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RESEARCH

Protective effects of insulin-like growth factor (IGF-1) in doxorubicin-induced experimental liver and kidney injury

Doksorubisin ile oluşturulan deneysel karaciğer ve böbrek hasarında insülin benzeri büyüme faktörünün (IGF-1) iyileştirici etkilerinin araştırılması

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Abstract

Purpose: This study aimed to investigate the protective effects of insulin-like growth factor-1 (IGF-1) against doxorubicin (DOX)-induced liver and kidney damage in rats using biochemical, immunohistochemical, and histopathological methods.

Materials and Methods: A total of 32 Wistar albino rats were randomly assigned into four groups: Control, DOX (4 mg/kg/week), IGF-1 (1 µg/kg daily), and DOX + IGF-1 (DOX 4 mg/kg/week + IGF-1 1 µg/kg daily). After the four-week experimental protocol, blood, liver, and kidney tissues were collected under anesthesia. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), urea, and creatinine were analyzed biochemically. Total antioxidant status (TAS) and total oxidant status (TOS) in liver and kidney tissues were measured using ELISA. Gene expression levels of HIF-1α and iNOS were evaluated by real-time PCR. Histopathological and immunohistochemical (Caspase-3, TNF-α, and FGF-2) evaluations were conducted on liver and kidney tissues.

Results: DOX significantly increased serum ALT, AST, BUN, urea, and creatinine levels and elevated TOS while reducing TAS in tissues). HIF-1α expression was markedly upregulated (+5.50-fold), indicating oxidative damage. IGF-1 administration reduced serum ALT, AST, BUN, urea, and creatinine levels, increased TAS, and decreased TOS. Expression levels of HIF-1α and iNOS approached those of the control group. DOX caused significant histopathological damage and increased Caspase-3 and TNF-α expressions in the liver and Caspase-3 and FGF-2 expressions in the kidney. These alterations were ameliorated by IGF-1 treatment.

Conclusion: The findings suggest that IGF-1 exerts protective effects against DOX-induced hepatorenal

Öz

Amaç: Bu çalışmanın amacı, biyokimyasal, immünohistokimyasal ve histopatolojik yöntemler kullanılarak sıçanlarda doksorubisin (DOX) kaynaklı karaciğer ve böbrek hasarına karşı insülin benzeri büyüme faktörü-1'in (IGF-1) koruyucu etkilerinin araştırılmasıdır.

Gereç ve Yöntem: Toplam 32 Wistar albino sıçanı rastgele dört gruba ayrıldı: Kontrol, DOX (4 mg/kg/hafta), IGF-1 (günlük 1 µg/kg) ve DOX + IGF-1 (DOX 4 mg/kg/hafta + IGF-1 1 µg/kg günlük). Dört haftalık deneysel protokolden sonra, anestezi altında kan, karaciğer ve böbrek dokuları toplandı. Alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), kan üre azotu (BUN), üre ve kreatinin serum seviyeleri biyokimyasal olarak analiz edildi. Karaciğer ve böbrek dokularındaki toplam antioksidan durum (TAS) ve toplam oksidan durum (TOS) ELISA kullanılarak ölçüldü. HIF-1α ve iNOS gen ekspresyon seviyeleri gerçek zamanlı PCR ile değerlendirildi. Karaciğer ve böbrek dokularında histopatolojik ve immünohistokimyasal (Kaspaz-3, TNF-α ve FGF-2) değerlendirmeler yapıldı.

Bulgular: DOX, serum ALT, AST, BUN, üre ve kreatinin seviyelerini önemli ölçüde artırdı ve dokularda TAS'ı azaltırken TOS'u yükseltti. HIF-1α ekspresyonu belirgin şekilde yukarı düzenlendi (+5,50 kat), bu da oksidatif hasarı gösteriyordu. IGF-1 uygulaması serum ALT, AST, BUN, üre ve kreatinin seviyelerini düşürdü, TAS'ı artırdı ve TOS'u azalttı. HIF-1α ve iNOS ekspresyon seviyeleri kontrol grubundakilere yaklaştı. DOX, karaciğerde önemli histopatolojik hasara ve artan Kaspaz-3 ve TNF-α ifadelerine ve böbrekte Kaspaz-3 ve FGF-2 ifadelerine neden oldu. Bu değişiklikler IGF-1 tedavisiyle iyileştirildi.

