ORIGINAL ARTICLE

Green Tea's Medicinal Benefits in Reducing Methotrexate's Harmful Effects on the Kidneys

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Abstract

Background: Hundreds of millions of people worldwide consume tea every day because it is a pleasant, well-liked, affordable, socially acceptable, and safe beverage. Drinking green tea is linked to a lower incidence of degenerative diseases and illnesses. Green tea has now been included to other vitamin and nutritional supplements as a dietary supplement. **Aims of Study**: The purpose of our study was to assess how methotrexate (MTX) administration affected the kidneys and how green tea protected them. **Materials and Methods:** There were 24 male rats split up into three groups. There were eight rats in each group, and the control untreated group received nothing. The MTX group got one dose (20 mg/kg) MTX intraperitoneally. The last group of rats was given a daily dosage of 36 mg/kg body weight of green tea through a gastric tube for 9 weeks after receiving the same injection of MTX as the second group. At the end of the research, renal functioning and histological alterations were assessed. **Results:** The second group's renal architecture was clearly altered, and their serum levels of uric acid, creatinine, and blood urea nitrogen were all elevated. When compared to the MTX group, the kidney's histological image improved in the green tea and MTX group. **Conclusion:** All aspects of evidence pointed to oxidative stress as the source of MTX-induced nephrotoxicity, and green tea's anti-inflammatory and antioxidant properties protect the kidney from this damage. In the group receiving green tea and MTX, the kidneys' natural structure was largely restored.

Key words: Green tea, kidney, methotrexate, nephrotoxicity, oxidative stress, pathology

INTRODUCTION

The kidney is an essential organ that filters blood to eliminate wastes, preserves electrolyte and water balance, and controls blood pressure.[1]

The two bean-shaped, purplish-brown kidneys are located behind the peritoneum. Anticancer medications, especially chemotherapy treatments, have a high toxicity level that might result in kidney damage that cannot be repaired. While there are numerous chemotherapeutic treatments available today to treat a wide range of malignancies, their side effects have created some obstacles to their usage and the complete eradication of cancer. Nephrotoxicity, one of the most prevalent adverse effects of anticancer medications, is linked to the drug's ability to

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cause kidney damage, either temporary or permanent, and potentially risk the lives of cancer patients, worsening their condition.[2] Research has indicated that nephrotoxicity, which is mostly brought on by anticancer medications and is linked to a number of adverse effects as well as mortality, is the reason for acute renal injury in 60% of clinical cases.[3] Simple blood tests can be used to diagnose nephrotoxicity. Blood tests are used to assess nephrotoxicity.

Antineoplastic medication methotrexate (MTX) is used to treat a variety of illnesses, including cancer and inflammatory conditions. Long-term usage of this medication resulted in several negative effects on the kidney, lung, bone marrow liver, testis, and brain, among other organs.[4]

Nephrotoxicity is more common than other adverse effects due to medication excretion from the kidneys through active transport procedures and glomerular filtration. Basically, this adverse effect limits how much MTX can be used for treatment. The most frequent way that MTX causes damage is through oxidative stress, which is brought on by inflammation and produces reactive oxygen species (ROS). Several medications are utilized to mitigate the possible adverse effects of MTX due to its anti-inflammatory and antioxidant properties.[5] Natural items, such as green tea, have been demonstrated to offer protection against the deleterious effects of oxidative stress.[6] Green tea's source plant, *Camellia sinensis*, has a lot of polyphenolic flavonoids, which are potent antioxidants. Research has demonstrated the potent antioxidant, antiapoptotic, anti-inflammatory, and auto-antigen-inhibitory properties of green tea polyphenol epigallocatechin gallate.[7] We proposed that dietary antioxidants could help prevent MTX-induced renal damage due to the close relationship between oxidative stress and renal dysfunction. Many studies based on a similar hypothesis have demonstrated that melatonin and other agents can prevent MTX-induced renal damage.[8] The purpose of the current study was to assess green tea's ability to protect the kidneys from MTX -induced nephrotoxicity. Studying the structure of the kidney and assessing renal function biomarkers in plasma allowed for the completion of this investigation.

MATERIALS AND METHODS

Our study complied with guidelines for the use and care of animals in research, and it was approved by PSA University's Al-Kharj Ethical Committee (SCBR-139-2023). This experimental investigation was completed between September 2023 and August 2024. 24 healthy, 10-week-old albino rats weighing 250–290 g were employed in our study and were acquired from PSA University's animal house.

The animal care facility maintained them on a regular diet. Rats were housed under strict observation for acclimatization for approximately 14 days before the start of the investigation. The animals were split up into three groups, each with eight rats. Green tea was prepared, according to Maity and his associates, by immersing 15 g of instant green tea powder in 1 L of boiling distilled water for 5 min.[9] 1.5% green tea extract was made by filtering the mixture. Rats were given this solution as their only supply of drinking water. The dosage of MTX utilized was 0.5 mg/kg. Two intraperitoneal injections of a vial containing 50 mg/5 mL (Ebewe, Austria) were administered to the rats biweekly based on body weight.

