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Research Article

Silymarin Ameliorates Acrylamide-Induced Hyperlipidemic Cardiomyopathy in Male Rats

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Acrylamide (AA) is a well-known potent carcinogen and neurotoxin that has been recently linked to atherosclerotic pathogenesis. The present study is aimed at investigating the protective effect of silymarin (SIL) as an antioxidant against AA-induced hyperlipidemic cardiomyopathy in male rats. The obtained results showed that animals exposed to AA exhibited a significant increase in the levels of cardiac serum markers, serum total cholesterol, triglycerides, low-density lipoprotein cholesterol, and very-low-density lipoprotein cholesterol with a significant decrease in high-density lipoprotein cholesterol. Furthermore, AA intoxication significantly increased the malondialdehyde level (a hallmark of lipid peroxidation) and reduced antioxidant enzyme activities (i.e., superoxide dismutase, catalase, and glutathione peroxidase). SIL administration significantly attenuated all these biochemical perturbations in AA-treated rats, except for the decreased high-density lipoprotein cholesterol. Our results were confirmed by histopathological assessment of the myocardium. In conclusion, this study demonstrated a beneficial effect of SIL therapy in the prevention of AA-induced cardiotoxicity by reversing the redox stress and dyslipidemia in experimental animals.

1. Introduction

Acrylamide (AA) is a small vinylic compound. It has the chemical structure CH_2=CO–NH_2. The primary use of AA is to produce polyacrylamides that are used in wastewater treatment, dye synthesis, gel chromatography, textile processing, electrophoresis, pharmaceutical industry, photography, tapes, and gels [1–3]. AA is also found in tobacco smoke and is primarily produced in fried, roasted, grilled, and baked foods at a temperature above 120°C [4, 5]. In the Maillard reaction, the NH_2 of asparagine and the carbonyl group of glucose react together to produce acrylamide [6]. Previous studies have shown that AA is absorbed in a very rapid and effective manner through the gastrointestinal system [7, 8]. AA can undergo cellular biotransformation by a cytochrome P450 enzyme (CYP2El) into a more potent epoxide derivative (glycidamide) that has more reactivity toward DNA and proteins than the AA itself [9]. AA is easily transported throughout the body because of its very small size and hydrophilicity [10]. Experimentally, the administration of AA may have diverse toxic effects, including carcinogenicity [11] and neurotoxicity [12], and the probability of human exposure to AA has increased worldwide. Thus, the toxicity of AA to animals and humans needs to be urgently studied to counteract or reduce these effects.

Heart disease is the leading cause of mortality and morbidity in the middle- and high-income countries [13]. There is strong evidence that high ingestion rates of AA elicit cardiac developmental disorders and may be an underlying risk factor for cardiogenesis in fetuses [14]. In regard to the cardiac

toxicity, prolonged exposure to AA could alter the properties of rat cardiomyocytes, including cell morphology, contraction patterns, and cell–cell communication [15]. It has been reported that AA can provoke oxidative stress through an abrogation of the antioxidant defense system, as well as by generating excessive reactive oxygen species (ROS) [16]. Indeed, oxidative stress may play a crucial role leading to cardiac and vascular abnormalities in different types of cardiovascular diseases (CVDs) [17]. Alternatively, AA can induce cardiac complications (e.g., pathogenesis) by altering lipid metabolism pathways and disrupting myocardial energy [18]. Furthermore, AA in the blood could modify lipoproteins via putative interactions and promote the exacerbation of the atherogenic process [19]. Earlier literature indicated that the increased production of ROS is a driving force in the development of atherosclerosis and hyperlipidemia [20]; therefore, we hypothesized that inhibiting oxidative stress may be an effective strategy for the treatment of AA-induced lipotoxic cardiomyopathy.

