BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Protective effects of silymarin against paclitaxel-induced cardiac toxicity

[Efectos protectores de silymarin contra toxicidad cardiaca inducida por paclitaxel]

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Section Pharmacology

Received: 11 November 2023
Accepted: 4 January 2024
Accepted corrected: 16 January 2024
Published: 30 September 2024

Citation:
Aktaş I, Gur FM, Martínez JL, Bilgiç S, Körkoca H
Protective effects of silymarin against paclitaxel-induced cardiac toxicity
Bol Latinoam Caribe Plant Med Aromat
23 (5): 749 - 759 (2024)
https://doi.org/10.37360/blacpma.24.23.5.47

Abstract: In this study, the protectivity of silymarin (SY) against the harmful effects of paclitaxel (PX) on the heart was investigated. PX was administered 2 mg/kg intraperitoneally to the PX group, 100 mg/kg SY was administered by gavage to the SY group, and both drugs were administered to the PX + SY group as other groups. Treatment with SY significantly decreased cardiac troponin I (cTn-I), brain natriuretic peptide (BNP), creatine kinase isoenzyme MB (CK-MB) and lactate dehydrogenase (LDH) levels. In the PX group; the decrease in glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) levels and the increase in malondialdehyde (MDA) levels were significantly normalized with SY administration. Histologically; heart injury was significantly reduced in the PX + SY group compared to the PX group. As a result, it was determined that SY, which has antioxidant, anti-apoptotic and anti-inflammatory effects, could protect the heart tissue from the toxic effects of PX.

Keywords: Heart damage; Paclitaxel; Rat; Silymarin; Oxidative stress

Resumen: En este estudio, se investigó la protección de la silimarina (SY) contra los efectos nocivos del paclitaxel (PX) en el corazón. Se administraron 2 mg/kg de PX por vía intraperitoneal al grupo de PX, se administraron 100 mg/kg de SY por sonda al grupo de SY y ambos fármacos se administraron al grupo de PX + SY como a otros grupos. El tratamiento con SY disminuyó significativamente los niveles de troponina I cardíaca (cTn-I), péptido natriurético cerebral (BNP), isoenzima MB de creatina quinasa (CK-MB) y lactato deshidrogenasa (LDH). En el grupo PX; la disminución de los niveles de glutatión (GSH), catalasa (CAT) y superóxido dismutasa (SOD) y el aumento de los niveles de malondialdehído (MDA) se normalizaron significativamente con la administración de SY. Histológicamente; la lesión cardíaca se redujo significativamente en el grupo PX + SY en comparación con el grupo PX. Como resultado, se determinó que SY, que tiene efectos antioxidantes, antiapoptóticos y antiinflamatorios, podría proteger el tejido cardíaco de los efectos tóxicos del PX.

Palabras clave: Daño al corazón; Paclitaxel; Rata; Silimarina; Estrés oxidativo

INTRODUCTION

Cancer is one of the most important diseases of our age, which is becoming more and more widespread in the world. There are many chemotherapeutic agents in the treatment of cancer. One of these is paclitaxel (PX) obtained from the *Taxus brevifolia* tree (Massey *et al*., 2019). It should also be specified that commercial PX is obtained by a hemisynthesis process from the derivative 10-deacetylbaccatin, obtained from the aerial parts of the genus *Taxus*, given the complexity of its isolation (in terms of yield) and synthesis (it is not viable economically) (Sah *et al*., 2020). It is especially used in the treatment of breast, ovarian, pancreatic, esophageal, colon, head and neck cancers (Sarosy & Reed, 1993; Xie *et al*., 2002; Guo *et al*., 2005; Stage *et al*., 2018; Zang & Kagan, 2018). PX is a microtubule stabilizing pharmacological agent that promotes polymerization and prevents microtubules from separating (Singla *et al*., 2002; Bang *et al*., 2019). With this effect of PX on microtubules, it inhibits cell proliferation and causes the death of rapidly proliferating tumor cells (Zasadil *et al*., 2014). However, PX stimulates apoptosis by increasing mitochondrial reactive oxygen species (ROS) and cytochrome-c release (Varbiro *et al*., 2001). PX might negatively affect healthy cells as well as cancer cells (Rowinsky *et al*., 1993). In previous studies, depending on the PX application; side effect such as bone marrow suppression, hypersensitivity, nephro, cardio and hepatotoxicity, central and peripheral neurotoxicity were reported (Huizing *et al*., 1995; Thornton *et al*., 2008; Xie *et al*., 2015; Zang & Kagan, 2018; da Costa *et al*., 2020; Gür *et al*., 2022). In some studies, it has been determined that PX application causes histopathological changes such as diffuse edema, hemorrhage, congestion, degeneration, apoptosis, necrosis and hyaline exudate accumulation in the heart tissue (Saad *et al*., 2004; Malekinejad *et al*., 2016; Khaled *et al*., 2022). In some other studies, it has also been reported that PX increases tumor necrosis factor-alpha (TNF-α) secretion by macrophages and TNF-α expression in various tissues (Bogdan & Ding, 1992; Saad *et al*., 2004; Gür & Bilgiç, 2023).

