Efficacy and Bioavailability of Silymarin on Plasma S100B Level in Cardiotoxicity-induced Rats

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Cardiotoxicity is a cause of death by drug overdose. Silymarin, a cytoprotective agent used in this research to protect against induced-cardiotoxic effects in Albino Wister rats; exhibited signs of heart damage, such as elevated levels of S100B, troponin I, and CK-MB. Seventy albino Wister rats of both genders were divided randomly with each group having 10 rats. Silymarin-treated, cardiotoxic-induced, and control groups were treated for ten days. The bioavailability of silymarin was assessed, and evaluation of the efficacy of silymarin on the biomarker S100B and cardiac biomarkers (Troponin I, and CK-MB), also the histopathological assessments of the heart, liver, and kidney, in addition to the coefficient correlation of the studied biomarkers were analyzed. Research's outcome indicated that the sets subjected to silymarin presented substantial differences in rat weight and food consumption, compared to a decrease in cardiotoxic clusters. S100B plasma level was increased in cardiotoxic groups, reduced in those subjected to silymarin, and eliminated by pretreatment with silymarin. Troponin I & CK-MB expressively elevated significantly in cardiotoxic prompted rats, which declined with silymarin treatment and were prohibited in pretreatment by silymarin. The shielding characteristic of silymarin detected in end organs, like the liver, kidneys, and hearts when exposed to the cardiotoxic agent clozapine was extremely significant. The consequences of the histopathological examination of this study illustrated silymarin's cardioprotective effects. A significant positive coefficient correlation of S100B with troponin I & CK-MB was recorded. In conclusion, silymarin reduces and prevents to a larger extent the cardiotoxicity brought about by clozapine and averts heart injury. The cardioprotective efficacy of silymarin is explained by its new mechanism of action as decreasing S100B, troponin I & CK-MB levels with a strong significant correlation to each other. The cardioprotective efficacy of silymarin gives promise for monitoring the cardiotoxicity adverse drug reaction induced by drugs.

Keywords: Cardiotoxicity; Cardiac function; S100B protein; Silymarin.

The concept of direct cardiac toxicity refers to the structural and functional changes that can occur in the cardiovascular system as a result of exposure to medicines[1]. This phenomenon can be affected by drugs such as cancer drugs, antibiotics, and antipsychotics. Most drugs that are used for treating various diseases have cardiotoxicity side effects[2]. A cardiac adverse drug reaction (ADR) is a broad category of effects that can include myocardial infarction, heart failure, thrombosis, pericarditis, and arrhythmias. The underlying mechanisms are believed to include the disturbance of ionic processes, the induction of cellular damage by mitochondrial dysfunction, and hypercoagulability[1]. The main mechanisms that cause this are hypoxia, oxidative anxiety, and the production of free radicals[3].

Clozapine is an atypical antipsychotic indicated medicinally for treatment-resistant schizophrenia. The pharmacological activities of clozapine are binding to dopamine D2 and D4 receptors, and anti-á-adrenergic, anti-muscarinic, anti-histaminic, and anti-serotoninergic effects[4]. It is known to increase the mortality rate due to heart inflammation. The presence of these conditions can obscure the supervision of patients, which can affect the clinical results[5, 6]. Clozapine is associated with the risk of myocarditis and cardiomyopathy[7]. Detecting cardiotoxicity in people administering clozapine who are at considerable risk can be done by measuring the levels of clozapine-N-oxide formation, and N-oxidation relative to N-desmethylation ratios during treatment by clozapine[8].

Milk thistle is native to the Compositae family and is regarded as a type of liver disease treatment[9]. It is carefully studied for its ability to aid in treating liver illness[10]. According to Theophrastus, the plant has been used as a universal herb since the fourth century BC. The dried seeds of the plant contain various nutrients, such as flavonoids[11].

Silymarin (SM) is a compound that contains four flavonolignans: silydianin, isosilychristin, silybin, and taxifolin[12]. It proves its cardiac protective effects during the coronary artery bypass graft (CABG) operation, due to its anti-inflammatory and antioxidant effects[13]. When exposed to acrolein-induced cardiotoxicity, the compound's protective mechanisms were confirmed in a mouse model[14].

The integrity of the Ca^{2+} channel is maintained by the presence of various proteins, such as those belonging to the Calmodulin superfamily[15]. The S100B protein is mainly concentrated in the astrocytes. It binds to calcium and is a vital component of the nervous system. The level of S100B protein in biological fluids can be regarded as a reliable indicator of distress in the nervous system. Recent studies have shown that the S100B protein is a damage-associated molecular pattern molecule.

The high concentration of the S100B protein can trigger tissue reactions that are related to damage[16]. The presence of S100B protein has been shown to play a role in various cellular processes. These include the regulation of cell differentiation, protein phosphorylation, and Ca^{2+} homeostasis. The large concentration of this protein in cells allows it to interact with other targeted proteins[17, 18].

An assessment of the following was conducted for the first time to evaluate the impacts of silymarin efficacy as cardioprotective on S100B and cardiac function biomarkers levels in the induced-cardiotoxic model by clozapine in rats.

a. The efficacy of different doses of silymarin as protection against induced cardiotoxicity by evaluation of the biomarker S100B and cardiac function biomarkers (troponin I, CK-MB).

b. The Bioavailability of silymarin.

c. Examination of minute histopathology of body organs tissues such as heart, liver, and kidney.

d. The correlation of the biomarkers S100B, troponin I, and CK-MB levels amongst themselves to cardiotoxicity.

MATERIALS AND METHODS

Materials

The chemical/ reagents used were rats, S100B, Elisa kit (AZ chemicals from Germany), CK-MB (Casabiotech from The USA), Troponin (Life Diagnostics from The USA), Dimethyl sulfoxide (Laboratory Rasayan from India), Ethanol absolute, Formaldehyde 37 – 40 %, Methanol, Phosphate buffer, acetonitrile, and HCL (AZ chemicals from Germany), Sodium chloride 0.9% w/v (KRxS from Germany), silymarin powder, Silybin, naringenin (internal standard, IS), Clozapine (Sigma–Aldrich, USA). Reference standards; Chroma Dex (Santa Ana, CA).

