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Prevention of methotrexate-induced nephrotoxicity by concomitant administration of garlic aqueous extract in rat

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Background/aim: Methotrexate (MTX) has been widely used for treatment of cancer and rheumatoid arthritis, but its use has been limited by its nephrotoxicity. This study was carried out to determine whether garlic exerts a protective effect against MTX-induced nephrotoxicity.

Materials and methods: Nephrotoxicity was induced in rats after a single i.p. injection of MTX (20 mg/kg). Garlic extract (1 mL/100 g b.w.) was given orally for 7 days before and after MTX administration. Serum samples were collected to evaluate urea, creatinine, sodium, phosphorous, potassium, and calcium. Reduced glutathione, catalase, adenosine deaminase, nitric oxide, and malondialdehyde were measured in renal tissue. Tubular injury was evaluated by histopathological examination.

Results: MTX increased urea and creatinine levels and led to imbalances in some electrolytes. It also depleted renal antioxidant enzyme levels and increased malondialdehyde, adenosine deaminase, and nitric oxide levels. Histopathological examination showed glomerular and tubular alterations. Pretreatment with garlic significantly improved renal function and increased renal antioxidant enzyme activities. Furthermore, garlic reduced renal oxidative stress and prevented alterations in renal morphology.

Conclusion: Garlic treatment has a reversible biochemical and histological effect upon MTX-induced nephrotoxicity.

Key words: Garlic extract, methotrexate, nephrotoxicity, oxidative stress

1. Introduction

Methotrexate (MTX) is a folic acid antagonist, which is widely used to treat a variety of disorders such as leukemia, lymphoma, osteosarcoma, and autoimmune diseases. In addition, it is the most common antirheumatic drug used for the treatment of rheumatoid arthritis and other rheumatic disorders (1). However, MTX toxicity limits its use. Relatively high doses of MTX have been associated with gastrointestinal, renal, nervous, hepatic, and bone marrow toxicity (2). Since more than 90% of MTX is excreted via the kidneys, nephrotoxicity is the most common toxic effect of treatment (3,4). Renal dysfunction induced by MTX delays its elimination and sustained elevation of plasma MTX can result in enhancement of MTX's other toxicities (5,6). MTX-induced toxicity appears to be a consequence of the interaction of many factors: dosing schedule, length of treatment, patients' risk factors, type of disease, and presence of genetic and molecular apoptotic factors (7). The pathogenesis of MTX-induced nephrotoxicity is thought to arise through two primary mechanisms. The first is crystal nephropathy, which occurs via precipitation of MTX and its metabolites within the renal tubules. Nephropathy initially manifests as an asymptomatic elevation in serum creatinine levels and then progresses to tubular necrosis. The second mechanism is due to a direct toxic effect of MTX on renal tubules by increasing reactive oxygen species (ROS) production in the kidney, with subsequent cellular injury (8). It has been reported that MTX increases the level of hydrogen peroxide and other free radicals released by stimulated polymorphonuclear neutrophils, leading to toxicity and cellular damage (9). MTX blocks the conversion of dihydrofolates to tetrahydrofolates by inhibiting the dihydrofolate reductase enzyme. Consequently, MTX changes the cellular folate concentration, which in turn affects various folate-metabolizing enzyme activities (10). Several studies have demonstrated that some agents, including vitamin E (11), nicotinamide, methionine (12),

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melatonin (13), caffeic ester (14), and curcumin (15), can prevent tissue damage caused by MTX.

Garlic (*Allium sativum*) and its preparations are common food spices and herbal medicines, which are used worldwide. Garlic has many pharmacological properties and medical applications: it lowers serum and hepatic cholesterol (16), inhibits bacterial growth (17), and reduces oxidative stress (18). The biologic effects attributed to garlic include the reduction of risk factors for cardiovascular diseases and cancer, stimulation of immune function, enhanced detoxification of foreign compounds, and hepatoprotective, antimicrobial, and antioxidant effects (19). Raw garlic homogenate (aqueous extract of garlic) is the major preparation of garlic, commonly used in various scientific studies. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in aqueous garlic extract. When garlic is chopped or crushed, the alliinase enzyme present in garlic is activated and acts on alliin (present in intact garlic) to produce allicin. In addition, allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate, and γ-L-glutamyl-S-alkyl-L-cysteine are important sulfur-containing compounds present in garlic homogenate (19).

