doi.org/10.36721/PJPS.2024.37.3.REG.591-599.1

Ascorbic acid mitigates doxorubicin-induced spleen injury in rats: Histopathological and immunohistochemical insights

Arzu Gezer[1]*, Mustafa Ozkaraca[2], Ebru Karadağ Sari[3], Gürsel Bedir[4], Pelin Aydın[5], Hasan Asker[6] and AM Abd El-Aty[5,7]

[1]Vocational School of Health Services, Atatürk University, Erzurum, Turkey
[1]Pharmaceutical Research and Development, Graduate School of Natural and Applied Sciences, Atatürk University, Erzurum, Turkey
[2]Department of Pathology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey
[3]Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey
[4]Department of Histology and Embryology, Ataturk University, School of Medicine, Erzurum, Turkey
[5]Department of Pharmacology, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[6]Department of Histology and Embryology, Faculty of Medicine, Uşak University, Uşak, Turkey
[7]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: This study assessed the protective potential of ascorbic acid against doxorubicin-induced spleen tissue damage in rats. Twenty-eight male *Sprague-Dawley* rats were divided into four groups. The control group received saline every other day at a dose of 1mL throughout the experiment. The ascorbic acid group was administered 50mg/kg of ascorbic acid daily for 10 days. The doxorubicin group received a single dose of 15mg/kg of doxorubicin on day 7. The ascorbic acid + doxorubicin group received both 50mg/kg of ascorbic acid daily for 10 days and a single dose of 15mg/kg of doxorubicin on day 7. After the experiment, splenic tissue samples were examined histopathologically and immunohistochemically. Histopathological analysis revealed edema, destruction, and degeneration in the doxorubicin group, but these changes were alleviated in the ascorbic acid-treated group, approaching control group levels. Immunohistochemical analysis showed increased $CD4^+$ and $CD8^+$ cell immunopositivity in the ascorbic acid + doxorubicin group compared to the doxorubicin group. Biochemical tests indicated that doxorubicin reduced superoxide dismutase activity and increased malondialdehyde levels, whereas ascorbic acid mitigated these effects. The findings suggest that ascorbic acid may have a protective role against doxorubicin-induced spleen injury in rats.

Keywords: Ascorbic acid, $CD4^+$, $CD8^+$, spleen, doxorubicin, splenotoxicity.

INTRODUCTION

Cancer ranks among the foremost contributors to global mortality, morbidity and various agents are widely used to treat it (Debela *et al.,* 2021). Doxorubicin (DOX), a broad-spectrum antineoplastic anthracycline drug, is one of the most widely used chemotherapeutic drugs (Ma *et al.,* 2022). Doxorubicin has demonstrated efficacy as a chemotherapeutic agent in the treatment of soft tissue sarcomas, thyroid, ovarian, breast, lung and stomach cancers, as well as non-Hodgkin's and Hodgkin's lymphoma, pediatric cancers, and multiple myeloma (Songbo *et al.,* 2019, Sohail *et al.,* 2021). DOX acts by forming complexes by intercalating between DNA and base pairs in cells, stabilizing the DNA-topoisomerase II complex and causing DNA damage. DOX also causes oxidative damage by producing free radicals that can damage DNA (Johnson-Arbor & Dubey, 2019, Songbo *et al.,* 2019). However, DOX not only targets tumor cells but also exerts cytotoxic effects on healthy tissues and growing cells by leading to oxidative stress and the manufacturing of proinflammatory cytokines (Peter *et al.,* 2022). Side effects such as peripheral neuropathy, hepatotoxicity, cardiotoxicity and myelosuppression limit the use of DOX, despite its broad spectrum of use (Corremans *et al.,* 2019; Thotakura *et al.,* 2021). These unwanted side effects can significantly affect a patient's health and quality of life. Hence, DOX needs to be used in combination with protective agents to reduce its adverse effects (Singh *et al.,* 2023).

There is growing interest in the use of natural compounds with antioxidant activity as possible therapeutic agents and immunostimulants (Gezer *et al.,* 2023). The use of combined therapy involving phytochemicals exhibiting antioxidant and anti-inflammatory characteristics has been discovered to provide defense against chemotherapy-induced oxidative injury and immune modulation (Liu *et al.,* 2021). Ascorbic acid (AA), also known as vitamin C, is a vitamin that plays a vital role in many important body processes, such as the normal functioning of tissues, the immune system and iron absorption. AA, an essential micronutrient, consists of six carbons and is related to hexoses. Today, AA is industrially produced from D-glucose (Loi *et al.,* 2020). AA has positive effects on DOX-induced hepatotoxicity (Hatamkhani *et al.,* 2020) and cardiotoxicity (Rawat *et al.,* 2021). In addition, it has the ability to prevent oxidative stress, dyslipidemia and electrolyte imbalance (Ifeanacho *et al.,* 2021). These findings make clear that

**Corresponding author:* e-mail: a.gezer25@hotmail.com

AA may have protective potential against chemical-induced toxicity.

The immune system aids the body in adjusting and protecting itself from assaults by detrimental agents and disease-inducing pathogens. Splenic tissue is among the secondary lymphoid organs in which antigens from the bloodstream are released by lymphocytes and has an important function in the immune response (Lewis *et al.,* 2019). T and B lymphocytes lead to a long-term specific immune response through the production of cytokines and chemokines. Mature T lymphocytes differentiate into $CD4^+$ (T helper cells) and $CD8^+$ (cytotoxic cells), which are involved in regulating immune responses, and these cells activate phagocytes to eliminate pathogens (Lewis *et al.,* 2019, Daniel *et al.,* 2023).