Methods

Rat and housing

The experiment used Seventy Albino rats, both male and female with weights of between 150-400 grams and an average age of 12-16 weeks. They were taken care of in ideal laboratory settings in the university's faculty of pharmacy, Isra University. In quadruple, the rats were accommodated in cages where they could last at least one week before the start of the study. They were luminated at 12 hours intervals and the temperatures were maintained plus or minus 25 degrees and a humidity of between 10 and 50 percent. Rodent chow and tapped water were also available. The research was approved by an

institutional Committee for Ethics in Animal Use from the University Faculty of Pharmacy (Protocol n. 001.11.2018) and was done according to the general ethical guidelines by N.I.H Publications No. 85-23, revised 1985).

Experimental design /study groups

A total of 70 albino Wister rats of both genders were divided randomly with each group having 10 rats. 3 silymarin doses were administered in a solution of 1 ml IP and DMSO (Dimethyl sulfoxide) as a solvent to groups 1 to 3. Groups 4-5 were cardiotoxic-induced and treated, while group 6 served as cardiotoxic-induced and 7 as negative control. The following is their randomization chat (randomized statistically by Microsoft Excel) in doses of milligrams per kilogram per day, for ten days.

(G1): 80 of silymarin.

(G2): 140 of silymarin.

(G3): 200 silymarin.

(G4): 25 Clozapine I.P. was injected in ten days and trailed by 140 silymarin (10 days).

(G5): 140 of silymarin (10 days), and then received 25 Clozapine I.P. (10 days).

(G6): 25 Clozapine I.P.

(G7): 0.5 ml of normal saline (control group).

Dosage and Trial Preparation

The solution of SM was prepared by reconstitution of SM powder with DMSO daily under sterile conditions before injection. The rats were anesthetized with ether (diethyl ether) solution (20 ml of ether on cotton in a jar) which required to render them unconscious, approximately 5 min. (3ml) the blood sample was withdrawn from the retro-orbital plexus by use of a capillary tube and transferred to an EDTA tube. Centrifugation of blood was instantly done for 10 minutes (1600RPM). Separation of plasma from the blood was done using the pipette to an Eppendorf covered by parafilm maintained at -20 degrees. The weights of the subjects were figured out by pre- and pro-research exercises. Every group except for controls had SM solution administered IP. The controls were injected with normal saline for the negative and clozapine for the positive control and left for ten days while receiving treatment dosages as per the schedule. On the tenth day, they were anesthetized using an ether solution. The process of withdrawing blood and centrifuging at 1600RPM for ten minutes was repeated and the separation and storage were

at -20 degrees. A high concentration of ether was used to sacrifice the rats and their organs were removed by dissection and dried using filter paper. The organs and tissues were then stored in a 10% buffered formalin solution for histopathological sectioning studies. Rats in cardiotoxic clusters; blood samples were collected, and plasma was prepared. The rats were then sacrificed, and hearts were excised immediately, weighed, and prepared for histopathology analysis.

Bioavailability of silymarin

SM contents were ascertained by use of an HPLC-system (LC-2010A HT) with an Agilent Eclipse XDB-C18- column; (5 mm, 4.6_250 mm). The mobile phase; is methanol & pure water (46:54, v/v); at a flow rate of 0.8 ml per minute. Monitoring of effluent was conducted at 288 nm. Standardized silymarin, naringenin [the internal standard (IS) for quantification], Silybin concentrations in plasma samples were analyzed. Samples were added to IS (naringenin, 20 ng/mL in acetonitrile). It was then vigorously mixed for around ten minutes. Centrifugation was then performed at a rate of 16,000 x g for around five minutes. Finally, 5 ¼L of supernatant aliquots were directly injected into an LC- system for analysis. The pharmacokinetic parameters: such as maximum-plasma-concentration (C_{max}), time of C_{max} (T_{max}), and area under plasma-concentration-time curve (AUC_{0-t}) were calculated.

Evaluation of cardiac biomarkers

Assessment of the biomarkers; S100B, Troponin I, and CK-MB from the plasma was done as per the manufacturer's instructions of Enzyme linked Immune Sorbent Assay.

Rat S100B assay

The quantitative sandwich immunoassay technique was utilized for the analysis. An antibody specific to S100B was pre-coated on a microplate. A pipette was used to place samples and standards into the wells, and an immobilized antibody was then bound to any S-100B protein. A biotin-conjugated antibody for S100B was then added to the wells after unbound substances were removed. Following a wash, a substrate solution was then added to the wells.

The intensity of the dye was measured after the development of the substance stopped. The concentration of S100B that could be detected was typically less than 0.78. The sensitivity of this

test, which was determined as the Lower Limit of Detection (LLD) could be differentiated. The value of 20 replicates of zero-standard added using three standard deviations was then determined.

Troponin I assay

The procedure utilized two antibodies that can recognize Troponin's epitope, which is characterized by a protease-resistant nature. One

