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Effects of adenosine triphosphate, Lacidipine, and Benidipine on 5-fluorouracil-induced kidney damage in rats

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Abstract. – OBJECTIVE: In the present study, the protective effects of adenosine triphosphate (ATP), Benidipine, and Lacidipine on potential kidney damage induced by 5-fluorouracil (5-FU) were investigated in rats.

MATERIALS AND METHODS: Totally 48 rats were divided into 8 groups: healthy (HG), 5-FU (FUG), ATP+5-FU (AFU), Benidipine+5-FU (BFU), Lacidipine+5-FU (LFU), ATP+Benidipine+5-FU (ABFU), ATP+Lacidipine+5-FU (ALFU) and Benidipine+Lacidipine+5-FU (BLFU). In a 10-day period, ATP (4 mg/kg) was administered intraperitoneally, and Benidipine (4 mg/kg) and Lacidipine (4 mg/kg) were administered orally once a day. On days 1, 3, and 5, 5-FU (100 mg/kg) was administered intraperitoneally one hour after the drug was administered. Afterward, the rats were euthanized, and kidney tissues were removed. An analysis of malondialdehyde, total glutathione, superoxide dismutase, and catalase was performed on tissues, as well as a histopathological examination. A creatinine and blood urea nitrogen analysis were performed on blood samples.

RESULTS: It was revealed that 5-FU decreased the amount of total glutathione, superoxide dismutase, and catalase activities in rat kidney tissues and increased malondialdehyde. Further, increased serum creatinine and blood urea nitrogen levels, as well as histopathological examination of kidney tissues, were found in the 5-FU group. ATP+Benidipine and ATP treatments were the most effective in preventing both biochemical and histopathological changes induced by 5-FU. A treatment with Benidipine improved biochemical and histopathologic data, but not to the same extent as a treatment with ATP+Benidipine and ATP. As a result of Lacidipine+ATP combination, 5-FU-induced biochemical changes in kidney tissue were partially inhibited, but the degree of histopathologic damage remained unchanged. Neither Benidipine+Lacidipine nor Lacidipine showed a protective effect on both biochemical changes and histopathologic damage.

CONCLUSIONS: It may be possible to prevent nephrotoxicity by adding ATP + Benidipine or ATP to 5-FU treatment.

Key Words:
Adenosine triphosphate, Benidipine, Lacidipine, 5-fluorouracil, Oxidative damage, Kidney.

Abbreviations

5-FU, 5-fluorouracil; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; ATP, adenosine triphosphate; ROS, reactive oxygen species; LPO, lipid peroxidation; HG, healthy group; FUG, 5-FU alone group; AFU, ATP+5-FU group; BFU, benidipine+5-FU group; LFU, lacidipine+5-FUgroup; ABFU, ATP+benidipine+5-FU group; ALFU, ATP+lacidipine+5-FU group; BLFU, Benidipine + Lacidipine+ 5-FU group; ip, intraperitoneally; tGSH, total GSH; BUN, blood urea nitrogen; ELISA, an enzyme-linked immunosorbent assay; GLDH, glutamate dehydrogenase; NADH, nicotinamide adenine dinucleotide hybrid; NAD, nicotinamide adenine dinucleotide; H&E, hematoxylin-eosin.

Introduction

As an antimetabolite, 5-fluorouracil (5-FU) is a pyrimidine derivative chemotherapeutic agent[1]. The anticancer effect of 5-FU is associated with inhibition of DNA and RNA synthesis[2,3]. 5-FU is currently used as a treatment for cancer of the gastrointestinal tract, the breast, the head, and neck, as well as skin cancers1. 5-FU, however, is associated with serious side effects such as gastrointestinal, hematologic, and cardiac complications[4,5]. The nephrotoxicity of 5-FU is another factor that limits its use[6]. 5-FU is known to cause oxidative damage to kidney tissue by increasing

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malondialdehyde (MDA) levels and decreasing antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT)7. Furthermore, 5-FU has been reported to decrease intracellular adenosine triphosphate (ATP) levels[4]. According to Spasojević et al[8], the adverse effects of 5-FU on the heart may be associated with a decrease in ATP concentration. Literature has indicated[9] that 5-FU increases intracellular Ca^{2+} ion concentrations. Increasing intracellular Ca^{2+} levels and lowering ATP and ATPase levels are two important steps in the pathogenesis of oxidative damage[10]. Based on the information provided in the literature, calcium channel blockers and ATP may be useful in treating renal injury induced by 5-FU.

ATP, which we will test the effect on 5-FU against possible kidney damage, is a nucleoside triphosphate consisting of adenine, ribose sugar, and three phosphate groups[11]. According to the literature[12,13], ATP is a major source of energy for the synthesis of antioxidants against reactive oxygen species (ROS). According to Kocaturk et al[14], ATP protects renal tissue from oxidative stress by preventing the accumulation of oxidants and the depletion of antioxidants. Another drug that we investigated the effect of 5-FU against possible renal toxicity was Benidipine, which is an antihypertensive drug that blocks L-, N-, and T-type Ca^{2+} channels, and it is a dihydropyridine derivative[15]. Benidipine has been demonstrated[16] to have renoprotective properties due to the inhibition of L- and T-type Ca^{2+} channels, and its antioxidant effects on the kidney are primarily related to the inhibition of T-type Ca^{2+} channels. Lacidipine, which we investigated as a potential countermeasure to 5-FU, is a selective L-type Ca^{2+} channel blocker[17]. Lacidipine is reported to possess antioxidant properties by inhibiting lipid peroxidation in living organs and tissues[18]. Furthermore, Lacidipine has been known to have a gastroprotective effect by suppressing the increase in MDA, the toxic product of lipid peroxidation (LPO), and the decrease in GSH and SOD[19]. This information obtained from the literature suggests that ATP, Benidipine, and Lacidipine may be useful in the treatment of 5-FU-associated renal toxicity. The extent to which these drugs may be effective in treating 5-FU-associated renal toxicity is unknown, however. The purpose of our study was to investigate and compare the effects of ATP, Benidipine, and Lacidipine against possible kidney damage induced by 5-FU in rats.

