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Article

Effect of Alcoholic Extract of Pomegranate Peel on Kidney Enzyme Levels, Histological Changes, in Male Albino Rats Treated with Methotrexate

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Abstract: Methotrexate (MTX) is highly nephrotoxic and acts via oxidative stress and inflammatory cascades. Pomegranate peel extract (PPE) contains punicalagins and exhibits potent antioxidant activity which is beneficial to the kidney. To assess the protective effects of pomegranate peel Extract (PPE) against methotrexate (MTX) induced renal damage in rats. Male Wistar rats were given PPE (400 mg/kg) for 10 days including one MTX injection (20 mg/kg) on day 7. Renal histology and serum biomarkers were, in turn, examined. following MTX administration, there was a marked increase in levels of blood urea (155% of baseline controls; 96.09±1.80 mg/dL) and blood creatinine (185% of baseline controls; 1.94±0.08 mg/dL; $p<0.001$). In MTX-treated kidneys, histopathological examination showed severe acute tubular necrosis, glomerular congestion and inflammatory cells infiltration. However, urea and creatinine levels in PPE pre-treatment group were significantly decreased to 54.24±1.76 mg/dL and 1.02±0.03 mg/dL respectively ($p<0.01$) thus these pathological alterations. In this study, the intervention resulted in a 43-47% decrease in nitrogenous waste products and significantly reduced renal architecture changes, which included absence of tubular dilations and preserved brush borders. The quantitative data demonstrates PPE ability to reverse the biochemical and structural changes caused by MTX, thereby demonstrating effective restoration of the renal filtration barrier and tubular secretory function. PPE is a potent nephro-protective agent that restores the renal markers and maintains the integrity of the renal tissues. It is an effective adjunctive therapy in clinical situations for reducing the side effects of chemotherapy.
Keywords: Creatinine, Kidney Enzyme, Methotrexate, Pomegranate Peel, Urea

Introduction

Methotrexate (MTX) is a folate antagonist antimetabolite drug which has been a cornerstone in clinical medicine for decades. It was used to treat malignancies, but continues to be a key drug in the treatment of acute lymphoblastic leukemia, lymphomas, and some solid tumors. It is not only used in oncology, but also as a disease modifying anti-rheumatic drug (DMARD) in autoimmune diseases such as rheumatoid arthritis and severe psoriasis [1]. MTX acts by inhibiting dihydrofolate reductase (DHFR) which results in the inability to convert dihydrofolate to tetrahydrofolate, which is needed to produce thymidylate and purine nucleotides. This depletion halts the synthesis of DNA and cellular

growth, especially of rapidly growing cells. However, its lack of selectivity may lead multi-organ toxicity, so a reduction in dosage, or even stopping the medication, may be necessary [2].

High dose MTX is associated with nephrotoxicity. Excretion of MTX is primarily in the kidneys, where 90% is excreted unchanged by glomerular filtration and active tubular secretion. MTX and its metabolite 7-hydroxymethotrexate may precipitate in the renal tubular environment and result in obstructive uropathy, especially in the presence of an acidic environment. In recent studies it has been emphasized that MTX-induced renal damage is not limited to physical obstruction, but also encompasses significant biochemical alterations. Oxidative stress, defined as overproduction of Reactive Oxygen Species (ROS) and reduction of antioxidant defenses, is an important mechanism involved [3]. MTX-induced renal injury is characterized by intricate interplay between oxidative stress, inflammation and apoptosis. Exposures result in decreases of endogenous antioxidants, such as glutathione (GSH) and superoxide dismutase (SOD). This oxidative imbalance leads to signaling pathways such as the nuclear factor-kappa B (NF-κB) pathway that results in the production of pro-inflammatory cytokines. Moreover, MTX has been shown to induce mitochondrial dysfunction, which leads to the release of cytochrome c and subsequent activation of the caspase system, thereby triggering the apoptotic cascade. Such programmed cell death is controlled by the Bcl-2 family of proteins, which is biased toward pro-apoptotic markers [4].

