PSM Biological Research ISSN(Online): 2517-9586

Research Article 2020 | Volume 5 | Issue 1 | 14-21

Article Info

Open Access

Citation: Othman, S.S., Ali, S.M., El- Deeb, N.M., 2020. Protective Effect of *Silybum marianum* Extract against Doxorubicin Induced Toxicity in Male Rats. PSM Biol. Res., 5(1): 14-21.

Received: November 18, 2019

Accepted: December 27, 2019

Online first: January 17, 2020

Published: January 29, 2020

***Corresponding Author:**
Sarah Samir Othman

Email:
sarahosman2014@yahoo.com

Scan QR code to see this publication on your mobile device.

Protective Effect of *Silybum marianum* Extract against Doxorubicin Induced Toxicity in Male Rats

Sarah Samir Othman[1*], Safaa Mohamed Ali[2], Nehal M. El- Deeb[3]

[1]Pharmaceutical Bioproducts research department, [2]Nucleic Acid research department, [3]Biopharmaceutical products research department, Genetic engineering and biotechnology research institute, City of Scientific Research and Technological Applications, New Borg El-Arab City 21934, Alexandria, Egypt.

Abstract:
Silybum marianum (Milk thistle) has been used in the treatment of various diseases including liver diseases and the prevention and treatment of cancer and has also been known to be used as food. Therefore, in the present research, the safety usage of milk thistle extract and its ability to alleviate doxorubicin-induced toxicity in rats was examined. Male Wistar rats were divided into 4 groups, 1st group was used as control, the 2nd, 3rd, and 4th groups administrated plant extract (100 mg/ml/Kg, orally), doxorubicin (15 mg/kg, i.p.) and plant extract plus doxorubicin respectively for 15 days except for doxorubicin which was injected on the 14th day. Data of present research proved the ability of milk thistle extract to alleviate doxorubicin-induced toxicity on various biochemical and hematological parameters, regulation of COX1 gene expression level as well as decreasing plasma and liver TBARS and increasing total antioxidant capacity, reduced GSH, GST, and GPx that minimized oxidative stress induced by the drug. In conclusion, milk thistle extract could be used during doxorubicin chemotherapy treatment as a natural source to avoid the side effects induced by this chemotherapeutic agent.

Keywords: Milk thistle, liver diseases, male Wistar rats, doxorubicin, chemotherapy.

INTRODUCTION

Doxorubicin commercially known as Adriamycin is a chemotherapeutic drug used to treat cancer. This includes lymphoma, acute lymphocytic leukemia, breast cancer, and bladder cancer. It is frequently used together with other chemotherapeutic agents. Doxorubicin is injected through the vein (The American Society of Health-System Pharmacists, 2017). Doxorubicin induces oxidative stress (Mai *et al*., 2016). Most chemotherapeutic agents' major mechanism for cytotoxicity to normal cells is due to their oxidative damage (Joshi *et al.,* 2001).

The popularity of complementary and alternative medicine (CAM) has increased primarily herbal medicine (Shahzad *et al.,* 2017; Iqbal and Ashraf, 2019a; Iqbal and Ashraf, 2019b). Its acceptance among physicians has increased despite the ongoing debate of integrating it into mainstream healthcare (Joos *et al.*, 2011).

Mediterranean milk thistle, blessed milk, Mary thistle, milk thistle, Scotch thistle, and variegated thistle are common names for *Silybum marianum* (Natural Resources Conservation Service, 2015). This practically characteristic thistle has glistening light green leaves with white veins and purple to red flowers. Initially a local of Southern Europe through to Asia, it is currently originated all over the globe. It has been used in Europe to prevent hepatic and biliary disorders, protect liver and kidneys from toxicity induced by medications as well as being an antidote for Amanita mushroom poisoning. The German Commission E recommends it for the treatment of dyspeptic complaints, toxin-induced liver damage, and hepatic cirrhosis and as supportive therapy for chronic inflammatory liver conditions (El-Kamary *et al.*, 2009).

