Beneficial effects montelukast, cysteinyl–leukotriene receptor antagonist, on renal damage after unilateral ureteral obstruction in rats

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ABSTRACT

Introductıon: Ureteral obstruction is a common pathology and caused kidney fibrosis and dysfunction at late period. In this present, we investigated the antifibrotic and antiinflammatory effects of montelukast which is cysteinyl leukotriene receptor antagonist, on kidney damage after unilateral ureteral obstruction(UUO) in rats.

Materıals and Methods: 32 rats divided four groups. Group 1 was control, group 2 was sham, group 3 was rats with UUO and group 4 was rats with UUO which were given montelukast sodium (oral 10 mg/kg/day). After 14 days, rats were killed and their kidneys were taken and blood analysis was performed. Tubular necrosis, mononuclear cell infiltration and interstitial fibrosis scoring were determined histopathologically in a part of kidneys; nitric oxide(NO), malondialdehyde(MDA) and reduced glutathione(GSH) levels were determined in the other part of kidneys. Urea-creatinine levels were investigated at blood analysis. Statistical analyses were made by the Chi--square test and one-way analysis of variance (ANOVA).

Results: There was no difference significantly for urea-creatinine levels between groups. Pathologically, there was serious tubular necrosis and fibrosis in group 3 and there was significantly decreasing for tubular necrosis and fibrosis in group 4(p<0.005). Also, there was significantly increasing for NO and MDA levels; decreasing for GSH levels in group 3 compared the other groups(p<0.005).

Conclusıon: We can say that montelukast prevent kidney damage with antioxidant effect, independently of NO.

INTRODUCTION

Chronic kidney diseases, which lead to end--stage kidney failure, are associated with changes in kidney structure and fibrosis regardless of the underlying cause. Urinary tract obstruction is characterized by tubular atrophy or dilation, tubular cell death by apoptosis and necrosis, interstitial

leukocyte infiltration, and increased interstitial matrix deposition (1). The acute phase of obstructed kidney in unilateral ureteral obstruction (UUO) is characterized by dramatic changes in glomerular filtration rate, renal blood flow, and interstitial edema (2, 3).

Such an obstruction might be observed after benign prostatic hyperplasia; renal, ureteral, or bladder calculi; urethral stricture; and neoplasm of the bladder, prostate, or urethra (1). The hydrostatic pressure, which is the result of the blockage, initiates renal injuries. The injuries are characterized by tubular dilatation or atrophy, inflammatory infiltration of leucocytes, fibroblast activation, proliferation, increase in matrix proteins, and progressive interstitial fibrosis with the loss of renal parenchyma. Unilateral ureteral obstruction (UUO) is an experimental rat model of renal injury that imitates the process of obstructive nephropathy in an accelerated manner(1).

Reactive oxygen species (ROS) are a recently recognized mechanism in the pathogenesis of UUO in experimental studies. Increased lipid peroxidation has been reported in renal cortexes of UUO animals. It has been shown that oxidative stress in UUO contributes to the development of tubulointerstitial lesions and renal fibrosis. Various factors with complex cellular and molecular interactions have also been proposed as possible causes that lead to tubulointerstitial lesions and renal fibrosis (4-7).

Nitric oxide (NO) acts as an intercellular messenger and regulates cellular functions such as vasorelaxation and inflammation. NO has an important role in the elimination of pathogens and tumor cells; however, overproduced NO is oxidized to ROS, resulting in the disruption of cell signaling and uncontrolled systemic inflammation (8, 9). Malondialdehyde (MDA) is one of the important markers of lipid peroxidation (10). Excessive MDA produced as a result of tissue injury and DNA damage could combine with free amino groups of proteins, resulting in the formation of MDA-modified protein adducts. Glutathione (GSH) is the major intracellular antioxidant with multiple biological functions, including the maintenance of the thiol moieties of proteins and the reduced from of many other biologically active molecules (11).

Leukotrienes, the products generated by the 5-lipoxygenase pathway are particularly important in inflammation; indeed, leukotrienes increase microvascular permeability and are potent chemotactic agents (12). Moreover, inhibition of 5-lipoxygenase indirectly reduces the expression of TNF-alpha (a cytokine that plays a key role in inflammation), and there are a number of studies demonstrating the role of leukotrienes as mediators of the gastric damage induced by ethanol and some other noxious substances(13, 14). Cysteinyl leukotrienes(CysLT), leukotrienes C4, D4, and E4 (LTC4, LTD4, LTE4) are secreted mainly by eosinophils, mast cells, monocytes and macrophages, and they exert a variety of actions which emphasize their importance as pathogenic elements in inflammatory states(15, 16).