The present study was performed to elucidate the possible protective effect of oral administration of garlic against MTX-induced nephrotoxicity.

2. Materials and methods

2.1. Chemicals

MTX was obtained from Mylan Pharmaceuticals Company (USA). A urea (BUN) kit was purchased from Biomed Diagnostics (Egypt). A creatinine kit was purchased from Spectrum Diagnostics (Egypt). Sodium, potassium, calcium, phosphorus, reduced glutathione (GSH), catalase (CAT), malondialdehyde (MDA), adenosine deaminase activity (ADA), and nitric oxide (NO) were estimated using commercial kits (Bio-Diagnostic, Egypt). All the other chemicals were of the highest analytical grade and purchased from Sigma-Aldrich Company (USA).

2.2. Preparation of garlic extract

Garlic extract was prepared according to Suru (20). In brief, fresh bulbs of garlic were purchased from a local market in Beni-Suef, Egypt. The cloves were peeled and about 100 mL of chilled distilled water per 100 g of garlic was added, and the garlic was crushed in a mixing machine. The resulting slurry was squeezed and filtered through a fine cloth and the filtrate was frozen until use.

2.3. Animals and treatments

Forty Wistar Albino male rats (100–120 g), obtained from the Egyptian Organization for Biological Products and Vaccine, were used for the experiment. All rats were housed at room temperature and had free access to water at all times. They were fed a commercial diet during the experiment. All experiments were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Beni-Suef University, Egypt. Rats were allowed to acclimatize for 1 week prior to treatment and were equally distributed into four groups. In the first (control) group, rats were given i.p. injections of saline. In the second (GE) group, rats received garlic extract orally (1 mL/100 g body weight daily). In the third (MTX) group, rats were given a single i.p. dose of MTX (20 mg/kg). In the fourth group (GE+MTX), rats received garlic extract (1 mL/100 g body weight, orally) daily for 7 successive days, followed by a single i.p injection of MTX (20 mg/kg) followed by garlic administration for 7 consecutive days. Dosage and route of MTX administration were determined from those described by previous authors (13,14,21–23). The dose of garlic extract had been shown previously to provide protection in other models (20).

Blood samples were collected from the orbital venous plexus under light anesthesia to obtain sera, which were stored at –20 °C for measuring blood urea nitrogen (BUN), creatinine, sodium, potassium, calcium, and phosphorus. The rats were then euthanized by cervical dislocation and their renal tissue was quickly removed and washed using cold saline solution, homogenized in 5 mL of phosphate buffer saline (pH 7.4), and centrifuged at 3000 rpm for 15 min. The obtained supernatant was collected and stored at –20 °C for biochemical analysis, including GSH, CAT, MDA, ADA, and NO activities. A part of the kidney was fixed in 10% neutral formalin to be used for histopathological studies.

2.4. Laboratory analysis

2.4.1. Assessment of BUN and serum creatinine

Urea was determined as described previously (24). Creatinine was measured by buffered kinetic Jaffe reaction method as described previously (25).

2.4.2. Determination of serum sodium, potassium, phosphorus, and calcium

Serum sodium, phosphorous, and calcium levels were measured by colorimetric method (26–28). Serum potassium ions concentration was measured by turbidimetric method (29).

2.4.3. Determination of GSH, CAT, and lipid peroxidation in renal tissue

GSH and CAT activities were determined by spectrophotometric method (30,31). Lipid peroxidation level was measured by the thiobarbituric acid reaction (32).

2.4.4. Determination of renal ADA

ADA enzyme activity was measured as described previously (33). One unit of enzyme activity is defined as

the amount of enzyme that catalyzes the conversion of 1 μmol of adenosine to inosine in 1 min at 37 °C.

2.4.5. Determination of renal NO content

NO content was measured based on the Griess reaction (34).

2.5. Histological and histochemical examination

Renal tissues of control and treated groups were processed as previously described (35). Samples were fixed in 10% formaldehyde and processed routinely for embedding in paraffin. Tissue sections of 5 mm in thickness were stained with hematoxylin and eosin and examined under light microscope. The periodic acid–Schiff technique (PAS) was used to stain mucopolysaccharides. The bromophenol blue method was used to demonstrate the total protein content (36). The sections were examined and observed under light microscope.

