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RESEARCH ARTICLE

Caspase-3 and CD68 Immunoreactivity in Lymphoid Tissues and Haematology of Rats Exposed to Cisplatin and L-carnitine

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Summary

This study was performed to determine caspase-3 and CD68 activities in lymphoid tissues of rats exposed to Cisplatin (CDDP) induced toxicity and L-carnitine (L-Car) protection. Group control was received single-dose intraperitoneal (i.p.) 1 ml isotonic saline, Group L-Car received single-dose injections of L-carnitine 500 mg/kg bw. i.p., Group CDDP received a single-dose injection of CDDP 7 mg/kg bw i.p. all at once, Group L-Car + CDDP received single doses of L-carnitine 500 mg/kg bw i.p. for 10 consecutive days and following a single-dose i.p. injection of CDDP 7 mg/kg bw i.p. When compared to the control group, the numbers of total WBC were decreased while a relative increase in lymphocyte percentages was observed in the CDDP-treated group. On the other hand, relative decreases in neutrophil and monocyte percentages were observed in the CDDP-treated group compared to control group. Conversely, L-Car treatment with CDDP injections caused significantly increased the number of total WBC. Caspase-3 immunopositivity was found in varying degree in lymphoid tissues. L-carnitine supplementation to diet decreased apoptotic effect of cisplatin. CD68 positive cells were found similar in control and L-Car groups. While CD68 positivity was found in low degree in CDDP group, it was moderate in L-Car + CDDP group. It is concluded that cisplatin increases apoptosis and decrease macrophage activity in lymphoid tissues. This both effects were found especially in lymph node in high degree and thymus in low degree.

Keywords: *Carnitine, Caspase-3, Cisplatin, CD68, Lymphoid organ*

Sisplatin ve L-karnitin'e Maruz Kalmış Ratların Lenfoid Dokularında Caspase-3 ve CD68 Immunoreaktivitesi ve Hematolojisi

Özet

Bu çalışmada sisplatin (CDDP) toksikasyonuna maruz kalmış ve dietlerine L-carnitine ilave edilmiş ratların lenfoid dokularında Caspase-3 ve CD68 aktivitesinin belirlenmesi ve elde edilen sonuçların hematolojik bulgularla karşılaştırılması amaçlanmıştır. Bu amaçla kontrol grubunu oluşturan ratlara tek doz peritoniçi (i.p.) izotonik salin, L-car grubuna tek doz 500 mg/kg (i.p.) L-carnitine, CDDP grubuna tek doz 7 mg/kg (i.p.) CDDP, ve son olarak CDDP + L-car grubuna 10 gün boyunca tek doz 500 mg/kg (i.p.) L-carnitine ve akabinde tek doz 7 mg/kg (i.p.) CDDP uygulandı. Deneme sonrası elde edilen bulgular doğrultusunda, kontrol grubu ile kıyaslandığında CDDP grubunda lenfosit oranında artış varken toplam WBC oranı azalmıştı. Diğer tarafdan nötrofil lökosit ve monosit oranlarında da CDDP grubunda kontrol grubuna oranla azalma görüldü. Bu bulguların aksine L-car+CDDP grubunda toplam WBC, nötrofil lökosit ve lenfosit miktarında CDDP grubuna oranla artış tespit edildi. Caspase-3 immunopozitifliği lenfoid dokularda değişen derecelerde tespit edilirken, diyete L-carnitine ilavesi ile sisplatinin apoptotik etkisinde azalma görüldü. CD68 pozitiflik oranı kontrol, L-car ve L-car + CDDP gruplarında birbirine yakın ve orta şiddette bulunurken CDDP grubunda CD68 pozitiflik oranı daha düşük düzeyde tespit edildi. Elde edilen bulgular ışığında, sisplatinin lenfoid dokularda apoptozisi arttırdığı, makrofaj aktivitesini ise azalttığı, her iki etkinin özellikle lenf düğümünde daha belirgin, timusda ise daha az etkide olduğu sonucuna varıldı.

Anahtar sözcükler: *Caspase-3, Cd68, Lenfoid organ, L-karnitin, Sisplatin*

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INTRODUCTION

Cisplatin (cis-diammine-dichloro-platinum, CDDP) is broadly used for the treatment of several tumors because its antitumor activity [1-5]. In spite of antitumoral activity CDDP has serious side effect especially on kidney [6-9] and liver [10-12] tissues. Cisplatin causes glomerular and interstitial hyperemia, degeneration and necrosis of tubular epithelium, interstitial fibrosis and tubular dilatation in kidney [9]. Similarly, congestion, hepatocyte degeneration and fibrosis have been reported in liver tissue [10-12]. It has been suggested that CDDP toxicity could be attributed free radical-mediated oxidative damage [7].

