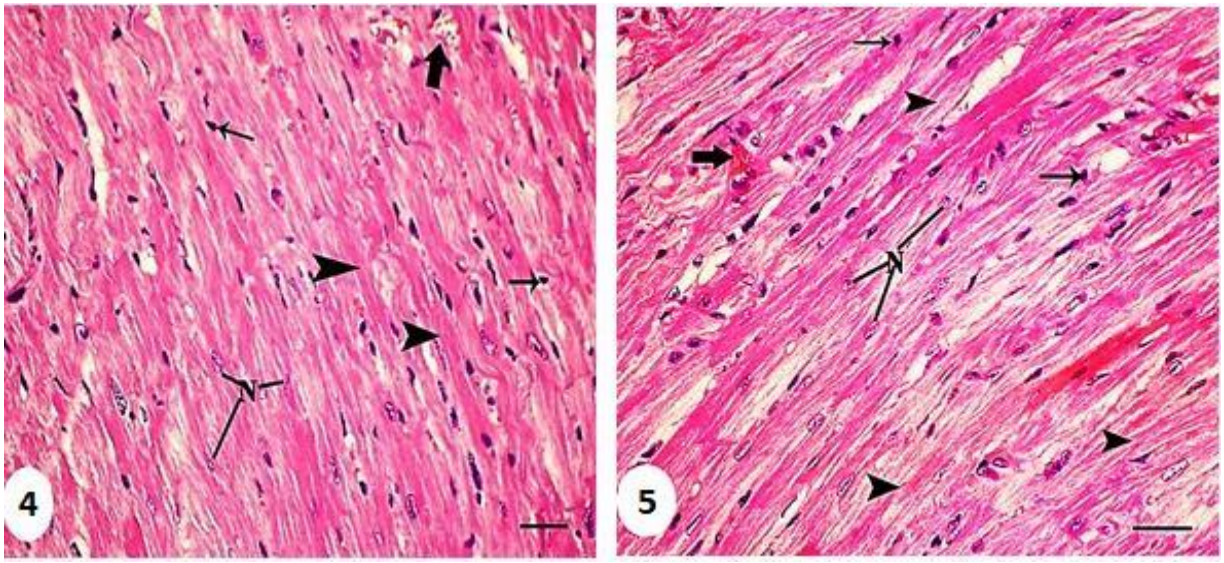


Fig. 3. L.S. of the cardiac muscle of a mouse injected i.p. with CCl_4 at a dose of 1 ml/kg/bw twice weekly for 6 weeks (GIII) showing more disarrangement of cardiomyocytes with marked sarcoplasmic vacuolation and pyknotic nuclei (thin arrows), necrosis of myocardial cells (thick arrows), and congestion of blood vessel (zigzag arrow). H&E, scale bar = 12.5 μm

Mice groups who received M.T.O daily for four weeks (GV and GVI) showed an obvious improvement in myocardial cells, where most of the muscle fibers looked normal with intact oval nuclei and reduction of the inflammatory leukocytes **(Figs. 4&5).**



Figs. 4&5. Longitudinal sections of the cardiac muscle of mice groups received daily M.T.O at a dose 1ml/kg/bw/mouse for 4 weeks; showing the approximately normal histological appearance of cardiomyocytes (arrowheads), normal oval central nuclei (N), and few pyknotic nuclei (thin arrows). Reduction of the blood congestion and leucocytes are seen (thick arrow). H&E, scale bar = 12.5 μm.

1b. Masson's trichrome staining in cardiac muscle:

Longitudinal sections of the cardiac muscle fibers of the normal & control mice groups (GI & GII) exposed normal distribution of delicate collagen fibers in the endomysium between the muscle fibers and around the blood vessels (Fig. 6).