2.6. Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5 (GraphPad Software, USA). Groups of data were compared with an analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison test. Values of $P < 0.05$ were regarded as significant. Results are expressed as mean ± SEM.

3. Results

Table 1 demonstrates the effect of MTX on renal functional parameters. It was evident that MTX-treated rats had a significant rise in BUN and serum creatinine levels in comparison with the control group. A complete reversal of renal functional changes induced by MTX was achieved by pretreating rats with the garlic extract.

As seen in Table 1, there was a significant decrease in sodium and increase in potassium levels in the MTX group when compared with the control group. Sodium level in the GE+MTX group was significantly increased compared with the MTX-only group. Regarding potassium, there was no significant change between the GE+MTX group and the control group. With respect to phosphorus, significant elevation was detected in the MTX group and in the GE+MTX rats compared to the control group. There was no significant change in calcium levels between different groups.

The effect of MTX, garlic extract, and their combination on the levels of oxidative stress biomarkers GSH, CAT, MDA, ADA, and NO in kidney tissues are shown in Table 2. A single dose of MTX resulted in significant decrease in GSH and CAT activities and an increase in ADA, NO, and MDA compared to the control group. Pretreatment with garlic increased GSH and CAT levels in kidney tissue in comparison with the MTX group. The level of MDA in GE+MTX rats was significantly decreased compared to the MTX group, but it failed to return to levels similar to the control group. Furthermore, pretreatment with garlic completely reversed the increase in ADA level induced by MTX to control values, whereas NO levels were only insignificantly greater than the control.

3.1. Histopathological findings

Microscopic examination of the control rat kidneys showed a normal structure of renal corpuscles and renal tubules. The renal corpuscle consists of a tuft of blood capillaries (glomerular tuft) surrounded by Bowman's capsule. The latter is composed of two layers. The first is the outer parietal layer lined with simple squamous epithelial cells and the second is an inner visceral layer lined with podocytes. The space between the two layers is called the urinary space. The renal tubules consist of proximal convoluted tubules lined with large pyramidal cells with apical brush borders, distal convoluted tubules lined with cuboid cells, the loop of Henle, and collecting tubules lined with simple cuboid cells (Figure 1A).

Table 1. Effect of garlic, methotrexate, and their combination on serum urea, creatinine, and some electrolyte levels.

Parameters	Control	GE	MTX	GE+MTX
Urea (mmol/L)	6.15 ± 0.37	5.99 ± 0.88	10.66 ± 0.34[a,b]	4.30 ± 0.95[c]
Creatinine (mg/L)	0.24 ± 0.01	0.23 ± 0.01	0.31 ± 0.01[a,b]	0.25 ± 0.02[c]
Sodium (mmol/L)	136.8 ± 1.3	135.7 ± 1.2	122.7 ± 2.7[a,b]	133.0 ± 1.2[c]
Potassium (mmol/L)	1.20 ± 0.52	1.30 ± 0.02	1.61 ± 0.29[a,b]	1.43 ± 0.21
Phosphorus (mg/dL)	2.50 ± 0.11	2.27 ± 0.13	4.04 ± 0.32[a,b]	3.63 ± 0.33[a,b]
Calcium (mg/dL)	12.77 ± 0.02	12.28 ± 0.73	11.33 ± 0.88	11.63 ± 0.67

Data are expressed as means ± SD with dissimilar superscript letters (significantly differing at $P < 0.05$): a) significantly different from control value; b) significantly different from the garlic group, and c) significantly different from the methotrexate-treated group.

Table 2. Effect of garlic, methotrexate, and their combination on ADA and NO levels, kidney enzymes antioxidant activities, and MDA (as lipid peroxidation).

Parameters	Control	GE	MTX	GE+MTX
GSH (mmol/g)	0.19 ± 0.01	0.18 ± 0.01	0.06 ± 0.01[a,b]	0.25 ± 0.03[c]
Catalase (U/g)	1.95 ± 0.01	1.90 ± 0.02	0.97 ± 0.01[a,b]	1.87 ± 0.02[c]
MDA (nmol/g)	16.47 ± 0.79	16.84 ± 0.36	51.62 ± 1.6[a,b]	31.23 ± 1.7[a,b,c]
ADA (U/L)	13.9 ± 0.06	12.0 ± 1.00	42.00 ± 0.56[a,b]	12.57 ± 0.34[c]
NO (μmol/L)	0.89 ± 0.20	0.92 ± 0.21	2.20 ± 0.14[a,b]	1.86 ± 0.21

The data are expressed as means ± SD with dissimilar superscript letters (significantly differing at $P < 0.05$): a) significantly different from control value; b) significantly different from the garlic group, and c) significantly different from methotrexate-treated group.