Sonuç: Bulgular, IGF-1'in muhtemelen antioksidan, anti-inflamatuar ve anti-apoptotik özellikleri yoluyla DOX

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toxicity, possibly through its antioxidant, anti-inflammatory, and anti-apoptotic properties.
Keywords:. Apoptosis, doxorubicin (DOX), inflammation, insulin-like growth factor-1 (IGF-1), kidney damage, liver damage

kaynaklı hepatorenal toksisiteye karşı koruyucu etkiler uyguladığını göstermektedir.
Anahtar kelimeler: Apoptoz, doksorubisin (DOX), inflamasyon, insülin-benzeri büyüme faktörü 1 (igf-1), böbrek hasarı, karaciğer hasarı

INTRODUCTION

Doxorubicin (DOX) is a widely used anthracycline antibiotic with potent antitumor activity. It is commonly administered in the treatment of various cancers, including lung, testicular, prostate, and breast malignancies, and is considered one of the most effective chemotherapeutic agents available [1,2]. The anticancer effects of DOX are primarily mediated through its ability to intercalate into DNA, inhibit topoisomerase II, and trigger apoptosis in malignant cells. However, its clinical use is often limited by severe side effects such as cardiotoxicity, hepatotoxicity, and nephrotoxicity[3].

The therapeutic efficacy of doxorubicin stems from its ability to selectively target rapidly dividing cancer cells, making it a cornerstone of many cancer treatment regimens. However, its application is often limited by its toxicity to non-cancerous tissues, particularly the liver and kidneys, which play crucial roles in drug metabolism and excretion. The liver and kidneys are especially susceptible to damage from doxorubicin due to their high drug-processing functions, ultimately compromising organ function and limiting therapeutic outcomes[4].

The mechanisms of doxorubicin-induced hepatotoxicity and nephrotoxicity are multifaceted but are largely driven by oxidative stress. Doxorubicin generates reactive oxygen species (ROS), which contribute to mitochondrial dysfunction, cellular damage, and an imbalance in redox homeostasis. These processes ultimately lead to a reduction in antioxidant enzyme levels inflammatory responses, apoptosis, and tissue injury [4, 5, 6]. A comprehensive understanding of these mechanisms is essential for developing strategies to mitigate the toxic effects of doxorubicin while preserving its anticancer properties.

In light of the aforementioned limitations, various studies have focused on the use of natural antioxidants and anti-inflammatory agents to protect against the oxidative damage caused by doxorubicin [7, 8]. Among these, insulin-like growth factor-1 (IGF-1) has gained attention due to its antioxidant, anti-inflammatory, and anti-apoptotic properties [9]. IGF-1 is a hormone with structural similarity to insulin and plays a vital role in cell growth, differentiation, and repair. It has been shown to reduce inflammation, inhibit proinflammatory cytokine expression, and protect cells from oxidative stress by neutralizing ROS, thereby preventing tissue damage [10, 11].

Given these properties, IGF-1 holds promise as a therapeutic agent for mitigating the toxic side effects of doxorubicin. The aim of this study is to investigate the protective effects and underlying mechanisms of IGF-1 in doxorubicin-induced liver and kidney damage. By shedding light on these mechanisms, the study seeks to contribute to the development of more effective therapeutic strategies that can minimize the toxic side effects of doxorubicin while maintaining its potent antitumor efficacy.

Therefore, we hypothesize that IGF-1 administration can alleviate doxorubicin-induced hepatotoxicity and nephrotoxicity by modulating oxidative stress, inflammation, and apoptosis pathways. This study is expected to provide novel insights into the protective role of IGF-1 and contribute to the existing literature by proposing a potential therapeutic approach to reduce doxorubicin-related organ toxicity.

MATERIALS AND METHODS

Experimental protocol

In this study, 32 Wistar albino male rats (2-3 months old) were used. The animals were housed under standard care conditions (temperature 22°C, humidity 50%, dark/light cycle). No restrictions were made on feed and drinking water. All applications were performed in accordance with the Guide for the Care and Use of Laboratory Animals after obtaining approval from the Pamukkale University Animal Experiments Local Ethics Committee (PAUHDEK-2021/06- 05.04.2022).