Apoptosis is a gene-regulated mechanism resulted with some histopathological changes such as DNA damage, chromatin condensation and shrinkage of cell [13]. The most important member of caspase family is caspase-3 and it is responsible for many biochemical mechanisms of apoptosis that leads to the cleavage of nuclear and cytosolic material, chromatin condensation, fragmentation of DNA, and apoptotic bodies [14,15]. CD68 is a glycoprotein which is expressed on monocytes/macrophages. Its expression was largely used for the detection of monocytes and macrophages which includes peritoneum, lungs, spleen and liver and this expression appeared to correlate with macrophage activation [16-18].

L-carnitine (L-Car) is a vitamin-like cofactor. It serves in the transfer of long chain fatty acids into the mitochondria and in the β-oxidation of fatty acids [19,20]. There are many studies in nephrotoxicity or hepatotoxicity of CDDP and ameliorative effect of many antioxidant such as royal jelly [9,12], alpha lipoic acid [8], rosiglitazone [6], melatonin [21], silibini [22], vitamin C [23] and ellagic acid [24]. In these studies, cisplatin toxicity has been demonstrated in kidney, liver, testes and heart tissues. But there is no specific and comprehensive study related to cisplatin toxicity on lymphoid tissues. The effected of lymphoid tissue is important as well as liver or kidney. Because similar damage in lymphoid tissues will lead to insufficient immune response and this side effect probably will cause secondary infection.

In the present study, it is aimed the determination of caspase-3 and CD68 immunoreactivity in CDDP -induced toxicity to lymphoid tissues and the effect of L-carnitine supplementation.

MATERIAL and METHODS

Animals, Housing and Experimental Design

Adult female Sprague Dawley rats, 180±20 g, were provided from Atatürk University, Experimental Research Centre, Turkey. The animals were divided into 4 groups, each with 6 rats, and were named according to their experimental treatment. Group control; received single-dose intraperitoneal (i.p.) injections of 1 ml isotonic saline for 10 consecutive days. Group L-Car; received single-dose injections of L-carnitine (500 mg/kg bw i.p. Santa Farma, İstanbul, Turkey) for 10 consecutive days. Group CDDP; received a single-dose injection of CDDP (7 mg/kg bw i.p. Ebewe and Liba, respectively, Istanbul, Turkey) all at once. Group L-Car + CDDP; received single doses of L-carnitine (500 mg/kg bw i.p.) for 10 consecutive days following a single-dose i.p. injection of CDDP (7 mg/kg bw i.p.). The doses of CDDP and L-carnitine used in this study were selected in accordance with Al-Majed [25]. The animals were kept in metal cages at a temperature of 22-24°C and a 12-h light/dark cycle during the study. They were fed with standard commercial rat food and tap water. All experimental process in this study was approved by the Local Ethics Board of Animal Experiments in Atatürk University (Protocol number: 2008, 2/11).

Blood Sampling and Hematological Analysis

All rats were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) intramuscularly, and then killed by cervical dislocation 24 h after the last injection and trunk blood was collected into heparinized tubes. From each sample, ten blood films were prepared and air-dried, and five of these were stained with May Grunwald-Giemsa's stain. Leukocytes (WBC) were counted by the haemocytometric method, and percentage rates of leukocytes were determined with the May Grunwald-Giemsa's staining method [26].

Immunohistochemistry

Lymphoid tissue samples from thymus, spleen and lymph nodes were fixed in 10% buffered neutral formalin solution. After the routine alcohol-xylol process, tissue samples were embedded in paraffin, sectioned in 5-6 µm and stained with Mallory's triple modified by Crossman. For immunohistochemistry, primary antibodies monoclonal caspase-3 (dilution: 1/25, Biovision- 3015-100, BioVision Inc, CA, USA); monoclonal mouse anti-CD68 (Clone KP1, 08-0125, Invitrogen Corporation, Camarillo, CA, USA) and biotinylated secondary antibody (Dako, Universal LSAB Kit-K0690, Dako, Carpinteria, CA, USA) were used. The binding sites of antibody were visualized with DAB (Sigma, D5905, Sigma-Aldrich Company, Gillingham, UK) and evaluated by high-power light microscopic examination (Olympus BX52 with DP72 camera system). All immunohistochemical staining were estimated with an image processing system (Olympus, DP2-BSW). For each specimen, caspase 3 and CD68 were examined in 10 randomly selected areas of approximately X40 objective. The scores were derived semi-quantitatively using light microscopy on the preparations from each animal and were reported as follows: none: –, mild: +, moderate: ++, severe: +++, and very strong: ++++.

Statistical Analysis

For statistical analysis, differences between the groups were tested by the analysis of variance (ANOVA) followed by Duncan's post hoc test using SPSS 17.0 for Windows XP (SPSS Inc., Chicago, III). A value $P<0.05$ was considered significant. All data were expressed as mean averages, ± S.E.M.

