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Effect of royal jelly on chronic liver and kidney damage induced by cadmium chloride in rats*

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Summary

Forty eight rats were used to evaluate the protective effect of royal jelly (RJ) against chronic liver and kidney damage induced by cadmium chloride ($CdCl_2$). In the histopathology of the $CdCl_2$ group, the findings of inflammation and necrosis in liver sections, inflammation in kidney sections, proximal tubule degeneration and adiposity in the glomerulus were similar to the histopathological findings in the $CdCl_2$ + RJ group. In immunohistochemical staining, it was determined that Caspase-3, Iba-1, KIM-1 and Tnf-a immunoreactivities in the liver and kidney tissue sections of the $CdCl_2$-only group (Group III) were significantly increased compared to the control groups (Groups I, II). In the $CdCl_2$ + RJ applied group (Group IV), Caspase-3, Iba-1, KIM-1 and TNF-α immunoreactivities were observed to be partially reduced compared to the $CdCl_2$ (Group III) group. It was determined that AST, ALT, glucose, triglyceride, cholesterol and creatinine levels increased in the $CdCl_2$ group and decreased in the $CdCl_2$ + RJ group. While MDA levels increased in the group given only $CdCl_2$ (Group III) compared to the control groups (Groups I, II), GSH levels decreased. In the $CdCl_2$ + RJ group (Group IV), it was determined that MDA levels decreased and GSH levels increased compared to the $CdCl_2$ (Group III) group. While the amounts of cadmium, zinc, copper, iron, magnesium and calcium in the liver tissue homogenates increased significantly in the $CdCl_2$ group, it was observed that they decreased numerically in the $CdCl_2$ + RJ group. This result shows that royal jelly application against $CdCl_2$ toxicity does not prevent liver and kidney tissue damage, but can bring serum biochemical parameters, lipid peroxidation and trace element levels closer to control group levels.

Keywords: cadmium chloride, liver, kidney, rat, royal jelly

Cadmium (Cd) has been reported as the seventh most toxic heavy metal in the "Agency for Toxic Substances and Disease Registry" (ATSDR) classification. In previous studies, it has also been determined that soluble Cd salts (such as $CdCl_2$) cause toxicity by accumulating in the kidney, liver, lungs, brain, testis, heart and central nervous system. Studieshave determined that cadmium affects various processes such as indirect oxidative stress in DNA, inducing apoptotic mechanisms and blocking DNA repair mechanisms, increasing lipid peroxidation, and triggering various processes such as protein oxidation (55).

This study aimed to evaluate the protective effect of royal jelly (RJ) against lesions that may occur in liver and kidney tissues in chronic toxicity caused by $CdCl_2$. Liver and kidney tissue sections taken from rats were evaluated by histopathological and immunohistochemical examination. AST, ALT activities, Albumin, Glucose, Triglyceride, Cholesterol, BUN, Creatinine, Malondialdehyde (MDA) and Glutathione (GSH) levels were measured in the serum samples. Cadmium (Cd), zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) levels were determined in liver tissue homogenates.

Material and methods

Chemicals. Cadmium chloride ($CdCl_2$) used in the study was obtained from Sigma Aldrich (202908), and Royal Jelly (AS) was commercially obtained from Sbs Bilim Bio Çözümler San. Trade Inc. (BEEO).

Animals, diets and experimental design. The experimental study was approved by Erciyes University Animal

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Experiments Local Ethics Committee on 07.10.2020 number 20/143. In the study, 48 Wistar Albino male rats weighing 200-250 grams, eight weeks old, obtained from Erciyes University Experimental Research Application and Research Center (DEKAM) were used. Rats were fed with pellet feed ad libitumfour in each cage, under the appropriate conditions of the research center [controlled temperature (21 ± 2°C), humidity (50 ± 5%), air exchange (12 cycles per hour), and 12 hours light, 12 h dark.]

Four groups of rats were formed, with 12 animals in each group. The first group was kept as the control group (Group I), and only 0.9% NaCl was administered intraperitoneally (IP) at a dose of 0.2 mL/kg-bw. In the second group (Group II), royal jelly was administered by gavage at a daily dose of 200 mg/kg-bw for six weeks. In the third group (Group III), $CdCl_2$ was administered only intraperitoneally (IP) thrice a week at 5 mg/kg-bw for six weeks. In the fourth group (Group IV), royal jelly was administered at a daily dose of 200 mg/kg-bw by gavage for six weeks, while $CdCl_2$ at a dose of 5 mg/kg-bw was administered intraperitoneally three times a week.

In studies on rats the following authors investigated the histopathological effects of Cd toxicity on the liver and kidney by giving $CdCl_2$ in intraperitoneally: Prozialeck et al. (41) 12 weeks (0,6 mg/kg/day), Yadav and Khandelwal (50) 24 weeks (0.5 mg/kg/day) subcutaneously, Olajide et al. (38) 3 weeks (10 mg/kg/day), Renugadevi and Prabu (43) 4 weeks; Kong et al. (28) 5 weeks (5 mg/kg/day), orally; Jemai et al. (24) 25 days (2 mg/kg/day with an interval of 5 days), and Seif et al. (46) 30 days (3.5 mg/kg/day) investigated the histopathological effects on liver and kidney by giving $CdCl_2$ in intraperitoneally. Considering the common histopathological findings in these studies and because it is 1/15 of the LD50 (13), a dose of 5 mg/kg/day $CdCl_2$ was administered to the experimental groups (Groups III, Group IV) intraperitoneally for 6 six weeks. Almeer et al. (2, 3) administered 6.5 mg/kg $CdCl_2$ intraperitoneally to rats in their study and administered royal jelly at 85 mg/kg and 250 mg/kg doses by gavage. Except for cadmium chloride (20, 23, 25, 26, 37, 52), royal jelly was used at doses of 100 mg/kg, 150 mg/kg, 200 mg/kg and 300 mg/kg in toxicity studies. In our study, the dose of royal jelly was 200 mg/kg, selected according to the studies (2, 3, 20, 23, 25, 26, 37, 52) on this subject, and applied to the experimental groups (Groups II, IV) by gavage every day for six weeks.

Collection and processing of samples. After the final applications of the rats in the study groups, thoracic and abdominal cavities were opened under anesthesia for 24 hours, intracardiac blood samples were taken into anticoagulant tubes and necropsied. The blood samples were centrifuged at 3000 rpm for 10 minutes and their serums were separated. All tissue samples were placed in a 10% buffered neutral formalin solution for light microscopic examination. Part of the liver tissue was stored at –80°C in Erciyes University Technology Research and Application Center to determine the ratios of cadmium and other elements.

Following fixation in neutral formalin solution (10%), liver tissue specimens were thoroughly rinsed overnight under tap water. Then, all tissue samples were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax and sectioned (thickness, 5 μm), for histopathological evaluation. After staining with hematoxylin and eosin sections were examined with a light microscope. After that, all liver sections were semi-quantitatively evaluated for inflammation and necrosis and were graded as 1 (mild, < 33% of liver cells), 2 (moderate, 33% to 66% of liver cells), and 3 (severe, > 66% of liver cells) and the values obtained in each group were calculated for averages with percentages. Kidney sections were semi-quantitatively evaluated for inflammation, tubular degeneration and formation of fat vacuoles in the glomeruli were graded as 1 (mild, < 33% of kidney tissues), 2 (moderate, 33% to 66% of kidney tissues), and 3 (severe, > 66% of kidney tissues) and the values obtained in each group were calculated for averages with percentages.

