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Evaluation of the healing and protective properties of adipose-derived mesenchymal stem cells from cisplatin-induced liver and kidney damage

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Abstract. – OBJECTIVE: The occurrence of nephrotoxicity and hepatotoxicity as a result of cisplatin administration is a major concern in clinical practice. This study examined the potential protective effects of administering mesenchymal stem cells (MSCs) on the renal and hepatic damage caused by cisplatin. Moreover, the study investigated the potential protective effects of administering Adipose-Derived Mesenchymal Stem Cells (ADMSC) to counteract the harmful effects of cisplatin-induced kidney and liver damage.

MATERIALS AND METHODS: Male Sprague-Dawley rats were divided into three groups: normal control, cisplatin + saline, and cisplatin + ADMSC. Cisplatin was administered to induce toxicity, and ADMSC was administered intravenously as a potential therapeutic intervention. Biochemical parameters and histopathological changes were assessed in the kidney and liver tissues. Statistical analyses were performed using a one-way ANOVA.

RESULTS: Cisplatin increased malondialdehyde (MDA), tumor necrosis factor alfa (TNF-alfa), IL-6, alanine transaminase (ALT), creatinine, Galectin-3, Tissue growth factor beta 1 (TGF-beta 1), compared to the normal control group. Cisplatin-MSC reduced these levels. Histopathology showed that cisplatin caused kidney tubular epithelial necrosis, luminal necrotic debris, tubular dilatation, interstitial inflammation, liver sinusoidal and central vein dilatation, congestion, necrosis, and cytoplasmic vacuolization. ADMSC administration significantly reduced histopathological changes.

CONCLUSIONS: These findings highlight the potential therapeutic benefits of mesenchymal stem cell (MSC) administration in mitigating cisplatin-induced nephrotoxicity and hepatotoxicity. MSC treatment demonstrated protective effects by reducing oxidative stress, inflammatory markers, and histopathological alterations. Further investigations are warranted to elucidate the precise mechanisms underlying these protective effects and evaluate their clinical implications for managing cisplatin-induced organ damage.

Key Words:
Cisplatin, Hepatotoxicity, Nephrotoxicity, Adipose mesenchymal stem cell.

Introduction

Despite a 33% decline in cancer-related fatalities since 1991, cancer remains the second leading cause of death after cardiovascular disease[1]. In addition, treatment-related toxicity continues to be a significant problem.

Cisplatin is an inorganic platinum derivative and one of the most effective chemotherapeutic agents widely used in treating various solid organ tumors such as lung, ovarian, bladder, sarcoma, stomach, and testicular cancers, among others[2-4]. It induces DNA damage and triggers apoptosis by affecting DNA repair mechanisms in cancer cells[3]. However, nephrotoxicity, which occurs in approximately 30% of patients, limits the clinical application of cisplatin. Renal insufficiency is marked by increased creatinine and blood urea nitrogen (BUN) levels, reduced renal blood flow, hypomagnesemia, hypokalemia, and proteinuria. These symptoms may necessitate discontinuing or reducing the dosage of a particular drug[5]. Acute kidney injury caused by cisplatin is asso-

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ciated with tubular epithelial cell proliferation and migration, fibrosis, and apoptosis[6]. Although hepatotoxicity is not commonly observed at low doses of cisplatin, it has been reported[7] to occur at high doses. The primary cause of cisplatin-induced hepatotoxicity is reported to be oxidative stress resulting from increased reactive oxygen species[8].

The lectin Galectin-3 (Gal-3) bonds specifically to β--galactosides[9]. It is expressed in numerous tissues, such as the heart and kidneys[10]. It is predominantly released during monocyte-to-macrophage differentiation[11]. The significance of this phenomenon cannot be overstated, as it plays a crucial role in many biological processes such as cell proliferation, apoptosis, pre-mRNA splicing, differentiation, transformation, angiogenesis, inflammation, fibrosis, and host defense[12]. The acute inflammatory response entails the activation and adhesion of neutrophils, chemotaxis of monocytes, opsonization of apoptotic neutrophils, and activation of mast cells[13]. Galectin-3 is an indicator of organ fibrosis, such as cardiac fibrosis[14]. It has been demonstrated[15] to play an essential role in acute tubular injury and subsequent phases of kidney regeneration.

The malondialdehyde (MDA) level has been established as a reliable indicator of oxidative stress and antioxidant status in individuals diagnosed with cancer[16]. MDA is a prevalent marker of oxidative stress, a physiological state arising from an inequity between the generation of free radicals and the cellular ability to eliminate them. Excessive oxidative stress has been linked[17] to various diseases, including cancer, neurodegenerative disorders, cardiovascular disease, and diabetes.

ADMSCs are self-renewing, multipotent cells with differentiation and immunomodulatory properties. On the other hand, adipose-derived stem cells (ASCs) are a subset of ADMSCs that can be readily isolated from adipose tissues and possess many of the same regenerative properties as other ADMSCs[18-20]. Given their ability to modulate inflammation, enhance tissue regeneration, and low immunogenicity, ADMSCs have gained recognition in cell-based therapies[21]. ADMSCs act in two ways: they are a potent chemoattractant that goes to damaged tissues by this feature; they affect the healing of the tissues with exosomes by increasing tissue supportive substances, such as growth factors, where they are located. Secondly, they activate anti-inflammatory mechanisms regardless of location. The homeostasis provided by the peritoneal cavity allows mesenchymal stem cells to live for a long time. Thus, the duration of action of ADMSCs can be prolonged[21].

Currently, no chemotherapy agent is as potent as cisplatin in anticancer efficacy and has fewer side effects. Therefore, it is challenging to abandon the clinical use of cisplatin. Consequently, researchers have been motivated to develop new treatment options to reduce cisplatin-induced acute toxicities to prevent the destructive effects. To the best of our knowledge, there are no studies on the use of ADMSC in both liver and kidney toxicity of cisplatin.