All experimental records were properly documented, archived, and stored in compliance with institutional and ethical standards, ensuring traceability and data integrity. The animals were divided into four groups as control, doxorubicin, IGF-1 and DOX+IGF-1.

Intraperitoneal saline was administered to the control group. Intraperitoneal doxorubicin was administered to the doxorubicin group at a dose of 4 mg/kg/week. Intraperitoneal IGF-1 was administered to the IGF-1 group at a dose of 1 μg/kg every other day. Doxorubicin and IGF-1 were administered to the DOX+IGF-1 group at a dose of 4 mg/kg/week and 1 μg/kg, respectively [29]. After 28 days of drug administration, tissue and blood samples were taken under ketamine xylazine anesthesia. Parts of the kidney and liver were stored at (-20 °C) for biochemical analysis. The remaining parts were fixed in 10% buffered formaldehyde for immunohistochemical and histochemical pathological analyses. The rest of the other fractions were fixed in 10% buffered formaldehyde for immunohistochemical and histochemical pathological analyses.

Biochemical analysis

After the blood samples were centrifuged at 3500 rpm for 15 minutes, the serum levels of ALT, AST, Creatinine, BUN and UREA specific to liver and kidney damage were measured using the Beckman-Coulter AU5800 autoanalyzer (Beckman Coulter, America).

Oxidative damage analysis

Tissues were mechanically homogenized using a homogenizer (DLAB D-160). The homogenate was centrifuged at 5000 rpm for 5 min to obtain the supernatant. Total antioxidant/oxidant (TAS/TOS) were measured by ELISA using the supernatant samples obtained after tissue homogenization. TAS/TOS (RelAssay Diagnostic®, Turkey), kits were used according to the manufacturer's instructions. Oxidative stress index (OSI) was calculated using the formula 'TOS (μmol H2O2 equivalent/L) / TAS (mmol Trolox equivalent/L) x 100'.

Gene expression analyses

RT-PCR device was used for gene expression analyses. Total RNA was isolated from liver and kidney tissues using the One Step RNA Reagent (BioBasic BS410A) kit. cDNA synthesis was performed using the OneScript Plus cDNA synthesis kit (Abm, Cat #G236) according to the manufacturer's kit protocol. Gene expression analyses (HIF-1α and iNOS) were performed using the BlasTaq 2X qPCR MasterMix (Abm, Cat #G891) kit with StepOnePlus Real-Time PCR (Applied Biosystems). The primer sequences used were obtained from Bio Basic Canada and are given in Table I.

Histopathological evaluation

After euthanasia, liver and kidney tissues were fixed in 10% formaldehyde solution. Routine tissue tracking procedures were performed ((Leica ASP300S; Leica Microsystem, Nussloch, Germany) and then embedded in paraffin blocks. 5 μm thick sections were taken from each block. For histopathological examination, preparations were stained with hematoxylin-eosin (HE) and examined under a light microscope (Olympus CX21) and microscopic digital photographs were taken with an Olympus DP26 model camera and transferred to a computer environment.

Immunohistochemical evaluation

While sections were taken for histopathological examination, two different serial sections were taken for immunohistochemical evaluations and transferred to Poly-L-lysine slides. Sections were stained with caspase-3, TNF-α and FGF-2 (respectively Anti-caspase-3 Antibody (E-8), Santa cruz- 7272; Anti-TNFα Antibody (52B83), Santa cruz- 52746; Anti-FGF-2 Antibody (C-2), Santa cruz -74412: 1/100 dilution) expressions were evaluated using the streptavidin-biotin method. EXPOSE 'Mouse and Rabbit Specific HRP/DAB Detection' immunohistochemistry kit (ab80436) (Abcam, Cambridge, UK) was used as the secondary antibody. Diaminobenzidine (DAB) was used as the chromogen. For immunohistochemical analysis, sections were examined separately for both antibodies. A scoring system ranging from (0) to (3) was used to evaluate the intensity of the immunohistochemical reaction of the cells with the markers. For evaluation, 10 different fields were examined in each section under 40X objective magnification. Morphometric analyses and microphotography were performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical analysis

One-way ANOVA and post-hoc Bonferroni and Tukey tests were used in the SPSS (25th version) package program for statistical analysis of the data.