Montelukast (MK-0476), a selective reversible cys-leukotriene-1 receptor (LTD4 receptor) antagonist is used in the treatment of asthma and is reported to reduce airway eosinophilic inflammation in this disease (17-19). CysLT1 receptor antagonists or biosynthesis inhibitors have been reported to ameliorate ethanol-induced gastric mucosal damage and experimental colitis (13, 20, 21).

Based on these findings, we investigated the antifibrotic and antiinflammatory effects of montelukast on kidney damage after UUO in rats by measuring MDA, NO and GSH levels and the myeloperoxidase activity.

MATERIAL AND METHODS

ANIMALS

Male Wistar Albino rats, weighing 200 to 250 g and 6 to 7 weeks old, were housed in clean plastic cages in a temperature and humidity controlled facility under a constant 12-hour light/12-hour dark photoperiods with free access to food and water. The Institutional Animal Care and Use Committee approved the use of animals and the experimental protocol, and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council.

TREATMENT AND EXPERIMENTAL PROTOCOLS

One week after acclimatization, UUO was induced. Briefly after induction of general anesthesia by intraperitoneal injection of thiopental (100 mg/kg), the abdominal cavity was exposed via midline incision and the left ureter was ligated at 2 points with 3-0 silk. The sham-operated rats had their ureters manipulated but not ligated. All rats were given amikacin sulfate (6 mg/kg, intramuscularly route) before operation. The rats were randomly divided into four groups, each consisting of eight animals. Rats with no operation (Group-1) received no treatment and served as controls. Rats in group 2 underwent unilateral ureteral ligation and received no treatment. Group 3 rats underwent sham operation and received no treatment with montelukast sodium (ML). Rats in Group-4 were subjected to unilateral ureteral ligation and received ML (p.o. 10mg/kg/day) (22).

At 14 days after UUO, all rats were sacrificed by high-dose ketamine. Kidneys were reached with an abdominal midline incision. Left kidney was immediately excised and separated from the surrounding tissues, washed twice with cold saline, and stored at -80ºC to determine the levels of renal malondialdehyde (MDA), GSH, and NO. A portion of the left renal tissue was stored in formol solution for the histopathologic examinations. Paraffinized tissue samples were examined for leukocyte infiltration and renal fibrosis. Urea-creatinine levels were investigated at blood analysis. Renal impairment was assessed by serum urea and creatinine levels, as well as by the kidney histology. Serum urea and creatinine levels were determined with an autoanalyzer (Syncron LX20, Ireland) by using commercial Becman Coulter diagnostic kits.

MEASUREMENT OF TISSUE LIPID PEROXIDATION LEVEL

Frozen kidney samples were homogenized in Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. MDA, which is formed as an end product of the lipid peroxidation, served as an index of the intensity of oxidative stress. MDA, referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrophotometer, as described previously (23). The MDA level was expressed as mmol/g wet tissue.

MEASUREMENT OF TISSUE GSH LEVEL

Reduced GSH was estimated by the method of Moron et al. (24), where the color developed was read at 412 nm. Protein concentrations in all samples were measured using the method of Lowry et al. (25). Results were reported as mmol/g wet tissue.

MEASUREMENT OF TISSUE NO LEVEL

Total nitrite was quantified by the Griess reaction after incubating the supernatant with Escherichia coli nitrate reductase to convert NO^3 to NO^2. Griess reagent (1 mL 1% sulfanilamide, 0.1% naphthyl-ethylenediamine hydrochloride, and 2.5% phosphoric acid; Sigma Chemicals) was then added to 1 mL of supernatant (26). The absorbance was read at 545nm after 30-minute incubation. The absorbance was compared with the standard graph of $NaNO^2$, obtained from the reduction of $NaNO^3$ (1-100 mmol/L). The accuracy of the assay was checked in two ways; the inter- and intraassay coefficients of variation were 7.52% and 4.61%, respectively. To check conversion of nitrate to nitrite (recovery rate), predetermined amounts of nitrate were added to control plasma samples; these samples were deproteinized and reduced as above.