No pronounced histological alteration was noticed in the examined sections of GE rat kidneys, while massive degenerative changes in both renal corpuscles and renal tubules were observed in cross-sectioned kidneys of the MTX group. Some of the renal corpuscles appeared shrunken with atrophied glomeruli and widening of the urinary space (Figure 1B). Moreover, marked congestion of glomeruli and cortical blood vessels with vacuolar degeneration were observed in podocytes and mesangial cells with pyknotic nuclei (Figures 1C and 1D). The most prominent feature of most examined sections was degeneration and complete loss of cellular architecture of renal tubules. The cytoplasm of most affected tubular epithelial cells showed vacuolar degeneration with pyknotic nuclei, loss of their apical brush borders with marked dilatation of renal tubular lumina, focal areas of peritubular lymphocytic infiltration (Figure 1D), and interstitial edema (Figure 1E).

The lumina of most of the degenerated renal tubules contained exfoliated epithelial cells, cellular debris, and hyaline casts (Figure 1F). The renal architecture of GE+MTX sections was greatly improved and more or less similar to the controls. The structure and size of the renal corpuscles with their glomerular tufts appeared normal. Moreover, most of the epithelium of renal tubules was normal and contained vesicular nuclei and apical brush borders (Figure 1G).

3.2. Histochemical results

The examined cross-sectioned kidneys of both control and GE rats stained by bromophenol blue method had cellular cytoplasm that stained homogeneously dense blue, indicating normal protein content in both renal tubules and glomeruli (Figure 2A). A marked reduction of the protein content in the renal tubular cells was observed in the MTX rats, indicated by the faint blue coloration in the cytoplasm of affected cells (Figure 2B). In GE+MTX samples, sections showed improvement in the protein content in most of the renal tubular epithelium, which was similar to the control group (Figure 2C).

The mucopolysaccharides were stained using the PAS technique. Strongly PAS-positive materials were concentrated in the glomerular tuft, brush borders, and basement membrane of the renal tubules of control and GE rats (Figure 2D). In the MTX group, the mucopolysaccharides were greatly depleted in the glomerular tuft and the basement membrane as indicated by decreasing in the stain ability of PAS-positive materials while the affected degenerated brush borders showed a negative reaction (Figure 2E). Marked improvement and increase in the mucopolysaccharide content was noticed in the rat renal sections in the GE+MTX group, which more or less resembled the control group (Figure 2F).

4. Discussion

Nephrotoxicity is a major complication of MTX administration. The aim of the current study focused on the possible protective and curative role of garlic against MTX-induced renal damage using biochemical and oxidative determinations and histopathology of the kidney.

Our data revealed that MTX has a direct toxic effect upon the kidney, as indicated by increased BUN and serum creatinine. Creatinine concentration is more significant than BUN level in the earlier phases of kidney disease. On the other hand, BUN begins to rise only after a marked renal parenchymal injury (22). Elevation of BUN does not usually occur until the glomerular filtration rate (GFR) has diminished to less than 50% of normal (37). For assessment of renal function, sodium, potassium, phosphorous, and calcium ion levels were estimated. Our data showed electrolytic disturbance manifested by hyponatremia and hyperkalemia in the MTX rats. Lower value of serum sodium indicates the inability of the kidney

