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RESEARCH ARTICLE

The Effect of L- Carnitine Administration on Doxorubicine Induced Hepatoxicity and Nephrotoxicity in Rabbits [1]

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Summary

This study was designed to investigate the protective effect of L-carnitine (LCAR) on doxorubicin (DOX) associated damage in liver and kidney. A total of 21 healthy albino New-Zealand rabbits were divided into 3 groups. Rabbits in group I (n=8) received DOX at the dose of 0.6 mg/kg/day body weight (BW) intraperitoneally (IP) for 6 days, group II (n=7) received DOX at the dose of 0.6 mg/kg/day BW, IP and LCAR at the dose of 1000 mg/kg/day BW IP for 6 days and group III (n=6) received LCAR at dose of 1000 mg/kg/day BW IP for 6 days. Blood samples from auricular vein were taken from all animals at the beginning of the experiment (before the drug administration) and 2 hours after the drug administration daily for 6 days. Histopathological examination of all animals was carried out at the end of the study. Severe alterations in liver and kidney of rabbits given only DOX were noticed on histopathology examination. This was supported by marked increases in serum concentrations of ALT, AST, creatinin, and urea in this group when compared to other groups. The results of histopathological and biochemical analysis revealed that parenteral LCAR has a potential role in protection against adverse effect of DOX on liver and kidney.

Keywords: *Doxorubucine, L-carnitine, Hepatoxicity, Nephrotoxicity, Rabbit*

L-Carnitin Uygulamasının Tavşanlarda Doksorubisin Kaynaklı Hepatopatoksisite ve Nefrotoksisite Üzerine Etkisi

Özet

Bu çalışma doksorubisin (DOX) kaynaklı karaciğer ve böbrek hasarlarında L-carnitinin koruyucu etkilerinin araştırılması için tasarlanmıştır. Toplam 21 adet sağlıklı Yeni Zelanda ırkı albino tavşan 3 gruba ayrıldı. I. Gruptaki tavşanlara (n=8) 0.6 mg/kg/canlı ağırlık (CA) dozunda DOX intraperitoneal (IP) olarak 6 gün boyunca verildi, II. Gruptaki hayvanlara (n=7) 0.6 mg/kg/CA dozunda DOX, IP olarak ve 1000 mg/kg/CA dozunda LCAR, IP olarak 6 gün boyunca verildi ve III. Gruptakilere (n=6) ise 1000 mg/kg/CA dozunda LCAR, IP olarak 6 gün boyunca verildi. Çalışmanın başlangıcında (ilaç uygulamadan önce) ve ilaç uyguladıktan iki saat sonra 6 gün boyunca tüm hayvanların vena auricularislerinden kan örnekleri alındı. Çalışmanın sonunda ise tüm hayvanların histopatolojik muayeneleri yapıldı. Sadece DOX uygulanan tavşanlarda histopatolojik muayenelerde karaciğer ve böbreklerde şiddetli hasarlar tespit edildi. Diğer gruplara nazaran DOX grubundaki serum ALT, AST, üre ve kreatin konsantrasyonlarındaki önemli artış histopatolojik bulguları teyit etti. Histopatolojik ve biyokimyasal analizler paranteral LCAR uygulamasının karaciğer ve böbrekteki DOX kaynaklı yan etkilere karşı olası koruyucu role sahip olduğunu ortaya koymuştur.