The use of natural bioactive compounds with antioxidant and anti-inflammatory properties are potential prevention measures. Pomegranate (Punica granatum L.) has been known to possess health benefits in traditional medicine for a long time. The current research is directed towards the pomegranate peel which is a by-product of juice industry and contains bioactive polyphenols. These comprise hydrolyzable tannins such as punicalagins, flavonoids and phenolics acids that have been shown to have a higher antioxidant activity than the edible portions of the fruit [5]. Pomegranate peel extract (PPE) is nephroprotective and is attributed to its influence on cellular defense mechanisms. PPE is an effective free radical scavenger to prevent lipid peroxidation that contributes to maintaining the integrity of the renal cell membrane. The alcoholic extract of the seeds contains high levels of punicalagin and ellagic acid, which promote increased activity of antioxidant enzymes and mitigation of oxidative injury in models of acute kidney injury [6]. In addition, the phytochemical composition of PPE has strong anti-inflammatory potential to inhibit the process of systemic inflammation, that occurs under toxic drug exposure [7].

PPE's protective effects also involve regulating molecular signaling pathways. The adaptive response to oxidative stress is essential and requires the activation of a pathway called nuclear factor erythroid 2-related factor 2 (NRF2). Genes regulated by Nrf2 include the cytoprotective genes, such as heme oxygenase-1 (HO-1). The study demonstrates polyphenols of pomegranate stabilized and promoted nuclear translocation of Nrf2, enhancing the renal antioxidant defense system [8]. This molecular intervention inhibits events that result in chronic damage to tissues and fibrosis [9]. PPE also blocks nephrotoxic agent-induced apoptotic pathways. PPE promotes cell viability and maintains structural integrity in renal tubules by downregulating the pro-apoptotic gene Bax and upregulating the anti-apoptotic gene Bcl-2 [10]. As a dual agent in reducing oxidative stress and inhibition of programmed cell death, PPE is a strong candidate in reducing toxicity caused by MTX [11]. Structural changes after MTX treatment comprise tubular necrosis, glomerular congestion and inflammatory infiltration. These histological changes correlate with increased levels in the blood of urea and creatinine, indicative of reduced renal filtration. The goal of protective interventions is to maintain renal structure and restore biochemical parameters to physiological normal values. Experimental studies have shown that PPE has a unique bioavailability and high therapeutic index suitable for co-administration with chemotherapy.

Prasad is investigating the use of novel drugs against MTX toxicity. Many synthetic drugs have been tested, but many possess adverse effects that preclude long-term use. By contrast, standardized alcoholic PPE has a multi-targeted approach to addresses the oxidative, inflammatory and apoptotic components of renal injury . Male albino rats are the highly suitable experimental models for which renal physiology and response to antimetabolite drugs is well established. Drawing upon historical data and previous studies, pomegranate extracts have shown protective effects against different nephrotoxic insults such as heavy metals and chemotherapeutic drugs including Gentianin. These results indicate that the renoprotective properties of pomegranate is a generalized capacity to maintain renal function during stress [12]. Combined with molecular analysis, these properties will provide a comprehensive understanding of the potential of PPE as supportive therapy to MTX patients. This study aims to assess the effect of pomegranate peel extract on kidney function parameters, such as serum urea, creatinine levels, and to evalute the extent of protection by the extract at histological level of the renal tissues through light microscopy.

Methodology

Plant Collection

The pomegranate fruits were procured from a local market in Musayyib city in Babil Governorate. A professor specializing in plant taxonomy at University of Babil – College of Agriculture, identified the pomegranate and confirmed it. The pomegranate fruits were washed in clean, running tap water and the peels were carefully removed from the arils and internal membrane of the fresh fruit. The peels were then cut into small pieces (1-2cm^2) and washed with distilled water and air-dried in a well-ventilated, ambient temperature (22-25°C) and shaded area until a constant weight was attained. Then, the dried peels were ground to a fine powder in a commercial electric grinding machine.

