

Protective effects of resveratrol on kidney function tests and renal histopathology in carbon tetrachloride-induced renal toxicity in rats

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Abstract

In this study, it was aimed to investigate the protective effect of Resveratrol, which has a strong antioxidant effect on kidney tissues of rats experimentally induced with carbon tetrachloride with nephrotoxicity, by kidney function tests and histopathology. For this purpose, 32 male Wistar Albino rats were used. The subjects were randomly selected, 1st group control, 2nd group CCl₄, 3rd group Resveratrol. The 4th group was divided into 4 groups as CCl₄ + Resveratrol. At the end of the experiment, animals were sacrificed under anesthesia and kidney samples were taken in 10% formalin solution for histopathological analysis. In the histopathological examination, it was found that the rats in the control and Resveratrol groups had normal kidney histological structure. In CCl₄ group, severe hydropic degeneration in tubules epithelium, mild coagulation necrosis in tubules epithelium and severe hyperemia in the vessels were observed. When kidney tissues of rats were examined in CCl₄ + Resveratrol group, mild hydropic degeneration in tubules epithelium and mild hyperemia in vessels were observed. When the kidney tissues of the rats in the Resveratrol group were examined, it was observed that they had a normal histological appearance. As a result, it was determined that Resveratrol has a protective effect on kidney damage caused by CCl₄.

Keywords: Kidney; CCl₄; Rat; Resveratrol; Urea

1. Introduction

Carbon tetrachloride (CCl₄) is a fast evaporating, colorless, non-flammable and fragrant chemical. Since CCl₄ does not occur naturally, it is produced chemically [1]. CCl₄ can be ingested largely by inhalation as well as swallowing and skin [2]. Since CCl₄ is a good chemical solvent, it is used as an organic solvent in industrial areas, in obtaining seed extracts, in the synthesis of heat transfer element in cooling equipment, in oils, pesticide products, petroleum products. It is stated that those living close to textile and chemical factories, pesticide (pesticide) applicators, those working in waste centers, dry cleaners are exposed to CCl₄ and are at great risk [1,3,4]. If the CCl₄ level in the body exceeds certain levels, an increase in oxidative stress and consequently serious damage to components such as protein, lipid and nucleic acid in the cell occurs [5]. It has been determined as a result of studies that CCl₄ causes organ toxicities in tissues such as liver [6], heart [7], brain [8] and kidney [9]. In various recent studies, some flavonoids and natural ingredients are used to prevent the harmful effects of some toxic substances on the organs [10-29]. One of these substances is resveratrol. Resveratrol is a polyphenolic compound with many positive effects on health, especially since 1997. In addition to its anti-carcinogenic, anti-inflammatory, neuroprotective, anti-atherogenic, anti-thrombogenic and anti-liver fatty effects, it has been reported to increase insulin sensitivity and prolong life expectancy [30]. Resveratrol is especially abundant in the content of black mulberries, grapes and red wine [31]. The protective and therapeutic effects of resveratrol in nephrotoxicity models induced by various chemicals have been determined by many studies [32-34]. In the light of

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literature information, in this study, it was aimed to determine the effects of Resveratrol on kidney function tests and kidney histopathology in CCl₄-induced nephrotoxicity.

2. Material and methods

This study was carried out in accordance with ethical rules with the permission decision of Atatürk University Animal Experiments Local Ethics Committee no 2018/11. In our study, 32 male Wistar Albino rats 7-8 weeks old and weighing 250-300 g were used. 4 experimental groups were formed with 8 rats in each group. This study was presented as an oral presentation at the 4th International Scientific Research Congress in 2019.

Table 1 Experimental groups

Experimental groups	Number of animals	Application
Control	8	Distilled water was given for 14 days.
CCl ₄	8	On the 1st day, a single dose of CCl ₄ (2 ml / kg) was administered intraperitoneally.
Resveratrol	8	Resveratrol was administered orally at a dose of 30 mg / kg / day for 14 days.
CCl ₄ + Resveratrol	8	On day 1, a single dose of CCl ₄ (2 ml / kg) was administered intraperitoneally.

On the 15th day of the experimental protocol, after blood was taken from rats under anesthesia, they were decapitated and kidney tissues were taken. Urea, Creatine and BUN parameters in serum samples obtained from blood were analyzed with an auto analyzer.

2.1. Histopathological Examination

As a result of the necropsy, the kidney tissues taken for histopathological evaluation were detected in 10% formalin solution for 48 hours. It was embedded in paraffin blocks as a result of routine tissue follow-up procedures. 4 µm thick sections were taken from each block. The preparations prepared for histopathological examination were stained with hematoxylin-eosin (HE) and examined with light microscopy (Leica DM 1000, Germany). Sections were evaluated as none (-), mild (+), moderate (++) and severe (+++) according to immune positivity.