HISTOPATHOLOGICAL EXAMINATION

Histopathological evaluation was performed on kidney tissues. Paraffin-embedded specimens were cut into 6-µm thickness sections and stained with hematoxylin & eosin for examination under the light microscope using a conventional protocol (27) (BH-2; Olympus, Tokyo, Japan). A semi-quantitative evaluation of renal tissues was accomplished by scoring the degree of severity according to previously published criteria (28). All sections of kidney samples were examined for

tubular necrosis. Briefly, a minimum of 50 proximal tubules associated with 50 glomeruli were examined for each slide and an average score was obtained. Severity of lesion was graded from 0 to 3 according to the percentage of tubular involvement. Slides were examined and assigned for severity of changes using scores on scale in which (0) denotes no change; grade (1) changes affecting <25% tubular damage (mild); grade (2) changes affecting 25-50% of tubules (moderate); grade (3) changes affecting >50% of tubules (severe).

Histopathological evaluation was performed on left kidney tissues. Paraffin-embedded specimens were cut into 5-mm thick sections and stained with hematoxylin & eosin and Masson's trichrome for examination under the light microscope (BH-2; Olympus, Tokyo, Japan).

To evaluate leukocyte infiltration, the widening of interstitial spaces with focal leukocyte infiltration was assessed in five randomly chosen sections prepared from each kidney sample. For each section, the average number of leukocytes per 0.28 mm^2 was calculated from these leukocyte-infiltrated foci using a high-power microscopic field (x400).

In order to estimate the grade of interstitial fibrosis, the interstitial area that stained green with Masson's trichrome was evaluated as a percentage of the total examined area in five randomly chosen sections prepared from each kidney sample using an image analyzer (Leica; Leica Micros Imaging Solutions, Cambridge, UK). For each section, interstitial space widening with focal leukocyte infiltration and interstitial fibrosis was assessed in high-power fields (x400) to quantify the results. The Banff classification of kidney pathology was used for scoring the degree of mononuclear cell infiltration and interstitial fibrosis. The score was graded from 0 to 3, depending on the severity of histological characteristics (29).

Statistical analyses

Results of all groups were shown as mean values±standard deviation (SD). Statistical analyses of the histopathologic evaluation of the groups were carried out by the Chi-square test and biochemical data were analyzed by the one-way analysis of variance (ANOVA). The significance between two groups was determined by the Dunnett's multiple comparison test, and $P<0.05$ was accepted as statistically significant value.

RESULTS

There was no significant difference for urea-creatinine levels between groups (Table-1).

Tissue MDA levels significantly increased in Group-2 compared with Groups-1, 3, and 4 ($p<0.001$). Rats with ML administration (Group-4) showed reduced levels of lipid peroxidation as measured by MDA levels (Table-2). UUO also induced a significant increase in the tissue NO levels that have been prevented by ML (Table-2). The unilateral ureteral ligation was accompanied by a marked reduction in GSH level in the kidney tissues of rats ($p<0.001$), and treatment with ML partially elevated the GSH levels (Table-2).

Histopathologic examination was normal in rats with only sham operation (Group-3) and in rats with no operation (Group-1) (Figures 1A and B). In rats with UUO, there were mild and severe tubular necrosis in the proximal tubules compared control and sham groups (Figure-1C). In rats treated with UUO+ML, despite the presence of mild

Table 1 - Effects of UO alone and its combination with ML on plasma urea, creatinine levels in rats.

Parameters	Control	Sham	UO	UO+ML
Urea (mg/dL)	34±8.1	35.5±8.6	37.5±10.6	36.1±8.1
Creatinine (mg/dL)	0.44±0.1	0.47±0.2	0.51±0.2	0.48±0.1

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

tubular degeneration and less severe tubular necrosis, glomeruli maintained a better morphology when compared with UUO group (Figure-1D). Severe leukocyte infiltration was observed in the periglomerular and peritubular interstitium of the kidneys of the rats in group-2 with UUO (Figures 2A and B).

Quantitative analysis of the focal leukocyte infiltration area in the interstitium showed that leukocyte infiltration was significantly reduced in rats that received UUO+ML (Group-4) (Figure-2C). UUO caused a significant interstitial fibrosis in rats that received no treatment (Group-2), and the percentage area of interstitial fibrosis in the kidney of rats with UUO that received no treatment was significantly greater than that of rats with UUO that received ML (Group-4) (Figures 3A, B and C). These changes are summarized in Table-3.