Materials and Methods

Animals

During the experiment, 48 albino Wistar male rats (285-295 grams, 5-6 months old) were used. All animals were procured from Erzincan Binali Yildirim University Experimental Animals Application and Research Center. In a suitable laboratory environment, animals were fed with animal feed in groups of six each at 22°C room temperature, 12 hours of darkness, and 12 hours of light before the experiment.

Drugs

Among the chemicals, 5-Fluorouracil (1,000 mg 20 ml, solution for i.v. injection) was procured from Training and Research Hospital (Turkey) affiliated to the Ministry of Health, ATP was procured from Zdorove Narodu (Ukraine), Benidipine from Deva (Turkey), Lacidipine from Glaxo Smith Kline Drugs (Turkey) and ketamine from Pfizer İlaçları Ltd. Sti (Turkey).

Experimental Groups

Eight groups of rats were used in the experiments, including the healthy (HG), 5-FU alone (FUG), and ATP+5-FU treatment (AFU), Benidipine+5-FU (BFU), Lacidipine+5-FU (LFU), ATP+Benidipine+5-FU (ABFU), ATP+Lacidipine+5-FU (ALFU) and Benidipine+Lacidipine+5-FU (BLFU) groups.

Experimental Procedure

ATP was injected intraperitoneally (ip) at a dose of 4 mg/kg in the AFU (n=6). Benidipine was administered orally to the BFU (n=6) at a dose of 4 mg/kg with gavage. In the LFU (n=6) group, Lacidipine 4 mg/kg was given orally using gavage. At doses and methods outlined above, ATP+Benidipine was administered to the ABFU (n=6), ATP+Lacidipine was administered to the ALFU (n=6), and Benidipine+Lacidipine was administered to the BLFU (n=6). As a solvent, distilled water was given to the HG (n=6) and FUG (n=6). ATP, Benidipine, Lacidipine, and distilled water were administered once a day for ten days. On days 1, 3, and 5, 5-FU (100 mg/kg) was administered intraperitoneally one hour after the drugs and distilled water were administered. A high dose of ketamine (120 mg/kg) was administered at the end of this period in order to euthanize the animals. MDA, total GSH (tGSH), SOD, and CAT levels were measured in kidney tissues removed from euthanized animals.

Creatinine and blood urea nitrogen (BUN) levels were determined in blood samples taken from the tail vein before euthanasia.

Biochemical Analyses

Sample preparation

The tissue samples were washed with physiological saline and then placed in petri dishes. Liquid nitrogen was used to grind all tissues into powder. The levels of MDA, GSH, SOD, CAT, and protein were determined by homogenizing the tissue samples.

MDA, GSH, SOD, CAT, and protein measurement

An enzyme-linked immunosorbent assay (ELISA) kit for experimental animals was used to measure the levels of MDA, GSH, and SOD in supernatants prepared from kidney tissue samples (product no. 706002, 703002, and 10009055 for MDA, GSH, and SOD, respectively, Cayman Chemical Company, Ann Arbor, Michigan, USA). CAT was determined using the method proposed by Goth[20]. Spectrophotometric determinations of protein were undertaken according to the Bradford method at 595 nm[21].

Creatinine measurement

A Roche Cobas 8000 autoanalyzer (Roche Diagnostics GmBH, Mannheim, Baden-Wurttemberg, Germany) was used for the quantitative determination of serum creatinine. This kinetic colorimetric assay was based on the Jaffe method[22]. In an alkaline solution, creatinine created a yellow-orange complex with picrate. A wavelength of 505 nm was used to measure this combination. There was a direct correlation between the rate of dye formation and the concentration of creatinine in the sample. "Rate-blanking" was used in the assay to minimize interference from bilirubin. To compensate for the non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, serum or plasma results were corrected to -26 µmol/L (-0.3 mg/dL). Creatinine+picric acid → yellow-orange complex (alkaline pH).

BUN measurement

On a Roche Cobas 8000 autoanalyzer (Roche Diagnostics GmBH, Mannheim, Baden-Wurttemberg, Germany), serum urea levels were quantitatively determined using spectrophotometric methods. BUN was calculated using the formula BUN = URE* 0.48. Kinetic testing with urease and glutamate dehydrogenase is based on the hydrolysis of urea to ammonium and carbonate ions. Urea was hydrolyzed by urease to form ammonium and carbonate (Urea + 2 H_2O→ (Urease) 2 NH_4+ + CO_3^{2}-). L-glutamate was produced by the reaction of 2-oxoglutarate with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme nicotinamide adenine dinucleotide hydride (NADH). In this reaction, for every mole of urea hydrolyzed, two moles of NADH were oxidized to nicotinamide adenine dinucleotide (NAD^+). NH^{4+} + 2-oxoglutarate + NADH → (GLDH) L-glutamate + NAD^+ + H_2O. A direct correlation was found between the concentration of urea in the sample and the rate of decrease in NADH concentration. A wavelength of 340 nm was used for the measurement.

Histopathological Analysis

Kidney tissue samples were first fixed with 10% formaldehyde and then washed under tap water for 24 hours. To dehydrate the samples, tissues were passed through a graded alcohol series. Kidney samples were then passed through xylene and embedded in paraffin. Sections were taken and stained with hematoxylin-eosin (H&E). The sections were photographed and analyzed using the DP2-SAL firmware program and a light microscope (Olympus Inc., Tokyo, Japan). For semiquantitative analysis, one central and five peripheral areas were selected from serial sections and degeneration criteria were scored for each sample. Histopathological changes in renal tissue were defined as the presence of glomerular degeneration, tubular vacuolization, inflammatory cells, capillary congestion, and hyaline casts. Each sample was scored for each criterion as follows: 0 indicated no damage; 1, slight damage; 2, moderate damage; 3, serious damage. Histopathological evaluation and scoring were performed by a pathologist blinded to the experimental groups.