Supraphysiologic doses of DOX are known to affect immunologic function. DOX may adversely affect quality of life by causing toxicity in normal tissues and suppressing the immune system (Shaldoum *et al.,* 2021, Rocca *et al.,* 2020, Bhagat *et al.,* 2022). The use of natural antioxidants to mitigate the toxic effects of chemotherapeutic drugs, leading to dose limitations, has become increasingly important (Ikewuchi *et al.,* 2021). Many agents with antioxidant properties show promise for preventing or reducing toxic effects without reducing the antitumor effect of DOX (Corremans *et al.,* 2019, Khan *et al.,* 2020).

The protective effect of AA, which is an exogenous antioxidant that plays an active role in strengthening the immune system, against DOX-induced splenic tissue damage was evaluated in this study. The AA of leukocytes is approximately ten times greater than that of plasma. This finding suggested that AA has functional roles in immune system cells. AA reportedly increases T- and B-cell differentiation and proliferation due to its gene regulatory effect (Agarwal *et al.,* 2022, El Deib *et al.,* 2021). These data suggest that AA may be useful against possible DOX-induced splenic damage. However, there are few reports on the toxicity of DOX in splenic tissue.

Thus, this study histopathologically, immunohisto-chemically and biochemically researched the protective effect of AA against DOX-induced splenic injury and dysfunction in rats.

MATERIALS AND METHODS

Ethics consideration

Ethics Committee approval was acquired from the Ataturk University Animal Experiments Local Ethics Committee, as per the decision dated 11/29/2022 and numbered 2022/13.

Chemicals

Doxorubicin was taken from Deva İlaç (İstanbul, Türkiye), xylazine for analgesia and sedation were obtained from Bayer (Rompun, İstanbul, Türkiye), ketamine was obtained from Pfizer (Ketalar, İstanbul, Türkiye) and ascorbic acid was taken from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and study design

The study animals, comprising 3-month-old male Sprague-Dawley rats (n=28) weighing 250±20g, were obtained from the Atatürk University Medical Experimental Application and Research Center Directorate. The animals were fed *ad libitum* under standard care conditions (at 50±2% room humidity and a temperature of 21°C) with a 12-hour light/dark circle.

The 28 rats were randomly distributed into 4 groups of 7 rats in each group:
Control group (n=7): Rats in this group were administered a physiological dose of 1mL of saline intraperitoneally (i.p.) every other day for a total of 5 days.
AA group (n = 7): Rats in this group were given 50mg/kg ascorbic acid by oral gavage daily for 10 days (Iqbal *et al.,* 2022).
DOX group (n=7): Rats in this group were given a one-time dose 15 mg/kg doxorubicin i.p. on day 7 (Jasim & Mutlag, 2023).
In the AA+DOX group (n=7), the rats in this group were administered both ascorbic acid at a dose of 50 mg/kg by oral gavage daily for 10 days and a one-time dose 15 mg/kg doxorubicin via the i.p. route on day 7.

Histopathological examination

On day 11, the rats were subjected to necropsy, and spleen tissue samples were gathered and preserved in a 10% formalin solution. The tissues underwent standard alcohol-xylene procedures and were inserted in paraffin. Then, sections of 5-µm thickness obtained from the paraffin blocks were stained using hematoxylin and eosin (H&E). The preparations were semiquantitatively evaluated under a light microscope for edematous and degenerative changes and were scored as absent (–), mild (+), moderate (++), or severe (+++) (Gezer & Sari, 2023).

Immunohistochemical examination

The 5-µm sections were transferred to polylysine slides, passed through a xylol and alcohol series, washed with phosphate-buffered saline (PBS), and then incubated in 3% H_2O_2 for 10min to inactivate the endogenous per oxidase. The tissues were treated with antigen retrieval solution at 500 W for 2 × 5 min to release the antigens. After protein blocking buffer was added, the tissues were washed with PBS and incubated with $CD4^+$ (Cat. No. sc-19641, Santa Cruz) and $CD8^+$ (Cat. No. sc-1177, Santa Cruz) at a dilution ratio of 1/100 at + 4°C overnight. Next, a large volume of the anti-polyvalent HRP detection system (Cat. No. TP-125-HL Thermo Fisher Scientific, Waltham, MA, USA) was employed as recommended by the manufacturer. 3-Amino-9-ethylcarbazole (AEC) was used as the chromogen. After staining with Mayer's

hematoxylin, the slides were masked with water-based adhesive and inspected under a light microscope. Immunopositivity in 6 different random areas was quantified (Gezer & Sari, 2023).

Biochemical examinations

Superoxide dismutase (SOD) activity was assessed following the protocol outlined by Mc Cord and Fridovich (Gezer *et al.,* 2023) and the enzyme activity was stated as U/mg protein. Malondialdehyde (MDA) analysis was conducted using the procedure described by Draper and Hadley (Gezer *et al.,* 2023), and the results are reported as nmol/g.

STATISTICAL ANALYSIS

The data collected were analyzed utilizing IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Differences between the groups according to the histopathological findings were determined applying the Kruskal-Wallis test, a nonparametric test, and the group causing the difference was confirmed applying the Mann-Whitney U test. Immunopositivity detected via immuno-histochemical examination was assessed using one-way analysis of variance (P<0.05).

RESULTS

Histopathological results

The splenotoxicity model was induced by injecting DOX. Relative to those of the control group, the DOX group exhibited a markedly reduced spleen weight-to-body weight ratio (P=0.001). However, this ratio was not markedly lower in the AA+DOX group (P>0.05). There was no statistically substantial distinction between the control group and the AA group (P>0.05), as shown in table 1 and fig. 1.

When edema in the spleen tissue, destruction of lymphoid cells and degeneration in the central arteriole were evaluated, considerable variations were observed between the experimental groups (table 2, P<0.05).

