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Research Article

Hepatoprotective effects of silymarin on cyclophosphamide-induced hepatotoxicity in male rats

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ABSTRACT

Background: Cancer is the primary cause of mortality and a significant medical problem affecting life expectancy. Cyclophosphamide (CP) is a chemotherapeutic and immunosuppressive medication that effectively treats cancers. Several side effects on the body, such as hepatotoxicity, limit its use. The study aims to assess the possible protective effect of silymarin on liver-induced hepatotoxicity caused by cyclophosphamide in rats.

Materials and methods: 30 adult male albino rats were randomly divided into three equal groups: control group, Cyclophosphamide group, and Cyclophosphamide plus silymarin group. At the end of the experiment, rats were sacrificed, and blood analysis was done to assay the liver functions. Also, fresh liver specimens were split and processed for further tissue and histopathological studies.

Results: Rats exposed to CP had significantly lower levels of antioxidant system and higher levels of liver enzymes, markers of free radicals, and apoptosis compared to the control group. While rats exposed to CP plus silymarin had higher levels of antioxidant system and lower levels of liver enzymes, markers of free radicals, and apoptosis compared to that in CP only group. The histopathological changes in the rats' liver on exposure to CP revealed dilated congested central veins and portal tracts; both showed infiltration of acute inflammatory cells. Some hepatocytes appear swollen, with the presence of hepatocytes with pyknotic nuclei. While rats exposed to CP plus silymarin had fewer structural changes than the only group.

Conclusion: Silymarin ameliorated the cyclophosphamide-induced hepatotoxicity in male rats.

Introduction

Cancer is the primary cause of mortality and a significant medical problem affecting life expectancy (1). In 2019, World Health Organization (WHO) estimations show that cancer is first or second among 183 countries' significant causes of death before the age of 70 and ranks third or fourth among 23 more countries (2).

Cyclophosphamide (CP) is a commonly used chemotherapeutic and immunosuppressive medication that effectively treats cancers such as breast cancer, ovarian cancer, lymphoma, lung cancer, and prostatic carcinoma, as well as several autoimmune illnesses (3). Although CP has a wide range of therapeutic uses, its use is limited by several side effects on the body, such as hepatotoxicity (4).

High dosages of CP can result in acute hepatotoxicity due to oxidative stress and promotion of the inflammatory cascade (5). Those harmful effects of CP are believed to be caused by the CP metabolites phosphoramide mustard and acrolein, produced by hepatic microsomal cytochrome P450 (CYP450) (6). Acrolein induces oxidative stress, lipid peroxidation, and cellular damage by disturbing the cellular antioxidant defense system and generating reactive free radicals. Additionally, it initiates the synthesis of pro-inflammatory mediators, causing hepatotoxicity (7).

Silymarin is one of the most potent hepatoprotective chemicals, a natural flavonoid produced from Milk thistle or Silybum marianum (a member of the Asteraceae family) (8). Silymarin has anti-inflammatory, anti-cancer, and antioxidant effects by scavenging free radicals and raising glutathione peroxidase levels (9, 10).

Numerous studies have investigated silymarin's protective effects in preventing various adverse impacts of anticancerous drugs (11, 12).

In this study, we aimed to investigate the possible protective role of silymarin against cyclophosphamide-induced hepatotoxicity.

Materials and methods

Chemicals

Cyclophosphamide (Endoxan 1gm vial) was purchased from Baxter S.P.A company (Roma, Italy) and dissolved by regular saline.
Silymarin was purchased from Madaus Germany (Legalon capsule 140mg).

Animals

In this study, thirty adult male Wistar albino rats weighing between 110 and 135gm were utilized.

Experimental groups

1. Rats were randomly divided into three groups (10 rats/each group):
2. Control group: rats were fed on standard rat chow pellets.
3. Cyclophosphamide group: rats were injected (IP) with CP, in a dose of (100mg/kg) only once on the first day of the experiment.

