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

Volkan Gelen 1,* and Emin Şengül 2

1 Department of Physiology, Veterinary Faculty, Kafkas University, Kars, Turkey.
2 Department of Physiology, Veterinary Faculty, Atatürk University, Erzurum, Turkey.

World Journal of Advanced Research and Reviews, 2021, 10(01), 156–161

Publication history: Received on 10 March 2021; revised on 13 April 2021; accepted on 15 April 2021

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

*Corresponding author: Volkan Gelen
Department of Physiology, Veterinary Faculty, Kafkas University, Kars, Turkey.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.
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. |
| CCl4                | 8                | On the 1st day, a single dose of CCl4 (2 ml / kg) was administered intraperitoneally. |
| Resveratrol         | 8                | Resveratrol was administered orally at a dose of 30 mg / kg / day for 14 days. |
| CCl4 + Resveratrol  | 8                | On day 1, a single dose of CCl4 (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 |
| CCl4                | 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 |
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.

Table 3 Histopathological findings in kidney tissues.

|                | Degeneration of the tubules epithelium | Necrosis of the tubules epithelium | Hyperemia of interstitial vessels |
|----------------|---------------------------------------|-----------------------------------|----------------------------------|
| Control        | -                                     | -                                 | -                                |
| CCl₄           | +++                                   | +                                 | +++                              |
| Resveratrol    | -                                     | -                                 | -                                |
| CCl₄ + Resveratrol | +                                  | -                                 | +                                |

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.
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 trichlormethyl peroxyl (CCl₃O₂) radical. The peroxyl 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 CCl₃ 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 toxicity 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

Acknowledgments

We would like to thank you for your histopathological evaluation, Associate Professor Dr. Serkan Yıldırım.

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.

References

[1] National Academies Press. Carbon tetrachloride toxicity. Environmental Medicine. 1995; 8: 249-266.