Throughout the trial, the control group of healthy animals did not receive any medication. For a period of 9 weeks, the MTX group received a single intraperitoneal injection of 0.5 mg/kg body weight 2 times a week. The last group of rats was given a daily dosage of 36 mg/kg body weight of green tea through a gastric tube for 9 weeks after receiving the same injection of MTX as the second group. On completion of the research, all animals had been killed by cervical dislocation, and blood samples were taken through retro-orbital plexus for biochemical and hematological analysis. Kidney specimens were prepared for light microscopy analysis by embedding them in paraffin, cutting them into 5 µm slices, dehydrating them in ethyl alcohol, cleaning them in xylene, and staining them with hematoxylin and eosin (H and E). For tests on blood urea nitrogen, creatinine, and uric acid, blood samples were taken from the retro-orbital plexus. The enzyme-linked immunosorbent assay test was utilized to determine the concentrations of serum tumor necrosis factor alpha and C-reactive protein in serum. Analytical statistics were carried out by the Statistical Packages for the Social Sciences. They were employed across various groups in order to compare changes in both immunohistochemistry and histopathology.

Table 1: Findings of the kidney function serum level analysis

Parameters	Control untreated	Methotrexate treated	Green tea and methotrexate
Uric acid	1.07±0.3	1.51±0.31	0.63±0.2
BUN	22.1±1.39	25.2±4.35	17.8±3.22
TNFA	-	1.81±0.73	0.52±0.37
Creatinine	0.47±0.01	0.70±0.12	0.39±0.01
CRP	-	1.79±0.68	-

BUN: Blood urea nitrogen TNFA: Tumor necrosis factor alpha, CRP: C-reactive protein

Table 2: Analysis of kidney marker immune-histochemical score results

Parameters	Control untreated	Methotrexate treated	Green tea and methotrexate
CRP	-	1.88±0.71	-
TNFA	-	1.72±0.71	0.42±0.41

TNFA: Tumor necrosis factor alpha, CRP: C-reactive protein

RESULTS

Table 1 displays biochemical assessments of renal functioning in all tested groups. When compared to the control group, the blood levels of creatinine, uric acid, and blood urea nitrogen were higher in the MTX group than in the first group. The third group's consumption of green tea considerably raised the plasma biochemical parameters to values that were almost normal compared to the first group. Furthermore, there was no response in the first group according to Table 2, which shows the immune-histochemical scoring of blood levels of tumor necrosis factor alpha and C-reactive protein. There was a significant expression of the inflammatory makers identified in the MTX group. On the other hand, the green tea group showed lower serum levels of tumor necrosis factor alpha and C-reactive protein.

Sections from various groups' histopathological analyses reveal the first group's normal renal structures [Figure 1]. In short, the control group's glomeruli had no obvious histological anomalies.

In comparison to the normal kidney in the control group, the renal tissue histopathological analysis of rats treated with MTX revealed moderate degenerative changes, shrinkage and disruption of glomeruli, mild feathery degeneration, and increased cytoplasmic granularity in the proximal convoluted tubules with tubular hypertrophy of the distal convoluted tubules [Figure 2]. MTX administration, however, resulted in renal corpuscle damage. Glomerulosclerosis was the most notable histopathological damage. Glomeruli showed the existence of glomerular tuft capsular attachment and the obliteration of the glomerular capillary tuft [Figure 2].

Ultimately, portions of the kidney in the green tea with MTX group resemble normal kidney structure once more [Figure 3].

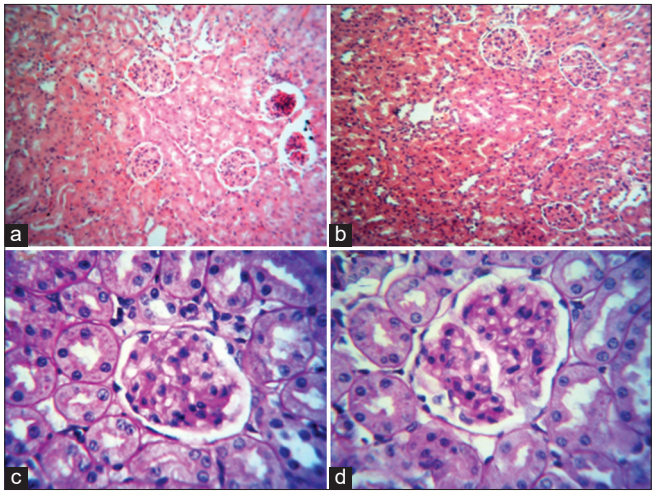

Figure 1: The kidneys from the first control group had a normal histological appearance, according to microscopical observations (hematoxylin and eosin) (a and b, ×200) (c and d, ×400)

DISCUSSION

One chemotherapeutic agent that is recommended for autoimmune illnesses, inflammatory myopathies, leukemia, and other ailments is MTX. Reactive oxygen species are frequently involved in the hepatotoxicity and nephrotoxicity adverse effects that are known to be associated with it.[10]