Preparation of Pomegranate Peel Ethanolic Extract (PPE)

The powdered pomegranate peel (100 g) was precisely weighed and extracted by maceration in 1000 mL of 70% ethanol (v/v) in a dark, closed glass bottle. Continuous stirring was performed using a magnetic stirrer at 150 rpm for 72 hrs at room temperature (25 °C). After maceration, particulate matter was removed by initial filtering of the mixture with a coarse filter paper, and then through Whatman No. 1 filter paper with a pore size of 11 μm. The ethanolic filtrate was concentrated on rotary evaporator by reducing pressure at 40 °C to yield crude extract. The crude extract was then lyophilized in a freeze dryer to obtain dry powdered extract. The last batch of PPE in powder form was packaged in amber glass vials in an airtight condition and kept at -20 °C until it until required for experimentation [13]. The percentage of dry weight yield of the extract, relative to the initial dry weight of the peel powder, was calculated using the following equation:

$$\text{Yield (\%)} = (\text{Weight of dry extract} / \text{Weight of initial dry peel powder}) \times 100$$

Quality Control of PPE

Before administration, the PPE was initially screened for the presence of major secondary metabolites including phenolics, flavonoids, and tannins, using a standard qualitative procedure. In addition, total phenolic content (TPC) was also evaluated using folin-ciocalteu method and total flavonoid content (TFC) was evaluated by aluminium chloride colourimetric method. The consistency and quality of the prepared extract was confirmed by these analyses in each batch [14].

Experimental Animals

Thirty two healthy adult male Wistar albino rats (8-10 weeks old) weighing between 200 and 250g. Animals were kept in standard polyacrylic cages (4 rats per cage), under strictly controlled environmental conditions, including temperature 25 ± 2 °C, relative humidity 50 ± 4% and light/dark

cycle of 12/12 hours. The rats were fed a standard commercial pellet diet and filtered tap water was provided ad libitum. All the animals were underwent a one-week acclimatization period before the start of the experiment [15].

Experimental Design and Treatment Protocol

The treatment protocol sought to assess the protective effect of PPE against nephrotoxicity caused by MTX for a 10-day period. The groups and their treatments were: group I (Normal Control) where rats received distilled water orally via gavage with distilled water (10 mL/kg BW) for 10 consecutive days. On the 7th day, they received a single intraperitoneal (i.p.) injection of normal saline (0.9% NaCl), serving as the vehicle group control, group II (PPE Control) rats were administered PPE dissolved in distilled water at a dose of 400 mg/kg body weight/day orally via gavage for 10 consecutive days. The rats of this group were given distilled water orally for 10 days as a control group to assess the safety and base-line effects of the extract alone, while group III MTX-Intoxicated, rats were administered MTX to induce intoxication for 10 days. On 7th day, nephrotoxicity was experimentally induced by a single dose of MTX (20mg/kg body weight, i.p.) in group IV (PPE + MTX Treatment) rats where they were pre-treated with PPE (400mg/kg body weight/day, oral) for six days. On the 7th day, a single i.p. injection of MTX (20 mg/kg body weight) was a administration of PPE. Subsequently, PPE treatment was continued for another three days (days 8, 9 and 10). The chosen MTX dose (20 mg/kg, i.p.) is a well-established model for the acute induction of nephrotoxicity in rats which has been associated with high oxidative stress and structural renal abnormalities. The dose of PPE (400 mg/kg, oral) used in the study was based on preliminary dose-response studies and previous studies which demonstrated PPE's optimal efficacy in protecting against oxidants and without any adverse effect in nephroprotection [16].

Blood Collection and Preparation

All rats were subjected to a 12-hour (overnight) fast with water ad libitum at the end of the experimental period (after the last treatment on day 10). Animals were then euthanized via isoflurane inhalation (2-3% in oxygen). Cardiac puncture blood samples (3-4 mL) were obtained from each rat, without anticoagulant. Blood was allowed to clot at room temperature for 30 minutes after which centrifuged at 3000rpm (~1500 × g) for 15 minutes at 4 °C in a refrigerated centrifuge. The serum supernatant was carefully separated and aliqouted into sterile Eppendorps tubes and stored at -80°C for biochemical analyses.

Biochemical Evaluation of Renal Function Markers

Renal function was broadly evaluated by measuring urea and creatinine, traditional markers of glomerular filtration rate and renal dysfunction, in the serum. Each biochemical measurement was performed manually on an automatic biochemistry analyzer per manufacturer's protocol.

1. Serum Urea

Urea concentration in the serum was spectrophotometrically determined by using commercial serum urea diagnostic kit (Randox Laboratories Ltd., UK) utilizing an enzymatic method with urease and GLDH enzyme. The absorbance was measured at 580 nm.