[2] Thrall KD, Vucelicke ME, Gies RA, Zanger RC, Weitz KK, Poet TS, Springer DL, Grant DM, Benson JM. Comparative metabolism of carbon tetrachloride in rats, mice, and hamsters using gas uptake and PBPK modeling. Journal of Toxicology and Environmental Health A. 2000; 60: 531-548.
[3] Kumar V, Abbas A, Fausto N. Pathologic basis of disease. 7th Ed. China: Elsevier Saunders. 2005.
[4] Abraham P, Wilferd G, Cathrine SP. Oxidative damage to lipids and proteins of the lungs, testis and kidney of rats during CCl4 intoxication. Clinica Acta. 1999; 289: 177–179.
[5] Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human diseases. Clinical Chemistry. 2006; 52: 601–623.
[6] Sreenivasamurthy B, Banjii D, Banjii O. Investigation on antioxidant and hepatoprotective activity of ethanolic leaf extract of Polygonum glabrum Wild on carbon tetrachloride-induced hepatotoxicity in rats. Spatula DD. 2012; 2: 199-205.
[7] Agbafor KN, Ezeali C, Akbugwo EI, Obiudu IK, Uraku AJ, Ogbsansi ME, Ugwu OPC. Cardioprotective effect of leaf and root extracts of Newbouldia laevis against carbon tetrachloride induced-cardiotoxicity in albino rats. European J Med. Plants. 2015; 9(3): 1-7.
[8] Venkatesan P, Satyan KS, Kumar MS, Pakash A. Protective effect by aqueous extract of Phyllanthus amarus linn; phyllanthin and nirocil against carbontetrachloride-induced liver and brain toxicity. Indian journal of pharmaceutical sciences. 2003; 65(3): 309.
[9] Kim S, Na JY, Song K, Kwon J. In vivo protective effect of phosphatidylcholine on carbon tetrachloride induced nephrotoxicity. Experimental and Toxicologic Pathology. 2016; 68(10): 553-558.
[10] Gelen V, Şengül E, Gedikli S, Atila G, Uslu H, Makav M. The protective effect of rutin and quercetin on 5-FU-induced hepatotoxicity in rats. Asian Pac J Trop Biomed. 2017; 7: 647–53.
[11] Gelen V, Şengül E, Yıldırım S, Atila G. The protective effects of naringin against 5-fluourouracil-induced hepatotoxicity and nephrotoxicity in rats. Iran J Basic Med Sci. 2018; 21: 404–10.
[12] Şengül E, Gelen V, Gedikli S, Özkanlar S, Gür C, Çelebi F, et al. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in rats. Biomed Pharmacother. 2017: 92: 303–7.
[13] Gelen V, Şengül E, Gedikli S, Gür C, Özkanlar S. Therapeutic effect of quercetin on renal function and tissue damage in the obesity induced rats. Biomed Pharmacother. 2017; 89: 524–8.
[14] Sengul E, Gelen V, Yildirim S, Tekin S, Dag Y. The Effects of Selenium in Acrylamide-Induced Nephrotoxicity in Rats: Roles of Oxidative Stress, Inflammation, Apoptosis, and DNA Damage. Biol Trace Elem Res. 2021; 199: 173–84.
[15] Gelen V, Şengül E. Antioxidant, anti-inflammatory and antiapoptotic effects of naringin on cardiac damage induced by cisplatin. Indian J Tradit Knowl. 2020; 19: 459–65.
[16] Karamese M, Guvendi B, Karamese SA, Cinar I, Can S, Erol HS, et al. The protective effects of epigallocatechin gallate on lipopolysa ccharide-induced hepatotoxicity: An in vitro study on Hep3B cells. Iran J Basic Med Sci. 2016; 19: 483–9.
[17] Gedikli S, Gelen V, Sengul E, Ozkanlar S, Gur C, Agirbas O, et al. Therapeutic Effects of Melatonin On Liver And Kidney Damages In Intensive Exercise Model Of Rats. Endocrine, Metab Immune Disord Targets. 2015; 15: 308–14.
[18] Sengul E, Gelen V, Gedikli S. Cardioprotective Activities of Quercetin and Rutin in Sprague Dawley Rats Treated with 5-Fluorouracil. J Anim Plant Sci. 2020; 31: 423–31.
[19] Gelen V, Şengül E, Cinar DA. The effects of rutin and quercetin on ECG parameters in 5-FU-induced cardiotoxicity rat model. World Journal of Advanced Research and Reviews. 2021; 09(03): 253–257.
[20] Karamese M, Aydìn H, Gelen V, Sengul E, Karamese SA. The anti-inflammatory, anti-oxidant and protective effects of probiotic mixture on organ toxicity in a rat model. Future Microbiol. 2020; 15: 401–12.
[21] Sengul E, Gelen V. Protective effects of naringin in indomethacin-induced gastric ulcer in rats, GSC Biological and Pharmaceutical Sciences. 2019; 8(2): 6-14.
[22] Gelen V, Sengul E, Yıldırım S, Celebi F, Cinar A. Effects of rutin on bladder contractility and histopathology in cyclophosphamide-induced hemorrhagic cystitis in rats. Atatürk University J Vet Sci. 2018; 13(3): 337–346.
[23] Gelen V, Gelen SU, Celebi F, Cinar A, Yıldırım S, Eser G. The protective effect of Lactobacillus rhamnosus, Lactobacillus fermentum and lactobacillus brevis against cisplatin-induced hepatic damage in rats. Fresenius Environ. Bull. 2019; 28: 7583–7592.
[24] Uslu GA, Gelen V, Uslu H, Özen H. Effects of cinnamonum cassia extract on oxidative stress, immunoreactivity of iNOS and impaired thoracic aortic reactivity induced by type II diabetes in rats. Brazilian J. Pharm. Sci. 2018; 54: 1-9.

[25] Karamese M, Aydin H, Sengul E, Gelen V, et al. Te Immunostimulatory Efect of Lactic Acid Bacteria in a Rat Model, Iranian journal of immunology: 2016; 13(3): 220–228.

[26] Gedikli S, Ozkanlar S, Gür C, Sengul E, Gelen V. Preventive effects of quercetin on liver damages in highfat diet-induced obesity. Journal of Histology & Histopathology. 2017; 4: 7.

[27] Kara A, Gedikli S, Sengul E, Gelen V, Ozkanlar S. Oxidative stress and autophagy. Free Radicals Dis, InTech. 2016.