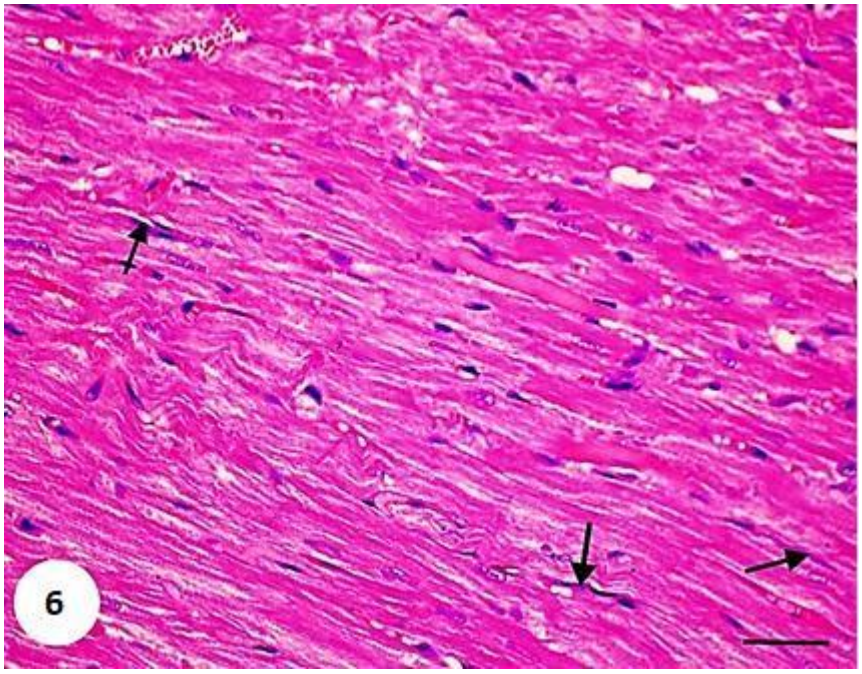
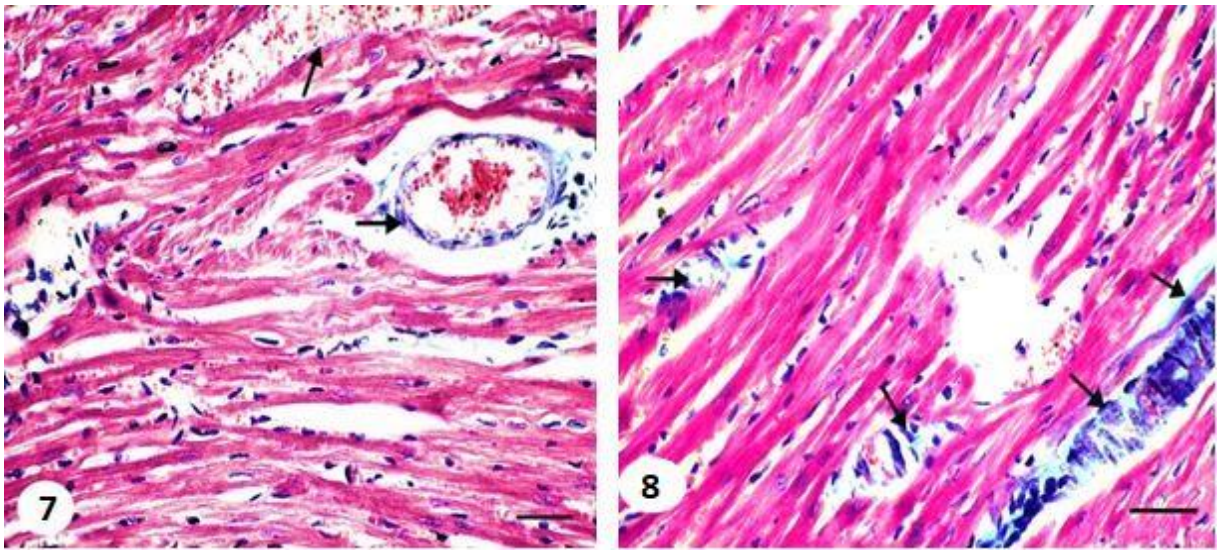


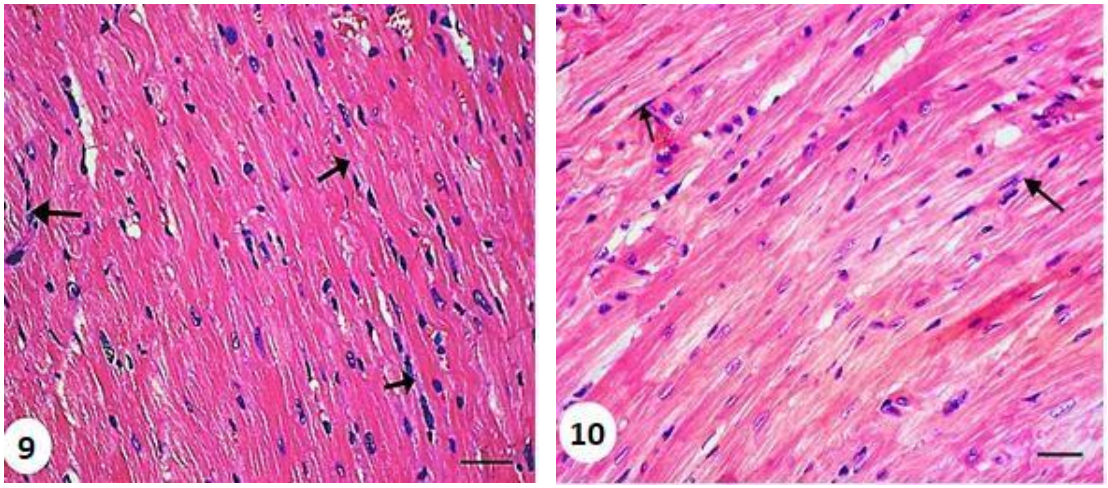
Fig. 6. Longitudinal section of the cardiac muscle of a normal mouse showing normal thin delicate distribution of collagen fibers in endomysium between the muscle fibers and around the blood vessels (arrows). Masson's trichrome, scale bar= 12.5 μm.

The mice groups (GIII & GIV) injected with CCl_4 (1ml/kg/bw, i.p.) twice weekly for 4 and 6 weeks respectively, expressed excess deposition of collagen fibers in the endomysium of cardiomyofibers and around the blood vessels (Figs. 7&8).