Medicinal plants and their active ingredients are known to produce beneficial therapeutic effects against drug-induced toxicity [21, 22]. Among these compounds, silymarin (SIL) is produced from leaves, seeds, and fruits of *Silybum marianum* (milk thistle), which belongs to the *Asteraceae* family [23]. SIL contains flavonolignans, such as taxifolin, silychristin, silydianin, silybin A, silybin B, isosilybin A, and isosilybin B [24]. SIL has multiple pharmacological properties, including antioxidant, hepatoprotectant, anti-inflammatory, antibacterial, antiallergic, antimutagenic, antiviral, antineoplastic, and antithrombotic activities and has vasodilatory actions [25, 26]. It was suggested that SIL dietary supplement could prevent free radical-related diseases as a natural antioxidant [27]. SIL received attention during recent years owing to its potential effect as a cardioprotective agent [28, 29]. SIL can modulate ischemic reperfusion-induced myocardial infarction in rats [30]. As previously reported, SIL shows a wide range of mechanisms in preventing the CVDs by increasing enzymatic antioxidant capacity, mitochondrial enzymes, and expression of Nrf2 and decreasing lipid peroxidation products, expressions of NOX4, low-density lipoprotein, total cholesterol, and triglyceride level in the blood, thus preventing cardiac dysfunction and dyslipidemia [31]. Therefore, the aim of the present study is to investigate (for the first time) the protective effect of SIL against AA-induced cardiotoxicity and distortion of lipidemic status in rats. In addition, the influence of SIL on myocardial oxidative injury was investigated. To our knowledge, no treatment is currently devised or known for AA cardiotoxicity.

2. Materials and Methods

2.1. Animals. The current study involved 24 adult male Wistar rats weighing approximately 180–200 g at 3 months of age. The rats were supplied by the Animal House of King Faisal University, KSA. All animals were housed in plastic cages (five rats/cage). Cages were kept under the same standard laboratory conditions of temperature (25°C), humidity (60%), and lighting (12 h light/12 h dark) for one week of acclimatization before the beginning of the experiment. The rats were fed with standard commercial rat chow and had free access to water *ad libitum* throughout the experiment.

2.2. Chemicals. Acrylamide (AA) and silymarin (SIL) were procured from Sigma Chemicals (St. Louis, MO, USA). All other chemicals and reagents used in the experiments were of analytical grade from commercial suppliers.

2.3. Experimental Design. The rats were randomly divided into four groups of five rats each and treated for 11 days as follows:

(i) Control group: rats received a daily intraperitoneal (i.p.) administration of saline.
(ii) AA group: rats received a daily i.p. dose of AA (50 mg/kg).
(iii) SIL group: rats received a daily i.p. dose of SIL (160 mg/kg).
(iv) AA+SIL group: rats received a daily i.p. dose of AA (50 mg/kg) and SIL (160 mg/kg).

Twenty-four hours after the last dose, animals were anesthetized with light ether and sacrified. The selected doses were based on the previous work of Mehri et al. [32]. All the animal experimental procedures in this study were performed according to the guidelines of the Research Ethics Committee at King Faisal University (approval reference no: KFU-REC/2019-03-02). No mortality occurred during the experimental period. Signs of AA toxicity included a change in activity and abnormal gait deficits. After laparotomy, blood was collected from trunk vessels, and then, the serum was separated and stored at −20°C until further biochemical estimations of total lactate dehydrogenase (LDH), total creatine phosphokinase (CPK), and blood lipid fractions. A portion of left ventricular tissue was excised immediately for histopathological investigation, and the remaining heart was preserved at −80°C until the biochemical analysis of lipid peroxides (measured as malondialdehyde, MDA) and enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

2.4. Detection of Oxidative Markers and Antioxidant Enzymes. The heart tissues were placed in ice-cold phosphate buffer (0.1 M, pH 7.4) and then homogenized (10% w/v) using a homogenizer machine. Next, the supernatant was obtained by centrifugation for 30 min (4°C) at 5000 rpm. Standard protocols were used to estimate myocardial MDA [33], SOD [34], CAT [35], and GPx [36]. The amount of protein in the sample was measured by the method of Lowry et al. [37] using bovine serum albumin as the standard protein.

2.5. Assessment of Serum CPK and LDH Levels. Functions of the heart were assessed by measuring the levels of CPK and LDH in serum using commercially available diagnostic kits (Abcam Chemicals, Tokyo, Japan). Respective diagnostic kits were used to estimate CPK and LDH levels and expressed in mU/ml.

Table 1: Effect of silymarin on cardiac lipid peroxidation production and antioxidant enzyme activities in rats treated with acrylamide.