Immunohistochemically, in order to determine apoptosis, necrosis and macrophage activity in liver tissue, the Avidin Biotin-Peroxidase Complex (ABC) technique was applied according to the standard procedure prescribed in the commercial kit (Zymed, Histostain Plus Kit, California, USA). Anti-caspase-3 (active) (Novus NB100-56113) (dilution ratio 1/1000), TNF-α (Abcam 183896) (dilution ratio 1/500), Iba-1 (Wako 019-19741) (dilution ratio 1/1000) and KIM-1 (R&D Systems GmbH AF3689) (dilution ratio 1/50) were used as primary antibodies. As a negative control, PBS was applied to the liver tissues and, as a positive control, primary antibodies were applied to the control tissues recommended by the manufacturers.

AST, ALT, albumin, glucose, triglyceride, cholesterol, BUN, and creatinine levels of serum samples separated from blood samples were determined using commercial kits (Roche Cobas Kit-Switzerland) in a Roche Cobas 8000 brand autoanalyzer at the Clinical Biochemistry Department of Erciyes University Medical Faculty Hospitals Central Laboratory. Serum MDA levels were determined using Sun Red Bio 201-11-0157, SOD levels Sun Red Bio 201-11-0169, and GSH levels in an ELISA device (μQuant Bio-Tek) using Sun Red Bio 201-11-7122 kits. In the liver tissue Cadmium (Cd), magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu) and zinc (Zn) levels were determined by the induced coupled plasma mass spectroscopy (ICP-MS) method at Erciyes University Technology Research and Application Center (TAUM).

Statistical analysis. Statistical analysis of biochemical data: While evaluating the data with the Kolmogorov-Smirnov test in terms of conformity with the normal distribution; Duncan and Bonferroni tests were used for pairwise comparisons while evaluating the biochemical data with the Kolmogorov-Smirnov test regarding conformity with the normal distribution. Liver and kidney damage scoring analyzes, body weight, liver and kidney weight analyzes were determined by Kruskal Wallis test The Kruskal-Wallis test determined liver and kidney damage scoring analyses, body weight, and liver and kidney weight analyses. Data are presented with mean ± standard error and median (1^{st} quarter to 3^{rd} quarter) summary statistics. Statistical analyzes analyses were performed with Stata/SE 17.0 for Windows (64-bitx86-64) software (StataCrop LLC 4905 Lakeway Drive Collage College Station, TX77845, USA). Significance The significance level was accepted as $P < 0.05$.

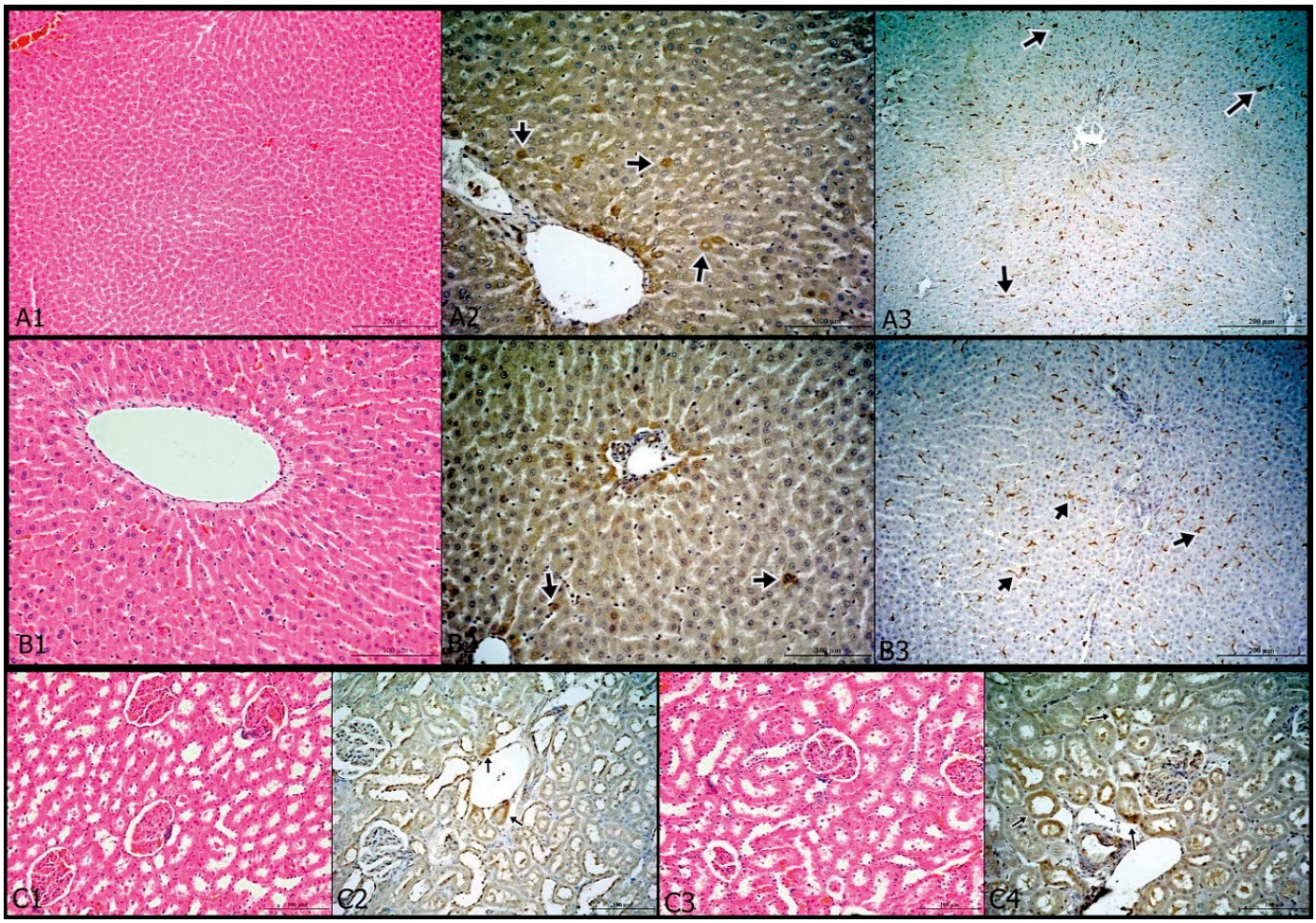

Fig. 1. Normal histological appearance of liver tissues of rats in Group I (A1) (200 µm) and Group II (B1) (100 µm), H × E. Positive appearance of Caspase-3 in Group I (A2) and Group II (B2) and Iba-1 in Group I (A3) and Group II (B3), 100 µm, liver, ABC-P. Normal histological appearance of kidney tissues of rats in Group I (C1) (100 µm) and Group II (C3) (100 µm), HxE. Positive appearance of Caspase-3 in Group I (C2) and Group II (C4) (100) µm, kidney, ABC-P

Results and discussions

Macroscopic-histopathological and immunohistochemical findings. Control (Group I) and Royal Jelly (Group II) Groups: It was observed that the liver and kidneys of the rats in these groups weremacroscopically and microscopically normal (Fig 1. A1, B1, C1, C3). In the immunohistochemical examination of liver tissue sections of these groups: Caspase-3 activation in a small number of hepatocytes are undergoing apoptosis (Fig. 1. A2, B2) and Iba-1 brown staining in activated kupffer cells (Fig. 1. A3, B3); In kidney sections, a small number of kidney tubule epithelial cells exposed to apoptosis in immunohistochemical staining with Caspase-3, TNF-α and KIM-1 were detected as brown staining positive for a small number of Caspase-3 (Fig. 1. C2, C4). TNF-α and KIM-1 staining were negative (Tab. 3).

