

Determination of the Protective Activity of Pomegranate (*Punica granatum* L.) Peel Extract Against Mitomycin-C Induced Toxicity by Spectrophotometric, Electrophoretic Histological and Image Processing Methods

Evren Koç¹

¹ Department of Bioengineering, Faculty of Engineering and Architecture, Kafkas University, Kars, Turkey

Pınar Aksu Kılıç², Özlem Önen², Erhan Uluman²,

² Department of Biology, Faculty of Arts and Sciences, Kafkas University, Kars, Turkey

Ali Berkan URAL^{3*}

³ Department of Electrical Electronics Engineering, Circuit and Systems/Biomedical, Kafkas University, Kars, Turkey

*Corresponding author: berkan.ural@kafkas.edu.tr; uralberkan@gmail.com

Abstract- Mitomycin-C (MMC) is a chemotherapeutic agent used in cancer treatment but has serious side effects. Pomegranate (*Punica granatum* L.) peel is rich in antioxidants, phytochemicals, and other bioactive compounds, suggesting potential health benefits. This study aims to evaluate the potential protective effects of pomegranate peel extract against MMC-induced toxicity using spectrophotometric, electrophoretic, histological and image processing methods. In the study, 48 female albino mice (*Mus musculus*) were divided into six groups and the groups were determined as control, Mitomycin-C (MMC), 150 mg/kg pomegranate peel extract, 300 mg/kg pomegranate peel extract, MMC + 150 mg/kg pomegranate peel extract and MMC + 300 mg/kg pomegranate peel extract. Following the application, blood samples were collected from the mice to determine serum biochemical parameters using spectrophotometric methods and to analyze serum protein expressions via electrophoresis. Additionally, tissue samples from the small intestine and spleen were collected for histological examination. Significant increases in AST enzyme levels were observed in the MMC group, while lower AST enzyme levels were recorded in the MMC + pomegranate peel extract groups. In electrophoretic analyses, it was determined that protein expressions increased only in the MMC-treated group, whereas expressions decreased in the pomegranate peel extract-treated groups and the MMC + 150 mg/kg pomegranate peel extract-treated groups. Histological examinations demonstrated that pomegranate peel extract attenuated tissue damage in the small intestine and spleen caused by MMC. Moreover, Our study has been reinforced by experimental results supported in detail with visual data obtained through graphical representations of intensity distributions of gel images, generated using image processing methods in the MATLAB environment. This study indicates that pomegranate peel extract may have protective effects against MMC toxicity. It is proposed that pomegranate peel extract could be utilized to mitigate the side effects of Mitomycin-C during cancer treatment; however, additional research is required to validate these findings.

Keywords: *Punica granatum* L., Mitomycin-C, biochemical parameters, electrophoresis, histological examination, image processing

I. INTRODUCTION

In modern medicine, continuous research is conducted to develop effective treatment methods for combating cancer [1, 2]. Cancer remains a significant global health issue, with treatment options often being limited in the advanced stages of the disease. Therefore, the development of new and effective therapeutic agents for cancer treatment has become one of the primary objectives of the scientific community [3]. Mitomycin-C (MMC) is an antineoplastic agent that inhibits the growth and proliferation of cancer cells [4]. However, the use of MMC can result in various adverse effects, including chemotherapy-induced side effects and long-term toxicity risks [5, 6]. Therefore, the potential use of naturally derived compounds to reduce or prevent MMC toxicity has become an active area of research [7]. Pomegranate (*Punica granatum* L.) peel is an extract commonly used in traditional medicine and is associated with various health benefits [8, 9]. It is stated that various components of this plant possess antioxidant, anti-inflammatory, and anticancer properties [10, 11, 12]. The objective of this study is to assess the potential protective effects of pomegranate peel extract against Mitomycin-C (MMC) toxicity using spectrophotometric, electrophoretic, and histological techniques. The findings of this research may help us understand the potential of compounds derived from natural sources to reduce the toxicity of MMC. Therefore, evaluating the protective effects of pomegranate peel extract against MMC toxicity could be an important step in supporting efforts to develop new strategies for cancer treatment.

II. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extract

Pomegranate (*Punica granatum* L.) was obtained from the Antalya region. The peel sections of the fruit were separated and dried in a well-ventilated, shaded area. It was ground using an IKA A11 (Staufen, Germany) basic laboratory mill. Fifty grams of the ground plant material was placed in a Soxhlet apparatus cartridge, washed with ethanol as the extraction solvent, and subsequently transferred into a 500 mL Soxhlet extractor. The solvent was extracted for approximately 10 hours (10–15 siphon cycles) until it became clear. Following this, the liquid extracts were filtered using filter papers with a pore size of $<2\ \mu\text{m}$ and a diameter of 110 mm (Grade 589/3, Whatman, UK) to eliminate particles. The filtered extract was evaporated at 35–45 °C using a rotary evaporator. The remaining pomegranate peel extract in the flask was kept in a desiccator for 12 hours. The extract was transferred to an extract container and stored at 4 °C for use in the study [13].

2.2 Animal Material

The study utilized 48 female mice (*Mus musculus* var. albinos), aged 3–4 months. Ethical approval for the research was obtained from the Kafkas University Local Ethics Committee (2019/03). The animals were housed at a room temperature of $20 \pm 2^\circ\text{C}$, with a 12-hour light/dark cycle and 50% humidity, and were given ad libitum access to food. The mice were divided into the following groups, with eight mice in each group:

Group 1: Control group (mice were orally administered a saline solution for 15 days).