of these was immobilized on a microtiter well. The other was conjugated to horseradish peroxidase (HRP), and it was used for detection. Diluted and standard samples were then placed into the wells for one hour using an HRP conjugate. The Troponin molecules were then sandwiched between the detection and immobilization antibodies. Tetramethylbenzidine (TMB) was added to the
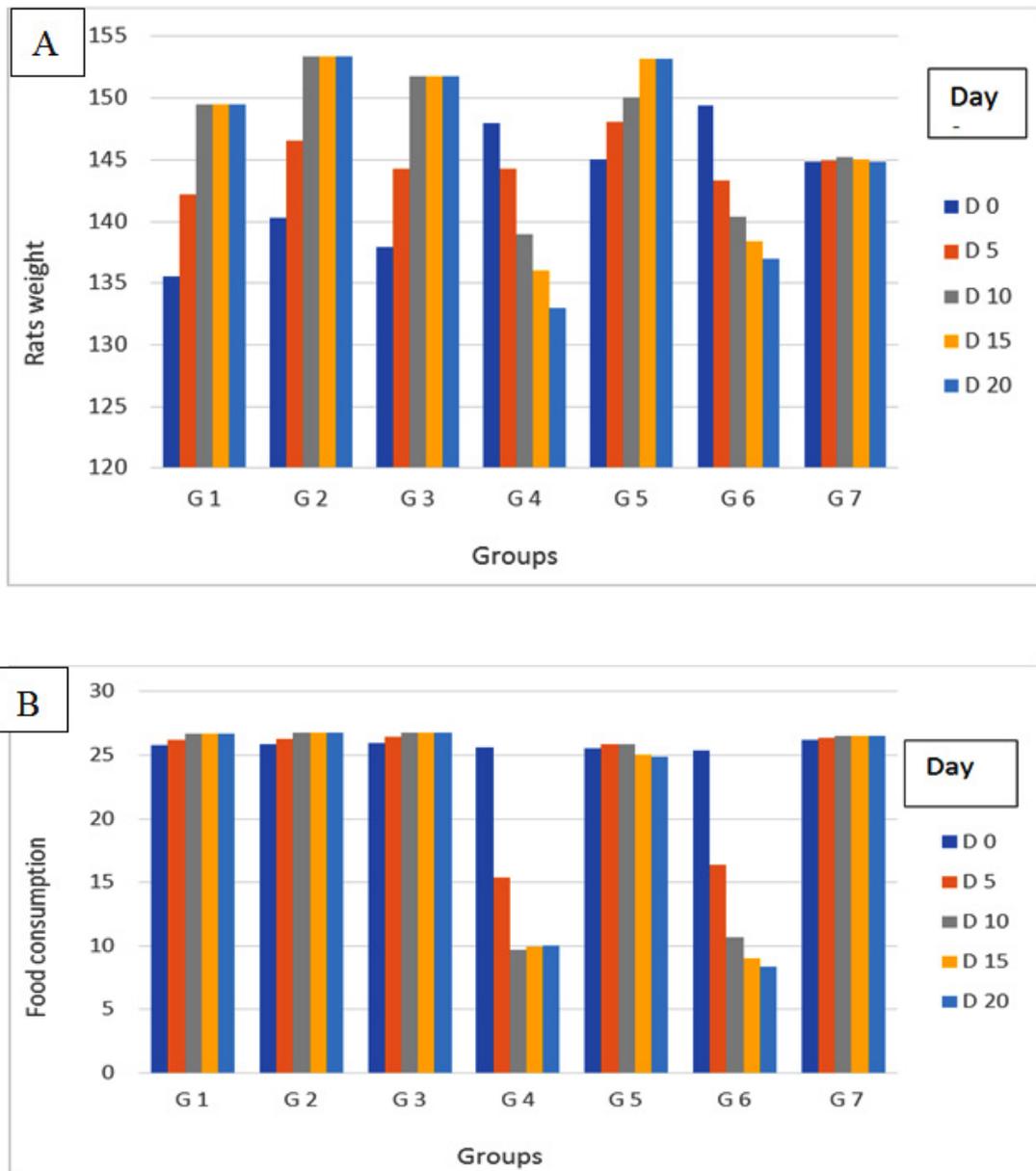

Fig. 1. The average rat's weight [A], and the food consumption in all studied groups during the study period [B]

wells for around 20 minutes. A blue-colored Troponin substance was then present, and its color was stopped by adding a stop solution. The absorbance at 450 nm was then measured. The Troponin concentrations were then calculated.

CK-MB assay

The activity of the CK-MB enzyme was estimated using a method that utilizes a quantitative sandwich immunoassay. This method involves coating an antibody specific to CK-MB on a microplate. A set of standards and samples was then placed into the wells. An immobilized antibody was then added to bind any CK-MB that was presented.

A biotin-conjugated anti-CK-MB antibody was added to the well after unbound substances were taken out. Following that, HRP was added to remove any avidin-enzyme reagent or substrate. As indicated earlier, the solution was added to wells, and the resulting color was proportional to the CK-MB's initial bounded quantity. The absorbance was then measured at 450 nm, and the intensity of the dye, and the concentration of CK-MB were calculated.

Histopathology assessments

The organs collected were immersed in 10% formalin and a clearing agent, Xylene, was presented to penetrate the tissues. Lastly, paraffin was introduced to end the process of embedding to create a block of paraffin. Sectioning of blocks then occurred via a microtome into 4-5 μm thick sections before hematoxylin staining for further scrutiny. Each ventricle slice was fixed in a 10% formalin solution before embedding in paraffin. The specimen was assessed for normal histopathological structures connected with a myocyte cardiotoxic agent. The ventricle specimens were evaluated for typical histopathological features associated with clozapine-induced cardiotoxicity including inflammation, myocyte vacuolar degradation, necrosis of myofibers, and interstitial fibrosis).