2.2. Statistical analysis

Statistical analysis of the quantitative data obtained in our study was performed using the SPSS 20 program. Results were evaluated using the Tukey test in One Way ANOVA. A value of P <0.05 was considered statistically significant.

3. Results

3.1. Kidney function tests

When the groups were compared in terms of kidney function tests, it was determined that urea, creatine and BUN levels were normal in the control group, but these values were increased in the CCl₄ group. In Resveratrol and CCl₄ + Resveratrol groups, all three values were determined to be similar to the control (Table 2).

Table 2 Serum urea, creatine and BUN values in the experimental groups (a, b: p <0.01, n = 8, statistically different groups are represented by different letters).

Experimental Groups	Urea (mg/dL)	Creatine (mg/dL)	BUN (mg/dL)
Control	34,12 ± 5.52 ^a	0,40 ± 0.07 ^a	15,11 ± 4.71 ^a
CCl ₄	55,46 ± 4.31 ^b	0,69 ± 0.09 ^b	35,49 ± 3.12 ^b
Resveratrol	35,42 ± 5.81 ^a	0,42 ± 0.06 ^a	18,53 ± 3.75 ^a

CCl ₄ + Resveratrol	39,88 ± 3.43 ^a	0,45 ± 0.09 ^a	20,33 ± 3.18 ^a
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3.2. Histopathological Findings

Control: When the kidney tissues were examined, it was observed that the cortex and medulla were in normal histological structure (Figure 1A). CCl₄: When the kidney tissues were examined, severe hydropic degeneration in the tubules epithelium, mild coagulation necrosis in the tubules epithelium and severe hyperemia in the vessels were observed (Figure 1B). Resveratrol: When the kidney tissues were examined, it was observed to have a normal histological appearance (Figure 1C). CCl₄ + Resveratrol: When the kidney tissues were examined, mild hydropic degeneration in the tubules epithelium and mild hyperemia in the vessels were observed (Figure 1D). It was found that the damage was significantly reduced compared to the CCl₄ group. Histopathological findings are summarized in Table 3.