Silymarin (SY) is a flavonoid derived from the seeds of the thistle plant (Silybum marinum) (Soto *et al*., 2004; Aktas & Bayram, 2020). It implies that SY is an isolated flavonoid, however it should be specified that they are flavolignans, and that silimaria is a mixture of flavolignans, of which silybins A and B stand out (between 50% and 60%) and their isomers isosilybins A and B (by 5%); silicristin (20%) and silidianin (10%) (Bijak, 2017). This agent is especially used in the treatment of gallbladder, liver diseases and amanita mushroom poisoning (Ding *et al*., 2001; Raskovic *et al*., 2011; Kocaman & Dabak, 2015). SY has anti-inflammatory, antioxidant, antiapoptotic, antiviral, anticarcinogenic and antifibrotic effects (Ramakrishnan *et al*., 2009; Shaker *et al*., 2010; Federico *et al*., 2017; Kocaman & Dabak, 2015; Aktaş & Armağan, 2019; Aktaş & Sevimli, 2020; Kim *et al*., 2020). Because of antioxidant effects, SY ameliorates tissue damage caused by chemical agents in liver, pancreas, kidney and heart tissues (Soto *et al*., 2004; Dashti-Khavidaki *et al*., 2012; Rafieian-Kopaie & Nasri, 2012; Aktaş *et al*., 2020; Aktas & Özgöçmen, 2020; Kim *et al*., 2020; Gür & Aktaş, 2022). SY also stimulates protein synthesis, affects lipid metabolism and stabilizes membrane phospholipids. In liver diseases; it reacts with ROS and converts them into less toxic and non-reactive structures. It increases the antioxidant activity of glutathione (GSH) and superoxide dismutase (SOD). In addition, it prevents the decrease in the density and functions of SOD and GSH and the deterioration of their structures. SY also treats cancer; modulates the imbalance between apoptosis and cell survival by regulating the cell cycle regulator (Raskovic *et al*., 2011).

Many drugs used in cancer treatment, including PX, might also cause damage to normal tissues. The side effects of chemotherapeutics negatively affect cancer treatment (Park *et al*., 2009). It has been reported that the application of antioxidants together with cancer drugs reduces the negative effects of these drugs on normal tissues (Padmavathi *et al*., 2006; Park *et al*., 2009; Salahshoor *et al*., 2019; Bilgic &Armagan, 2020; Bilgic *et al*., 2020; Gür *et al*., 2022). For the above reasons, in the present study, the protection of SY against the possible harmful effects of PX on the heart tissue was investigated.

MATERIAL AND METHODS

Chemicals

PX (100 mg/17 mL) was obtained as Actavis (Little Island, Cork, Ireland) and SY as Legalon fort (100 mg/kg capsule) from Madaus (Madaus, Istanbul, Turkey). Other chemicals used were obtained from

Sigma-Aldrich (USA).

