Evaluating Protective Effects of Silymarin on Liver and Cardiac Side Effects of Chemotherapy Drugs in Childhood Acute Lymphoblastic Leukemia

Mehdi Ghaderian MD¹, Nahid Reisi MD^{2,3,*}, Alireza Moafi MD², Safora Farasat MD²

- 1. Isfahan University of Medical Sciences, Isfahan, Iran.
- 2. Faculty of Child Growth & Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
- 3. Isfahan Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
- *Corresponding author: Dr. Nahid Reisi, Department of Pediatric Hematology and Oncology, Seyed-Al-Shohada Hospital, Isfahan University of Medical Sciences (IUMS), Isfahan, Iran. Email: reisi@med.mui.ac.ir

Received: 05 February 2017 **Accepted:** 02 April 2017

Abstract

Background: The protective effects of silymarin on liver and heart have been investigated and identified by many researchers. This study evaluates the protective effects of this substance on hepatotoxicity and cardiotoxicity of chemotherapy drugs in leukemic children.

Materials and Methods: In this study, 71 children, aging 2-14 years old, going through the maintenance phase of acute lymphoblastic leukemia (ALL) treatment at Children's Cancer Center of Isfahan University of Medical Sciences (Iran) in 2015 were studied. Two patients died and the remaining patients were divided into intervention and control groups. Intervention group received Mercaptopurin and Methotrexate plus Silymarin 140mg/day and control group were taking only Mercaptopurin and Methotrexate. Liver enzymes, Coagulation tests, Creatine kinase- Muscle and Brain (CK-MB), Ejection fraction and Systolic function (SF) were measured at the first, and 3 and 6 months' intervals from the commencement of study. The Data was analyzed by using SPSS and $P \le 0.05$ was considered as statistically significant. Associations between variables were analyzed using independent t-test, chi square, and repeated measures ANOVA test.

Results: Mean levels of Alanine aminotransferase in both groups increased significantly at 6 months interval (P=0.01, 0.01, for control and intervention groups respectively). Although increment was more among controls, there were no significant differences between the two groups (P= 0.85). Furthermore, Aspartate aminotransferase and Alkaline phosphatase increased in both groups and the increment was significant in the controls (P=0.001, 0.05, for control and intervention groups respectively). Coagulation tests prolonged in the first 3 months in both groups and the prolongation was more in control group. Changes in CK-MB were not significant in intervention and controls (P=0.07, 0.10, respectively) but ejection fraction and SF decreased in both groups, especially among controls.

Conclusion: It seems that Silymarin can be useful in prevention of hepatotoxicity and cardiotoxcity of chemotherapy drugs for ALL children.

Keywords: Acute lymphoblastic leukemia, Cardiotoxity, Hepatotoxicity, Silymarin

Introduction

Although chemotherapy drugs, especially anthracyclines, have numerous benefits in treatment of childhood lymphoblastic leukemia (ALL), the cardiac side effects have still limited their clinical use. Cardiac dysfunctions and disorders can range from asymptomatic diseases to severe heart failure (1-2) and include: systolic diastolic dysfunction, cardiomyopathy, arrhythmia, and pericardial effusion (1, 3-6). These

complications are mostly created by cumulative dose of drugs, but can be presented by administration of the first dose or during the treatment without considering drug dosage or even as late as 20 years from the initial exposure (7). Duration of drug use, total amount of the drug used during treatment procedure and simultaneous prescription of the drug with another medication can increase cardiac toxicity (8). Correlations have been reported in some studies between these

drugs and decreased cardiac function that may last many years (9,10). Trans-thoracic echocardiography is an important safe diagnostic way for evaluating the effects of these drugs on systolic and diastolic heart function (5, 11).