2. Serum Creatinine

The serum level of creatinine was determined by colorimetric method (modified Jaffe's method) in a ready-to-use commercial kit (Randox Laboratories Ltd., UK). The reaction rate of creatinine picrate complex formation was spectrophotometrically determined at 492 nm.

Histopathological Examination

The fixed in formalin right kidneys were embedded, sectioned and stained for routine histopathology. The tissue samples after fixation for 48 hours were dehydrated using ascending concentration of ethanol (70%, 80%, 90%, 95% and 100%), cleared in Xylene, and embedded in

paraffin wax. The resulting preparations were sliced in serial sections of a thickness of (4-5 μm) using a rotary microtome. These sections were deparaffinized, rehydrated and stained with a standard/routine Hematoxylin and Eosin (H&E) for general morphological evaluation. The stained slides were examined using a light microscope with a digital camera. Kidney sections were examined for pathological histological changes [17].

Statistical Analysis

All experimental data are presented as mean ± standard error of the mean (SEM). Data were analyzed with GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA, Version 9.0). Normality of data distribution was evaluated by Shapiro-Wilk test and homogeneity of variances was evaluated by Levene's test. One-way analysis of variance (ANOVA) and Tukey's test were used for multiple comparison analysis, when overall significance was observed. Any p-values <0.05 ($p<0.05$) were considered to indicate a statistically significant difference.

Results

Characterization of Pomegranate Peel Ethanolic Extract (PPE)

Extraction process which used 70% ethanol as a solvent system yielded a dark reddish-brown crude extract with recovery percentage of 18.42% (w/w). This yield was comparable to maceration techniques that give high yield of polyphenols from pomegranate by-products. Bioactive secondary metabolites were found to be present in high amount by quantitative analysis. The Total Phenolic Content (TPC) was determined as 142.5 ± 4.2 mg Gallic Acid Equivalents (GAE)/g dry extract and the Total Flavonoid Content (TFC) was found to be 24.8±1.5 mg Quercetin Equivalents (QE)/g (**Table 1**). Qualitative screening for preliminary confirmation of plant compounds indicated the presence of hydrolyzable tannins, especially punicalagins, which are well known for their strong radical scavenging activities. The lack of alkaloids in the extract further hints to the specificity of the ethanolic extraction for the polar antioxidant compounds.

Table 1. Quantitative and qualitative phytochemical profile of PPE

Parameters	Methodology	Findings
Extraction yield	Maceration (70% EtOH)	18.42% (w/w)
Total phenolic content	Folin-Ciocalteu assay	142.5 ± 4.2 mg GAE/g
Total flavonoid content	$AlCl_3$ colorimetric	24.8 ± 1.5 mg QE/g
Tannins/Punicalagins	$FeCl_3$ / TLC	Highly abundant (+++)

Evaluation of Serum Renal Biomarkers

Methotrexate (MTX) was systemically administered at 20 mg/kg i.p. which resulted in a state of acute azotemia, characterized by the dramatic increase in nitrogenous waste products. Serum urea levels surged by approximately 155% (96.09±1.80 mg/dL) compared to the control group (37.56±1.38 mg/dL, $p<0.001$). Likewise, serum creatinine which is a more sensitive indicator of glomerular filtration rate (GFR) rose significantly to 1.94±0.08 mg/dL from 0.68 ± 0.02 mg/dL ($p<0.001$). The biochemical profile clearly demonstrates that the renal filtration barrier and tubular secretory function is severely impaired.

These biochemical derangements were effectively prevented and/or reversed by prophylactic and therapeutic use of PPE (400 mg/kg) (**Table 2 & Fig. 1**). Serum urea and creatinine of the

PPE+MTX group were significantly decreased by 54.24±1.76 mg/dL and 1.02±0.03 mg/dL, respectively ($p< 0.01$). The antioxidant bioactive compound rich PPE decreased the biomarkers by 43-47% which indicates that it has enhanced the renal defense mechanism against MTX induced oxidative stress, possibly by preserving the integrity of basement membrane and restricting the obstructive uropathy due to MTX precipitation.