Group 2: Mitomycin-C (MMC) group. Mice in this group were administered 2 mg/kg Mitomycin-C (Sigma cat. No: M0440) intraperitoneally on day 15. On day 16, cervical dislocation was performed under ether anesthesia.

Group 3: 150 mg/kg Pomegranate (*Punica granatum* L.) Peel Extract. Mice in this group were orally administered 150 mg/kg pomegranate peel extract via gavage for 15 days.

Group 4: 300 mg/kg Pomegranate (*Punica granatum* L.) Peel Extract. Mice in this group were administered 300 mg/kg pomegranate peel extract orally via gavage for 15 days.

Group 5: 150 mg/kg Pomegranate (*Punica granatum* L.) Peel Extract + Mitomycin-C. Mice in this group were orally administered 150 mg/kg pomegranate peel extract via gavage for 15 days, followed by an intraperitoneal injection of 2 mg/kg Mitomycin-C at the end of day 15.

Group 6: 300 mg/kg Pomegranate (*Punica granatum* L.) Peel Extract + Mitomycin-C. In this group, mice were orally administered 300 mg/kg pomegranate peel extract via gavage for 15 days, and at the end of day 15, 2 mg/kg Mitomycin-C was administered intraperitoneally.

At the end of the experimental period, cervical dislocation was performed on all animals under ether anesthesia, and blood and tissue samples were collected.

2.3 Biochemical Analyses

Blood samples collected from the control and experimental groups were analyzed spectrophotometrically using commercial kits to determine serum aspartate aminotransferase (AST) levels (SEB214Ra Enzyme-Linked Immunosorbent Assay Kit) and alanine aminotransferase (ALT) levels (SEA207Ra Enzyme-Linked Immunosorbent Assay Kit).

2.4 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein concentrations of the samples were measured by the biuret method and SDS-PAGE was performed according to the methods of Laemmli (1970) and O'Farrell (1975) [14, 15]. Proteins were separated on slab gel measuring 10 × 10 cm with a thickness of 1 mm. Each sample was mixed with a standard sample buffer, and after the protein concentration was adjusted to 2 µg/µL, the sample was denatured by boiling in water for 3 minutes. Each well was loaded with 15 µL of serum sample and 10 µL of standard protein, and a voltage of 200 volts was applied until the bromophenol blue dye reached the bottom of the gel. After electrophoresis, the gels were stained for 20-30 minutes in a staining solution containing 0.125% Coomassie Blue R-250, 40% methanol, and 7% acetic acid in a water bath at 56°C. Excess stain was removed for 1 hour using a de-staining solution containing 5% methanol and 7.5% acetic acid, with the solution being changed every 20 minutes in a water bath at 56°C. In electrophoresis, Prestained Molecular Weight Marker (M.W. 26,600-180,000 Da) (Sigma-Aldrich, Cat no: SDS7B2, St. Louis, USA) was used as the protein standard.

2.5 Analysis of Gel Electrophoresis by Algorithm

For image analysis, the multiple minima found in the new image profile are related to the separation between each lane of the gel image. In this region, the number of white pixel values in the resulting array is seen to decrease, which helps in determining the number of stripes in the image. On the other hand, when the analysis is performed for each lane, the multiple maxima in the new image profile are associated with the position of the proteins, as they are represented by white. The locations of maximum intensity represent the presence of proteins, while locations of lower intensity indicate the absence of proteins.

Several parameters were selected and used in this procedure. These parameters, however, were algorithmically designed and implemented in MATLAB 2024a software, and the relevant flowchart is shown in Figure 1. Additionally, MATLAB software codes are given in detail in the Appendix section.

MATLAB 2024a Code Flowchart :

1. Image Acquisition
2. Image Resizing Process
3. Grayscale Image Acquisition
4. Conversion to Binary Image and Otsu Segmentation
5. Dilation-Erosion Process with a 5-pixel Mask in the Image
6. Image Inversion Process
7. Area Determination Process
 - 7.1. The process of automatically drawing the size of the image as rows and columns
 - 7.2. The process of determining the x and y coordinates of the exact center of the image when the image is in a vertical position and drawing a vertical line
 - 7.3. Drawing the intensity distribution graph obtained from this area using the Improfile code

2.6 Histological Analyses

The tissue samples obtained for histopathological examination were fixed with formalin for 24 hours, processed through increasing alcohol series, cleared, and embedded in paraffin. The 5 μ m thick sections obtained using a microtome were stained with Hematoxylin-Eosin (H&E) method, examined under a light microscope, and photographed.

2.7 Statistical Analyses

The Kolmogorov-Smirnov test was used to determine the normality of the data obtained from biochemical analyses. The statistical significance of the differences between groups for data which were showing normal distribution was determined using One-Way ANOVA followed by the post-hoc Tukey test.

All analyses were performed using SPSS 22 software.

III. RESULTS

3.1 Biochemical Results

The AST levels in the MMC group showed a statistically significant increase compared to the other groups ($P < 0.001$). In the MMC + 150 mg/kg PG group and the MMC + 300 mg/kg PG group, it was observed that pomegranate peel extract prevented the MMC-induced increase in AST levels ($P < 0.001$). No statistical difference was found between the groups in the levels of ALT, another liver enzyme, and total protein levels ($P > 0.5$).

