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Protective effect of green tea in methotrexate-induced nephrotoxicity

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ABSTRACT

Green tea consumption is associated with reduced disease prevalence, and degenerative disorders. Subsequently, green tea has been included as a dietary supplement with other nutritional and vitamin supplements. Our study aimed to evaluate the effect of methotrexate administration on the kidneys and the role of green tea in its protection. It was performed on twenty-one albino rats. The animals were distributed into three equal groups. Control group, Methotrexate group and Methotrexate-green tea group whose rats were injected with methotrexate and green tea for nine weeks. Renal functions, histopathological as well as immunohistochemical changes were evaluated at the end of the study. Increased Blood urea nitrogen, Uric acid and Creatinine serum levels and marked changes in kidney architecture were obvious in the second group. These changes were in the form of glomerulosclerosis, tubular cell necrosis hemorrhages as well as inflammatory infiltrations. There was a relatively return to the normal structure of kidney structure in the group treated with Green tea plus Methotrexate.

Keywords: Green tea, Methotrexate, Nephrotoxicity, Oxidative stress

1. INTRODUCTION

The kidneys are pair of bean–shaped purplish-brown organs situated behind the peritoneum. The kidneys are situated behind the peritoneum, lie on the posterior abdominal wall. They are responsible for blood filtering and urine formation. Each kidney possesses two poles (lower and upper), two borders (medial and lateral). The lateral border is smooth and convex. The medial one is concave and possesses a hilum at its middle. The hilum leads to a part within the kidney named sinus of the kidney (Standring, 2021). Methotrexate is an antitumor agent and can be used in the treatment of many diseases, such as inflammatory diseases and cancer. Long term use of this factor has resulted in many side effects on various organs including the kidneys, lung, liver, bone

marrow, testicle, and brain. Nephrotoxicity occurs more than other side effects due to drug excretion by the kidneys by glomerular filtration and active transport. Essentially, this side effect limits the use of methotrexate in treatment (Jakubczyk et al., 2020).

Food antioxidants are generally safe substances found in medicinal plants and have attractive effects in reducing oxidative stress (Hamden et al., 2009; Elzoghby et al., 2014). The antioxidants such as polyphenols and catechins that make up large groups of nutritional antioxidants are predominantly found in green tea (Adel et al., 2009; Higdon & Frei, 2003) Plus, to its antioxidant effect, these nutrients have anti-diabetic, antihypertensive, anti-atherosclerotic, anti-carcinogenic, anti-oxidant, anti-inflammatory functions (Heikal et al., 2013). So, green tea consuming is associated with a lower death rate, especially from cardiovascular diseases (Al-Attar & Abu Zeid, 2013). The protective mechanism of green tea utilization against diseases is unclear. Despite this, it has been suggested that the protective role of green tea may be invoked by the antioxidant effect of catechins and polyphenols (Kuriyama et al., 2006).

The current study aimed to evaluate the preservative effect of green tea on the kidney against nephrotoxicity induced by methotrexate. This study was achieved by studying the kidney structure, and evaluating biomarkers in plasma for renal function.

2. MATERIALS AND METHODS

Our studies was done according to animal research guide line for the use and care of animals for research and were authenticated by the Ethical Committee of PSA University, Al-Kharj (PSAU-2021 ANT 9/43PI). It is an experimental study achieved in the interval from March 2021 to February 2022.

In our research, 21 healthy, aged 10 weeks, albino rats (250-310 g.) were used and obtained from an animal house at PSA University. They were kept under standard diet feeding at the Animal Care Facility. For acclimation, rats were kept under supervision for about 14 days before the initiation of the study. Animals were divided into three groups consisting of seven rats. Healthy animals in control group injected with saline intraperitoneally and received orally methyl cellulose for 10 successive days. Methotrexate group were injected with a single dose of Methotrexate intraperitoneally (the dose 0.5 mg/kg body weight two times per week for nine weeks. The last group is Methotrexate-green tea whose rats were injected with methotrexate as in the second group followed by administration of green tea at a dosage of 36 mg/kg body weight /day by a gastric tube for nine weeks. One day (24 hours) later, all rats were anesthetized and sacrificed by cervical cutting. Samples of blood were collected from retro-orbital plexus for blood urea nitrogen, Creatinine and Uric acid analyses. Measurement of levels of Tumor necrosis factor alpha and Serum C-reactive protein in serum was achieved by using ELISA assay. The kidneys were dissected then fixed in 10% formalin for histopathological examinations.