Examination of the spleen tissues revealed a normal histologic appearance in the control and AA groups. The histopathological changes (edema, destruction of lymphoid cells, and degeneration of the central arteriole) detected in the DOX group were moderate, while they were mild in the AA+DOX group (figs. 1-2). (P<0.05).

Immunohistochemical results

Immunohistochemical staining of the spleen tissue from the two groups revealed significant differences in terms of $CD4^+$ and $CD8^+$ immunopositivity (table 3, P<0.05).

There was less $CD4^+$ and $CD8^+$ immunopositivity in the control and AA groups than in the DOX group. Nevertheless, the AA+DOX group had greater immunopositivity than the DOX group. When comparing $CD4^+$ and $CD8^+$ immunopositivity, the overall $CD4^+$ positivity was greater than the overall $CD8^+$ positivity (figs. 3-4). (P<0.05).

Biochemical results

Analysis of the SOD activity in our study revealed that the SOD activity in the DOX group was markedly lower than that in the control group (P<0.001). However, in the AA+DOX group, we observed a significant increase in SOD activity, which decreased due to DOX, upon the administration of AA (P<0.001) (see fig. 5).

Our findings revealed a substantial increase in the MDA concentration in the DOX group compared to that in the control group (P<0.001). In contrast, in the AA+DOX group, the elevated MDA levels induced by DOX were significantly reduced following AA administration (P<0.001) (fig. 6).

DISCUSSION

Anticancer treatment can damage healthy cells as well as cancer cells (Mosleh-Shirazi *et al.,* 2023). This damage can have adverse effects on many organs and may lead to undesirable physiological outcomes (Redrado & Fernández‐Moreira, 2023). Thus, side effects during or after cancer treatment are of concern. This necessitates clarifying the mechanisms of action for targeted therapeutics or identifying supplementary agents that can mitigate drug toxicity without compromising the effectiveness of the treatment (Wang *et al.,* 2023). In this study, immune system organs and spleen tissue were examined histopathologically and immunohisto-chemically to evaluate DOX-induced splenic injury and toxicity in DOX-treated experimental models. Additionally, the inquiry into the protective mechanism of AA against DOX was conducted using the examined parameters.

Immune system parameters are often used to assess the overall immune function of the body. Arsenic-exposed rats exhibit a decrease in the size and parameters of the spleen, which is ameliorated by AA (Maity *et al.,* 2023). The use of DOX is known to cause a reduce in the thickness of white pulp, red pulp and the margin region per volume (Dias *et al.,* 2024) and to weaken immunity by reducing the white pulp volume as well as the white pulp/red pulp ratio (Shaldoum *et al.,* 2021). The observed decrease in the percentage of spleen white pulp may be due to the suppression of lymphoproliferation and genotoxic effects and possible cytotoxicity induced by DOX (Karaulov *et al.,* 2019).

The decrease in splenic parameters observed in the present study was indicative of immunosuppression due to DOX treatment. This difference may have been caused

Table 1: Effect of ascorbic acid on the spleen weight/body weight ratio in patients with doxorubicin-induced splenotoxicity.

Groups	Spleen Weight/Body Weight %
Control	0.6529 ± 0.0116^{a}
AA	0.6575 ± 0.0479^{a}
DOX	0.5571 ± 0.0133^{b}
AA+DOX	0.6296 ± 0.0368^{a}

AA: ascorbic acid, DOX: Doxorubicin, AA+DOX: ascorbic acid+doxorubicin. [a, b]Differences between groups. (P<0.05).

Table 2: Comparison of histopathological changes among the groups.

Groups	Edema	Destruction in lymphoid cells	Degeneration in the central arteriole
Control	0.16 ± 0.40^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
AA	0.33 ± 0.5^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
DOX	1.66 ± 0.5^{b}	1.66 ± 0.40^{b}	1.83 ± 0.40^{b}
AA+DOX	0.83 ± 0.40^{c}	1.00 ± 0.00^{c}	1.12 ± 0.40^{c}

AA: ascorbic acid, DOX: doxorubicin, AA+DOX: ascorbic acid+doxorubicin. [a, b, c]Differences between groups (P<0.05).

Table 3: $CD4^{+}$ and $CD8^{+}$ immunopositivity in the spleen.

Groups	$CD4^{+}$	$CD8^{+}$
Control	48.56 ± 0.19^{aA}	40.41 ± 1.04^{aB}
AA	50.42 ± 0.57^{aA}	39.17 ± 0.26^{aB}
DOX	35.80 ± 1.64^{bA}	31.60 ± 1.93^{bB}
AA+DOX	42.23 ± 0.3^{cA}	36.04 ± 1.42^{cB}

AA: ascorbic acid, DOX: doxorubicin, AA+DOX: ascorbic acid+doxorubicin. [a, b, c]Different letters in the same column indicate differences between groups. [A, B]Different letters in the same row indicate intra-group differences. (P<0.05).

by DOX-induced apoptosis, decreased absolute T-cell counts, inhibition of lymphocyte stimulation, or suppression of lymphoproliferation (Akter *et al.,* 2022). In accordance with these findings, the present study showed that DOX disrupted the histological structure of the spleen. The spleen tissue of the DOX-treated rats exhibited sclerosis and hyalinosis in the central artery of the germinal center, a reduction in the size of the white pulp, and hyperplasia of interstitial fibrous tissue. DOX caused hypoplasia, edema, destruction of lymphoid cells, and degeneration of the central arteriole in the splenic tissue, while AA alleviated hypoplasia and prevented edema and destruction and degeneration in the spleen tissue. Vitamin C administration strengthens the immune system by activating lymphatic nodules in the central arterioles of rats (El Deib *et al.,* 2021). There are also many studies on the effects of AA on splenic tissue toxicity. However, there is no information on the preventative action of AA against DOX-induced damage.