Table 1: Liver enzyme levels and total protein levels of the experimental and control groups.

	Control	MMC	150 mg/kg PG	300 mg/kg PG	MMC + 150 mg/kg PG	MMC + 300 mg/kg PG
AST (U/L)	77.33 $\pm$ 5.05 ^c	124.00 $\pm$ 6.63 ^a	74.33 $\pm$ 8.21 ^c	78.50 $\pm$ 7.99 ^c	109.17 $\pm$ 10.78 ^b	110.00 $\pm$ 5.48 ^b

ALT (U/L)	15.67 ± 1.75	17.50 ± 3.27	17.83 ± 2.40	18.50 ± 4.51	18.67 ± 2.07	17.67 ± 5.72
Total Protein (g/L)	4.40 ± 0.43	4.72 ± 0.36	4.58 ± 0.29	4.43 ± 0.35	4.45 ± 0.10	4.73 ± 0.85

* Different letters on the same row indicate statistical significance ($P < 0.001$).

3.2 Electrophoretic Results

The configuration element value for erosion and dilation were set to 20 for lanes and 5 for bands, which enabled the determination of the total number of samples and bands per protein in the respective gel.

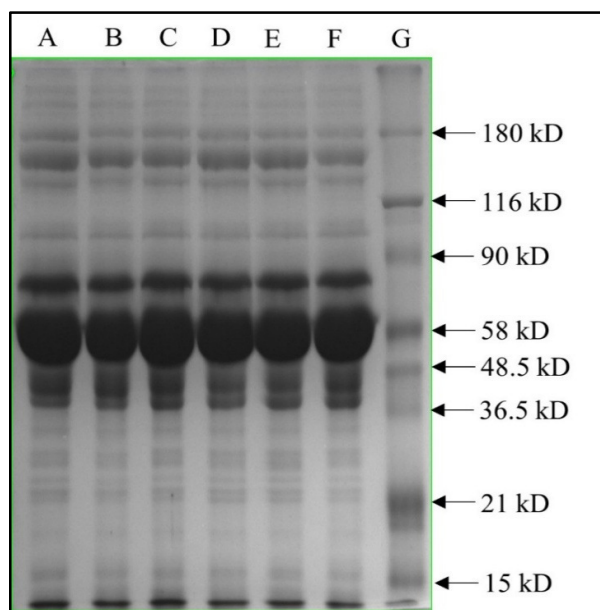


Figure 1) The gel image obtained from SDS-PAGE. **A.** Control, **B.** MMC, **C.** 150 mg/kgPG, **D.** 300 mg/kgPG, **E.** MMC + 150 mg/kg PG, **F.** MMC + 300 mg/kgPG. **G.** Standart proteins

The lanes used in the image represent protein concentrations at different levels. The images of the gel lanes were analyzed using the density profile shown in Figure. The gel image in Figure 1 below contains samples of serum protein at different concentrations.

Preliminary analyses were conducted on the image to verify whether the density profile plots could detect the presence and overexpression of proteins. Figure 2 shows the density profile of the molecular weight control or ladder (for lanes). In the gel image of Figure 1, columns A-G are sequentially related to the graphs a-g in Figure 2.

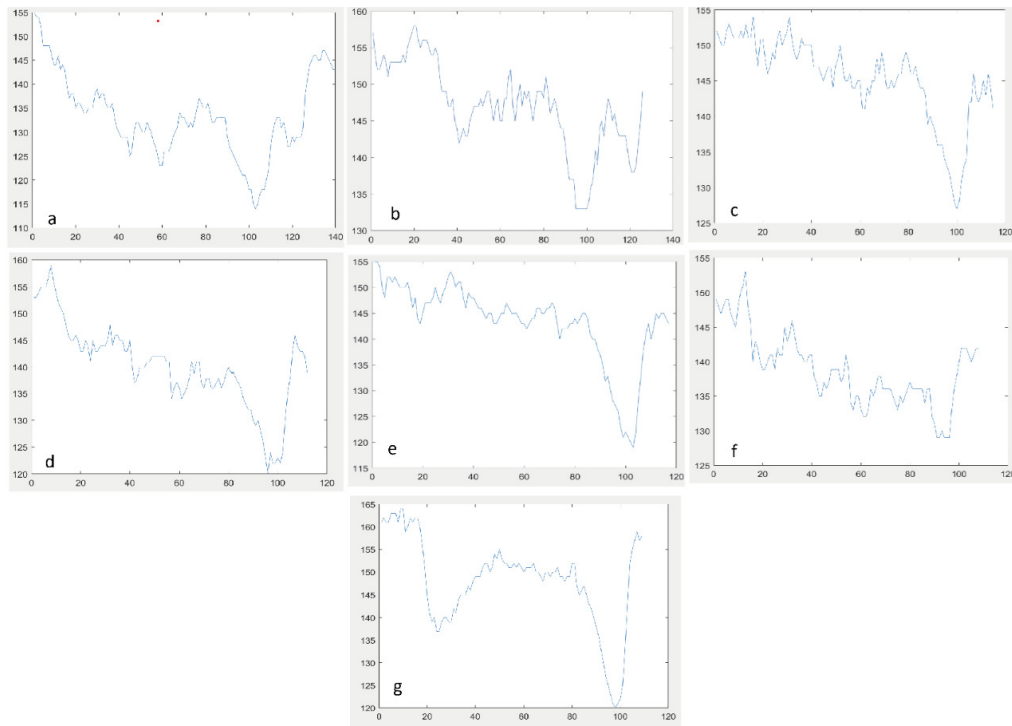


Figure 2) a. Control, b. MMC, c. 150 mg/kgPG, d. 300 mg/kgPG, e. MMC + 150 mg/kg PG, f. MMC + 300 mg/kgPGg. Standart proteins

The minimum values in the graph coincide to the positions of the protein bands of the sample lanes or the standard sample, which are used to determine the molecular weight of the analyzed samples in the rest of the gel.