Cyclophosphamide plus silymarin group: rats received a single intraperitoneal injection of cyclophosphamide in a dose of (100mg/kg) on the first day of the experiment, then received 50 mg/kg silymarin orally at a once-daily dose for six weeks.

Biochemical analysis

After the experiment, pentobarbital (50 mg/kg) was used to anesthetize all of the rats. Blood was drawn by decapitating the cervical region, and the sample was left undisturbed at room temperature for 15 to 30 minutes to allow it to clot. According to the manufacturer's instructions, ELISA analyzed serum liver enzymes (ALT, AST).

A specimen of liver tissue of rats of each group was dissected, homogenized in ice-cold sodium-potassium phosphate buffer (pH 7.4), centrifuged at 3000 rpm for 10 minutes, and then stored at −80 °C to estimate tissue levels of malondialdehyde (MDA), Superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (Cat) activity.

Histopathological analysis

A liver tissue specimen of rats of each group was fixed in formaldehyde 10%, underwent standard processing steps, and stained with either Hx.&E.(structural stain or caspase-3 immunostain (antiapoptotic stain) for further histological study by light microscope with a built-in camera (motion image).

Results

Effect of drug regimen on liver functions

Cyclophosphamide significantly increased serum ALT and AST compared to the control group. Conversely, significant reductions in ALT and AST were observed in the treated group with Cyclophosphamide plus silymarin compared to that in the Cyclophosphamide group (Table 1).

Table 1. Assay of the serum levels of liver enzymes in the study

Parameter/ Group	Control	CP	CP +Sily.
ALT(U/L)	29.2±1.8	82.54 ± 15.49*	39.81 ± 8.27·
AST (U/L)	27.4±2.7	71.4±10.92*	37.41± 6.37·

ALT: Alanine transaminase; AST: Aspartate transaminase; CP: cyclophosphamide; Sil.: Silymarin.
* significant increase compared to the control group.
· considerable decrease compared to the CP group.

Effect of drug regimen on the antioxidative system and free radicals

Rats exposed to CP had significantly lower levels of antioxidant system and higher levels of markers of free radicals compared to the control group. Rats exposed to CP plus silymarin had higher levels of antioxidant system and lower levels of markers of free radicals than those in the only group (Table 2).

Table 2. Assay of the levels of markers of oxidative/antioxidative in the study

Parameter/ Group	Control	CP	CP +Sily.
MDA (ng/ml wet tissue)	٤,٢٤ 85.71 ±	190.58 ± 16.25·	101.58 ± 7.35·
SOD (ng/ml)	6.81±0.57	3.45±0.82·	5.63±0.37·
GSH (ng/ml)	6.25±0.18	3.21±0.43·	5.38±0.19·
CAT (ng/ml)	147.28±7.71	82.17±9.49·	128.64±6.39·

MDA; malondialdehyde; SOD: Superoxide dismutase; GSH: reduced glutathione; CAT: catalase.
* significant increase compared to the control group.
· considerable decrease compared to the CP group.

Histopathological results

The structural changes in the rats' livers after exposure to CP revealed dilated congested central veins and portal tracts; both showed infiltration of acute inflammatory cells. Some hepatocytes appear swollen, with the presence of hepatocytes with pyknotic nuclei. Rats exposed to CP plus silymarin had fewer structural changes than the only group (Figure 1).

Apoptotic changes

The caspase-3 immune stain deposition in the hepatocytes was markedly deposited in rats exposed to CP compared to the control group. Rats exposed to CP plus silymarin had decreased caspase-3 immune stain deposition levels in the hepatocytes compared to that in the CP-only group (Figure 2).

Discussion

In the current study, we investigated the hepatotoxic effects of cyclophosphamide in rats and evaluated the possible protective effects of silymarin.

The liver function of albino rats showed deterioration on exposure to cyclophosphamide, as confirmed by increased serum ALT and AST compared to the control group. This was similar to the results of other studies, which revealed increased liver enzymes in CP groups compared to the control group (4, 13). The increased levels of liver enzymes are a direct indicator of Hepatocyte damage (14).

