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Effect of Livergol on the Liver Function and its Histopathological Changes in Doxorubicin-Treated Rats

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Abstract

Background: Doxorubicin (DOX) is an effective anti-cancer medicine with serious side effects on healthy organs, especially the liver.
Objectives: The present study aimed to investigate the histopathology and effects of Livergol (Liv), a product of *Silybum marianum*, on serum levels of hepatic parameters in long-term DOX-treated rats.
Methods: Sixty three male Wistar rats were divided into seven groups, namely the control, sham, and experimental groups 1-5, which received certain doses of DOX, Liv, and their combination for 2 months. To produce a cumulative dose, 10 doses of 2 mg/kg DOX were intraperitoneally administered once every 6 days. Moreover, 150 and 300 mg/kg daily doses of Liv were gavaged to the experimental groups 2-5. In addition, blood samples were taken, serum levels of hepatic functional factors and liver enzymes were measured, and hematoxylin-eosin staining was performed to evaluate liver histopathology. The results were analyzed between experimental and control groups by analysis of variance and Tukey tests and the significance level was considered at < 0.05.
Results: The serum levels of triglyceride, cholesterol, low-density lipoprotein, total and direct bilirubin, and liver enzymes in the DOX + Liv300 and Liv150 groups showed a significant decrease, compared to the DOX group. While high-density lipoprotein, albumin, and total protein showed a significant increase. Liver tissue in DOX + Liv300 and Liv150 groups did not show any damage. In addition, the serum level of liver enzymes, lipid profile, biochemical factors, and liver histopathology in the Liv300 and Liv150 groups were similar to those in the control group.
Conclusion: The findings demonstrated that the oral administration of Liv powder can prevent liver side effects of DOX in rats.

Keywords: Cholesterol, Doxorubicin, Liver enzymes, Livergol, Triglyceride

1. Background

Doxorubicin (DOX) is a broad-spectrum antitumor anthracycline antibiotic used to treat a wide range of cancers (1). It is structurally similar to Daunorubicin, a natural anti-cancer antibiotic first isolated from Streptomyces peucetius (2). The antitumor effects of DOX are primarily mediated by direct interactions with DNA. Despite the widespread use of this potent chemotherapy anthracycline, its mechanism of action is complex and controversial (3).

Research has demonstrated the following proposed mechanisms for DOX in cancer cells: A) interaction with DNA that inhibits protein synthesis and DNA transcription, B) production of free radicals leading to DNA damage, lipid peroxidation, and cell membrane injuries, C) binding to DNA and alkalinizing it, D) interference in the helicase activity and DNA opening, E) direct effect on the membrane bilayer and cell membrane disruption, and F) onset of DNA degradation by Topoisomerase (4-6). Furthermore, severe side effects have been reported for DOX, including nausea, emesis, irreversible degenerative cardiomyopathy, and hepatotoxicity (6).

Despite its cytostatic and cytotoxic effects on cancer cells, DOX accumulates in various organs, especially the liver. Indeed, the liver plays a major role in the metabolism and detoxification of medications and is the primary organ affected by chemotherapy. Inside hepatocytes, DOX undergoes redox cycling, leading to the generation of reactive oxygen species (ROS) (7, 8). The generated free radicals also decrease glutathione levels and damage DNA resulting in apoptosis (4).

Furthermore, it has been found that DOX treatment promotes systemic inflammation by releasing microbial endotoxins into circulation through the damaged intestine. These endotoxins stimulate pro-inflammatory pathways and enhance hepatic inflammation (6). The hepatic inflammation increases the production of ROS starting the redox cycle, which damages cellular lipids, proteins, and DNA, leading to cell death (8).

Liver health is related to low levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) enzymes; hence, their increased levels in the blood indicate liver damage (9). Moreover, excessive accumulation of fat and medications in liver cells also leads to inflammation and fibrosis and may progress to the stage of liver cirrhosis (10).

