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Original Research / Özgün Araştırma

Preventive effects of montelukast against acetaminophen-induced nephrotoxicity: An experimental study

Asetaminofenin indüklediği nefrotoksisiteye karşı montelukastın önleyici etkisi: Deneysel bir çalışma

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Özet

Amaç: Bir astım ilacı olan Montelukast, dokular üzerinde anti-inflamatuar etkiye sahiptir. Sıçan modellerinde montelukastın (MK) asetaminofen (APAP) ile indüklenen böbrek hasarı üzerindeki tedavi edici etkisini araştırmayı amaçladık.

Gereç ve Yöntemler: Yirmi dört sıçan rastgele her biri altı hayvandan oluşan dört gruba ayrıldı. APAP intraperitoneal olarak 1000 mg/kg/gün tek doz olarak uygulandı. Tedavi grubundaki MK dozu 10 mg/kg olup APAP sonrası oral gavaj ile uygulandı. Diğer gruplar APAP + Salin grubu ve kontrol grubuydu. Nefrotoksisiteyi belirlemek için doku malondialdehit (MDA), indirgenmiş glutatyon (GSH) ve Nitrik oksit (NO) seviyelerini ölçtük**.**

Bulgular: APAP grubundaki serum üre ve kreatinin seviyeleri kontrol ve APAP + MK gruplarındaki sıçanlara göre anlamlı derecede yüksek ölçüldü. APAP ile tedavi edilen sıçanlarda GSH seviyesi önemli ölçüde azaldı. Bununla birlikte, MK verilmesi tedavi grubunda GSH seviyesini önemli ölçüde arttırdı. Tek başına APAP ile tedavi edilen sıçanlardaki doku MDA seviyeleri kontrol grubu ve APAP + MK grubuna kıyasla önemli ölçüde yüksekti. NO seviyesi, APAP ile tedavi edilen grupta yükselmiş olarak ölçüldü. Bununla birlikte, MK tedavi grubundaki NO seviyeleri, APAP ile tedavi edilen gruptan önemli ölçüde düşüktü. Ayrıca, tek başına APAP grubuna kıyasla MK tedavi grubunda bazı morfolojik geri kazanımlar gözlendi.

Sonuç: MK'nin APAP kaynaklı renal toksisite ve disfonksiyonu üzerinde faydalı etkileri vardır. Ancak uygun kullanımı ve etkileri göstermek için klinik çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: asetaminofen, böbrek, montelukast, nefrotoksisite, oksidatif stres

Abstract

Objective: Montelukast, an asthma drug, has an anti-inflammatory effect on tissues. We aimed to investigate therapeutic effect of montelukast (MK) on acetaminophen (APAP) - induced renal damage in rat models.

Material and Methods: Twenty-four rats were randomly divided into four groups of six animals each. APAP was administered intraperitoneally as a single dose of 1000 mg/kg/day. In the treatment group, MK dose was 10 mg/kg and administered by oral gavage after APAP. The other groups were APAP + Saline group and the control group. We measured tissue malondialdehyde (MDA), reduced glutathione (GSH), and Nitric oxide (NO) levels to determine the nephrotoxicity.

Results: Serum Blood Urea Nitrogen (BUN) and creatinine levels were measured significantly higher in APAP group than rats in the control and APAP + MK groups. The level of GSH was significantly diminished in APAP-treated rats. However, the administration of MK significantly increased the level of GSH in the MK treatment group. Tissue MDA levels in rats treated with APAP alone were significantly higher compared to the control group and APAP + MK group. The level of NO was measured as elevated in APAP treated group. However, NO levels in the MK treatment group were significantly lower than APAP treated group. Furthermore, some morphological recoveries were observed in the MK treatment group compared to APAP alone group.

Conclusion: MK has beneficial effects on APAP-induced renal toxicity and dysfunction. However, clinical studies are needed to demonstrate appropriate use and effects.

Keywords: acetaminophen, kidney, montelukast, nephrotoxicity, oxidative stress

The study was approved by Istanbul University Animal Experiments Local Ethics Committee in 16/01/2015. Approval no is 20.
All research was performed in accordance with relevant guidelines/regulations, and informed consent was obtained from all participants.