Experimental groups	MDA nmol/g tissue	SOD U/mg protein	CAT U/mg protein	GPxm U/mg protein
Control	$22.40^{a} \pm 0.81$	66.60^{a} 1.03	$44.80^{a} \pm 1.50$	$61.60^{a} \pm 1.03$
AA	$62.40^{c} \pm 1.17$	$26.40^{c} \pm 0.75$	$17.20^{c} \pm 1.02$	$22.60^{c} \pm 0.68$
SIL	$24.18^{a} \pm 0.22$	$64.40^{a} \pm 1.36$	$45.80^{a} \pm 1.16$	$61.40^{a} \pm 1.17$
AA+SIL	$32.80^{b} \pm 1.07$	$46.60^{b} \pm 0.68$	$29.60^{b} \pm 1.03$	$33.0^{b} \pm 1.18$
One-way ANOVA F (p)	466.00 (<0.001*)	354.46 (<0.001*)	130.75 (<0.001*)	370.65 (<0.001*)

Data are expressed as the mean ± SE of five rats/group. Means within the same column with different superscript letters are significant. *: Statistically significant at $P \leq 0.05$. MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, GPx: glutathione peroxidase.

2.6. Determination of Serum Lipid Profile. Total amounts of cholesterol (TC), triglycerides (TGs), and high-density lipoprotein cholesterol (HDL-C) were assayed using kits purchased from Bio-Med Diagnostics (Bad Homburg, Germany) following the procedures and instructions provided by the supplier. The amounts of very-low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were calculated using Friedewald's formula [38]; the units were expressed in mg/dl.

2.7. Histopathological Studies. Specimens from the hearts of the control rats and treated rats were fixed in 10% formalin solution, dehydrated in ascending series of ethanol, cleared in xylene, and then embedded in paraffin wax. Sections were stained with H&E dye and examined under a light microscope. Six slides were prepared per experimental animal, each with three sections. The pathologist (A. M. Abdel-Moneim) performing the evaluation was blinded to the treatment assignments of various study groups. The criteria of myocardial lesions included myocardial disorganization, necrotic injury, leukocyte infiltration, and nuclear pyknosis [39, 40]. The severity of every pathological alteration was graded semi quantitatively as previously described [41].

2.8. Statistical Analysis. SAS statistical software, version 9.2 (SAS Institute Inc., Cary, North Carolina), was used to perform all statistical tests reported in this work. The obtained variables were compared using one-way analysis of variance (ANOVA) followed by LSD multiple range test. The difference was considered significant at $p < 0.05$.

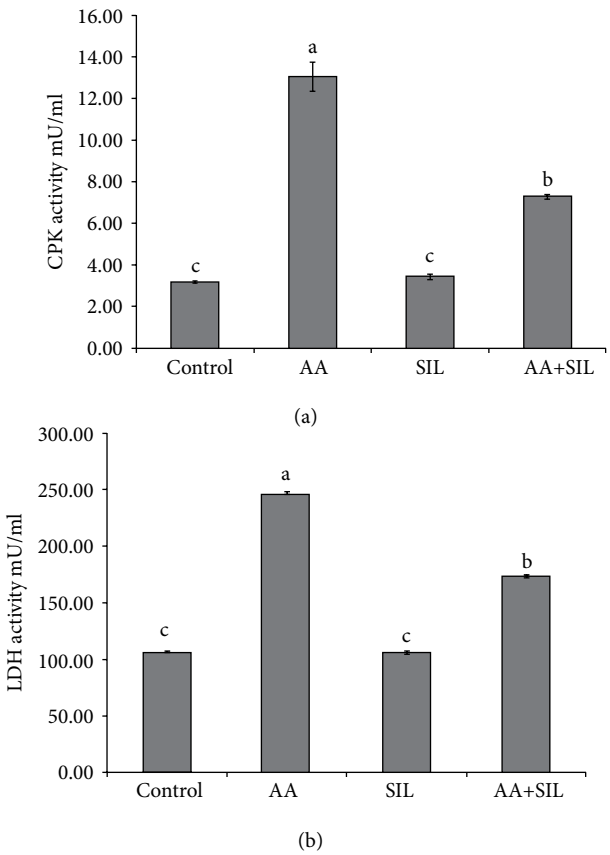

Figure 1: Effect of silymarin on serum cardiac markers in male rats treated with acrylamide. (a) Creatine phosphokinase (CPK), and (b) lactate dehydrogenase (LDH). Data are expressed as the mean ± SE of five rats/group. Bars with different letters show significant differences between the groups ($p \leq 0.05$).