Disturbance of the intestinal epithelium, the most frequent cause of adverse effects caused by DOX (20 mg/kg, i.p.), leads to leakage of gut microbial-related endotoxins, resulting in increased TLR4 signaling. This process causes immunotoxicity via systemic inflammation and multiple-organ damage (Chu *et al.,* 2020). The management of DOX at a dose of 18 mg/kg to albino rats has been shown to cause marked congestion and degenerative changes in the kidney, heart, liver, and testis (Moustafa *et al.,* 2021). In addition to its known side effects, DOX use is also associated with the risk of splenic injury (Shaldoum *et al.,* 2021, Rocca *et al.,* 2020). One mechanism thought to be responsible for the severe side effects of DOX is damage to the cellular elements accountable for triggering immune responses, increasing the likelihood of microbial infection and suppressing the immune system (Lubis *et al.,* 2019). It has been proven that the use of DOX in rats leads to a decrease in lymphocyte count and its toxic effects on the hematopoietic system are evidenced by decreases in erythrocyte, leukocyte, granulocyte, lymphocyte, and monocyte counts (Owumi *et al.,* 2021, Shaldoum *et al.,* 2021). DOX has been reported to be immunosuppressive in rats, as it decreases interleukin-10 (IL-10) levels and suppresses $CD8^{+}$ cytotoxic T cells (Wang *et al.,* 2021). The use of DOX in rats with tumors reportedly decreases splenocyte IL-2 and INF-γ production, lymphocyte proliferation, the $CD4^{+}/CD8^{+}$ ratio and natural killer cell cytotoxicity (Wang *et al.,* 2023).

Consistent with the results herein, the immunosuppressive effects of DOX, like $CD4^{+}$ and $CD8^{+}$ inhibition and a delay in the decline of composite tissue allografts, have been demonstrated (Lubis *et al.,* 2019). One study reported that DOX was found to impede lymphocyte proliferation, macrophage phagocytic capacity and activity, CD8+ cells and IL-10 (Chen *et al.,* 2021).

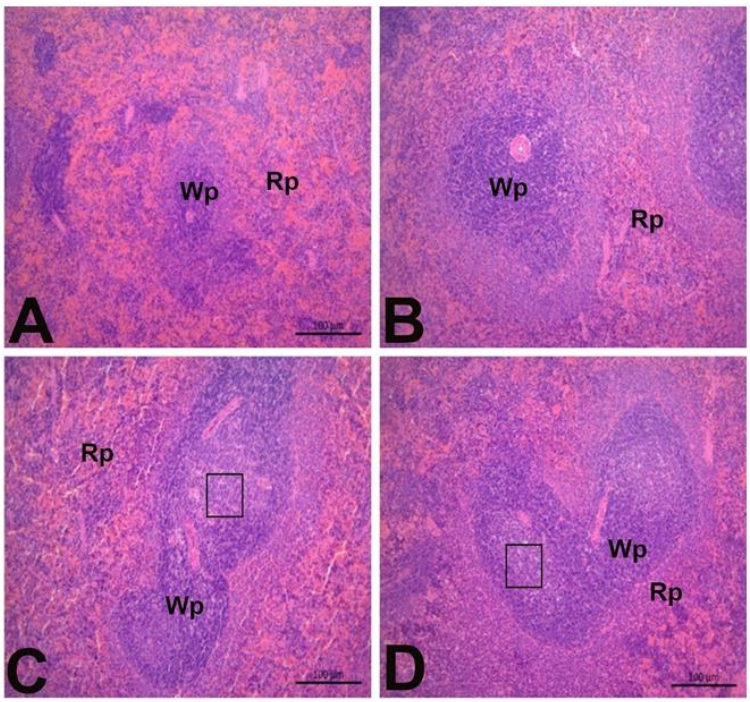

Fig. 1: A) Control group; normal view of red pulp (Rp) and white pulp (Wp); B) AA group; normal view of red pulp (Rp) and white pulp (Wp); C) DOX group; normal view of red pulp (Rp) with moderate edematous areas in white pulp (Wp) (ϒ); D) AA+DOX group; normal view of red pulp (Rp) with mild edematous areas in white pulp (Wp) (ϒ). Spleen. H&E.