Statistical Analysis

The statistical analysis was conducted using the SPSS Statistics 22 (IBM Corp., Armonk, NY, USA) software. First, the suitability of the biochemical data for normal distribution was confirmed by the Kolmogorov-Smirnov test. A one-way ANOVA test was used for analysis. For pairwise comparisons, the Games-Howel test was used for MDA, tGSH, SOD, and BUN, and the CAT and Tukey tests for creatinine, according to the results of Levene's test. The biochemical data were presented as mean±standard deviation. Due

to the semi-quantitative nature of histopathologic grading data, Kruskal-Wallis and Dunn's tests were used for analysis. The data were presented as a median (quartile 1-quartile 3). $p<0.05$ was determined as statistically significant.

Results

MDA, tGSH, SOD, and CAT Analysis Results in Kidney Tissue

As observed in Figure 1A and Table I, MDA levels in the kidney tissues of animals in the 5-FU were found to be increased compared to the HG group ($p<0.001$). ATP+Benidipine, ATP, ATP, ATP+Lacidipine, and Benidipine treatments were the best inhibitors of 5-FU-induced MDA increase, respectively ($p<0.001$). Lacidipine and Lacidipine+Benidipine did not inhibit the 5-FU-induced MDA increase ($p=1.000$). MDA values in the ATP+Benidipine group were similar to those of healthy animals ($p=0.609$).

Furthermore, tGSH levels were found to be lower in the 5-FU group compared to the healthy group ($p<0.001$). The groups with the best suppression of 5-FU-induced tGSH decrease were ABFU ($p<0.001$), AFU ($p<0.001$), BFU ($p<0.001$), and ALFU ($p=0.005$), respectively. Whereas tGSH data in the BLFU were similar to FUG ($p=1.000$), tGSH values in the LFU were even lower than FUG ($p=0.44$). The tGSH values between BFU and ALFU ($p=0.997$) and ABFU and HG ($p=0.960$) were similar (Figure 1B, Table I).

As presented in Figures 1C-D and Table I, SOD and CAT activities in the kidney tissues of the FUG were also lower than HG ($p<0.001$). In LFU and

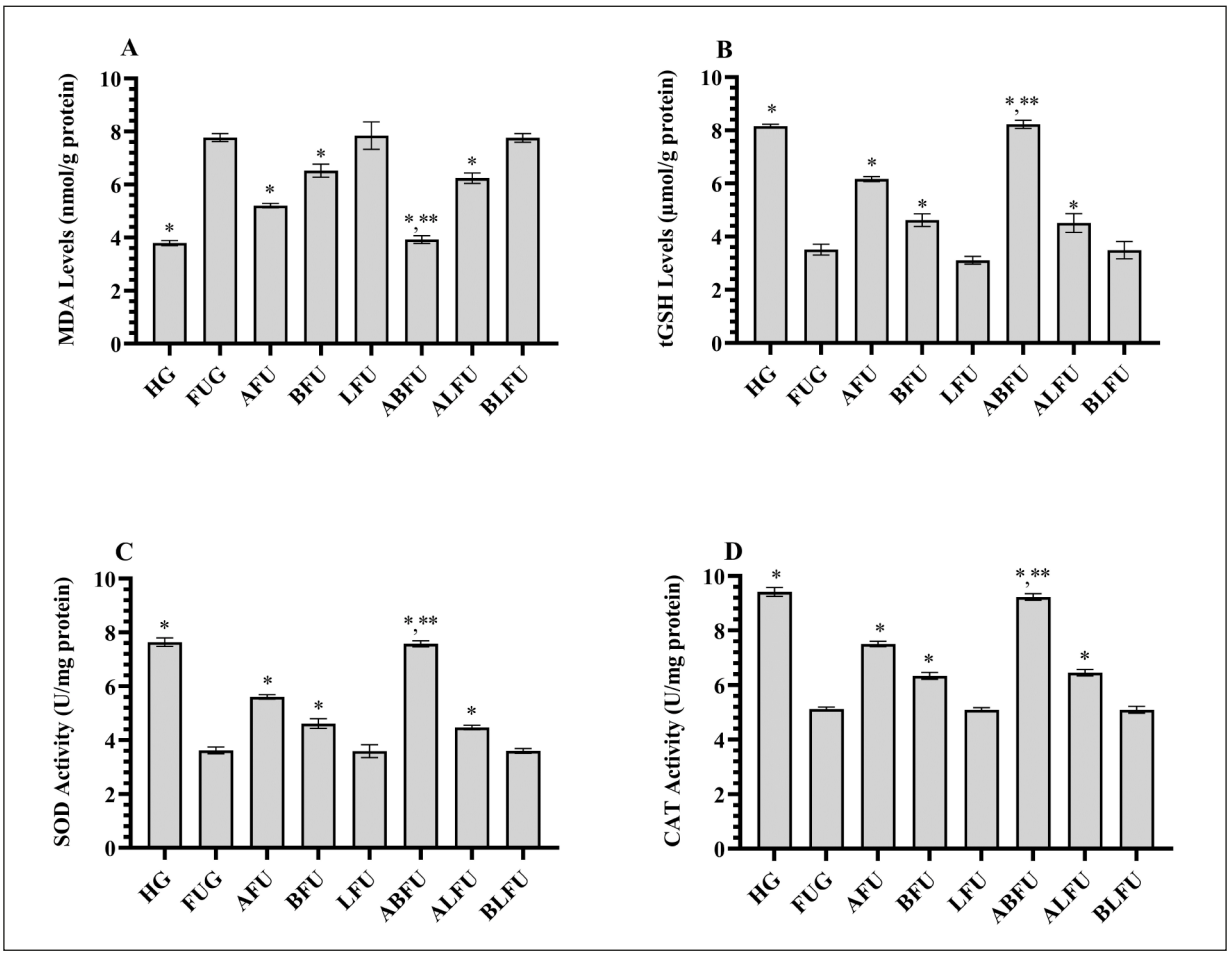

Figure 1. Analysis of MDA (**A**), tGSH (**B**), SOD (**C**), and CAT (**D**) data obtained from kidney tissues. *: $p<0.05$ *vs.* FUG, **: $p>0.05$ *vs.*. HG, MDA: malondialdehyde, tGSH: total glutathione, SOD: superoxide dismutase, CAT: catalase, HG: healthy group, FUG: 5-Fluorouracil group, AFU: ATP+5-Fluorouracil group, BFU: Benidipine+5-Fluorouracil group, LFU: Lacidipine+5-Fluorouracil group, ABFU: ATP+Benidipine+5-Fluorouracil group, ALFU: ATP+Lacidipine+5-Fluorouracil group, BLFU: Benidipine+Lacidipine+5-Fluorouracil group.