Milk thistle is a family Asteraceae, which is one of the most widely used medicinal plants. It has been useful in the treatment of a range of liver and gall bladder disorders, including hepatitis, jaundice and cirrhosis, and protection of liver poisoning from environmental toxins, including insect stings, snakebites, mushroom poisoning and alcohol and chemicals (Abenavoli *et al.*, 2010). *Silybum marianum* contains numerous hepatoprotective bioactive molecules such as lipids, in the form of linoleic, oleic and palmitic acid and flavonoids including quercetin, eriodictyol, taxifolin, and chrysoeriol. It was proved the constituents responsible for the activity are flavanolignans (flavanone derivatives) initially isolated as a mixture of addition products of a coniferyl alcohol, phenylpropanoid alcohol, and a 2, 3-dihydroflavonol, taxifolin. This mixture's name is silymarin and consists of silybin isosilibin, silychristin and silydianin, in addition to silimonin, isosilibinin and isosilychristin (Wu *et al*., 2009). Silymarin converts reactive oxygen species (ROS) to harmless molecules and stops the reduction in glutathione and superoxide dismutase concentrations (El-Shitany *et al.*, 2008). Silymarin was proved to increase the uptake and actions of chemotherapeutic agents as suggested by several in vitro and in vivo studies. In vitro and animal, data suggest that silymarin may increase the uptake and actions of chemotherapeutic agents. Preclinical investigations emphasized that silymarin increased daunomycin accumulation, potentiated doxorubicin activity, and inhibited the efflux of these drugs from cancer cells (Colombo *et al.*, 2011).

The present research aimed to use milk thistle extract as a mixture of antioxidants to alleviate oxidative stress-induced toxicity by doxorubicin.

MATERIALS AND METHODS

Chemicals

Doxorubicin was obtained from Sigma Chemical Company; St Louis, MO, USA. The dose selection for doxorubicin was used

according to the previous work of Mello et al. (2017).

Ethical approval

This research was carried out at the Pharmaceutical and Fermentation Industries Development Center in the City of Scientific Research and Technological Applications (SRTA-city) and approved by its ethics committee (IACUCs) l IACU # 11-1H-1019.

Safety assay of using milk thistle extract

Neutral red assay protocols were used to quantify the safety patterns of plant extract on preferable blood mononuclear cells (PBMCs) cells using neutral red assay protocols. Briefly, about, $6X10^4$ cells/ml cell suspension was seeded in 96 well plates and incubated at 37°C in 5% CO_2 incubator till semiconfluency. After 24 hrs., the exhausted media were discarded and replaced with serially diluted plant extract prepared in RPMI media. The inoculated plates were incubated for 48 hrs., the Cytotoxicity percentages of the plant extract were quantified using neutral red.

Preparation of plant extract

Silybum marianum extract prepared in which 100 gm wet plant was dissolved in 100 ml distilled water and boiled for 5 minutes, homogenized and filtered then lyophilized. The doses were prepared in which 1gm was dissolved in every 10 ml.

Experimental animals

Thirty-six male Wistar rats (140–170 g) were used in this study. Rats were kept on basal diet and tap water which were provided *ad libitum* and were kept under standard conditions which conformed to the National Institutes of Health (NIH) guidelines. Animals were divided into four groups after 14 days of acclimation, 9 rats each in which control was the first group, the second, third and fourth administrated the plant extract (100 mg/kg BW, orally), doxorubicin (15 mg/kg BW, IP), the plant extract plus doxorubicin respectively. The experiment was performed for 14 days, doses of the extract were given daily but doxorubicin was given on 14th day.

Blood collection and tissue preparation

24 hrs after the last injection, blood samples were collected from the sacrificed animals then placed on ice, then they were centrifuged for 20 min at 3000 rpm, the obtained plasma was stored at −80°C. Tissues samples were separately minced and homogenized in ice-cold sodium and potassium phosphate buffer (0.01 M, pH 7.4). The homogenate was centrifuged for 20 min at 4°C at 3,000 rpm and the supernatant was used.