Figs. 7&8. Longitudinal sections of the cardiac muscle fibers of mice (GIII&GIV) illustrate an increment and dense distribution of collagen fibers around the dilated congested blood vessels (arrows). Masson's trichrome, scale bar= 12.5 μm.

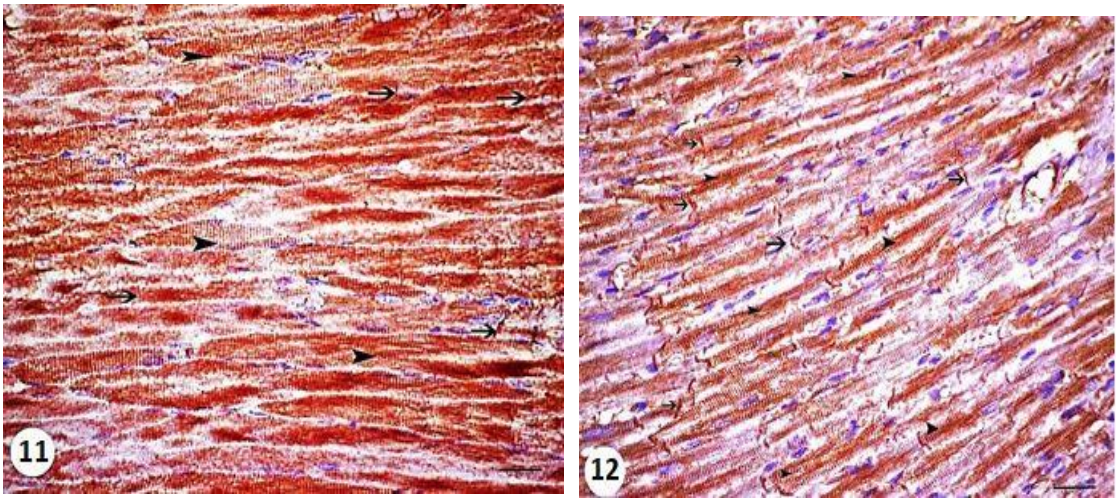
Significant improvements in the cardiomyocytes of treated mice with M.T.O at a dose of 1ml/kg/bw daily for 4 weeks (GV and GVI) were observed, since there was a marked decrease in the distribution of collagen fibers and appeared approximately similar to the control ones (Figs.9&10).



Figs. (9&10). Longitudinal sections of the cardiomyocytes of mice the administered with of M.T.O daily for 4 weeks (GV&GVI, respectively), showing approximately normal distribution of collagen fibers (arrows). Masson's trichrome, scale bar= 12.5 µm.

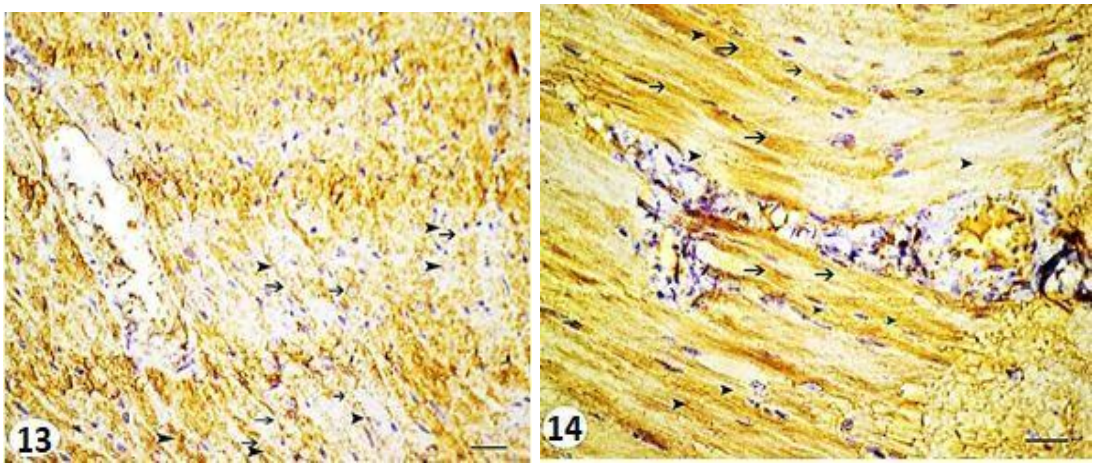
2. Immunohistochemical expression of desmin:

Immunostain against desmin expresses in cardiac muscle fibers of the ordinary mice group (GI) showed normal moderate to strong immunopositive reaction in the intercalated discs and Z lines (Fig.11). Similar results were demonstrated in the cardiomyocytes of control mice given M.T.O only at a dose of 1 ml/ kg /bw for 4 weeks (GII) (Fig.12).