Statistical analysis Variables were carried out by SPSS 15.0 and other statistical testes such as ANOVA. To compare histopathological as well as immunohistochemical changes, they were used among different groups. ($p < 0.05$ was significant).

3. RESULTS

Table 1 shows biochemical analyses of kidney functions in all studied groups. Blood urea nitrogen, Uric acid and Creatinine serum levels were increased in Methotrexate group compared with the control group when compared with the first group. Green tea intake in the third group significantly improved the plasma biochemical parameters to nearly normal levels for the first group. In addition, in table 2, Immunohistochemical scoring of Tumor necrosis factor alpha and C-reactive protein serum levels, there was no reaction in the first group. In Methotrexate group there was strong expression of mentioned inflammatory makers. In the other hand, small number of Tumor necrosis factor alpha and C-reactive protein serum levels were observed in green tea group ($P>0.05$). Histopathological examinations of sections of different groups disclose normal renal architectures of the first group (Fig. 1) In contrast, Methotrexate treated group, showed marked changes in kidney structures. These deteriorations were in the form of glomerulosclerosis, tubular cell necrosis hemorrhages as well as inflammatory infiltrations (Fig. 2). Finally, sections of the kidney of Green tea plus Methotrexate group relatively return to the normal appearance of kidney structure (Fig. 3).

Table 1 Results of analysis of serum levels of kidney function.

	First group	Methotrexate group	Green tea with Methotrexate group	P
Blood urea nitrogen	21.3 ± 1.42	24.3 ± 4.54	16.7 ± 3.11	0.009
Uric acid	1.07 ± 0.3	1.51 ± 0.31	0.63 ± 0.2	0.012
Creatinine	0.48 ± 0.01	0.60 ± 0.13	0.49 ± 0.01	0.028
Tumor necrosis	0.00 ± 0.00	1.71 ± 0.70	0.43 ± 0.42	0.003

factor alpha				
C-reactive protein	0.00 ± 0.00	1.89 ± 0.69	0.00 ± 0.00	0.002

Table 2 Results of analysis of Immunohistochemical scoring of kidney markers.

	First group	Methotrexate group	Green tea with Methotrexate group	P
Tumor necrosis factor alpha	0.00 ± 0.00	1.71 ± 0.70	0.43 ± 0.42	0.003
C-reactive protein	0.00 ± 0.00	1.89 ± 0.69	0.00 ± 0.00	0.002

