Protective Effect of Ellagic Acid on Paraquat-induced Kidney Hazards in Rats

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Keywords. ellagic acid, intoxication, kidney, paraquat

Introduction. Paraquat is a commonly used highly toxic herbicide. Despite many studies on detoxification of paraquat, an efficient and safe antidote has not been introduced for toxic cases in human being. The aim of this study was to investigate the effect of ellagic acid (EA) on paraquat-induced kidney hazards in rats.
Materials and Methods. Sixty rats were randomly assigned as controls and 5 treatment groups (n = 10 each) receiving EA only, paraquat at doses of 15 mg/kg and 45 mg/kg, and paraquat at the same doses plus EA. Paraquat was intraperitoneally injected and the EA was orally given. Kidney tissues were stained with hematoxylin-eosin for histopathologic investigation.
Results. Pathologic scoring showed that paraquat at the higher dose was associated with higher scores than the in the controls, EA group, and the high-dose paraquat group with EA treatment ($P < .001$ for all comparisons). It was noted that paraquat caused a serious damage in the kidney and the EA treatment significantly reduced the extent of the damage.
Conclusions. This study showed the protective effects of EA against paraquat-induced nephrotoxicity histologically. Ellagic acid provided significant improvement in glomerular and tubular structure.

IJKD 2017;11:23-8
www.ijkd.org

Original Paper

INTRODUCTION

Paraquat has been commonly practiced herbicide to control the weed in agriculture for many years.[1] It is an easily accessible and highly toxic chemical. Accidental or intentional intake of paraquat can be toxic, causing death. Paraquat toxicity can be grouped into slight toxicity (< 20 mg/kg), serious toxicity (20 mg/kg to 40 mg/kg), and fatal toxicity (> 40 mg/kg) causing death in a couple of days from the multiple organ failure.[2,3] Paraquat causes hazards by means of free oxygen radicals such as super oxide, hydrogen peroxide, and hydroxyl in DNA, protein, and lipid metabolisms of human.[1,4] These hazards can be responsible for kidney failure.[3,5]

There is a considerable awareness against antioxidants intake through vegetable diets. Ellagic acid (EA) or 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione is a polyphenol-like compound found in pomegranates, strawberries, and raspberries and has indications as an antioxidant, anticarcinogenic, and anti-inflammatory agent.[6,7] There are some reports that it has antiviral and antibacterial effects, as well.[8]

There is no commonly practiced treatment in paraquat intoxication. Prognosis of the patient depends on the quantity of paraquat intake and the time to take the patient to emergency unit for eliminating and inactivating the paraquat.[3,4]

There are a delicate balance between antioxidant

defense system and free radical production in the body. Oxidative stress results from an imbalance between the production of free oxygen radicals and elimination of these antioxidants.[9] There are defensive molecules what metabolize free oxygen radicals generated in the body, prevent the formation of free oxygen radicals, increase clearance of these radicals, repair, and prevent damage. These antioxidants in aerobic cells can be sourced exogenous and endogenous.[10] Measuring each antioxidant requires long time, expensive and complex techniques. Therefore, measuring of total antioxidant status (TAS) is accepted nowadays.[11]

The aim of this study was to investigate the protective effect of EA on paraquat-induced kidney hazards in terms of TAS.

MATERIALS AND METHODS

Animals

In our study, 60 female albino Wistar rats, each weighing 200 g to 250g, were randomly assigned to 6 groups, each comprising 10 rats. The experiments began a week after the rats' arrival in the laboratory in order to ensure their acclimatization. During the experiment, the rats were kept in standard humidity, 12 hours' daylight and 12 hours' darkness, and room temperature of 23°C, and they were fed standard rat feed.

The experiments were conducted at Konya University Experimental Medicine Research and Application Centre, Konya, Turkey, with the approval of the research ethics committee (number 2012-009).