Animals

In the application, Sprague-Dawley (28 pcs) female rat, 9 weeks old, weighing 230-280 g, were taken from Adiyaman University Experimental Animals Unit. After obtaining ethical approval, the study was started (2021/013). The animals were given fresh drinking water and oily feed every day. They were hosted at 50 ± 4% of humidity, 12:12 hours of light and dark, and 21 ± 3°C of temperature.

Experimental applications

1. Control (n=7): 1 mL of saline was given intraperitoneally (i.p.) for six days.
2. SY (n=7): 100 mg/kg SY was given as gavage for six days (Aktas & Ozgocmen, 2020).
3. PX (n=7): 2 mg/kg PX i.p. on days 0. 2. 4. and 6 of the study to implemented via (Bilgic & Ozgocmen, 2019).
4. PX + SY (n=7): In the study, PX and SY were applied together as their own groups.

At the end of the experimental procedures, all rats were anesthetized using intramuscular (i.m.) injections of ketamine hydrochloride (45 mg/kg) and xylazine (5 mg/kg). Sacrification of rats was performed using the spine dislocation method blood samples were centrifuged at 5000 xg for 15 minutes and the resulting sera were stored at -86°C for biochemical tests. Heart tissues were divided into two equal parts, one of them was placed at -86°C for biochemical tests and the other one was placed in fixation solution for histopathological tests.

Histopathological analysis

The heart tissues, which were fixed for 24 hours by immersion in a 10% buffered neutral formalin solution, were passed through alcohol and xylol series and embedded in paraffin. Tissue sections obtained by cutting paraffin blocks with a thickness of 5 µm using a microtome were subjected to routine histological procedures and stained with hematoxylin & eosin method (Balcıoglu *at al*., 2021; Gur *at al*., 2021). After staining, tissue sections were examined with an Olympus Bx53 light microscope and pictures of these tissue sections were photographed with the camera of the same microscope (DP 80).

Immunohistochemistry

Paraffin tissue blocks were cut at 5 µm with a microtome and the resulting sections were placed on polylysine-coated slides. Microwave irradiation antigen retrival technique (Gur *et al*., 2011, Gur & Timurkan, 2016a) was applied to the sections passed through the xylol and alcohol series, respectively. Then, tissue sections were incubated with anti-TNF-α (ab220210, Abcam, Cambridge, MA, USA) primary antibody diluted 1/200 with Phosphate Buffered Saline (PBS) for 16-20 hours at 4°C in a humid environment. Sections used as negative control were incubated with PBS instead of primary antibody. The staining was completed by performing the next procedures as stated in the previous studies (Gur & Timurkan, 2016b; Gur & Aktas, 2020). After staining, the photographs of the tissue sections examined with the above-mentioned microscope were taken with the same camera.

Relative heart weight and body weight

The weight of the subjects was determined at the beginning and end of the experiment. After the heart was washed with cold salt water, it was dried on a filter paper and its weight was determined. Relative heart weight; It was calculated using the formula below.

Relative Heart W. (%) = Heart W. x 100 Body W.

Biochemical tests

Serum biochemical biomarker

Creatine kinase isoenzyme MB (CK-MB) and lactate dehydrogenase (LDH) were determined by commercial kit (Biolabo, Maizy, France) and auto analyzer (Airone 200 RA; Crony Instruments, Rome, Italy). Cardiac troponin I (cTn-I) was determined by a commercial enzyme-linked immunosorbent assay (cTnI ELISA, DRG®; BiSino Biotechnology and Science Inc., Beijing, China). Brain natriuretic peptide (BNP) level was determined by enzyme immunoassay (Biosit Diagnostics, San Diego, CA, USA) (Bilgic *et al*., 2020).

Heart oxidative stress biomarker

Homogenization of heart tissue was conducted with a homogenizer (Turrax T 25, Wilmington, USA). Homogenates were obtained by centrifugation of samples (0.5-1.0 g) at 10,000 ×g for approximately 15 minutes at 4°C. Catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) parameters were determined using with a

spectrophotometer (UNICO Instruments C., Dayton, USA). Assay of protein density, Lowry et al. (1951) method (Lowry *et al*., 1951).