Anahtar sözcükler: *Doksorubusin, L-carnitin, Hepatopati, Nefropati, Tavşan*

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INTRODUCTION

Doxorubicin (DOX), a broad-spectrum anthracycline antineoplastic agent, is commonly used in human cancer cases. However, severe side effects associated with especially heart, liver and kidney limits its clinical use [1-4]. Therapeutic and adverse effect of the drug have been related to DNA damage or hindrance of DNA repair, the production of free radicals and lipid peroxidation, immune mediated cell damage and initiation of apoptosis leading to cell function abnormalities [2-8]. DOX use has therefore been associated with cardiotoxicity, hepatotoxicity and nefrotoxicity in various species [2,9,10]. Our previous data has shown severe cardiotoxic effect of DOX [9] and alteration in oxidant-antioxidant status [11] in rabbits. We also disclosed protective effect of L-carnitine against DOX induced cardiotoxicity [9] and its oxidative stress [11]. L carnitine, a natural nutrient and essential requirement for the β-oxidation of fatty acids in mitochondria to generate ATP [12] has been speculated to overcome these side effects through effective inhibition of mitochondrial injury induced by oxidative stress and mitochondria-dependent apoptosis of various types of cells [13-16] and by inhibiting production of cytotoxic substances such as reactive oxygen species (ROS) and other free radicals [6,11,17] and by enhancing gluthation peroxidase (GSH) [18]. Many anti-oxidants have been used to overcome DOX associated hepatotoxicity and nephrotoxicity [19-22] but there is a scarcity of literature existing on the use of LCAR for the same purpose [10].

This study was a part of project investigated cardiotoxicity induced by DOX and protective role of LCAR. In this part, coexisting hepatotoxicity and nephrotoxicity induced by DOX and use of LCAR in an attempt to avoid these side effects were investigated.

MATERIAL and METHODS

Experimental design

Animal material and study design have already been published [9]. Briefly a total of 21 healthy New Zealand albino rabbits were divided into three groups; DOX group (n=8) received doxorubicin (DOX) at the dose of 0.6 mg/kg bw intraperitonally (IP), DOX+LCAR group (n=7) received DOX at the dose of 0.6 mg/kg bw and L carnitine (LCAR) at the dose of 1000 mg/kg bw, IP, and LCAR group (n=6) received L-carnitine at the dose of 1000 mg/kg bw, IP. All injections lasted for 6 days. Approval of The Laboratory Animal Care and Use Committee of the Faculty of Veterinary Medicine was obtained for the experimental protocol.

Blood samples and biochemistry

Animals were examined and sampled before injections (day 0 as baseline value) and 2 h after daily injections. Blood samples were collected from auricular vein into plain tubes for determination of biochemical parameters related to liver and kidney. Serum AST, ALT, urea and creatinin were measured on a spectrophotometer (Tecan-spectra, Austria) using commercial kits (DDS®, Germany). Tests were carried out and the results were calculated as instructed by the manufacturers.

Necropsy and urine samples

All rabbits were subjected to necropsy at the end of the experiment. Routine histopathology was performed on liver and kidney tissues in all rabbits and urine samples were also collected at necropsy from all rabbits for analysis using commercial test strip (Combur Test 10, Roche).

Statistical analysis

Results were statistically analyzed using Duncan ANOVA on SPSS for windows 10.0 and expressed in the tables as mean and standard error.

RESULTS

Biochemistry

Biochemical changes of the groups are given in *Table 1*. All parameters of concern began to change on the 3rd day of DOX administration. The concentrations of particularly ALT and creatinin of DOX group started to significantly increase from the second day of the study ($P<0.05$). AST, ALT, urea and creatinin concentrations determined in DOX group were significantly higher than those of DOX+LCAR and LCAR group especially on day 5th and 6th of the experiment ($P<0.05$).

Histopathological findings

Liver

Macroscopic assessment disclosed that in rabbits of DOX group, livers were cyanotic with pale appearance at lobular edge, fragile, friable, and heamorrhagic on cross-section whereas in DOX+LCAR group livers had only mild degenerations and 3-4 yellowish focal points and they were normal in LCAR group.