Quantification of liver COX1

At the end of the *in vivo* study, RNA extraction kit purchased from Thermo scientific was used to extract total liver RNAs. Strand cDNA synthesis Thermo scientific purchased kit was used to synthesize cDNA. Real-time polymerase chain reaction (Rt-PCR) was conducted using β-actin as an internal control reference by Qiagen Syber Green master mix. Table (1) illustrates the list of used primers.

Table 1. The list of primers used in gene expression analysis.

Primers	Sequence
Cox-1	F. 5′-AGGAGATGGCTGCTGAGTTGG-3′
	R. 5′-AATCTGACTTTCTGAGTTGCC-3′
β-actin	F. 5'- GTGGGGCGCCCCAGGCACCA -3'
	R. 5'- CTCCTTAATGTCACGCACGATTTC-3'

Thiobarbituric acid reactive substances and antioxidants

Plasma and liver supernatant thiobarbituric acid-reactive substances (TBARS) were calculated using the Esterbauer and Cheeseman method (1990). Total antioxidant capacity was measured by Koracevic et al. (2001) method. Reduced glutathione content was estimated using Beutler et al. (1963) method.

Biochemical parameters

Kits from BioSystems (Spain) were used to examine total protein (Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2005), albumin (Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2012), urea, creatinine, LDH (lactate dehydrogenase), γ-GT (γ- Glutamyl transferase), ALT (alanine transaminase) and AST (aspartate transaminase) (Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2005) in stored plasma samples. Whereas thiobarbituric acid was purchased from Sigma Chemical Company, St Louis, MO, USA.

Hematological parameters

Blood samples were collected from the animals 24 h after the last dose and placed on ice. Heparin was used as an anticoagulant. Red Blood cells (RBC), Hemoglobin (HGB), Mean corpuscular hemoglobin (MCH), Hematocrit (HCT), Mean corpuscular volume (MCV) and platelets (PLT) were examined in non-coagulated blood using HA-VET CLINDIAG obtained from Clindiag Systems Co.

RESULTS

Safety assays

The neutral red assay protocol results (Table 2 and Figure1) indicated that the usage of *S. marianum* extract didn't record any significant toxicity on PBMCs after 48 hrs. The maximum used concentration (10 mg/ml) showed about 17.3 cytotoxicity percentage on PBMCs cellular proliferation. Also, by treating PBMCs cells with 1.25 mg/ml of plant extract, the cellular proliferation rate increase with percentage 157.82 (Figure 1).

Bodyweight and Biochemical parameters

There was no significant change in body weight. Treatment with *Silybum marianum* extract alone significantly increased albumin and decreased AST. On the other hand, injection with doxorubicin alone significantly decreased total protein and albumin and increased urea, creatinine, LDH, γ-GT. ALT and AST. Administration of *Silybum marianum* extract with doxorubicin was able to alleviate its toxicity on most of the estimated parameters.