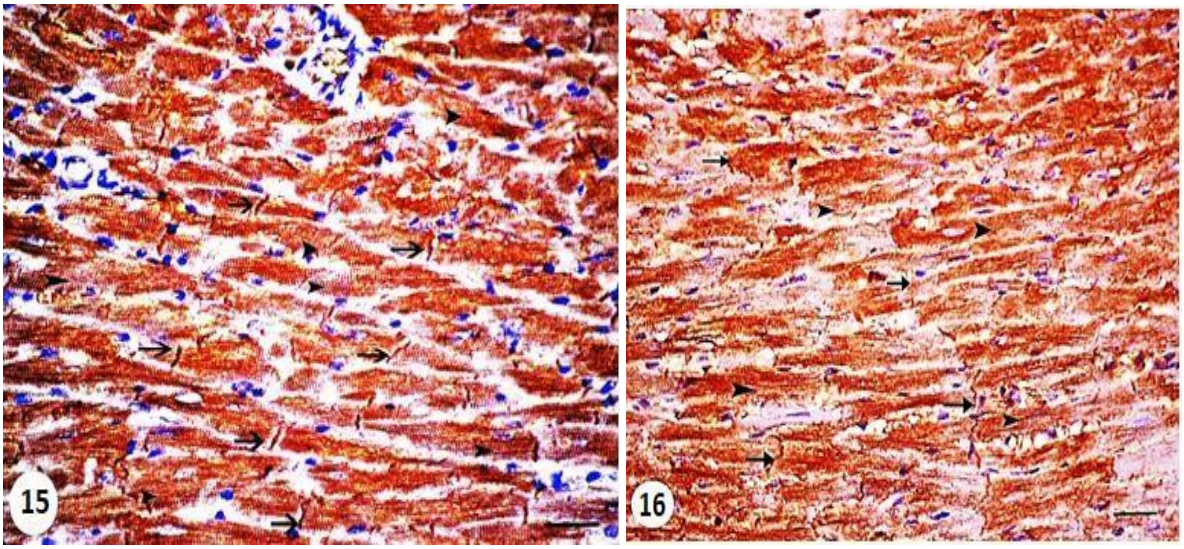
Figs.11&12. Longitudinal section of the cardiac muscle of a normal mouse (GI) **(Fig.11)** and control mouse (GII) treated with MT oil for 4 weeks **(Fig.12)** expressing moderate to strong immunopositive reaction to desmin in the intercalated discs (arrows) and Z lines (arrowheads). Desmin immunostain, scal bar =12.5 µm.

The intensity of the immunoreactivity to desmin was almost diminished in cardiomyocytes of the mice groups (GIII and GIV) that injected with CCl4 for 4&6 weeks. An obvious reduction of immunopositive reaction to desmin in intercalated discs and Z lines of cardiomyocytes mice group (GIII) (Fig.13), while in (GIV), the disappearance of desmin-immunoreaction in the intercalated discs and Z lines was cleared (Fig. 14).



Figs.13&14. L.S. of the cardiac muscle fibers of the mice injected with CCl_4 at a dose 1ml/kg/ bw twice a week for 4 & 6 weeks (GIII and GIV), respectively; expressing an obvious reduction of immunopositively reaction to desmin and appears faint brown color in intercalated discs (arrows) and in Z lines (arrowheads) in GIII (**Fig. 13),** showing a sharp decrease to no desmin expression with the disappearance of intercalated discs (arrows) and Z lines (arrowheads) in GIV (**Fig. 14)**. Desmin immunostain, scale bar =12.5 µm.

The sections of the cardiac muscle of the mice groups treated with CCl_4 and M.T.O (GV and GVI) expressed a marked recovery of strong immunoreaction intensity to desmin in the intercalated discs and Z lines approximately similar to the normal one (Figs. 15& 16).



Figs.15&16. L. S. of the cardiomyocytes of the mice groups treated with CCl_4 and M.T.O (GV and GVI) expressing approximately recovery of ordinary moderate to strong of desmin

immunoreactivity in the intercalated discs (arrows) and Z lines (arrowheads). Desmin immunostain, scale bar =12.5 μm.

Image analysis of the cardiac muscle positive cells by desmin immunostaining:

Image analysis of cardiomyocytes of the mice groups (GIII & GIV) injected with CCl4 for 4&6 weeks recorded a significant decrease in the areas of positive cells by desmin immunostain comparable of normal mice group (GI) (***P ≤ 0.001); Cardiomyocytes of the mice groups (GV&GVI) administered orally with M.T.O daily for 4 weeks expressed a significant increase in the areas of positive cells (***P ≤ 0.001), in comparison to GIII & GIV (Table 1 &Fig. 17).

Table1: Mean of area fraction of positive cells of the cardiac muscle (%) by desmin immunostain of all groups

Groups	**Mean ±SE**
GI (Normal)	34.47±3.52
GII (Control + M.T.O for 4 weeks)	51.89±3.07
GIII (mice injected with CCl4 for 4 weeks)	1.61 ±0.14 ###
GIV (mice injected with CCl4 for 6 weeks)	1.67 ±0.24 ###
GV (mice injected with CCl4 for 4 weeks + M.T.O for 4 weeks)	52.17±2.9 ***
GVI (mice injected with CCl4 for 6 weeks + M.T.O for 4 weeks)	50.7± 3.17 **

Data are expressed as mean ± SE. Significant at **P≤0.01 and ***P≤0.001
Significant change compared to normal group.
* Significant change between two related groups.