Statistics

SPSS version 24 was run to analyze the data. G*Power 3.1.9.4. software was used for Effect Size and Sample size calculations. Means and standard deviations were the main determinants in quantitatively explaining the validity. Statistical comparison between the means of two groups (i.e., means of two diverse groups or comparing means of one sample on two separate occasions) and autonomous samples was made by using the t-test. It determined the average values between the 2 groups. The change of means was ascertained using variance analysis. Pearson correlation coefficient helped in assessing the strength and the association between two numerical variables.
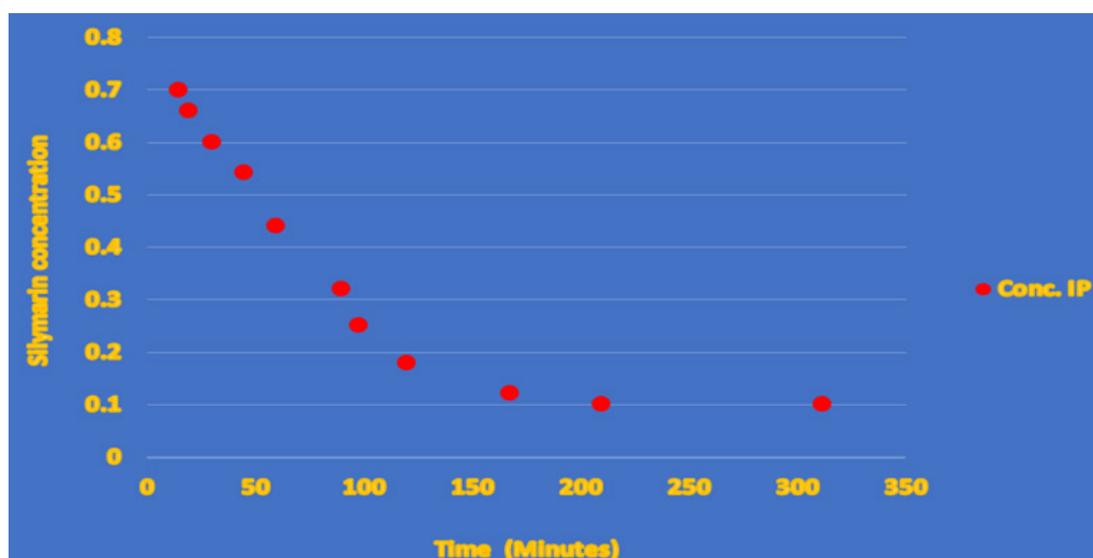
Fig. 2. Plasma-concentration-time profile of SM levels following administration, relative bioavailability, and pharmacokinetic parameters of silymarin concentration were 58.6±2.9 in the G1 (80 mg/kg), 74.9±2.1 in G2 (140 mg/kg) and 81.6±1.4 in the G3 (200 mg/kg). Cmax was 0.63±0.5 ì/ml, the time of Cmax (Tmax) was 1.50±0.4 hours, and the area under the plasma concentration-time curve (AUC0-t) was 2.12±0.53
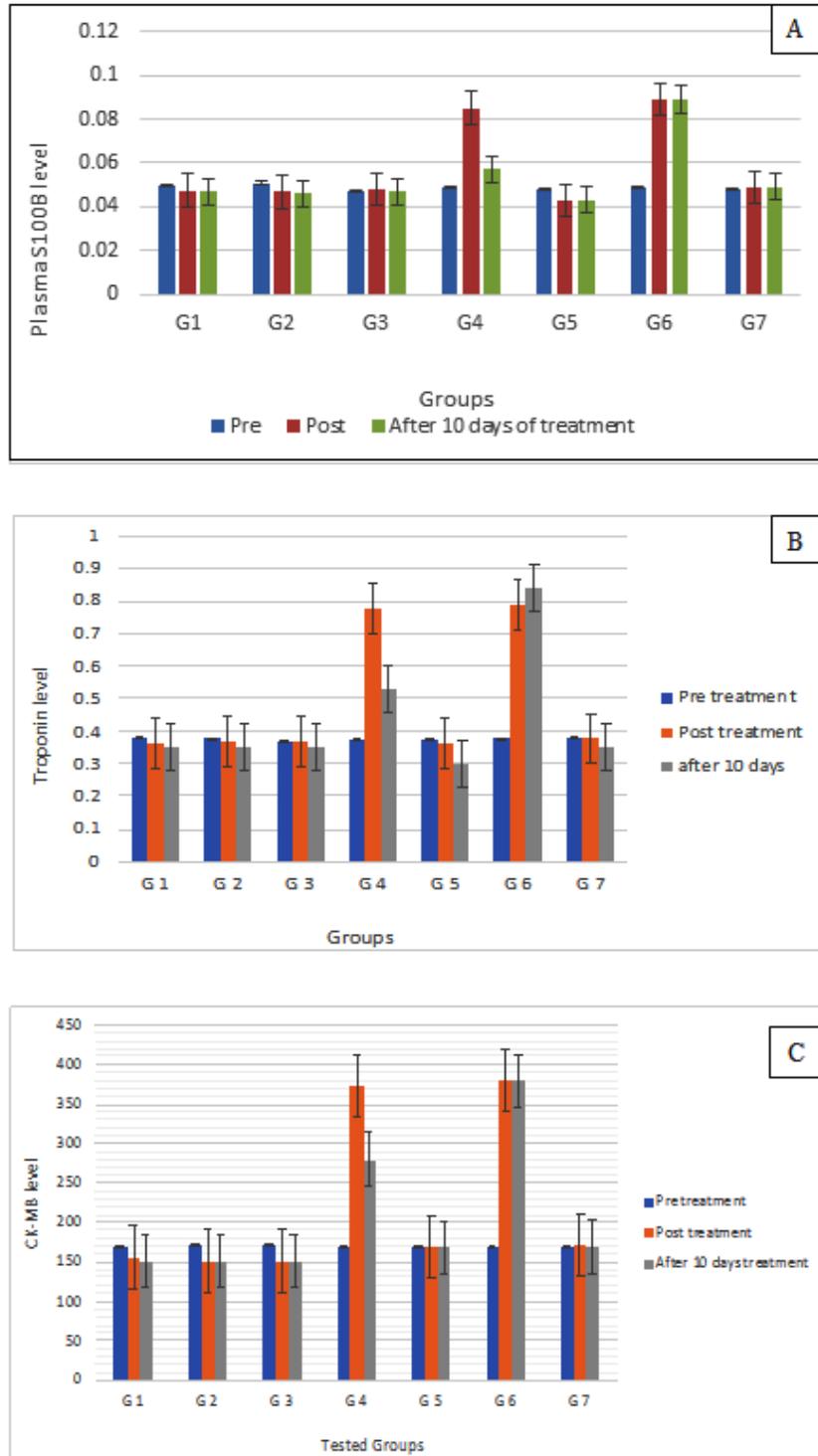
Fig. 3. Plasma cardiac biomarkers; S100 (A), Troponin I (B), and CK-MB (C) levels pre- and post-treatment in all tested groups

Study outcomes

Seventy domestic Wister albino rats of varying ages 12-16 weeks, with weights of between 150-400 grams were utilized. Both genders were involved in a ratio of 9: 5.

