ORIGINAL ARTICLE

The Therapeutic Value of Green Tea in Preventing the Negative Effects of Methotrexate on the Stomach

Ali Hassan A. Ali[1,2], Kamal R. El Baz[2], Ahmed Hassan Al-Rashidy[3,4], Abdulrahman Majed Helal Alanazi[5], Abdullah Mohammed Owaidh Almutairi[6], Abdulaziz Nasser S Almutairi[6], Salah Farhan Alanazi[6], Faisal Hamdan Borydan Alruwili[6], Mohammed Hamdan Borydan Alruwili[6], Khalid Faleh D. Alanazi[6], Maha Mohammed T. Alshalan[6], Nourah Mansour F. Alqasoumi[7]

[1]Department of Anatomy, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia, [2]Department of Anatomy, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, [3]Department of Pathology, College of Medicine, Taif University, Kingdom of Saudi Arabia, [4]Department of Pathology, Faculty of Medicine (Assiut), Al-Azhar University, Egypt, [5]College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia, [6]College of Medicine, Northern border university, Arar, Kingdom of Saudi Arabia, [7]College of Medicine, Almaarefa University, Riyadh, Saudi Arabia

Abstract

Aim: The purpose of the study was to determine how green tea contributed to the adverse effects on the stomach that methotrexate (MTX) mediated. **Materials and Methods:** Sixty adult male albino rats were split equally into three groups. The first group received intraperitoneal injections of normal saline twice a week for 9 weeks at a dosage of 0.5 mg/kg body weight. For 9 weeks, the second group received intraperitoneal injections of MTX hydrate at a dosage of 0.5 mg/kg body weight twice a week. The third group, on the other hand, was given green tea and MTX at an oral dosage of 36 mg/kg body weight/day in 0.2 mL distilled water for an additional 9 weeks. The stomachs of the sacrificed animals were processed, stained, and viewed under a microscope. Then, serum sample concentrations of interleukin (IL)-2 and IL-6 as well as glutathione peroxidase (GPx), glutathione (GSH), and superoxide dismutase (SOD) were measured. **Results and Discussion:** The histological findings demonstrated that green tea can significantly reverse the pathological alterations caused by MTX, which damages stomach tissue. Furthermore, green tea therapy exacerbated MTX-induced reductions in GSH content, GPx, and SOD activity and dramatically decreased the elevation of serum MDA, IL-2, and IL-6 contents that MTX had caused. **Conclusion:** Our findings suggest that green tea can moderately protect histological damage of the stomach in rats exposed to MTX, as well as reduce pro-inflammatory parameters and oxidative stress.

Key words: Green tea, methotrexate, oxidative stress, stomach

INTRODUCTION

Potential antimetabolite and folate antagonist methotrexate (MTX) has been used to treat autoimmune conditions such as rheumatoid arthritis and psoriasis, leukemia, lymphoma, and certain solitary tumors.[1] Diarrhea and vomiting are side effects of MTX treatment. Although the exact mechanism underlying MTX's harmful effects on tissues is unknown, one theory is that the deregulation of cellular antioxidant defense mechanisms is involved.[2]

Regrettably, serious adverse effects and toxic sequelae of many organs, including hepatotoxicity, cardiotoxicity,

Address for correspondence:
Ali Hassan A. Ali, Department of Anatomy, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia.
Phone: 00966115886171.
E-mail: alihassan3750@yahoo.com/a.ali@psau.edu.sa