Since DOX side effects limit its use as an

anticancer agent, interventions to minimize its toxicity have received much attention. There is currently no reliable treatment to prevent DOX-induced toxicity in non-target organs (11-12). Supplementation of the diet with creatine, amifostine, and dioscin has been suggested as a potential intervention to minimize or alleviate DOX-induced hepatotoxicity (13).

Livergol (Liv) tablet, containing milk thistle plant extract (*Silybum marianum)*, protects the liver, choleretic, acute and chronic hepatitis, liver cirrhosis, and fatty liver, and thereby reduces the liver side effects of drugs. *Silybum marianum*, commonly known as milk thistle, is the most extensively studied plant in the treatment of hepatic disorders (14). In traditional medicine, *Silybum marianum* powder or extract is used in the treatment of liver disorders, cardiovascular disease, and hypolipidemia (15). Many reports have shown that the use of silymarin can exert positive effects on reducing drug resistance (15, 16).

Milk thistle is a plant that is known as an increaser of bile flow (17). Its extract contains various chemicals, including flavonolignans, which are collectively called silymarin (16). Silymarin is a mixture of flavonoids that have been proven to have hepatoprotective and antioxidant effects. It has been effective in the treatment of liver poisoning and chronic liver diseases (15, 17).

2. Objectives

It seems that the conventional methods of cancer treatment are facing many issues and the use of new medicines as well as new methods of prescribing to treat cancer cells is necessary. Based on the antioxidant, anti-inflammatory, anti-apoptotic, and hepatoprotective properties of silymarin, this study aimed to investigate the protective effects of Liv on biochemical parameters of blood serum and liver histology.

3. Methods

3.1. Animals

This experimental research was carried out at Gachsaran Branch, Islamic Azad University, Fars, Iran in 2021. Sixty-three adult male Wistar rats were used with an approximate weight of 200-220 g and the age of 2.5-3.0 months old. The rats were placed in special polycarbonate cages (15×25×30 cm^3) with the standard space in the ambient temperature of 23-25 °C with 50-55% humidity and a 12 h light/dark cycle. The floor of the cages was covered with sawdust and cleaned every 3 days. All rats had free access to feed and water (*ad libitum*).

This research received an ethical approval code of IR.IAU.IAUG.REC.1399.021 and all ethical principles were observed regarding working with laboratory animals. After weighing, the animals were divided into 7 groups based on their weight range and placed in separate cages. The average weight of each group was labeled on the respective cage. The groups included the control, the sham receiving solvents, and five experimental groups receiving certain doses of DOX, Liv, and a combination of them (Table 1).

Table 1. Animal grouping and experimental treatments

Groups	Treatment materials (mg/Kg. of BW)
Control	-
Sham	Distilled water
Experimental I or DOX	20 mg/kg DOX
Experimental II or Liv150	150 mg/kg Liv
Experimental III or Liv300	300 mg/kg Liv
Experimental IV or DOX+Liv150	20 mg/kg DOX+150 mg/kg Liv
Experimental V or DOX+Liv300	20 mg/kg DOX+300 mg/kg Liv

DOX: Doxorubicin
Liv: Livergol
Doxorubicin was administered once every 6 days by intraperitoneal injection. Livergol was gavaged daily between 9-10 AM with an oral feeder for 2 months.

3.2. Drug Preparation

The DOX was purchased in powder from the pharmacy of Amir Shiraz Oncology Hospital, Shiraz, Iran. Its production sites in Iran are Sobhan Oncology Company and Minoo pharmaceutical Private Company. To reach 20 mg/ml concentration, it was diluted in sodium chloride solution and stored in the refrigerator at 4 °C. The usual dose of liposomal DOX is 20-40 mg every 3 weeks or 50 mg every 4 weeks per square meter of body surface area. The prescribed dose was selected based on previous studies (18).