INTRODUCTION

The kidney is a crucial organ and it has important roles in controlling the volume of body fluids, blood osmolality, acid-base balance, various electrolyte concentrations and removing toxins. In addition to environmental variables, some drugs also may affect these functions (1)no single animal model would be completely satisfactory because the etiology and development of renal failure are diverse. During recent years injection of uranyl nitrate has been found to be the most effective and easiest method to produce renal dysfunction in laboratory animals. Changes over the last 10 years in government regulations on the production and use of radioactive substances make the compound less available. There is, therefore, a need for a more accessible compound comparable to uranyl nitrate as an inducer of renal failure. The present study compares the effects of another known nephrotoxin, cisplatin, with uranyl nitrate in the rat. Cisplatin was chosen because of its ability to produce kidney damage and its identical site and mechanism of action on the kidneys as uranyl nitrate. In the present study, rats were given different i. v. doses of uranyl nitrate or cisplatin dissolved in 0.9% of saline solution. The effects of nephrotoxins were evaluated on the basis of changes in body weight, creatinine and blood urea nitrogen (BUN. N-acetyl-p-aminophenol (APAP) molecule is also called paracetamol or acetaminophen. Paracetamol has analgesic and antipyretic properties at therapeutic dosage (2). APAP is primarily metabolized with sulfuration and glucuronidation reactions in the liver. The final metabolites formed after these reactions are excreted by the kidneys. N-acetyl-p-benzoquinone imine (NAPQI), an extremely reactive intermediate, occurs after metabolization of APAP with the microsomal P-450 enzyme system (3). Overdose APAP causes cellular GSH depletion via excessive reaction between NAPQI and GSH. In this way, lipid peroxidation begins as a result of NAPQI binding to cellular proteins. These reactions lead to hepatic and renal damage (4). Although APAP toxicity has been well-defined in the liver, its effect on the kidney is not well known. The mechanisms of renal toxicity may be explained with activation of prostaglandin synthase and N-deacetylase enzymes in Cytochrome P450 (CYP450) pathway according to human and animal studies (3,5)that is, bioactivation, detoxication, chemoprevention, and chemoprotection. In addition, some pharmacological and clinical aspects are discussed briefly. A general introduction is presented on the biokinetics, biotransformation, and structural modification of paracetamol. Phase II biotransformation in relation to marked species differences and interorgan transport of metabolites are described in detail, as are bioactivation by cytochrome P450 and peroxidases, two important phase I enzyme families. Hepatotoxicity is described in depth, as it is the most frequent clinical observation after paracetamol-intoxication. In this context, covalent protein binding and oxidative stress are two important initial (Stage I).

Montelukast (MK), is an asthma drug, a selective reversible cysteinyl leukotriene-1 (CysLT1) receptor antagonist. It shows a reducing effect on airway eosinophilic inflammation in asthma (6). Wallace et al. showed that ethanol-induced gastric mucosal damages and colitis were improved by CysLT1 receptor antagonists (7). MK shows these effects with anti-inflammatory and antioxidant features. It has been stated that APAP tissue damage is closely related to increased Reactive oxygen species (ROS). Also, some studies showed that APAP-induced nephrotoxicity occurs as a result of lipid peroxidation (3).

Nitric oxide (NO) has an intercellular messenger role. It makes crucial missions like vasorelaxation and inflammation in the cell. Although important roles such as removing of pathogens and tumour cells; excessive NO converts to ROS by oxidation, causing to deterioration of cell signalling pathways and out of control inflammation (8)urinary biochemistry and urinary levels of oxalate, NO metabolites (nitrate and nitrite. One of the most important indicators of lipid peroxidation is Malondialdehyde (MDA). Glutathione (GSH) is the main intracellular antioxidant molecule. It has many crucial biological functions such as the reduction of some biologically active metabolites and the protection of the thiol part of proteins (9). Because of these features, MDA, GSH, and NO levels were used to determination of inflammatory and oxidative injury in many experimental studies.