The visible sharp and abrupt minimum regions in the lane intensity plots indicate the presence of the protein with the highest expression or concentration in the lanes. The background image formed by proteins in the total extract and varying serum protein concentrations in the samples can also be observed. In general, the regions corresponding to the multiple maxima in the graph cannot be directly associated with each distinct expression of serum proteins.

Considering the 7 standard protein lanes, it was observed that the maximum intensity value was obtained in the second lane, indicating the overexpression of serum protein in that specific sample. The lowest expressions were detected in lanes 3, 4, and 5, and maximum values of 153, 155, and 158 were obtained, respectively.

3.3 Histological Findings

Jejunum:

As observed in the control group samples, the jejunum, a section of the small intestine that constitutes the extensive absorption surface of the gastrointestinal tract in *M. musculus*, is organized into mucosa, submucosa, muscularis externa and serosa layers, progressing outward from the lumen. In the mucosa layer where there is loose fibrous connective tissue (lamina propria) covered with cylindrical epithelial cells and Lieberkühn glands, known as the digestive structure specific to the jejunum the epithelial cell membranes are not apparent, but the presence of nuclei can be observed and goblet cells can be easily distinguished. Underneath the muscularis mucosae layer, which appears as a thin smooth muscle layer, there is a submucosa layer consisting of loose fibrous connective tissue. At the bottom, there is the muscularis externa, a layer of muscle arranged in transverse and longitudinal muscle layers, and the serosa layer, which surrounds the outermost part as connective tissue (*Figure 3a-b*). Only the groups that treated pomegranate extract showed no changes in jejunal histology (*Figure 3c-d*). In the jejunal histology of the MMC-treated group, there is significant edema and necrosis in the Lieberkühn glands accompanied by prominent ruptures on the lumen-facing side of the mucosa, seen as the presence and absence of brush borders and even villi. Prominent lymphocyte infiltration is observed in the lamina propria and muscularis externa layers. Necrosis has reached striking levels between the submucosa and muscularis mucosa layers. Desquamation and necrosis are evident in the serosa layer (*Figure 3e*). In the jejunum histology of the MMC+150 mg/kg treated group, there was mild necrosis observed in the form of occasional separations in the mucosal epithelium. The lamina propria and Lieberkühn glands have a similar appearance to the control group. There is a very mild lymphocytic infiltration in the muscularis externa layer. The serosa layer has a normal appearance (*Figure 3a*). In the MMC+300 mg/kg pomegranate extract-treated group, unlike the MMC+150 mg/kg applied group, mild edema was observed around the Lieberkühn glands in the mucosa layer, with lymphocyte infiltration being slightly more pronounced in the muscularis externa layer. No necrosis was observed in the overall appearance (*Figure 3f-g*).