Treatment

The control group received 85 mg/kg of EA (Sigma E2250, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany); 2 groups received paraquat (1,1'-dimethyl-4,4'-bipyridinedilium ion, 200 g/L; Plaquat®20SL, Platin Chemistry, Istanbul, Turkey) at doses of 15 mg/kg and 45 mg/kg; and 2 other groups received same doses of paraquat plus 85 mg/kg of EA.

Paraquat was administered intraperitoneally. One hour was regarded as the time to take the patient to the emergency unit following the intoxication.[12] Thus, EA was orally given to the rats 1 hour after the intoxication. Ellagic acid was dissolved in corn oil and administered to animals at the single dose of 85 mg/kg. Then, 24 hours after the medication, the animals were sacrificed following anaesthesia with ketamine, 60 mg/kg (Ketalar, Parke Davis, Eczacibasi, Istanbul, Turkey), and xylazine, 5 mg/kg (Rompun, Bayer AG, Leverkusen, Germany). The kidney of each animal was excised for histopathological examinations and kept in the formaldehyde solution. Kidney tissues and blood serums were kept in Eppendorf tubes at -80°C until measuring the parameters of TAS, total oxidant status (TOS), and biochemical examination.

Histopathologic Examination

A histopathologist blinded to the groups evaluated tissue samples. After fixing in 10% neutral buffered formalin solution, kidney tissue samples were embedded in parafin, sectioned at 5 µ and then stained with hematoxylin-eosin for light microscopic examination. The histological scoring system was used to obtain quantitative data. Ten sections representing each group were analyzed, and histopathological changes were graded according to cortical involvement and the severity of the lesions as following points[13]: zero, no pathological changes; 1, slight degenerative changes in tubules and glomeruli with cortical involvement less than 25%; 2, mild degenerative changes with cortical involvement of 25% to 50%; 3, tubular and glomerular necrosis at different foci throughout the cortex with cortical involvement of 50% to 75%; and 4, extensive and marked necrosis throughout the cortex with cortical involvement more than 75%.

Biochemical Analysis

The TAS levels of the sera were determined using an automated measurement method based on bleaching of the characteristic color of a more s 2,2'-azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid] radical cation caused by antioxidants. Renal tissue samples were used for determination of TAS and TOS levels. Renal tissues were weighed and cut into small pieces. Renal tissues were homogenized in 10 volumes of ice-cold phosphate buffer solution (50 mM/L, pH 7.0) using a homogenizer (Ultra-Turrax T8 dispersing homogenizator, Staufen, Germany). The homogenate was centrifuged at 15.000 rpm for 10 minutes at 4°C to obtain supernatant. Supernatant samples were used for the determination of TOS and TAS levels.

Statistical Analyses

Only 4 groups (control, EA, and groups with high-dose paraquat) were included in statistical analysis to see the toxic effects of paraquat and protective effect of EA against this toxic insult. A general linear model analysis of variance was used for data analysis by the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, IL, USA). The mean separation was done using the Fisher exact test. A *P* value less than .05 was considered significant.

RESULTS

There were no significant differences between the control groups and the rats treated with EA regarding the TAS and TOS values of serum and kidney tissues or the pathological scoring of the kidneys (Tables 1 to 3). The TOS values were not different between the rats receiving each dose of paraquat and their counterparts that also received EA (Table 2). The TAS values of serum were also comparable between the rats receiving the two doses of paraquat ($P > .99$) and also between their counterparts that also received EA ($P = .42$). Ellagic acid medication, however, significantly increased the serum TAS values in both groups of rats that received paraquat compared to those without EA (Table 2).

There was no difference between the TOS values of the kidney tissues for the rats with lower dose (15 m/kg) of paraquat with and without EA treatment ($P = 0.98$), whereas the difference was significant with the higher dose (45 mg/kg; $P < .001$). In contrast to TOS values, pairwise comparison of TAS values showed no significant difference between the rats with and without EA at neither of the paraquat doses (Table 3).