We have shown a preventive effect of MK on gentamicin-induced nephrotoxicity in our previous experimental study (10). We consider that MK may show similar protective effect against APAP nephrotoxicity. Therefore, we examined the protective effect of MK on APAP-induced renal injury in experimental models.

MATERIAL AND METHODS

Animals and Drug Administration

Twenty-four male Wistar-Albino mouses (345-350 g) were included in the experiment. The rats were acclimated for one week before the experiment with 12 hours light and dark cycle, 20-24oC temperature and appropriate food and water. Istanbul University Animal Experiments Local Ethics Committee provided the ethical approval for the experimental study. (Date:16/01/2015. Approval no is 20) After one-week isolation period, 4 groups were randomly created as each one consists 6 rats;

Control group, only 0.9 % saline

APAP group

APAP + 0.9 % saline

APAP + MK treatment group

Normal saline was used to dissolve the APAP and it was injected intraperitoneally (i.p.) at a single dose of 1,000 mg/kg/day (3). The administration dose of APAP was based on prior studies (11). The MK dose was adjusted to 10 mg/kg by dilution with saline solution and applied by oral gavage 20 minutes after APAP injection (3).

A single 1 ml isotonic saline was administered to control group by intraperitoneally. A single dose 1000 mg/kg APAP was given to APAP group. The treatment group received MK after APAP. The other group received APAP + 0.9 % saline.

All surgical operations were done in general anaesthesia stimulated by intraperitoneal ketamine hydrochloride twenty-four hours after APAP administration. Blood samples from rats were used to evaluate biochemical parameters. Then, whole rats were sacrificed.

Kidneys were found following an abdominal mid incision and rapidly removed. Kidneys were irrigated twice with cold saline solution following separated from other tissues. One of the kidneys was stored at -80°C to evaluate tissue GSH, NO and MDA levels. Formol solution was used to preserve the remaining kidney for subsequent histopathological evaluation. Blood analysis were used to investigate urea–creatinine levels.

Histopathological Evaluation

Histopathological assessments were done according to 6 μm sections of kidney samples. Then, haematoxylin–eosin (H&E) stain was used to assess the sections under light microscopic. The criteria defined by Allen et al. were used during the semi-quantitative evaluation of the tissues in terms of scoring the severity (12). All sections were analysed in terms of tubular vacuolization, parietal hyperplasia and necrosis. While obtaining an average score, the least 50 glomerulus and proximal tubules were examined for each slide. The percentage of tubular damage was categorised as 4 group from 0 to 3. Grade 0 (none) means no changing on tubules. Grade 1 (mild) denotes tubular damages <25 %. Grade 2 (moderate) expresses tubular damage between 25-50 %. Grade 3 (severe) indicates tubular injury >50 %.

Kidney fibrosis was evaluated following Masson's trichrome stain. Specimens were categorised as 4 groups after staining. Absence of fibrosis was shown as (-). Fibrosis in <25 % of tissue (mild) was shown as (+). Fibrosis in between 25-50 % of tissue (moderate) was shown as (++). Fibrosis in >50 % of tissue (serious) was shown as (+++).

Biochemical Examinations

MDA is the final product of the lipid peroxidation. It is one of the indicators of the oxidative stress intensity. A buffer solution containing 1.5 % potassium chloride in teflon-glass was used to the homogenization of frozen kidney slides while obtaining 1:10 (w/v) whole homogenate. Since it is the reactive molecule of thiobarbituric acid, it was measured by thiobarbituric acid determination in spectrophotometer and defined as nmol/mg.

The method of Moron et al. which is based on the colour-changing at 412 nm, was used to measure reduced GSH (13). Described method of Lowry et al. was used to measure protein concentrations in tissues (14). GSH levels were expressed as nmol/mg wet tissue.

Griess test was used to assess the nitrite levels after incubating with nitrate. The absorbance was qualified at 545 nm after 30 minutes incubation.

Statistical Analysis

The values were presented as mean ± standard deviation (SD). Statistical analysis of the histopathological evaluations of the groups was performed by the chi-square test. To analyse biochemical data, ANOVA (one-way analysis of variance) test was used. The definition of significance between the two groups was done by Dunnett's multiple comparison test. Statistically significant value is $p<0.05$.