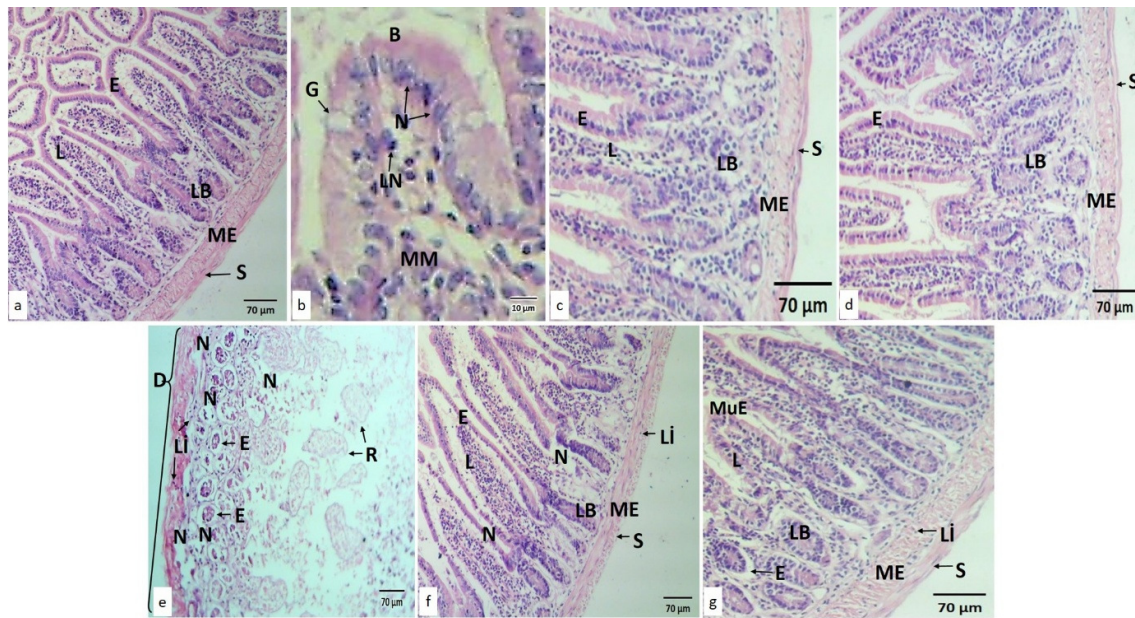


Figure 3) a. General structure of jejunum in control group, Mucosal epithelium (E), lamina propria (L), Lieberkühn (LB) glands, muscularis externa (ME) and serosa (S), **b.** General structure of jejunum in control group, Enterocytes with cylindrical nuclei (N), brush border (B), goblet cells (G), lymphocytes (LN), goblet (G) cells, muscularis mucosa (MM), **c.** 150 mg/kg pomegranate extract, **d**) 300 mg/kg pomegranate extract. Mucosal epithelium (E), lamina propria (L), Lieberkühn (LB) glands, muscularis externa (ME) and serosa (S), **e.** General structure of jejunum in MMC group. Ruptures (R), edema (E), intense desquamation (D) in apical epithelial cells and lymphocyte infiltration (LI) into lamina propria and muscularis mucosa, necrosis (N), **f.** MMC+150 mg/kg pomegranate extract group, **g.** MMC+300 mg/kg pomegranate extract group. Mucosal epithelium (MuE), lamina propria (L), Lieberkühn (LB) glands, edema (E), muscularis externa (ME) and serosa (S), lymphocyte infiltration (LI) into muscularis mucosa and necrosis (N), H&E.

Spleen:

In the control group samples, the spleen tissue of *Mus musculus*, which is one of the essential organs of the hematopoietic and immune systems, consists of red pulp and darker-colored white pulp, surrounded by a thin capsule. The white pulp contains a central blood vessel, known as the central arteriole, which is surrounded by a cluster of lymphocytes. The red pulp consists of sinusoidal spaces and a light lymphocyte aggregation with red blood cells (*Figure 4a*). No alterations were observed in the spleen histology of the groups treated only with pomegranate extract (*Figure 4b-c*). In the spleen histology of the MMC-treated group,

deformations were observed as significant separations in the areas separating the white pulp and red pulp, and distinct separations were also present within the red pulp. There are hemorrhagic foci in places within the white pulp. In the red pulp, findings of focal vasodilation and significant tissue damage (necrosis) are observed in some areas (*Figure 4d*). No differences were observed in the histology of the spleen between the MMC+150 mg/kg (*Figure 4e*) and MMC+300 mg/kg (*Figure 4f*) pomegranate extract-treated groups, and vacuolization was present in places in both groups.

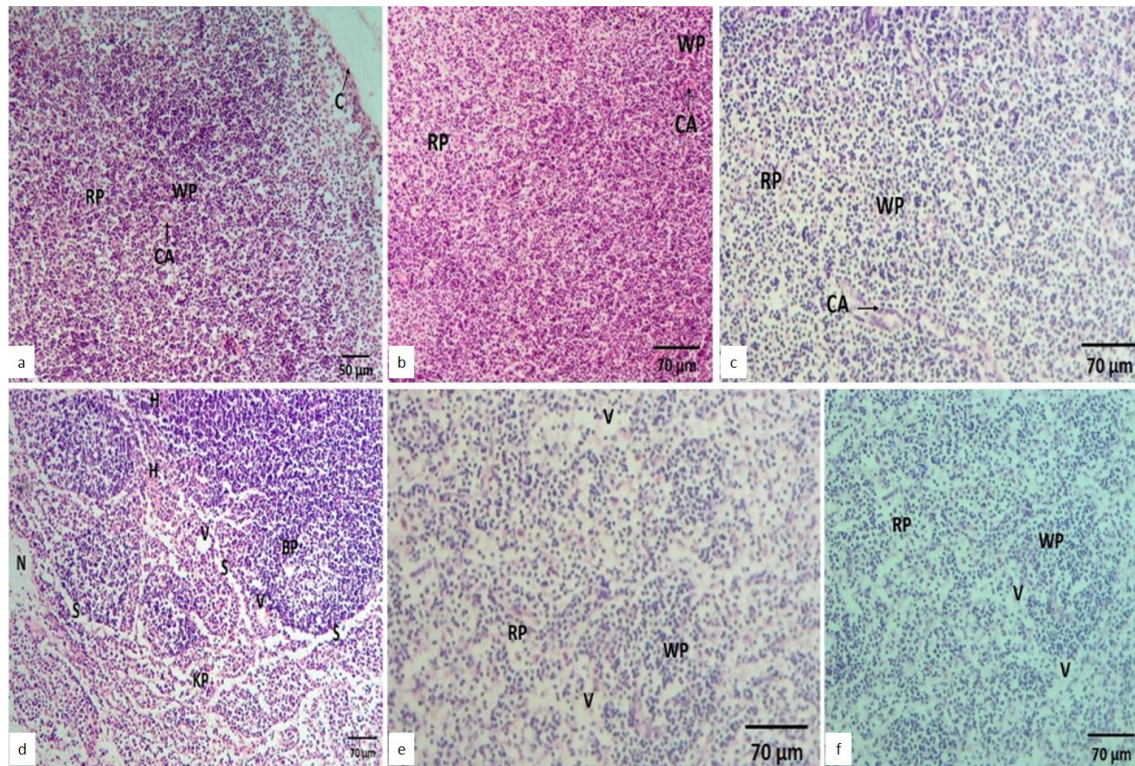


Figure 4 **a.** General structure of the spleen in the control group, **b.** 150 mg/kg pomegranate extract, **c.** 300 mg/kg pomegranate extract, **d.** General structure of the spleen in the MMC group, **e.** MMC+150 mg/kg pomegranate extract, **f.** MMC+300 mg/kg pomegranate extract. Capsule (C), white pulp (WP), red pulp (RP), central arteriole (CA), vacuolization (V), hemorrhage (H), separation (S), necrosis (N), H&E.