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nephrotoxicity, and intestinal toxicity, have occasionally hampered the effectiveness of MTX.[3] The cytotoxic action of MTX is not exclusive to cancer cells; it also affects healthy tissues with high proliferative activity, such as the alimentary system's mucous membrane and the bone marrow's hematopoietic cells.[4] One well-known complication linked to various standard-dose chemotherapy regimens that are typically used with MTX is gastrointestinal mucositis. The most common sites of mucositis seem to be the mouth and small intestine. According to certain research, MTX damaged the small intestine's mucosa, which resulted in GI ulcers, reduced absorption, nausea, vomiting, diarrhea, and stomatitis. Intestinal mucositis affects about 40% of people using MTX at conventional dosages; at high doses, this percentage has been reported to reach nearly 100%. After intestinal mucositis develops, there is less mucin in the slime layer and it is depleted.[5] Apart from diarrhea, other adverse effects include conspicuous weight loss, reduced absorption of nutrients, and limited capacity to withstand treatment, leading to the postponement of subsequent cycles or early drug discontinuation.[6] Based on the investigations, it is known that reactive oxygen species (ROS) contribute to the intestinal toxicity brought on by MTX. Generally speaking, nutritional antioxidants are harmless compounds that can be found in medicinal plants. They have appealing benefits in complementary medicine, one of which is a significant reduction in oxidative stress.[7] Green tea is very high in polyphenols and catechins, which make up important nutritional antioxidant groups.[8] These nutrient compounds have anti-diabetic, anti-atherothrombogenic, anti-oxidative, antihypertensive, anti-carcinogenic, anti-inflammatory, and anti-fungal properties in addition to their antioxidant effect.[9] Although the exact mechanism by which green tea consumption protects against chronic diseases is unknown, it has been proposed that the antioxidant properties of the tea's polyphenols and catechins may have a preventive role in these conditions. Furthermore, in rat models, the catechins in green tea inhibit the intestinal absorption of fat and lower serum lipid levels.[10] Therefore, our research demonstrated the therapeutic value of green tea in preventing MTX's harmful effects on adult male stomach albino rats.

MATERIALS AND METHODS

The Al-Kharj Ethical Committee of PSA University's rules for the use and care of animals in research were followed in the performance of our work (SCBR-137-2023). In addition, the protocol for this study was developed in accordance with the Laboratory of the International Committees for the Protection of Animal Rights' ethical guidelines. We began our research in March 2023 when the Ethical Committee released the IRB.

60 mature male albino rats weighing between 200 and 250 g each were picked up from the animal house at PSAU in KSA. They were kept in an animal housing.

The rats were kept in a controlled environment in the animal housing facility of the laboratory for experimental surgery and surgical research at PSA University. We bought 2.5 mg MTX tablets from Orion Corporation in Espoo, Finland. However Erythropoietin (5000 IU) was purchased from Germany, Mannheim city Company called Recormon, a Roche Diagnostics GmbH product. The third group received green tea orally through a gastric tube from Tecno Med Company in Saudi Arabia.

Conditions are all consistent with typical and customary laboratory conditions. Following 2 weeks of acclimation, the animals were divided equally into 3 groups, each with 20 rats. In the first group (G1) the animals assigned to the control one received intraperitoneal injections of normal saline twice a week for a total of 9 weeks at a dose of 0.5 mg/kg BW. For a total of 9 weeks, animals in the second group (G2) received intraperitoneal injections of MTX hydrate at a dose of 0.5 mg/kg BW twice a week. The final group of rats (G3) the animals in the MTX-green tea-managed group received IP injections of MTX hydrate at a dosage equivalent to that of group 2 for a comparable amount of time. After that, they received gastric tube administration of green tea at a dosage of 36 mg/kg BW/day in 0.2 mL distilled water for a further 9 weeks. Then, ether inhalation anesthesia has been given to the rats. Rats' left ventricles were used to draw blood samples, and the serum was separated by centrifugation at 1000 g for 10 min. The serum was then kept at −20°C until analysis. Samples of the stomach were taken out right away after the death. 10% neutral formalin was used to fix the stomach, and it was left at room temperature for 48 h. All experimental groups' fixed stomach tissues were dehydrated with increasing alcohols, cleaned with xylol, and embedded in paraffin wax. The activity of glutathione peroxidase, glutathione, and superoxide dismutase (SOD) was evaluated using a diagnostic kit manufactured by ZellBio Company.[11] SOD activity units (U) per milliliter are used to express the results. Using ELISA kits from the Abcam Company, the pro-inflammatory cytokines interleukin 2 and 6 in the serum were measured. Sections were cut at 5 µm and placed on slides coated with polylysine. Hematoxylin and eosin was used to stain the slides after the paraffin wax was removed using xylene and they were rehydrated using a series of graduated alcohols. Version 17 of the SPSS software program was used for data collection and analysis. F-test (ANOVA) analysis of quantitative data was used to compare the various groups. To calculate the correlation between various parameters, Pearson's coefficient was used. Furthermore, *P*-values obtained from the least significant difference test that was lower than 0.05 were considered statistically significant.

RESULTS

The control group's stomach fundus displayed thick mucosa that was muscularis mucosa-separated from the submucosa. Gastric glands are arranged in a dense layer, tall and straight,

in the lamina propria. Each one has a base, neck, and isthmus with little stomach pits. With basophilic cytoplasm and basally positioned nuclei, the principal cells (peptic) were positioned basally and gave the basal region of the gland its dark look. They also seemed low columnar [Figure 1].