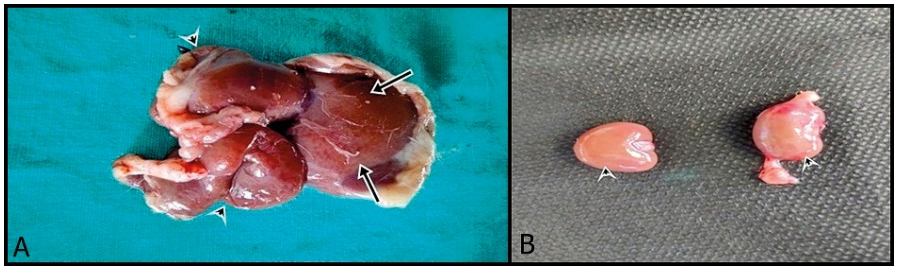

Fig. 2. The appearance of whitish-yellowish color changes in the liver (arrow) and the appearance of sticking of the lobes to each other (arrowhead) in the rats in Group III (A) and the appearance of sizeable yellowish-white color changes in the renal cortex (arrowhead) (B)

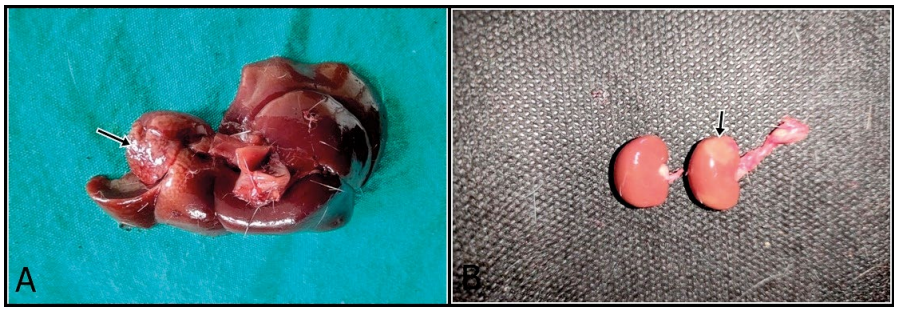

Fig. 3. The appearance of whitish-yellowish color changes in the liver of rats in Group IV (arrow) (A) and the appearance of whitish color changes in the renal cortex (arrow) (B)