This study assessed the structural changes in the liver on exposure to CP. We found dilated, congested central veins and portal tracts, which showed infiltration of acute inflammatory cells. Some hepatocytes appeared ruptured, and some were swollen, with the presence of hepatocytes with pyknotic nuclei. In agreement with our results, previous studies revealed the presence of hepatocytes with pyknotic nuclei, along with dilated, congested central veins and portal tracts. Some hepatocytes appeared ruptured, while others were enlarged (15, 16).

According to reports, the toxic action of CP on different body organs, including the liver, brain, and kidney, is mediated by the breakdown of antioxidant defense mechanisms by acrolein, a metabolite of CP that causes excessive production of free radicals (17-19). Moreover, the hepatic cytochrome P450 system is responsible for the metabolism of cyclophosphamide, which leads to sinusoidal obstruction syndrome and subsequent hepatic vein necrosis, blockage, and congestion (16).

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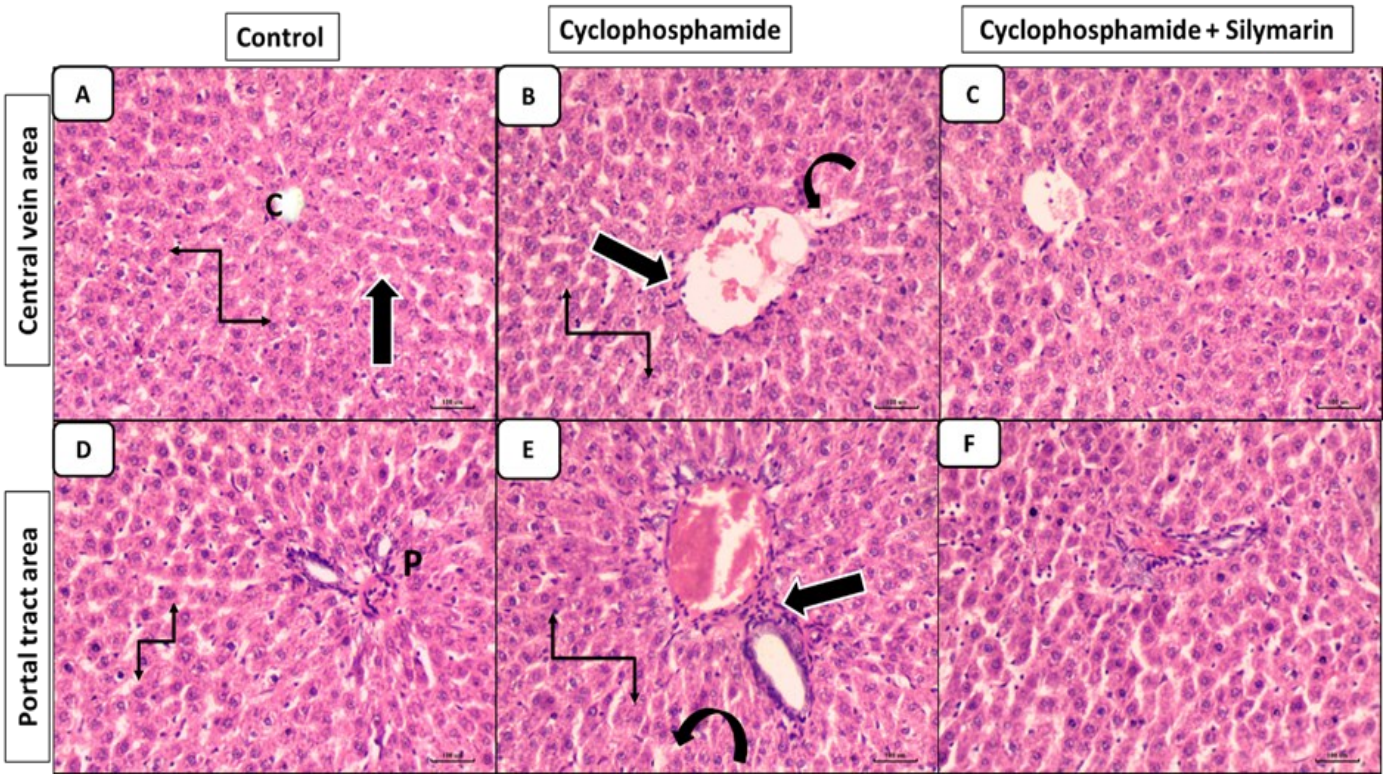