Table 1. *Serum biochemistry alteration associated with treatment groups (mean±standard error)*
Tablo 1. *Tedavi gruplarında serum biyokimya parametreleindeki değişiklikler (ortalama±standart hata)*

Parameters	Groups	Days							P
		0	1	2	3	4	5	6	
AST (U/dl)	DOX	5.62±1.97	6.53±2.60	6.25±2.23	7.88±5.16	6.59±1.97	7.25±2.26A	7.49±2.77A	
	DOX+LCAR	5.55±1.74	5.33±1.18	6.67±2.47	6.05±1.66	5.45±0.86	5.37±1.03AB	6.50±1.36AB	
	LCAR	5.15±0.52	4.49±0.53	5.14±1.03	5.19±0.95	4.59±1.05	4.19±1.04B	4.14±1.58B	
	P						**P<0.05**	**P<0.05**	
ALT (U/dl)	DOX	5.54±2.38b	5.59±2.42b	6.12±4.16b	6.36±3.33ab	6.78±3.19ab	8.27±1.54ab	10.41±6.94a	**P<0.05**
	DOX+LCAR	5.87±1.07	4.58±0.93	4.52±1.32	5.44±1.51	6.18±3.15	5.20±1.18	5.24±1.31	
	LCAR	4.95±1.14	5.80±1.55	5.14±1.13	5.33±2.11	5.42±1.19	4.56±1.37	4.76±1.68	
	P								
Urea (mg/dl)	DOX	34.87±13.38	34.37±6.64	34.13±14.33	35.53±5.69	38.61±8.45	39.40±3.58A	39.84±5.72A	
	DOX+LCAR	32.46±4.09	34.33±12.06	33.17±8.32	32.71±5.07	37.32±8.14	35.48±5.10AB	35.48±3.81AB	
	LCAR	34.19±8.41	30.32±4.01	32.90±7.35	32.90±13.18	32.90±5.04	31.29±5.54B	30.96±7.33B	
	P						**P<0.05**	**P<0.05**	
Kreatinin (mg/dl)	DOX	0.94±0.12b	1.29±0.29A,a	1.38±0.26A,a	1.39±0.19A,a	1.50±0.27A,a	1.30±0.14A,a	1.44±0.18A,a	**P<0.001**
	DOX+LCAR	0.88±0.12	1.12±0.27A	0.98±0.24B	1.07±0.44A	1.08±0.16B	1.04±0.42AB	1.01±0.17B	
	LCAR	0.82±0.14a	0.67±0.08B,ab	0.63±0.08C,ab	0.63±0.08B,ab	0.61±0.07C,b	0.68±0.21B,ab	0.54±0.13C,b	**P<0.05**
	P		**P<0.05**	**P<0.001**	**P<0.001**	**P<0.001**	**P<0.001**	**P<0.001**	

A,B,C: *Indicates differences in columns;* ***a,b,c,d:*** *Indicates differences in rows*

Microscopic evaluation of livers in DOX group revealed swollen and granulated cytoplasm of hepatocytes, dissociated Remark cords and loss of nuclei in some cells or heavy staining of nuclei in others and some nuclei were in variable size and stained with different degree of intensity. Accumulation of mononuclear cells in Kiernan's space especially around bile ducts was noted. Hepatocyte necrosis was markedly present around central veins *(Fig 1)*. Livers of DOX + LCAR group had less severe lesions when compared to DOX group where hepatocytes had granulated cytoplasm and disarrangement of Remark cords at less intensity. Nuclei of hepatocytes were in variable size and stained with different degree of intensity. A mild mononuclear cell accumulation was present in Kiernan's space and a mild hepatocyte necrosis was evident around central veins *(Fig 2)*. Microscopy of LCAR group revealed a normal liver apart from small vacuoles within cytoplasm of hepatocytes in peripheral lobules.

Kidney

Kidneys of rabbits in DOX group were swollen and had reddish pinpoint hollow and pale appearance of depressed area on cross-sections. The line between cortex and medulla was marked and cortex/medulla ratio increased in favor of medulla. The gross appearance of kidneys in DOX+LCAR group was near to normal and the kidneys were not affected in LCAR group.