Table 2. Serum urea and creatinine levels across experimental groups

Group (n=8)	Urea (mg/dL)	Creatinine (mg/dL)
Control	37.56 ± 1.38	0.68 ± 0.02
PPE Control	36.50 ± 0.54	0.63 ± 0.01
MTX	96.09 ± 1.80***	1.94 ± 0.08***
PPE+MTX	54.24 ± 1.76##	1.02 ± 0.03##

Data expressed as Mean ± SEM. *** $p < 0.001$ vs Control; ## $p < 0.01$ vs MTX group.

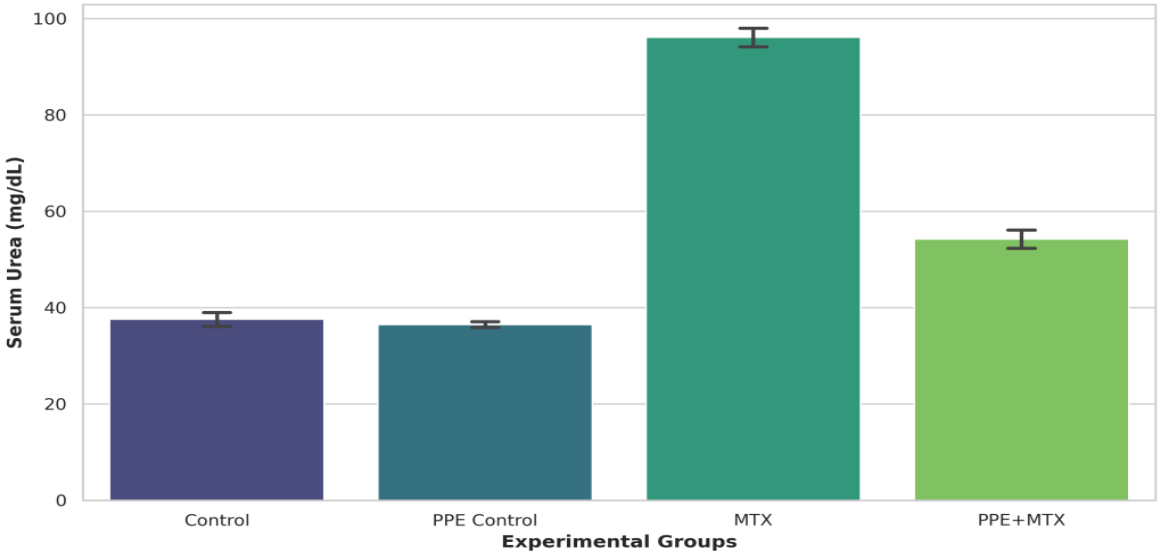

Figure 1. Effect of PPE on serum urea levels in MTX-treated rats

Histopathological Examination and Morphological Integrity

The biochemical data were confirmed by microscopy of the H&E stained kidney sections (**Fig. 2)**. The MTX-intoxicated group showed characteristic signs of acute nephrotoxicity: diffuse epithelial lining desquamation and loss of brush border of the proximal tubule (red arrows), large number of mononuclear inflammatory cells in the interstitium (blue arrows), and marked glomerular shrinkage with increase in the width of the bowman's space with vascular congestion (yellow arrows). The group PPE+MTX, on the contrary, showed an exceptionally preserved renal architecture. The majority of the tubules were intact with scant epithelial sloughing (green arrows). The decreased inflammatory cell infiltration, and recovery of glomerular morphology, indicates that phytochemicals in PPE (punicalagins) directly affect the renal parenchyma, inducing anti-apoptotic and anti-inflammatory activity.