This study investigates adipose-derived mesenchymal stem cells' protective effects and cellular mechanisms through the Galectin-3 pathway in preventing cisplatin-induced nephrotoxicity and hepatotoxicity.

Materials and Methods

Animals

This study used thirty adult female Wistar rodents weighing between 200 and 210 gr. The animals were confined in enclosures and maintained under standard conditions, such as 12-hour light/dark cycles and room temperature (22.2°C). Throughout the study, they were fed pellets and had unrestricted access to potable water. Unless specified otherwise, all chemicals were acquired from Sigma-Aldrich.

Ethical Issues

The study protocol was authorized by the Institutional Animal Care and Ethical Committee of the Demiroğlu Science University Istanbul, Türkiye (Ethical Permission Number: 16.03.2022/2723031514) and reported in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. All methods followed the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH) and Minimum Quality Threshold in Pre-Clinical Sepsis Studies (MQTiPSS) recommendations.

Experimental Procedure

The research included thirty rodents. The investigation included eight rats as a standard control group. This group was not administered any medication.

To create a model of cisplatin-induced liver and kidney toxicity, 20 rodents were given cisplatin (Koçak Farma, Istanbul, Türkiye) at

a dose of 2.5 mg/kg/day, twice weekly for four weeks (total 20 mg/kg). Two groups of cisplatin-treated rodents were established. The rodents in group 1 (n=10) received 1 ml/kg/day of 0.9% NaCl intraperitoneally (i.p.). In contrast, group 2 (mesenchymal stem cells; n=10) rodents received 2.0 X 10^6 cells/kg intraperitoneally (i.p.) twice weekly for four weeks. (total dose 16 X 10^6 cells/kg). Three of the rodents receiving cisplatin and saline perished during the experiment. When cisplatin was administered to rats, mesenchymal stem cells did not succumb.

At the end of the experiment, all rats were given anesthesia: 100 mg/kg Ketamine (Ketasole Richterpharma AG Austria) and xylazine 50 mg/kg (Rompun, Bayer, Germany) before being euthanized by cervical dislocation. Blood samples were collected through cardiac puncture for biochemical analysis, and samples of the kidney and liver were collected for immunohistochemistry and biochemical analysis.

Isolation of Mesenchymal Stem Cells (MSC) from Adipose Tissue

ADMSCs were isolated using the flank adipose tissue derived from rats. The adipose tissue was extracted and stored on ice after the rodents were administered anesthesia [ketamine 50 mg/kg (Ketasol, Richterpharma AG, Austria) and xylazine 10 mg/kg (Rompun, Bayer, Germany)]. The tissue was transported to the stem cell laboratory under sterile conditions. Small fragments of adipose tissue were treated with 0.2% collagenase type II (Gibco, Thermo Fischer Scientific, Waltham, MA, USA) at 37° Celsius for 40 minutes under stirring. Following tissue lysis, centrifugation was performed at 1,500 revolutions per minute for 5 minutes. The precipitate was resuspended in a solution consisting of 2 mL of Dulbecco's Modified Eagles Medium (DMEM) from Gibco (Thermo Fischer Scientific, Waltham, MA, USA) and 3 mL of DMEM supplemented with 10% fetal bovine serum (FBS) from Gibco (Thermo Fischer Scientific, Waltham, MA, USA), 1% penicillin, 1% streptomycin, and 2 mM L-glutamine from Invitrogen, Netherlands. The specimen was placed in an incubator at 37° Celsius, with a CO_2 concentration of 5% and a humidity level of 100%. The media was refreshed at the end of three days to achieve a confluence level of 85%. The cells underwent subculture using 0.25% trypsin (Gibco Thermo Fisher Scientific, Waltham, MA, USA) until the fourth passage, after which they were rendered inactive by adding an equivalent volume of DMEM. In preparation for potential cell transplantation, mesenchymal stem cells (MSC) at the fourth passage were cryopreserved at a concentration of 2,106 viable cells/mL in a solution consisting of 50% DMEM media, 40% FBS, and 10% dimethyl sulfoxide (DMSO; M.P. Bio) in cryovials that were correctly labeled and sterilized. These cryovials were subsequently stored in a nitrogen tank at -196°C. The examination of cell morphology and growth was conducted using an inverted microscope. The cells were subjected to a thawing process at 37°C after transferring from a nitrogen cylinder to facilitate their preparation for transplantation. Following 5-minute centrifugation at 1,500 rpm, the cellular component was resuspended in DMEM and stored in a CO_2 incubator at 37°C and 100% relative humidity until required.

MSC Characterization

Classification of cells immunofluorescence staining of CD13, CD29, and CD105 molecules was used to characterize mesenchymal stem cells in their second passage. For immunostaining, culture dish-grown cells were cleansed with PBS and fixed for 5 minutes in methanol at -10°C. The methanol was extracted after fixation, and the specimen was dried. The blocking serum (normal goat serum) was incubated with the cells for twenty minutes. After removing the blocking serum, the cells were rinsed with PBS three times – a straightforward method for isolating, cultivating, and identifying mesenchymal stem cells isolated from rat adipose tissue. Six hundred sixty cells were incubated for one hour with anti-CD13, anti-CD29, and anti-CD105 antibodies. The cells were incubated with a secondary antibody for 45 minutes following three rinsing in PBS. After rinsing the cells, a mounting medium was used to observe them under a fluorescence microscope. Every operation was carried out at ambient temperature.

Measurement of Plasma Lipid Peroxidation (MDA)

Malondialdehyde (MDA) levels were measured in plasma samples using the thiobarbituric acid reactive substances (TBARS) assay to evaluate lipid peroxidation. The plasma samples were treated with trichloroacetic acid and TBARS reagent, mixed, and incubated at 100°C for 60 minutes. Following a 20-minute centrifugation at 3,000 rpm and cooling on ice, the absorbance of the supernatant was measured at a wavelength of

535 nm. The MDA levels were calibrated using tetraethoxypropane as a reference substance.