Statistical evaluation of gene expressions was performed using the 'RT² Profiler™ PCR Array Data Analysis' program on the Gene Globe platform using the 2-ΔΔCT method. Duncan test was used to determine the differences between the groups in the analysis of histopathological and immunohistochemical scores. The significance value in all analyses was accepted as $p<0.05$. The sample size was determined based on a priori power analysis using G*Power version 3.1.9.7. Assuming an effect size of 0.86, a statistical power of 0.95, and a significance level of $\alpha = 0.05$, the analysis indicated that the planned group sizes would be sufficient to detect significant differences between groups.

RESULTS

Serum ALT, AST, BUN, Urea and Creatinine levels are given in Table I. When the doxorubicin group was compared with the control group, ALT, AST, BUN, Urea and Creatinine levels were statistically significantly increased ($p<0.05$). When the DOX+IGF-1 group was compared with the DOX group, serum ALT, AST, BUN, Urea and Creatinine levels were significantly decreased ($p<0.05$).

Table 1. Serum ALT, AST, BUN, Urea, Creatinine levels.

	ALT (U/L)	AST (U/L)	Creatinin (mg/dl)	BUN (mg/dl)	Ure (mg/dl)
CONTROL	38.15± 1.31^{b}	114.9± 0.96^{b}	0.21± 0.005^{b}	15.33± 0.57^{b}	32.71± 0.73^{b}
DOX	68.17± 1.39^{a}	325.7± 1.0^{a}	0.30± 0.2^{a}	26.22± 1.34^{a}	55.39± 0.93^{a}
IGF-1	39.24± 1.23^{b}	116.1± 0.7^{b}	0.22± 0.005^{b}	15.96± 0.82^{b}	32.97± 0.53^{b}
DOX+ IGF-1	55.12± 1.32^{ab}	132.1±0.94 ^{ab}	0.28± 0.01^{ab}	19.66± 0.56^{ab}	42.04± 0.55^{ab}

Data are expressed as mean ± SEM. Letters on the columns indicate statistical significance between groups (a: Compared to the control group and b: Compared to the Dox group; $p < 0.05$). ALT: Alanine Aminotransferase, AST: Aspartate alanine animotransferase, BUN: blood urea-nitrogen, UREA: Blood urea, DOX: Doxorubucin, IGF-1: insulin-like growth factor-1.

Changes in TAS and TOS levels related to oxidative damage in liver and kidney tissue are given in Table 2 and Table 3, respectively. In the DOX group, TOS levels were significantly increased and TAS levels were significantly decreased in liver and kidney tissue compared with the control group ($p<0.05$). When compared with the DOX group, TOS levels were significantly increased and TAS levels were significantly increased in the DOX+IGF-1 group ($p<0.05$). No significant change was observed in the IGF-1 group compared to the control group ($p>0.05$).

Table 2. Total antioxidant and total oxidant levels in liver tissue.

	TAS	TOS
CONTROL	1.619+ 0.008^{b}	11.857+ 1.102^{b}
DOX	1.507+ 0.007^{a}	22.501+ 0.902^{a}
IGF-1	1.567+ 0.011	14.497+ 1.169
DOX+ IGF-1	1.518+ 0.006^{b}	18.247 + 1.218^{b}

Data are expressed as mean ± SEM. Letters on the columns indicate statistical significance between groups (a: Compared to the control group and b: Compared to the Dox group; $p < 0.05$). DOX: Doxorubucin, IGF-1: insulin-like growth factor-1, TAS: Total antioxidant levels, TOS: total oxidant levels.

Table 3. Total antioxidant and total oxidant levels in kidney tissue.

	TAS	TOS
CONTROL	1.531+ 0.007^{b}	9.065+ 0.320^{b}
DOX	1.374+ 0.013^{a}	14.293 + 1.118^{a}
IGF-1	1.549+ 0.013	6.902+ 0.108
DOX+ IGF-1	1.483 + 0.003^{b}	10.707+ 0.206^{b}

Data are expressed as mean ± SEM. Letters on the columns indicate statistical significance between groups (a: Compared to the control group and b: Compared to the Dox group; $p < 0.05$). DOX: Doxorubucin, IGF-1: insulin-like growth factor-1, TAS: Total antioxidant levels, TOS: total oxidant levels.