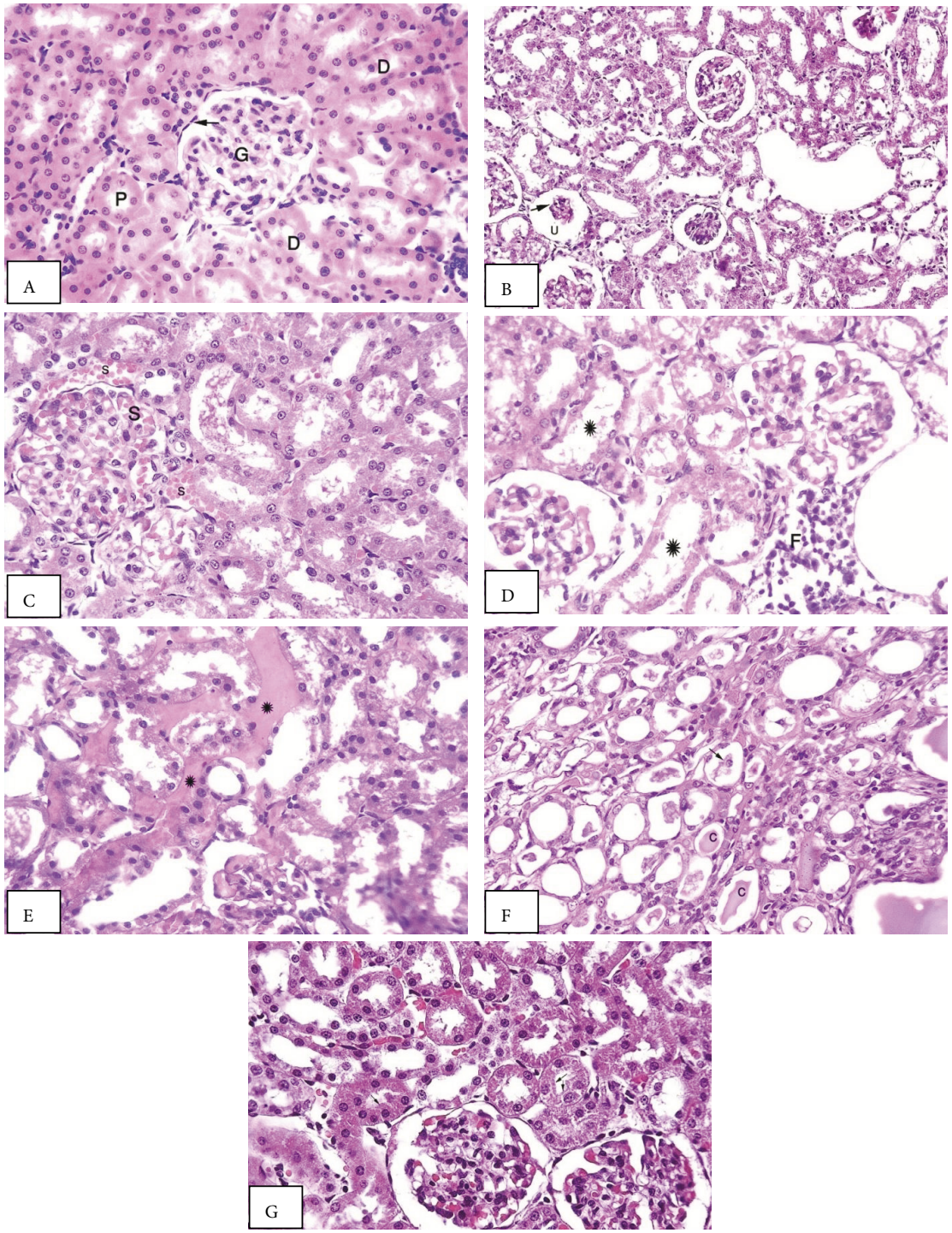

Figure 1. A- Control rat kidney showing normal renal architecture, including renal corpuscle surrounded by the outer parietal layer lined by simple squamous epithelium (an arrow) containing glomerular tuft (G) lined by mesangial cells and podocytes, proximal convoluted tubule (P), and distal convoluted tubules (D) **B**- MTX group showing renal corpuscle containing atrophied glomerular tuft (an arrow) with wide urinary space (U). **C**- MTX group showing severe congestion in the glomerular tuft and cortical blood vessels (S) with vacuolar degeneration in podocytes and mesangial cells. **D**- MTX group showing massive vacuolar degeneration in most of the tubular epithelial cells, loss of apical brush borders, severe dilatation of cortical renal tubules (*), and focal areas of peritubular lymphocytic infiltration (F). **E**- MTX group showing an interstitial edema (*). **F**- MTX group showing exfoliated cells, cellular debris (arrows), and hyaline casts (c) in the renal tubular lumina. **G**- A photomicrograph of cross-sectioned kidneys of the GE+MTX group; most of the renal architecture returned to normal, including renal corpuscles and renal tubular epithelium with their apical brush borders (arrows). H&E. 400×.