TNF-α (MyBioSource No.: MBS2507393), IL-6 (MyBioSource No.: MBS269892), and ALT (MyBioSource No.: MBS269614) plasma levels were measured using enzyme-linked immunosorbent assay (ELISA) reagents.

Determination of Creatinine Levels

The concentration of Creatinine (MyBioSource No.: MBS3809095) was measured spectrophotometrically using an automated analysis system. Concentrations of creatinine were expressed as mg/dl.

Liver and Kidney Biochemical Analysis

After decapitation, the organs were promptly removed and stored at 20° Celsius, pending biochemical analysis. The liver and kidney were homogenized with a glass homogenizer in 5 volumes of phosphate-buffered saline (pH 7.4), five times the obtained tissue volume, and centrifuged for 15 minutes at 5,000 g. The total protein concentration of the innerve homogenates was determined using Bradford's method and bovine serum albumin as a standard after collecting the supernatant. Using commercially available ELISA kits for rats, the levels of Galectin-3 (MyBioSource No.: MBS761093) and TGF-beta 1 (MyBioSource No.: MBS824788) in liver and kidney tissue supernatants were determined.

Histopathological Examination of Liver and Kidney

For histological examinations, each animal was anesthetized with ketamine (100 mg/kg, Alfamine®, Alfasan International B.V., Holland) and xylazine (10 mg/kg, Alfazyne®, Alfasan International B.V., Holland) and perfused with 200 ml of 4% formaldehyde in 0.1 M phosphate buffer saline (PBS). Formalin-fixed liver and kidney sections (4 mm) were stained using hematoxylin and eosin. All sections were photographed using an Olympus C-5050 digital camera attached to an Olympus BX51 microscope.

An observer blinded to the study group evaluated ten microscopic fields per section at a magnification of 20x utilizing a computerized image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc., USA). This evaluation of the kidney assessed the degree of tubular epithelial necrosis, luminal necrotic debris, tubular dilatation, hemorrhage, and interstitial inflammation. The evaluation was conducted by assigning ratings to a predetermined scale. score 0=0-5%, score 1=6-20%, score 2=21-40%, score 3=41-60%, score 4=61-80%, score 5=81-100%

Histological photomicrographs were examined using the liver scoring system to evaluate liver damage. This study investigated the extent of sinusoidal and central vein dilatation, congestion, necrosis, and cytoplasmic vacuolization. At a magnification of 20x, five random sections and ten random fields were examined for each rodent. Data were scored as 0-5%=score 0; 6-20%=score 1; 21-40%=score 2; 41-60%=score 3; 61-80%=score 4; and 81-100%=score 5.

Statistical Analysis

Data were analyzed with SPSS Package Program version 26.0 (IBM Corp., Armonk, NY, USA). Number, percentage, mean, and standard deviation were used to present descriptive data. A paired samples *t*-test was used to compare the means of independent variables without a dependent variable. A value of $p<0.05$ was accepted for the *p*-significance level.

Results

Biochemical Results

The results presented in Table I demonstrate the effects of ADMSCs on various parameters related to cisplatin-induced liver and kidney damage. The study included a normal control group (n=8), a cisplatin + saline group (n=10), and a cisplatin + ADMSCs group (n=10) (Table I).

The levels of malondialdehyde (MDA), a marker of oxidative stress, were significantly increased in the cisplatin + saline group compared to the normal control group (141.3±7.5 nM *vs.* 54.8±4.3 nM, $p<0.001$). However, treatment with ADMSCs in the cisplatin + MSC group significantly reduced MDA levels (83.4±8.1 nM, $p<0.01$) (Table I).

The pro-inflammatory cytokines TNF-α and IL-6 showed similar trends. The cisplatin + saline group exhibited significantly higher levels of TNF-α (108.6±5.8 pg/ml *vs.* 26.2±3.2 pg/ml, $p<0.001$) and IL-6 (125.2±6.3 ng/ml *vs.* 1.42±0.9 ng/ml, $p<0.001$) compared to the normal control group. Treatment with ADMSCs attenuated the increase in TNF-α (53.6±4.4 pg/ml, $p<0.01$) and IL-6 (87.3±1.6 ng/ml, $p<0.001$) (Table I).

Liver and kidney function markers, such as ALT and creatinine, also showed significant dif-

Table I. Results were presented as mean ± SEM. Statistical analyses were performed using the Kruskal-Wallis H test and the post-hoc Tukey test.

	Normal control group (n = 8)	Cisplatin + Saline group (n = 10)	Cisplatin + MSC group (n = 10)
MDA (nM)	54.8 ± 4.3	141.3 ± 7.5*	83.4 ± 8.1$^{\#}$
TNF-alfa (pg/ml)	26.2 ± 3.2	108.6 ± 5.8*	53.6 ± 4.4$^{\#}$
IL-6 (ng/ml)	1.42 ± 0.9	125.2 ± 6.3**	87.3 ± 1.6$^{\#}$
ALT (IU/L)	40.9 ± 6.4	91.5 ± 8.9**	67.3 ± 5.5$^{\#}$
Creatinine (mg/dl)	0.43 ± 0.06	0.75 ± 0.08**	0.61 ± 0.1$^{\#}$
Kidney Galectin-3 Level (pg/mg protein)	11.06 ± 0.8	29.1 ± 1.6 *	15.1 ± 1.4$^{\#}$
Liver Galectin-3 Level (pg/mg protein)	6.5 ± 0.2	37.8 ± 1.1**	13.7 ± 2.05$^{\#}$
Kidney TGF-Beta 1 Level (pg/mg protein)	18.5 ± 3.09	113.2 ± 87.5**	54.5 ± 6.2$^{\#\#}$
Liver TGF-Beta 1 Level (pg/mg protein)	9.7 ± 1.1	33.5 ± 5.04*	19.4 ± 2.8$^{\#}$