Table I. Analysis of biochemical data obtained from kidney tissues.

Groups (n=6/each group)	Biochemical parameters					
	MDA	tGSH	SOD	CAT	Creatinine	BUN
	Mean±Standard deviation					
HG	3.79±0.09*	8.16±0.07*	7.64±0.16*	9.42±0.16*	1.13±0.07*	39.17±3.19*
FUG	7.77±0.15	3.51±0.20	3.62±0.12	5.12±0.07	2.75±0.09	181.17±7.25
AFU	5.21±0.09*	6.17±0.10*	5.61±0.09*	7.51±0.10*	1.93±0.12*	86.00±6.16*
BFU	6.53±0.25*	4.62±0.24*	4.61±0.18*	6.34±0.13*	2.41±0.11*	127.17±13.60*
LFU	7.85±0.52	3.11±0.15	3.59±0.24	5.09±0.08	2.92±0.13	199.67±7.50
ABFU	3.93±0.15*,**	8.23±0.15*,**	7.58±0.11*,**	9.23±0.12*,**	1.25±0.06*,**	48.33±7.12*,**
ALFU	6.24±0.20*	4.51±0.36*	4.47±0.09*	6.46±0.12*	2.49±0.09*	125.17±13.21*
BLFU	7.76±0.17	3.49±0.33	3.60±0.09	5.09±0.13	2.84±0.11	202.50±21.38
F(7,40)	295.137	523.696	843.735	1434.522	302.895	197.463
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*: $p<0.05$ *vs.* FUG, **: $p>0.05$ *vs.* HG, MDA: malondialdehyde, tGSH: total glutathione, SOD: superoxide dismutase, CAT: catalase, BUN: blood urea nitrogen, HG: healthy group, FUG: 5-Fluorourasil group, AFU: ATP+5-Fluorourasil group, BFU: benidipin+5-Fluorourasil group, LFU: lasidipin+5-Fuorourasil group, ABFU: ATP+benidipin+5-Fluorourasil group, ALFU: ATP+lasidipin+5-Fluorourasil group, BLFU: benidipin+lasidipin+5-Fluorourasil group. Statistical analysis was done with one-way ANOVA, and pairwise comparisons were made with Games Howel or Tukey tests. $p<0.05$ was determined as statistical significance.

BLFU, these values were close to FUG ($p=1.000$). In addition, ATP+Benidipine, ATP, Benidipine=ATP+Lacidipine treatments inhibited the decrease in SOD and CAT, respectively ($p<0.001$). SOD and CAT data obtained from the ABFU were similar to those obtained from the healthy animals ($p>0.05$).

Creatinine and BUN Analysis Results in Serum

Creatinine levels in the blood serum of the FUG were found to be increased compared to the HG ($p<0.001$). The treatments that suppressed this increase best were ATP+Benidipine, ATP, Benidipine=ATP+Lacidipine, respectively ($p<0.001$). Creatinine levels in LFU and BLFU were not different from the FUG ($p>0.05$). Creatinine levels of ABFU and HG were determined to be similar ($p=0.439$) (Figure 2A, Table I). Similarly, an increase in BUN levels was found with 5-FU treatment ($p<0.001$). The groups in which the increase in BUN was best prevented were ABFU, AFU, ALFU, and BFU, respectively ($p<0.001$). BUN levels in the BLFU were similar to FUG ($p=0.405$), whereas BUN levels in the LFU were even higher

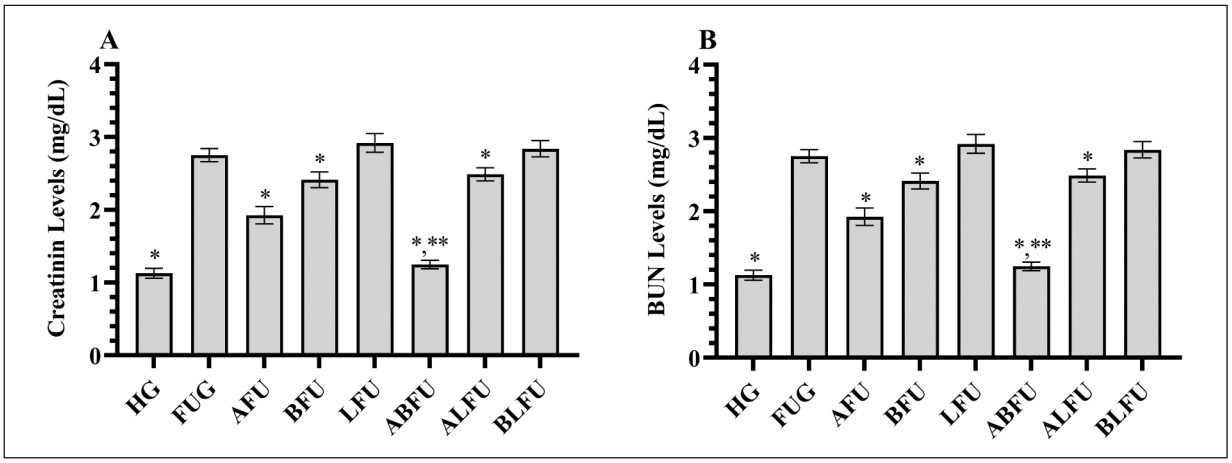

Figure 2. Analysis of creatinine (**A**) and BUN (**B**) data obtained from serum. *: $p<0.05$ *vs.* FUG, **: $p>0.05$ *vs.* HG, BUN: blood urea nitrogen, HG: healthy group, FUG: 5-Fluorouracil group, AFU: ATP+5-Fluorouracil group, BFU: Benidipine+5-Fluorouracil group, LFU: Lacidipine+5-Fluorouracil group, ABFU: ATP+Benidipine+5-Fluorouracil group, ALFU: ATP+Lacidipine+5-Fluorouracil group, BLFU: Benidipine+Lacidipine+5-Fluorouracil group.

than FUG (p=0.021). Benidipine and ATP+Lacidipine treatments suppressed the increase in BUN at a similar level (p=1.000). The lowest BUN levels among the treatment groups belonged to the ABFU, and the BUN data of ABFU and HG were close to each other (p=0.212) (Figure 2B, Table I).