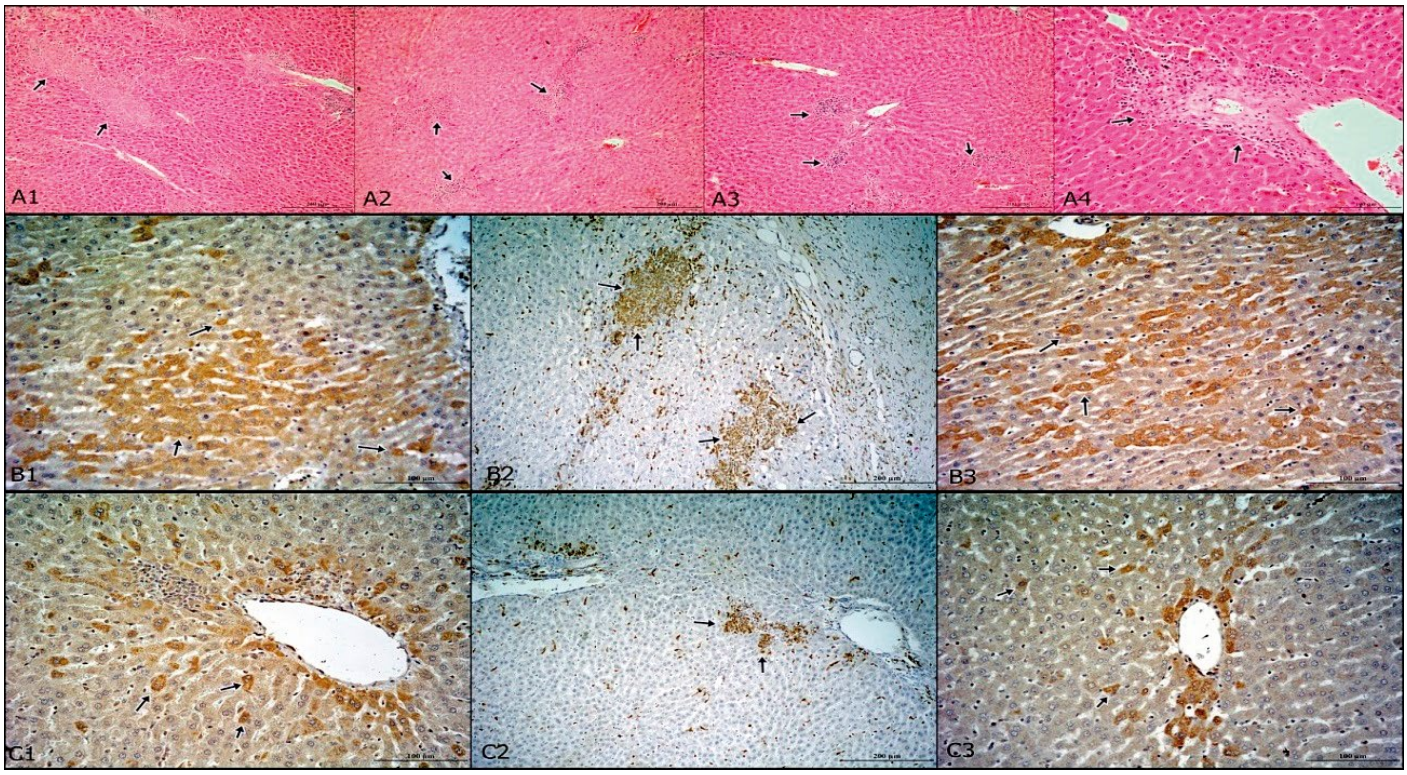

Fig. 4. Liver sections of rats in Group III and Group IV. (A1) Appearance of lymphocyte-rich mononuclear cell infiltration areas (arrows) within necrotic areas (Group III), (200 µm), H × E. (A2) Appearance of large necrotic areas (arrows) with a pink homogeneous appearance (Group III) (200 µm) H × E. (A3) Appearance of lymphocyte-rich mononuclear cell infiltration areas (arrows) within necrotic areas (Group IV), (200 µm), H × E. (A4) Appearance of large necrotic areas (arrows) with a pink homogeneous appearance (Group IV) (100 µm) H × E. (B1) Immunopositive (arrows) view of Caspase-3 activation in hepatocytes, (Group III) (100 µm), ABC-P. (B2) Immunopositive Iba-1 (arrows) appear of Kupffer cells, (Group III), (200 µm), ABC-P. (B3). Immunopositive (arrows) appearance of TNF-α in hepatocytes, (Group III), (100 µm), ABC-P. (C1) Immunopositive (arrows) view of Caspase-3 activation in hepatocytes, (Group IV) (100 µm), ABC-P. (C2) Immunopositive Iba-1 (arrows) appear of Kupffer cells, (Group IV), (200 µm), ABC-P. (C3). Immunopositive (arrows) appearance of TNF-α in hepatocytes, (Group IV), (100 µm), ABC-P

Cadmium Chloride Group (Group III): In the systemic necropsies performed on rats, the livers increased in volume and yellowish color changes in which the lobes adhered to each other; yellowish-white comprehensive color changes were detected in the cortex in the kidney (Fig. 2. A, B).

In the histopathological examination of liver tissue sections belonging to the group, large necrotic areas with pink homogeneous appearance in the parenchyma and most of them are randomly distributed in these areas, some of them are close to the portal areas; lymphocyte-rich mononuclear cell infiltration areas were seen (Fig. 4. A1, A2). Remark cords and sinusoids were disturbed in areas of necrosis. In the histopathological examination of kidney tissue sections, mononuclear cell infiltration areas were noted, mainly in the cerebral intertubular areas and a small part in the vessel's periphery (Fig. 5. A1). Lesions ranging from degenerative to necrotic (coagulation necrosis) were observed in the epithelium of the proximal tubules close to these infiltration sites (Fig. 5. A2). Hyperemic glomeruli and the formation of rounded fat vacuoles with sharp edges were observed in podocyte cells (Fig. 5. A3).

At the end of the study, in the immunohistochemical staining of the sections prepared from the liver tissue of the rats in this group (Group III), Caspase-3 in hepatocytes, Iba-1 in activated kupffer cells and TNF-α in necrotic hepatocytes, intense brown positive staining (Fig. 4. B1, B2, B3), Caspase-3 in apoptosis in the tubular epithelium in kidney sections, KIM-1 in degeneration and in those with necrosis, it was noted that TNF-α was stained intensely brown (Fig. 5. C1, C2, C3). In the $CdCl_2$ (Group III) group, liver Caspase-3, Iba-1 and TNF-α immunreactivity scores, kidney Caspase-3, KIM-1 and TNF-α scores, a statistically significant increase was detected compared to the control (Group I) and royal jelly (Group II) groups ($P < 0.001$) (Tab. 3).

Cadmium Chloride + Royal Jelly (Group IV) Group: In the systemic necropsies performed on rats, the macroscopic findings of the rats in the group given cadmium chloride (Group III) were similar (Fig. 3. A, B).

In the histopathological examination of liver tissue sections belonging to the group, mononuclear cell infiltration areas rich in lymphocytes (Fig. 4. A3) and focal necrosis foci that turned into a pink homogeneous mass were noted in the portal regions (Fig. 4. A4). In kidney tissue sections, mononuclear cell infiltration area located in the intertubular areas of the cortex (Fig. 5. B1), necrotic changes were noted in the proximal

tubular epithelium (Fig. 5. B2). Podocyte cells in the glomerulus of a rat were determined to have rounded fat vacuoles with sharp edges (Fig. 5. B3).

At the end of the study, in the immunohistochemical staining of the sections prepared from the liver tissue of the rats in this group it was determined that hepa-