Table 2 - Effect of ML on the Levels of Malondialdehyde, Glutathione, and Nitric Oxide in Each Rat Group.

| Parameters	Kontrol	Sham	UO	UO + MLS
NO (nmol/g wet tissue)	30.3±9.3	30.3±10.2	63.2±15.2[a]	36.3±9.3[b]
MDA (nmol/g wet tissue)	2.6±0.7	2.7±0.7	5.1±1.2[a]	2.9±0.9 [b]
GSH (umol/g wet tissue)	2.3±0.8	2.2±0.7	1.1±0.5[c]	2.1±0.7[d]

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

[a] Significantly different from sham (p<0.001); [b] Significantly different from UO group (p<0.001); [c] Significantly different from sham (p<0.05);

[d] Significantly different from UO group (p<0.05).

Abbreviations: **NO =** nitric oxide; **MDA =** malondialdehyde; **GSH =** reduced glutathione.

Figure 1 – A = normal tubulus and glomerules in kidney kortex H&Ex100 (control group). B = normal tubulus and glomerules in kidney kortex H&Ex100 (sham group). C = severe tubular necrosis, tubular degeneration and epithelial vacuolization in the proximal tubules H&Ex100(UUO group). D = mild epithelial vacuolization in the proximal tubules and normal glomerules H&Ex100 (UUO+ML treated group).
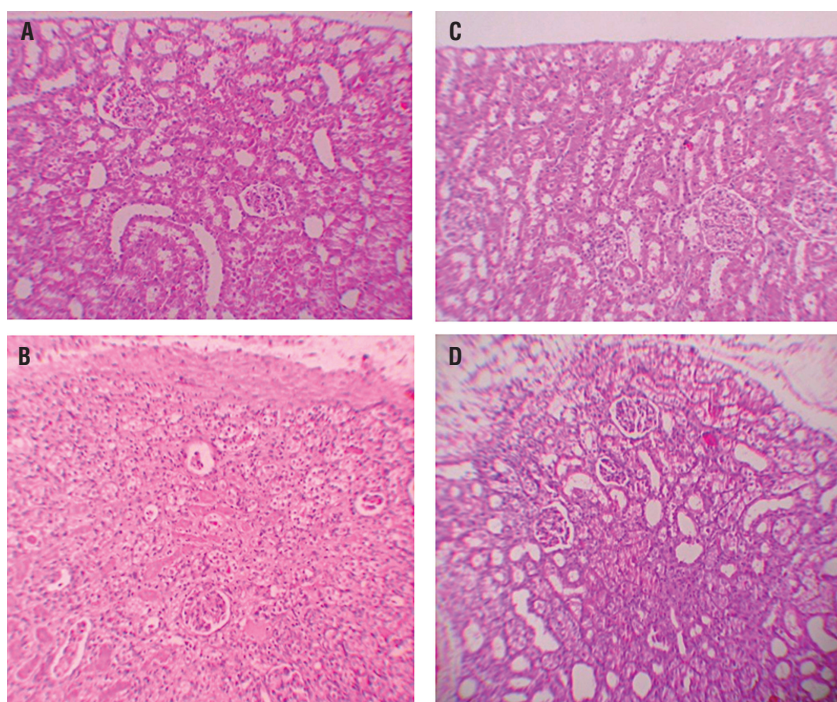
Figure 2 – A = Normal kidney morphology in a sham group. B = Leukocyte infiltration was observed in the peritubular interstitium of the UUO. C = Leukocyte infiltration was reduced in the ML-treated group (hematoxylin & eosin,*400).
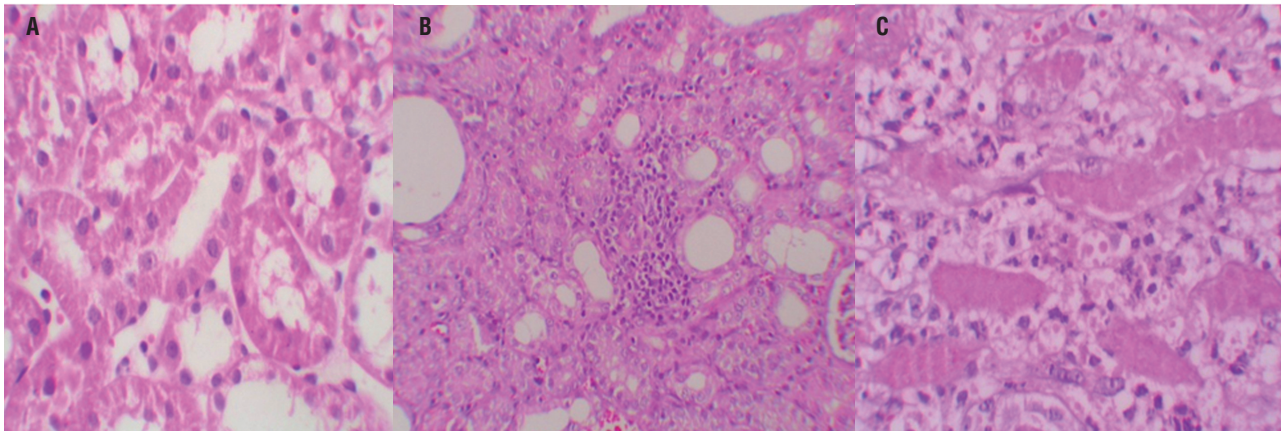