The efficacy of different doses of silymarin as cardioprotection for cardiotoxicity-induced rats

**Silymarin efficacy on the rats' weight and food consumption**

Figure 1A shows that in all the groups the weight of the rats as measured daily increased after the tenth day except for the cluster in groups 4 and 6, but this was determined to be insignificant statistically. The daily average food intake for the groups was slightly increased after treatment with
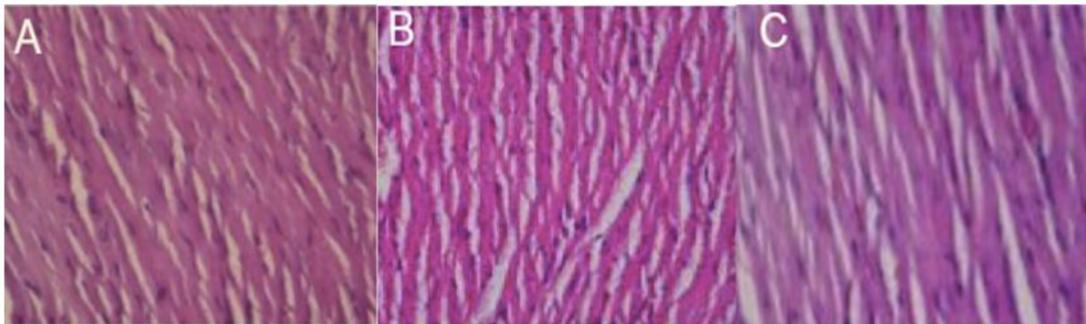
Fig. 4. Sections showed normal myocardium architecture of silymarin-treated groups; 1 (A), 2 (B), and 3 (C), Hematoxylin, and eosin x400
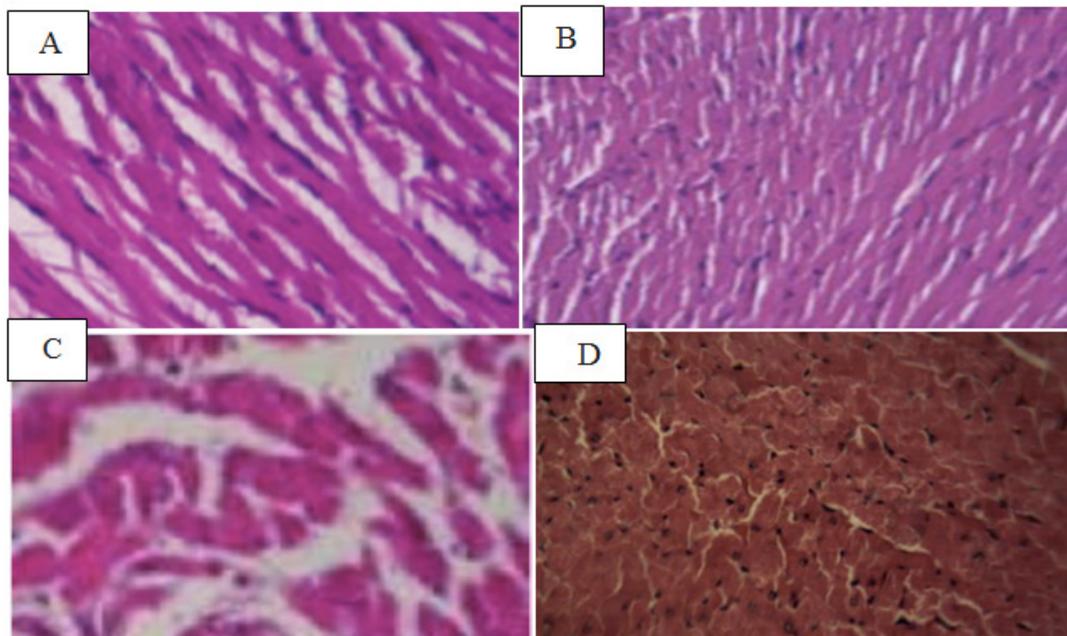
Fig. 5. A: Heart tissues of group 4 treated with clozapine then by silymarin, showed less inflammation compared to group 6. Hematoxylin and eosin x400. B: Heart tissues of group 5, which were treated with silymarin first and then treated with clozapine, the section shows normal myocardium architecture. Hematoxylin and eosin x400. C: Heart tissues of clozapine-induced cardiotoxicity group 6; there is interstitial edema and moderate interstitial inflammation, showed mixed inflammation at the periphery of the myocardium and in papillary muscle with focal necrosis. Hematoxylin and eosin x400. D: Heart tissues of normal saline control rat group 7; show a normal histological pattern. Hematoxylin and eosin x400.
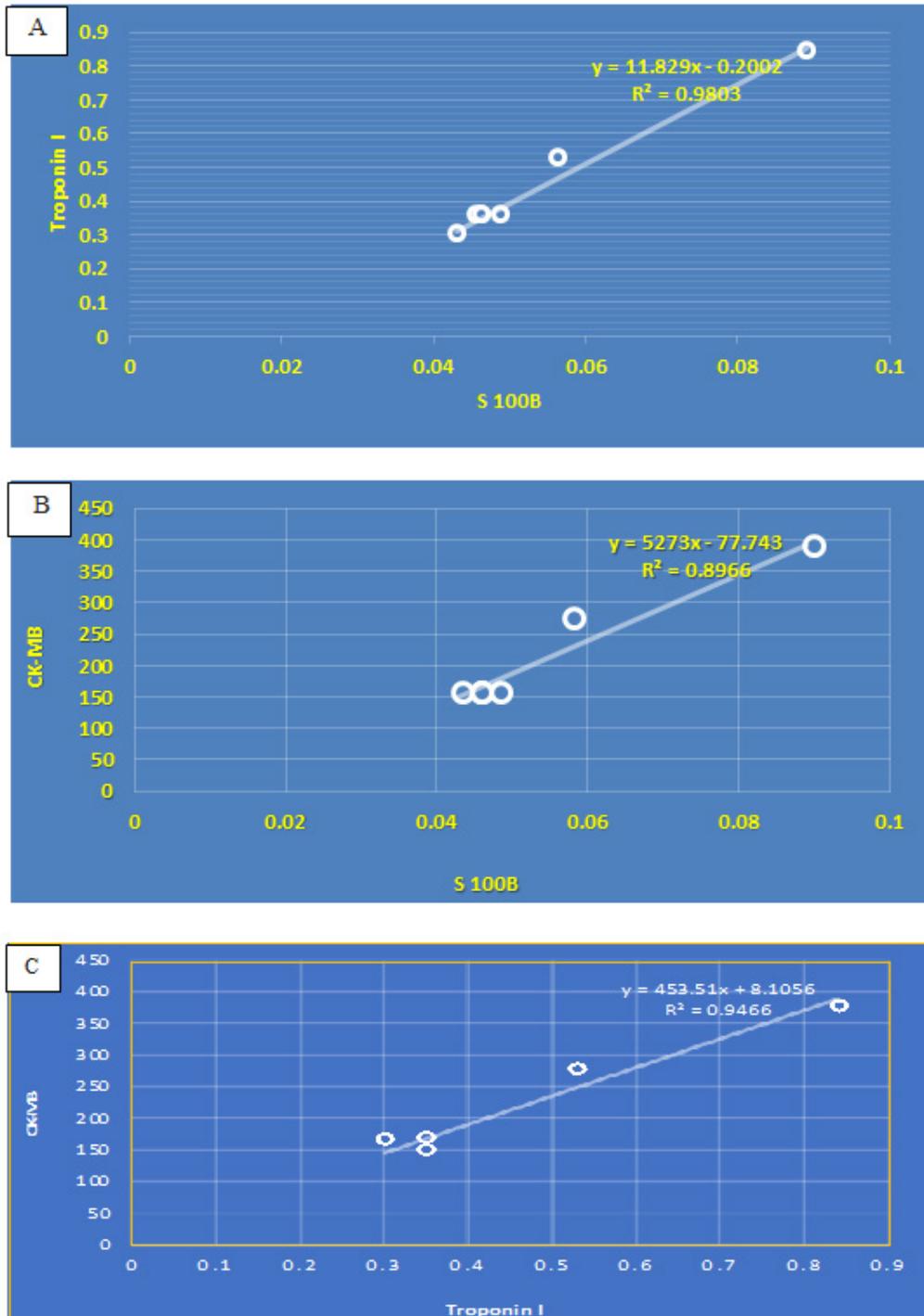
Fig. 6. A: The correlation study between plasma S100B concentrations and Troponin I level, a significantly very strong positive correlation of plasma S100B & Troponin I in groups 4, 5, and 6. B: The correlation study between plasma S100B concentrations and CK-MB levels, a very strong positive correlation of plasma S100B conc. & CK-MB in groups 4, 5, and 6. C: The correlation study between plasma Troponin I concentrations and CK-MB levels showed a strong positive correlation between Troponin I & CK-MB in groups 4, 5, and 6