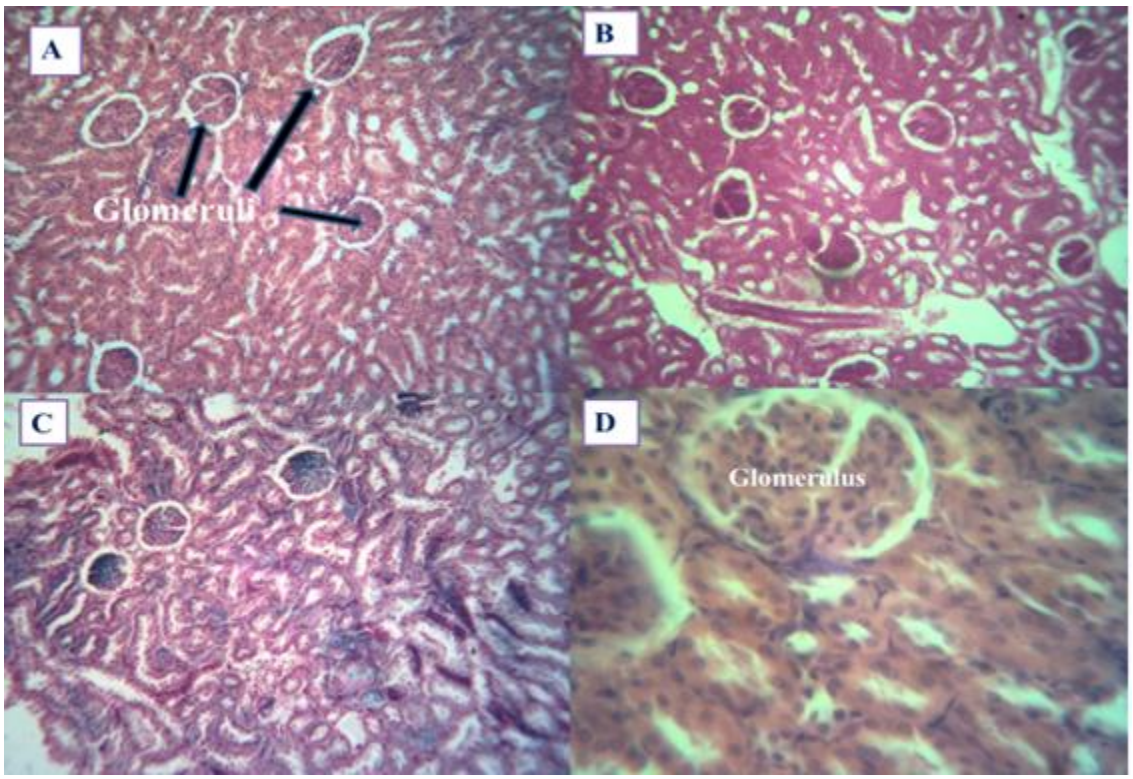

Figure 1 A, B, C and D demonstrate normal histological appearance of the kidney of first control group. Narrow Bowman's capsule surrounding the glomeruli (A, B, and C H&E X200, D X400).

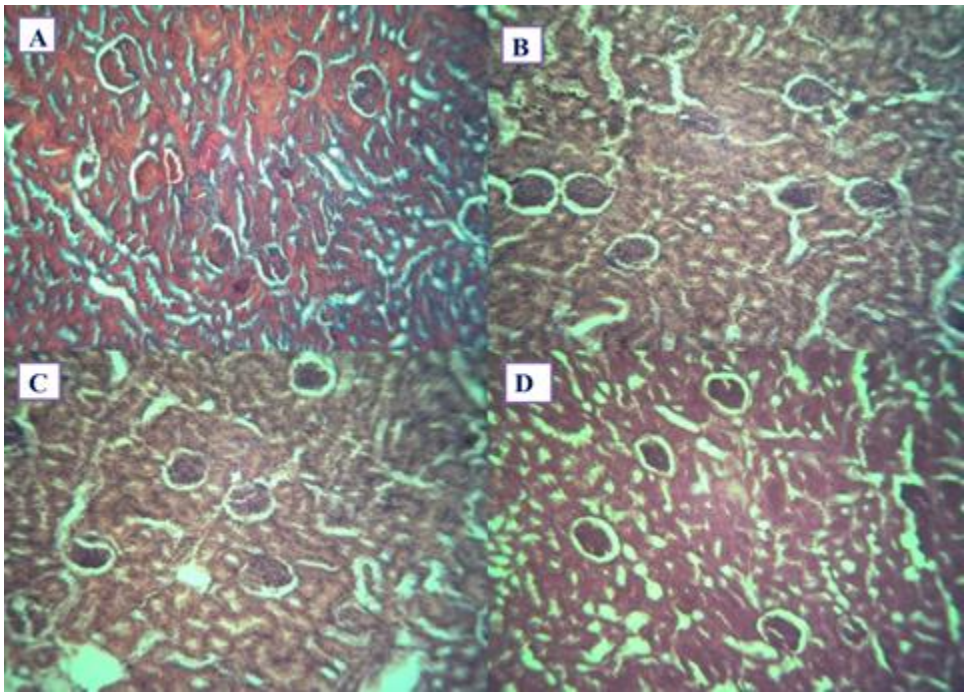

Figure 2 A, B, C and D demonstrate sections of the kidney of Methotrexate group with alteration of normal histology. Glomerulosclerosis, hemorrhage and necrosis are seen.