MDA: Draper and Hadley's (1990) method was used for the analysis. The reaction of TBA with MDA occurs at 95°C, 15 minutes and pH 3. The pink pigment formed at maximum absorption at 532 nm. It was then measured with the spectrophotometer (Draper & Hadley, 1990).

GSH: Ellman's (1959) method was utilized for the analysis. The reaction developed with the chemicals added to the sample and a yellow-green color was formed. Measurement is made at 410 nm absorbance (GSH) with the spectrophotometer (Ellman, 1959).

SOD: Marklund and Marklund method (1974) was used for the analysis. Autoxidation of pyrogallol was inhibited. Enzyme activity was completed at 440 nm in 3 minutes. The activity of SOD was determined as U/mg Hb (Marklund & Marklund, 1974).

CAT: Ten percentages tissue homogenates are formed by centrifugation at 8500 xg in 0.9% NaCl for 3 minutes at 4°C. The measurement was conducted by assaying the hydrolysis of H2O2 with phosphate buffer at pH 7.0. Its level was determined as nmol/mg protein at 240 nm absorbance (Aebi, 1984).

Statistical analysis

Statistical analysis was performed using SPSS 20.0. Data are given as mean ± standard error of mean. Normality was measured with the Shapiro-Wilk test. Parametric in biochemical parameters comparison of values within and between groups, was conducted with directional LSD and ANOVA. In addition, the Crustal-Wallis test was used for histopathological results. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Relative difference of the body weight and relative heart weight

The results are reported in Table No. 1. It was observed that the final weights of the SY, PX, and PX + SY groups decreased significantly compared to the initial weights ($p<0.03$). Relative heart weight was increased significantly in the PX group compared to the control, SY, and PX + SY groups ($p<0.015$). SY treatment produced a significant decrease in the PX-stimulated relative heart weight ($p<0.004$).

Table No. 1
Changes in the relative heart weight, the mean body weight, and relative difference

Stage of study	Control	SY	PX	PX + SY
Rel. heart. W. (mg/ 100 g B.W.)	$3.50 \pm 0.03^{c,d}$	$3.51 \pm 0.04^{c,d}$	$4.70 \pm 0.02^{a,b,d}$	$3.60 \pm 0.01^{a,b,c}$
Initial body weight (g)	249 ± 4	250 ± 3	251 ± 3	252 ± 0
Final body weight (g)	$264 \pm 3^{c,d}$	$240 \pm 3^{c,d}$	$215 \pm 3^{a,b,d}$	$225 \pm 4^{a,b,c}$
Rel. dif. of body weight (%)	5 ± 1^{c}	6 ± 1	$13 \pm 1^{a,d}$	11 ± 2^{d}
p ANOVA	0.004	0.022	0.015	0.004

Data are means ± SEM, n=7. The mean body weight is expressed in grams. PX, paclitaxel; SY, silymarin; Rel. Kid. W, relative heart weight. [a]significantly different from control at $p<0.05$, [b]significantly different from SY at $p<0.03$, [c]significantly different from PX treated rats at $p<0.02$, [d]significantly different from PX + SY treated rats at $p<0.01$.

Biochemical results

Serum biochemical biomarkers

We measured LDH, CK-MB activities, and cTn-I and BNP levels in serum; the results are reported in Table No. 2. LDH and CK-MB activities were significantly increased in the PX group compared to the control group ($p<0.01$). LDH and CK-MB activities decreased significantly in the PX + SY group compared to the PX group ($p<0.03$). The cTn-I and BNP levels were increased significantly in the PX group compared to the control group ($p<0.02$), while the cTn-I and BNP levels were decreased significantly in the PX + SY group compared to the PX group ($p<0.02$) (Table No. 2).