RESULTS

As shown in *Table 1*, when compared to the control group, the number of total WBC was decreased (7.75±0.95 versus 4.30±1.25); while a relative increase in lymphocyte percentages (65.50±4.50 versus 78.50±5.00) were observed ($P<0.05$) in the CDDP-treated group. On the other hand, relative decreases in neutrophil and monocyte percentages were observed ($P<0.05$) in the CDDP-treated group compared to control group (25.50±2.50 versus 10.50±3.50 and 5.50±1.50 versus 2.50±0.50, respectively).

Conversely L-Car treatment with CDDP injections caused significantly increased the number of total white blood cells, and neutrophil and monocyte percentages (4.30±1.25 versus 6.50±0.85, 10.50±3.50 versus 20.50±2.50, and 2.50±0.50 versus 4.00±1.50, respectively) when compared with CDDP alone ($P<0.05$). However, a noticeable decrease in lymphocyte percentages was observed ($P<0.05$) in the L-Car treated with CDDP group compared to CDDP group (78.50±5.00 versus 70.55±3.50) *(Table 1)*.

After the semi-quantitative assessment of spleen sections, caspase 3 immunopositivity was found same intensity except in CDDP groups. This positivity significantly increased in lymphoid tissues which were exposed to cisplatin. There was mild immunopositivity in control *(Fig. 1a)*, L-car *(Fig. 1b)* and L-car + CDDP groups *(Fig. 1d)*. Positive immunostaining was moderate in CDDP group *(Fig. 1c)*. Similarly, positive immunoreactions for caspase-3 were observed in thymus sections. Caspase-3 reactivity was mild in groups of control *(Fig. 2a),* L-car *(Fig. 2b)* and L-car + CDDP *(Fig. 2d)*. Immunoreaction was observed more higher than these 3 groups in CDDP group *(Fig. 2c)*. When immunopositive staining was in moderate degree in CDDP group *(Fig. 3c),* immunostaining of caspase 3 was mild also, in lymph node sections of control *(Fig. 3a),* L-car *(Fig. 3-b)* and L-car + CDDP *(Fig. 3d)* groups. Apoptotic cells were characterized by chromatin condensation and picnosis and mainly observed in both red and white pulp of spleen, sinusoids of lymph nodes and thymus. Apoptotic cells were observed in the sinusoids of lymph nodes and less in follicles. While apoptotic staining was in high degree in lymph node sections**,** it was less in spleen and thymus, respectively. Thymus was found as more resistant lymphoid organ to cisplatin induced apoptosis. L-Car treatment with CDDP injections caused decrease apoptotic effect of CDDP *(Table 2)*.

When thymus sections was examined, CD68 *positivity was found mild in control group (Fig. 4a), none or mild in group of L-carnitine (Fig. 4b), CDDP (Fig. 4c) and L-Car + CDDP (Fig. 4d)*. CD68 staining in spleen was similar and marked in control, L-Car and L-Car + CDDP groups. When this positivity was mild in CDDP group *(Fig. 5c),* it was mild or moderate in control *(Fig. 5a),* L-Car *(Fig. 5b)* and L-Car + CDDP *(Fig. 5d)* groups. CD68 immunoreactivity was in

Table 1. *Leucocytes values of Control, L-Car, CDDP and L-Car + CDDP treated groups*
Tablo 1. *Kontrol, L-car, CDDP ve L-Car + CDDP gruplarında lökosit değerleri*

Parameters	Groups			
	Control	L-Car	CDDP	L-Car + CDDP
WBC ($mm^3/10^3$)	7.75±0.95	8.15±1.10	4.30±1.25[a]	6.50±0.85[b]
Lymphocyte (%)	65.50±4.50	67.55±2.50	78.50±5.00[a]	70.55±3.50[b]
Neutrophil (%)	25.50±2.50	23.50±3.00	10.50±3.50[a]	20.50±2.50[b]
Monocyte (%)	5.50±1.50	6.00±1.50	2.50±0.50[a]	4.00±1.50[b]

Each value represents the mean ± SEM of 6 animals
Significantly different from ***a:*** *Control group,* ***b:*** *L-Car + CDDP group ($P < 0.05$),* ***L-Car:*** *L-Carnitine,* ***CDDP:*** *Cisplatin*

Table 2. *Semi-quantitative assessment of caspase 3 immunopositivity in different lymphoid tissues of Control, L-Car, CDDP and L-Car + CDDP treated groups*
Tablo 2. *Kontrol, L-car, CDDP ve L-Car + CDDP gruplarında farklı lenfoid dokularda kaspaz 3 immunopozitiflik değerlendirmeleri*

Lymphoid Tissue	Groups			
	Control	L-Car	CDDP	L-Car + CDDP
Spleen	+	+	++	+
Thymus	+	+	++	+
Lymph node	+	+	+++	++