Oxidative stress-induced renal damage primarily occurred at the histopathological level, as demonstrated by the gross examination and histopathological findings of our study, which included hemorrhages, tubular cell necrosis, inflammatory cell infiltrations, glomerulosclerosis, and proteinous materials in tubules in the MTX group. MTX caused significant degenerative alterations in a prior research, including renal tubular injury,

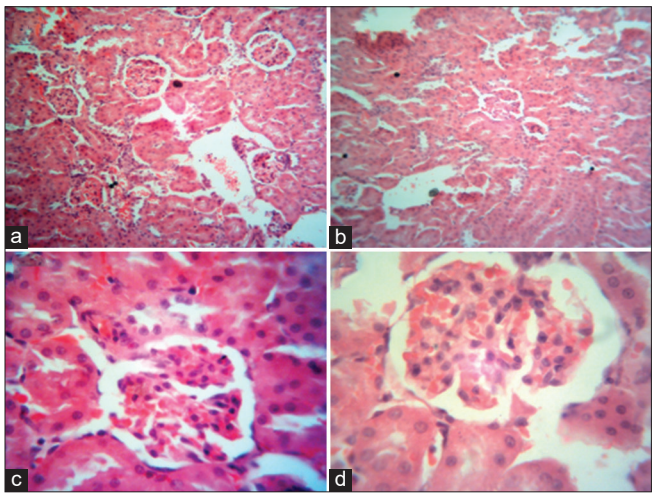

Figure 2: Microscopic observations of the kidneys with varying magnifications in the methotrexate group. Hemorrhages and proteinous materials in tubules, as well as noticeable glomerulosclerosis (hematoxylin and eosin) (a and b, ×200) (c and d, ×400)

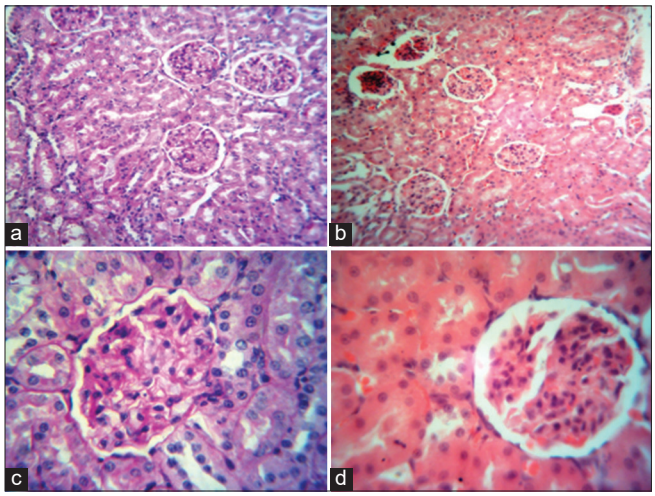

Figure 3: Sections of the kidneys in the green tea plus methotrexate group comparatively recover to their usual look, according to microscopic examinations of the kidneys (hematoxylin and eosin) (a and b, ×200) (c and d, ×400)

dilatation, tubular degeneration, and tubular cell edema.[4] Urea and creatinine, two biomarkers of renal function, were taken into account in this investigation. The kidneys eliminate metabolic products such as creatinine and urea from the bloodstream. Increase in their plasma level is an evidence of decrease in renal function.[11] This study's findings regarding the rise in creatinine and plasma urea are consistent with earlier MTX reports.[12] Green tea's nephron-protective properties and enhanced renal function are demonstrated by its reduction of plasma urea and creatinine levels. Plant resources include a higher concentration of antioxidant molecules. By reducing oxidation processes, it shields the organism from the damaging effects of free radicals, as stated in its definition.[13] Green tea is thought to have potent antioxidants. One of the components of green tea, polyphenols, contributes to its potent antioxidant qualities by preventing geno-toxicity and mutagenicity in addition to its antioxidant processes.[14] Research verifying the elevated antioxidant potential of tea beverages suggest that catechins are a significant contributor to this attribute. The leaves of the *C. sinensis* plant are a clear source of catechin, one of the polyphenol families that are good for human health. However, other researches back up the claim that tea is the primary food source of antioxidants for humans on a regular basis.[15] According to earlier studies, one toxicological mechanism of MTX nephrotoxicity has been identified as oxidative stress. The production of ROS and free radicals is a major contributor to this toxicity.[16] In this study, similar results were noted, and the mechanisms underlying MTX damage and green tea's mitigating effects were assessed.

CONCLUSION

Our research indicates that green tea may be able to guard against MTX-induced nephrotoxicity. Green tea may also have anti-inflammatory and free radical-scavenging properties as part of its defense mechanism. Therefore, green tea can be applied as a co-treatment with MTX for guarding against oxidative degradation of tissue.

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AVAILABILITY OF DATA AND MATERIALS

The data are available on request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-139-2023).

CONFLICTS OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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