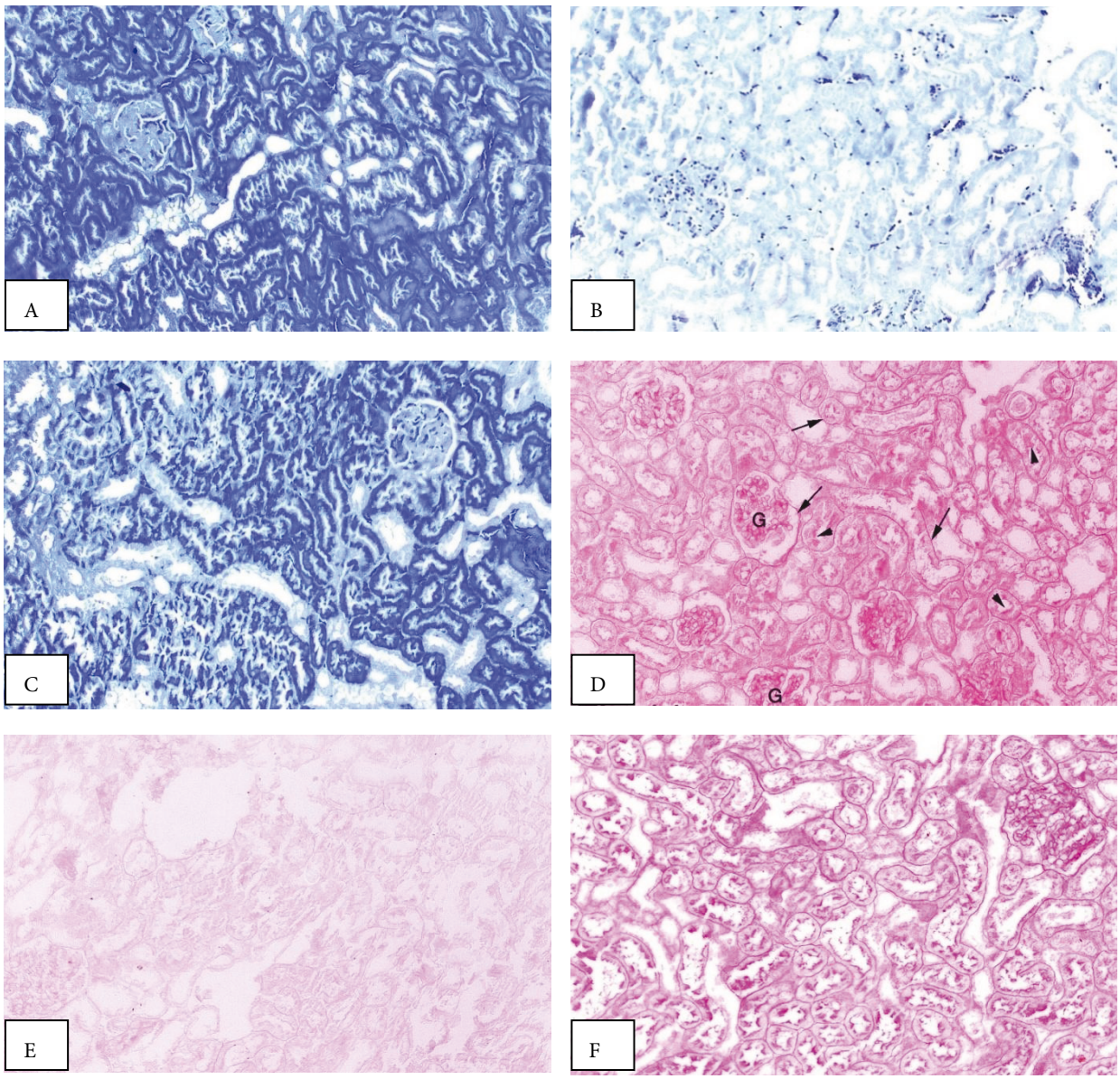

Figure 2. A- A photomicrograph of control rat kidneys stained by bromophenol method showing normal protein content, indicated by dense blue staining. **B**- A photomicrograph of kidneys of the MTX group stained by bromophenol method showing a marked reduction in protein content, indicated by faint blue staining. **C**- A photomicrograph of kidneys of the GE+MTX group stained by bromophenol method showing great improvement in protein content. **D**- A photomicrograph of PAS-stained control rat kidneys showing strong PAS-positive reaction in glomerular tuft (G), apical brush borders (head arrows), and basement membrane of renal tubules (arrows) **E**- A photomicrograph of kidneys of the MTX group showing marked decreased PAS-positive staining. **F**- A photomicrograph of PAS-stained kidneys of the GE+MTX group showing strong similarity to the PAS-positive stains of the control group. 200×.