To reach the cumulative dose of 20 mg/kg, from the start of the experiment, 2 mg/kg DOX was administered once every 6 days for 2 months in the form of 10 intraperitoneal administrations on days 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60. The Liv tablets containing extract of *Silybum marianum* (14) were purchased from the pharmacy in doses of 70 and 140 mg in packs of 30 tablets (Goldaru Company, Iran, health license codes: 1228055713 and PR001001120). The Liv was dissolved in normal saline and doses of 150 and 300 mg/kg were gavaged to experimental groups from 9 to 10 AM every day. The extract was fed to the rats 1 h after DOX injection from the first day of the study for two months.

3.3. Animal Treatment

At the end of the 2-month experimental period, all animals were weighed again. Moreover, under ether anesthesia, blood was drawn from their hearts and also their livers were removed. Blood samples were kept at 37 °C for about 20-30 min to agglutinate. They were centrifuged at 5000 rpm for 15 min and the isolated serums were kept at -20 °C to be examined later. Serum levels of liver enzymes (ALT, AST, and

ALP) and blood biochemical factors (triglycerides, cholesterol, Low-density lipoprotein (LDL), High-density lipoprotein (HDL), total protein, albumin, total bilirubin, and direct bilirubin) were measured using a fully automatic auto-analyzer (Technicon RA-1000, USA) and standard kits (Pars Azmoon, Iran).

3.4. Histological Study

After washing in sterile plates, the livers of each group were sampled separately, transferred to 1.5 ml vials containing 1 ml of Traysol, and stored in an ice pack. After fixation with 10% formalin, dehydration with certain concentrations of ethanol, clarification with Xylene, and replacement with paraffin, the liver samples were molded separately (19). A semi-automatic microtome (model: CUT 6062, Slee MAINZ, Germany) was used to prepare 4-5 µm thick sections from the molds. A large number of suitable sections were selected from each sample in different groups, attached on slides, stained by the hematoxylin-eosin method, studied, and compared by light microscopy (20).

3.5. Statistical Analysis

Findings were analyzed in SPSS software (version 21), using the one-way analysis of variance, and Tukey post hoc test. The level of significant statistical differences between experimental groups relative to the control and the positive control (experimental I or DOX) was considered at < 0.05 (21).

4. Results

Based on the results in experimental group I, DOX increased the amount of AST, ALT, and ALP enzymes by 129.6±14.81 (65.7%), 104.00±3.77 (190%), and 32.19±425.4 U/L (32.19%), respectively. The results of the statistical analysis of serum levels of liver enzymes (ALT, AST, and ALP) are summarized in Table 2. As seen, the levels of all three enzymes in experimental group I (receiving DOX) underwent a significant increase, compared to the control group (P<0.05). Moreover, serum levels of ALT, AST, and ALP enzymes in experimental groups II and III were not significantly different from those of the control group. Conversely, their levels in experimental groups IV and V showed a significant decrease, compared to the DOX experimental group (P<0.05).

The results for serum levels of triglycerides, cholesterol, LDL, and HDL are presented in Table 3.

The consequences of one-way analysis of variance and Tukey post hoc test showed that serum levels of triglycerides, cholesterol, and LDL in experimental group I (receiving DOX) increased significantly, while HDL levels declined significantly, compared to the control group. Similarly, serum levels of triglycerides, cholesterol, LDL, and HDL in the experimental groups II and III (Liv150 and Liv300) showed no significant.

Table 2. Comparison of the mean (±standard deviation) of three liver enzymes in different experimental groups

Groups	AST (SGOT) (U/L)	ALT (SGPT) (U/L)	ALP (U/L)
Control	78.20±12.90	35.80±2.72	321.80±17.10[b]
Sham	80.20±12.99	36.00±2.60	330.00±18.40
Experimental I or DOX	129.60±14.81	104.00±3.77	425.40±32.87
Experimental II or Liv150	80.30±10.10	32.80±4.70	319.80±17.80
Experimental III or Liv300	87.00±2.42	34.90±3.20	320.00±17.40
Experimental IV or DOX+Liv150	97.20±18.25	54.24±7.19	341.80±26.40
Experimental V or DOX+Liv300	98.20±17.16	51.80±6.99	339.80±17.40
P-value*	<0.001**	0.048**	<0.001**

DOX: Doxorubicin, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase
* One-way analysis of variance, Tukey's Post hoc test (P<0.05).
** Experimental I or DOX group was significantly higher than others. Experimental IV or DOX+Liv150 and experimental V or DOX+Liv300 groups were significantly lower than experimental I or DOX group.