3. Results

3.1. SIL Inhibits AA-Induced Cardiac MDA and Protects Antioxidant Enzymes. The effects of AA and/or SIL on MDA, CAT, SOD, and GPx levels are depicted in Table 1. Oxidative stress caused by AA resulted in a significant ($p < 0.05$) rise in the value of MDA level and a significant ($p < 0.05$) reduction in the activities of SOD, CAT, and GPx enzymes relative to the control. SIL significantly ($p < 0.05$) alleviated the MDA increase in AA-treated rats. Further, the antioxidant enzyme activities were all statistically significantly ($p < 0.05$) greater in the group treated with AA+SIL compared with the AA treatment group.

3.2. SIL Prevents AA-Induced Elevation in Serum Markers. The enzymatic activities of CPK (Figure 1(a)) and LDH (Figure 1(b)) were substantially ($p < 0.05$) higher in the AA group relative to the control. AA-treated rats administered SIL had significantly ($p < 0.05$) reduced CPK and LDH activities compared to those of the AA-intoxicated group.

3.3. Preventive Effect of SIL against AA-Induced Dyslipidemia. As given in Table 2, AA produced a significant ($p < 0.05$) increase in the values of TC, TGs, LDL-C, and VLDL-C compared to those of the control group. In addition, it was found that exposure to AA significantly ($p < 0.05$)

Table 2: Effect of silymarin on lipid profile in rats treated with acrylamide.

Experimental groups	TC (mg/dl)	TGs (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control	$86.20^{c} \pm 0.66$	$58.80^{c} \pm 1.11$	$47.20^{a} \pm 0.97$	$35.40^{c} \pm 0.98$	$11.08^{c} \pm 0.35$
AA	$174.60^{a} \pm 1.21$	$133.40^{a} \pm 0.81$	$33.20^{b} \pm 0.97$	$107.20^{a} \pm 0.86$	$26.12^{a} \pm 1.09$
SIL	$84.60^{c} \pm 1.03$	$59.60^{c} \pm 0.75$	$47.0^{a} \pm 0.95$	$35.20^{c} \pm 0.49$	$11.48^{c} \pm 0.43$
AA+SIL	$102.80^{b} \pm 0.73$	$85.20^{b} \pm 0.86$	$35.0^{b} \pm 1.64$	$56.60^{b} \pm 1.29$	$17.80^{b} \pm 0.58$
One-way ANOVA F (p)	2064.68 (<0.001*)	1529.98 (<0.001*)	41.52 (<0.001*)	1278.43 (<0.001*)	108.31 (<0.001*)

Data are expressed as the mean ± SE of five rats/group. Means within the same column with different superscript letters are significant. *: Statistically significant at $P \leq 0.05$. TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, and VLDL-C: very-low-density lipoprotein cholesterol.