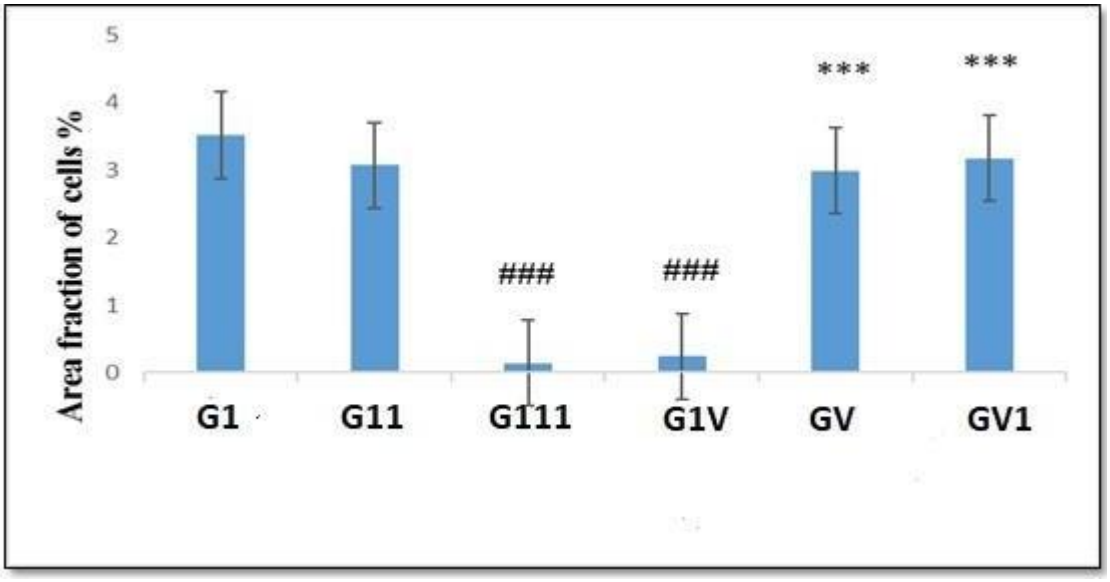


Fig. 17: Mean of area fraction of positive cells of cardiac muscle by desmin immunostain (%) of all groups.Significant at ***P ≤0.001.

Discussion

In the present study, the histological examination of the cardiac muscle sections of the control mice group, showed typical myocardial architecture. The myocardium is striated and organized in a linear array that branched and anastomosed in a specific shape giving the appearance of a sheet. The cardiac muscle fibers are connected by intercalated discs, representing that the fibril is dividing, combining, and then spreading again. The intercalated disc represents the intracellular junction between two cardiac muscle cells (cardiomyocytes). The rich capillaries supply intercellular areas. The myocytes possess centrally located oval nuclei and homogeneous acidophilic sarcoplasm. The cardiac muscle sections of the control mice group given MT oil showed results similar to those in the standard group with approximately typical cardiac muscle architecture.

Cardiac muscle fibers of CCl_4-intoxicated mice groups (GIII & GIV) revealed a loss of cardiac muscle architecture, demonstrating many cardiomyocytes with remarkable disorganization and fragmentation of myofibrils, necrosis appearance in myocardial cells, vacuolar degeneration, leucocytes recruitment, loose of cross striation of cardiomyocytes, disappearance of intercalated discs in many fibers. The nuclei appeared irregular in shape and other with pyknotic nuclei. Marked congestion with dilatation of the myocardial blood vessels was also observed. Moreover, the cardiac muscle sections of cirrhotic mice group showed more disarrangement of cardiomyocytes with marked sarcoplasmic vacuolation, necrotic and pyknotic nuclei, and congestion of blood vessels. Resemble observations were seen by many authors **(Al-Rasheed *et al.*, 2014; Chang *et al*., 2014; Sahreen *et al*., 2014).** CCl_4-induced myocardial toxicity in rats by the increase in lipid peroxidation caused degeneration and changes in the architecture of cardiomyocytes and interstitial edema. Moreover, CCl_4 caused a decrease in troponin-I and lactate dehydrogenase immunoreactivities, increasing tumor necrosis factor-alpha (TNF-α) and apoptosis by caspase-3 immunoreactivities on the cardiac tissue **(Yildiz *et al.*, 2022)**.

he present findings showed that the mice groups who treated with CCl4 and received M.T.O showed and obvious improvements in myocardial cells, and most of the muscle fibers looked normal form with normal nuclei and less disorganization of myofibrils. Similar results are recorded by **Al-Rasheed *et al.* (2014)** demonstrated that, administration of silymarin 24 h post CCl_4 injection showed mild improvement in the cardiac histological picture and few areas of fibrosis. Actually, **Razavi and Karimi (2016)** suggested that oxidative stress has been as one of the molecular mechanisms involved in drug-induced cardiac toxicity, and they showed in their study that silymarin has a broad spectrum of cardiac protective activity against toxicity induced by some chemicals including metals, environmental pollutants, oxidative agents, and anticancer drugs. **Kumaş *et al.* (2016)** concluded that silymarin has an antioxidant and antiapoptotic effect on heart tissue. **Taleb and his colleagues (2018)** explained that silymarin shows a wide range of mechanisms in preventing the cardiovascular diseases like hypertension, atherosclerosis, ischemia, vascular

dysfunction, cardiotoxicity, cardiomyopathies, and heart failure by increasing enzymatic antioxidants, mitochondrial enzymes, and expression of nuclear factor-erythroid factor 2-related factor 2.