Table 3. Effect of experimental treatments on the levels of different lipids in the blood of Doxorubicin-treated rats

Groups	TG (mg/dL)	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control	36.12±9.60	64.90±7.21	10.75±1.20	37.03±2.60
Sham	38.30±10.41	66.80±6.79	11.20±1.14	36.19 ±2.41
Experimental I or DOX	348.60±15.98	100.40±9.30	17.31±1.89	29.41±3.61
Experimental II or Liv150	37.94±11.83	66.40±6.71	10.98±1.74	38.18±3.56
Experimental III or Liv300	36.37±9.37	65.60±8.11	11.23±1.58	37.20±4.24
Experimental IV or DOX+Liv150	50.72±16.23	52.60±7.43	12.22±1.80	33.20±3.62
Experimental V or DOX+Liv300	57.60±15.83	51.60±8.26	12.80±1.72	32.20±4.57
P-value*	<0.001**	0.037**	0.048**	0.044***

DOX: Doxorubicin, TG: Triglyceride; Chol.: Cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.
* One-way analysis of variance, Tukey's Post hoc test (P<0.05).
** Experimental I or DOX group was significantly higher than others. Experimental IV or DOX+Liv150 and experimental V or DOX+Liv300 groups were significantly lower than experimental I or Dox group.
*** Experimental I or DOX group was significantly lower than others. Experimental IV or DOX+Liv150 and experimental V or DOX+Liv300 groups were significantly higher than experimental I or DOX group.

Table 4. Comparison of the mean (±standard deviation) of some blood biochemical parameters in different experimental groups

Groups	Albumin (g/dL)	Total Protein (g/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Control	4.20±0.24[a]	6.90±0.28[a]	0.048±0.011[b]	0.038±0.006[b]
Sham	4.32±0.25[a]	6.88±0.31[a]	0.050±0.009[b]	0.037±0.006[b]
Experimental I or DOX	2.94±0.40[b]	5.62±0.53[b]	0.150±0.027[a]	0.061±0.008[a]
Experimental II or Liv150	4.23±0.24[a]	6.95±0.32[a]	0.046±0.010[b]	0.03 ±0.006[b]
Experimental III or Liv300	4.35±0.18[a]	7.10±0.46[a]	0.047±0.013[ab]	0.03 ±0.007[b]
Experimental IV or DOX+Liv150	3.34±0.37[b]	6.12±0. 40[b]	0.088±0.007[ab]	0.045±0. 007[b]
Experimental V or DOX+Liv300	3.22±0.32[b]	6.24±0.60[b]	0.081±0.007[ab]	0.044±0.004[b]
P-value*	0.025**	0.041**	0.048***	0.044***

DOX: Doxorubicin.
* One-way analysis of variance, Tukey's Post hoc test (P<0.05).
** Experimental I or DOX group was significantly lower than others. Experimental IV or DOX+Liv150 and Experimental V or DOX+Liv300 groups were significantly higher than Experimental I or DOX group.
*** Experimental I or DOX group was significantly higher than others. Experimental IV or DOX+Liv150 and Experimental V or DOX+Liv300 groups were significantly lower than Experimental I or DOX group.

difference at the level of P<0.05, compared to the control group. However, serum levels of triglycerides, cholesterol, and LDL in experimental groups IV and V decreased significantly, and the level of HDL showed a significant increase, compared to the experimental group I (P<0.05).