Figure 3 – A = Normal kidney morphology in a sham group. B = severe fibrosis was observed in the peritubular interstitium of the UUO. C = mild fibrosis was reduced in the ML-treated group (masson & trichrome,*400).
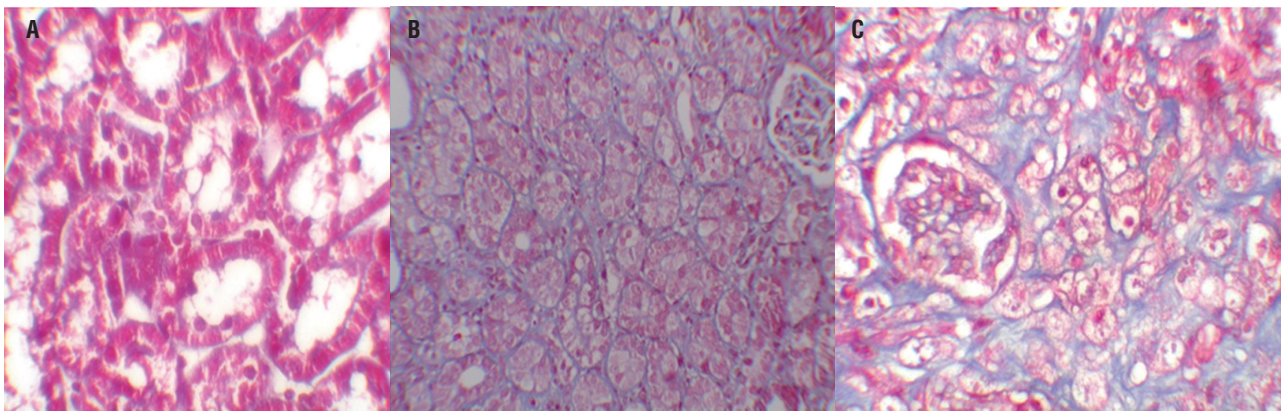
Table 3 - Semiquantitative analysis of tubular necrosis, interstitial fibrosis, mononuclear cell infiltration in control, Sham, UO, and UO+ML treated rats.

	Tubular necrosis					Interstitial fibrosis				Mononuclear cell infiltration			
	n	0	1	2	3	0	1	2	3	0	1	2	3
Control	8	8	0	0	0	8	0	0	0	7	1	0	0
Sham	8	8	0	0	0	8	0	0	0	7	1	0	0
UO[a]	8	0	1	4	3	0	2	3	3	0	2	2	4
UO+ML[b]	8	1	5	1	1	2	5	1	0	1	6	1	0

Score 0 = no degeneration, 1 = mild degeneration, 2 = moderate degeneration, and 3 = severe degeneration

[a] Statistical significant difference from the Sham group

[b] Statistical significant difference from the UO group and $P < 0.05$

DISCUSSION

The present study confirmed through a quantitative survey the protective role of ML on renal tissue damage after the induction of UUO in rats. Our results showed that the obstructed kidney had significantly higher tissue MDA, NO levels, and lower GSH levels along with more fibrosis. The current data demonstrate UUO structural and functional alterations in the kidney with a concomitant increase in proinflammatory cytokines in the blood. The CysLT1 receptor antagonist montelukast, on the other hand, reduced the severity of injury, depressed the concentration of these cytokines and increased the antioxidative capacity.