The gene expression findings in liver tissue are given in Table 4. HIF-1α expressions in liver tissue increased in the Doxorubicin-applied group compared to the control group. IGF-1 application in the DOX+IGF-1 group caused a decrease in the HIF-1α expression level. iNOS expression decreased in the Doxorubicin-applied group compared to the control group, and IGF-1 application caused an increase in the expression level in the DOX+IGF-1 group. The gene expression findings in kidney tissue are given in Table 5. The HIF-1α and iNOS expressions in kidney tissue increased in the Doxorubicin-applied group compared to the control group. IGF-1 application caused a decrease in the expression level in the DOX+IGF-1 group.

Table 4. HIF-1α and iNOS mRNA expression changes in liver tissue.

	HIF-1α	iNOS
CONTROL	1	1
DOX	5.50	0.95
IGF-1	1.97	1.55
DOX+ IGF-1	4.28	0.99

The relative mRNA expression levels (fold change) of HIF-1α and iNOS of the liver tissues. *Statistically significant differences compared to the control ($p < 0.05$). Real-Time PCR data were analyzed using the ΔΔCT method via the RT 2 Profiler™ PCR Array Data Analysis program (Qiagen). Student's t-test was used for pairwise comparisons, and significance was set at $p < 0.05$. Mean ± SD. DOX: Doxorubucin, IGF-1: insulin-like growth factor-1, HIF-1α: Hypoxia-inducible factor, iNOS: Inducible nitric oxide synthase

Table 5. HIF-1α and iNOS mRNA expression changes in kidney tissue.

	HIF-1α	iNOS
CONTROL	1	1
DOX	2.60	5.13
IGF-1	0.95	1.85
DOX+ IGF-1	0.90	3.63

The relative mRNA expression levels (fold change) of HIF-1α and iNOS of the kidney tissues. *Statistically significant differences compared to the control ($p < 0.05$). Real-Time PCR data were analyzed using the ΔΔCT method via the RT 2 Profiler™ PCR Array Data Analysis program (Qiagen). Student's t-test was used for pairwise comparisons, and significance was set at $p < 0.05$. Mean ± SD. DOX: Doxorubucin, IGF-1: insulin-like growth factor-1, HIF-1α: Hypoxia-inducible factor, iNOS: Inducible nitric oxide synthase.

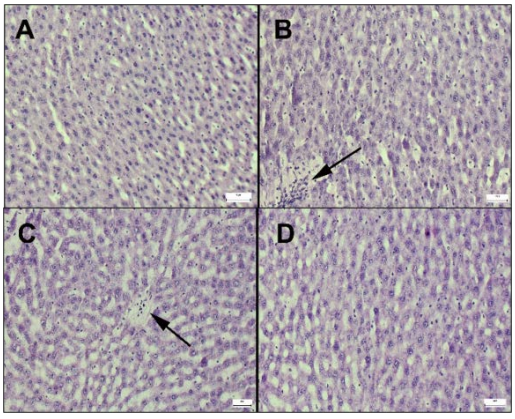

Figure 1. Histopathological findings in liver tissue. (A) Normal tissue histology in the control group, (B) significant inflammatory cell infiltrations in the DOX group (black arrow), (C) decreased inflammatory cell infiltrations in the DOX+ IGF-1 group (black arrow), (D) normal liver histological appearance in the IGF-1 group (H&E staining, bars=50μm).

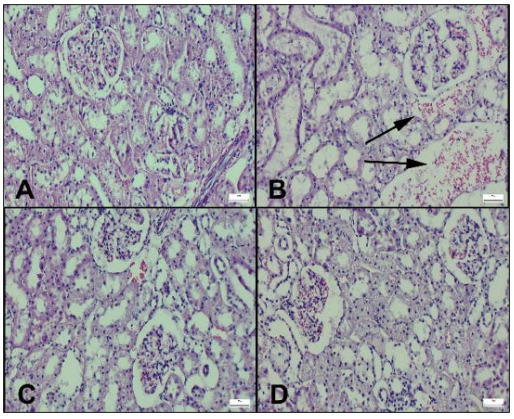

Figure 2. Histopathological findings in kidney tissue. (A) Normal tissue histology in the control group, (B) Obvious bleeding in the DOX group (black arrow). (C) Decrease in pathological findings in the DOX+IGF-1 group, (D) Normal kidney histological appearance in the IGF-1 group (H&E staining, bars=50μm).