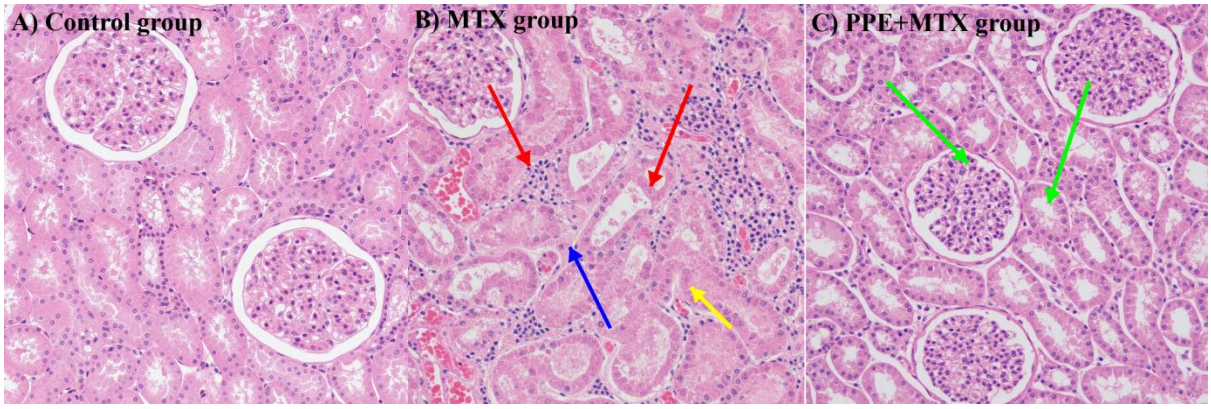

Fig. 2. Representative photomicrographs of renal tissues (H&E, 400x). (A) Normal architecture in control; (B) Severe MTX-induced injury showing necrosis (red), inflammation (blue), and glomerular atrophy (yellow); (C) PPE-mediated structural recovery showing preserved tubular integrity (green)

Discussion

The present study has been carefully assessed the nephroprotective potential of an alcoholic extract of pomegranate peel (PPE) against methotrexate (MTX) induced renal toxicity in male albino rats. Our results clearly show that the injection of MTX at a dose of 20 mg/kg ip triggers a severe form of acute kidney injury (AKI) with drastic increase in the serum urea and creatinine levels, and remarkable histopathological changes in the renal parenchyma. These findings are consistent with the vast literature on MTX nephrotoxicity. MTX is excreted primarily by the kidneys and the renal tubules may get obstructed by MTX and its metabolites (including 7 hydroxyl methotrexate), which can crystallise in the acidic environment and damage cells directly [18]. The azotemia noted in the MTX treated group is a biochemical marker of dysfunction of glomerular filtration rate (GFR) and renal dysfunctioning, which is a common complication of chemotherapeutic nephrotoxicity [19].

To understand the mechanistic basis, it can be stated that MTX induced renal damage is orchestrated primarily with the complex interplay of oxidative stress, inflammation and apoptosis. It is known that methotrexate acts as a folate antagonist and its cytotoxic action is mediated through the inhibition of the enzyme Dihydrofolate Reductase (DHFR) which is involved in the biosynthesis of the purine and pyrimidine nucleotides. This inhibition is not only a hindrance in the ability of rapidly dividing cells to synthesize DNA, but it also causes increases in levels of dihydrofolate that can be redirected to formation of reactive oxygen species (ROS) [1]. The oxidative burst occurs and the body's own anti-oxidant defense systems are overwhelmed causing a series of harmful events to ensue. In particular, MTX exposure has been linked to a decrease in critical antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels, which worsens oxidative damage through lipid peroxidation and protein carbonylation [20]. The significant increase in serum urea and creatinine in the MTX-intoxicated group along with the significant decrease in serum urea and creatinine in PPE co-administered group strongly indicate that the rich antioxidants profile of PPE plays a pivotal role in ameliorating these biochemical derangements. The pomegranate peel is a rich source of bioactive polyphenols including punicalagins and ellagic acid which are known for their strong free radical scavenging activity and their ability to boost cell antioxidant capacities [21]. These compounds are able to directly scavenge ROS, chelate metal ions that can participate in Fenton reactions, and induce the expression of endogenous antioxidant enzymes, improving the redox balance in renal cells [5].

PPE's protective effects are not limited to antioxidant properties, but also involve modulation of inflammatory pathways. Oxidative stress induced by MTX is a strong inflammatory stimuli to the

kidney. Excessive production of ROS triggers important pro-inflammatory molecular pathways, particularly the nuclear factor-kappa B (NF-κB) pathway. Once activated, NF-κB can increase the levels of various inflammatory cytokines including tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) and also the production of chemokines, which attract inflammatory cells to the wound site [22]. Our study showed marked inflammatory cell infiltration in MTX group which was significantly reduced in PPE + MTX group in the histological examination. PPE's anti-inflammatory properties are likely due to its polyphenolic components that are known to inhibit NF-κB activation and the production of inflammatory cytokines, reducing the inflammatory cascade [23]. For example, punicalagin is shown to prevent the phosphorylation and degradation of IκBα, an important step in the activation of NF-κB, resulting in a decrease in nuclear translocation and nuclear activity of NF-κB, and thereby reducing the production of pro-inflammatory mediators [24].