On microscopic evaluation of kidneys, DOX group had atrophic glomerulus and markedly enlarged glomerular gap, swollen tubular epithelium and less intense staining of cytoplasm of epithelial cells, tubular necrosis in patches and protein rich material in some tubular lumens, intertubular haemorrhagia and

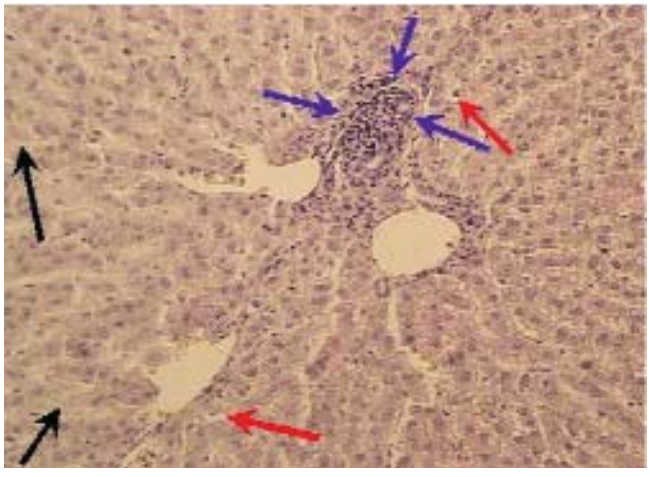

Fig 1. Microscopic view of livers in DOX group. Vacuoles in cytoplasm of hepatocytes (red arrows), necrotic hepatocytes (black arrows) mononuclear cell infiltration (blue arrows). Haemotoxylin & Eosin Staining

Şekil 1. DOX grubu tavşanların karaciğerlerinin mikroskobik görünümü. Sitoplazmalarında vakuoller bulunan hepatositler (Kırmızı oklar), Nekrotik hepatositler (Siyah oklar), Mononükleer hücre infiltrasyonu (Mavi oklar). Hematoksilen&Eozin Boyama

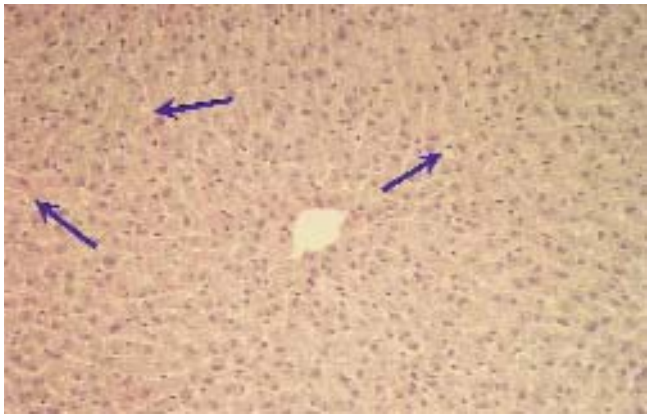

Fig 2. Microscopic view of livers in DOX+LCAR group. Small vacuoles in cytoplasm of hepatosytes (arrows). Haemotoxylin & Eosin Staining

Şekil 2. DOX&LCAR grubu tavşanların karaciğerlerinin mikroskobik görünümü. Hepatositlerin sitoplazmalarında küçük vakuoller (oklar). Hematoksilen& Eozin Boyama

a few mononuclear cells in renal interstitium (*Fig 3* and *4*). The lesions were mild in DOX + LCAR group where enlarged glomerular space with an increased number of mesengial cells, mild intertubular haemorraghia and a few swollen and granulated cytoplasm of tubular epithelium *(Fig 5)*. Kidneys of LCAR group were histologically normal except for small vacuoles in cytoplasm of a few tubular epithelium and slightly pinkish fluid in tubular lumen.