Heart tissue oxidative stress biomarkers

The PX group exhibited a significantly higher level of MDA ($p<0.03$) and a significantly lower level of GSH ($p<0.02$), SOD ($p<0.04$) and CAT ($p<0.02$) activities compared to the control group. SY

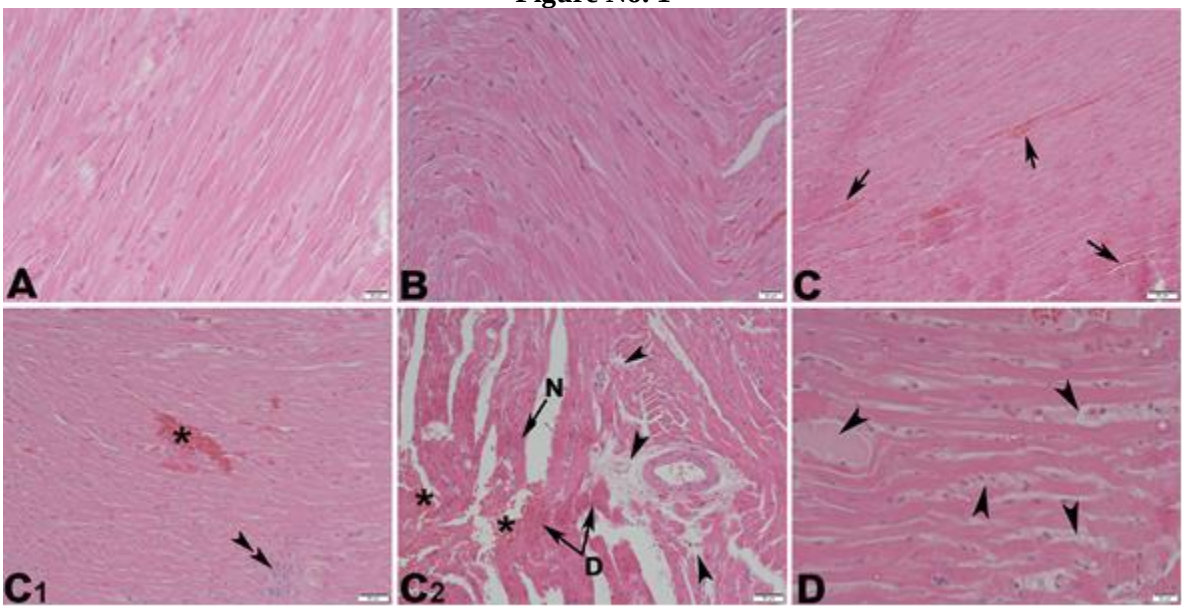
treatment produced a significant decrease in the PX-stimulated MDA level (p<0.04). Also, SY treatment significantly increased the reduced SOD and CAT activities and GSH levels caused by PX (p<0.05) (Table No. 2).

Table No. 2
Serum biochemical and cardiac tissue oxidative stress biomarkers

	Control	SY	PX	PX + SY
Serum biochemical biomarkers				
Serum LDH (U/l)	**487.15 ± 4.26^{c,d}**	**494.84 ± 2.90^{c,d}**	**889.00 ± 5.78^{a,b,d}**	**541.90 ± 2.87^{a,b,c}**
Serum CK-MB (U/l)	**650.01 ± 4.76^{c,d}**	**651.48 ± 3.51^{d}**	**911.00 ± 42.48^{a,d}**	**648.58 ± 2.47^{a,b,c}**
cTn-I (pg/ml)	**1.00 ± 0.042^{c,d}**	**1.02 ± 0.04^{c,d}**	**1.79 ± 0.04^{a,b,d}**	**1.10 ± 0.05^{a,b,c}**
BNP (pg/ml)	**70.72 ± 1.87^{c,d}**	**71.74 ± 2.69^{d}**	**81.43 ± 1.11^{a,b,d}**	**70.86 ± 2.69^{a,b,c}**
Cardiac tissue oxidative stress biomarkers				
GSH (µmol/g)	**0.18 ± 0.02^{b,c,d}**	**0.18 ± 0.03^{c,d}**	**0.14 ± 0.02^{a,b,d}**	**0.18 ± 0.01^{a,b,c}**
MDA (nmol/g tissue)	**0.25 ± 0.03^{c,d}**	**0.26 ± 0.02^{c}**	**0.40 ± 0.03^{a,b,d}**	**0.31 ± 0.03^{a,c}**
SOD (U/g)	**3.68 ± 0.27^{b,c,d}**	**3.70 ± 0.03^{c,d}**	**2.85 ± 0.21^{a,b,d}**	**3.64 ±0.27^{a,b,c}**
CAT (K/g)	**0.10 ± 0.032^{c,d}**	**0.10 ± 0.03^{c,d}**	**0.06 ± 0.01^{a,b,d}**	**0.7 ± 0.01^{a,b,c}**