In addition, MTX nephrotoxicity is correlated with high levels of programmed cell death (apoptosis) within the renal tubular epithelial cells in the kidneys. Mitochondrial dysfunction may be caused directly by oxidative stress/inflammation and result in the release of pro-apoptotic factors in the cytoplasm, including cytochrome c. This, in turn, triggers the activation of a group of proteases called the caspase cascade that is responsible for dismantling the cell [25]. Our histological data, which showed normal renal structure in PPE+MTX group compared to severe tubular necrosis in MTX-intoxicated group, further confirmed the anti-apoptosis effect of PPE. Pomegranate extracts have been seen to alter the expression of the genes related to apoptosis; they have been shown to decrease the expression of pro-apoptotic proteins such as Bax and caspase-3 and increase that of anti-apoptotic proteins such as Bcl-2 [26]. This rebalancing of Bcl-2 family proteins renders the mitochondrial apoptotic pathway inactive and ensures cellular viability as well as structural integrity in the renal tubules [27]. This combination of reducing oxidative stress, suppressing inflammation and inhibiting apoptosis has a synergic effect which explains why all these effects are seen and thus PPE acts as a multi-target approach to counter MTX induced cytotoxicity and hence renoprotective effects are observed.

The systemic actions of MTX include inhibition of renal aquaporins and inhibition of renal ion transporters that further complicated the clinical picture regarding nephrotoxicity. In recent studies, it was demonstrated that MTX-induced oxidative stress is associated with the downregulation of the expression of aquaporin-1 (AQP1) and aquaporin-2 (AQP2) in the renal cortex and medulla, respectively, which is responsible for decreased tubular water reabsorption and polyuria [28]. Interestingly, we noted recovery of renal function markers in our PPE-treated group, which indicates that the polyphenols in pomegranate might also affect expression and/or localization of these vital transporters; further molecular studies are needed to see how PPE may affect this. Furthermore, hydrolyzable tannins in PPE have been associated with regulation of the canonical Nrf2/HO-1 pathway, a major master regulator in the adaptive response towards oxidative stress. PPE increases the nuclear accumulation of Nrf2, which increases the production of hemin oxygenase-1 (HO-1) and other phase II detoxifying enzymes that will create a powerful armory against electrophilic metabolites of MTX [8].

Based on our findings, it is revealed that the antioxidant action of natural antioxidants against the damage of organs caused by chemotherapeutic agents is a growing theme around the world. In the same vein, Morsy et al., showed that different phytochemicals might be effective in reducing MTX-mediated renal inflammation through the same pathway in which we have studied PPE (TLR4/NF-κB axis) [29]. Moreover, Aladaileh et al., found that punicalagin, as a pure compound, effectively reversed MTX nephrotoxicity by modulates the oxidative and apoptotic markers,

supporting our findings and suggesting the relevance of standardized extracts in therapeutic use [26]. The marked decrease in serum urea and creatinine and the preservation of glomerular and tubular structures by the histological examination suggest the use of PPE as an effective natural medicine for reducing the renal side effects of MTX.

Conclusion

Pomegranate peel alcoholic extract (PPE) showed an excellent nephroprotective activity on methotrexate (MTX) induced toxicity in rats. Here it was observed that prophylactic use of PPE at 400 mg/kg resulted in a dramatic improvement of renal function with significantly reduced levels of serum urea and creatinine, and the maintenance of histological integrity of renal tubules and glomeruli. This efficacy has been attributed to the presence of the extract's large number of bioactive polyphenols, especially punicalagins, which through multi-targeted molecular mechanisms, inhibit oxidative stress; suppress inflammatory pathways and, prevent apoptosis in renal epithelial cells. Nevertheless, additional clinical trials are needed to establish safe and effective doses in humans and to confirm long-term safety and efficacy, which would be necessary for its use as an adjunct in the treatment of oncological and autoimmune conditions.

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