The mechanisms of anthracyclines cardiotoxicity are multi-factorial. These drugs "induce multiple forms of cellular injuries by free radical production, alter nucleic acid biology by intercalation into DNA, and modulate intracellular signaling that lead to cell death and disruption of homeostatic process, such as sarcomere maintenance" (7). So substances, such as silymarin and its isolated components, can be protective for cardiomyocytes because of their antioxidant effects and cell membrane stabilizing as well as radical scavenging potency (12, 13).

One of the other complications that most commonly are observed in the treatment of childhood ALL is hepatic side effects. aminotransferases Serum elevation with combination associated a ofmethotrexate and 6MP used maintenance phase of the therapy has been commonly used (14). Five-fold elevation of these enzymes causes drug interruption (15) that can increase the risk of relapse in childhood ALL (16). Moderate rise in bilirubin or reduced levels of coagulation factors may be seen, however, there is a low risk of permanent liver damage and within few weeks after discontinuing of drug administration, the enzymes usually are normalized (17). Mercaptopurine influenced by age and metabolism. genotype and drug response, is related to cell (RBC) metabolite blood concentrations. In patients, aged 6 years or less, steady-state is lower than the older patients and these children require higher doses to achieve clinical improvement. Hepatotoxicity, induced by 6-MP, is strongly related to concentration of 6methylmercaptopurine nucleotide MMPN) in red blood cells. A 6-MMPN threshold of 5000 pmol/8 × 108 RBC has been reported with hepatotoxicity (18).

MTX was reported to be more hepatotoxic than 6-MP and it was witnessed that its chronic low-dose may lead to hepatic cirrhosis and fibrosis in the maintenance phase of ALL treatment. MTX is usually the first drug to be adjusted or discontinued when a hepatotoxicity occurs and the enzymes abnormalities can resolve within one month after the cessation of therapy (14, 18).

Therefore, the of hepato use cardioprotective substances, such Silymarin, which is an herbal extract of silybum marianum plant with known hepatoprotective and cardioprotective effects may be effective for these patients (13). This substance has been used for years for its protective effects against liver and biliary diseases, toxin and druginduced hepatitis, and cirrhosis. It can reduce the duration of therapy in acute hepatitis and can improve the liver enzyme profile in chronic hepatitis (12, 19, 20). The protective effect of silymarin on liver and heart has been more related to anti-oxidant effects which eliminate free radicals. On the other hand, it acts as a potent antioxidant by scavenging free radicals of the body. In addition, this substance can increase RNA polymerase activity in the nucleus of hepatocytes and can initiate ribosomal protein synthesis improves hepatic cell re-generation and can inhibit lipid peroxidation, reduce glutathione oxidation, and enhance liver detoxification (13).Cardioprotective activities of Silymarin were primarily shown in sodium fluoride and Cisplatininduced cardiotoxicity in rat models (21, 22) and then, during ischemia-reperfusion injuries and inflammations due to coronary artery bypass grafting surgeries in rats and humans (23, 24). These effects are due to replenishing endogenous antioxidant enzymes. suppressing neutrophil infiltration and reducing serum malondialdehyde as end product of myocardial lipid peroxides (13).

Based on the above information, the present study evaluated the protective

effects of Silymarin on livers and hearts of a small group of Iranian leukemic children undergoing chemotherapy using Livergol tablet containing 70 or 140 mg Silymarinmade in Iran.

Materials and Methods

This study was conducted in a 12-month In this clinical trial (IRCT2017071935175N1), 71 children, aged 2-14 years old, in the maintenance phase of the treatment of ALL were studies. They had received antracycline 25mg/m2 /week/4 times in induction phase and 3 times in reinduction phase (totally 175mg/m2). All of the patients were selected from Children's Cancer Center (at Seyed-Al-Shohada Hospital) of Isfahan University of Medical Sciences, located in the central area of Iran in 2015. The Research Council and Ethics Committee of School of Medicine approved this study (Code: 394213, IR.MUI.REC.1394.3.213 written informed respectively) and consents were obtained from the parents who agreed with participation of their children in this study.