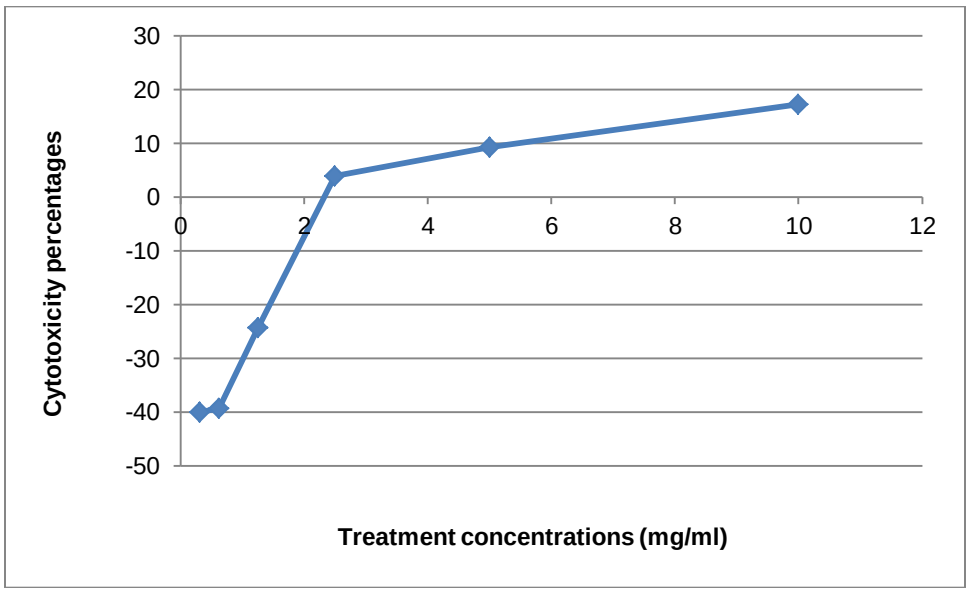

Fig. 1. Cytotoxicity results of milk thistle extract

Table 2. Effect of Silybum marianum extract administration on various parameters.

Parameters	Group 1	Group 2	Group 3	Group 4
Body weight(gm)	150±5.03^{ab}	155±7.99^{ab}	127±5.91^{b}	154±2.57^{ab}
Biochemical				
Total protein (g/L)	75.2±0.66^{a}	76.8±0.67^{a}	62.6±1.71^{b}	63.9±1.42^{bc}
Albumin (g/L)	42.6±1.13^{b}	57.7±1.41^{a}	19.3±0.32^{d}	26.4±1.27^{c}
Urea (mg/dl)	29.5±2.63^{cd}	28.7±2.71^{d}	42.5±2.89^{a}	35.3±3.12^{bc}
Creatinine(mg/dl)	0.91±0.02^{c}	0.92±0.01^{c}	2.40±0.14^{a}	1.74±0.12^{b}
LDH(U/L)	170±2.97^{c}	169±6.92^{c}	435±24.4^{a}	311±16.9^{b}
γ-GT(U/L)	24.3±0.44^{c}	22.4±0.71^{c}	35.5±0.67^{a}	29.6±1.11^{b}
ALT(U/L)	30.3±1.56^{c}	28.3±1.13^{c}	64.1±3.77^{a}	51.3±1.80^{b}
AST(U/L)	27.3±1.62^{b}	24.4±0.93^{c}	38.1±1.45^{a}	36.4±0.89^{a}
Antioxidants				
Plasma				
TAC(mM/L)	1.01±0.05^{b}	1.92±0.04^{a}	0.69±0.03^{d}	0.84±0.02^{c}
TBARS(nmol/ml)	0.72±0.02^{c}	0.36±0.01^{d}	2.60±0.11^{a}	1.55±0.02^{b}
GSH(µmole/ml)	4.56±0.16^{b}	5.07±0.18^{a}	2.88±0.07^{c}	4.39±0.21^{b}
GPx(U/ml)	8.18±0.18^{ab}	8.63±0.29^{a}	7.56±0.12^{c}	7.84±0.19^{bc}
GST(µmol/hr/ml)	1.04±0.08^{ab}	1.18±0.06^{a}	0.64±0.05^{d}	0.93±0.04^{bc}
Liver				
TAC(mM/L)	0.77±0.03^{b}	0.94±0.01^{a}	0.57±0.02^{d}	0.68±0.02^{c}
TBARS(nmol/gm wet tissue)	27.8±0.53^{c}	19.2±1.32^{d}	61.7±1.32^{a}	29.0±1.53^{b}
GSH(^{µmole}/gm wet tissue)	6.44±0.16^{b}	7.41±0.39^{a}	4.85±0.22^{c}	5.36±0.21^{c}
GPx(U/mg protein)	30.8±0.98^{a}	31.7±0.93^{a}	24.2±1.12^{b}	28.6±1.24^{a}
GST(µmol /hr/mg protein)	0.98±0.04^{bc}	1.16±0.08^{a}	0.78±0.04^{d}	0.95±0.05^{c}
Hematological				
Plasma RBC's(10^{6} /µL)	7.53±0.09^{ab}	7.43±0.14^{b}	5.78±0.25^{c}	7.95±0.21^{ab}
HGB(g/dL)	10.1±0.19^{c}	11.5±0.26^{b}	8.60±0.26^{d}	12.5±0.23^{a}
HCT(%)	35.2±0.53^{c}	38.0±1.52^{b}	29.4±1.66^{d}	39.5±1.77^{a}
MCV(fL)	46.6±1.75^{c}	48.4±2.37^{ab}	43.2±1.34^{c}	50.7±0.26^{a}
MCH(Pg)	12.9±0.54^{c}	13.9±0.29^{ab}	13.7±0.76^{ab}	15.4±0.53^{a}
PLT(10^{3}/µL)	356±17.6^{a}	332±18.7^{a}	170±16.6^{c}	284±32.7^{b}