Montelukast, one of the selective reversible CysLT1 receptor antagonists, is used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies(30). It is reported that montelukast can reduce eosinophilic inflammation in the airways (31-33).

Besides, CysLT1 receptor antagonists or biosynthesis inhibitors ameliorate ethanol-induced gastric mucosal damage (13, 34), experimental colitis (35), and wound healing (36, 37).

Recently, Sener et al. (22) have reported that montelukast has protective effects on chronic renal failure-induced multiple organ injury. They attributed this to montelukast's ability to inhibit neutrophil infiltration and apoptosis. They also suggested that montelukast balances the oxidant–antioxidant status and regulates the generation of proinflammatory mediators. In a different study, it has been shown that montelukast reversed ischemia reperfusion-induced oxidant responses and improved microscopic damage and renal functions (31).

Apoptotic cell death has been reported to play an important role in UUO-induced renal damages (38). The lack of investigation on whether ML has affected the apoptotic cell death because of UUO may be a limitation of this study. However, in a recent study, curcumin and melatonine which is an antioxidant and antiinflammatory agent like ML, has been reported to prevent UUO-mediated apoptotic cell death and reduce the UUO related renal damage. While we believe that ML can reduce the UUO-induced renal damage by a similar

mechanism that prevents apoptosis-related cell death, there is a need for further study on that subject for verifications.

The pathogenesis of renal fibrosis caused by UUO involves infiltration of the kidney by inflammatory cells including monocytes, activation and possible transformation of intrinsic renal cells, and interactions between infiltrating and resident cells. NF-kB is activated during renal obstruction, and inhibition of NF-kB activity has been demonstrated to prevent renal fibrosis induced by obstruction (39, 40). In the present study, in agreement with these findings, ML treatment prevented renal fibrosis in UUO rats.

In obstructive nephropathy, it is known that particularly by treatment of UUO, some functions of the kidney can be regained. However, by recovery from the ureteral obstruction, reperfusion damage may occur in the renal tissue as a result of the elevated renal blood flow.

Ischemia-reperfusion (IR) injury is one of the underlying causes of acute renal failure and ROS along with NO, and it plays important roles in mediating cell damage during IR injury (41, 42). Inflammatory cells, neutrophils, are potent cells for production of ROS that are highly produced during IR injury. Renal IR causes tissue injury by way of oxygen radicals and disturbs balance between oxidants and antioxidants in tissue (43). Rodriguez-Reynoso et al. found that exogenous melatonine (MLT) preserved renal function, increased GSH levels, reduced lipid peroxidation, and prevented the rise in NO levels induced by renal IR (44).

They also indicated that MLT treatment reduced histological kidney injury. In accordance with the increase in toxic oxygen metabolites, the renal MDA level was also significantly increased, indicating the presence of enhanced lipid peroxidation due to IR injury, while the levels of tissue glutathione were declined, demonstrating the depletion of the antioxidant pool.

Several studies have demonstrated that IR in the kidney is associated with lipid peroxidation, which is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (45-47). As montelukast reduced the oxidative injury on cellular structures, the level of the intracellu-

lar antioxidant glutathione, which is otherwise oxidized when inactivating free radicals, was not changed. Thus, it appears that the anti-oxidative effect of montelukast on lipid peroxidation does not involve the expenditure of tissue GSH stores, but the antioxidant pool is further supported by the action of montelukast. Moreover, IR-induced reduction in total antioxidant capacity was also reversed by montelukast treatment (31).

As shown in our study, ML has a protective role against both renal damage arising because of UUO and the reperfusion damage occurring as a result of the treatment of obstruction that gives way to elevated renal blood flow.

In conclusion, the results reported here indicate that ML exerts a preventive effect on UUO-induced kidney damage in rats by reducing oxidative stress. We therefore propose that ML supplementation therapy can be used for kidney protection in patients with UUO, such as ureteral stones. Hovewer, further animal and clinical studies are needed to confirm our suggestion.

CONFLICT OF INTEREST

None declared.

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