Scores obtained by pathologic examination through showed that higher dose of paraquat resulted in higher scores than that in the control and EA groups, as well as the rats with the same dose of paraquat that also received EA ($P < .001$ for all comparisons). In microscopic examination, normal kidney structure was observed in the controls (Figure 1) and the EA group (Figure 2). In the rats with low-dose paraquat, rare tissue necrosis and hemorrhage was recorded (Figure 3). However, significant tissue injury was observed in the rates

Table 1. Pathologic Scoring of Kidney Specimens From Rats Groups

Pathologic Score	Control	Ellagic Acid*	Paraquat, 45 mg/kg†	Paraquat, 45 mg/kg, and Ellagic Acid‡
0	8	7	0	0
1	2	3	0	9
2	0	0	6	1
3	0	0	4	0

*$P = .62$ compared to the controls
†$P < .001$ compared to the controls
‡$P < .001$ compared to the paraquat, 45 mg/kg, group

Table 2. Serum Levels of Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) of Rats Groups

Groups	Control	Ellagic Acid*	Paraquat, 15 mg/kg	Paraquat, 15 mg/kg, and Ellagic Acid†	Paraquat, 45 mg/kg	Paraquat, 45 mg/kg, and Ellagic Acid‡
TOS, mmol H_2O_2 equivalent/g protein	253.80 ± 124.85	287.52 ± 125.39	269.24 ± 91.76	403.08 ± 90.90	294.35 ± 106.33	320.28 ± 151.10
TAS, mmol trolox equivalent/g protein	0.90 ± 0.10	0.86 ± 0.01	0.85 ± 0.06	1.15 ± 0.06	0.84 ± 0.06	1.23 ± 0.12

*$P > .99$ for TOS and $P = .95$ for TAS compared to the controls
†$P = .31$ for TOS and $P < .001$ for TAS compared to the paraquat, 15 mg/kg, group
‡$P > .99$ for TOS and $P < .001$ for TAS compared to the paraquat, 45 mg/kg, group

Table 3. Kidney Tissue Levels of Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) of Rats Groups

Groups	Control	Ellagic Acid*	Paraquat, 15 mg/kg	Paraquat, 15 mg/kg, and Ellagic Acid†	Paraquat, 45 mg/kg	Paraquat, 45 mg/kg, and Ellagic Acid‡
TOS, mmol H_2O_2 equivalent/g protein	0.37 ± 0.02	0.38 ± 0.04	0.23 ± 0.01	0.24 ± 0.03	0.15 ± 0.03	0.28 ± 0.04
TAS, mmol trolox equivalent/g protein	21.98 ± 4.00	21.85 ± 3.95	27.91 ± 3.28	29.46 ± 3.24	29.22 ± 4.20	30.95 ± 3.72

*$P = .98$ for TOS and $P > .99$ for TAS compared to the controls
†$P = .98$ for TOS and $P = .98$ for TAS compared to the paraquat, 15 mg/kg, group
‡$P < .001$ for TOS and $P = .97$ for TAS compared to the paraquat, 45 mg/kg, group

that received the higher dose with tubular and glomerular necrosis (Figure 4). Administration of EA improved tissue structure in all paraquat groups. Glomeruli and tubules were better preserved with low-dose paraquat and EA (Figure 5) and high-dose paraquat and EA groups (Figure 6).

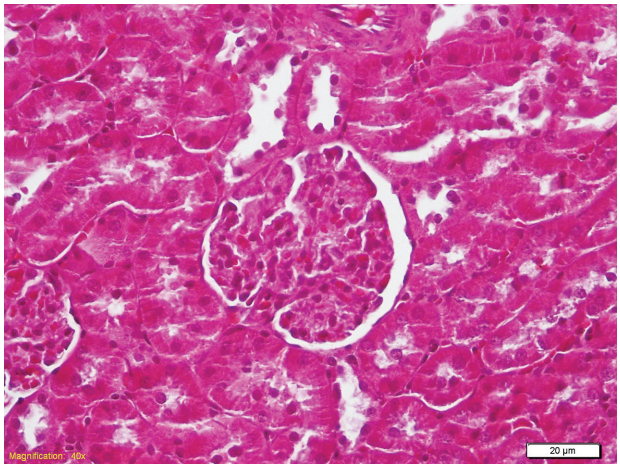

Figure 1. Normal kidney histology in control group.