RESULTS

The degree of the tubular necrosis and biochemical results are shown in Tables 1, 2 and 3. Death or any extraordinary signs were not detected in groups. The results of the APAP and APAP + 0.9 % saline group were similar in terms of biochemical and histopathological parameters.

Tissue MDA levels in APAP alone group were significantly higher compared to control and APAP + MK groups. ($p<0.001$) This increase was prevented by MK treatment. The GSH levels were found statistically significantly lower in APAP group. However, MK treatment significantly increased the GSH levels in APAP + MK group. NO levels in APAP group were higher. However, NO levels were found significantly lower in the MK treatment group.

Serum urea and creatinine levels in APAP alone group were higher compared to both of APAP + MK and control groups. ($p< 0.001$) In the case of MK being added to APAP treatment, serum urea and creatinine levels improved.

According to histopathologic examination, there was no pathologic changing in the control group. According to light microscopic evaluation, regular morphology consisting normal glomeruli and tubules were seen in the control group (Figure 1). APAP-treated rats had obvious morphological changes such as tubuloepithelial deterioration and necrosis (grade of tubular necrosis:2–4) (Figure 2). Cortical interstitial congestion and cellular debris were determined only in the tissues of APAP-treated rats. Moderate epithelial vacuolization and tubular degeneration were found in the proximal tubules of rats treated with APAP + MK. MK treatment group had better tubular morphology and lower cellular desquamation compared to APAP group (Grade of tubular necrosis: 0-2) (Figure 3). Severe tubular vacuolization, degeneration and necrosis were seen in the tissues of APAP + 0.9 % saline group (Figure 4). According to Masson trichrome staining, statistically significant difference was not determined between groups in terms of kidney fibrosis (Table 3 and

Table1. NO, MDA, GSH levels and Kidney functions in groups

Parameters	Control	APAP	p value[a]	APAP+MK	p value[b]	APAP+ Ve
Urea (mg/dL)	33 ± 7.8	107 ± 13.9[a]	**<0.001**	42.8 ± 6.9[b]	**<0.001**	105 ± 13.4
Creatinine (mg/dL)	0.42 ± 0.1	2.08 ± 0.4[a]	**<0.001**	0.98 ± 0.09[b]	**0.013**	1.97 ± 0.4
NO (nmol/mg protein)	13.9 ± 5.1	88.3 ± 41.6[a]	**<0.001**	14.5 ± 5.7[b]	**<0.001**	83.1 ± 28.3
MDA (nmol/mg protein)	41.8 ± 10.2	83.6 ± 24.9[a]	**<0.001**	37.3 ± 19.6[b]	**<0.001**	82.4 ± 50.1
GSH (umol/mg protein)	39.1 ± 20.4	15.2 ± 3.3[a]	**<0.001**	37.6 ± 9.3[b]	**<0.001**	14.1 ± 1.7

Values are expressed as mean ± SD for six rats in each group.

[a] *Significantly different from control.*

[b] *Significantly different from APAP group ($p < 0.05$).* ***NO:*** *nitric oxide,*

MDA: *Malondialdehyde,* ***GSH:*** *glutathione,* ***APAP:*** *N-acetyl-p-aminophenol,* ***MK;*** *Montelukast,* ***Ve:*** *Vehicle*

Table 2. Semiquantitative analysis of tubular necrosis, tubular vacuolization, parietal cell hyperplasia in groups

		Tubular necrosis				Tubular vacuolization				Parietal cell hyperplasia			
	n	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Control	6	6	0	0	0	5	1	0	0	6	0	0	0
APAP[a]	6	0	1	3	2	0	0	4	2	0	1	5	0
APAP+MK[b]	6	5	1	0	0	2	4	0	0	4	2	0	0
APAP+Ve	6	0	0	2	4	0	0	0	6	0	0	5	1

Score 0: no degeneration, ***1:*** mild degeneration, ***2:*** moderate degeneration, and ***3:*** severe degeneration
a Statistically significant difference from the control group, ***b*** Statistically significant difference from the APAP treated group and $p<0.05$. ***APAP:*** N-acetyl-p-aminophenol, ***MK:*** Montelucast, ***Ve:*** Vehicle