L-Car: *L-Carnitin,* ***CDDP:*** *Cisplatin,* ***+:*** *mild,* ***++:*** *moderate,* ***+++:*** *severe,* ***++++:*** *very strong*

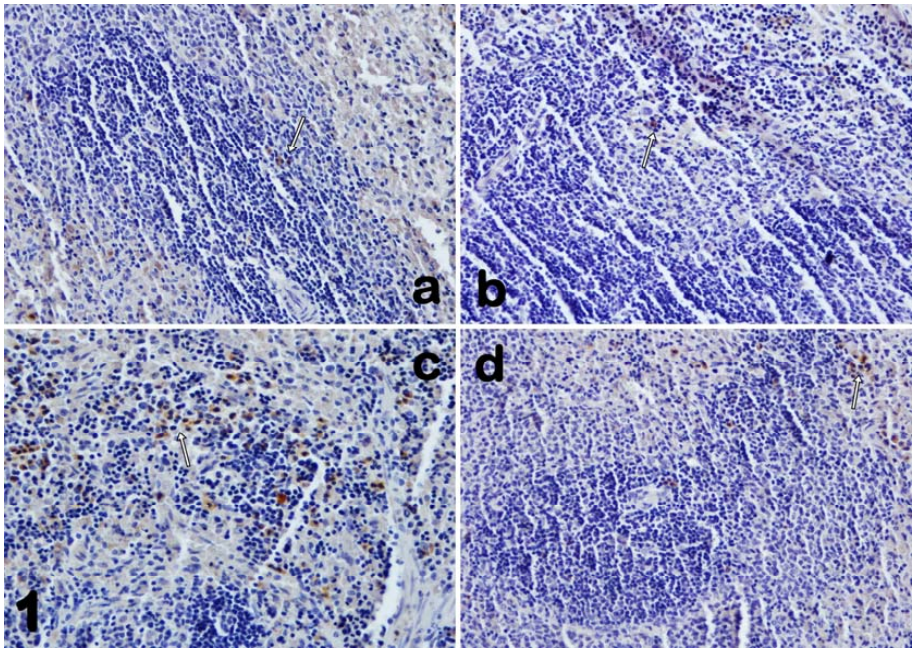

Fig 1. Spleen, **a.** Caspase 3 positive cells in spleen of control group**.** Weak immunoreactivity in white and red pulp (arrow), **b.** Weak immunoreactivity in spleen of L-Car group. It is similar to control group in severity of immunopositivity (arrow), **c.** Mild immunopositivity in spleen of CDDP group (arrow). Some lymphocytic cells have picnotic nuclei, **d.** Weak immunopositivity in spleen of L-Car + CDDP group and affected cells (arrow). Original magnification: x400. Streptavidin-biotin peroxidase staining

Şekil 1. Dalak, **a.** Kontrol grubunda kaspaz 3 pozitif hücreler. Kırmızı ve beyaz pulpada zayıf immunoreaktivite (ok), **b.** L-car grubunda control grubuna benzer zayıf immunoreaktivite (ok), **c.** CDDP grububnda hafif immunoreaktivite (ok), **d.** L-Car+CDDP grubunda zayıf immunopozitivite (ok). Orjinal büyütme: x400. Streptavidin-biotin peroxidase boyama

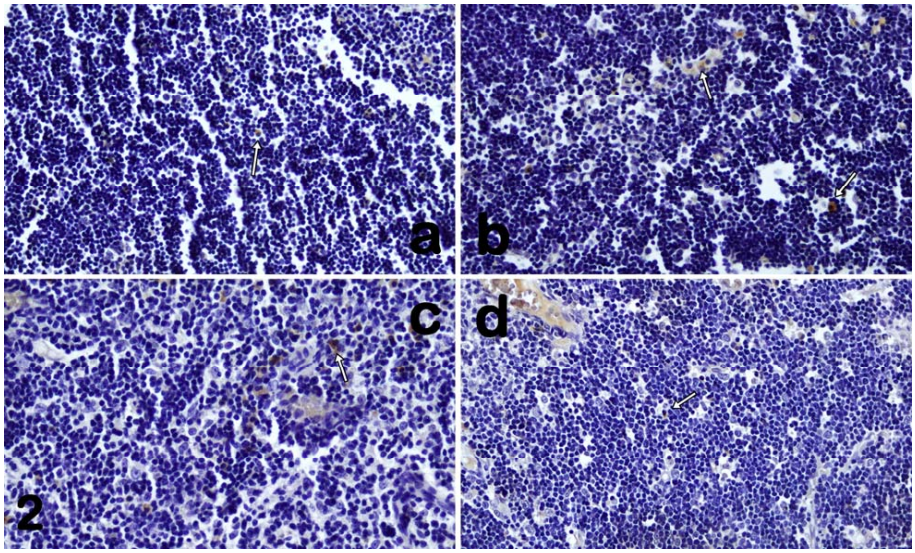

Fig 2. Thymus, **a.** Weak immunopositivity (arrow) in thymus of control group, **b.** Weak immunopositivity (arrows) in thymus of L-Car group, **c.** Mild immunopositivity (arrow) in thymus of CDDP group, **d.** Weak immunopositivity (arrow) in thymus of L-Car + CDDP group. Original magnification x400. Streptavidin-biotin peroxidase staining