Data are presented as Mean ± S.E, S.E: Standard Error. Mean values within a row not sharing common superscript letters (a, b, c, d) were significantly different, $p<0.05$. Group 1: control group, Group 2: Plant extract group, Group 3: Doxorubicin group, Group 5: Plant extract + doxorubicin group.

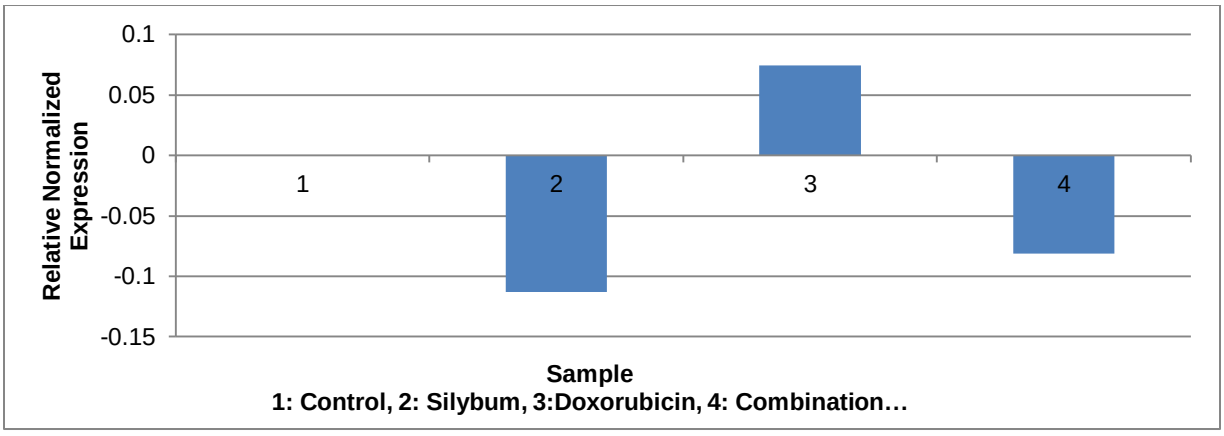

Fig. 2. Gene expression results in liver COX1 gene.

Hematological parameters

Silymarin administration significantly increased HGB and HCT whereas RBC, HGB, HCT, and PLT were decreased in rats treated with doxorubicin while MCH increased. Treatment with *Silybum marianum* extract with doxorubicin minimized its toxic effect on these measured parameters.

Oxidative stress and antioxidants

Doxorubicin injection significantly increased plasma and liver TBARS and decreased their TAC, GSH, GST, and GPx. Whereas *Silybum marianum* extract administration overcame the harmful effect of oxidative stress induced by doxorubicin via decreasing plasma and liver TBARS and increasing TAC and various antioxidants in rats.

Gene Expression

COX-1 expression was increased upon treatment with doxorubicin whereas Silymarin treatment decreased this elevation (Figure 2).

DISCUSSION

Rašković et al. (2011) suggested that silymarin prevented the dramatic loss in body weight than the group treated with doxorubicin alone although this decrease was not significant. They concluded that doxorubicin causes irreversible heart failure and confirmed the ability of silymarin to minimize the increase in myocardial excitability caused by the drug.

Rašković et al. (2011) explained that the semiquinone form of doxorubicin causes the production of free radicals via interacting with molecular oxygen, these free radicals are responsible for its cardio and hepatotoxicity.