IV. DISCUSSION AND CONCLUSION

This study contains important results regarding the protective effects of pomegranate peel extract against the toxicity induced by MMC. The extract emphasizes the protective potential of pomegranate peel extract against Mitomycin-C (MMC)-induced toxicity, specifically focusing on enzyme levels, protein expressions, and tissue damage. These findings suggest that pomegranate peel extract may play a significant role in the chemotherapy process by reducing the side effects associated with chemotherapeutic agents such as MMC. However, further research is needed to determine the mechanism of action of this substance and optimal dosages for clinical applications. The protective effects of pomegranate peel extract against MMC-induced toxicity may be attributed to its antioxidant properties as evidenced by its ability to scavenge free radicals and reduce oxidative stress in various models [16, 17]. A study conducted by Nameni and Alavi (2021) [18] demonstrated a significant increase in superoxide dismutase and catalase levels, as well as a reduction in C-reactive protein levels, in response to pomegranate peel extract [18]; this is consistent with the lower AST enzyme levels observed in the MMC + pomegranate peel extract groups compared to the MMC group. Similarly, other studies have demonstrated that pomegranate peel extract may ameliorate liver damage by reducing AST and ALT levels [19]. It is stated that AST levels elevation due to liver damage may be caused by increased hepatocyte enlargement and enzyme production [20]. In the current study, significant increases in aspartate aminotransferase (AST) enzyme levels were observed in the MMC-treated group. Elevated AST levels are commonly indicative of liver damage or systemic stress, suggesting that MMC administration could potentially result in hepatotoxicity or widespread physiological distress. Conversely, lower AST levels were observed in the groups treated with the combination of MMC and pomegranate peel extract, suggesting that the harmful effects induced by MMC may be mitigated through the antioxidant or anti-inflammatory properties of pomegranate peel extract, providing protective effects on liver function and against systemic damage. This finding aligns with previous research, which demonstrates the ability of pomegranate peel extract to mitigate the toxic effects induced by various agents, as shown in studies conducted on rats and obese individuals [11, 22].

The potential effects of MMC and pomegranate peel extract treatments on serum protein electrophoresis profiles were evaluated. Serum protein electrophoresis is an important method for determining biochemical changes by analyzing the fractional distribution of proteins. When SDS-PAGE images were analyzed using the MATLAB software program, an

increase in protein expression was observed, especially in the MMC-treated group. In contrast, a decrease in protein expression was noted in the groups treated with pomegranate peel extract and the group treated with 150 mg/kg pomegranate peel extract in combination with MMC. This suggests that MMC may potentially have an enhancing effect on cellular responses and that the expression levels of proteins increase in association with this situation. MMC is a chemotherapeutic agent that triggers DNA damage and affects the cell cycle by targeting cancer cells. An increase in protein expression may indicate that MMC is associated with cellular stress responses and DNA repair mechanisms. In this context, it can be suggested that the increased protein levels represent an adaptive response aimed at regulating the cellular damage repair processes. Pomegranate peel extract, known for its antioxidant properties, has the potential to mitigate cellular damage and oxidative stress. The effect of pomegranate peel on reducing protein expressions may indicate that this extract initiates a mechanism to repair pre-existing cellular damage or modulates cellular stress responses. Particularly when applied in combination with MMC, it is possible that pomegranate peel extract may more effectively reduce this damage and preserve cellular homeostasis. While the increase in protein expression observed with MMC treatment may indicate a cellular stress response or repair process, the reduction in protein expression seen with the combination of pomegranate peel extract and MMC reveals that this extract has a balancing and protective effect on cellular responses. This type of interaction may suggest that pomegranate peel extract could be used as a potential adjuvant in cancer treatment; however, these findings need to be validated in larger studies.

Histological examinations provided further confirmation of the biochemical and electrophoretic findings. It was found that pomegranate peel extract alleviated tissue damage caused by MMC in both the small intestine and spleen. It is suggested that pomegranate peel extract provides protection against MMC-induced histopathological changes, potentially by reducing oxidative stress or inflammation in these tissues. This protective effect is consistent with previous studies suggesting that pomegranate peel extract possesses significant antioxidant and anti-inflammatory properties.

According to the literature, some plant extracts have been reported to possess immune system-boosting and antioxidant properties, and these extracts have been informed to protect the structure of the small intestine from damage caused by certain harmful chemicals [22, 23, 24, 25, 26, 27]. It is believed that plant extracts may be utilized in clinical applications to reduce tissue damage in the small intestine. The antioxidant activity of pomegranate peel

extract may play a significant role in alleviating tissue damage caused by MMC, as demonstrated through histological examinations in a study conducted on female albino rats [28]. Even in the present study, in animals treated with MMC, significant epithelial losses in the mucosal epithelium, degeneration progressing to necrosis within the digestive glands, and marked separations between the submucosal and muscularis mucosal layers, indicative of necrosis, were observed as signs of cellular death in the jejunum. Lymphocyte infiltration into the lamina propria and muscularis mucosa suggests that immune mechanisms are actively engaged in efforts to facilitate tissue repair. The absence of the epithelial layer and brush border indicates a potential disruption in the absorptive function of the jejunum in this group of mice.