*p<0.01, **p<0.001 (different from control group), $^{\#}p$<0.05, $^{\#\#}p$<0.001 (different from cisplatin and saline group). MDA: malondialdehyde, TNF-alfa: Tumor Necrosis Factor alfa, ALT: alanine transaminase, TGF-Beta 1: Transforming growth factor beta 1.

ferences. The cisplatin + saline group exhibited higher levels of ALT (91.5±8.9 IU/L *vs.* 40.9±6.4 IU/L, p<0.01) and creatinine (0.75±0.08 mg/dl *vs.* 0.43±0.06 mg/dl, p<0.001) compared to the normal control group. Treatment with ADMSCs resulted in a significant reduction in ALT (67.3±5.5 IU/L, p<0.05) and creatinine (0.61±0.1 mg/dl, p<0.01) (Table I).

The expression levels of Galectin-3 in both kidney and liver tissues were significantly increased in the cisplatin + saline group compared to the normal control group (kidney: 29.1±1.6 pg/mg protein *vs.* 11.06±0.8 pg/mg protein, p<0.01; liver: 37.8±1.1 pg/mg protein *vs.* 6.5±0.2 pg/mg protein, p<0.001). However, treatment with ADMSCs in the cisplatin + MSC group led to a significant reduction in Galectin-3 levels in both kidney (15.1±1.4 pg/mg protein, p<0.01) and liver (13.7±2.05 pg/mg protein, p<0.01) tissues. (Table I).

Similarly, the levels of TGF-β1, a profibrotic cytokine, were significantly increased in the cisplatin + saline group compared to the normal control group (kidney: 113.2±8 pg/mg protein) (Table I).

Histopathological Slices Results

Figure 1a-b shows the histology of a normal liver from the control group. No significant abnormalities or lesions were observed. The liver architecture appears intact, with well-preserved hepatocytes and a normal arrangement of central veins and sinusoids. Figure 1c-d demonstrates the liver histology of the cisplatin and saline group. Vacuolar changes can be observed in the pericentral hepatocytes, indicated by intracellular vacuoles. Additionally, there is evidence of central venous and sinusoidal dilatation (*) and hepatocyte necrosis (arrow). These findings indicate liver damage induced by cisplatin treatment.

Figure 1e-f shows the liver histology of the cisplatin and ADMSCs group. Treatment with ADMSCs improved liver histology compared to the liver of the cisplatin and saline groups. The central venous and sinusoidal dilatation (*) and hepatocyte necrosis (arrow) are clearly reduced, which suggests that ADMSCs protect the liver from damage caused by cisplatin.

The histopathological study of the liver tissues backs up the results shown in Tables II and III. It shows that treatment with MSCs reduced the histological changes that occur when cisplatin damages the liver, such as changes in the vacuoles, enlargement of the central veins and sinusoids, and death of hepatocytes.

Figure 2A-B shows the histology of a normal kidney from the control group. The glomeruli (G) and tubules (t) appear normal and well-preserved without significant abnormalities. The kidney architecture is intact, indicating a healthy kidney. Figure 2C-D displays the kidney histology of the cisplatin and saline group. Tubular cell necrosis is observed, indicated by damaged and disintegrated tubular cells (arrow).

Additionally, there is evidence of interstitial lymphocytic infiltration (*), suggesting an inflammatory response in the kidney due to cisplatin treatment. Figure 2E-F shows the kidney histology of the cisplatin and MSC group. Treatment with adipose-derived mesenchymal stem cells (MSCs) improved kidney histology compared to the cisplatin and saline group. It is clear that the tubular dilatation (*) and tubular cell necrosis (arrow) have decreased. This means that MSCs