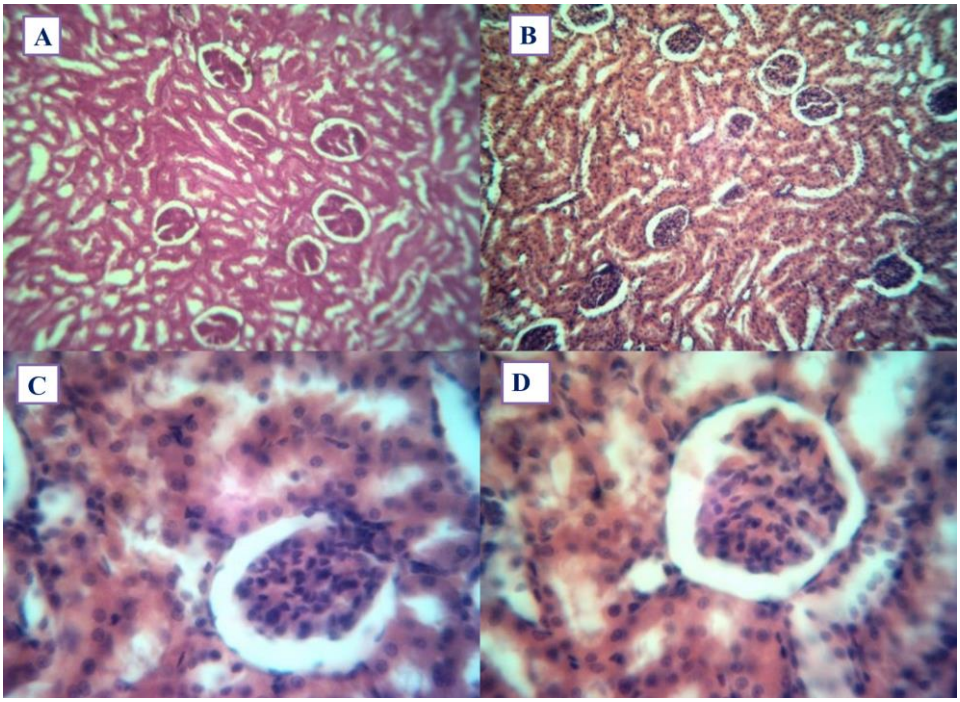

Figure 3 A, B, C and D demonstrate sections of the kidney of Green tea plus Methotrexate group relatively return to the normal appearance of kidney structure. (A, B, H&E X200, C and D X400).

4. DISCUSSION

Antioxidant compounds are more abundant in plant resources. According to its definition, it is characterized by decreasing oxidation processes, protecting the organism from the of free radical's effects (Halliwell & Gutteridge, 1995; Jakubczyk et al., 2020). Green tea is considered as strong antioxidants. Polyphenols are one of the contents of green tea that, apart from its antioxidant mechanisms, also has the ability to prevent genotoxicity and mutagenicity of compounds, which contributes to its powerful antioxidant properties (Beltz et al., 2006; Ganapathy & Srivastava, 2007).

Methotrexate is one of the primary drugs for cancer chemotherapy being a widely used therapeutic drug in the management of rheumatoid arthritis (Selga et al., 2008; Stamp et al., 2007). Our study revealed various renal structural, biochemical alterations in the group of mice treated with Methotrexate. Studies confirming the high antioxidant capacity of tea drinks indicate that catechins are the important source of this potential. Catechin is one of the of polyphenol groups, which has a beneficial effect on human health, and are clearly found in the leaves of Camellia sinensis. On the other hand, other researchers support the idea that tea can be considered the main source of antioxidants in daily human food (Pastoriza et al., 2017; Benzie & Szeto, 1999).

Biomarkers of renal function: urea and creatinine were considered in our work. Urea and creatinine are metabolic products that the kidneys remove from the circulation. An increase in their plasma level is an indication of loss of kidney function (Olayinka et al., 2016; George et al., 2014). The increase in plasma urea and creatinine observed in our study is consistent with previous work on methotrexate (Gressier et al., 1994). The impairment of the level of urea and creatinine in the plasma by green tea is an indication of the improvement of function of the kidney and the green tea role in the protective effect of the kidney (Uz et al., 2005).

Another study used a single dose of Methotrexate, and found similar biochemical results on the deterioration of kidney function caused by Methotrexate (Armagan et al., 2015). Similarly, a previous study showed that taking methotrexate caused an increase in creatinine and blood urea levels. Nephrotoxicity was induced by a single dose of methotrexate that improved all markers of biochemical and oxidative stress (Asci et al., 2017).

5. CONCLUSION

We can conclude that our findings suggest that green tea has the potential role to protect against nephrotoxicity induced by Methotrexate. In addition, the protection mechanism of green tea may involve free radical scavenging and anti-inflammatory activities. Therefore, green tea can be used as a co-therapy with Methotrexate for protection against oxidative damage to tissue. Cancer patients should eat more food sources that contain green tea during methotrexate treatment.

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Authors' Contributions

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Nasser Awadh W Almutairi and Faisal Mohammed Al Dawsari–participated in the study design and wrote the first draft of the manuscript. Abdullah buqaysh S Alshabani, Yousef Saad Almutairi, and Meshal Tami Alotaibi collected and processed the samples. Musab Khalid Alotaibi, Nasser Salman ALSaloom, Abdulaziz Ali Al Talib and Mohammad Fahad Alajmi participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

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 (PSAU-2021 ANT 9/43PI).

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Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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