Figure 1. Photomicrographs of the liver sections of the study groups showing: the control group {A, D} showed standard hepatic architecture; central vein (C), portal tract (P); hepatocytes (double-sided arrow); blood sinusoids (thick arrow). The cisplatin group {B, E} appears with dilated congested central vein and portal tract with infiltration by inflammatory cells (thick arrow), dilated congested blood sinusoids (curved arrow), hepatocytes with pyknotic nuclei (double-sided arrow). While rats exposed to CP plus silymarin {C, F} had fewer structural changes than the CP-only group (HX.&E. stain, X400, scale bar 100um).

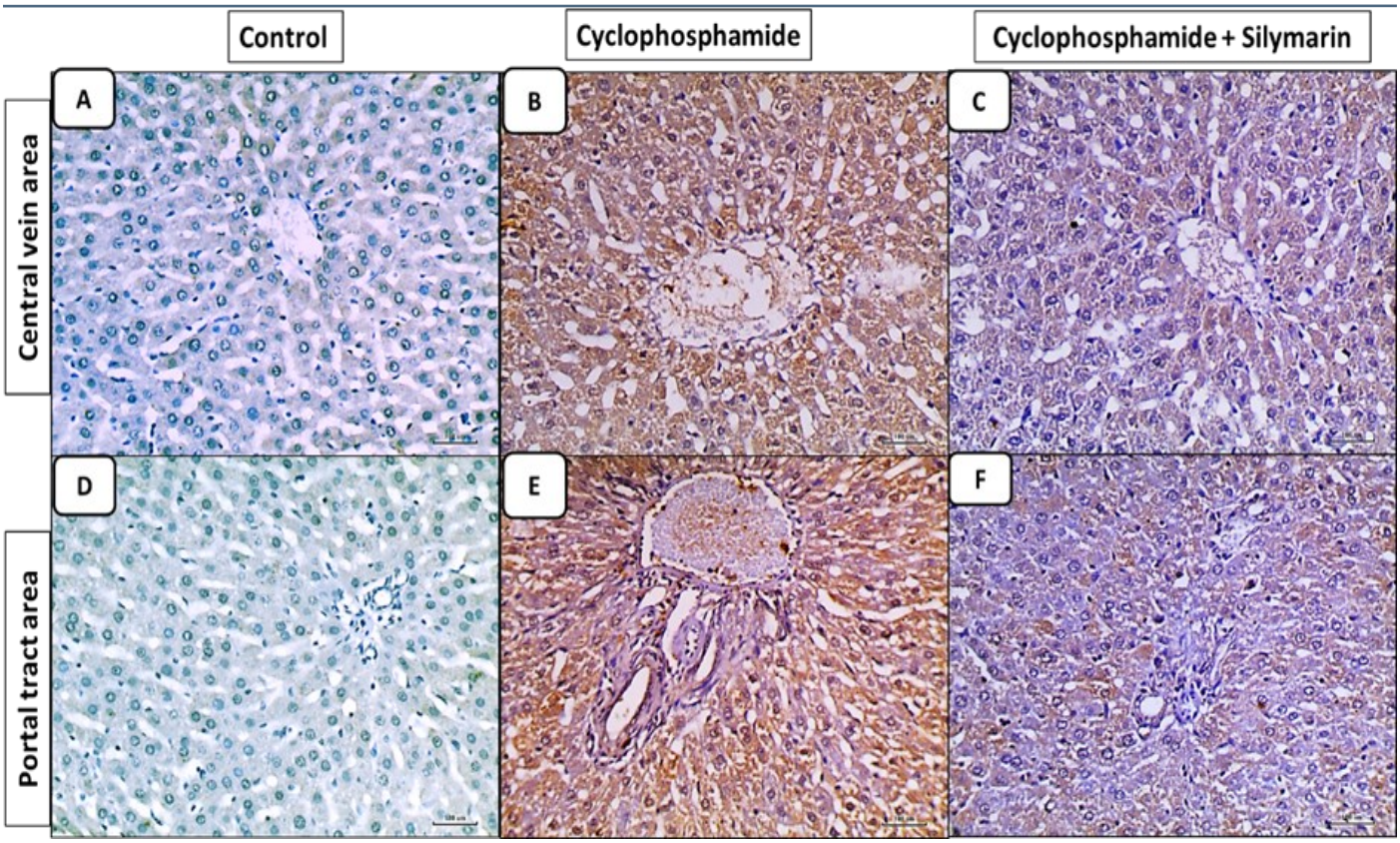

Figure 2. The caspase-3 immune stain deposition (brown color) in the hepatocytes was markedly deposited in rats exposed to CP compared to the control group. At the same time, rats exposed to CP plus silymarin had decreased caspase-3 immune stain deposition levels in the hepatocytes compared to that in the CP-only group (Caspase-3 immune stain, X400, scale bar 100um).

Moreover, the hepatic cytochrome P450 system is responsible for the metabolism of cyclophosphamide, which leads to sinusoidal obstruction syndrome and subsequent hepatic vein necrosis, blockage, and congestion (16).

In this study, we found that rats exposed to CP had lower levels of antioxidant system and higher levels of free radicals markers than the control group. This coincides with the results of other studies on rats exposed to CP (19, 20).

Under oxidative stress, tissue cells may become more permeable to the mitochondrial membrane, which could release cytochrome c into the cytosol and trigger the caspase cascade and apoptosis (21).