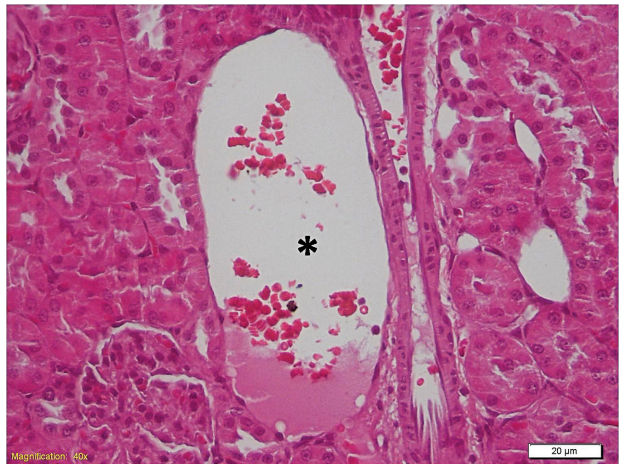

Figure 4. Tissue necrosis in the rats with 45 mg/kg of paraquat.

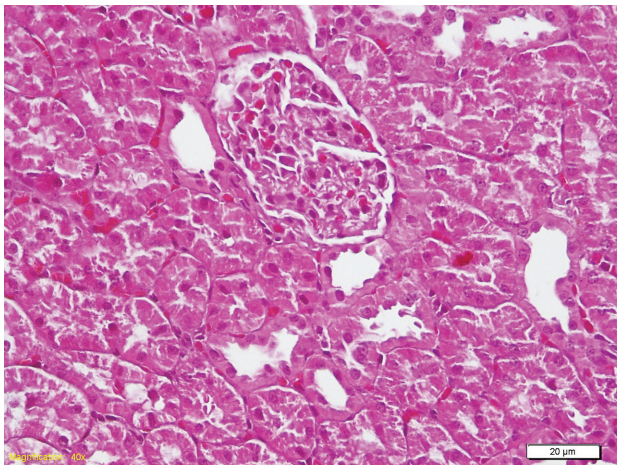

Figure 2. Normal glomerular structure in ellagic acid group.

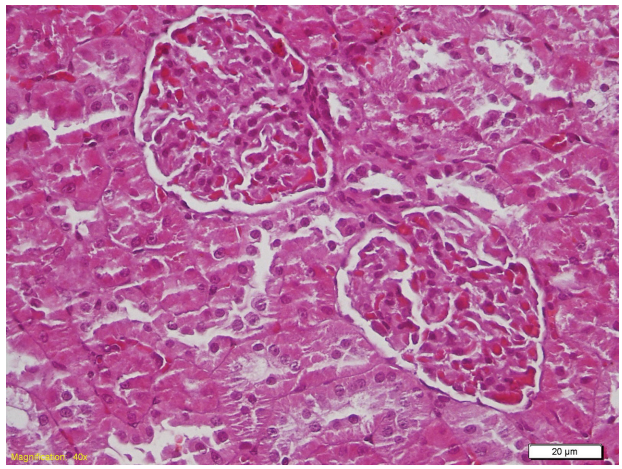

Figure 5. Improved tubular and glomerular histology in the rats with 15 mg/kg of paraquat and ellagic acid.