Younis *et al. (*2020) concluded that silymarin has cardio-protective potential against CCl_4 induced injuries owing to its antioxidant constituents. Additionally, the cardioprotective activities of silymarin were primarily shown in cisplatin-induced cardiotoxicity in rat models. These effects are due to replenishing endogenous antioxidant enzymes, suppressing neutrophil infiltration, and reducing serum malondialdehyde as the end product of myocardial lipid peroxide. Silymarin treatment protects against reperfusion damage and inflammation by confirming anti-inflammatory and antioxidant actions. Silymarin antioxidant properties are considered responsible for its cardio-protective activities **(Zalat *et al*., 2021). Wang *et al*. (2021**) illustrated the incidence of obesity-related complications such as cardiovascular disease and type2 diabetes is high. Their study explored the effects of silybin on protein expression in obese mice, and they concluded that silybin could protect cardiac function by inducing the protein expression of tropomyosin 1 and myosin light chain 2 myosin heavy chain1 in the adipose tissue of obese mice.

CCl_4 is mainly caused liver tissues disorder and indued liver fibrosis and cirrhosis as well as the increment of collagen fibers distribution **(El-Desouki *et al*., 2022)**, The authors demonstrated that the CCl_4 expressed intense positive for α-fetoprotein (AFP) immunoreactivity to the hepatocyte's cytoplasm of the fibrotic or cirrhotic mice liver and expressed intense AFP more dyeable in the hepatocytes of cirrhosis than in liver fibrosis, and a significant increase in serum AFP levels measurement as compared to the normal. Similarly, **Mizejewski (2015)** recorded that AFP was positive in liver cirrhosis and highly significant in hepatocellular carcinoma (HCC). Administration of milk thistle (*Silybum marianum*) oil (M.T.O) to fibrotic and cirrhotic mice groups expressed amelioration and an obvious improvement of the majority of histological hepatocytes, and appeared with a decline or negative AFP immunostain or measurement as compared to fibrotic and cirrhotic mice groups **(El-Desouki *et al*., 2022)**. Moreover, **El-Desouki *et al.* (2024)** illustrated that the fibrotic and cirrhotic livers of mice induced by CCl4 revealed overexpression immunoreaction and increased the cytoskeletal intermediate filament vimentin proteins,(important for cell morphology, motility and growth(. The treatment with M.T.O to either fibrotic or cirrhotic group expressed an improvement and decline in vimentin filaments in the hepatic tissues.

The present study revealed that the cardiac muscle fibers of normal mice group stained with Masson's trichrome exposed normal distribution of delicate collagen fibers in the endomysium between the muscle fibers and around the blood vessels as well as that observed in the cardiac muscle of control mice group received orally M.T.O. Cardiomyocyte sections of CCl_4-injected to mice expressed excess deposition of collagen fibers in the endomysium of cardiomyofibers and around the blood vessels. Similar results were seen by **El-Desouki *et al.* (2012)** who elucidated

the thickness of collagen fibers stained by azan, gradually increased, and became compact dense in the cardiac muscle fibers of stressed rats, and they were more obvious around the blood vessels.

Furthermore, in the present work, significant improvements in the cardiac muscle damage of the mice given M.T.O were observed since there was a marked decrease in the distribution of collagen fibers and appeared approximately similar to the control ones. In agreement, **Al-Rasheed *et al*. (2014)** and **Chang *et al*. (2014)** declared that the normal untreated rats showed typical cardiac muscle fibers and normal collagen fibers. They demonstrated that the myocardial damage induced by CCl_4 is observed by focal areas with massive degeneration and scattered areas of fibrosis stained in blue with Masson's trichrome. Administration of silymarin 24h post CCl_4 injection showed improvement in the cardiac histological picture and few areas of fibrosis. Administration of silymarin with chlorogenic acid or/and melatonin showed recovery of most normal cardiac tissue and normal collagen.

Moreover, **Attia *et al.* (2017)** elucidated the cardioprotective effect of silymarin against doxorubicin-induced cardiotoxicity in rats followed pathological cardiomyocytes examination by Masson's trichrome stain. They illustrated a marked reduction of collagen deposition and significant improvement in cardiomyocytes of rats post-treatment with silymarin. **Gillessen and Schmidt (2020)** recorded that silymarin acts as a free radical scavenger, by regulating the enzymes responsible for the development of cellular damage, fibrosis, and cirrhosis. Moreover, **Elyasi (2021)** concluded in a study includes cleansing and detoxification after chemotherapy for adverse reactions of cancer treatment like constituents protected rat heart microsomes and mitochondria against doxorubicin-induced lipid peroxidation, so it seems that silymarin may prevent doxorubicin-mediated cardiotoxicity.