Many methods were used to assess tissue apoptosis, such as morphological, immunohistochemical, biochemical, immunological, and molecular biology (15). This study used caspase-3 immunostain to evaluate apoptosis in hepatic tissue. We found that caspase-3 immune stain deposition (brown color) in the hepatocytes was markedly deposited in rats exposed to CP compared to the control group. At the same time, rats exposed to CP plus silymarin had decreased caspase-3 immune stain deposition levels in the hepatocytes compared to that in the only group. In agreement with our results, according to literature reports, the rate of apoptotic cells was much higher in the tissues (liver, kidney, lung, testis) of rats given CP alone (15, 17, 22, 23).

Thus, developing a safe and effective antioxidant to prevent liver injury caused by CP is crucial. In the present study, silymarin was used as an antioxidant, and we found that rats exposed to CP plus silymarin had higher levels of antioxidative markers such as GSH, SOD, and CAT and lower levels of MDA (free radicals' markers). Similar results to our study were recorded in rats' liver, kidney, and testis exposed to CP plus silymarin compared to CP only(10, 11, 24). In this study, the antioxidant property of silymarin exposure plus CP led to ameliorating the structural pathological changes that appeared due to only CP exposure. This was in agreement with the results reported by several studies assessing silymarin treatment concomitant to treatment with antineoplastic drugs such as CP, 5-fluorouracil, and Doxorubicin (24-26).

Conclusion

Furthermore, the enhancement in liver structure after silymarin treatment was associated with enhancement in liver enzymes. This coincides with several literatures studying silymarin's effects on various anticancerous drugs-induced hepatotoxicity or nephrotoxicity (15, 26, 27).

From the above findings, we found that Silymarin ameliorated the cyclophosphamide-induced hepatotoxicity in male rats through antioxidative and antiapoptotic properties.

Declaration of Competing Interests

The authors declare no competing interests

Data sharing plans

Data will be available upon request

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