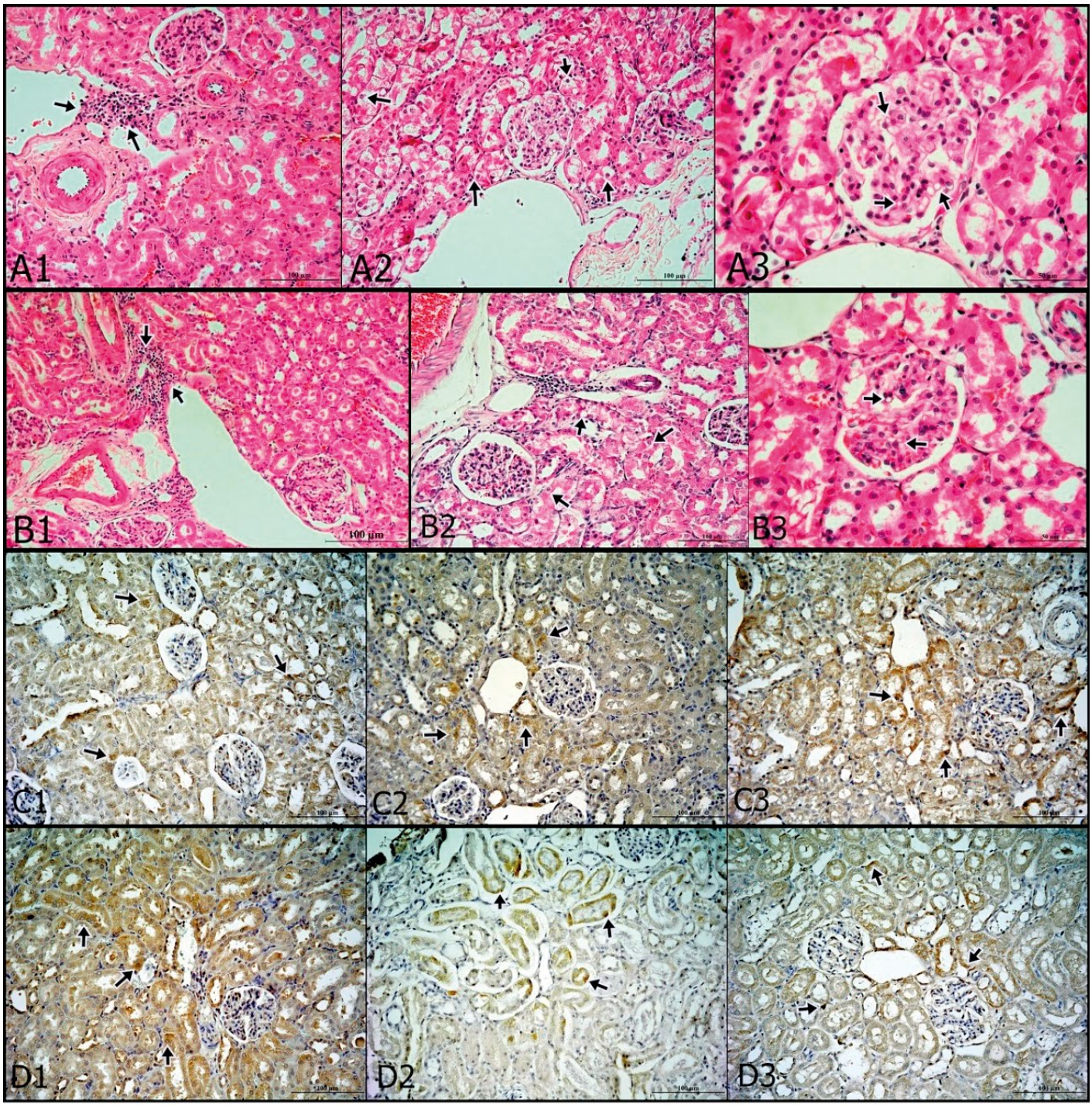

Fig. 5. (A1). Appearance of mononuclear cell infiltration areas (arrows) in intertubular kidney sections of rats in Group III, (100 µm), H × E. (A2) Appearance of degeneration (arrows) of the epithelium of proximal tubules close to infiltration sites in Group III (100 µm), H × E. (A3) Hyperemia in glomeruli and appearance of formation of fat vacuoles with sharp edges (arrows) in podocyte cells in Group III (50 µm), H × E. (B1). Appearance ofmononuclear cell infiltration areas (arrows) in intertubular kidney sections of rats in Group IV, (100 µm), H × E. (B2) Appearance of degeneration (arrows) of the epithelium of proximal tubules close to infiltration sites in Group IV (100 µm), H × E. (B3) Hyperemia in glomeruli and appearance of formation of fat vacuoles with sharp edges (arrows) in podocyte cells in Group IV (50 µm), H × E. (C1) Appearance of Caspase-3 positive (arrows) areas (Group III) (100 µm) ABC-P. (C2) Immunopositive (arrows) appearance of KIM-1 in the proximal tubule epithelium, (Group III) (100 µm) ABC-P. (C3) Immunopositive (arrows) appearance of TNF-α in the tubular epithelium, (Group III) (100 µm) ABC-P. (D1) Appearance of Caspase-3 positive (arrows) areas (Group IV) (100 µm) ABC-P. (D2) Immunopositive (arrows) appearance of KIM-1 in the proximal tubule epithelium, (Group IV) (100 µm) ABC-P. (D3) Immunopositive (arrows) appearance of TNF-α in the tubular epithelium, (Group IV) (100 µm) ABC-P

tocytes Caspase-3, activated Kupffer cells Iba-1 and TNF-α in necrotic hepatocytes (Fig. 4. C1, C2, C3) stained brown positive. In kidney sections, it was noted that Caspase-3 was positive in cells that underwent apoptosis in tubule epithelium, KIM-1 in degenerated epithelial cells, and TNF-α brown in cells with necrosis (Fig. 5. D1, D2, D3).

The change in the overall body weights of the rats is shown in Table 1. When the liver and kidney weights were measured, an increase was determined compared to the control group (Tab. 1). In the study, liver inflammation and necrosis scores were in the control (Group I) and royal jelly (Group II) groups; in the kidney, the scores for inflammation, degeneration, and glomerular adiposity were zero. In the $CdCl_2$ (Group III) and $CdCl_2$ + AS (Group IV) groups, both liver inflammation and necrosis scores, kidney inflammation, degeneration and glomerular adiposity scores, a statistically significant increase was detected compared to the control (Group I) and royal jelly (Group II) groups ($P < 0.001$) (Tab. 2). Only numerical decreases were observed in the immunoreactivities of Caspase-3, Iba-1, TNF-α in the liver tissue of the $CdCl_2$ + AS (Group IV) group, and Caspase-3, KIM-1, TNF-α in the kidney tissue and the $CdCl_2$ (Group III) group rats (Tab. 3).

Analysis of liver and kidney parameters, oxidative stress biomarkers and antioxidant enzymes in serum. Significantly increased ($P < 0.001$) serum AST, ALT, glucose, total cholesterol, triglyceride and creatinine levels due to liver damage in groups (Groups III, IV) administered $CdCl_2$. In rats given royal jelly (Group IV) together with $CdCl_2$, those in the group administered only $CdCl_2$ (Group III) while no statistically significant difference was observed compared to the levels of the population, it was determined that it only decreased numerically (Tab. 4).

Tab. 1. Changes in body, liver and kidney weights of rats at six weeks

Groups	Measurement Median (25%-75% Percentage)							
	1. Week	2. Week	3. Week	4. Week	5. Week	6. Week	*Liver (gr)	*Kidney (gr)
Group I (N = 12)	215 gr (213-218)	253 gr (251-254)	275 gr (273-278)	278 gr (276-279)	282 gr (281-282)	283 gr (281-284)	8.0^{c} (7.0-9.0)	2.0^{ab} (2.0-3.0)
Group II (N = 12)	240 gr (240-240)	260 gr (260-260)	273 gr (271-274)	283 gr (279-286)	284 gr (281-287)	288 gr (285-290)	10.0^{b} (9.0-10.0)	2.0^{ab} (2.0-3.0)
Group III (N = 12)	285 gr (283-288)	288 gr (286-289)	284 gr (279-288)	279 gr (275-283)	278 gr (273-282)	277 gr (273-281)	15.0^{a} (15.0-16.0)	3.0^{a} (2.5-4.0)
Group IV (N = 12)	260 gr (260-260)	255 gr (255-255)	244 gr (244-245)	250 gr (249-251)	254 gr (253-255)	249 gr (247-251)	13.0^{a} (12.0-14.5)	3.0^{a} (2.5-3.0)
Statistical Significance Check (Friedman test)	P = 0.973						P < 0.001	

Explanation: *a-b – the difference between groups with different letters in each line is significant

Tab. 2. Statistical significance control of liver and kidney tissue damage scores of thegroups

Variables	Groups Median (25%-75% Percentage)				Statistical Significance Check (Kruskal Wallis Test)
	Group I (N = 12)	Group II (N = 12)	Group III (N = 12)	Group IV (N = 12)	
Liver Inflammation	0^{b} (0-0)	0^{b} (0-0)	1.5^{a} (1.0-2.5)	1.0^{a} (1.0-1.0)	P < 0.001
Liver Necrosis	0^{b} (0-0)	0^{b} (0-0)	1.5^{a} (0.5-2.0)	1.0^{a} (0-1.0)	P < 0.001
Kidney Inflammation	0^{b} (0-0)	0^{b} (0-0)	1.0^{a} (0-2.0)	1.0^{a} (0-1.0)	P < 0.001
Tubular Degeneration	0^{b} (0-0)	0^{b} (0-0)	2.0^{a} (2.0-2.0)	1.0^{a} (1.0-1.0)	P < 0.001
Fat vacuoles in the glomeruli	0^{b} (0-0)	0^{b} (0-0)	1.0^{a} (0.5-1.5)	1.0^{a} (0-1.0)	P < 0.001

Explanations: $^{a-b}$ – the difference between groups with different letters in each line is significant

Tab. 3. Statistical significance check of immunohistochemical scores of liver and kidney tissues