On the other hand, as is known, the spleen is one of the central organ for initiating primary immune responses, particularly in the activation of B lymphocytes and the production of antibodies [29]. Observed findings, including vacuolization, hemorrhage, separations within and between the pulps, and necrosis, are reported to result as secondary effects of vascular damage in the spleen [30, 31] and serve as indicators of cell and tissue death. The reduction in severe findings observed in the spleen structure after pomegranate extract administration, with only minimal vacuolization noted, suggests that the treated pomegranate extract supports the tissue's self-repair mechanisms and, in this regard, promotes the renewal of immune/hematopoietic systems.

It has been documented that certain plant extracts suppress immune responses and reduce the effects of diseases [32]. Besides, it has been shown that plant extracts protect the body against the harmful effects of xenobiotics, which are defined as cancer-causing agents [33, 34]. Even in another study, it was thought that all these effects may be due to phytochemicals found in plants. The results have shown that the use of certain plant extracts at specific doses leads to an increase in antibody response [29].

In conclusion, the observed protective effects of pomegranate peel extract can be characterized as a complementary therapeutic agent in alleviating the side effects of MMC. Due to findings such as the decrease in AST levels and the preservation of tissue integrity, it has been concluded that pomegranate peel extract aids in reducing the systemic toxicity of MMC.

REFERENCES

- [1] J. Baranwal, B. Barse, A. DiPetrillo, *et al.*, "Nanoparticles in cancer diagnosis and treatment", *Materials*, vol. 16, p. 5354, 2023.
- [2] X. Kong, P. Gao, J. Wang, *et al.*, "Advances of medical nanorobots for future cancer treatments", *J. Hematol. Oncol.*, vol. 16, p. 74, 2023.
- [3] J. Mateo, L. Steuten, P. Aftimos, *et al.*, "Delivering precision oncology to patients with cancer", *Nat. Med.*, vol. 28, pp. 658–665, 2022.
- [4] L. Xie, L. Cheng, Y. Wei, "Mitomycin C enhanced the antitumor efficacy of Rocaglamide in colorectal cancer", *Pathol. Res. Pract.*, vol. 243, p. 154350, 2023.
- [5] G. Talwar, R. Daniel, T. McKechnie, *et al.*, "Radiotherapy alone versus chemoradiotherapy for stage I anal squamous cell carcinoma: a systematic review and meta-analysis", *Int. J. Colorectal Dis.*, vol. 36, pp. 1111–1122, 2021.
- [6] P. Valério, J.P. Barreto, H. Ferreira, *et al.*, "Thrombotic microangiopathy in oncology—a review", *Transl. Oncol.*, vol. 14, p. 101081, 2021.
- [7] M. Pourmadadi, A. Ghaemi, M. Shaghghi, *et al.*, "Macromolecules and nanomaterials loaded with mitomycin C as promising new treatment option: Cancer drug nanoformulation: A literature review", *J. Drug Deliv. Sci. Technol.*, vol. 87, p. 104835, 2023.
- [8] W.A. Al-Megrin, "In vivo study of pomegranate (*Punicagranatum*) peel extract efficacy against *Giardia lamblia* in infected experimental mice", *Asian Pac. J. Trop. Biomed.*, vol. 7, pp. 59–63, 2017.
- [9] N. Maphetu, J.O. Unuofin, N.P. Masuku, *et al.*, "Medicinal uses, pharmacological activities, phytochemistry, and the molecular mechanisms of *Punicagranatum* L. (pomegranate) plant extracts: A review", *Biomed. Pharmacother.*, vol. 153, p. 113256, 2022.
- [10] E. Eroglu Ozkan, M.F. Seyhan, O. Kurt Sirin, *et al.*, "Antiproliferative effects of Turkish pomegranate

(Punicagranatum L.) extracts on MCF-7 humanbreastcancer celllineswithfocus on antioxidantpotentialandbioactivecompoundsanalyzedby LC-MS/MS", *J. FoodBiochem.*, vol. 45, 2021.

[11] Y. Korkmaz, H. Gungor, A. Demirbas, *et al.*, "Pomegranatepeelextract, N-AcetylcysteineandtheircombinationwithOrnipuralalleviateCadmium-inducedtoxicity in rats", *J. Vet. Med. Sci.*, vol. 85, pp. 990–997, 2023.

[12] A. Minutolo, A. Gismondi, R. Chirico, *et al.*, "AntioxidantPhytocomplexesExtractedfromPomegranate (Punicagranatum L.) Using HydrodynamicCavitation Show PotentialAnticancer Activity In Vitro", *Antioxidants*, vol. 12, p. 1560, 2023.

[13] E. Uluman, P.A. Kilicle, "Theinvestigation of thepossibleantigenotoxic in vivoeffects of pomegranate (Punicagranatum L.) peelextract on mitomycin-C genotoxicity", *Turk. J. Vet. Anim. Sci.*, vol. 44, no. 2, pp. 382–390, 2020.