Results of the total protein, albumin, total bilirubin, and direct bilirubin tests are tabulated in Table 4. Based on the findings, there was a significant decline in the levels of total protein and albumin. Furthermore, the levels of total bilirubin and direct bilirubin increased significantly in the experimental group I (DOX recipient), compared to the control group (P<0.05). In contrast, serum levels of all four biochemical factors in experimental groups II and III showed no significant difference, compared to the control group (P<0.05). Besides, the levels of total protein and albumin in experimental groups DOX + Liv300 and DOX + Liv150 elevated significantly in comparison to the experimental group I, while serum levels of total and direct bilirubin showed a significant decrease (P <0.05).

The results of histopathological studies of the liver are shown in Figure 1. There were no

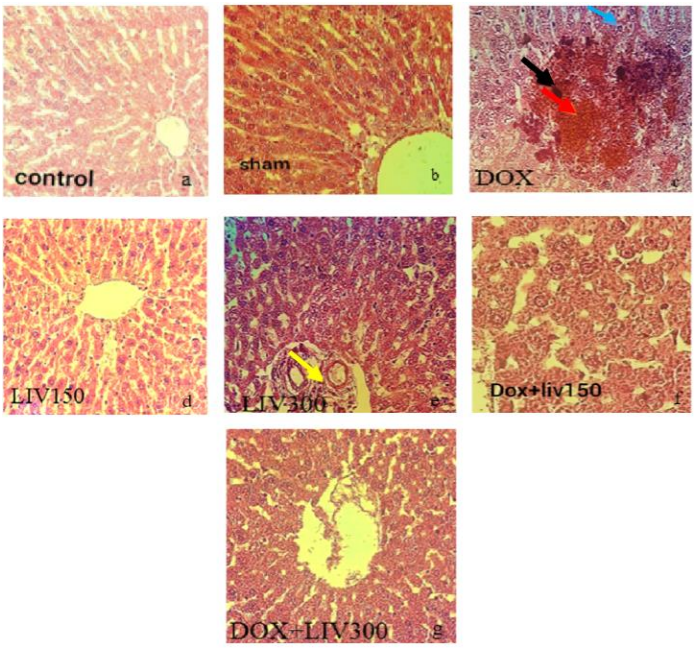

Figure 1. Photomicrographs of rat liver tissues in different experimental groups (Black arrow: necrosis; Red arrow: bleeding; Blue arrow: hepatocyte destruction; Yellow arrow: lymphocyte infiltration).
(a) The control and (b) sham groups: Histology of liver tissue, space around the port duct, pagination and filaments of liver tissue, and order of hepatic sinusoids are completely normal. There are no signs of necrosis, apoptosis, steatosis, congestion and lymphocytic infiltration, decreased port space, or local inflammation.
(c) DOX group: Severe liver tissue damage with necrosis in different areas, hepatocyte destruction, and bleeding are seen. There is also a loss of sinusoid order and a decrease in the number of hepatocytes.
(d) LIV150 group: Steatosis, necrosis, inflammation, bleeding, and lymphocyte infiltration are not seen, and sinusoids and hepatocytes appear normal.
(e) LIV300 group: The order of the sinusoids is disturbed. The appearance of liver hepatocytes is not very normal. Lymphocyte infiltration (yellow arrow) is also seen.
(f) Experimental group IV (DOX+LIV150): a decrease in the order of the sinusoids and signs of steatosis or necrosis, inflammation, infiltration of lymphocytes, and bleeding are seen.
(g) Experimental group V (DOX+LIV300): Except for moderate hepatocyte order and damage to the hepatocyte wall, no signs of severe tissue damage are seen (Hematoxylin-eosin staining; magnification: ×400).

pathological and damage changes, including necrosis, apoptosis, steatosis, or inflammation in the photomicrograph of the control and sham groups (Figures 1a and 1b). Nevertheless, severe histological changes in experimental group I (DOX) showed signs of necrosis, steatosis, and destruction of cells in different tissue areas.

There was a loss of sinusoid order and a decrease in the number of hepatocytes as well (Figure 1c). No signs of liver damage or bleeding were seen in any of the liver tissue samples of the Liv150 group (Figure 1d). However, traces of lymphocytic infiltration and loss of sinusoidal order were seen in the LIV300 group (Figure 1e). The appearance of the liver structure was normal and there was no sign of tissue damage in the liver micrographs of experimental group IV (DOX+Liv 150; Figure 1f) and experimental group V (DOX+Liv300; Figure 1g).