Variables		Groups				Statistical Significance Check (Kruskal Wallis Test)
		Group I (N = 12)	Group II (N = 12)	Group III (N = 12)	Group IV (N = 12)	
Liver	Caspase-3	1.0^{b} (1.0-1.0)	1.0^{b} (1.0-1.0)	3.0^{a} (2.0-3.0)	2.0^{ab} (1.0-2.0)	P < 0.001
	Iba-1	1.0^{b} (1.0-1.0)	1.0^{b} (1.0-1.0)	3.0^{a} (2.5-3.0)	2.0^{ab} (1.0-2.0)	P < 0.001
	TNF-α	0.0^{b} (0.0-1.0)	0.0^{b} (0.0-1.0)	3.0^{a} (2.0-3.0)	2.0^{ab} (1.0-2.0)	P < 0.001
Kidney	Caspase-3	1.0^{b} (0.75-1.25)	1.0^{b} (0.75-1.0)	2.0^{a} (2.0-3.0)	1.5^{ab} (1.0-2.0)	P < 0.001
	KIM-1	0.0^{c} (0.0-0.25)	0.0^{c} (0.0-0.25)	2.0^{a} (2.0-3.0)	1.5^{ab} (1.0-2.0)	P < 0.001
	TNF-α	0.0^{c} (0.0-1.0)	0.5^{c} (0.0-1.0)	3.0^{a} (2.0-3.0)	2.0^{ab} (1.0-2.0)	P < 0.001

Explanations: a-c – the difference between groups with different letters in each line is significant

Tab. 4. Statistical analysis of serum biochemical parameters

Variables	Groups				Statistical Significance Check (Kruskal Wallis Test)
	Group I (N = 12)	Group II (N = 12)	Group III (N = 12)	Group IV (N = 12)	
Glucose (mg/dL)	195.45 ± 11.04^{a}	174.20 ± 6.50^{a}	249.75 ± 6.97^{b}	240.50 ± 10.90^{b}	P < 0.001
Cholesterol (mg/dL)	69.2 ± 2.11^{ab}	67 ± 0.9^{a}	81.73 ± 4.44^{c}	76.5 ± 3.91^{bc}	P < 0.05
Creatinine (mg/dL)	0.35 ± 0.01^{b}	0.29 ± 0.01^{a}	0.39 ± 0.01^{c}	0.37 ± 0.02^{bc}	P < 0.001
Albumin (g/dL)	3.84 ± 0.05^{c}	3.71 ± 0.08^{bc}	3.51 ± 0.10^{a}	3.58 ± 0.04^{ab}	P < 0.05
AST (U/L)	92.5^{b} (84.25-111.)	78.5^{b} (72-91.75)	359^{a} (246-427)	336.5^{a} (227.77-452)	P < 0.001
ALT (U/L)	54^{b} (49.25-58)	49.5^{b} (46.25-55.5)	108.20^{a} (101-129)	94.25^{a} (87.49-119.75)	P < 0.001
Triglyceride (mg/dL)	55^{b} (47.75-65.5)	66^{b} (52-82)	138^{a} (101-145)	80^{ab} (61-99)	P < 0.001
MDA (µmol/L)	0.63 ± 0.03^{bc}	0.43 ± 0.03^{a}	1.1 ± 0.01^{e}	0.81 ± 0.06^{d}	P < 0.001
GSH (µmol/L)	184.23 ± 14.08^{b}	182.12 ± 4.0^{b}	133.76 ± 8.78^{a}	141.11 ± 1.52^{a}	P < 0.001

Explanations: a-e – the difference between groups with different letters in each line is significant

Tab. 5. Trace element parameters in liver tissue of control (Groups I and II) and trial (Groups III and IV)

Trace element	Groups				Statistical Significance Check (Kruskal Wallis Test)
	Group I (N = 12)	Group II (N = 12)	Group III (N = 12)	Group IV (N = 12)	
Cd (ppm)	–	–	192.7 (175.8-223.8)	191.6 (152-204)	P = 0.289
Mg (ppm)	49.31 ± 1.54^{a}	59.52 ± 3.28^{ab}	88.29 ± 6.79^{c}	84.43 ± 1.59^{c}	P < 0.001
Fe (ppm)	86.45 ± 2.93^{ab}	66.41 ± 3.38^{a}	127.41 ± 15.51^{c}	112.14 ± 12.16^{bc}	P < 0.001
Cu (ppm)	2.10 ± 0.03^{a}	1.75 ± 0.10^{a}	4.70 ± 1.18^{b}	3.26 ± 0.95^{ab}	P < 0.001
Zn (ppm)	9.87 ± 0.21^{ab}	12.26 ± 2.65^{bc}	18.55 ± 1.99^{d}	15.89 ± 1.07^{cd}	P < 0.001
Ca (ppm)	44.8^{b} (30.5-78.8)	103.4^{ab} (80.8-194)	179.68^{c} (61.6-313.1)	175.6^{a} (123-231.9)	P < 0.001

Explanations: a-d – The difference between groups with different letters in each line is significant

There was a statistically significant difference in serum MDA levels between the control and royal jelly groups (Groups I, II) and those treated with $CdCl_2$ (Groups III, IV). A significant increase was observed in the $CdCl_2$-administered groups (Groups III, IV) compared to the control groups (Groups I, II) at these levels ($P < 0.001$). In addition, between the $CdCl_2$ group (Group III) and the $CdCl_2$ + RJ (Group IV) group, it was also determined that giving royal jelly simultaneously with $CdCl_2$ decreased MDA levels statistically ($P < 0.001$). In the groups administered $CdCl_2$ (Groups III, IV), significantly decreased serum GSH levels ($P < 0.001$) due to liver damage. With the simultaneous administration of royal jelly with $CdCl_2$ (Group IV), a numerical increase was observed, which was not statistically significant, compared to the levels in the group that was administered only $CdCl_2$ (Group III) (Tab. 4).

Trace element analyses in liver tissue homogenates. While the amount of cadmium in the liver tissue homogenates of the control and royal jelly groups (Group I, II) rats was below the measurable level, increased significantly in the $CdCl_2$ (Group III) group and the $CdCl_2$ + RJ (Group IV) group. However, there was no statistically significant difference between these two groups (Groups III, IV) given $CdCl_2$ regarding the amount of cadmium. The amount of Fe determined in the liver tissue homogenates of the rats in the groups given $CdCl_2$ (Group III, IV) was found to be higher than that the of control groups (Groups I, II). However, from the groups given cadmium no statistically significant, but numerically, between the $CdCl_2$ + RJ (Group IV) group and $CdCl_2$ (Group III) group, with simultaneous administration of royal jelly (Group IV) with $CdCl_2$, decreases in the amount of Fe, Mg, Cu, Ca and Zn were detected (Tab. 5).