Şekil 2. Timus, **a.** Kontrol grubunda zayıf boyanma (ok), **b.** L-car grubunda zayıf boyanma (oklar), **c.** CDDP grubunda zayıf boyanma (ok), **d.** L-Car+CDDP grubunda zayıf immunopozitivite (ok). Orjinal büyütme: x400. Streptavidin-biotin peroxidase boyama

high degree in lymph node sections. Positivity was moderate to severe in control *(Fig. 6a),* L-car *(Fig. 6b)* and L-car + CDDP *(Fig. 6d)* groups, while it was mild in CDDP group *(Fig. 6c)*. When compared to the control and L-Car groups, CD68 staining was prominently decreased in CDDP-treated group. Conversely, L-Car treatment with CDDP injections caused significantly increase in the staining of CD68. This reaction was more prominent in lymph node sections when compared to spleen and thymus *(Table 3)*.

Table 3. *Semi-quantitative assessment of CD68 immunopositivity in different lymphoid tissues of Control, L-Car, CDDP and L-Car + CDDP treated groups*

Tablo 3. *Kontrol, L-car, CDDP ve L-Car + CDDP gruplarında farklı lenfoid dokularda CD68 immunopozitiflik değerlendirmeleri*

Lymphoid Tissue	Groups			
	Control	L-Car	CDDP	L-Car + CDDP
Spleen	++	++	+	+
Thymus	+	+	+	+
Lymph node	+++	+++	+	++

L-Car: *L-Carnitin,* ***CDDP:*** *Cisplatin,* ***+:*** *mild,* ***++:*** *moderate,* ***+++:*** *severe,* ***++++:*** *very strong*

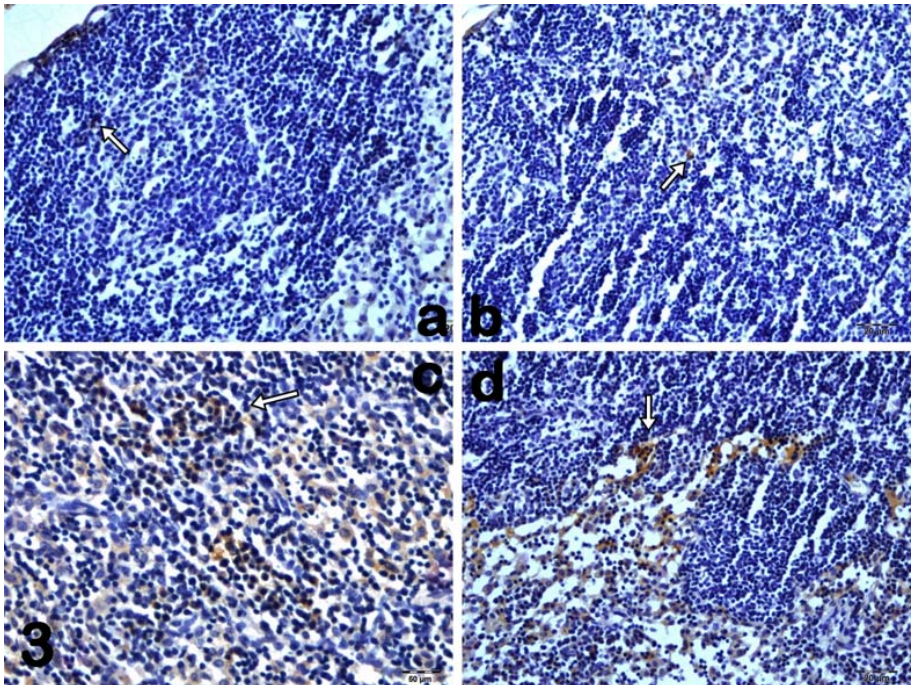

Fig 3. Lymph node, **a.** Weak immunopositivity (arrow) in lymph node of control group, **b.** Weak immunopositivity (arrow) in lymph node of L-Car group, **c.** Moderate immunopositivity (arrow) in lymph node of CDDP group, **d.** Mild immunopositivity (arrow) in lymph node of L-Car + CDDP group (arrow). Original magnification x400. Streptavidin-biotin peroxidase staining