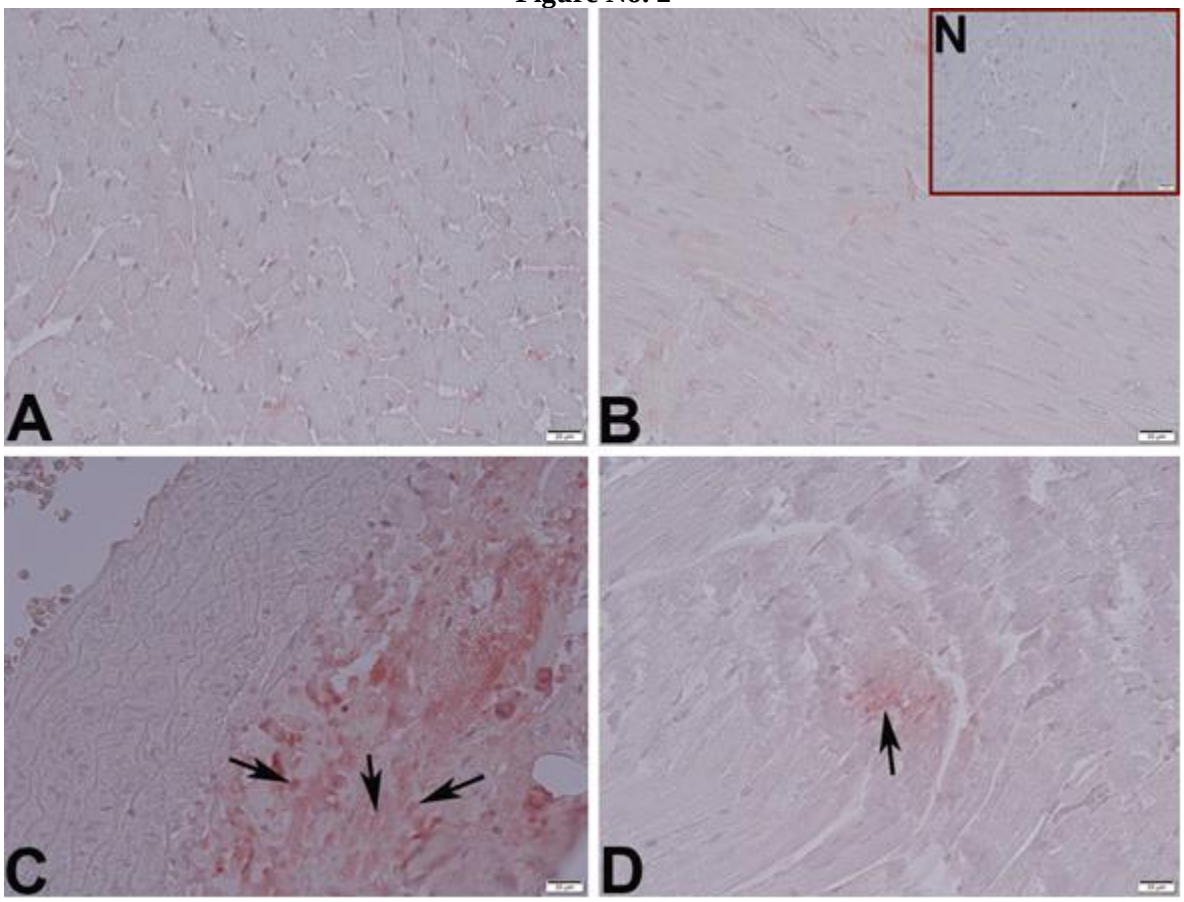
Data are means ± SEM, n=7. PX, paclitexel; SY, silymarin; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB; cTn-I, cardiac troponin I; BNP, brain natriuretic peptide; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase. ^{a}significant difference from control group, ^{b}significant difference from SY group, ^{c}significant difference from PX group, ^{d}significant difference from PX + SY.