In the histopathological examination of the liver tissue, normal histological appearance was observed in the Control and IGF-1-only groups. In the Doxorubicin-administered group, when compared to the control, significant hyperemia, degeneration in hepatocytes and inflammatory cell infiltrations consisting mainly of lymphocytes were observed. A decrease in the pathological findings observed in the damage was found in the DOX+IGF-1 group (Figure 1).

In the histopathological examination of the kidney tissue, significant hyperemia, mild to moderate hemorrhages, congestion in capillaries, expansion of Bowman capsules, glomerular atrophy or in some cases disappearance, tubular degeneration, protein precipitates in the renal tubules, exfoliated cells, cystic dilatation in the tubules, inflammatory cell infiltrations, vacuolization in the cell cytoplasm of the proximal tubules and microvillous degeneration were observed in the Doxorubicin-administered group compared to the control group. These pathological findings, which are indicators of damage, decreased in the DOX+IGF-1 group. Normal histological appearance of renal glomeruli and cortical tubules was found in the control and IGF-1-only groups (Figure 2).

In the immunohistochemical evaluation in liver tissue, caspase-3 and TNF-α expressions increased in the DOX group. The most prominent caspase-3 and TNF-α expressing cells were seen as hepatocytes. There was a very low level of expression increase or no expression was observed in the control and IGF-1 groups (Figure 3 and Figure 4). Immunohistochemical evaluation scores in liver tissue are shown in Table 6. In the immunohistochemical evaluation in kidney tissue, caspase-3 and FGF-2 expressions increased in tubular cells. Caspase-3 and FGF-2 expressions decreased in the DOX+IGF-1 group after IGF-1 treatment. There was little or no expression increase in the control and IGF-1 groups (Figure 5 and Figure 6). Immunohistochemical evaluation scores in kidney tissue are shown in Table 7.

Table 6. Caspase-3 and TNF-α immunohistochemical scores in liver tissue.

	Caspase-3	**TNF-α**
CONTROL	0.14± 0.14[a]	0.14± 0.14[a]
DOX	1.28± 0.48[b]	1.28± 0.48[b]
IGF-1	0.14± 0.14[a]	0.14± 0.14[a]
DOX+ IGF-1	0.57± 0.20[a]	0.42± 0.20[a]
P value	< 0.001	< 0.001

The difference between the means of groups with different letters is statistically significant. Data are given as mean±standard error. DOX: Doxorubucin, IGF-1: insulin-like growth factor-1, TNF-α: Tumor necrosis factor.

Table 7. Caspase-3 and TNF-α immunohistochemical scores in kidney tissue.

	Caspase-3	**FGF-2**
CONTROL	0.14± 0.14a	0.14± 0.14a
DOX	1.85± 0.89b	1.57± 0.53b
IGF-1	0.14± 0.14a	0.14± 0.14a
DOX+ IGF-1	0.85± 0.69a	0.57± 0.53a
P value	< 0.001	< 0.001

The difference between the means of groups with different letters is statistically significant. Data are given as mean±standard error. DOX: Doxorubucin, IGF-1: insulin-like growth factor-1, FGF-2: Fibroblast growth factor 2.

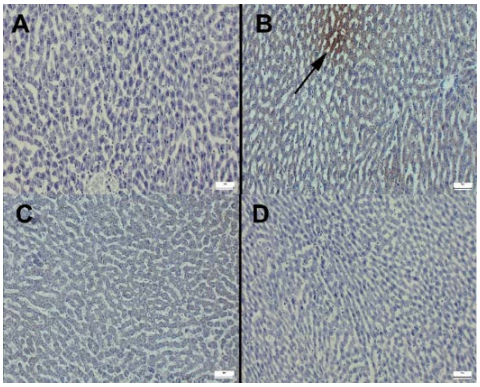

Figure 3. Caspase-3 immunohistochemistry findings in liver tissue. (A) Negative expression in the control group. (B) Significant expression in hepatocytes in the DOX group (black arrow), (C) Decreased expression in the DOX+IGF-1 group, (D) Negative expression in the IGF-1 group (Streptavidin biotin peroxidase method, bars=50um.)