Histopathologic Findings

It was evident from the examination of the renal tissues of the healthy group that the renal corpuscles, capillaries of the glomeruli, and the borders of the Bowman's interval were smooth. The structures of the proximal and distal tubules, epithelial cells within these structures, and interstitial areas were found to be regular and normal in appearance (Figure 3A, Table II). The histopathological analysis of the FUG group revealed severe signs of degeneration. There was an intense congestion of the blood vessels throughout the tissue. A severe dilatation of the bowman space was observed in the renal corpuscles, as well as an irregular and collapsed vascular network within the glomeruli. The interstitial areas of the tissue displayed a marked increase in inflammatory cells, and a significant accumulation of hyaline material was found within the tubules. Furthermore, cells in the distal and proximal tubules displayed vacuolization, increased eosinophilia, and cellular loss. A detachment of tubule epithelial cells from their basement membranes was also observed (Figure 3B, Table II). According to the analysis of the renal tissues of the AFU group, no abnormalities were observed in the glomerular vascular network, and the Bowman interval was within normal limits. Comparatively to the FUG group, the vascular congestion and the accumulation of hyaline material in the tubules were significantly less intense. The borders of proximal and distal tubule cells were clearly visible. Occasionally, inflammatory cells were observed in the interstitial spaces (Figure 4A, Table II). Several renal corpuscles in the BFU-treated group displayed evidence of dilated Bowman's intervals, but generally, the glomerular vascular network appeared normal. A moderate level of vascular congestion was found in the interstitial spaces. Vacuolation of epithelial cells and accumulation of hyaline material continued to occur in renal tubule structures. The separation and loss of tubule cells were moderately severe compared to the FUG-treated group (Figure 4B, Table II). According to the histological images of the LFU group, similar findings were observed in the FUG group. There was severe damage to the entire kidney tissue. Bowman's interval dilatation and glomerular capillary collapse were severe. It was remarkable to observe congestion in the vascular structures, intense inflammatory cells in the interstitial areas, and accumulation of hyaline material in the tubules. The tubule cells showed similar separation and necrotic changes as those in the FUG group (Figure 4C, Table II). When the

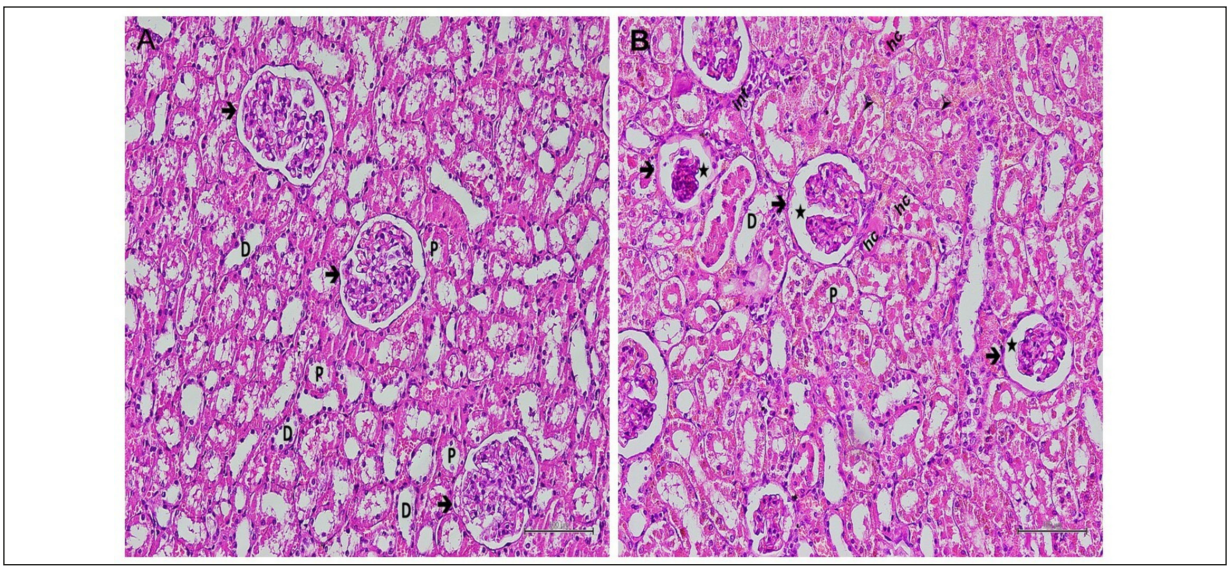

Figure 3. A, Kidney tissue belonging to HG. →: Renal corpuscle, P: proximal tubule, D: distal tubule x200 (H&E). **B**, Kidney tissue belonging to FUG. →: Renal corpuscle, P: proximal tubule degeneration, D: distal tubule degeneration, ⋆: Bowman dilatation-collapsed capillary, ►: congestion, inf: Inflammatory cell, hc: hyaline cast x200 (H&E). HG: healthy group, 8 FUG: 5-Fluorourasil group.

Table II. Analysis results of histopathological grading data obtained from animal groups.