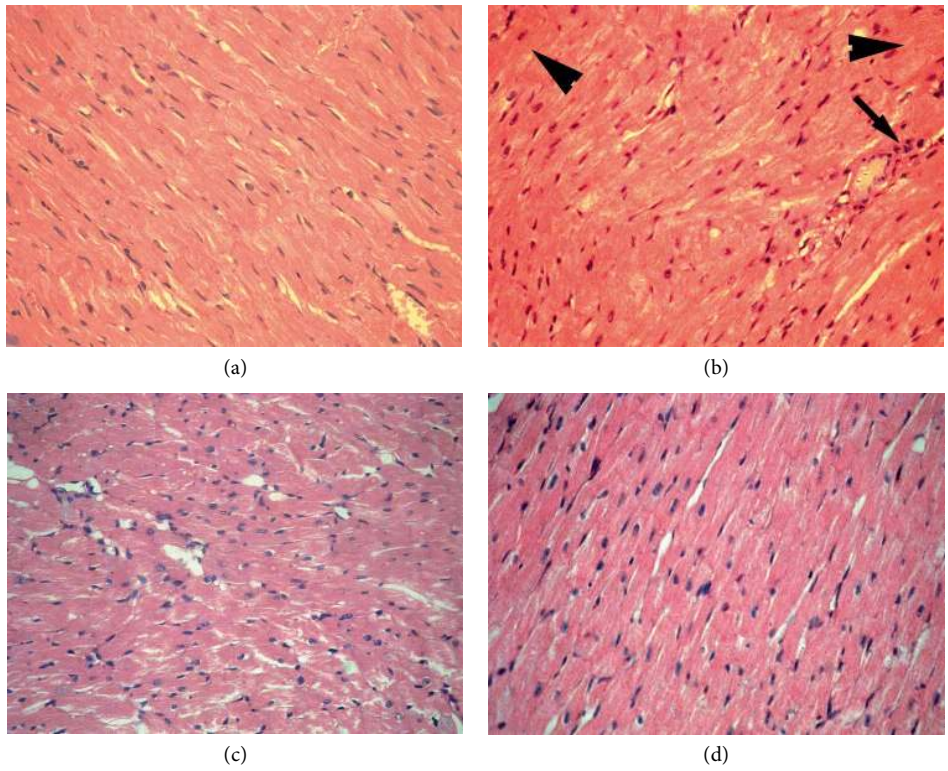

Figure 2: Representative light micrographs of myocardium tissues. (a) Control group revealing normal morphological features of cardiomyocytes. (b) AA group showing degeneration and necrosis (arrowheads) in the muscle fibrils. Inflammatory cells (arrow) can also be seen. (c) SIL group showing normal architecture of the myocardium. (d) AA+SIL group showing reduced muscle fiber degeneration without inflammatory cells. H&E staining (original magnification: 400x).

reduced the HDL-C level compared to that of the control. However, treatment of rats with AA and SIL significantly ($p < 0.05$) lowered the quantities of TC, TGs, LDL-C, and VLDL compared to those of the AA group. In the meantime, there was no significant change ($p > 0.05$) in HDL-C levels in AA+SIL group compared to AA alone group.

3.4. SIL Improves Cardiac Histopathology. The cardiac tissue of control rats showed normal branched myofibers with obvious striations and centrally located mononuclei (Figure 2 (a)). Pathological examination revealed obvious cardiomyocyte injuries of AA-treated rats, such as irregularity in myofibrils (i.e., wavy appearance), sporadic necrotic degeneration, and focal infiltration of inflammatory cells (Figure 2 (b)). In necrotic areas, striation disappeared completely, and the sarcoplasm exhibited deep eosinophilic staining. In addition, pyknosis of myocyte nuclei was occasionally observed. The SIL group did not show any histological alterations in cardiac muscular tissue (Figure 2 (c)). In the AA+SIL group, we observed a decrease in damage in cardiac cell structure (Figure 2 (d)) compared to that of the group treated with AA only. The semi quantitative scoring of pathological changes is summarized in Table 3.

4. Discussion

Although many reports have shown that AA is a potent carcinogen with neurotoxic action, there are few reports of its cardiovascular effects in animal models. Our study found that

Table 3: Effect of silymarin on severity of myocardial lesions in rats treated with acrylamide.

Histopathological changes	Control	AA	SIL	AA+SIL
Disruption of cardiac muscle organization	−	+++	−	++
Necrosis	−	+++	−	++
Inflammatory cell infiltrate	−	++	−	+
Nuclear pyknosis	−	++	−	+

Scores obtained from tissue sections of five rats/group. Scores: −(normal), + (mild change), ++ (moderate), and +++ (severe levels).