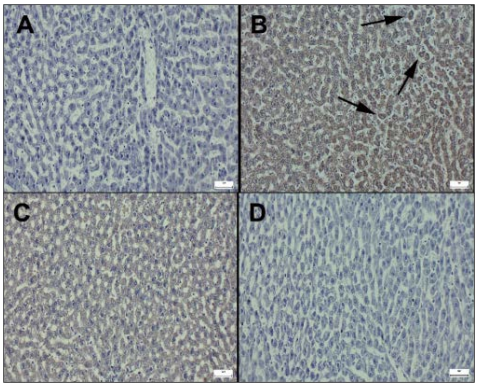

Figure 4. TNF-α immunohistochemistry findings in liver tissue. (A) Negative expression in the control group, (B) significant expression in hepatocytes in the DOX group (black arrow), (C) decreased expression in the DOX+IGF-1 group, (D) negative expression in the IGF-1 group (Streptavidin biotin peroxidase method, bars=50um.)

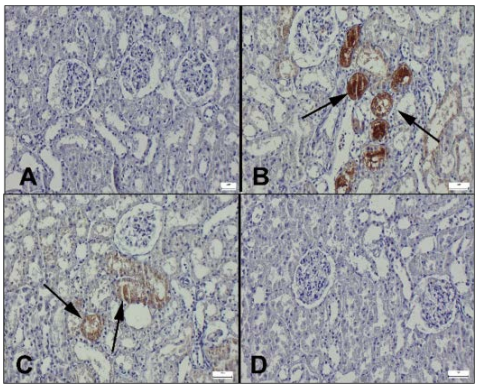

Figure 5. Caspase-3 immunohistochemistry findings in kidney tissue. (A) Negative expression in the control group, (B) significant expression in tubular cells in the DOX group (black arrow), (C) decreased expression in the DOX+IGF-1 group (black arrow), (D) negative expression in the IGF-1 group (Streptavidin biotin peroxidase method, bars=50um.)

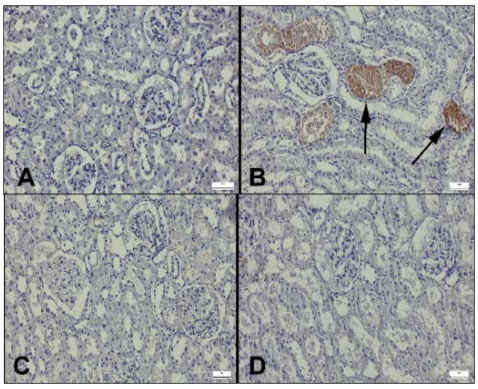

Figure 6. FGF-2 immunohistochemistry findings in kidney tissue. (A) No expression in the control group, (B) Significant expression in tubular cells in the DOX group (black arrow). (C) Decreased expression in the DOX+IGF-1 group, (D) Negative expression in the IGF-1 group (Streptavidin biotin peroxidase method, bars=50um.)

DISCUSSION

As chemotherapy agents are commonly used in cancer therapy, the resulting dose-limiting toxicities and their negative impact on patients' quality of life have attracted increasing attention. Therefore, antioxidant and anti-inflammatory compounds are being investigated against the adverse reactions of chemotherapeutic drugs[12].

The present study aimed to evaluate the effect of IGF-1 on doxorubicin-induced hepatorenal toxicity. DOX is known to be a powerful and effective anticancer agent, but the therapeutic application of DOX has been greatly limited, especially due to its

severe cardiotoxicity, hepatotoxicity, and nephrotoxicity. [13]. Similar to the studies conducted with DOX [14,15,16] it was determined that AST, ALT, BUN, urea, and creatinine levels increased in this study. ALT and AST are enzymes necessary for the mutual conversion of sugar and protein in the body, and when the liver is damaged, the serum levels of ALT and AST increase significantly. [17]. Therefore, an increase in the serum levels of ALT and AST suggests hepatic injury. Serum BUN, Urea and creatinine levels are the main indices for evaluating renal function in clinical practice [18]. Therefore, the increase in these biochemical indices suggests that DOX causes acute damage to the liver and kidney. IGF-1 treatment significantly reduced serum levels of AST, ALT, BUN, urea, and creatinine, thereby ameliorating DOX-induced liver and kidney injury.