Group II (treated with MTX) revealed the sloughing off of surface epithelial cells into the lumen, the desquamation of gastric glands, and the shrinking of gastric pits. In the submucosa, inflammatory cellular infiltration and congested blood arteries were observed. Vacuolated cytoplasm was seen in certain parietal cells, while deep acidophilic cytoplasm and darkly pigmented nuclei were seen in others. Sections had varying degrees of lesions on the mucosa. Widening between the fundic glands and several regions of tissue loss were visible in some sections. In the lamina propria as well as in the spaces between fundic gland cells, congested dilated blood veins were observed. The gland's basal region, on the other hand, was dilated and had a thinned lining epithelium. The morphology of the chief cells varied; some had highly pigmented, flat nuclei, while others had foamy, vacuolated cytoplasm. There were several atrophied glands in the basal region. Certain portions of the lamina propria showed evidence of mononuclear cellular infiltration. Others displayed follicle-shaped aggregates of lymphoid cells in the lamina propria that extended to the fundic gland's basal region [Figure 2].

Examined stomach sections of the third group (treated with green tea) revealed that the fundus histological structure was essentially the same as that of the control group. The fundic glands were positioned normally, and the mucosa seemed undamaged. The surface columnar cells that made up the fundic gland's lining cells had normal-looking cytoplasm and nuclei [Figure 3]. Compared to the control group, the MTX group had a considerably reduced serum concentration of the SOD enzyme. Once more, Table 1 shows that the green tea treatment dramatically increased this value in comparison to the MTX group. Serum IL-6 levels were low in the control group and significantly higher in the MTX group, as shown in Table 2 illustrates. On the other hand, green tea therapy dramatically reduced the rise in IL-6. The analysis of the data revealed that the control group had a serum IL-2 level of 288.78 ± 2.32, which was statistically significant when compared to the MTX group.

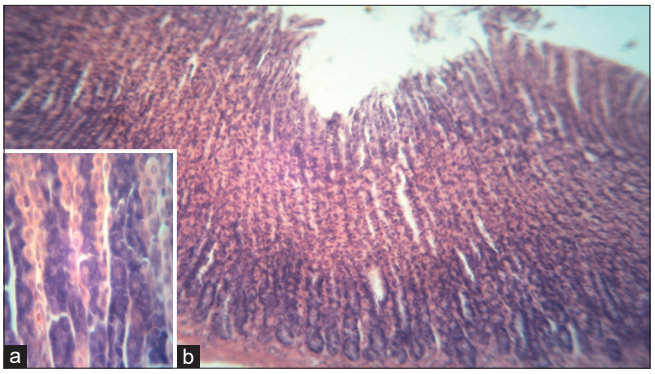

Figure 1: An image captured under a microscope of a portion of the control group's stomach reveals many straight, tubular glands that are perpendicular to the surface. (a) H&E, ×400. (b) H&E, ×200

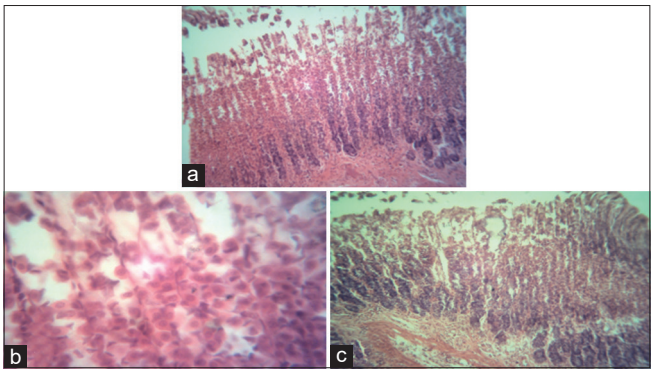

Figure 2: A photomicrograph of a portion of the stomach fundic mucosa in the group receiving methotrexate demonstrates several regions of tissue loss that are expanding between the fundic gland. Certain parietal cells exhibit cytoplasmic vacuolization. (a) H&E, ×200. (b) H&E, ×400 (c) H&E, ×200