Table 3. Analysis of kidney fibrosis in groups

	n	(-)	(+)	(++)	(+++)
Control	6	6	0	0	0
APAP	6	3	1	2	0
APAP+MK	6	2	4	0	0
APAP+Ve	6	2	2	2	0

Score (-): no fibrosis, ***(+):*** mild fibrosis, ***(++):*** moderate fibrosis, and ***(+++):*** serious fibrosis.
No statistical difference between groups ($p>0.05$). ***APAP:*** N-acetyl-p-aminophenol, ***MK:*** Montelucast, ***Ve:*** Vehicle

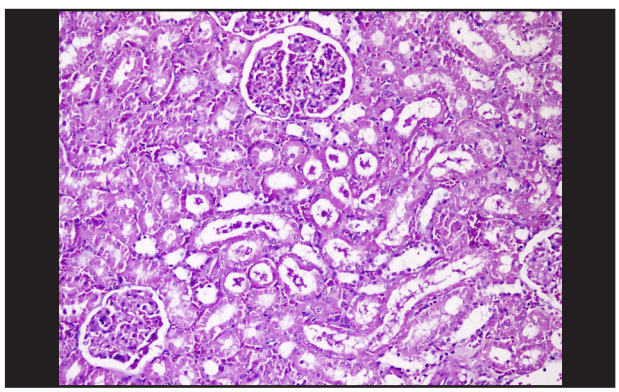

Figure 1. Normal tubules and glomeruli in kidney cortex of control group (H&E x 200)

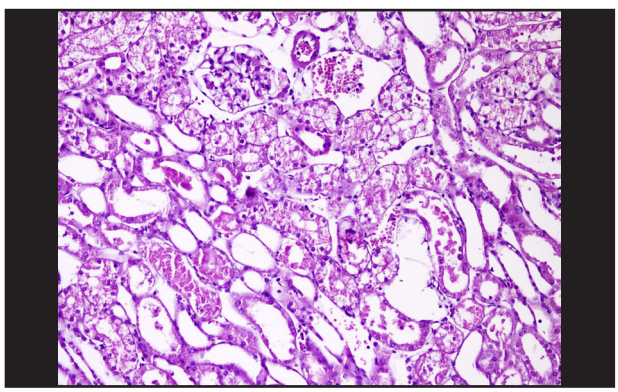

Figure 2. Severe tubular necrosis, tubular degeneration, and epithelial vacuolization in the proximal tubules of APAP group (H&E x 200).

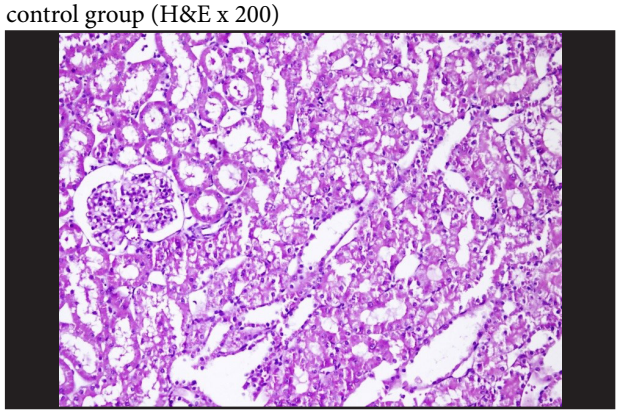

Figure 3. Mild tubular necrosis, tubular degeneration, and mild-moderate epithelial vacuolization in the proximal tubules of APAP + MK group (H&E x 200).