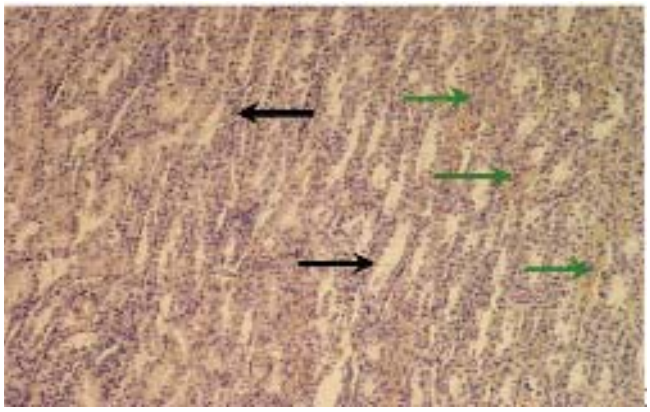

Fig 3. Microscopic view of kidneys in DOX group. Large intertubular haemorrhagic areas (green arrows), tubular epithelial degeneration (black arrows). Haemotoxylin & Eosin Staining

Şekil 3. DOX grubu tavşanların böbreklerinin mikroskobik görünümü. Geniş intertubuler kanama alanları (Yeşil oklar), Epitelleri dejenere olmuş tubuluslar (Siyah oklar). Hematoksilen&Eozin Boyama

Urine analysis

Urine of rabbits in DOX group was foamy, cloudy, dense and dark yellow. The specific gravity and pH were 1.000-1.005 and 8.5, respectively. Protein and

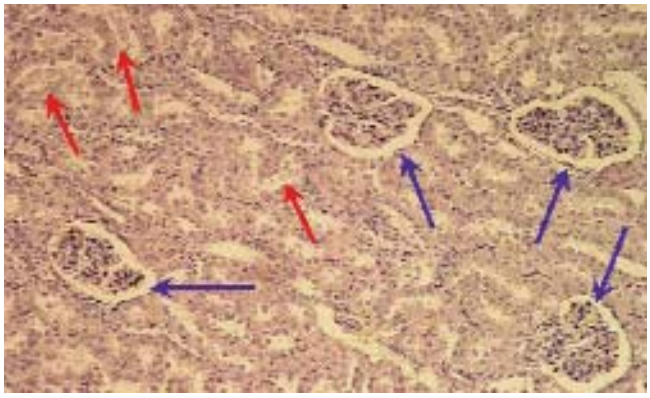

Fig 4. Microscopic view of kidneys in DOX group. Enlargement of glomerular space (blue arrows), mass in tubular lumen (red arrows). Haemotoxylin & Eosin Staining

Şekil 4. DOX grubu tavşanların böbreklerinin mikroskobik görünümü. Glomerular yumakta atrofi ve glomerular boşlukta genişleme (Mavi oklar), Tubul lümenlerinde kitle (Kırmızı oklar), Hematoksilen&Eozin Boyama

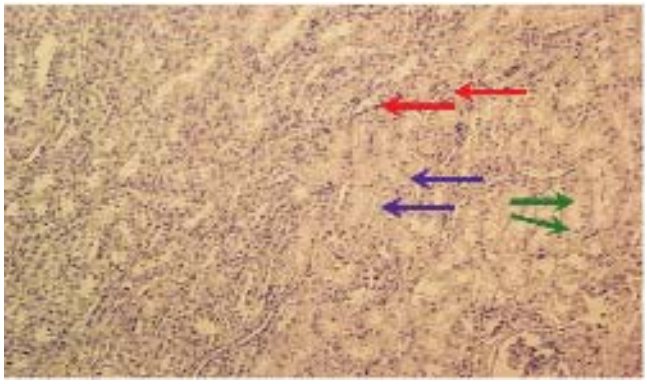

Fig 5. Microscopic view of kidneys in DOX+LCAR group. Intertubular hemorrhagia (red arrows), cloudy swelling of tubular epithelial cells (blue arrows), large vacuole in tubular lumen (green arrows). Haemotoxylin & Eosin Staining

Şekil 5. DOX&LCAR grubu böbreklerinin mikroskobik görünümü. İntertubuler kanama alanları (Kırmızı oklar), Tubulus Epitellerinde bulanık şişkinlik (Mavi oklar). Tubulus lümenlerinde geniş vakuoller (Yeşil oklar). Hemotoksilen&Eozin Boyama

leukocytes were present on strip analyses. Sediment analyses of urine also disclosed erythrocyte, leukocyte and renal epithelial cells. Urinanalyses of DOX + LCAR group was less severe when compared to DOX group. Urine was clear, runny and had no particles in. The specific gravity was 1.010-1.020 and pH was 6.5-8.5. Trace protein was noted and no cells were present in urine sediments. The urine parameters were normal in LCAR group.