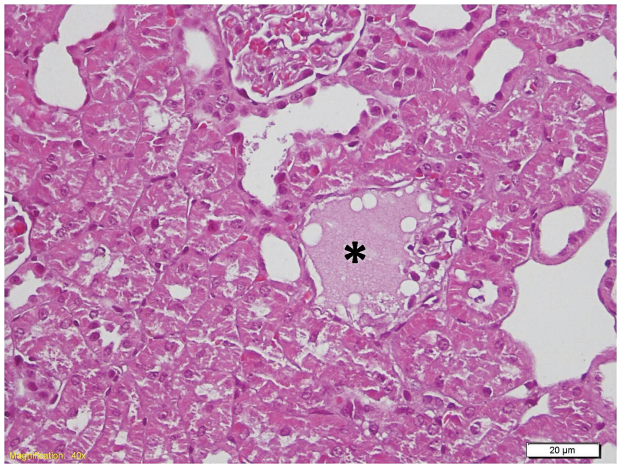

Figure 3. Glomerular necrosis (arrow) in the rats with 15 mg/kg of paraquat.

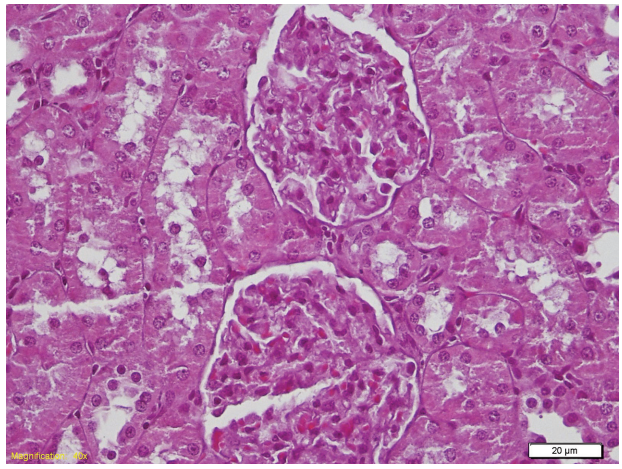

Figure 6. Preserved tubules and a glomeruli in the rats with 45 mg/kg of paraquat and ellagic acid.

DISCUSSION

Many reports showed that paraquat causes the kidney hazards.[14-17] Researchers reported that paraquat has a tendency to accumulate in the lung and kidney.[18,19] Similarly, Afzali and Gholyaf pointed out the fatal kidney hazard-induced by paraquat.[14] It is well known that paraquat-induced toxicity appears trough-released free oxygen radicals.[19-21] Thus, protective effects of many antioxidants were investigated.[15-17,20] Unfortunately, an efficient medication has not been reported, yet.

Uzar and coworkers demonstrated that EA did not affect the TOS levels whereas TAS levels significantly increased.[22] In our current study, we found an increase in TOS level in kidney tissues induced by high dose of paraquat and a considerable decrease in TOS level upon EA treatment. Another significant outcome of this study is the protective effect of EA treatment through increasing the TAS level of serum comparing the paraquat groups only. The level of TOS decreases while the level of TAS increases by treating of EA. These results show the protective effect of EA on paraquat-induced kidney hazards.

Similar to the biochemical results, pathologic scoring through hematoxylin-eosin staining strengthened the recovery effect of EA in paraquat intoxication cases. Paraquat toxicity was previously shown by many researchers in terms of histopathology.[15-17] Similar to our results, hemorrhage and necrosis in both glomeruli and tubules and degenerative changes in proximal tubules were shown in the previous studies. The protective effects of EA against nephrotoxicity induced by different agents were shown previously.[23]

CONCLUSIONS

Our study is the first report showing the protective effects of EA against paraquat-induced nephrotoxicity histologically. Microscopic photos also clearly provided conservative effects of EA in glomerular and tubular structures. Thus, EA can be suggested a promising medication for paraquat intoxication cases. This animal experiment, however, is based on a limited sample. Further studies are yet to be carried out to confirm our results.

CONFLICT OF INTEREST

None declared.

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Received March 2016
Revised July 2016
Accepted August 2016