silymarin, while groups 4, and 6 had less food intake, but were statistically insignificant, as shown in Figure 1B.

The Bioavailability of silymarin

It was figured out by measurements of plasma C_{max} and T_{max} from the groups treated with different doses of silymarin. Silybinin (isomeric-compound), was revealed as two-peaks noticed at about 19 & 21 min. The standard curves were linear (r=0.987). The mean silybinin conc. was 40.82±2.4, and silymarin was 68.67±1.7. The silymarin concentration was 58.6±2.9 in the first cluster (1) treated with 80 mg/kg, 74.9±2.2 in cluster (2); treated with 140 mg/kg silymarin, and 81.6±1.4 clusters (3); treated by 200 mg/kg silymarin. The highest concentration of plasma (C_{max}) was 0.63±0.5ì/ml, and the time of C_{max} (T_{max}) was 1.50±0.4 hours. and $AUC_{(0-t)}$ was 2.12±0.53 for 140 mg/kg of silymarin, figure 2.

The Efficacy of silymarin on cardiac biomarkers S100B

Compared to the other groups**,** plasma S100B level in groups 4, and 6 was notably extremely significant numerically. Prior treatment with SM in the induced-cardiotoxic groups had different results; at a dosage of 140 mg per kg, writing down a significant reduction of S100B level compared to cardiotoxic cluster 6 and control, with 95% CI (p = 0.002), as illustrated in figure 3 (A).

Cardiac Troponin

Plasma troponin I was highly significantly increased with the cardiotoxic-induced group 6 (p=0.0001) and a highly significant increase in group 4 after induction of cardiotoxicity, then a significant decrease after treatment with silymarin, while in silymarin-treated groups was decreased with the dose 140 mg per kg; p< 0.001, no significant difference in group 5 compared with the positive-control, while negative-control; showed highly significant difference compared to cardiotoxic groups as showed in figure 3 (B).

Cardiac CK-MB

Assessment of plasma CK-MB illustrates significant differences in their levels among the cardiotoxic groups 4, 5, and 6 [p=0.007]. Plasma CK-MB was highly significantly increased in group 6 [p=0.0001] and a highly significant increase in group 4 after induction of cardiotoxicity, then a significant decrease after treatment with silymarin, while in silymarin treated groups was decreased

with the 140 mg/kg dose (p < 0.001), comparison to cardiotoxic groups showed highly significant difference, while comparing to negative control showed no significant difference Figure 3 (C).