The inclusion criteria were diagnosis of childhood ALL based on bone marrow morphology and immunophenotype, age range of 2-14 years old, and therapy in the maintenance phase of childhood ALL (at least 6 months). Previous history of liver and heart diseases and being under any other kind of medication except chemotherapy drugs included exclusion criteria of the study. To calculate the sample size, the standard formula suggested for clinical trials was used by considering a= 0.05 and the power of the test equal to 80%. Finally, a total of 20 cases in each group were estimated and they increased to 49 samples control group to cover 20% anticipated dropout. $n=((z 1+ z 2)^2 ([2s]^2)/d^2$ z = 1.96, z = 0.84, d = two-tailed t-testof the difference between means for ALT and AST parameters (0.8*s).

Of all the patients included in this study (n=71) two patient died. The remainder of

the patients (n=69) were divided into two intervention (n=20) and control (n=49) groups. In intervention group, the patients were taking MTX: 20mg/m2/week/po [EBEWE Pharma Ges.m.b.h. Austria] and 6MP: 75mg/m2/day/po [KOREA United Pharma Inc] at least 6 months (25) plus Silymarin: 140mg / day (26) in 2 divided doses by using Livergol herbal tablet containing 70 mg Silymarin [Goldaru Pharma Com, Iran]. Patients in control group were taking only 6MP and MTX with a similar dose in interventions.

Data collection

At the beginning phase, and within 3 and 6 months' intervals from the commencement of the study, patients' blood was tested for liver enzymes: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), coagulation tests: ProthrombinTime (PT), activated Partial Thrombin Time (aPTT), International Normal Ratio (INR(and CK-MB (as a marker of heart injury). Liver enzymes were measured by biochemical methods (Elitech kit, Man Company under license of France, Iran), coagulation studies were done using Fisher kit, Netherlands, and CK-MB were also measured by biochemical methods (Parsazmoon kit, Karaj, Iran).

Ejection Fraction (EF) and SF were measured in all patients by echocardiography at the first and at intervals of 3 and 6 months from the commencement of the study. All data was recorded in specific checklists.

Statistical Analyses

Data analyses were performed using SPSS (version 22). Continuous software variables were reported as the mean \pm SD, and P value ≤ 0.05 was considered as statistically significant. Changes in ALT, AST, ALP, CK- MB, PT, aPTT, INR, EF, and SF were compared in two groups at first, at two different time intervals, the first 3 and the first 6 months of study. The association between variables investigated using independent t-test, chiand repeated measures square test,

ANOVA test. A two-sided α level of 0.05 was used to assess the statistical significance.

Results

71 patients participated in this study two of whom (2.8%) died and the remainder were divided as intervention and control groups. It needs to be noted that distribution of age and sex in groups did not show any significant differences (Table Measurement of ALT in the two groups showed that the mean levels of ALT in and control groups increased significantly during 6 months (P-value= 0.01, 0.01, respectively), and that the ALT increment was more in control than case group, however, comparisons between the two groups revealed that there were no significant differences after 3 and 6 months of the study (Table II).

Furthermore, changes in the mean levels of AST are shown in Table II. The results showed that unlike ALT, AST increment was only significant in controls after 3 and 6 months, and there were no significant differences in comparison of ALT between two groups, after 3 and 6 months.

Alkaline phosphatase changes in the control group had similar AST changes during 6 months (P-value=0.04,) but changes in the case group and comparison s between the two groups were not significant (Table II).

According to the ANOVA test results, PT, aPTT, and INR increased in the first 3 months in both groups, but the increment was only significant in control group (P-value= 0.01, 0.03, and 0.04, respectively). Despite the increase in CK-MB at the first 2 months in both groups, along as in CK-MB.

Despite the increase in CK-MB at the first 3 months in both groups, changes in CK-MB at the end of study in cases and controls were not significant (P-value= 0.07, 0.10, respectively).