In DOX-induced toxicity studies, it was reported that doxorubicin caused the formation of oxygen species in liver and kidney tissues, oxidative damage and apoptotic cell death [19]. In line with the literature, a significant increase in total oxidant status (TOS) and a significant decrease in total antioxidant status (TAS) were observed in the doxorubicin-administered group compared to the control group. On the other hand, following IGF-1 administration, TOS and TAS levels approached those of the control group.

Although the exact mechanism of action of IGF-1 is not fully understood, it has been shown to reduce oxidative damage in liver and kidney tissues, increase antioxidant enzyme levels, and improve nitric oxide bioavailability and mitochondrial function[11]. IGF-1 may act as an effective antioxidant and free radical scavenger that inhibits ROS production.

Moreover, it is still uncertain whether the protective effects of IGF-1 on liver and kidney functions are solely due to its antioxidant activity. Therefore, expressions of iNOS and HIF-1α were evaluated in liver and kidney tissues to investigate the inflammatory response in DOX-induced hepatorenal toxicity in rats. According to the literature, iNOS expression increases during DOX-induced toxicity, but this overexpression may diminish in the later stages of damage [20].

Increased HIF-1α expression can reduce ROS production by redirecting cellular metabolism toward glycolysis, while decreased HIF-1α expression may lead to increased ROS formation due to endoplasmic reticulum stress[21]. In contrast, decreased expression of HIF-1α may result in high reactive oxygen species mediated by endoplasmic reticulum stress [22]. In this study, increased expression levels of both iNOS and HIF-1α were observed in liver and kidney tissues in the doxorubicin-treated group. Conversely, IGF-1 treatment resulted in expression levels closer to those of the control group, indicating a potential recovery effect.

Histopathological evaluations in DOX-induced nephrotoxicity models have reported lymphocytic infiltration, tubular degeneration, glomerular atrophy, and bleeding in the renal interstitium [23]. Similarly, hepatotoxic models have shown severe hyperemia, vascular bleeding, inflammatory cell infiltration, hydropic degeneration, coagulation necrosis, serosal thickening, and degeneration in hepatocytes[24, 25].

In this study, in line with previous reports, doxorubicin-induced kidney damage was characterized by marked hyperemia, expansion of Bowman's capsules, glomerular atrophy, tubular degeneration, inflammatory cell infiltration, and vacuolization in the cytoplasm of proximal tubular cells. Liver damage was evidenced by hyperemia, hepatocyte degeneration, and lymphocytic infiltration. IGF-1 treatment was found to significantly improve these histopathological alterations.

In the immunohistochemical evaluation, consistent with previous studies, a marked increase in caspase-3 and TNF-α expression was observed in the liver and kidney tissues of the doxorubicin-treated group [26,27]. In the DOX + IGF-1 group, a significant reduction in the expression of these apoptotic and inflammatory markers was noted. FGF-2 is one of the most potent angiogenic factors and has also been shown to act as a strong renal tubular regenerative growth factor. However, excessive accumulation of FGF-2 in renal tissues may lead to proliferative and fibrogenic lesions[28].

In this study, FGF-2 expression was increased in doxorubicin-induced renal injury, whereas IGF-1 treatment was able to reduce this expression and mitigate the damage. Collectively, these findings suggest that IGF-1 can prevent doxorubicin-induced histopathological and immunohistochemical damage.

This study has certain limitations. Although IGF-1 showed protective effects against doxorubicin-induced liver and kidney damage, the exact molecular mechanisms remain unclear. The study focused mainly on oxidative stress, inflammation, and apoptosis, and did not evaluate other relevant

pathways such as autophagy or endoplasmic reticulum (ER) stress. Further studies are needed to explore the broader mechanisms of IGF-1 action in more detail.

In conclusion, the present study demonstrates the protective role of IGF-1 against acute DOX-induced liver and kidney toxicity in a rat model. IGF-1 administration significantly reduced liver and kidney dysfunction, as well as histopathological damage caused by DOX. The underlying mechanism appears to involve the attenuation of oxidative stress, inflammation, and apoptosis, potentially through modulation of the HIF-1α/iNOS signaling pathway. hese findings indicate that IGF-1 may serve as a promising therapeutic agent for alleviating and preventing DOX-induced organ toxicity in clinical settings. Future studies should validate these findings in larger animal models and under clinical-like conditions. In addition, it is important to investigate the effects of IGF-1 on other biochemical pathways and to evaluate dose optimization and long-term safety.

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