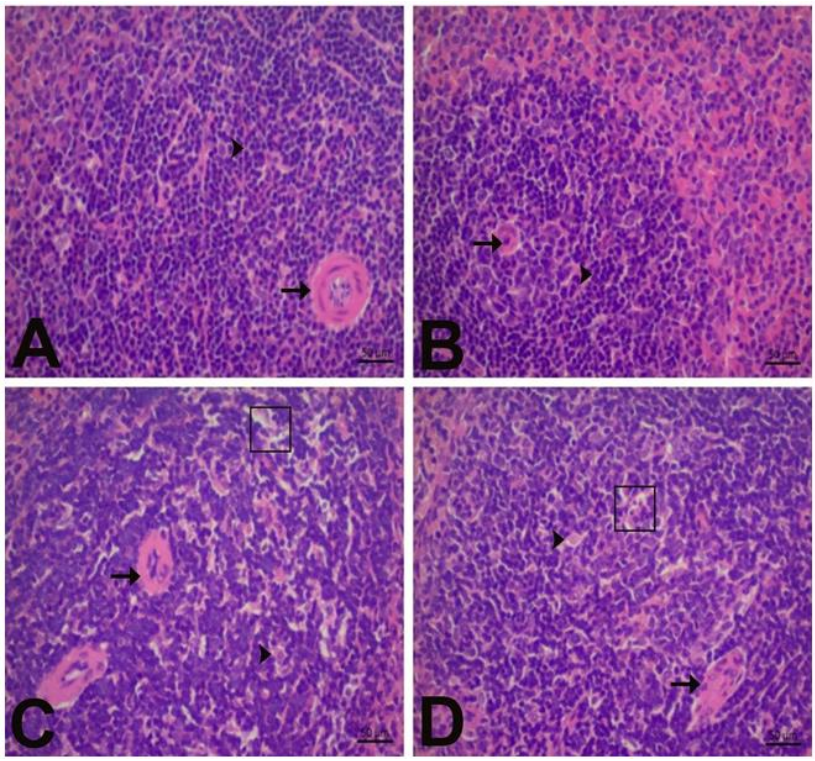

Fig. 2: A) Control group; central arteriole in its normal view (arrow) and lymphoid cells (arrowhead); B) AA group; central arteriole in its normal view (arrow) and lymphoid cells (arrowhead); C) DOX group; moderately edematous areas (ϒ), degenerative central arteriole (arrow), and destruction in lymphoid cells (arrowhead); D) AA+DOX group; mildly edematous areas (ϒ), degenerative central arteriole (arrow), and damage in lymphoid cells (arrowhead). Spleen. H&E.

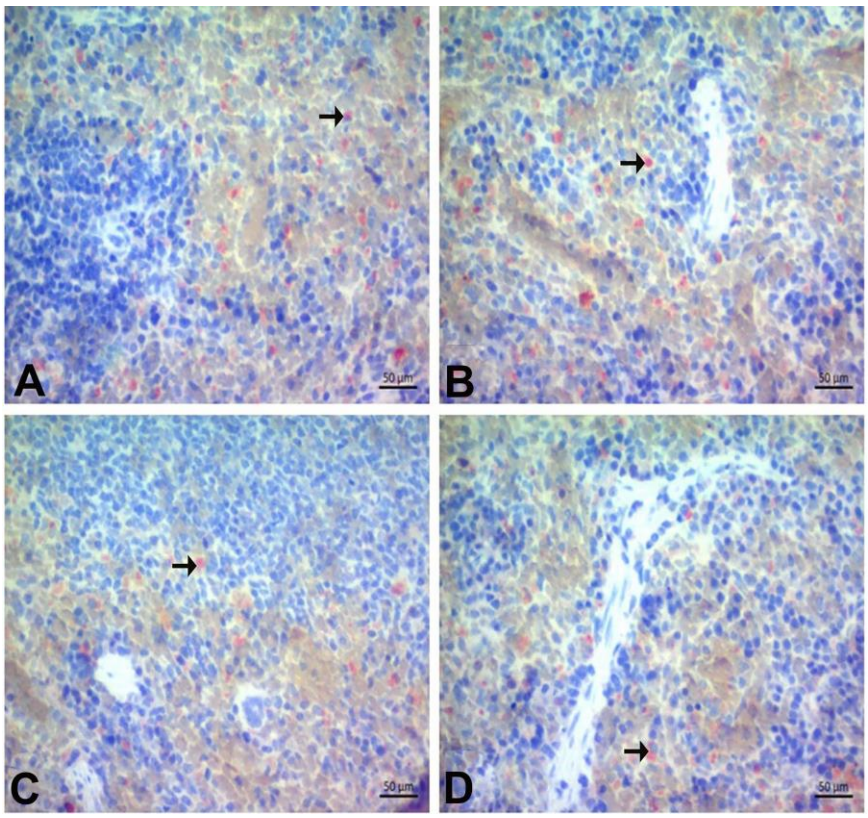

Fig. 3: A) Control group and B) AA group; $CD4^+$ immunopositivity, C) DOX group; decrease in $CD4^+$ immunopositivity, D) AA+DOX group; increase in $CD4^+$ immunopositivity. Spleen. IHC.

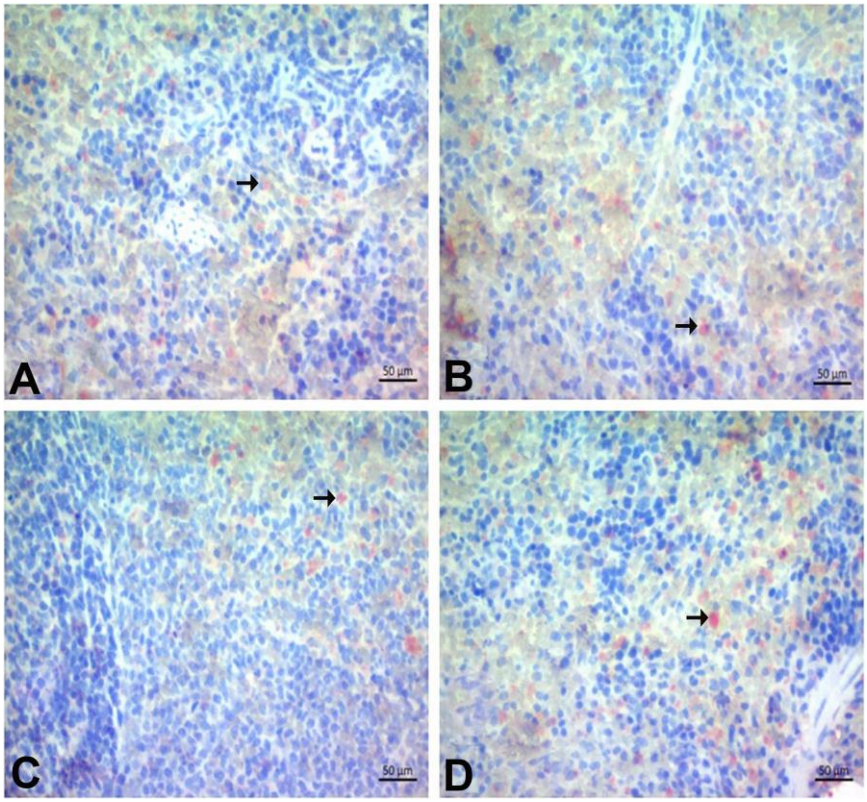

Fig. 4: A) Control group and B) AA group; $CD8^+$ immunopositivity, C) DOX group; decrease in $CD8^+$ immunopositivity, D) AA+DOX group; increase in $CD8^+$ immunopositivity. Spleen. IHC.