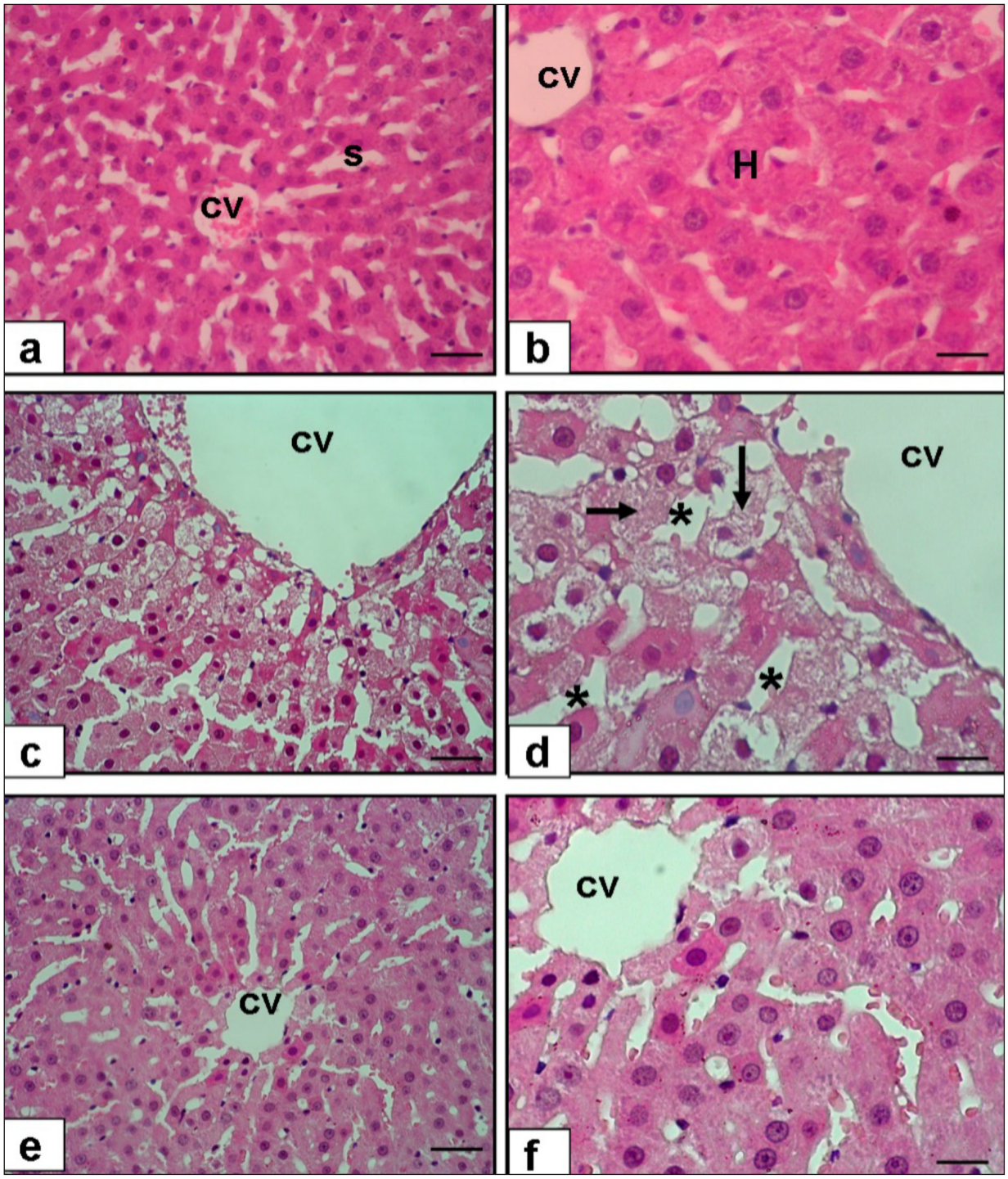

Figure 1. Liver histopathology H&E (× 10 and × 40 Magnification, scale bar: 100 µm), (**a-b**) Normal liver (control group), (**c-d**) Cisplatin+Saline group liver show vacuolar changes in pericentral hepatocytes, central venous (cv) and sinusoid dilatation (*) and hepatocyte necrosis (arrow), (**e-f**) Cisplatin+MSC group liver decreased central venous and sinusoid dilatation (*) and hepatocyte necrosis (arrow).

are protecting the kidneys from damage caused by cisplatin.

The histopathological study of the kidney tissues shown in Figure 3 backs up the results shown in Figure 3. It shows that treatment with MSCs reduced the histological changes that occur because of cisplatin-induced kidney damage, including tubular cell necrosis and interstitial lymphocytic infiltration.

Table II. Histopathological results in liver tissue were presented as mean ± SEM.

Liver	Group	Mean ± SEM	*p**
Sinusoidal and central vein dilatation	Normal control Group (n=8) Cisplatin + Saline Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Saline Group (n=10)	0.4 ± 0.1 3.5 ± 0.5 0.9 ± 0.2 3.5 ± 0.5	**< 0.001** **< 0.01**
Congestion	Normal control Group (n=8) Cisplatin + Saline Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Saline Group (n=10)	0.3 ± 0.1 2.7 ± 0.4 0.8 ± 0.1 2.7 ± 0.4	**< 0.001** **< 0.01**
Necrosis	Normal control Group (n=8) Cisplatin + Saline Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Saline Group (n=10)	0.2 ± 0.1 2.8 ± 0.5 1.1 ± 0.2 2.8 ± 0.5	**< 0.001** **< 0.001**
Cytoplasmic vacuolization	Normal control Group (n=8) Cisplatin + Saline Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Saline Group (n=10)	0.2 ± 0.1 3.5 ± 0.4 1.2 ± 0.3 3.5 ± 0.4	**< 0.001** **< 0.05**

*: Kruskal-Wallis H test and post-hoc Tukey test were used.

Histopathological Findings in Kidney Tissue

Figure 3 presents the histological findings in the kidney and liver tissues, specifically in kidney tissues, assessing tubular epithelial necrosis, luminal necrotic debris, tubular dilatation, and interstitial inflammation.

The cisplatin + saline group exhibited significantly higher levels of tubular epithelial necrosis (2.4±0.2, $p<0.01$), luminal necrotic debris (3.6±0.3, $p<0.01$), tubular dilatation (3.8±0.3, $p<0.01$), and interstitial inflammation (4.1±0.4, $p<0.01$) compared to the normal control group. However, treatment with ADMSCs in the cisplatin + MSC group

Table III. Histopathological results in kidney tissue were presented as mean ± SEM.

Liver	Group	Mean ± SEM	*p**
Tubular epithelial necrosis	Normal control Group (n=8) Cisplatin + Salin Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Salin Group (n=10)	0.2 ± 0.1 2.4 ± 0.2 1.5 ± 0.4 2.4 ± 0.2	**< 0.001** **< 0.01**
Luminal necrotic debris	Normal control Group (n=8) Cisplatin + Salin Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Salin Group (n=10)	0.3 ± 0.1 3.6 ± 0.3 1.1 ± 0.2 3.6 ± 0.3	**< 0.001** **< 0.001**
Tubular dilatation	Normal control Group (n=8) Cisplatin + Salin Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Salin Group (n=10)	0.2 ± 0.1 3.8 ± 0.3 1.9 ± 0.3 3.8 ± 0.3	**< 0.001** **< 0.05**
Interstitial inflammation	Normal control Group (n=8) Cisplatin + Salin Group (n=10) Cisplatin + MSC Group (n=10) Cisplatin + Salin Group (n=10)	0.2 ± 0.1 4.1 ± 0.4 1.4 ± 0.1 4.1 ± 0.4	**< 0.001** **< 0.01**

*: Kruskal-Wallis H test and post-hoc Tukey test were used.