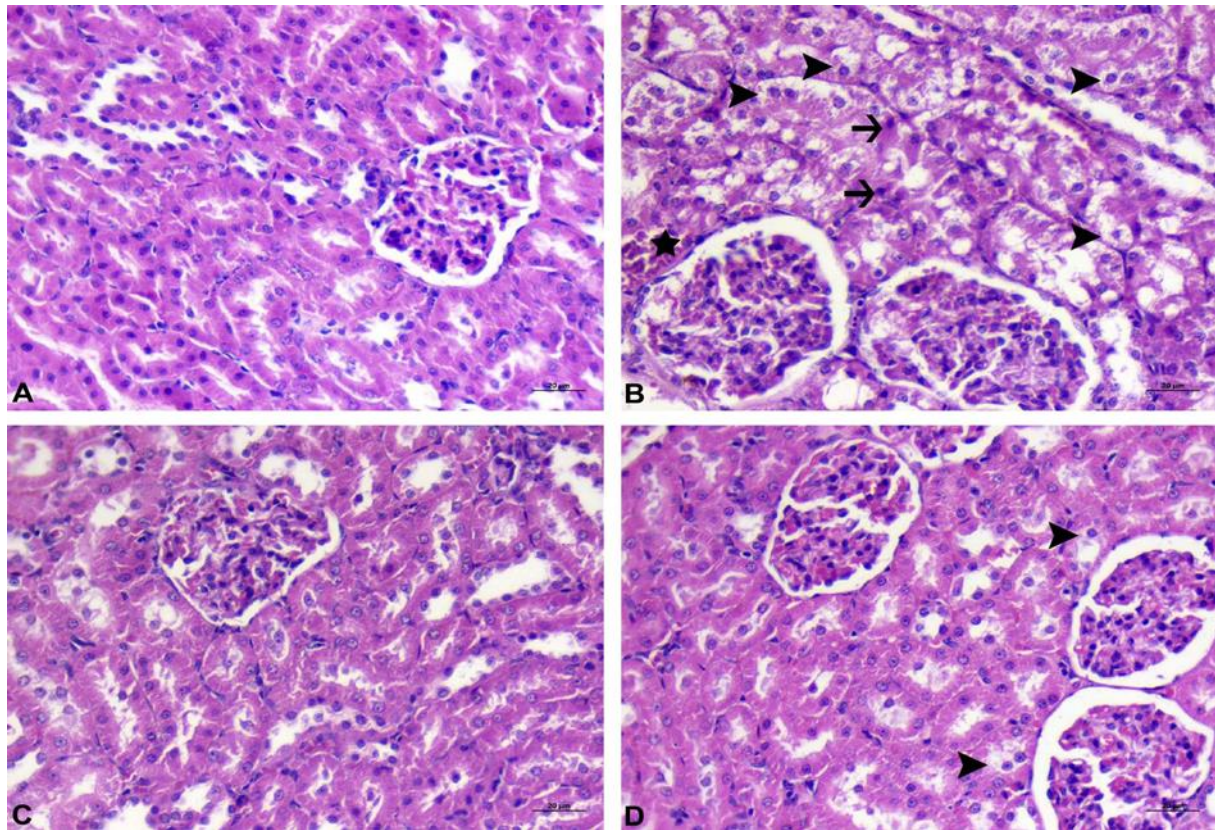


Figure 1 Renal tissue, in Control (A) and Resveratrol (C) groups, in normal histological appearance, in CCl₄ group (B), mild necrosis in tubules epithelium (arrows), severe hydropic degeneration in tubules epithelium (arrowheads), hyperemia (asterisks) (C), CCl₄ + Resveratrol group (D), mild hydropic degeneration of tubules epithelium (arrowheads) (D), H&E, Bar: 20µm.

Table 3 Histopathological findings in kidney tissues.

	Degeneration of the tubules epithelium	Necrosis of the tubules epithelium	Hyperemia of interstitial vessels
Control	-	-	-
CCl ₄	+++	+	+++
Resveratrol	-	-	-
CCl ₄ + Resveratrol	+	-	+

4. Discussion

Carbon tetrachloride (CCl₄) is known to be biohazardous. It is a toxic substance commonly used in animal models in experimental studies to induce nephrotoxicity. CCl₄ is metabolized by cytochrome P450 2E1 and forms trichloromethyl radicals. These radicals have the ability to initiate lipid peroxidation by separating hydrogen from polyunsaturated fatty acids [35]. The resulting trichloromethyl (CCl₃) radical combines with oxygen to form the trichloromethyl peroxy (CCl₃O₂) radical. The peroxy radical causes the cell's membrane structure to deteriorate [36]. Carbon tetrachloride is mostly used in experimental studies because it damages kidney tissues [37]. The renal dysfunction and pathogenesis induced by CCl₄ are not fully known. Generally, it has been shown that CCl₄ causes damage to the liver tissue at first, followed by deterioration of kidney functions [38]. Biochemical analyzes such as serum urea, creatinine and BUN related to kidney function are important to detect kidney damage. Urea is the final product formed as a result of the metabolism of proteins. Absorption of this substance is carried out within the renal tubules. Creatine is a body waste produced by muscle metabolism. It is absorbed from the blood by glomerular filtration and excreted in the urine [39]. In other studies, when lower doses of CCl₄ were administered to rats intraperitoneally and orally to create nephrotoxicity, higher urea and creatinine levels were found in measured serum samples compared to control groups [40]. In our study, similar to some studies, it was found that serum urea level increased in the group in which nephrotoxicity was created with CCl₄ compared to the control groups. It was determined that there was a decrease in all three values in the resveratrol group. This shows that resveratrol prevents CCl₄ induced renal dysfunction.

It has been reported that CCl₄ alone causes significant histopathological damage in the kidney tissue [35]. Kidney damage due to CCl₄ has been characterized by widespread vacuolar degeneration and congestion in the tubular epithelium [41]. In another study, when kidney tissue damage caused by CCl₄ intoxication was examined, significant glomerular and tubular damage was detected [42]. When kidney damage induced by CCl₄ was examined, it was shown that kidney tissue Bowman's capsule and glomerular degeneration, cellular vacuolization, necrotic areas [43] and significant inflammatory changes. In addition, epithelial hydropic changes in kidney tissue, degenerative changes, dense cortical damage, focal glomerular necrosis, hypocellularity and shrinkage of glomeruli, tubular dilatation, epithelial cell vacuolization and necrotic areas, glomerular basement membrane thickening, interstitial cell infiltration and some tubules have been shown [44, 45]. In our study, in accordance with the findings of the investigators, significant glomerular and tubular changes in kidney tissue were detected in the CCl₄ group. Degeneration, necrosis in tubules and prominent hyperemic areas in tubulo-interstitial regions were detected. However, these findings were found to be reduced in the Resveratrol group.

5. Conclusion

As a result, in this study, it was determined by kidney function tests and kidney histopathology that CCl₄ caused kidney damage. It was determined that resveratrol application significantly reduced kidney damage caused by CCl₄.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Statement of ethical approval

This study was carried out in accordance with ethical rules with the permission decision of Atatürk University Animal Experiments Local Ethics Committee no 2018/11.

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