Echocardiography results indicated both EF and SF decrease in the second 3 months in case and control groups (Table II). Although these changes were not significant during 6 months, according to the ANOVA test, the decline was higher in control group. In this study, two-fold ALT increase was more in cases than controls, but five-fold or more ALT increase based on age which led to discontinuation of treatment was more among controls [Table III].

Table I. Demographic variables in intervention and control groups

Variable		Control	Intervention
Gender	Boy	29(65.5%)	12(60%)
	Girl	20(34.5%)	8(40%)
	Total	49(100%)	20(100%)
Age	Mean age ±SD	6.4±3.1	6.7±3.1

Table II. ALT, AST, ALP, PT, aPTT, INR, EF, and SF in interventions and controls at the beginning, 3, and 6 months intervals of the study

Variable	Time	Control Group	Intervention Group	P- value
ALT	At the beginning	33.8±3.7	32.8±6.2	0.88
	After 3 months	122.9±32.4	44.3±9.9	0.1
	After 6 months	52.6±7.9	50±11.4	0.85
	P- value	0.007	0.009	
AST	At the beginning	35.3±2.2	35.4±3.7	0.91
	After 3 months	87.7±14.9	37.7±4.7	0.28
	After 6 months	50.9±5.3	49.5±9.5	0.88
	P- value	0.001	0.11	
ALP	At the beginning	400.7±20	357.8±46.1	0.33
	After 3 months	450±26.8	400.1±48.5	0.35
	After 6 months	446.1±23.5	400.8±43.7	0.33
	P- value	0.046	0.45	
PT	At the beginning	12.9±0.27	15.8±2.4	0.26
	After 3 months	15.9±0.55	16.8±2.5	0.72
	After 6 months	13.8±0.44	16±2.5	0.42
	P- value	0.009	0.139	
aPTT	At the beginning	33.3±1.3	32.4±3	0.79
	After 3 months	43±3	46±9	0.74
	After 6 months	35±1.7	37.4±3.3	0.67
	P- value	0.031	0.27	
INR	At the beginning	1.1±0.05	1.27±0.18	0.41
	After 3 months	1.4±0.07	1.24±0.07	0.17
	After 6 months	1.2±0.06	1.18±0.05	0.62
	P- value	0.045	0.28	
EF	At the beginning	68±7.14	70±6.21	0.46
	After 3 months	69.2±6.43	72.5±4.6	0.15
	After 6 months	65.6±9.3	71.9±5.9	0.04
	P- value	0.147	0.304	
SF	At the beginning	38.2±5.4	38.7±4.5	0.77
	After 3 months	37.3±5	40.7±3.96	0.07
	After 6 months	36.1 ±5.6	39.9±4.6	0.08
	P- value	0.406	0.25	

Table III. Increasing two and five-fold of ALT and AST in case & control groups

Variable	Control Group	Intervention Group		
	Multiplied by 5	Multiplied by 2	Multiplied by 5	Multiplied by 2
ALT	10(20.4%)	15(30.6%)	2(10%)	3(15%)
AST	5(10.2%)	16(32.6%)	0	2(10%)

Discussion

As mentioned earlier, side effects and toxicity of cytotoxic drugs is a problem in the treatment of malignancies. Toxicity of these drugs is due to their inability to differentiate between normal malignant cells. Thus, cytoprotective compounds play a very important role in ameliorating the toxicity associated with these drugs. Compounds as dexrazoxane, glutathione, mesna, etc. have been extensively investigated in the past (27). ingredients with cytoprotective effects, however, may also be effective in ameliorating side effects of chemotherapy drugs.