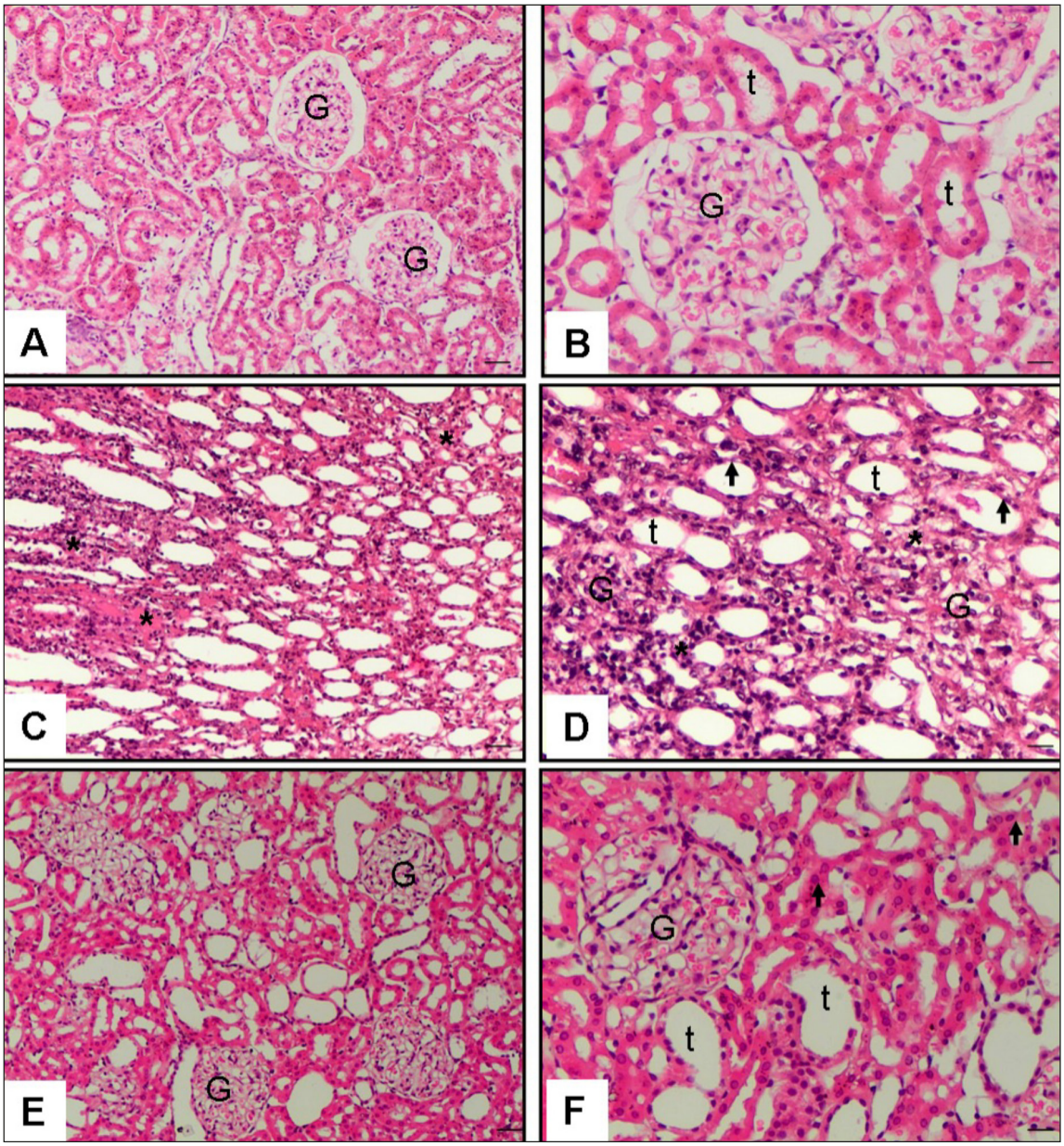

Figure 2. Kidney histopathology H&E (× 10 and × 40 Magnification, scale bar: 100 µm), (**A-B**) Normal kidney (control group), glomeruli (G), tubules (t), (**C-D**) Cisplatin+Saline group kidneys have tubular cell necrosis (arrow), interstitial lymphocytic infiltration (*), (**E-F**) Cisplatin+MSC group kidney decreased on tubular dilatation (*) and tubular cell necrosis (arrow).

resulted in a significant reduction in tubular epithelial necrosis (1.5±0.4, $p<0.01$), luminal necrotic debris (1.1±0.2, $p<0.001$), tubular dilatation (1.9±0.3, $p<0.01$), and interstitial inflammation (1.4±0.1, $p<0.01$) (Figure 3).

The results show that treatment with ADMSCs prevented kidney damage caused by cisplatin. This was shown by the reduced number of histological changes linked to tubular injury, luminal debris, tubular dilatation, and interstitial inflammation.

Histopathological Findings in Liver Tissue

The histological results in the liver tissue are shown in Figure 3. It mainly shows dilated sinusoidal and central veins, as well as congestion, necrosis, and cytoplasmic vacuolization.