[14] U. Laemmli, "Cleavage of structuralproteinsduringtheassembly of thehead of bacteriophage T4", *Nature*, vol. 227, pp. 680–685, 1970.

[15] P. O'Farrell, "High resolution two-dimensionalelectrophoresis of biologicalpropertiesandsignificance", vol. 88, pp. 497–501, 1975.

[16] K.N. Chidambaramurthy, G.K. Jayaprakasha, R.P. Singh, "Studies on Antioxidant Activity of Pomegranate (Punicagranatum) PeelExtract Using in VivoModels", *J. Agric. FoodChem.*, vol. 50, pp. 4791–4795, 2002.

[17] O.A. Fawole, N.P. Makunga, U.L. Opara, "Antibacterial, antioxidantandtyrosinase-inhibitionactivities of pomegranatefruitpeelmethanolicextract", *BMC Complement. Altern. Med.*, vol. 12, p. 200, 2012.

[18] F. Nameni, R. AliakbarAlavi, "TheEffect of HydroethanolicExtract of PomegranatePeelsand High-intenseInterval Training on C-reactive Protein, CatalaseandSuperoxideDismutase in Rats", *Intern. Med. Today*, vol. 27, pp. 182–197, 2021.

[19] T. Sahloul, D. El-Bushuty, N. Shanshan, *et al.*, "Effect of PomegranatePeelsandJuice on HypercholesterolemicRats", *J. Home Econ.-MenofiaUniv.*, vol. 32, pp. 122–141, 2022.

- [20] M.M. Jumaal, E.Z. Naji, "Study of the effect of Ethanolic extract of pomegranate peels on some blood serum biochemical parameters in alloxan induced diabetes rats", *Tikrit J. Agric. Sci.*, vol. 21, pp. 138–151, 2021.
- [21] Y. Nozohour, R. Golmohammadi, R. Mirnejad, *et al.*, "Antibacterial activity of pomegranate (Punica granatum L.) seed and peel alcoholic extracts on Staphylococcus aureus and Pseudomonas aeruginosa isolated from health centers", *J. Appl. Biotechnol. Rep.*, vol. 5, pp. 32–36, 2018.
- [22] H. Amagase, B.L. Petesch, H. Matsuura, *et al.*, "Recent advances on the nutritional effects associated with the use of garlic as a supplement", *J. Nutr.*, vol. 131, pp. 955S–962S, 2001.
- [23] F.G. Apaydin, M. Uzunhisarcikli, A. Aslantürk, *et al.*, "Bisphenol A-induced histopathological alterations on small intestine tissues of rats: The protective role of taurine and curcumin", *J. Inst. Sci. Technol.*, vol. 8, pp. 43–47, 2018.
- [24] A. Arslan, F. Ozcicek, F.K. Cimen, *et al.*, "Protective effect of resveratrol against methotrexate-induced oxidative stress in the small intestinal tissues of rats", *Int. J. Clin. Exp. Med.*, vol. 8, pp. 10491–10497, 2015.
- [25] T. Horie, S. Awazu, Y. Itakura, *et al.*, "Alleviation by Garlic of Antitumor Drug-Induced Damage to the Intestine", *J. Nutr.*, vol. 131, pp. 1071S–1074S, 2001.
- [26] G.O. Omotoso, J.N. Muonagolu, B.U. Enaibe, "Histological evaluation of the jejunum and ileum of rats after administration of high dose garlic aqueous extract", *Int. J. Health Sci.*, vol. 6, pp. 135–140, 2012.
- [27] A. Shirpoor, H. Barmaki, M.K. Ansari, *et al.*, "Protective effect of vitamin E against ethanol-induced small intestine damage in rats", *Biomed. Pharmacother.*, vol. 78, pp. 150–155, 2016.
- [28] F.A. Shaheen, M.A. Hammam, F.M. Al-Shouny, *et al.*, "Evaluation the protective effect of garlic and green tea on lipid profile and some hormones in obese rats", *Menoufia J. Agric. Biotechnol.*, vol. 8, pp. 139–147, 2023.

- [29] H. Sulisty, D.W. Kurniawan, L. Rujito, "Biochemical and histopathological effects of green tea nanoparticles in ironized mouse model", *Res. Pharm. Sci.*, vol. 12, pp. 99–106, 2017.
- [30] S.A. Elmore, "Enhanced Histopathology of the Spleen", *Toxicol. Pathol.*, vol. 34, pp. 648–655, 2006.
- [31] A.W. Suttie, "Histopathology of the Spleen", *Toxicol. Pathol.*, vol. 34, pp. 466–503, 2006.
- [32] M.L.B. Ahui, P. Champy, A. Ramadan, *et al.*, "Ginger prevents Th2-mediated immuneresponses in a mouse model of airway inflammation", *Int. Immunopharmacol.*, vol. 8, pp. 1626–1632, 2008.
- [33] K. Nirmala, T.P. Krishna, K. Polasa, "Modulation of xenobiotic metabolism in ginger (*Zingiber officinale* Roscoe) fed rats", *Int. J. Nutr. Metab.*, vol. 2, pp. 56–63, 2013.
- [34] G. Udo-Affah, E.O. Kebe, P.P. Obasee, *et al.*, "Extract of ginger (*Zingiber officinale*) on the histology of the spleen using adult male rats", *J. Biol. Agric. Healthc.*, vol. 4, pp. 259–267, 2014.