Experimental group IV (DOX+Liv150) underwent a decrease in the order of the sinusoids and signs of steatosis or necrosis, inflammation, infiltration of lymphocytes, and bleeding. In experimental group V (DOX+LIV300), except for moderate hepatocyte order and damage to the hepatocyte wall, no signs of severe tissue damage were observed (Hematoxylin-eosin staining; magnification: X 40).

5. Discussion

The experimental results showed that acute administration of 20 mg/kg DOX in experimental group I increased the amount of AST, ALT, and ALP enzymes by 65.7%, 190%, and 32.19%, respectively, in comparison to the control group. Moreover, the use of Liv caused a significant decrease in liver enzymes. Livergol increased the levels of HDL, Albumin, and total protein, while having an opposite effect on other blood biochemical parameters in this experiment. Furthermore, it is noteworthy that it reduced liver damage and improved its function.

Similar increases in hepatic enzyme levels resulting from DOX injection have been observed in other studies (3, 5, 6). According to the American Association for the Study of Liver Disease, AST and ALT are good markers for liver health and disease (22). The most effective and valid biochemical tests for the assessment of hepatotoxicity are the serum activity levels of aminotransferases, such as aspartate aminotransferase and alanine aminotransferase (6, 7). High serum levels of these two enzymes indicate damage to hepatocytes, and their increased activity can signify the presence of active liver disease.

Alkaline phosphatase is another biomarker of liver damage that is mainly increased in diseases related to the secretion and excretion of bile (23). Changes in these three enzymes indicate the severity of the hepatic damage and the need for its treatment (24). In agreement with the results of the present study, it has been found that high doses of DOX can induce extensive hepatic toxicity, and low doses cause changes, such as hepatic portal degeneration, increased apoptosis, and peritoneal fibrosis (25, 26). Inflammatory disorders of liver cells lead to an acute increase in the blood levels of transaminases (27).

The most important cause of DOX-induced hepatotoxicity seems to be related to oxidative stress (4), which induces the expression of proinflammatory cytokines and leads to cell death. Accordingly, normal liver function is disrupted, DOX excretion is delayed, and its plasma concentrations rise, leading to increased systemic toxicity and intensified liver malfunction (11, 18).

Among the pharmacological effects, silymarin's ability to protect liver cells is more important than other properties. Silymarin can provide various antioxidant mechanisms, including inhibition of lipid peroxidation, stimulation of ribosomal RNA polymerase, protection of hepatocytes against toxins, and inhibition of cytokinins, such as TNF-α (tumor necrosis factor-alpha). The researchers have reported that silymarin can inhibit the oxidative stress produced in sucrose-fed rats and improve the cholesterol and lipoproteins pattern in plasma (15, 24).

In recent years, researchers have made significant efforts to identify, prevent, and treat DOX-induced cytotoxicity, particularly in liver and heart tissues (27). High-fat diet results in non-alcoholic fatty liver, and treatment with combined *Ziziphus jujube*, *Cichorium intybus*, and *Silybum marianum* extracts improves lipid profile, liver enzyme activity, and liver tissue damage in rats (11, 14-17).

Based on the findings of the present study, Liv powder can protect the liver against the harmful effects of DOX. The serum levels of liver enzymes, total bilirubin, direct bilirubin, triglyceride, cholesterol, and LDL showed a significant decrease in experimental groups IV (DOX+Liv150) and V (DOX+Liv300). However, the levels of HDL, total protein, and albumin increased relative to the DOX group. The results of clinical trials involving more than 500 patients with non-alcoholic fatty liver disease have shown that silymarin consumption significantly reduced liver enzymes and regenerated liver tissue (28).