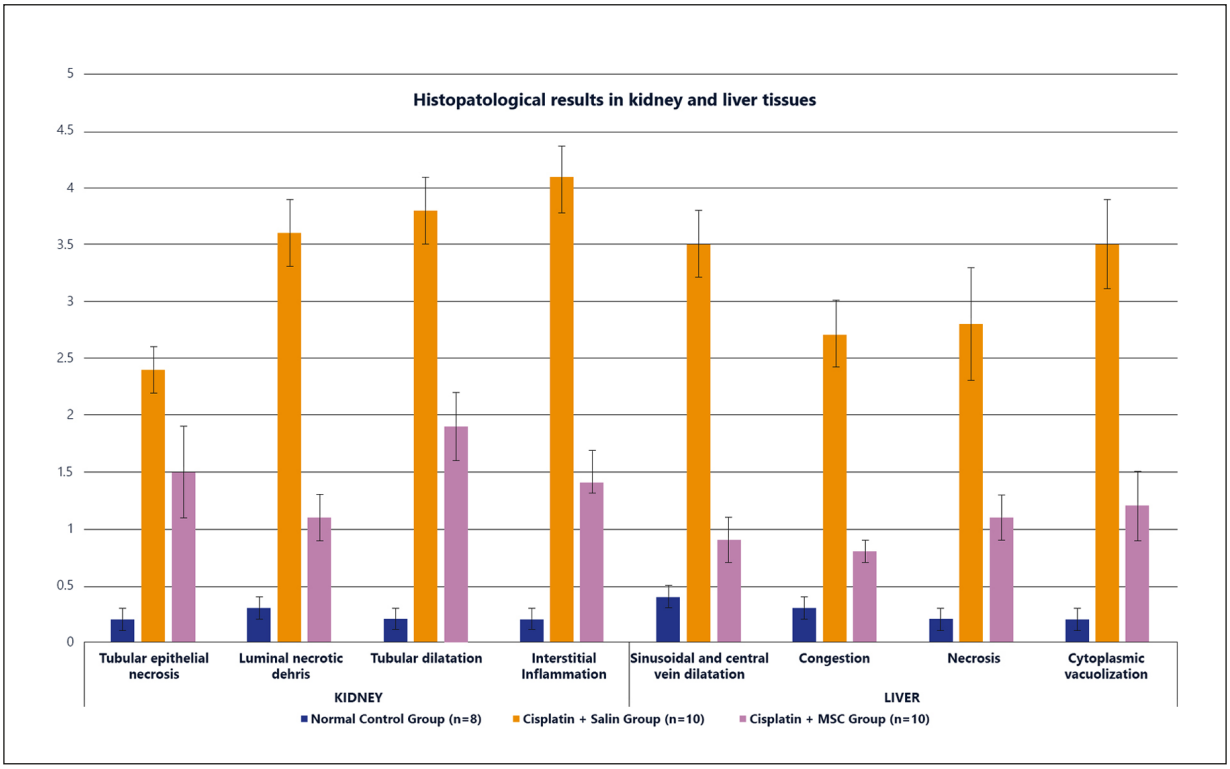

Figure 3. Histopathological results in kidney and liver tissues were presented as mean ± SEM. Statistical analyses were performed using the Kruskal-Wallis H test and the post-hoc Tukey test. Tubular epithelial necrosis in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.01); luminal necrotic debris in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.001); tubular dilatation in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.05); interstitial inflammation in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.01); sinusoidal and central vein dilatation in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.01); congestion in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.01); necrosis in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.001); cytoplasmic vacuolization in the cisplatin + saline group was significantly higher than the control group (p<0.001) and the cisplatin + MSC group (p<0.05).

The cisplatin + saline group exhibited significantly higher levels of sinusoidal and central vein dilatation (3.5±0.5, p<0.01), congestion (2.7±0.4, p<0.01), and necrosis (2.8±0.5, p<0.01) compared to the normal control group. However, treatment with ADMSCs in the cisplatin + ADMSCs group resulted in a significant reduction in sinusoidal and central vein dilatation (0.9±0.2, p<0.01), congestion (0.8±0.1, p<0.05), necrosis (1.1±0.2, p<0.01), and cytoplasmic vacuolization (1.2±0.3, p<0.01) (Figure 3).

These findings indicate that treatment with ADMSCs protected against cisplatin-induced liver damage, as evidenced by the attenuation of histological abnormalities associated with sinusoidal and central vein dilatation, congestion, necrosis, and cytoplasmic vacuolization.

Discussion

This study demonstrated that administering adipose-derived mesenchymal stem cells could mitigate cisplatin's biochemical and histological toxicity in the kidneys and liver.

One of the pathogenic causes of cisplatin-induced renal and liver toxicity is well-known[22] to be the production of free radicals leading to oxidative stress. Cisplatin generates reactive oxygen species, including hydroxyl radicals, hydrogen peroxide, singlet oxygen, and superoxide ions. Increased reactive oxygen species inhibit antioxidant enzymes, producing lipid peroxidation and diminished membrane peroxidation-protective enzyme activity. Increased oxidative stress is responsible for DNA damage and cisplatin's

toxicity. Due to the prevalence of numerous mitochondria in hepatocytes[23], cisplatin is metabolized predominantly in the kidneys, followed by the liver. MDA, a marker of oxidative stress, increased substantially in the blood of cisplatin-treated rats.

In rats that received ADMSC in addition to cisplatin, MDA levels were lower than in those who received cisplatin alone. Curcumin, which has antioxidant and anti-inflammatory properties, substantially decreased MDA levels in rodents treated with cisplatin compared to the control group, according to a study by Birici et al[24]. The results of this study indicate that ADMSCs may also reduce reactive oxygen species and have a protective effect on cells.

Cytokines are potent regulators secreted by various cell types and cellular activities[25], particularly in the immune system. TNF-α is a protein closely associated with apoptosis and inflammatory responses[26]. Proinflammatory cytokines are cytokines released by the immune system in response to various stresses or stimuli, such as infections, injuries, and other pathological conditions. These cytokines initiate and regulate the inflammatory process, enhancing the immune response. TNF-α and IL-6 are well-known examples of proinflammatory cytokines. These proinflammatory cytokines are released from the liver into the bloodstream during hepatotoxic damage[27]. ADMSCs, when administered, can migrate to the damaged liver and kidney tissues. They can sense and respond to the inflammatory and damaged microenvironment.

Previous studies[7,28,29] have shown that cisplatin stimulates the production of proinflammatory cytokines like TNF-α, IL-1, and IL-6. In this study, cisplatin treatment substantially increased mice's proinflammatory cytokines TNF-α and IL-6 levels. However, in mice treated with ADMSCs along with cisplatin, the levels of these cytokines decreased. This decrease indicates that ADMSCs can reduce the proinflammatory cytokine increase, thereby reducing irreversible damage such as fibrosis in the liver and kidneys.