Figure No. 1



Hematoxylin & eosin staining of heart tissue sections of control (A), SY (B), PX (C, C1, C2), PX + SY (D) groups. Aserix, hemorrhage; Arrow, congestion; Arrowhead, edema; double arrowhead, mononuclear cell infiltration; N, necrosis (A, B, Dx400; C, C1, C2x200)

Figure No. 2



Immunohistochemical localisation of TNF-α in heart tissue sections of control (A), SY (B), PX (C), PX + SY (D) groups. Arrows, TNF-α positive myocytes. N, Negative control (A, B, C, D, N×400)

Histopathological analysis

In contrast to the control and SY groups, which showed a normal histological structure, many histopathological changes occurred in the PX group (Figure No. 1A, 1B, 1C, 1C1, 1C2). Histopathological findings; congestion, edema, hemorrhage, apoptosis, mononuclear cell infiltration, and degeneration characterized by hypereosinophilia were observed in the heart tissue of PX group (Figure No. 1C, 1C1, 1C2). On the other hand, the histological structure of the heart tissue in rats in the PX + SY group was almost the same as in the control group, except for edema (Figure No. 1D).

Immunohistochemical analysis

When TNF-α expressions in the heart tissues of control and PX group rats were examined, it was determined that both groups were TNF-α-negative (Figure No. 2A, 2B). TNF-α-positive areas were observed as focal foci in the heart tissues of PX group rats (Figure No. 2C). In the PX + SY group, the number of TNF-α-positive staining areas and the intensity of staining observed in PX group rats were significantly reduced compared to the PX group (Figure No. 2D). Sections reserved as negative controls were TNF-α-negative (Figure No. 2).

DISCUSSION

Relative heart weight increased in the PX group compared to the control. There is a relationship between PX exposure and change in heart weight. Lower mean body weight of PX compared to control group Park *et al.*, (2009) coincides with his study (Park *et al.*, 2009). In the PX + SY group, SY administration produced an increase in mean body weight and a decrease in relative heart weight. Has

shown a curative effect on the biosystem of SY. In the study of Nasr (2013), body weight loss decreased after SY treatment following cisplatin administration, and this result is consistent with our results (Nasr, 2013). In the study of Aktaş & Armağan (2019), the decreased body weight in the rats given valproic acid was normalized with SY, which is consistent with the present study. These results show us that SY treatment prevents weight loss by reducing energy loss, increasing energy input, and improving storage (Aktaş &Yahyazadeh, 2022).

According to the serum results obtained from rats exposed to PX in our study; CK-MB, cTn-I, BNP, LDH values increased compared to the control group. In the PX + SY group samples, this situation was reversed and approached to normal. Increased lipid peroxidation in heart tissue causes degeneration of myocyte cell membranes and causes cardiac enzymes to leak and increase more than normal (Aktas & Ozgocmen, 2020). CK-MB is an isoenzyme of creatine kinase. It is found in 15% of the myocardium. Myocardial cell death causes CK-MB elevation. Its level in the circulation increases in acute myocardial infarction cases (Kurapati & Soos, 2020). In the study conducted by Saad *et al*. (2004), PX increased CK-MB and LDH parameters, which are cardiac parameters in rats, and this information is in line with the present study. BNP parameter is used as an important biomarker for individuals with cardiac hypertrophy, heart failure and hypertension. BNP is secreted from cardiomyocytes in response to cardiac stress and ischemia. In addition, the increase in BNP is considered an important factor in cardiorenal disease (Okamoto *et al*., 2019). Troponin is a protein complex found in striated (including heart) muscle. CK-MB, BNP, LDH and cTn-I are indicators of human heart muscle damage (Cooper *et al*., 2014; Malekinejad *et al*., 2016). Malekinejad *et al*. (2016), reported that CK-MB levels increased in rats treated with PX, and a significant decrease in CK-MB levels was observed with royal jelly application. In the study conducted by Nasr (2013) serum BNP, LDH, cTn-I and CK-MB levels, which are markers of cardiac damage, increased with doxorubicin (DOX) application. This increase is indicative of developing cellular damage and deterioration in functional integrity. Treatment with SY brought these increased values closer to normal. Raskovic *et al*. (2011), found in their study that serum CK and LDH parameters increased significantly in DOX-treated rats compared to the control group. It was also observed that the parameters approached normal values by giving SY to these rats. This result is consistent with the present study. According to the mechanism of these events, the cell membrane contains various polyunsaturated fatty acids. The attack of free radicals converts multiple fatty acid side chains into lipid peroxides. This membrane leakage develops as a result of the rupture of the damaged membrane (Malekinejad *et al*., 2016).