Silymarin acts as a strong antioxidant via three different pathways including direct elimination of free radicals (Taleb *et al*., 2018), stopping the production of free radicals by inhibiting its associated enzymes (Zhu *et al*., 2014) and maintaining optimal redox state of the cell by activating a wide range of enzymatic and non-enzymatic antioxidants through transcription factors (Surai, 2015).

Silymarin was used as a strong antioxidant to overcome the oxidative stress-induced toxicity by doxorubicin. The obtained results are consistent with Abdel Maksoud et al. (2019) who explained that *Silybum marianum* has hepatoprotective effects through anti-inflammatory and antioxidants mechanisms. They also agree with Morales-González et al. (2015) who concluded the antioxidant activity of silymarin alarm its aptitude to reduce the free radicals that are produced from toxic substances metabolisms such as acetaminophen, carbon tetrachloride, and ethanol.

The invention of free radicals is identified to break cellular membranes and reason lipoperoxidation. Hepatic glutathione was enhanced by Silymarin which may cause the contribution to the antioxidant protection of the liver and increases protein production in hepatocytes by motivating the activity of RNA polymerase I.

Chemotherapeutic agents induce oxidative stress in patients which is responsible for its various side effects (Yokoyala *et al.,* 2017). Silymarin (active extract from milk thistle seeds) is known to have an antioxidant effect and offers protection against oxidative stress (Taleb *et al.,* 2018).

Our results are collinear with Kolarovic et al. (2010) who explained that Silymarin interacts with cell membranes increasing their resistance to harmful influences, mostly through changes in their physicochemical properties. They added that It also combine with free radicals converting them into less reactive and toxic compounds. Silymarin was also proved to inhibit lipid peroxidation and stimulating the production of glutathione.

CONCLUSION

Patients treated with doxorubicin can take milk thistle extract as an additional supplement to overcome the side effects of chemotherapy.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

Abdel Maksoud, H.A., Abdel Magid, A.D., Mostafa, Y.M., Elharrif, M.G., Sorour, R.I., Sorour, M.I., 2019. Ameliorative effect of liquorice extract versus silymarin in experimentally induced chronic hepatitis: A biochemical and genetical study. Clin. Nutr. Exp., 23: 69e79.

Abenavoli, L., Capasso, R., Milic, N., Capasso, F., 2010. Milk thistle in liver diseases: past, present, future. Phytother. Res., 24(10): 1423e32.

Beutler, E., Duron, O., Kelly, B.M., 1963. An improved method for the detection of blood Glutathione. J. Lab. Clin. Med., 61: 882–888.

Colombo, V., Lupi, M., Falcetta, F., Forestieri, D., D'Incalci, M., Ubezio, P., 2011. Chemotherapeutic activity of silymarin combined with doxorubicin or paclitaxel in sensitive and multidrug-resistant colon cancer cells. Cancer Chemoth. Pharm., 67:369–379.

El-Kamary, S.S., Shardell, M.D., Abdel-Hamid, M., Ismail, S., El-Ateek, M., Metwally, M., Mikhail, N., Hashema, M., Mousab, A., Aboul-Fotouhe, A. *et al*., 2009. A randomized controlled trial to assess the safety and efficacy of silymarin on symptoms, signs and biomarkers of acute hepatitis. Phytomed., 16:391–400.

El-Shitany, N.A., El-Haggar, S., El-Desoky K., 2008. Silymarin prevents adriamycin-induced cardiotoxicity and nephrotoxicity in rats. Food Chem. Toxicol., 46: 2422–2428.

Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Meth. Enzymo., 186: 407–421.

Iqbal, M.N, Ashraf, A., 2019a. *Silene inflata* Sm: a Potential Source of Novel Therapeutic Agents. PSM Biol. Res., 4(2): 97-99.

Iqbal, M.N., Ashraf, A., 2019b. Withania somnifera: Can it be a Therapeutic Alternative for Microbial Diseases in an Era of Progressive Antibiotic Resistance? Int. J. Nanotechnol. Allied Sci., 3(1): 16-18.