DISCUSSION

This part of the study investigated prevention of DOX induced hepatotoxicity and nephrotoxicity in

rabbits by using LCAR as adjunct remedy. DOX is a well known and widely used antineoplastic drug. DOX has been associated with a time and dose dependent cradiotoxicity in human and in laboratory animals [9,23,24] and hence has been used at dose rate of 0.6 mg/kg bw for only 3 days followed by a 3 week off period. We evaluated possible use of DOX at the same dose for 6 consecutive days without attaining likely side effects on liver and kidney by concurrently utilizing LCAR in rabbit model.

Biochemical, urinanalyses and histopathological findings obtained in this study indicated hepato-toxicity and nephrotoxicity associated with DOX use in rabbits as previously reported [2,3,9,20,24]. Lesions obtained at necropsy and microscopic evaluations were in parallel with those reported earlier [24-27]. Cytotoxic effect of DOX on various organs is believed to be associated with its DNA intercalation and cell membrane lipid binding activities. DOX-induced apoptosis is thought to be another fundamental part of the cellular mechanism of action responsible for its therapeutic or toxic effects [20,28]. DOX is therefore believed to achieve toxic effect through initiation of disorders in oxidant-antioxidant systems that eventually result in tissue injury and accordingly organ dysfunctions. Toxic agents such as DOX have been verified to induce liver damage through inflammatory process, oxidative stress and lipid peroxidation [19] similar processes have also been reported for kidney damage [10,20,29]. Apoptotic processes in liver and kidney tissue after a single dose of DOX was already described [29-31]. It was also confirmed that the therapeutic doses of DOX enhance lipid peroxidation in microsomes and mitochondria in the liver, especially in the presence of Fe^{+3} ions [32]. Similarly we previously demonstrated oxidant effect of DOX in the rabbits studied in the present study [11]. These adverse effects limited use of DOX and thus approaches to minimize these toxic effects have been matter of subject by researchers. This search led to development of various ways of diminishing DOX toxicity by optimizing dosages, use of analogous remedy or more importantly and commonly combining DOX with antioxidants [5]. Many studies exist about use of antioxidants such as vitamin E, erdosteine, cystathionine, and catechin along with DOX [2,19,21,22] but use of LCAR for this purpose has only recently been appreciated and studied, especially in preventing liver and kidney damage due to DOX.

In the present study use of LCAR as adjunct drug to DOX resulted in decreased toxic effect of DOX. This was confirmed by biochemical and histopathological findings as serum ALT, AST, urea and creatinin concentrations were significantly lower even close to baseline values in LCAR+DOX group than DOX group and similarly histopathologic lesions in liver and kidney were markedly less severe in LCAR+DOX group. The protective effect of LCAR may be attributed to enhancing β-oxidation to generate ATP, thus minimizing the toxic effects of free forms of long-chain fatty acids in mitochondria [10,13]. Furthermore, it has been reported that carnitine strongly inhibits mitochondrial membrane permeability transition and apoptosis of various types of cells through inhibition of sphingomyelin-ceramid pathway [13-16,28,33]. DOX has also been reported to inhibit carnitine palmitoyltransferase that plays an important role in beta oxidation of long chain fatty acids in mitochondria so exogenous administration of LCAR may overcome this affect. LCAR might also have exerted its protective effect through enhancement of antioxidant system as reported earlier [10,11].

In conclusion the results clearly indicated that L-carnitine substantially inhibited the doxorubicin-induced injury of the kidney and liver and, hence, this compound may have therapeutic potential in cancer patients receiving chemotherapy.

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