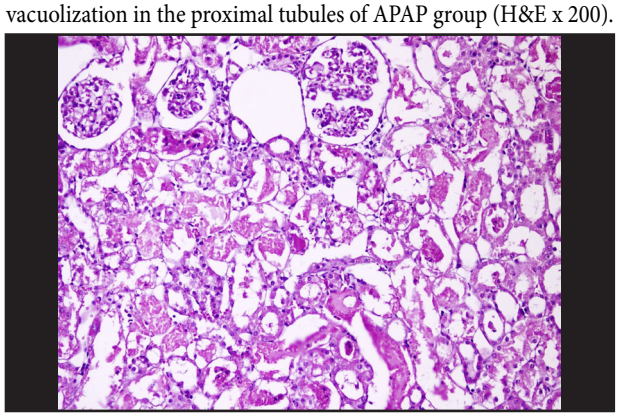

Figure 4. Severe tubular necrosis, tubular degeneration, and epithelial vacuolization in the proximal tubules of APAP+ Ve group (H&E x 200).

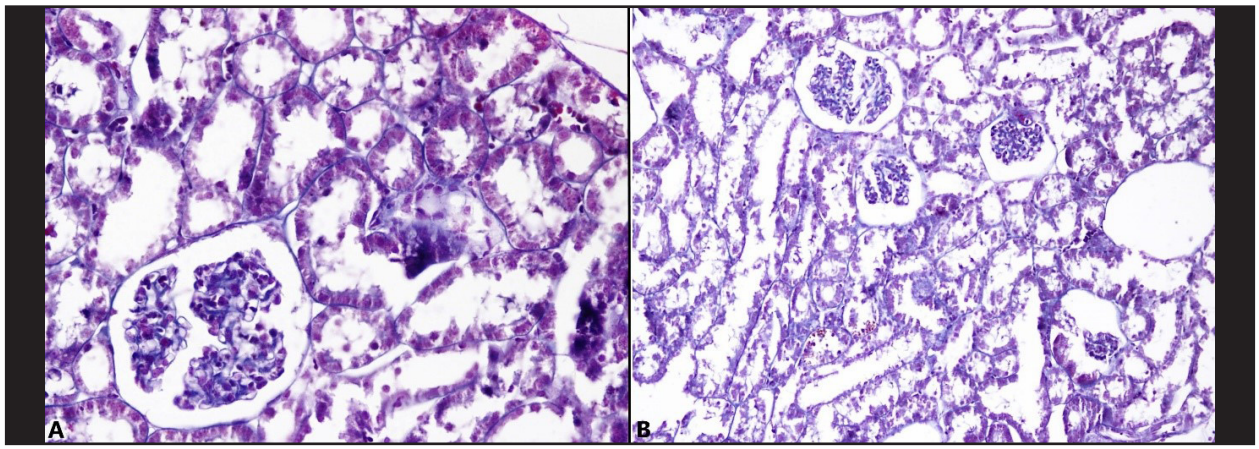

Figure 5/A. Mild fibrosis in interstitium of APAP group (Masson's trichrome x 400).

5/B. Moderate fibrosis in interstitium of APAP+MK group (Masson's trichrome x 400).

Figure 5).

DISCUSSION

Nephrotoxicity is also a crucial problem during APAP intoxication in addition to hepatotoxicity. Acute kidney injury is seen in 1-2 % of APAP overdose cases (15). Proximal tubules have been shown as a target for APAP toxicity due to absorption and secretion activities (16)acetaminophen can be toxic to the kidneys in patients who are glutathione depleted (chronic alcohol ingestion, starvation, or fasting. As APAP may induce acute kidney damage, we consider that the research of therapy of APAP induced nephrotoxicity is a notable topic. The purpose of the present study is to explore the preventive effect of MK on APAP nephrotoxicity in rats.

Although some studies showed that kidney damage begins 3-5 days after chronic use of APAP, the damage was occurred 24 hours after high dose APAP administration in some experimental studies (11). In accordance with this, we detected acute nephrotoxicity after a single dose of APAP in biochemical and histopathological findings.

Serum Blood Urea Nitrogen (BUN) and creatinine levels give us precious information about kidney functions. Increased BUN levels usually indicate the glomerular damage. Creatinine, a metabolite of creatine, is eliminated from the body via urine. Similarly, elevated creatinine levels indicate disturbed kidney functions. Cases requiring haemodialysis after APAP overdose have been reported in the literature (17). In present study, creatinine and BUN levels were found to be high in the APAP group. Our results were also in accordance with previous studies which reported enhanced urea and creatinine levels following APAP induced kidney injury (11,18)all the rats were sacrificed with a high dose of ketamine. Urea and creatinine levels were measured in the blood, and the levels of malondialdehyde (MDA. The levels were measured as lower in the MK treatment group.