Groups (n=6/each group)	Glomerular degeneration	Tubular vacuolization	Inflammatory cells	Capillary congestion	Hyaline casts
	Median (Quarter 1 - Quarter 3)				
HG	0 (0-0)*	0 (0-0)*	0 (0-0)*	0 (0-0)*	0 (0-0)*
FUG	3 (2-3)	3 (2-3)	3 (2-3)	3 (3-3)	3 (2.5-3)
AFU	1 (0-1)*,**	1 (0-1)*,**	1 (0-2)*,**	1 (1-2)*	1 (1-1)*,**
BFU	2 (1-2)*	2 (1-2)*	2 (1-2)*	2 (1-3)*	2 (1-2.5)*
LFU	3 (2-3)	3 (2-3)	3 (3-3)	3 (2.5-3)	3 (2-3)
ABFU	0 (0-0)*,**	0 (0-1)*,**	0 (0-1)*,**	0 (0-0.5)*,**	0 (0-0)*,**
ALFU	2 (2-3)	2 (1.5-3)	2 (2-2.5)	2 (2-3)	2 (2-3)
BLFU	3 (2.5-3)	3 (2-3)	3 (2-3)	3 (3-3)	3 (2-3)
H	229.664	227.236	220.684	215.821	224.838
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

*: $p<0.05$ *vs*. FUG, **: $p>0.05$ *vs*. HG, HG: healthy group, FUG: 5-Fluorourasil group, AFU: ATP+5-Fluorourasil group, BFU: benidipin+5-Fluorourasil group, LFU: lasidipin+5-Fuorourasil group, ABFU: ATP+benidipin+5-Fluorourasil group, ALFU: ATP+lasidipin+5-Fluorourasil group, BLFU: benidipin+lasidipin+5-Fluorourasil group. Statistical analysis was done with the Kruskal-Wallis test, and pairwise comparisons were made with Dunn's test. $p<0.05$ was determined as statistical significance.

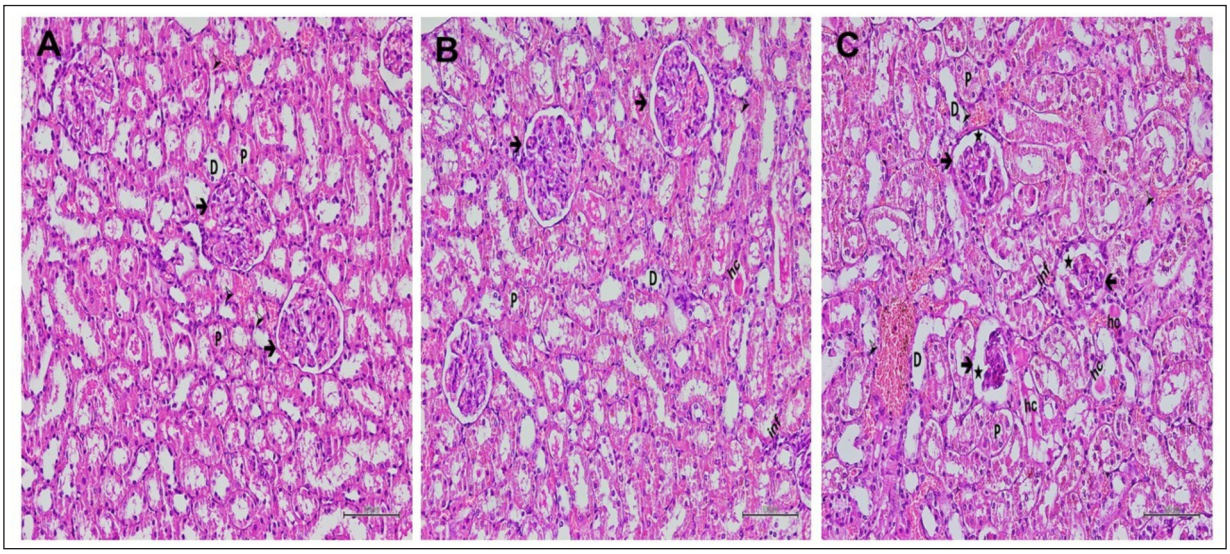

Figure 4. A, Kidney tissue belonging to AFU. →: Renal corpuscle, P: proximal tubule, D: distal tubule, ►: congestion x200 (H&E). **B**, Kidney tissue belonging to BFU. →: Renal corpuscle, P: proximal tubule, D: distal tubule, ►: congestion, inf: Inflammatory cell, hc: hyaline cast x200 (H&E). **C**, Kidney tissue belonging to LFU. →: Renal corpuscle, P: proximal tubule degeneration. **D**, distal tubule degeneration, ⋆: Bowman dilatation-collapsed capillary, ►: congestion, inf: Inflammatory cell, hc: hyaline cast x200 (H&E). AFU: ATP+5-Fluorourasil group, BFU: benidipin+5-Fluorourasil group, LFU: lasidipin+5-Fuorourasil group.

kidney tissue of the ABFU group was evaluated in general, it was found that all structures were similar to those of the healthy group. There were no abnormalities observed in the renal corpuscles, glomeruli, proximal and distal tubules, or interstitial connective tissue. Healing was evident throughout the tissue (Figure 5A, Table II). There were moderate degenerative findings in tissue samples treated with ALFU. A dilatation of the Bowman's interval was observed in some of the renal corpuscles. It was evident, however, that the tubules had experienced cellular loss and separation. In moderate severity, hyaline material continued to accumulate in the tubules (Figure 5B, Table II). As with the FUG- and LFU-treated samples, severe damage findings were observed in the BLFU group as well. The presence of congestion and inflammatory cells in the vascular structures in the interstitial areas was noticeable throughout the tissue. A remarkable degree of

vacuolization and tubular necrosis was observed in the proximal and distal tubules. The tubule lumens were found to be filled with hyaline material. The glomeruli were found to have a collapsed vascular network with irregular borders. There was evidence of dilated Bowman spaces in the renal corpuscles (Figure 5C, Table II).

Discussion

5-FU is an antineoplastic agent widely used in the treatment of various malignancies. However, it has been reported in the literature[23] that its clinical use is limited due to its toxic effects in various organs, including the kidney. Previous studies[24] have reported that 5-FU decreases ATP production by inhibiting the Krebs cycle. There is also information in the literature[14] that ATP reduction may cause an increase in intracellular Ca^{2+} concentration and cell toxicity. Therefore, in the present study, the protective effects of ATP, Benidipine and Lacidipine against possible renal damage induced by 5-FU in rats were investigated biochemically and histopathologically. According to the results of our biochemical analysis, 5-FU caused an increase in MDA, a decrease in tGSH, and a decrease in the activities of SOD and CAT in rat kidney tissues. The increase in serum creatinine and BUN levels, as well as a histopathological examination of renal tissues, also contributed to damage in the 5-FU group. The ATP+Benidipine and ATP treatment was the most effective in preventing both biochemical and histopathological changes induced by 5-FU. Biochemical and histopathological data were improved by Benidipine treatment, although not to the same extent as by ATP+Benidipine and by ATP alone. Benidipine+Lacidipine, as well as Lacidipine treatments, failed to show a protective effect on both biochemical and histopathological changes. Lacidipine+ATP partially inhibited 5-FU-induced biochemical changes, while the level of histopathologic damage in kidney tissue did not change.