AA has been shown to be effective at preventing and reversing lead acetate toxicity in the spleens of rats. AA also has protective effects against mercuric chloride toxicity in the spleen of experimental animals (Ahmed *et al.,* 2023). It is known that arsenic causes a decrease in $CD4^+$ and $CD8^+$ counts in spleen tissue, and AA increases $CD4^+$ and $CD8^+$ counts (Maity *et al.,* 2023). Consistent with these findings, AA attenuated the DOX-induced decrease in $CD4^+$ and $CD8^+$ T lymphocytes by increasing their immunostimulatory activity. This was probably due to the antioxidant and genetic protective effects of AA, which improved the lymphocyte count and reduced the inflammatory result in the spleens of the DOX-treated rats.

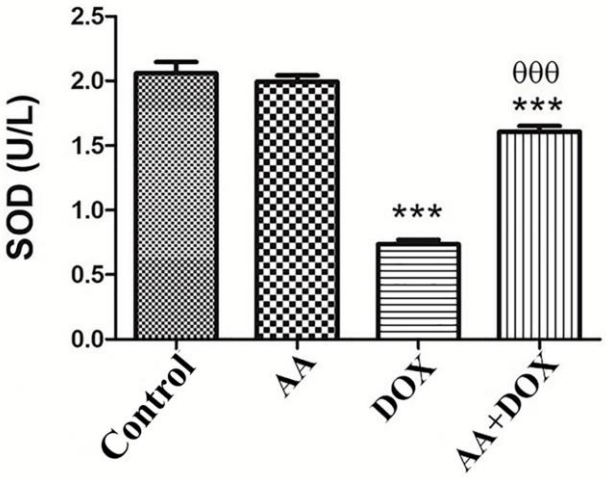

Fig. 5: SOD enzyme activity. AA: ascorbic acid, DOX: doxorubicin, AA+DOX: ascorbic acid+doxorubicin. *(Comparison according to the healthy group), *: indicates $P<0.05$ **:$P<0.01$ and ***:$P<0.001$. θ (Comparison by spleen ınjury group), θ: $P<0.05$ θθ: $P<0.01$ θθθ: $p<0.001$.

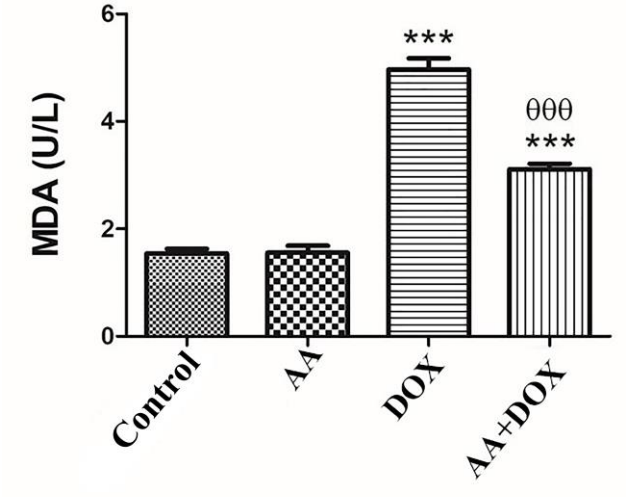

Fig. 6: MDA enzyme activity. AA: Ascorbic acid, DOX: doxorubicin, AA+DOX: Ascorbic acid + doxorubicin. *(Comparison according to the healthy group), *: indicates $P<0.05$ **:$P<0.01$ and ***:$P<0.001$. θ (Comparison by spleen ınjury group), θ: $P<0.05$ θθ: $P<0.01$ θθθ: $p<0.001$.

Our study revealed that SOD activity was decrease in the AA+DOX group than in the DOX group, indicating that the histological damage induced in the spleens of DOX rats correlated with decreased SOD antioxidant enzyme activity. Additionally, the MDA levels were greater in the DOX group than in the AA+DOX group. Elevated levels of MDA, a marker of lipid per oxidation, suggest cellular damage. These findings align with a study by Erbaş *et al*. (2024), which also reported that histological damage induced in the spleen of DOX rats was associated with increased lipid peroxide content and decreased SOD antioxidant enzyme activity.

Dysfunction of organs and components of the immune system can weaken an organism's defense mechanisms, especially in the case of uncontrolled inflammation. Inflammation usually occurs as a defense mechanism, providing protection against different pathogens and initiating repair and regeneration processes following tissue damage. However, if inflammation progresses undetected, chronic inflammation can occur, possibly damaging the immune response as well as other systems in the body. Therefore, maintaining a balanced inflammatory balance and under control are important (Galler *et al.,* 2021).

CONCLUSION

In conclusion, DOX treatment affected the concentration of $CD4^+$ and $CD8^+$ cells and the histologic structure of the spleen tissue in rats. These findings indicate that coadministration of AA with DOX may reduce DOX-induced spleen tissue toxicity, immunotoxicity and inflammatory responses and prevent cellular damage. Therefore, AA can be considered a chemoprotective agent against DOX-induced spleen toxicity. Nevertheless, additional investigation is required to gain a deeper understanding of the basic molecular processes the protective impact of AA in rats treated with DOX and to more effectively evaluate the potential hazards associated with DOX.