This study evaluated the effects of Silymarin as an herbal cytoprotective (13, 28-31) on livers and hearts of leukemic children during the 6 months maintenance therapy. The findings revealed that although there were no significant differences in comparing the two groups after 3 and 6 months of study, increased liver enzymes levels were less in patients who received Silymarin than the control group. Studies that assessed the effects of herbal medicine showed the efficacy of this substance in decreasing liver enzymes and mortality of liver disease (32, 33). Saller et al., evaluated 106 liver disease patients who were treated with Silymarin and placebo and showed more reduction and normalization of liver enzymes in patients treated with Silymarin than the placebo (34). Although most of studies suggested that Silvmarin is effective in reducing liver enzymes, Vargas-Mendusa et al., showed that the levels of liver enzymes are not different in patients treated with Silymarin (35). These differences in results of studies may have occurred due to the dosage of drug, stage of liver disease, and different planning in studies unraveling the need to review articles. In the present study, lack of significant difference in the amount of liver enzymes in comparison between the two groups after 6 months may be due to length of the study or dosage of drug and

longer studies with more doses of Silymarin recommended.

In addition to decrease in liver enzymes, protective effects of Silymarin in the fall of clotting factors was reported in intoxicated dogs (36). Vargas-Mendusa et al., which evaluated the hepatoprothective effects of Silymarin, showed that using this substance can improve Prothrombin Time. In present study also patients received Silymarin had fewer changes in coagulation tests especially for Prothrombin Time.

Another problem in the treatment of malignancies is cardiotoxicity secondary anthracyclines. Echocardiographic evidence of left ventricular contractile abnormalities was seen in up to 65% of patients with a history of a childhood malignancy treated with doxorubicin (37). In a study on 14,358 five-year survivors of childhood malignancies, patients that used <250 mg/m2 anthracyclinehad 2.4-fold higher risk of developing congestive heart failure compared to those patients who did not receive (38). Free radical formation caused by doxorubicin metabolism and formation of doxorubicin-iron complexes that catalyze conversion of hydrogen peroxide to hydroxyl radical and result in the generation of reactive oxygen species are the responsive of cardiotoxicity. In animal models probucol and carvedilol due to antioxidant properties have been shown protective effects against doxorubicininduced left ventricular dysfunction. In human in vitro experiments and some clinical studies have also shown beneficial effects of antioxidant therapy on cardiac function (39).

Because of the interaction of doxorubicin with non-heme iron in the body iron chelating agents may be effective in prevention of antracyclines cardiotoxicity. Protective effects of Dexrazoxane as an iron chelator in reducing the incidence of congestive heart failure have been shown from a long time ago (40) therefore Silymarin as an iron chelating agent (41)

may be effective on cardiotoxicity of these drugs.

In this study, EF was decreased in the second quarter in both groups and although these decreases were not significant, the amount of decline was more in controls than the interventions. Animal studies showed the protective effects of silymarin against antracyclines cardiotoxicity (42, 43) but there is lack of sufficient data in human therefore, the need for these studies is clear.

Conclusion

In conclusion, it seems that using Silymarin in leukemic children has protective effects on their liver and heart and can decrease the side effects of chemotherapy drugs.

The limitations of this study were small sample size and short duration of study. Therefore longer studies with larger sample size are recommended in the future.

Acknowledgments

The authors acknowledge of all patients and their parents for their kind cooperation and also appreciate Mr. Akbar Hasanzadeh for statistical analysis.

Conflict of interest

All authors declare that they have no conflict of interest.

References

- 1.Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. N Engl J Med 1998;339(13):900-5.
- 2.Lipshultz SE, Alvarez JA, Scully RE. Anthracycline associated cardiotoxicity in survivors of childhood cancer. Heart 2008;94(4):525-33.
- 3.Monsuez JJ, Charniot JC, Vignat N, Artigou JY. Cardiac side-effects of cancer chemotherapy. Int J Cardiol 2010;144(1):3-15.