There is a balance between antioxidants and ROS to maintain a healthy metabolism. If this balance is tipped in favor of harmful radicals, oxidative stress occurs. This situation may cause lipid peroxidation, which causes biomolecular damage. MDA; It is one of the lipid peroxidation products, which is an important element of oxidative stress (Tanbek *et al*., 2017). The level of oxidative stress is regulated by enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidant systems (Ohkawa *et al*., 1979; Shoji & Koletzko, 2007; Birben *et al*., 2012; Arora & Singh, 2014). In the present study, it was determined that the MDA level of the PX group increased significantly compared to the control group, while the levels of SOD, CAT and GSH decreased. SY treatment produced a significant decrease in the PX-stimulated MDA level. Also, SY treatment significantly increased the reduced SOD and CAT activities and GSH levels caused by PX. SY probably achieves these effects through its antioxidant and free radical scavenging properties. Aktaş & Özgöçmen (2020) stated in their study that the increased MDA and decreased GSH due to the effect of valproic acid returned to normal as a result of SY application. The data obtained in this study are consistent with our study. Rao & Viswanath (2007) found that myocardial infarction in the heart was effective in reducing the levels of antioxidant markers (GSH, CAT and SOD). Therefore, in our study SY therapy reversed the oxidation damage caused by PX in cardiac tissues by inhibiting lipid peroxide formation and blocking oxidative reactions. SY may be a potential treatment for attenuating and preventing the complications of PX in clinical practice.

In cases of toxicological changes in the myocardium, antioxidant enzymes effectively scavenge free radicals that damage cells. In addition, these enzymes convert harmful radicals into low-

toxicity or non-toxic products. If toxicity in heart tissue exceeds antioxidant capacity, antioxidant enzyme activities are suppressed and may cause oxidative stress (Saad *et al*., 2004; Malekinejad *et al*., 2016; Khaled *et al*., 2022). PX application in heart tissue; It causes pathological changes such as edema, bleeding, congestion, inflammatory cell infiltration, apoptosis and necrosis. The findings obtained in the PX group in the current study are consistent with these literatures and prove the toxic effects of PX on heart tissue. In the PX + SY group, no pathological changes were observed except for edema and mild congestion. This result suggests that SY can be used effectively as a preventative against the toxic effects of PX on the heart. In the immunohistochemical analyzes performed in the current study, it was observed that the heart tissue of the control and SY group rats was TNF-α-negative. An intense TNF-α-positive staining was observed in the PX group compared to the other groups. This finding is in line with publications reporting that PX increases TNF-α-expression in the liver, brain, and other tissues (Bogdan & Ding, 1992; Saad *et al*., 2004; Gür & Bilgiç, 2023). In the PX + SY group, the TNF-α-positive immunostaining intensity decreased significantly compared to the PX group and approached the control group. This result is in line with studies reporting that the effect of PX on TNF-α expression in different tissues can be reduced by antioxidant protective agents (Gür & Bilgiç, 2023).

CONCLUSION

When the biochemical and histopathological findings obtained in the present study were evaluated together, it was determined that PX caused damage at the molecular and histopathological level by increasing oxidative stress in the heart tissue. It was determined that SY greatly improved the negative effects of PX on the heart tissue, especially with its antioxidant effects. These results obtained; shows that the combined use of PX with SY during cancer treatment can protect the heart tissue from the negative effects of PX.

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