REFERENCES

Agarwal A, Basmaji J, Fernando SM, Ge FZ, Xiao Y, Faisal H, Honarmand K, Hylands M, Lau V and Lewis K (2022). Parenteral vitamin C in patients with severe infection: A systematic review. *NEJM Evidence,* **1**(9): EVIDoa2200105.

Ahmed RM, Mwaheb MA, Elmahdi MH, Abd Elguaad MMK, Eldosoki DE, Mohamed EA, Helal A and Gaber SN (2023). The effect of ascorbic acid on histopathological, biochemical, pharmacological and

immunological toxicity of chronic lead acetate exposure on the spleen in a rat model. *Egypt Pharm. J.*, **22**(1): 129-142.

Akter R, Neelotpol S and Kabir MT (2022). Effect of *Allium sativum* methanol extract in amelioration of arsenic-induced toxicity in Swiss albino mice. *Phytomed. Plus*, **2**(1): 100192.

Bhagat A, Shrestha P and Kleinerman ES (2022). The innate immune system in cardiovascular diseases and its role in doxorubicin-induced cardiotoxicity. *Int. J. Mol. Sci.*, **23**(23): 14649.

Chen Y, Jin H, Song Y, Huang T, Cao J, Tang Q and Zou Z (2021). Targeting tumor associated macrophages: A potential treatment for solid tumors. *J. Cell. Physiol.*, **236**(5): 3445-3465.

Chu X, Zhang Y, Xue Y, Li Z, Shi J, Wang H and Chu L (2020). Crocin protects against cardiotoxicity induced by doxorubicin through TLR-2/NF-κB signal pathway *in vivo* and *vitro*. *Int. Immunopharmacol.*, **84**: 106548.

Corremans R, Adão R, De Keulenaer GW, Leite Moreira AF and Brás Silva C (2019). Update on pathophysiology and preventive strategies of anthracycline induced cardiotoxicity. *Clin Exp Pharmacol Physiol.*, **46**(3): 204-215.

Daniel L, Counoupas C, Bhattacharyya ND, Triccas JA, Britton WJ and Feng CG (2023). L-selectin-dependent and-independent homing of naive lymphocytes through the lung draining lymph node support T cell response to pulmonary *Mycobacterium tuberculosis* infection. *PLoS Pathog.*, **19**(7): e1011460.

Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC and Manyazewal T (2021). New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Med.*, **9**: 20503121211034366.

Dias FCR, De Torres SM, De Figueiredo Pereira M, Morais DB, da Silva WE, De Silva Junior VA (2024). Bioconjugate based on cisplatin and bacterial exopolysaccharide with reduced side effects: A novel proposal for cancer treatment. *J. Trace Elem. Med. Biol.*, **83**: 127374.

El Deib MM, El-Sharkawy NI, Beheiry RR, Abd-Elhakim YM, Ismail SA, Fahmy EM, SaberT and Saber TM (2021). Boldenone undecylenate disrupts the immune system and induces autoimmune clinical hypothyroidism in rats: Vitamin C ameliorative effects. *Int. Immunopharmacol.*, **99**: 107939.

Erbaş E, Gelen V, Kara H, Gedikli S, Yeşildağ A, Özkanlar S and Akarsu SA (2024). Silver nanoparticles loaded with oleuropein reduce doxorubicin-induced testicular damage by regulating endoplasmic reticulum stress and apoptosis. *Biol. Trace Elem. Res.*, pp.1-12.

Galler KM, Weber M, Korkmaz Y, Widbiller M and Feuerer M (2021). Inflammatory response mechanisms of the dentine-pulp complex and the periapical tissues. *Int. J. Mol. Sci.*, **22**(33): 1480.

Gezer A and Sari EK (2023). Investigation of apoptotic and autophagic effects of chronic roflumilast use on testicular tissue in rats by immunohistochemical and immunofluorescence methods. *Iran. J. Basic Med. Sci.*, **26**(3): 276.

Gezer A, Laloglu A and Bolukbaş MK (2023). Protective effects of alpha lipoic acid against ionizing radiation-induced hepatotoxicity in rats. *EAJM*, **55**(2): 104.

Hatamkhani A, Shirani D, Rassouli A, Bokaei S and Dezfoulian O (2020). Evaluation of the effects of ascorbic acid on Doxorubicin-induced hepatotoxicity in mice. *J. Vet. Res. Majallah-i Taḥqiqat-i Dāmpizishki University*, **75**(3).

Ifeanacho MO, Ikewuchi JC, Ikewuchi CC, Nweke PC, Okere R and Nwate TL (2021). Prevention of doxorubicin-induced dyslipidaemia, plasma oxidative stress and electrolytes imbalance in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens*. *Sci. Afr.*, **11**: e00636.

Ikewuchi CC, Ifeanacho MO and Ikewuchi JC (2021). Moderation of doxorubicin-induced nephrotoxicity in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens. Porto Biomed. J.*, **6**(1): e129.

Iqbal S, Hameed U, Kamran M, Faisal L, Moeen S and Huda N (2022). Effect of ascorbic acid on methylphenidate induced decreased spermatogenesis in albino rats. *Annals of Abbasi Shaheed Hospital and Karachi Medical & Dental College*, **27**(1): 40-45.

Jasim IAJ and Mutlag SH (2023). Possible cardioprotective effect of iraqi ammi majus seed extract on doxorubicin induced cardiotoxicity in mice. *Hist. Med.*, **9**(2): 119-126-119-126.

Johnson-Arbor K and Dubey R (2019). Doxorubicin. *In*: StatPearls. Treasure Island, FL., 2018.

Karaulov AV, Renieri E, Smolyagin AI, Mikhaylova IV, Stadnikov A, Begun D, Tsarouhas K, Djordjevic AB, Hartung T and Tsatsakis A (2019). Long-term effects of chromium on morphological and immunological parameters of Wistar rats. *Food Chem Toxicol.*, **133**: 110748.