Şekil 3. Lenf düğümü, **a.** Kontrol grubunda zayıf immunopozitivite (ok), **b.** L-car grubunda zayıf immunopozitivite (ok), **c.** CDDP grubunda orta şiddette immunopozitivite (ok), **d.** L-Car + CDDP grubunda hafif immunopozitivite (ok). Orjinal büyütme: x400. Streptavidin-biotin peroxidase boyama

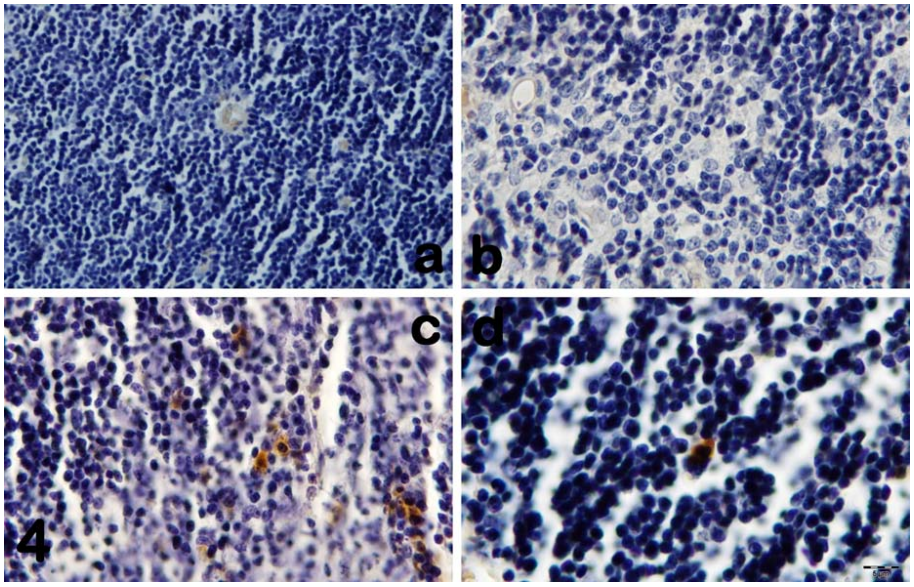

Fig 4. Thymus, **a.** Group control. None of CD68 staining, **b.** Group L-car. Mild CD68 staining, **c.** Group CDDP. Mild or moderate positivity in CD68, **d.** L-Group Car + CDDP. None or mild positivity in CD68 immunostaining. Original magnification: a=X400, b,c,d=x1000. Streptavidin-biotin peroxidase staining

Şekil 4. Timus, **a.** Kontrol grubu. Negatif CD68 boyanma, **b.** L-car grubu. CD68 hafif boyanma, **c.** CDDP grubu. Hafif/orta yoğunlukta CD68 aktivitesi, **d.** L-car + CDDP grubu. Hafif CD68 boyanma. Orjinal büyütme: a=X400, b,c,d=x1000. Streptavidin-biotin peroxidase boyama

DISCUSSION

Cisplatin, broadly used in tumor therapy, has some side effect and its toxicity has been determined in many studies. In those studies, degenerative, necrotic and fibrous tissue proliferative effect of cisplatin was described [9,12]. Besides, apoptotic effect of cisplatin on kidney and liver tissues has been studied in detail [12,27-29]. Despite of many detailed investigation about the toxicity on kidney and liver tissue any specific report could not been attained in current literature on the apoptotic effect on lymphoid tissues. Similarly, CD68 immunoreactivity in this toxication has not been studied. The present study was undertaken to determine the apoptotic changes and CD68 immunoreactivity in lymphoid tissues exposed to CDDP. CDDP induced apoptosis were observed in varying degree in lymphoid tissues in this study. This effect was found significantly increased, particularly, in lymph node sections and less in spleen and thymus, respectively. It is emphasized that thymus was less influenced from this toxication. Apoptotic cells were characterized by shrinkage and chromatin condensation in these areas. Apoptotic caspase enzymes are known as initiators and effectors. Effector caspase enzymes activate the initiators which trigger the process of apoptosis [14,30]. Caspase 3 is responsible for many biochemical mechanisms of apoptosis which leads to cleavage of nuclear and cytoplasmic substrates, chromatin condensation, DNA fragmentation and apoptotic bodies [14,15]. Carnitine adding to diet decreased the apoptotic effect of cisplatin. CD68 expression was largely used for the