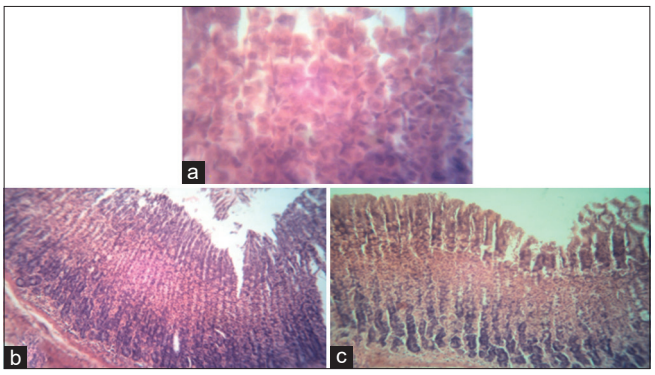

Figure 3: An image of the glandular gastric mucosa from the green tea-treated group demonstrates a nearly normal gastric mucosa. The mucosa appeared unharmed, and the fundic glands are positioned correctly. (a) H&E, ×400. (b) H&E, ×200 (c) H&E, ×200

DISCUSSION

We looked at whether green tea administration could lessen the harm caused by MTX treatment to the stomach tissue. In comparison to the control group, we observed a notable enlargement of the stomach glands as well as erosion and loss of epithelium on the mucosal surface in the MTX group. These findings are consistent with a number of recent investigations that shown a considerable rise in gastric mucus in rats given different natural chemicals as treatments for drugs that caused gastric mucosal damage.[12] Chemotherapy treatment has several side effects, primarily in the gastrointestinal tract, when used for various cancer types. These side effects lead to treatment-related morbidity. The stomach epithelium is more susceptible to chemotherapy and

Table 1: Impact of green tea on methotrexate-induced decrease in antioxidant serum parameters

Groups	Superoxide dismutase (U/mL)	Glutathione peroxidase (U/mL)	Glutathione (nmol/mL)
G1 (Control)	55.64±6.44	89.43±11.69	149.49±14.44
G2 (Methotrexate)	14.31±3.22	50.07±6.57	75.95±8.67
G3 (Methotrexate+green tea)	42.97±5.12	69.23±8.39	96.03±11.39

Table 2: Green tea's impact on methotrexate-induced serum cytokine content was assessed using two different metrics

Groups	Serum interleukin 6	Serum interleukin 2
G1 (Control)	322±29.32	288.78±2.32
G2 (Methotrexate)	520.16±74.22	411.49±3.33
G3 (Methotrexate+green tea)	332.13±5.32	245.13±5.69

has a faster turnover rate.[13] Because of all these findings, which point to the necessity of using herbal therapy to lessen the effects of MTX, green tea was used in this study. Ginger's impact on peptic ulcers was shown in earlier research using animal models.[14] Furthermore, it is yet unknown how green tea protects against stomach harm. Therefore, the current study's goal was to investigate green tea's potential to mitigate MTX-induced stomach damage in a rat model. Our results corroborated those of other authors who examined the detrimental effects of repeatedly administering 5-fluorouracil therapy in rat stomachs, which resulted in morphological and functional abnormalities and enhanced the mucosal sensitivity to acid.[15] We propose that the treatment of MTX increases the vulnerability of stomach cells to oxidative stress. We also propose that green tea's inhibition of oxidative stress may help shield the stomach from MTX-induced injury. When ROS are produced, different transcription factors, including nuclear factor-Kb (NF-B), are activated. These results in increased transcription of genes linked to the development of mucositis and set off a series of reciprocal biological events. Pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF), are encoded by these genes. It is feared that cytokines particularly TNF and IL-6 are major contributors to intestinal inflammation.[16] According to research conducted *in vitro*, a modest amount of IL-2 has been identified as a critical growth factor that is required to sustain autoimmunity. In addition, the combination of MTX and IL-2 in rheumatoid arthritis patients led to positive improvements in immunological and clinical responses, and the administration of low doses of IL-2 has novel and useful therapeutic efficacy in these patients, according to the results of a recent clinical trial study conducted by precedes study.[17] Thus, we assess the levels of IL-2 and IL-6 in the serum of several research cohorts. The results showed that the animal group receiving MTX had significantly higher levels of these pro-inflammatory cytokines in their serum as compared to normal levels.

CONCLUSIONS

In short, the goal of the current study was to evaluate if green tea could help lessen the harm that MTX causes to the stomach in an animal model. The results of this study demonstrated that green tea can reduce several forms of histological, pro-inflammatory, and biochemical damage. Supplementing with green tea may help lessen the detrimental pathological impact on the stomach. It can be added to food as a supplement to help avoid stomach damage and diseases.

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INFORMED CONSENT

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with the Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-137-2023).

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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