In previous studies[23], oxidative stress was considered responsible for renal damage induced by 5-FU. Oxidative stress indicates an imbalance between the excessive formation of oxidants and the scavenging of these radicals by antioxidants[25]. Previous studies[26] reported that 5-FU treatment causes an increase in ROS in the kidney, leading to toxic effects such as oxidative damage, necrosis, and apoptosis. It was difficult to quantify the amount of circulating free radicals because ROS were unstable molecules with a short half-life. Therefore, oxidation end products were used to assess redox status[27]. MDA was one of the end products of LPO and is currently recognized as one of

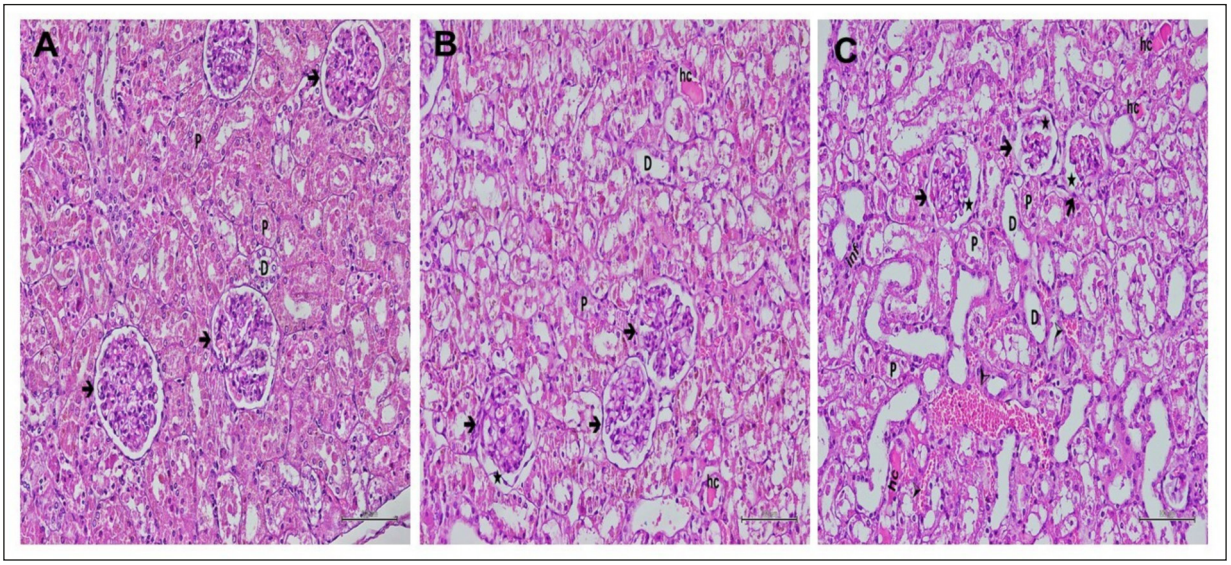

Figure 5. **A**, Kidney tissue belonging to ABFU. →: Renal corpuscle, P: proximal tubule, D: distal tubule x200 (H&E). **B**, Kidney tissue belonging to ALFU. →: Renal corpuscle, P: proximal tubule degeneration, D: distal tubule degeneration, ⋆: Bowman dilatation-collapsed capillary, hc: hyaline cast x200 (H&E). **C**, Kidney tissue belonging to BLFU. →: Renal corpuscle, P: proximal tubule degeneration, D: distal tubule degeneration, ⋆: Bowman dilatation-collapsed capillary, hc: hyaline cast, ►: congestion, inf: Inflammatory cell, hc: hyaline cast x200 (H&E). ABFU: ATP+benidipin+5-Fluorourasil group, ALFU: ATP+lasidipin+5-Fluorourasil group, BLFU: benidipin+lasidipin+5-Fluorourasil group.

the most important indicators of oxidative stress[28]. Oxygen radicals, which increase oxidative stress, interact with unsaturated fats and lead to lipid peroxidation and, ultimately, MDA production[29]. Our biochemical results revealed that 5-FU administration increased MDA formation in kidney tissue. A similar increase in MDA levels in renal tissues was reported by Rashid et al[23] and Gelen et al[7]. In our study, the best suppressors of MDA increase were ATP+Benidipine, ATP, ATP+Lacidipine, and Benidipine, while Lacidipine and Benidipine+Lacidipine treatments were ineffective. According to the literature[30], ATP prevented LPO by creating an antioxidant effect on reactive oxygen species. In an experimental study[14], ATP was shown to prevent an increase in MDA in an oxidative kidney damage model. Similarly, we found that rats treated with ATP and Benidipine had the lowest levels of MDA in our study. In addition, Kocaturk et al[14] demonstrated that ATP and Benidipine together inhibited bevacizumab-induced increases in MDA in renal tissue more effectively than ATP and Benidipine alone. It was also reported that Benidipine suppressed ischemia-reperfusion injury-induced MDA increase in renal tissue[31]. Our study results revealed that although Benidipine significantly suppressed the increase in MDA, the combination of Benidipine and Lacidipine was ineffective. Moreover, the MDA level of the Lacidipine group was even higher than that of the 5-FU group. However, there are reports[32] in the literature affirming that Lacidipine could suppress the increase in MDA in case of oxidative damage. Nevertheless, our findings suggested that Lacidipine had a toxic rather than a protective effect on renal tissue.