In addition, it can prevent the body from embarking on a path leading to an excessive inflammatory response triggered by an increase in these cytokines. Moreover, the statistically lower decrease of IL-1 and IL-6 relative to TNF-α suggests that ADMSCs exert pressure on the primary inflammatory trigger mechanism *via* TNF-α.

Serum levels of ALT are used as a marker to assess liver damage because this enzyme is normally localized in the cytoplasm and is only released into circulation in response to hepatocellular damage. In our study, the ALT levels of cisplatin-treated mice were significantly higher than those of the control group. In contrast, the increase in ALT levels was reduced in rodents treated with ADMSCs and cisplatin. Similar to the decrease in inflammatory markers, this finding indicates that liver functions have improved due to the decline in ALT levels.

TGF-beta 1, a cytokine, regulates cell proliferation, differentiation, and function, as documented in literature[30]. It is synthesized by a diverse range of cellular phenotypes comprising immunocytes, epithelia, and fibrocytes. It has been linked to the formation of fibrosis in organs, including the liver, lungs, heart, and kidneys[30,31]. In this study, the levels of TGF-beta 1 in the liver and kidneys were significantly increased after cisplatin administration. However, in mice treated with ADMSCs along with cisplatin, the levels of TGF-beta 1 were lower compared to those treated with cisplatin alone. Galectin-3 can act as a chemoattractant, promoting the recruitment and migration of ADMSCs to the injured sites. Galectin-3, upon binding to its receptors on immune cells, can modulate the inflammatory response. It can influence the activation and function of immune cells, such as macrophages and T cells, potentially promoting an anti-inflammatory phenotype. By reducing inflammation, ADMSCs can help mitigate the damaging effects of cisplatin.

Galectin-3 plays a role in various biological processes, including but not limited to cell proliferation, apoptosis, transformation, angiogenesis, inflammation, fibrosis, and host defense, as documented in literature[12]. Research[32,33] has indicated that the inhibition of Gal-3 can potentially mitigate the onset of kidney disease. Galectin-3 represents a promising therapeutic target for the management of renal disorders. The research conducted by Boutin et al[34] reveals a positive correlation between the degree of Gal-3 and the severity of acute kidney injury and the minimal estimated glomerular filtration rate (eGFR). They also made recommendations for the use of Galectin-3 monitoring in intensive care settings[35,36]. The histological findings of cisplatin-induced kidney toxicity can include tubular cell death, tubular atrophy, tubulointerstitial fibrosis, chronic inflammation, and glomerulosclerosis[37].

The cisplatin-treated group displayed a significant increase in tubular epithelial necrosis, luminal necrotic detritus, tubular dilation, and

interstitial inflammation compared to the control group. In the cisplatin + ADMSC group, however, the severity of these pathological changes decreased significantly.

In cisplatin-induced liver toxicity, it is common to observe hepatocellular vacuolation, sinusoidal dilation, and cytoplasmic alterations around the central vein[37]. The hepatic tissue of mice treated with cisplatin exhibited a marked increase in sinusoidal and central vein dilation, congestion, necrosis, and cytoplasmic vacuolization. Nevertheless, the aforementioned pathological modifications exhibited a significant reduction in rodents subjected to cisplatin and ADMSC treatment. Elevated levels of serum Galectin-3 have been associated[35] with heightened susceptibility to hepatocellular carcinoma (HCC), liver failure, liver cirrhosis, and chronic active hepatitis B.

This study found that administering ADMSCs reduced liver and kidney damage caused by cisplatin[36]. The protective effect was linked to a reduction in the expression of Galectin-3, a protein involved in inflammation and tissue injury. Galectin-3 has been linked to the pathogenesis of numerous diseases, including liver and kidney damage, and its modulation by ADMSCs is thought to account for the antioxidant, anti-inflammatory, and regenerative effects observed in this study.

Indeed, stem cells, particularly MSCs, show potential to protect against cisplatin-induced liver and kidney damage due to their ability to modulate inflammation, oxidative stress, and apoptosis. It is believed[19] that ADMSCs secrete various bioactive molecules, such as growth factors and cytokines, that can directly or indirectly modulate Galectin-3 expression and function. The anti-inflammatory, antioxidant, and tissue-repair properties of these secreted factors contribute to the protective effects of ADMSCs[21]. In addition to the Galectin-3 pathway, ADMSCs can exert protective effects through other mechanisms. It has been shown[21,37] that they promote tissue regeneration, reduce oxidative stress, inhibit apoptosis, and modulate immune responses, all of which can contribute to the prevention of cisplatin-induced organ damage.

Limitations

The present study is subject to certain limitations. The effects of cisplatin in rats may be more toxic than in humans. In addition, the behavior of ADMSCs may differ in humans. Consequently, the results obtained in an experiment on animals are not immediately applicable to the human context. Further studies will provide more detailed information on the subject.

Conclusions

This study demonstrates the significant renoprotective and hepatoprotective effects of ADMSC administration against cisplatin-induced liver and kidney damage. ADMSCs have been shown to reduce MDA, creatinine, and ALT levels and decrease pathological changes in renal and hepatic histological examinations. Specifically, it has been observed that ADMSCs achieve this effect by reducing Galactin-3 levels, indicating the involvement of this pathway. Further research is needed to determine the potential beneficial role of ADMSCs in preventing and treating hepatic and renal diseases.

Authors' Contributions

Oytun Erbaş, Ejder Saylav Bora, Müslih Ürün, and Hüseyin Acar contributed equally during the study and made critical revisions related to the relevant intellectual content of the manuscript.

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Conflict of Interest

No conflict of interest.

Informed Consent

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Ethics Approval

Ethical approval Animal Ethics Committee of Demiroğlu Science University Istanbul, Türkiye (Ethical Permission Number: 16.03.2022/ 2723031514) and reported in compliance with the ARRIVE guidelines.

Availability of Data and Materials

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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