Joos, S., Musselmann, B., Szecsenyi, J., 2011. Integration of complementary and alternative medicine into family practices in Germany: Results of a national survey.

Joshi, S., Kuszynski, C.A., Bagchi, D., 2001. The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. J Curr. Pharm. Biotechno., 2: 187-200.

Kolarovic, J., Popovic, M., Zlinská, J., Trivic, S., Vojnovic, M., 2010. Antioxidant activities of celery and parsley juices in rats treated with doxorubicin. Molecules, 15:6193–6204.

Koracevic, D., Koracevic, G. *et al.,* 2001. Method for the measurement of antioxidant activity in human fluid. J. Clin. Pathol., 54: 356-361.

Mai, Y., Jessica Yu, J., Bartholdy, B., Xu-Monette, Z.Y., Knapp, E.E., Yuan, F., Chen, H., Ding, B.B., Yao, Z., Das, B.,

Zou, Y., Young, K.H., Parekh, S., Ye, B.H., 2016. An oxidative stress-based mechanism of doxorubicin cytotoxicity suggests new therapeutic strategies in ABC-DLBCL. Blood, 128: 2797-2807.

Mello, M.B., Machado, C.S., Ribeiro, D.L., Aissa, A.F., Burim, R.V., Alves da Cunha, M.A., Barcelos , G.R.M., Antunes, L.M.G., Bianchi, M.L.P., 2017. Protective effects of the exopolysaccharide Lasiodiplodan against DNA damage and inflammation induced by doxorubicin in rats: Cytogenetic and gene expression assays. Toxicol., 376: 66–74.

Morales-González, J.A., Madrigal-Santillán, E., Morales-González, Á., Bautista, M., Gayosso-Islas, E., Sánchez-Moreno, C., 2015. What is Known Regarding the Participation of Factor Nrf-2 in Liver Regeneration? Cells, 4(2): 169–177.

Natural Resources Conservation Service PLANTS Database, 2015. *"Silphium marianum".* USDA. Retrieved 15 November 2015.

Rašković, A., Stilinović, N., Kolarović, J., Vasović, V., Vukmirović, S., Mikov, M., 2011. The Protective Effects of Silymarin against Doxorubicin-Induced Cardio-toxicity and Hepatotoxicity in Rats. Molecules, 16(10): 8601–8613.

Shahzad, M.I., Ashraf, H., Iqbal, M.N., Khanum, A., 2017. Medicinal Evaluation of Common Plants against Mouth Microflora. PSM Microbiol., 2(2): 34-40.

Surai, P.F., 2015. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants (Basel, Switzerland), 4 (1): 204–247.

Taleb, A., Ahmad, K.A., Ihsan, A.U., Qu, J., Lin, N., Hezam, K., Koju, N., Hui, L., Qilong, D., 2018. Antioxidant effects and mechanism of silymarin in oxidative stress induced cardiovascular diseases. Biomed. Pharmacother., 102: 689–698.

The American Society of Health-System Pharmacists, 2017. Doxorubicin Hydro-chloride. Archived from the original on 11 October 2016. Retrieved 12 January 2017.

Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2005. 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co.

Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2012. 5th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co.

Wu, J.W., Lin, L.C., Tsai, T-H., 2009. Drugedrug interactions of silymarin on the perspective of pharmacokinetics. J. Ethnopharmacol., 121(2): 185e93.

Yokoyama, C., Sueyoshi,Y., Ema, M., Mori, Y., Takaishi, K., Hisatomi, H., 2017. Induction of oxidative stress by anticancer drugs in the presence and absence of cells. Oncol. Lett., 14: 6066–6070.

Zhu, S.Y., Dong, Y., Tu, J., Zhou, Y., Zhou, X.H., Xu, B., 2014. *Silybum marianum* oil attenuates oxidative stress and ameliorates mitochondrial dysfunction in mice treated with D-galactose. Pharmacogn. Mag., 10: S92–S99.