[28] Kükürt A, Gelen V, Başer ÖF, Deveci HA, Karapelahvan M. Thiols: Role in Oxidative Stress-Related Disorders. 2021.

[29] Gelen V, Çelebi F. In vitro investigation effects of 4-Hydroxyacetophenone on rat thoracic aorta's vasomotor activity. 2018; 10(3): 319-324.

[30] Clapier VR. Potentiating exercise training with resveratrol. J Physiol. 2012; 590(14): 3215-3216.

[31] Park CE, Kim MJ, Lee JH, et al. Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. Exp Mol Med. 2007; 39(2): 222-229.

[32] Al Dera HS. Protective effect of resveratrol against aluminum chloride induced nephrotoxicity in rats. Saudi medical journal. 2016; 37(4): 369.

[33] Hao Q, Xiao X, Zhen J, Feng J, Song C, Jiang B, Hu Z. Resveratrol attenuates acute kidney injury by inhibiting death receptor mediated apoptotic pathways in a cisplatin induced rat model. Molecular medicine reports. 2016; 14(4): 3683-3689.

[34] Zendeboodi S, Esmaiil A, Movahed A, Fatemikia H, Jamshidi A, Nazari M, Seyedian R. The attenuative effects of oral resveratrol on renal changes induced by vanadium injection in rats. Journal of Renal Injury Prevention. 2019; 8(2).

[35] Adewole SO, Salako AA, Doherty OW, Naicker T. Effect of melatonin on carbon tetrachloride-induced kidney injury in wistar rats. African Journal of Biomedical Research. 2007; 10: 153 – 164.

[36] Çetin E, Çetin N. Protective effect of ghrelin against the oxidative brain and kidney injuries induced by carbon tetrachloride in rats. Ataturk Üniversitesi Veteriner Bilimleri Dergisi. 2011; 6(3): 195-200.

[37] Güven A. ve Yılmaz S. The effect of carbon tetrachloride (CCL4) and ethanol (C2H5OH) on the determination of levels glutathione peroxidase, catalase, glucose-6-phosphate dehydrogenase and lipid peroxidation liver and kidney in goose. Kaşkas Üniversitesi Veteriner Fakültesi Dergisi. 2005; 11(2): 113-117.

[38] Rincon AR, Covarrubias A, Pedraza-Chaverri J, Poo JL. Armendariz-Borunda J. and Panduro, A. 1999. Differential effect of CCl4 on renal function in cirrhotic rats. Experimental and Toxicologic Pathology. 1999; 51: 199-20.

[39] Anderson T. Cecil Essentials of Medicine 4th edition. W.B. Saunders. 1999; 184-186.

[40] Hamed MA, Ali SA, El-Rigal NS. Therapeutic potential of ginger against renal injury induced by carbon tetrachloride in rats. The Scientific World Journal. 2012; 1-12.

[41] El-kholy TA, Hassanen NHM, Abbas HY. Protection of the mushroom (shiihake "Lentinus-edodes") against carbon-tetrachloride-induced renal injury in rats. Life Sciences Journal. 2020; 10(1): 1701-1708.

[42] Makni M, Chtourou Y, Garoùi EM, Boudawara T, Fetoui H. Carbon tetrachloride-induced nephrotoxicity and DNA damage in rats: Protective role of vanillin. Human and Experimental Toxicology. 2012; 31: 844-852.

[43] Manjrekar AP, Jisha V, Bag PP, Adhikary B, Pai MM, Hegde A, Nandini M. Effect of phyllanthus niruri Linn treatment on liver kidney and testes in CCl4 induced hepatotoxic rats. Indian Journal of Experimental Biology. 2008; 46: 514-520.

[44] Morakinyo, AO, Oludare, GO., Anifowose, AA. and Adegoke OA. Protective effects of alpha lipoic acid on carbon tetrachloride-induced liver and kidney damage in rats. British Journal of Pharmacology and Toxicology, 2012;3: 21-28.

[45] Özturk, F., Ucar, M., Ozturk, I.C., Vardi, N. and Batcioglu, K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology, 2003;62: 353-356.