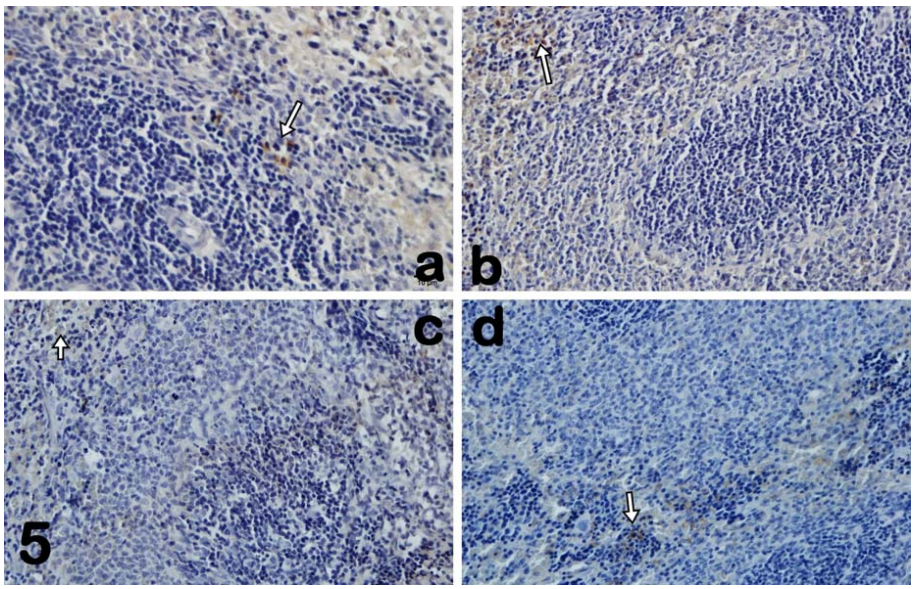

Fig 5. Spleen, **a.** Group control. Mild or moderate CD68 positivity (arrow), **b.** Group L-Car. Mild or moderate CD68 positivity (arrow), **c.** Group CDDP. Mild positivity in CD68 (arrow), **d.** Group L-Car + CDDP. Mild or moderate positivity in CD68 immunostaining (arrow). Original magnification: x400. Streptavidin-biotin peroxidase staining

Şekil 5. Dalak. **a.** Kontrol grubu. Hafif/orta yoğunlukta CD68 boyanma (ok), **b.** L-car grubu. Hafif/orta yoğunlukta CD68 boyanma (ok), **c.** CDDP grubu. Orta yoğunlukta CD68 aktivitesi (ok), **d.** L-car + CDDP grubu. Hafif/orta yoğunlukta CD68 boyanma (ok). Orjinal büyütme: x400. Streptavidin-biotin peroxidase boyama

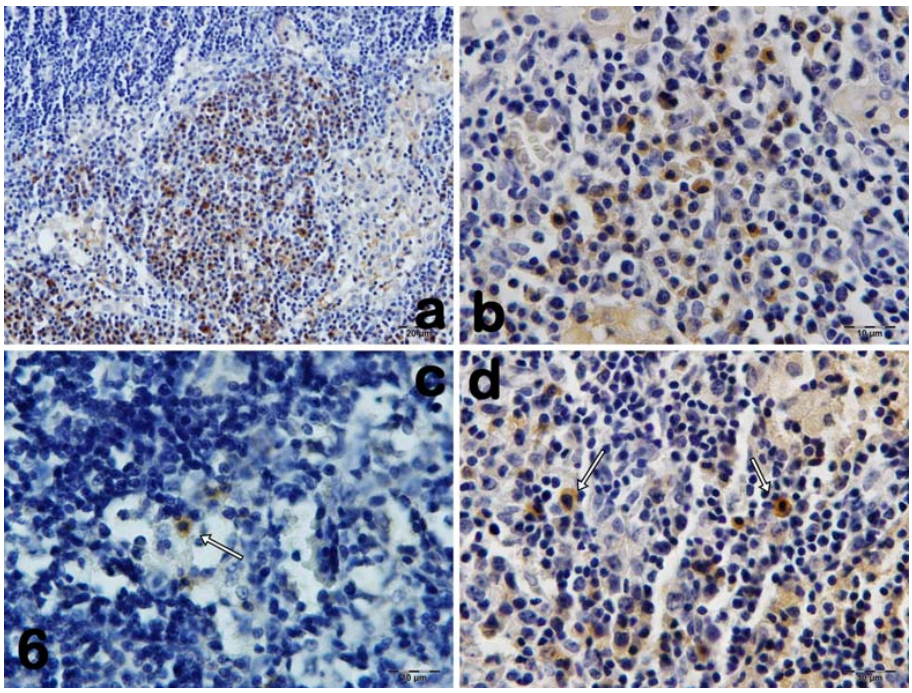

Fig 6. Lymph node, **a.** Group control. Moderate or severe CD68 positivity, **b.** Group L-Car. Moderate or severe CD68 positivity, **c.** Group CDDP. Mild CD68 positivity (arrow), **d.** Group L-Car + CDDP. Mild or moderate CD68 immunopositivity (arrows). Original magnifications: **a** = x400, **b,c,d** = x1000. Streptavidin-biotin peroxidase staining