Following systemic cadmium toxicity, the liver is the primary target organ (11). In the hepatocyte, Cd forms complexes with small peptides and proteins through glutathione (GSH) or sulfhydryl groups, including the high-affinity metal-binding protein families metallothionein-I (MT-I) and metallothionein-II (MT-II) (5). Metallothionein (MT) binds cadmium in the hepatic cytosol, converting it to the inactive form (CdMT complex). Chronic cadmium toxicity causes nephrotoxicity and hepatotoxicity (11). Over time, CdMT is transported from the liver to the kidneys with liver damage (11). CdMT is filtered from the blood and reabsorbed by the kidney's proximal tubules (17). In our study, liver tissue sections of rats given cadmium chloride (Group III) showed large necrotic areas, mononuclear cell infiltration mainly consisting of lymphocytes, focal hemorrhage areas and hyperemia in the vessels, and dilatation due to partial disruptions in the remark cords. These findings are similar to Renugadevi and Prabu (43) and Seif et al. (46). In the histopathological examination of the kidneys of rats given $CdCl_2$ (Group III), mononuclear cell infiltrations,

necrotic and degenerative changes in the proximal tubular epithelium; Renugadevi and Prabu (43), Seif et al. (46), Kong et al. (28), while showing similarities with the findings; Hyperemia in the glomeruli and the formation of sharp-edged fat vacuoles in podocyte cells differ from the findings of these investigators and are likely due to dose, route of administration, and duration of the study. In our study, the histopathological changes in the kidney and liver tissues of the rats in the $CdCl_2$-administered group (Group III) suggest that they may develop as a result of insufficient antioxidant defense system and increased oxidative stress, as reported by Fujiwara et al. (19). Royal jelly is a nutrient secreted from the hypopharyngeal and mandibular glands of worker honeybees (47), and its biological effects include antioxidant (30), anti-inflammatory (18), and hypoglycemic (7) effects. Studies evaluating the hepatoprotective (2) or nephroprotective (3) effect of royal jelly against $CdCl_2$ toxicity are minimal. In our study, focal necrosis and mononuclear cell infiltration areas occurring in the liver tissue of rats in the group (Group IV) administered royal jelly together with $CdCl_2$ were found; on the other hand, in kidney tissue sections, mononuclear cell infiltration areas, necrotic and degenerative changes in tubular epithelial cells; the similarity of the rats in the $CdCl_2$-only group (Group III) with histopathological changes occurring in liver and kidney tissue sections; contrary to the studies of Almeer et al. (2, 3), it suggests that royal jelly has no nephroprotective and hepatoprotective effects against $CdCl_2$ toxicity. The increase in Caspase-3 and TNF-α immunoreactivity in the immunohistochemical staining of liver tissue sections of the rats in the $CdCl_2$ group is similar to the findings of Fouad et al. (15) and the increase of Caspase-3 activation is similar to the findings of Fouad and Jresat (16). In addition, the increase in Iba-1 activation is similar to the study findings of Sauer et al. (45) and Mantur et al. (34). In our study, Caspase-3 and TNF-α immunohistochemical staining on kidney tissue sections of rats treated with $CdCl_2$, the increase of Caspase-3 and TNF-α activities is in line with the findings of Fouad and Jresat (16). Positive staining of KIM-1 in kidney tissue sections of rats in the $CdCl_2$ group (Group III) also overlaps with the immunohistochemical findings in the study of Prozialeck et al. (41).

In our study, in our immunohistochemical findings, Caspase-3 and TNF-α activations in the liver tissues of rats administered royal jelly together with $CdCl_2$ (Group IV) were partially reduced compared to rats in the group administered only $CdCl_2$ (Group III), which is consistent with the findings of Almeer et al. (2). The literature review found no studies evaluating the Iba-1 activation of royal jelly in $CdCl_2$ toxicity. The increased Iba-1 activation in the $CdCl_2$ group (Group III) was partially decreased in the rats (Group IV) treated with $CdCl_2$ and royal jelly. In our study; in the Caspase-3 immunohistochemical staining of kidney tissue sections of rats; the fact that Caspase-3 positive areas in the kidney tissues of rats given $CdCl_2$ together with royal jelly (Group IV) decreased compared to those of the rats in the group only $CdCl_2$ administered (Group III) is similar to the findings of Almeer et al. (3). The literature review found no study in which TNF-α and KIM-1 activation were evaluated in kidney tissue sections of rats given royal jelly and $CdCl_2$. In our study, TNF-α and KIM-1 immunohistochemical stainings showed that the increased immunoreactivity in $CdCl_2$ group rats was partially decreased in the rats in the group (Group IV) to which royal jelly was administered simultaneously.

In our study, as a result of experimental $CdCl_2$ toxicity, it was determined that there was a decrease in the body weights of the rats in the $CdCl_2$ applied groups (Groups III, IV) compared to the rats in the control groups (Groups I, II); while this finding overlaps with the findings of Liu et al. (31) and Aqeel et al. (4), we think that the decrease in body weight may be due to the decrease in food intake of rats as a result of toxicity to $CdCl_2$, as stated in the studies of Luo et al. (32). It was determined that the liver and kidney weights of the rats in the $CdCl_2$ applied groups (Groups III, IV) were also increased compared to the control groups (Groups I, II); this increase in organ weights is similar to the findings of El-Demerdash et al. (13), Chen et al. (9), Fan et al. (14) and Liu et al. (31). In our study, there was no significant change in the body weights of the rats in the group administered royal jelly with $CdCl_2$ (Group IV) compared to the rats in the group administered only $CdCl_2$ (Group III). In our study, there was no significant change in the body weights of the rats in the group administered royal jelly with $CdCl_2$ (Group IV) compared to the rats in the group administered only $CdCl_2$ (Group III) differ with the findings of these researchers (2). In our study, no significant difference was found in the liver and kidney weights of the rats in the group administered only $CdCl_2$ (Group III) and in the group given royal jelly with $CdCl_2$ (Group IV). Supporting our macroscopic and histopathological findings, we believe that the antioxidant-effective royal jelly does not completely wholly prevent liver and kidney damage and does not reduce weight gains due to hepatomegaly in the liver (31) and hypertrophy in the kidney (10), which occur in $CdCl_2$ toxicity.

In our study, the increase in serum AST and ALT levels of rats in the $CdCl_2$-treated groups (Groups IV, V) compared to the control groups (Groups I, II), it is compatible with the studies of Renugadevi and Prabu (43), Fan et al. (14) and Yang et al. (51). Royal jelly application (Group IV) reduced these values compared to the group in which only $CdCl_2$ was applied (Group III); it is similar to the studies of Almeer et al. (2), Hamza et al. (21) and Omar et al. (39). When serum AST and ALT levels that increase as a result of $CdCl_2$ intoxication are evaluated together with our histopathological findings, it may result from hepatocellular damage due