Microscopically appearance and histopathology study

The microscopically histopathological section of all groups showed the tissue architecture. The heart-to-body weight was measured for all groups [H/B wt. ratio (g/Kg) x10^{-3}] for the groups 1-7 as follows; 2.88 ±0.18, 2.86±0.1, 2.87±0.11, 3.3±0.2, 2.95±0.05, 3.97±0.11, 2.90±0.21, respectively.

Histopathology of the heart

Histopathological studies of heart sections of the studied groups; silymarin-treated groups (G1, 2, and 3) showed normal cardiac sections, figure 4 (A, B, and C).

Group 4 treated first with clozapine and then silymarin showed less inflammation than the clozapine-induced cardiotoxicity group (6). At the same time, the group (5), which was treated with silymarin first and then clozapine showed no cardiotoxicity, no cellular infiltration, or inflammation when compared to rats whose cardiotoxicity induction was done by clozapine (G 6); Inflammation abrasions were noted in both heart ventricles for the rats for which induction was done, there were signs of myocardial inflations of the cells, and myocarditis in the heart section. 5 (A-D).

Histopathology of the Rat's Liver and Kidney

Histopathological sections of the rat's liver for the tested groups showed no morphological changes, and normal architecture, the portal tract showed no fibrosis and no inflammation, and parenchyma showed no inflammation, no necrosis or apoptosis, and no bile stasis.

The microscopic features of the rat's kidney for all groups showed normal architecture, the sections showed normal glomerulus, normal tubular, and no inflammation, or necrosis. The interstitium is normal, with no fibrosis, or inflammation, and a normal vessel.

Correlation study

The correlation study of plasma biomarker S100B levels to the studied parameters was estimated and analyzed according to their correlation coefficient with significance, and the correlation of Troponin I to CK-MB. It showed

that there is a significantly very strong positive-correlation of plasma S100B & Troponin I in groups 4, 5, and 6, with correlation-coefficient {r = 0.990, *p* < 0.001}, as showed in figure 6 (A), while the correlation results showed that there was a very strong positive-correlation of plasma S100B conc. & CK-MB in groups 4, 5, and 6, the correlation-coefficient {r = 0.981, *p* < 0.001}, as shown in figure 6 (B). also, the correlation results showed that there is an extraordinarily strong positive-correlation of plasma Troponin I conc. & CK-MB in groups 4, 5, and 6, the correlation-coefficient {r = 0.984, *p* < 0.001}, as shown in figure 6 (C).

DISCUSSION

Evaluation of the contribution of cardiotoxicity induced by clozapine to cardioprotection by SM has been conducted for the first time in this study. Polypharmacy methods are to blame for the increased cardiac and cardiovascular problems. Drugs administered together with therapy worsen cardiac complications. Silymarin has a wide range of pharmacological effects including antioxidant activity[19], anti-cancer effects against several human carcinoma cell lines[20, 21], and stimulation of protein-synthesis, cell-regeneration from toxic liver damage, liver cirrhosis, and chronic inflammatory liver diseases[22, 23], anti-inflammatory, immune-modulation effects, and neuroprotective[24], and cardioprotection[13, 25].

The main components of silymarin are silybin A and B accounting for 60–70% [26], the silymarin bioavailability in the silymarin-treated groups showed that Silybin, which is an isomeric compound, appeared as two peaks were detected at about 19, and 21 min., the standard curves were linear, silymarin concentration was high compared to silibinin with significance outcome. With a rise in dosage, the concentration of silymarin corresponded with the maximum concentration of plasma C_{max} as well as the corresponding time T_{max} and the area under the concentration of plasma curve AUC having compatibility with earlier studies.

Protein S100B is a calcium-tying protein in the semicircular lining of human heart cells[27, 28], Continuous rise in the level of the protein characterizes its spread to the damaged tissues which clinically reflects an injury like hypoxia[29],

trauma[30-32]. Notably, protein serum level is top in numerous, neurologic, and circumstances[33-35].

Focus on cardioprotective impacts of silymarin as a priority of the research being the first one having been undertaken with its relation to the plasma level of S100B protein. The plasma level was high in the rats induced with cardiotoxicity and the positive controls when a comparison was made with the other clusters of rats. The level of plasma was seen to be reduced when subjects were predisposed to silymarin in comparison to rats injected with cardiotoxic negative and positive controls at 95% CI, P equals 0.002).

Explanations are given in terms of research and clinical perspective for S100B, splicing operation of the artery side[36], endarterectomy of the carotid[37], and ischemia of myocardial[38]. The cardioprotection ability of silymarin was well demonstrated in this research where groups that were prior treated with it indicated reduced plasma levels. Serum S100B protein concentrations elevated in a key depression and following acute or chronic injection of antidepressants[33, 39, 40], Inducing cardiotoxicity with clozapine has the level of serum increase while there was notably no observed elevation of the level of plasma in the clusters of rats studied.

Surgery pretreatment shields heart tissues from reperfusion damage, inflammation, and antioxidants when splicing coronary arteries in humans, Prior treatment of CK-MB and troponin I notably reduced silymarin[41]. In group 4 Troponin I was observed to elevate after inducing the rats with clozapine cardiotoxic but on silymarin treatment the level significantly decreased. while silymarin pretreated (group 5) showed a substantial variance explaining the cardioprotection nature of silymarin against the cardiotoxicity induction by clozapine, this study concurs with the one previously studied by Altaei (2012)[13, 41].