to conserve sodium. Tubular epithelial injury may result in decreased epithelial transport activity, which leads to a decrease of tubular sodium reabsorption. Increased potassium levels may be due to the reduced excretion of potassium aggravated by the leakage of intracellular potassium into the blood stream due to renal tubular epithelium injury (38).

MTX may act as a direct toxin to the tubular epithelium or it may precipitate within the tubular lumen, leading to intratubular obstruction (39). The damaged renal tubular cells will interfere with tubular cell renewal and otherwise impair functions, causing a decline in the GFR. Furthermore, these cells could slough into the tubular lumen, resulting in a local slowing of the tubular fluid flow

and aggravating MTX precipitation in those tubules. MTX and its metabolites are poorly soluble at acidic pH levels. An increase in urine pH from 6.0 to 7.0 increases the solubility of MTX and its metabolites by 5- to 8-fold (40). Urinary alkalinization may be one of the possible underlying mechanisms of garlic extract that reduces the risk of acute kidney injury induced by MTX therapy. Furthermore, it could explain the reduced tubular cast that was seen in MTX-treated rats. Alkalinization of urine only reduces the renal damage and does not prevent it completely, suggesting that other mechanisms may also be involved in MTX-induced renal damage (2). ROS are generally considered to be key mediators of MTX-induced damage to the kidney (22). Previous studies have demonstrated increased oxidative stress in the kidneys of MTX-treated rats (14). Furthermore, MTX has been reported to reduce the methionine synthesis, antioxidant enzymes such as CAT, glutathione peroxidase, and superoxide dismutase (41). The reduction in antioxidant defense system caused by MTX may be a reason for increased ROS.

In this study, the significant decrease in the renal GSH and CAT activities in the MTX group may be the consequence of great oxidative stress, suggested by a marked increase in the level of lipid peroxidation. MTX affects energy metabolism in mitochondria and decreases the availability of NADPH in cells. NADPH is used by GSH to maintain the reduced state of cell GSH (42). The enzyme level of CAT is higher in proximal tubules to prevent excessive oxidant stress (43). The free radicals may induce the inactivation and consumption of CAT in renal tissue in MTX-treated rats. Our data show that MDA, a reliable marker of oxidative damage to lipids, was significantly increased in renal tissue of the MTX group compared to the control group. Free radicals have been implicated in MTX-induced lipid peroxidation (9). In addition, depletion of renal GSH is one of the reasons for lipid peroxidation (42).

According to the present study, pretreatment with garlic extract restored the function of the kidney, as indicated by decreased levels of BUN and creatinine levels induced by MTX. This could be attributed to garlic's antioxidant properties (44).

Moreover, in the GE+MTX group, administration of garlic led to increased renal GSH and CAT activities and reduced MDA levels compared with the MTX group. The oxidation preventive potential of garlic is possibly related to its high content of antioxidant sulfur compounds (45).

The possible mechanism by which garlic augmented the GSH level may be dietary cysteine, a rate-limiting substrate in the biosynthesis of GSH that is prevalent in garlic (46). The antioxidant components of the garlic may act as sacrificial antioxidants, sparing the depletion of GSH and CAT caused by MTX-induced oxidative stress.

In this study, it is pertinent to note that pretreatment with garlic extract significantly altered MTX-induced increases in MDA levels; however, MDA levels did not return to those of the control group. The direct cause of lipid peroxidation may be the formation of peroxynitrite from superoxide anion and nitric oxide (47). Yüncü et al. (48) found that an aqueous garlic extract halted MTX-induced MDA increases in the small intestine by preserving cellular integrity. Furthermore, raw garlic homogenate augmented the endogenous antioxidants, along with a reduction of lipid peroxidation in the rat heart, liver, and kidney in a dose-dependent manner (49).