Numerous studies have attributed DOX-induced hepatotoxicity to the overproduction of free radicals (1, 3, 23), which leads to lipid peroxidation, mitochondrial damage, and cytotoxicity (29). Free radicals can bind to proteins and lipids, and pick up a hydrogen atom from unsaturated lipids. In this way, they stimulate lipid peroxidation resulting in endoplasmic reticulum modification and decreased protein synthesis. The most important cause of DOX hepatotoxicity and heart tissue toxicity appears to be related to free radical production, which disturbs the balance of oxidants/antioxidants (7, 8, 30).

Laboratory studies have demonstrated that silymarin present in the methanolic extract of thistle

seeds improves LDL excretion, declines cholesterol synthesis in liver cells, prevents complications caused by high cholesterol, and reduces the formation of atherosclerosis plaques in rats with hypercholesterolemia (8, 14). Moreover, results of some previous research have indicated that silymarin lowers LDL cholesterol and blood triglycerides and increases HDL cholesterol levels. In a previous study, the effects of silymarin supplementation were examined on the glycemic index and lipid profile of type 2 diabetic patients (15, 28). Findings of the above-mentioned study showed that silymarin has a significant effect on the reduction of triglyceride, LDL, blood sugar, serum insulin, and insulin resistance as well as the increase of HDL levels (16, 17, 28).

The histopathological results of this study also confirm the occurrence of structural liver damage following the long-term use of DOX. During chemotherapy with DOX, a high concentration of DOX accumulates in the liver and is metabolized. Concomitant use of DOX and Liv in the experimental groups of DOX + Liv150 and DOX + Liv300 prevented DOX-induced structural liver disorders. Histologically, cell necrosis, cell inflammation, fat vacuole accumulation, disintegration in the portal space, and the formation of large spaces between cells were observed in the DOX group, compared to the control group. However, such symptoms were absent in the groups receiving DOX along with 150 and 300 mg/kg Liv. Similarly, clinical experiments have shown that silymarin has a protective effect on the liver against acute viral hepatitis and alcohol toxicity, including liver cirrhosis (14, 19). It is also effective in the treatment of non-alcoholic fatty liver, which is common throughout the world (10, 14, 16).

In a previous study, Picro-Sirius Red staining was used to assess hepatic tissue, collagen deposition in the liver, and serum enzyme levels (27). Results of the aforementioned study showed liver damage and reported a significant difference in fibrosis between the study groups. Liver fibrosis usually occurs when chronic damage or inflammation leads to the accumulation of scar tissue, which can be manifested by increased collagen deposition (1, 5, 11).

In addition, the results of the present study showed that oral administration of Liv tablets had no negative effects on hepatic tissue and its functional tests in experimental groups II and III. Based on the above-mentioned findings, it can be concluded that Liv tablets (containing a high level of pure silymarin) are a good option for optimal protection against DOX-induced hepatotoxicity and in case of combination with other recommended methods can lead to significant beneficial results in the recovery of cancer patients and restoration of their liver function.

It should be mentioned that one of the limitations of this study was the small number of rats and the infections caused during the experiment. It is suggested to increase the length of the treatment period and use higher doses for remedy.

6. Conclusion

The findings of the present study confirmed the results of previous studies regarding that DOX has many side effects on liver function and structure. They also showed that oral consumption of 150 and 300 mg/kg Liv extract has no apparent side effects and can protect the liver against the toxicity of DOX and possibly other hepatotoxic medications.

Further research is required in the field of molecular pathology induced by DOX to develop the necessary strategies for the prevention of its toxicity. The Liv extract can be a candidate for the prevention of DOX-induced liver injury., Given the positive effects of Liv on the blood biochemical and histological characteristics of the liver in the present study, it is suggested to use gene expression tests in future research to collect more reliable data.

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Footnotes

Conflicts of Interest: The authors have no conflict of interest to declare.
Authors' Contributions: M.N. designed the article. H.J. and M.S. performed the laboratory tests.
Ethical Approval: Animal investigations were approved by the Ethics Committee of the animal house of the Gachsaran Branch of Islamic Azad University with the code of IR.IAUG.REC.1399.021
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