Kadoglou *et al. (*2022) approved in a study on cardiovascular protective properties of silibinin/silymarin that silymarin treatment decreased cardiac fibrosis and collagen deposition whereas, silibinin has also been proven to reduce the hypertrophic response in H9c2 rat embryonic heart cells induced by phenylephrine. Many authors concluded in their studied that milk thistle and its constituents, especially silymarin act as cardioprotectant mainly depend upon its ability to reduce oxidative stress and consequent cytotoxicity by removing the free radicles. Essentially, silymarin particularly silybin or silibinin is known to be a very strong antioxidant and free radical scavenger **(Khazaei *et al*., 2022).**

In the present study, the expression of desmin immunostain in the cardiac muscle fibers of the ordinary mice group showed normal moderate to strong immunopositive reaction in the intercalated discs and Z lines**.** Similar results were demonstrated, in the control cardiomyocytes of mice given M.T.O only. However, the intensity of the immunoreactivity to desmin of cardiac muscle fibers was almost decline in CCl_4 treated mice groups, and it decreased sharply with time in GIV due to the disappearance of intercalated discs and Z lines. The cardiac muscle sections of mice

injected with CCl4 and treated with M.T.O expressed a marked recovery of immunoreaction to desmin at intercalated discs and Z lines, approximately similar to the normal one.

Studies with desmin-deficient mice, which showed dilated cardiomyopathy, have seen that desmin intermediate filaments play a significant role in stabilizing the organization of the sarcolemma into costameres (**Capetanaki *et al.*, 2007).** Since proper muscle needs tightly link energy production and demand; thus, mitochondria abnormalities are pathological features among desmin-related cardiomyopathies. The earliest and most prominent features of desmin-deficient mice pathology are mitochondrial structural perturbations, arising before any other cardiac dysfunction **(Milner *et al.*, 2000).** Mitochondrial defects included loss of proper morphology followed by cardiomyocyte death, inflammation, fibrosis, and calcification, all leading to widespread myocardial degeneration, dilated cardiomyopathy, and heart failure. So, desmin's presence is significant for cristae structure, respiratory function, mitochondrial permeability transition pore activation, sensitivity to oxidative stress, and proper mitochondrial membrane potential **(Diokmetzidou *et al.*, 2016).** The balance between reactive oxygen species (ROS) and antioxidants is disturbed when excessive amounts of free radicals are produced or antioxidant capacity is decreased. This disturbance is known as oxidative stress, and it plays an important role in cardiac pathophysiology **(Vichova and Motovska, 2013).**

El-Desouki *et al.* (2012) displayed obviously loss of desmin immunostain in intercalated discs and periphery of Z lines in cardiomyocytes of rats stressed for different durations (5, 15, and 30 days), where the treatment of stressed-rats with diazepam for 30 days revealed a remarkable improvement and recovery of desmin almost up to the control form. Moreover, **El-Desouki *et al.* (2017b)** elucidated weak desmin immunostain in intercalated discs and Z-lines of cardiac myofibres of diabetic rats, while after treatment with herbal *Moringa oleifera* leaves extract, the cardiomyofibres restored approximately regular desmin. Furthermore**, El-Desouki *et al.* (2018b)** exhibited the aged rabbit's cardiac muscle expressed an intense immunostain to desmin, and the administration of vitamin E as an antioxidant medication improved cardiac muscle architecture.

Cardiomyopathies are caused by desmin (DES) mutations. DES mutations cause multiple pathomechanisms leading to the death of cardiomyocytes and contributing accordingly to disease progression. The majority of pathogenic DES mutations caused abnormal desmin filaments. Because desmin is a scaffolding protein connecting different cell organelles, the cellular pathomechanism *in vitro* and *in vivo* are diverse and affect different cellular compartments **(Brodehl *et al.*, 2013&2018).** Moreover, the disruption of desmin organization, regardless of the genetic cause, could affect the proper function of the entire network leading to multiple defects which can enhance by the presence of toxic aggregates leading to different myopathies and cardiomyopathies (**Tsikitis *et al.*, 2018; Heffler *et al.* 2020).**

In conclusion, milk thistle oil most likely has a beneficial role in the amelioration of mice cardiac muscle injuries. So, this study suggests that milk thistle oil, a natural herb in the form of oil, may be valuable in the treatment of humans who expose to cardiotoxicity, and more studies are needed under controlled laboratory conditions to test this hypothesis, as a promising result to overcome cardiac muscle diseases, and their complications.

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الدور المفيد لزيت نبات شوك الجمل)سليبين المراريانية(لتحسين الخلل في العضلة القلبية المرتبطة بتلف الكبد المستحث تجريبيا في الفئران المعق