There is evidence in the literature[7] that 5-FU-induced kidney damage was associated with an increase in oxidants and a decrease in antioxidants. Hence, both tGSH (non-enzymatic) levels, as well as SOD and CAT (enzymatic) activity, were investigated in this study. According to our analysis, 5-FU treatment decreased kidney tGSH levels. 5-FU was reported to decrease tGSH levels in the kidney in a study similar to ours[14]. A decrease in GSH resulted in oxidative stress being induced in tissues. GSH detoxified radicals such as hydrogen peroxide and lipid peroxide by donating electrons to them because of its free thiol group[27]. Furthermore, GSH was an antioxidant that required ATP for its synthesis[33]. Overall, ATP treatment suppressed the decrease in tGSH levels across all treatment groups. The highest tGSH levels were obtained in the ATP and ATP+Benidipine groups, similar to Kocaturk et al[14]. The tGSH levels were also higher in the Benidipine group than in the 5-FU group. Previous studies[34] have also shown that Benidipine was able to maintain GSH levels in oxidative renal[14] and cardiac injury. The use of Lacidipine with 5-FU further decreased tGSH levels compared to the 5-FU group. However, Yigiter et al[35] found that Lacidipine was able to partially suppress the decrease in tGSH levels in renal tissues.

In addition to the decrease in tGSH levels, SOD and CAT activities were also decreased with 5-FU administration. SOD and CAT were ROS-scavenging enzymes that eliminated both superoxide anion and hydrogen peroxide with a gradual reaction and produced molecular oxygen[36]. In an experimental study[26] on mice, similar to our findings, SOD and CAT activities in the kidneys of 5-FU-treated mice were found to be lower than in healthy mice. Zhao et al[37] also found that both the amount of ATP and the decrease in SOD and CAT activities were parallel in kidney tissues exposed to oxidative damage. According to our study results, ATP-containing protocols preserved SOD and CAT activities the most. In the literature, there was a study[14] on the effect of ATP use on renal SOD and CAT activities in oxidative kidney injury, and the results were similar to our findings. Benidipine was able to partially protect SOD and CAT activities, although not as much as ATP. It was previously reported[14,38] that Benidipine prevented the suppression of SOD and CAT activities in oxidative tissues such as the kidney, heart, etc. However, our biochemical results showed that both Benidipine+Lacidipine and Lacidipine in combination with 5-FU had no ameliorative effect on SOD and CAT activities. Kamal[39] found that Lacidipine suppressed gentamicin-induced decrease in SOD and CAT activities, which is in contrast to our findings. There was also evidence in the literature that Lacidipine exhibited antioxidant activity[18]. On the other hand, there were also reports showing that Lacidipine preferentially dilated the afferent arteriole in the kidney but not the efferent arteriole, leading to an increase in intraglomerular pressure and a decrease in antioxidants as a result of impaired oxidative balance[38,40]. Elbanan et al[41] found that increasing GSH levels and restoring SOD and CAT enzyme activities with exogenously administered products in oxidative damage of the kidney resulted in a nephroprotective effect.

In our study, oxidant and antioxidant levels, as well as serum creatinine and BUN levels, were also investigated. Our analysis results indicated that 5-FU increased serum creatinine and BUN levels. It was previously reported[26,42] that 5-FU induced an

increase in creatinine and BUN, which are markers of renal function. According to our biochemical results, creatinine and BUN increases were suppressed in the groups treated with ATP. Koo et al[43] also reported the ameliorative results of oxidized ATP treatment in renal dysfunction due to renal ischemia-reperfusion injury. We found that renal function parameters improved in Benidipine-treated animals, although not as much as ATP. In the literature, it was reported[14] that Benidipine exhibited a renoprotective effect by decreasing intraglomerular pressure due to blocking all 3 Ca^{2+} channels (L, N, T), and it was superior to only L-type Ca channel blockers (Lacidipine, etc.) in this respect. There are studies in the literature that demonstrate the renoprotective properties of Benidipine and that it inhibits the increase in serum creatinine and BUN levels due to bevacizumab[14] and ischemia-reperfusion[31]. In addition, Lacidipine and Benidipine+Lacidipine treatments did not prevent the increase in creatinine and BUN levels. In fact, BUN levels were even higher in the Lacidipine group than in the 5-FU group. However, it was reported[32] in the literature that Lacidipine attenuated the increase in creatinine and BUN levels with cyclosporine use and exhibited renoprotective properties, which was not consistent with our findings.

The kidney tissues obtained from the experimental groups were also examined histopathologically. Histopathologic findings were consistent with our biochemical results. It was found that 5-FU also caused damage to the histologic structures of the kidney tissues. ATP+Benidipine and ATP treatments were the treatment protocols that prevented this damage the best, and renal tissues in these groups had an almost normal histologic appearance. Previous studies[14,43] have also shown that ATP treatment ameliorates oxidative damage in renal tubules. Benidipine prevented histopathologic damage weaker than ATP and ATP+Benidipine. In a preclinical study[44], Benidipine was reported to reduce glomerulosclerosis and tubulointerstitial lesion scores in rat kidneys. Lacidipine, on the other hand, did not have a protective effect on histologic structure as in biochemical findings. Yiğiter et al[35] also found no protective effect of Lacidipine on tubule and glomerular damage, inflammation, and apoptosis in oxidative kidney injury.

Conclusions

Our study revealed that the use of 5-FU induced oxidative stress and tissue damage in renal tissues, and increased serum creatinine and BUN levels reflected the deterioration in renal function. ATP was more successful than Benidipine and Lacidipine in preventing oxidative kidney damage. The best results were obtained in the ATP+Benidipine group, and all biochemical and histopathologic data were similar to those of healthy animals. ATP-containing groups were more successful than Benidipine-treated groups in preventing 5-FU-induced damage, while Lacidipine treatments were completely ineffective. According to the results of our biochemical and histopathologic analyses, it could be concluded that especially ATP+Benidipine and ATP treatments had protective properties in 5-FU-induced kidney damage.

Conflict of Interest
The authors declared no conflict of interest.

Ethics Approval
The experiments were performed after obtaining approval from Erzincan Binali Yıldırım University Animal Experiments Local Ethics Committee (Meeting date: 30.03.2023, Decision number: 10).

Informed Consent
Not applicable.

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Authors' Contributions
The study was designed by TD, HS. TD, HS, DA, and SB wrote and edited the manuscript. BS and RM performed the experimental procedures. TBT and MG collected and analyzed the experimental data. SB performed the statistical analysis. All authors read and approved the final manuscript.

Availability of Data and Materials
All relevant data are included in the paper and its supporting information files.

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