In this study, MTX-treated rats showed high activities of adenosine deaminase and nitric oxide. ADA is an important aminohydrolase that plays a part in purine metabolism. It catalyzes the conversion reactions of adenosine to inosine and deoxyadenosine to deoxyinosine (50). In agreement with the current study, Uz et al. (51) hypothesized that ADA and NO might play an important role in the pathogenesis of MTX-induced oxidative renal damage. High ADA activity reflects accelerated purine turnover and high salvage pathway activity (52). Mitochondrial membrane dysfunction leads to impaired ATP metabolism with increased production of purine degradation products, such as adenosine and inosine, which are substrates for ADA (53). Released adenosine may further deaminate to inosine with the action of ADA.

Exposure of epithelial cells to oxidant stress leads to an elevation in NO release and nitrite production and to decreases in cell viability (54). NO seems to worsen renal injuries because of its free radical nature; through its reaction with the superoxide radical, it probably generates the very cytotoxic peroxynitrite that could damage the tubular cells, resulting in renal failure (55).

Garlic administration in the present study significantly reduced high levels of ADA activity. Several authors (49,56–58) attributed the protective action of garlic extract to its antioxidative role.

With regard to NO, the level of NO in the GE+MTX rats was lower than in MTX rats. Dirsch et al. (59) demonstrated that allicin and ajoene, two compounds present in crushed garlic, can reduce nitrite accumulation via a reduction of the inducible form of NO synthase expression, an enzyme induced by an inflammatory environment and that promotes peroxynitrite formation.

In this study, we observed severe histological alterations due to MTX toxicity, including degenerative changes in glomeruli and the renal tubules. Most glomeruli appeared shrunken and atrophied with increasing of the urinary space. Glomerular atrophy may be attributed to a decrease in the glomerular filtration of the drug as a result of capillary constriction (60).

In the present study, severe congestion appeared in the glomerular tufts and the renal blood capillaries, with interstitial edema that may be attributed to an increase in renal blood vessel permeability caused by a high dose of MTX (61). The tubular epithelium, especially the proximal and distal convoluted tubules, showed great degeneration as vacuolation accompanied by pyknosis and complete loss of apical brush borders. Some authors (61–63) presented similar findings. They stated that MTX has a lethal effect on the renal tubular epithelium due to its direct toxic damage. Dilatations of the renal tubules with accumulation of hyaline casts, cellular debris, and focal peritubular infiltration of inflammatory cells was seen in the present study. The increase of MTX in the body leads to an accumulation of MTX crystals in the nephron and dilatation of the renal tubules, resulting in renal toxicity and dysfunction (64).

Regarding the histochemical study, our results clearly indicated a massive reduction in total protein and mucopolysaccharides in the renal tissue of the MTX group. The decrease in total protein may due to the damage that occurred in the rough endoplasmic reticulum and complement ribosomes (65). Toxic doses of some drugs lead to disruption of lysosomal membranes, leading to liberation of hydrolytic enzymes in the cytoplasm and ultimately resulting in marked lysis of cytoplasmic organelles (60). This result was observed by Awasthi et al. (66), who found that the elevation of lysosomal activities was accompanied by a decrease in the total protein and nucleic acid contents in response to organophosphorus insecticides. Moreover, MTX treatment resulted in a great reduction in mucopolysaccharides, as indicated by a weak PAS reaction in the affected degenerated brush borders of renal tubules as well as the deformed basement membranes of both glomeruli and renal tubules. Similar observations were reported in toxicants causing nephrotoxicity, such as carbon tetrachloride (67) and piroxicam (60). Jahovic et al. (68) used PAS staining for studying the effect of MTX on the small intestine; they reported a negligible PAS reaction in the surface and glandular epithelium due to damaged microvillar structures. Administration of garlic extract ameliorated the histological damages caused by MTX.

In conclusion, according to our findings, which were in parallel with our histopathological evidence, MTX induced a significant increase in BUN and serum creatinine levels and a disturbance in some serum electrolyte levels as well as the activities of MDA, ADA, and NO in renal tissues. Furthermore, the resulting decrease of renal GSH and CAT activities suggested remarkable damage to the kidneys. Treatment with garlic extract restored the activities of GSH and CAT with concomitant reduction in MDA levels. Moreover, it ameliorated the glomerular and tubular alterations of MTX nephrotoxicity. The protective effect of garlic against MTX-induced renal damage is associated with its antioxidant and ROS-scavenging properties.

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