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يهدف هذا العمل إلى دراسة دور زيت نبات شوك الجمل في تحسين التغيرات في نسيج عضلة القلب الناتجة عن الحقن برابع كلوريد الكربون من باستخدام صبغ الهيماتوكسيلين واألوسين وكذلك صبغ الماسون الثالثي الكروم للكشف عن ألياف الكوالجين وكذلك في بروتين الديسمين desmin من خالل استخدام تقنية الكيموهستوجي المناعي . تم استخدام ستون فارا من ذكور الفئران البيضاء البالغة . وتم تقسيمها إلى ست مجموعات. المجموعة ألولى: وهي المجموعة العادية والتي تلقت الغذاء والماء بدون اي معالجة لمدة شهر، المجموعة الثانية: وهي المجموعة الضابطة والتي تم معالجتها بزيت نبات شوك الجمل فقط يوميا عن طريق الحقن في التجويف البروتوني لمدة أربع أسابيع بجرعة مل/ كجم/ وزن الجسم ، اما المجموعة الثالثة والرابعة : فقد تم حقنها برابع كلوريد الكربون + زيت الزيتون بنسبه ١ : ١مرتين أسبوعيا في التجويف البروتوني بجرعة ١مل/كجم من وزن الجسم ولمده أربعة وستة أسابيع علي التوالي ، المجموعة الخامسة و السادسة: وهما

المجموعتان المحقونتان برابع كلوريد الكربون ولمدة أربعة وستة أسابيع ثم تم معالجتهما بزيت شوك الجمل يوميا بجرعة ١مل/كجم من الجسم ولمدة أربع أسابيع. أوضحت النتائج النسيجية لعضلة القلب للفئران العادية والضابطة من المجموعة الأولى والثانية بعد فحصهما بالمجهر الضوئي أن بنية عضلة القلب طبيعية حيث ظهرت مخططة ومنظمة في مجموعة خطية تتفرع بشكل معين مما يعطي مظهر الصنيحة. وترتبط ألياف عضلة القلب ببعضها البعض عن طريق أقراص مؤسمة ، ويمثل القرص البيني تقاطعًا داخل الخلايا بين خليتين عضليتين القلب ، وتمتلك الخلايا العضلية أنوية في بيضاوية ذات موقع مركزي وسارکوبالزم حمضي الصبغة ومتجانس ، ويتم تغذية المناطق بين الخلايا عن طريق الشعيرات الدموية . أما العضلة القلبية للفئران المجموعة الثالثة والرابعة المحقونة برابع كلوريد الكربون لمدة ٤و٦ أسابيع على التوالي تؤد أظهرت تمتك وفؤدان بنية عضلة القلب مع عدم انتظام في ألياف العضلة وظهور نخر بها، وتكون فجوات بالسارکوبالزم واختفاء الأقراص البينية في العديد من الياف عضلة القلب. وكذلك ظهرت الأنوية غير طبيعية ، منها الداكن ومنها المتحلل، كما لوحظ احتقان وتمدد للأوعية الدموية وتسلل لكريات الدم البيضاء اللمفاوية خارج الوعية. أما المجموعتان الخامسة والسادسة المعالجة بزيت شوك الجمل للفئران المريضة بالنلوف والتشمع تؤد أظهرت تحسنًا لحالة إف عضلة القلب واسترجاع أغلبها إلى تركيبه واض الطبيعي .

وباستخدام صبغ الماسون ثالثي الكروم، تؤد ظهرت الألياف الكوالجينية لعضلة القلب لمجموعة الفئران العادية والضابطة التي تؤت زيت زيت شوك الجمل نقط بتوزيعا الطبيعي الرقيق في بطانة النسيج الضام بين ألياف العضلة القلبية وحول الألوعية الدموية . أما مجموعات الفئران التى حقنت برابع كلوريد الكربون لمدة ٤و٦ أسابيع على التوالي تؤد أظهرت توزيعا زائدًا لألياف الكوالجين في بطانة النسيج الضام لعضلة القلب وحول الوعية الدموية. أما بعد المعالجة بزيت شوك الجمل لهذه الفئران تؤد أظهرت تحسنا ملحوظا في توزيع ألياف الكوالجين وكانت مشابه تقريبًا لألياف الطبيعية. أما النتائج الكيموسيجية المناعية لعضلة القلب للفئران من المجموعة الأولى باستخدام دالات الديسمن desmin تؤد أظهرته معتدل إلى قوي في الأقراص البينية وخطوط Z الموجودة فى ألياف عضلة القلب الطبيعية. ولوحظت نتائج مماثلة كذلك في خلايا عضلة القلب في المجموعة الضابطة)المجموعة الثانية(للفئران التي أعطيت زيت زيت شوك الجمل فقط. وعلى الجانب الآخر، فإن شدة ظهور الديسمن تؤد تضاءلت وضعفت الأقراص البينية وخطوط Z لمجموعتي الفئران الثالثة والرابعة ، حيث ظهر الديسمن منخفضًا انخفاضا واضحا في المجموعة الثالثة ، و يكاد يكون معدومًا وذلك بسبب اختفاء الأقراص البينية وخطوط Z ف المجموعة الرابعة ، بينما أظهرت مجموعتي الفئران المريضتين والمعالجتين)الخامسة والسادسة(بزيت شوك الجمل زيادة ملحوظة واستعادة ظهور الديسمن تقويا في عضلة القلب للشكل الطبيعي تقريبًا.

نستنتج من هذه الدراسة أن زيت شوك الجمل ربما له دورًا منيدًا ونفعا في تحسين الأضرار والخلل الملحق بعضلة القلب ، لذلك توصى النتائج بمزيد من الدراسة والبحث عن إمكانية تناول زيت نبات شوك الجمل لتحسين بنية ووظائف القلب.