Excessive oxidative activity causes cellular damage and lipid peroxidation. The last product of lipid peroxidation and one of the indicators of oxidative damage is MDA (19)as well as both nuclear and mitochondrial DNA. Melatonin achieves this widespread protection by means of its ubiquitous actions as a direct free radical scavenger and an indirect antioxidant. Thus, melatonin directly scavenges a variety of free radicals and reactive species including the hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite anion, and peroxynitrous acid. Furthermore, melatonin stimulates a number of antioxidative enzymes including superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase. Additionally, melatonin experimentally enhances intracellular glutathione (another important antioxidant. Some studies demonstrated that MK reduces the myeloperoxidase and MDA levels with

its antioxidant effect in rat models (20). Similarly, we found that MDA levels in the MK treatment group were lower than the APAP group. The primer function of GSH is the protection of the cellular components against oxidative damage. It provides the protection by supplying protein and lipid integrity in normal cellular metabolism (21). We measured a reduction in GSH levels in parallel with the increased MDA levels in the APAP group. Nitric oxide is one of the potent radicals and has been shown to have an important role in the mechanism of APAP-induced kidney injury (22). This study showed that renal NO levels in the APAP group were higher than the control and MK treatment groups.

MK, which is a selective reversible CysLT1 receptor antagonist, has been shown to regulate the oxidant-antioxidant balance and pro-inflammatory mediators in previous studies (23,24)the protective effect of montelukast (ML. We can say that MK might have a protective effect against to APAP induced kidney damage considering beneficial impacts in the levels of GSH, MDA and NO, which are indicators of oxidative damage. Furthermore, some studies indicated that MK showed reducing ischemia/reperfusion injury in various organs with angiogenic properties (25,26 ± 1, 23 ± 2, 24 ± 2 and 24 ± 4% at 24, 28, 36, 48 and 72 h after remote IPC, respectively ($P < 0.05$; $n = 826$) sham group (operation without clamping. This feature also may be related to the therapeutic effect of MK on APAP induced nephrotoxicity.

CYP-450 enzyme systems are predominantly localised in the proximal tubules of the kidney (27). Therefore, nephrotoxicity that occurs during CYP-450-mediated bioactivations is definitely localized in the proximal tubules (28). In addition, proximal tubules have been shown as a target for APAP in different studies (22,29).

This study demonstrates clear evidence of nephrotoxicity after overdose APAP administration based on histopathological findings. The most prominent histopathological change is acute tubular necrosis. The renal histological changes occurring after overdose APAP administration are also consistent with the previous study (30).

There are some limitations in our study. Other oxidative stress parameters such as catalase, superoxide dismutase and myeloperoxidase levels were not measured.

CONCLUSION

MK, a CysLT1 receptor antagonist, has protective effects on APAP-induced nephrotoxicity. It shows this effect with its free radical scavenger agent feature. Further clinical studies are needed to indicate the definite mechanism and effect of MK on APAP-induced nephrotoxicity.

Conflict of Interest

The authors declare to have no conflicts of interest.

Financial Disclosure

The authors declared that this study has received no financial support.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The study was approved by Istanbul University Animal Experiments Local Ethics Committee (Approval no: 20, Date: 16/01/2015) and written informed consent was received from all participants. The study protocol conformed to the ethical guidelines of the Helsinki Declaration.

Author Contributions

Conception and design; Ötünçtemur A, Çakır SS, Can O, Data acquisition; Can O, Eraldemir C, Çekmen M, Data analysis and interpretation; Eraldemir C, Çekmen M, Vural Ç, Can O, Drafting the manuscript; Can O, Çakır SS, Critical revision of the manuscript for scientific and factual content; Ötünçtemur A, Çakır SS, Statistical analysis; Çakır SS, Can O, Supervision; Ötünçtemur A, Eraldemir C, Çakır SS.

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