Şekil 6. Lenf düğümü. **a.** Kontrol grubu. Orta/şiddetli yoğunlukta CD68 boyanma, **b.** L-car grubu. Orta/şiddetli yoğunlukta CD68 boyanma, **c.** CDDP grubu. hafif yoğunlukta CD68 aktivitesi (ok), **d.** L-car + CDDP grubu. Hafif/orta yoğunlukta CD68 boyanma (oklar). Orjinal büyütme: **a** = x400, **b,c,d** = x1000. Streptavidin-biotin peroxidase boyama

detection of monocytes and macrophages and this expression appeared to correlate with macrophage activation [16-18]. In the present study, CD68 immunoreactivity was found in high degree in lymph node sections. It was less in spleen and there was almost no in thymus. This positivity was prominently decreased in CDDP-treated group when compared to the control and L-Car groups, CD68. Conversely, L-Car treatment with CDDP injections caused significantly increase in the staining of CD68. Suppression of macrophages due to CDDP toxicity may be the reason for this. Thus, haematological results support this mechanism. L-carnitine, a vitamin-like cofactor was reported to be protective for cellular membranes via the detoxification of acetyl groups and free CoA [31]. It serves in the transfer of long chain fatty acids in to the mitochondria [19,20]. In present study, carnitine supplementation decreased the apoptotic effect of CDDP in lymphoid tissue.

The success of a chemotherapy is dependent not only on effectively removing tumor cells but also on reducing the related immunosuppressive complications that are primarily caused by apoptosis of circulating leucocytes cells (leucopenia). This danger situation interferes with a patient's response to chemotherapy by causing severe immunosuppressive conditions, as reflected in a lower WBC count. In the present work, we also analyzed changes in total leucocytes counts and percentage rates of leukocytes by haemocytometric method and May Grunwald-Giemsa's staining method (26), respectively. In our study, analysis of white blood cells, and neutrophil

and monocyte percentages showed that CDDP treatment induced the decrease in circulation. The main mechanism of cisplatin myelotoxicity is unknown; however, there may be two mechanisms have been shown both in vitro and in vivo studies. These include firstly, it's toxicity is associated closely with platinum DNA interstrand bifunctional N-7 adducts at d(GpG) and d(Apg) [32], and other mechanism, CDDP caused the mitochondrial oxidative stress with resultant energetic metabolism impairment, and the release of reactive oxygen species in the cells [33]. Evans et al.[34] and Nowrousian and Schmidt [33] reported similar results that after a single dose of cisplatin treatment in mice, the bone marrow derived colony-forming units (CFUs) were depleted significantly. Das et al.[35] recently reported that cisplatin treatment exerts significant toxicity on the hematopoietic stem cell fraction in vitro.

Antioxidants such as glutathione and metallothioneins are found to prevent CDDP-induced toxicity [10,36]. Hence, the clinical uses of antioxidants have been suggested to reduce CDDP-induced myelosuppression [37,38]. In our study, L-Car treatment were prevented this tendency to decrease in CDDP-treated rat circulation. Previous researchers have demonstrated the enhancement of immune responses by L-carnitine supplementation [39-41]. Also, Deufel [42] informed that leukocytes are enriched in carnitine; and also Monti et al.[43] informed that L-car protect to cells against toxicity of reactive oxygen species. Moreover, Famularo et al.[44] have demonstrated antiapoptotic effects of L-car. On the other hand Abd-Allah et al.[45] stated that L-Car halts apoptosis and myelosuppression induced by carboplatin in rat bone marrow cell cultures. The hematostimulatory and antiapoptotic nature of L-Car [39,44] may participate in preventing chemotherapy-induced leukocyte apoptosis [46]. Various natural or synthetic antioxidant agents have been well used in order to prevent or ameliorate CDDP and other chemotherapeutic agents-induced bone marrow toxicity [47]. Ghosh et al.[48] informed that Neem (Azadirachta Indica) leaf preparation prevents leukocyte apoptosis mediated by cisplatin plus 5-fluorouracil, treatment in swiss mice. The same author et al.[49] informed that pretreatment with neem (Azadirachta indica) leaf preparation in Swiss mice diminishes leukopenia and enhances antitumor activity of cyclophosphamide. On the other hand, Das et al.[50] stated that squalene exerts a cytoprotective effect on bone marrow and against platinum-induced bone marrow toxicity in mice. Perez-Cruz et al.[51] informed that Vitamin C inhibits FAS-induced apoptosis in monocytes and U937 cells.

It is concluded that CDDP increase apoptosis in lymphoid tissues, and also L-Car showed a cytoprotective effect on leucocytes against CDDP-induced toxicity in rat. Apoptotic effect of CDDP was found in lymph node in high degree and thymus in low degree. Carnitine supplementation to diet decreased apoptotic effect of cisplatin.

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