Khan TH, Ganaie MA, Alharthy KM, Madkhali H, Jan BL and Sheikh IA (2020). Naringenin prevents doxorubicin-induced toxicity in kidney tissues by regulating the oxidative and inflammatory insult in Wistar rats. *Arch. Physiol. Biochem.*, **126**(4): 300-307.

Lewis SM, Williams A and Eisenbarth SC (2019). Structure and function of the immune system in the spleen. *Sci. Immunol.*, **4**(33): eaau6085.

Liu YQ, Wang XL, He DH and Cheng YX (2021). Protection against chemotherapy-and radiotherapy-induced side effects: A review based on the mechanisms and therapeutic opportunities of phytochemicals. *Phytomed.*, **80**: 153402.

Loi M, De Leonardis S, Mulè G, Logrieco AF and Paciolla C (2020). A novel and potentially multifaceted dehydroascorbate reductase increasing the antioxidant

systems is induced by beauvericin in tomato. *Antioxidants*, **9**(5): 435.

Lubis MR, Haryani R, Safriana S and Satria D (2019). Ethanolic extract of herb Pugun tanoh (Picria fel-terrae Lour.) modulates TCD4+ and TCD8+ cell profile of Doxorubicin-induced immuno-suppressed rats. *Open Access Maced. J. Med. Sci.*, **7**(22): 3774.

Ma N, He F, Kawanokuchi J, Wang G and Yamashita T (2022). Taurine and its anticancer functions: *In vivo* and *in vitro* study. *Adv. Exp. Med. Biol.,* **1370**: 121-128.

Maity J, Pal P, Pal R and Mukhopadhyay PK (2023). Co-administration of L-ascorbic acid and α-tocopherol alleviates arsenic-induced immunotoxicities in the thymus and spleen by dwindling oxidative stress-induced inflammation. *Biol. Trace Elem. Res.,* **202**(5): 2199-2227.

Mosleh-Shirazi S, Kasaee SR, Dehghani F, Kamyab H, Kirpichnikova I, Chelliapan S, Firuzyar T, Akhtari M and Amani AM (2023). Investigation through the anticancer properties of green synthesized spinel ferrite nanoparticles in present and absent of laser photothermal effect. *Ceram. Int.*, **49**(7): 11293-11301.

Moustafa S and Ali M (2021). The probable protective effect of quercetin against doxorubicin-induced hepatotoxicity in adult albino rats: a biochemical and immunohistopathological study. *Ain Shams Med. J.*, **37**(2): 78-97.

Owumi SE, Nwozo SO, Arunsi UO, Oyelere AK and Odunola OA (2021). Co-administration of luteolin mitigated toxicity in rats' lungs associated with doxorubicin treatment. *Toxicol. Appl. Pharmacol.,* **411**: 115380.

Peter S, Alven S, Maseko RB and Aderibigbe BA (2022). Doxorubicin-based hybrid compounds as potential anticancer agents: A review. *Mol.,* **27**(14): 4478.

Rawat PS, Jaiswal A, Khurana A, Bhatti JS and Navik U (2021). Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomed. Pharmacother.,* **139**: 111708.

Redrado M and Fernández Moreira V (2023). The role of metallodrugs in cellular senescence. *Eur. J. Inorg. Chem.,* **26**(4): e202200593.

Rocca C, Pasqua T, Cerra MC and Angelone T (2020). Cardiac damage in anthracyclines therapy: Focus on oxidative stress and inflammation. *ARS.,* **32**(15): 1081-1097.

Shaldoum F, El-Kott AF, Ouda MMA and Abd-Ella EM (2021). Immunomodulatory effects of bee pollen on doxorubicin induced bone marrow/spleen immunosuppression in rat. *J. Food Biochem.,* **45**(6): e13747.

Singh M, Kadhim MM, Turki Jalil A, Oudah SK, Aminov Z, Alsaikhan F and Farhood B (2023). A systematic review of the protective effects of silymarin/silibinin against doxorubicin-induced cardiotoxicity. *Cancer Cell Int.,* **23**(1): 88.

Sohail M, Sun Z, Li Y, Gu X and Xu H (2021). Research progress in strategies to improve the efficacy and safety of doxorubicin for cancer chemotherapy. *Expert Rev. Anticancer Ther.,* **21**(12): 1385-1398.

Songbo M, Lang H, Xinyong C, Bin X, Ping Z and Liang S (2019). Oxidative stress injury in doxorubicin-induced cardiotoxicity. *Toxicol. Lett.,* **307**: 41-48.

Thotakura N, Panjeta A, Negi P, Preet S and Raza K (2021). Doxorubicin-loaded mixed micelles for the effective management of skin carcinoma: *in vivo* anti-tumor activity and biodistribution studies. *AAPS PharmSciTech.,* **22**: 1-12.

Wang H, Zhang G, Fan W, Wu Y, Zhang J, Xue M and Li (2023). Clinical significance of peripheral blood lymphocyte subtypes and cytokines in patients with hepatocellular carcinoma treated with TACE. *Cancer Manag Res.,* **14**: 451-464.

Wang L, Li P and Feng K (2023). EGCG adjuvant chemotherapy: Current status and future perspectives. *Eur. J. Med. Chem.,* **250**: 115197.

Wang Y, Yu J, Luo Z, Shi Q, Liu G, Wu F, Wang Z Huang Y and Zhou D (2021). Engineering endogenous tumor- associated macrophage – targeted biomimetic nano-RBC to Reprogram tumor immunosuppressive microenvironment for enhanced chemo-immunotherapy. *Adv. Mater. Lett.,* **33**(39): 2103497.