- 4.Shan K, Lincoff AM, Young JB. Anthracycline-induced cardiotoxicity. Ann Intern Med 1996;125(1):47-58.
- 5.Bu'Lock FA, Mott MG, Oakhill A, Martin RP. Left ventricular diastolic function after anthracycline chemotherapy in childhood: relation with systolic function, symptoms, and pathophysiology. Br Heart J 1995;73(4):340-50.
- 6. Zuppinger C, Timolati F, Suter TM. Pathophysiology and diagnosis of cancer drug induced cardiomyopathy. Cardiovasc Toxicol 2007;7(2):61-6.
- 7.Geisberg CA, Sawyer DB. Mechanisms of anthracycline cardiotoxicity and strategies to decrease cardiac damage. Curr Hypertens Rep 2010;12(6):404-10.
- 8. Keizer H, Pinedo H, Schuurhuis G, Joenje H. Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. Pharmacol Ther 1990;47(2):219-31.
- 9.Myers C. The role of iron in doxorubicin-induced cardiomyopathy. Semin Oncol; 1998;25(4 Suppl 10):10-4.
- 10.Ganame J, Claus P, Uyttebroeck A, Renard M, D'hooge J, Bijnens B, et al. Myocardial dysfunction late after low-dose anthracycline treatment in asymptomatic pediatric patients. J Am Soc Echocardiogr 2007;20(12):1351-8.
- 11.Lebaron S, Zeltzer LK, Lebaron C, Scott SE, Zeltzer PM. Chemotherapy side effects in pediatric oncology patients: drugs, age, and sex as risk factors. Med Pediatr Oncol 1988;16(4):263-8.
- 12.Dixit N, Baboota S, Kohli K, Ahmad S, Ali J. Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. J pharmacol 2007;39(4):172-79.
- 13.Moayedi Esfahani BA, Reisi N, Mirmoghtadaei M. Evaluating the safety and efficacy of silymarin in β-thalassemia patients: a review. Hemoglobin 2015;39(2):75-80.
- 14.Kinga PD, Perryb MC. Hepatotoxicity of Chemotherapy. Oncologist 2001;6:162-76.

- 15.ALL IC-BFM 2009 A Randomized Trial of the I-BFM-SG FOR the Management of Childhood non-B acute Lymphoblastic Leukemia. Final Version of Therapy Protocol from August-14-2009. 16.Schmiegelow Schrøder K, H, Gustafsson G, Kristinsson J, Glomstein A, Salmi T, et al. Risk of relapse in childhood acute lymphoblastic leukemia is related to **RBC** methotrexate and mercaptopurine metabolites during maintenance chemotherapy. Nordic Society for Pediatric Hematology and Oncology. J Clin Oncol 1995;13:345-51. 17. Schmiegelow K, Nielsen SN, Frandsen Nersting Mercaptopurine/Methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction. J Pediatr Hematol Oncol 2014;36(7):503-17. 18. Adam de Beaumais T, Fakhoury M, Medard Y, Azougagh S, Zhang D, Yakouben K, et al. Determinants of mercaptopurine toxicity in paediatric acute lymphoblastic leukemia maintenance
- 19. Feher J, Lengyel G. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Curr Pharm Biotechnol 2012; 13(1): 210-7.

Clin

J

Pharmacol

- 20.Mayer KE, Myers RP, Lee SS. Silymarin treatment of viral hepatitis: a systematic review. J Viral Hepat 2005; 12(6): 559-67.
- 21. Nabavi SM, Nabavi SF, Moghaddam AH, Setzer WN, Mirzaei M. Effect of silymarin on sodium fluoride-induced toxicity and oxidative stress in rat cardiac tissues. An Acad Bras Cienc 2012; 84(4): 1121-6.
- 22.El-Awady el SE, Moustafa YM, Abo-Elmatty DM, Radwan A. Cisplatininduced cardiotoxicity: Mechanisms and cardioprotective strategies. Eur J Clin Pharmacol 2011; 650(1): 335-41.
- 23.Rao PR, Viswanath RK. Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial

- infarction in albino rats. Exp Clin Cardiol 2007; 12(4): 179-87.
- 24. Altaei T. Protective effect of silymarin during coronary artery bypass grafting surgery. Exp Clin Cardiol 2012; 17(1): 34-8.
- 25.Lanzkowsky Ph. Manual of pediatric hematology and oncology. 5th ed. Academic Press (Elsevier Inc): USA; 2011.
- 26.Mayo Clinic [homepage on the Internet]. Milk thistle(Silybum marianum) Dosing -. [updated: 2013 Nov 01; cited 2017 June 15] Available from: http://www.mayoclinic.org/drugs-supplements/milk-thistle/dosing/hrb-
- supplements/milk-thistle/dosing/hrb-20059806
- 27. Links M, Lewis C. Chemoprotectants: a review of their clinical pharmacology and therapeutic efficacy. Drugs 1999 Mar;57(3):293-308.
- 28.Kren V, Walterová D. Silybin and silymarin--new effects and applications. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2005;149(1):29-41.
- 29.Borsari M, Gabbi C, Ghelfi F, Grandi R, Saladini M, Severi S, et.al. Silybin, a new iron-chelating agent. J Inorg Biochem 2001;85(2-3):123-9.
- 30.Adibi A, Shayganfar A, Moayedi BS, Gharagozloo M, Maraashi J, Maracy M, et al. Therapeutic effects of deferoxamine and silymarin versus deferoxamine alone in β-thalassemia major based on findings of liver MRI. J Res Med Sci 2012; 17(Spec 1): S73-S78.
- 31.Feher J, Lengyel G. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Curr pharm biotechnol 2012;13(1):210-7.
- 32.Bode JC, Schmidt U, Dürr H. [Silymarin for the treatment of acute viral hepatitis? Report of a controlled trial .(author's transl)]. Med Klin 1977;72(12):513-8.
- 33.Fallah Huseini H, Alavian S, Toliat T, Jamshidi A, Heshmat R, Naghdi Badi H, et al. The efficacy of herbal medicine Khar Maryam (Silybum marianum (L.)

therapy.

Br

2011;71(4):575-84.

- GAERTN.))on liver cirrhosis in chronic hepatitis B patients. JMP 2005;1(13):1-6. 34.Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs 2001;61(14):2035-63.
- 35. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González A, Esquivel-Soto J, Esquivel-Chirino C, García-Luna Y, et al. Hepatoprotective effect of silymarin. World J Hepatol 2014;6(3):144-9.
- 36.Floersheim G.L, Eberharda M, Tschumia P, Duckerta F. Effects of penicillin and silymarin on liver enzymes and blood clotting factors in dogs given a boiled preparation of Amanita phalloides. Toxicol Appl Pharmacol 1978;46(2):455–62.
- 37.Grenier MA, Lipshultz SE. Epidemiology of anthracycline cardiotoxicity in children and adults. Seminars in oncology 1998;25(4 Suppl 10):72–85.
- 38.Mulrooney DA, Yeazel MW, Kawashima T, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer retrospective analysis of

- the Childhood Cancer Survivor Study cohort. BMJ 2009;339:b4606.
- 39.Volkova M , Russell R. Anthracycline Cardiotoxicity: Prevalence, Pathogenesis and Treatment. Curr Cardiol Rev 2011;7(4): 214–20.
- 40.Kwok JC1, Richardson DR. The cardioprotective effect of the iron chelator dexrazoxane (ICRF-187) on anthracyclinemediated cardiotoxicity. Redox Rep 2000;5(6):317-24.
- 41.Moayedi B, Gharagozloo M, Esmaeil N, Maracy MR, Hoorfar H, Jalaeikar M. A randomized double-blind, placebo-controlled study of therapeutic effects of silymarin in β-thalassemia major patients receiving desferrioxamine. Eur J haematol 2013;90(3):202-9.
- 42.Rašković A, Stilinović N, Kolarović J, Vasović V, Vukmirović S, Mikov M. The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules 2011;16(10):8601-13.
- 43. Stilinović N. Influence of silymarin and doxorubicin on the myocardial function in rats. Med pregl 2008;61(1-2):95-8.