AA induced cardiotoxicity after i.p. injection. AA-induced cardiotoxicity was evidenced by the increase in serum CPK and LDH activities and confirmed by noticeable myopathic lesions in the form of necrotic disruptions, loss of cross striations, and cellular infiltration. In parallel, marked hyperlipidemia occurred along with pronounced elevation of myocardial MDA content. Oxidative stress deterioration is considered a major contributor in triggering and progressing AA-induced myocardial dysfunction [42]. AA exposure accelerates the level of ROS by disturbing oxidant–antioxidant balance and suppressing the antioxidant defense system [43]. It was previously known that compared to other tissues, the heart is selectively sensitive to ROS-induced damage because of its highly oxidative metabolism and lower abundance of antioxidant defenses [44]. A large amount of ROS can extensively damage cardiac tissue in several aspects, including destruction of cell membrane permeability, mitochondrial and bioenergetic failure, upregulation of proteins that mediate apoptosis, and alterations in the calcium (Ca^{2+}) homeostasis causing Ca^{2+} overload that leads to sarcomeric disarray and myofibril deterioration [45, 46]. Therefore, mitigation of cardiotoxicity and protection of the heart against oxidative stress using antioxidative drugs have previously been suggested [47]. Herein, oxidative damage was assured by increased lipid peroxidation (LPO) and decreased SOD, CAT, and GPx in the AA-treated group. SOD acts as the first line of antioxidant defense against superoxide radicals, which catalyzes dismutation of two superoxide radicals to H_2O_2 and O_2, while CAT and GPx act to support antioxidant enzymes by converting H_2O_2 to H_2O, thereby providing protection against ROS [48]. The observed decrease in the antioxidant enzyme activities can be explained on the basis of the exhaustion of endogenous cellular antioxidants in combating AA-induced overproduction of ROS. Previous studies have reported that SIL has antioxidant characteristics in many types of oxidative stress-mediated damage [28, 49]. In the present study, SIL therapy reduced the levels of the reactive aldehyde (i.e., MDA) and upregulated antioxidant enzymes in AA-treated rats. Thus, free radical scavenging could be a mechanism by which SIL causes a reduction in AA cardiotoxicity.

Elevated serum levels of CPK and LDH have been indicated for cardiac tissue dysfunctions because these enzymes are normally located in the cytoplasm of cardiomyocytes and leakage occurs into the plasma or serum after myocardial injury [50]. CPK promotes the reversible phosphotransfer between the ATP/ADP and creatine/phosphocreatine systems [51]. CPK is highly expressed in vertebral excitable tissues that require large energy fluxes [52]. LDH is found in nearly all cells. Its function is to catalyze the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. LDH is expressed extensively in the heart muscle and is released during tissue damage, so it is used as a marker of common injuries and disease [53]. In this study, administration of AA increased the activities of CPK and LDH in serum. AA may cause membrane injury by initiating LPO which results in loss of function and integrity of myocardial membranes with subsequent leakage of these markers into the blood. Cotreatment with SIL restored the activities of CPK and LDH and reduced their serum level in AA-treated rats. These findings are consistent with the improved cardiac histology in the AA+SIL group and may be attributed to the protective role of SIL against myocardial LPO and its stabilizing action on the cardiac muscle membrane, thereby restricting the leakage of these enzymes into the serum [54]. Similar results were reported with SIL in a model of adriamycin-induced cardiotoxicity [55].

AA is known to result in disruption of blood and cellular lipid levels [56, 57], which are well-known risk factors for CVDs [58]. In the present study, the cardiomyopathy induced by AA was associated with a significant increase in lipid panel (TC, TGs, and LDL-C) as well as a decrease in HDL-C. AA induces free radical production [18], which may cause cellular cholesterol accumulation due to (1) an increase in cholesterol biosynthesis and esterification, (2) a decrease in cholesteryl ester hydrolysis, and (3) a reduced cholesterol efflux [59]. In light of the present results, the occurrence of hypertriglyceridemia may result from the poor expression of lipolytic activity in the vascular bed during AA treatment. Recently, Lee and Pyo [60] reported that AA promoted adipocyte differentiation and intracellular lipid accumulation via regulation of the expression of adipogenesis-related genes and AMPK and MAPK signaling. Experimental studies in rodents have shown that lipid-lowering agents with antioxidant properties were able to protect the myocardium against cardiotoxicity conditions induced by other drugs/pharmaceuticals (e.g., doxorubicin and cyclophosphamide) [50, 61]. Our results showed that SIL treatment in AA-intoxicated rats caused an overall reduction of the elevated serum lipids (TC and TGs) and improvements in the lipoprotein profile. These results are in harmony with the studies of Iliskovic and Singal [62], Krečman et al. [63], and Radjabian and Huseinim [64].

5. Conclusions

This study demonstrated that SIL protects against AA-induced cardiotoxicity through decreasing the levels of cardiac markers, attenuating LPO products, increasing antioxidant status, and reducing histopathological damages in the myocardium. Our data also indicate that SIL was beneficial in restoring the lipidemic status in AA-challenged rats, suggesting that it may be considered an effective drug for potential treatment of hyperlipidemic cardiomyopathy.

Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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