to cadmium, as reported by Refaie et al. (42), royal jelly, which has the effect of reducing the levels of these enzymes this suggests that it may be due to the glycoprotein it contains stimulating liver regeneration and hepatocyte formation (2). In our study, the decrease in serum albumin levels of rats (Groups III, IV) in the $CdCl_2$ applied groups compared to the control groups (Groups I, II) overlapped with the results of these researchers (13, 29) suggests that this decrease may occur due to the decrease in the ability of the liver to synthesize protein, as reported by El-Demerdash et al. (13). Studies evaluating the effect of royal jelly on serum albumin levels could not be found in literature reviews. At the same time it was determined that the serum albumin levels in the group (Group IV) treated with royal jelly and $CdCl_2$ were increased compared to the rats in the group treated with only $CdCl_2$ (Group III). This suggests that royal jelly may be due to its protective effect on cell membranes and preventing lipid peroxidation, as reported by Almeer et al. (2) reported. In our study, a significant increase in serum glucose levels of rats administered $CdCl_2$ (Groups III, IV) compared to the control group (Groups I, II) rats; this finding is similar to the findings of Chapatwala et al. (8) and Trevino et al. (49). It suggests that the increase may be due to the increase in glucogenolysis and gluconeogenesis as reported by Chapatwala et al. (8) and Matsuda and DeFronzo (35). In addition, it was determined that the serum glucose levels of the rats administered royal jelly with $CdCl_2$ (Group IV) were decreased compared to the rats in the group administered only $CdCl_2$ (Group III). Caixeta et al. (7) stated that the effect of royal jelly may be due to its hypoglycemic effect by increasing antioxidant activity. It increased serum cholesterol and triglyceride levels due to experimental $CdCl_2$ toxicity in rats (1, 44), and royal jelly administered together with $CdCl_2$ decreased these values (21, 39) have been reported. Our study showed increased serum cholesterol and triglyceride levels of rats in $CdCl_2$-administered groups (Groups III, IV) compared to control groups (Groups I, II); With the findings of Afolabi et al. (1) and Samarghandian et al. (44), the decrease in rats administered $CdCl_2$ together with royal jelly (Group IV) compared to the group administered only $CdCl_2$ (Group III) is similar to the study findings of Hamza et al. (21) and Omar et al. (39). This increase in cholesterol and triglyceride levels suggests that it may result from increased cholesterol biosynthesis and decreased lipoprotein lipase activity, as reported by Hirano et al. (22). The decrease in cholesterol and triglyceride levels is by reducing royal jelly's lipid peroxidation, as Ghanbari et al. (20) reported, suggests that it may be due to its protective effect against cadmium. In studies evaluating the serum BUN and creatinine levels of rats administered $CdCl_2$ (27, 40), these values increased; it has been reported that royal jelly decreased these values when applied with $CdCl_2$ (39). In our study; serum BUN and creatinine values of the rats in the $CdCl_2$ applied groups (Groups III, IV) were increased compared to the control groups (Groups I, II), with the findings of the researchers (27, 40). The reduction of BUN and creatinine in the group given $CdCl_2$ with royal jelly (Group IV) is similar to the study findings of Omar et al. (39). This increase in BUN and creatinine levels as a result of cadmium may have occurred as a result of damage to glomerular and renal tubular cells, as the kidneys could not fulfill their excretory function, as stated in the studies of Chen et al. (9), Kim et al. (27); the decrease in these values in the group treated with royal jelly (Group IV) compared to the group administered only $CdCl_2$ (Group III) suggests that it may be because royal jelly reduces lipid peroxidation by showing an antioxidant effect, as reported in the studies of Ghanbari et al. (20).

It has been reported that serum MDA levels increased in liver damage caused $CdCl_2$ toxicity (12, 44) and decreased in rats treated with royal jelly and $CdCl_2$ (2,39). In our study: Significantly increased serum MDA levels in rats in the $CdCl_2$ administered groups (Groups III, IV) compared to the rats in the control groups (Groups I, II) with the findings of El-Boshy et al. (12), Samarghandian et al. (44) the decrease in the group administered royal jelly with $CdCl_2$ (Group IV) compared to the group administered only $CdCl_2$ (Group III) is similar to the findings of Almeer et al. (2) and Omar et al. (39). This increase in MDA levels, as Stohs and Bagachi (48) reported, increased oxidative stress due to $CdCl_2$ may be related to lipid peroxidation; the reducing effect of royal jelly on serum MDA levels may be due to the antioxidant activity of royal jelly, which reduces lipid peroxidation by scavenging hydroxyl radicals, as reported by Kanbur et al. (25). Studies report that serum GSH levels decrease due to experimental $CdCl_2$ toxicity (12). It has been reported that royal jelly increases serum GSH levels when used with $CdCl_2$ (3, 39). In our study; decreased serum GSH levels of rats in $CdCl_2$ administered groups (Groups III, IV) compared to control groups (Groups I, II) El-Boshy et al. (12) while it is compatible with the data in his studies; the increase in serum GSH levels of rats in the group (Group IV) administered $CdCl_2$ with royal jelly compared to the group administered only $CdCl_2$ (Group III) Almeer et al. (3) and Omar et al. (39) are similar to the findings. The decrease in GSH levels as a result of experimental $CdCl_2$ toxicity is because that royal jelly contains glutathione precursor cysteine and free amino acids such as cysteine, glycine, aspartic acid and valine, which have an essential role in the liver detoxification system (25).

In our study, it was determined by the ICP-MS method that the amount of cadmium increased in the liver tissue homogenates in the groups (Groups III, IV) treated with $CdCl_2$. This finding is consistent with the study of Zhang et al. (54) with the ICP-MS method that the groups that administered cadmium increased the

amount of cadmium in liver tissue homogenates compared to the control groups. The fact that the amount of cadmium in liver tissue homogenates did not change in the group in which royal jelly (Group IV) was administered together with $CdCl_2$ in our study differs from the study of Omar et al. (39), who said that the amount of cadmium decreased. In our study, the amount of zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) in liver tissue homogenates in rats in $CdCl_2$ applied groups (Groups III, IV) compared to control group rats (Groups I, II) was determined by the ICP-MS method that it increased significantly. Our findings are consistent with the findings of studies determining the amount of zinc, copper (54), iron (33), calcium (6, 36), magnesium (6). It is thought that these essential metal ions (such as Zn, Cu, Fe, Ca and Mg) that increase in cadmium toxicity are due to the adverse effects of enzymatic cell reactions by displacing cadmium in metalloenzymes (48).

Studies evaluating the amounts of zinc, copper, iron, calcium and magnesium in liver tissue homogenates in $CdCl_2$ toxicity of royal jelly could not be found in the literature review; the increased amounts of zinc, copper, iron, calcium and magnesium in the liver tissue homogenates of the rats in the $CdCl_2$ applied group (Group III) by the ICP-MS method; In the studies of Zhai et al. (53), the decrease in liver tissue homogenates of rats in the groups treated with royal jelly (Group IV) together with $CdCl_2$. It is thought that it may occur due to the chelating, antioxidant effects and lipid peroxidation-reducing effects of redox-active transition metals that prevent the formation of hydrogen peroxide and hydroxyl radicals, which is reported according to the content of royal jelly.

Although the positive effect of royal jelly (200 mg/kg/day) used in experimental chronic $CdCl_2$ toxicity on the histopathological changes in the liver and kidneys was not observed in our study, it is thought that the generally evaluated biochemical parameters, lipid peroxidation, antioxidant enzyme activities and trace element levels may be partially healing. However, this improvement is not reflected in the damage in the liver and kidney tissuliver and kidney tissue damage.

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