The myocardium comprises numerous marker enzymes such as troponin and CK-MB, and once metabolically spoiled; it disposes of its contents into the extracellular fluid. The original increase in cardiac troponins after myocardial infarction happens concurrently with CK-MB, but it endures for longer than other enzymes[42]. Amplified actions of troponin, CK-MB could be noted in the plasma of clozapine-induced cardiotoxicity rats during the study. Prior injection of silymarin

expressively dropped the release of biomarker enzymes. Assessment of plasma CK-MB displayed a substantial difference in levels of the cardiotoxic induced clozapine in clusters 4 and 5; A notably high rise at the positive cardiotoxic control cluster 6 while a high noteworthy raise in cluster 4 after inducing cardiotoxicity. After silymarin treatment, there was a substantial decline, while the previously treated cluster declined significantly in group 5. This concurs with earlier studies by Altaei, (2013)[13, 41]. CK-MB, and troponin I biomarkers levels increased, signifying an exhibition of myocardial necrosis—cellular injury with loss of purposeful integrity or cell membrane permeability[43]. High plasma CK-MB levels submit the existence of an injury inside the heart or necrosis when the comparison is done to the plasma troponin & CK-MB points in other clusters with the control clusters writing down a heart injury because of clozapine injected cardiotoxicity and demolishing cells. its deterioration in silymarin-treated clusters demonstrates silymarin cardioprotective. An increase in CK MB followed by an increase in the wet weight of the heart confirms the existence of cardiac hypertrophy and edema. The cardioprotective effects produced from the prior silymarin treatment are associated with the significance of reduced actions of troponin and CK-MB.

Researchers defined potentially deadly myocarditis, pericarditis, and eventual death as associates of clozapine injection[44]. This research concurs with the observation. scratches were found in both the left and right ventricles in comparison with the control subjects. The experiential examination of microscopical histopathology appeared normal in the end organ tissues building from silymarin-treated clusters. Heart-to-body weight was normal, while cardiotoxic-induced rats by clozapine showed heart injury and inflammation. The heart sections of the clozapine-induced cardiotoxicity (group 4) that were treated first by clozapine and then by silymarin showed less inflammation than the clozapine-induced cardiotoxicity group six (positive control); minimal myocardial damage which was characterized by mild interstitial edema and focal degeneration and necrosis of myofibers, while the pretreated silymarin group (5), which treated by silymarin first then treated by clozapine showed no cardiotoxicity.

Clozapine administration is known to produce free radicals via its quinine metabolites, which react with oxygen resulting in the enhanced production of reactive oxygen species (ROS). This ROS, the highly toxic by-products of aerobic metabolism are known to respond to cell membranes and macromolecules which enhance the creation of lipid peroxides, leading to tissue damage. Lipid peroxidation is an essential pathogenic issue in the necrosis of myocardium while the buildup of lipid hydroperoxides shows cardiac tissue injury [45-47]. Silymarin injection could lower lipid peroxide levels [25]. The prevention of damage to antioxidant balance in the regulation of inflammatory mediators is done by silymarin[26]. Inhibition of cellular penetration in prior silymarin-treated clusters and its purpose leading to in condensed production of responsive oxygen species, during ischemia, contributing to the cardioprotective nature of silymarin when subjected to myocardial ischemia and oxidative pressure by potential antioxidant action because of its capability to counteract the creation of free radicals by a high content of flavonoids.

Assessment of histopathological constraints in end organs like the kidneys wrote down an ordinary architecture, with no parameters of inflammation or destruction in all studied groups of rats. The results can explain silymarin hepato- and reno-defensive activities when subjected to clozapine. Silymarin has been hailed by researchers due to its capability to protect the liver from hepatoxic drugs and further occurrence of liver cancer[48]. This study agrees with that in its action as a hepatoprotective agent. The histopathology analysis of rat liver in cardiotoxicity-induced groups by clozapine showed normal architecture, and no inflammation or necrosis.

Koçarslan A. (2016)[49] revealed an intraperitoneal silymarin injection decreases the chances of oxidative anxiety thereby protecting end organs like the liver from severe supraceliac abdominal reperfusion damage in the subjects.

Significance was noted as a strongly optimistic association in plasma S100B & Troponin I, CK-MB concentrations levels from clusters 4-6. Again, an examination of the correlation coefficient for plasma Troponin I and CK-MB points exhibited a solid confident link. This indicates that there is a correlation among the three biomarkers and may be

effective in diagnosing heart damage or necrosis.

The distinct types of protein sensors that are involved in membrane repair are likely to coordinate their responses to diverse types of injuries. Defects in the repair genes are known to contribute to various forms of heart disease and muscle disease. The plasma membrane is a vital component of maintaining a healthy cellular environment and ensuring that cells survive. The influx of Ca^{2+} following membrane rupture is a signal that initiates the repair process. Multiple mechanisms have been identified that involve the recruitment of sensor proteins that are dependent on Calcium[50].

The loss of S100A1 in the cell environment can result in the dysfunction of the Ca^{2+}-controlled networks. This can lead to the failure of cardiomyocytes and endothelial cells. In addition, the lack of this receptor in ischemic myocardium can affect the cardiac fibroblasts' function[51], the present study agrees with that.

Acute inflammation during the initial stages of wound healing generates factors that are essential for tissue repair, but a prolonged inflammatory phase may lead to cell destruction and a changed constitution of the extracellular matrix with subsequent failure of epithelialization[52]. Pretreatment with silymarin normalized the clozapine-induced cardiotoxicity and the elevation of plasma levels of the diagnostic biomarker, suggesting that silymarin could maintain the membrane integrity, restricting the leakage of these biomarkers.

Silymarin could stabilize the myocardial membrane and associated enzymes which are connected to properties of anti-oxidant and anti-inflammatory. The authors agree with the evidence in the research that elucidates the instrument of silymarin antioxidant which is aligning its cardioprotective ability. Deterrence of cardiotoxicity by silymarin and shielding of myocardial necrosis can be described by antioxidant and anti-inflammatory significance impacts on S100B, troponin, and CK-MB levels.

CONCLUSION

The efficacy of silymarin presented cardioprotection for cardiotoxicity-induced rats by clozapine was proved for the first time in this research. A substantial alteration was observed in the plasma biomarkers S100B, troponin I and CK-MB exhibited a significant difference as compared to the baseline and controls. Also were proven to be supportive indicators in clozapine-induced cardiotoxicity accomplishment or myocardial wounds. The relationship of plasma S100B to cardiac biomarkers disclosed a significant very strong positive correlation. The cardioprotective efficacy of silymarin gives promise for preventing the cardiotoxicity adverse drug reaction and cardiac irreversible damage induced by drugs.

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