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Review Article

Methotrexate induced nephrotoxicity: possible underlying mechanisms and promising natural protective agents in experimental models

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ABSTRACT

Methotrexate (MTX) stands as a noteworthy example of a potent anticancer and anti-rheumatic drug that has gained approval from the FDA for its efficacious treatment of various cancer types and non-oncologic diseases such as rheumatoid arthritis and psoriasis. Its remarkable ability to inhibit cellular proliferation by curtailing the synthesis of purine and pyrimidine has solidified its place in the medical field. Nonetheless, the panorama is not devoid of challenges, with the clinical utilization of MTX being somewhat constrained due to an array of associated toxicities, most prominently nephrotoxicity an adversity that can be both devastating and unfortunately common. The challenges associated with MTX nephrotoxicity arise due to its significant dependence on renal excretion for clearance, making the kidneys highly vulnerable to its effects. The objective of this review is to elucidate the potential mechanisms underlying MTX-induced nephrotoxicity and to discuss promising phytochemical protective strategies investigated experimentally within the past two years. This exploration aims to provide a comprehensive understanding of the challenges associated with MTX usage, particularly its impact on renal function, and to highlight emerging strategies for mitigating nephrotoxic effects.

Keywords: *Nephrotoxicity, Methotrexate, Oxidative stress, Apoptosis, Inflammation.*

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1. Introduction

Due to its great potency and effectiveness in rheumatoid arthritis patients, MTX is an FDA-approved folic acid antagonist that is also suggested for the treatment of juvenile idiopathic arthritis **[1]**. Moreover, MTX is one of the main chemotherapeutic options for treating different types of tumors in today's world. Aminopterin was initially considered the parent compound for MTX. It was first used successfully to treat childhood leukemia **[2]**. The objective of this review is to elucidate the potential mechanisms underlying MTX-induced nephrotoxicity and to discuss promising phytochemical protective strategies investigated experimentally within the past two years. This exploration aims to provide a comprehensive understanding of the challenges associated with MTX usage, particularly its impact on renal function, and to highlight emerging strategies for mitigating nephrotoxic effects.

2. Pharmacodynamics of MTX as a chemotherapeutic agent and immunosuppressant

Regarding its usage in chemotherapy and immunosuppression in autoimmune illnesses, MTX has a unique mode of action. MTX is well known as a competitive inhibitor of dihydrofolate reductase, so it prevents the production of tetrahydrofolate, which is important for the generation of folate cofactors needed for *de novo* synthesis of purine and pyrimidine **[3]**. In addition, MTX induces inhibition of amino imidazole carboxamide ribonucleotide (AICAR) tras-formylase, thus AICAR levels increase intracellularly leading to hindrance in adenosine and guanine metabolism eventually resulting in adenosine accumulation **[4]**. Adenosine induces an anti-inflammatory response by repressing T-cell activation, down-regulating B-cells, sensitizing CD-95 T-cells, and inhibiting the binding of interleukin-1β (IL-1β) to the cell surface **[5]**.

3. Pharmacokinetics of MTX

3.1. Absorption

After oral administration, MTX is readily absorbed by the proximal jejunum **[6]**. MTX has a T_{max} of 1 to 2 h and a bioavailability of 64-90%, which falls at oral dosages beyond 25 mg due to saturation of the carrier-mediated transport of MTX **[7]**.

3.2. Distribution

Low-dose MTX is distributed throughout the body mostly in extravascular tissue compartments with a half-life of one hour, including the kidneys, liver, and synovia. About 35 to 50% of MTX binds to albumin **[8]**.

3.3 Metabolism

In the liver tissues, MTX is converted to MTX polyglutamate by the enzyme folylpolyglutamate synthase. The glutamyl chains of MTX-polyglutamates are hydrolyzed by gamma-glutamyl hydrolase, reverting them to MTX. Additionally, 7-hydroxy MTX is created from a small quantity of MTX **[9]**.

3.4 Excretion

More than 80% of MTX is excreted unaltered, and only around 3% of it is excreted as the 7-hydroxylated metabolite. The majority of MTX is eliminated in the urine, with 8.7-26% of an intravenous dose appearing in the bile **[10]**. With higher dosages, MTX clearance declines and varies greatly between people **[11]**.

4. Mechanisms involved in MTX-induced nephrotoxicity

MTX-induced nephrotoxicity, the most significant side effect of MTX, happens due to the buildup of MTX and its byproducts in kidney tubes or because MTX directly harms these tubes **[12]**.

4.1. Oxidative Stress

Reactive oxygen species (ROS) play a dual role, participating not only in cellular apoptosis but also in regulating various cellular functions, including the inhibition of cell proliferation. MTX-induced oxidative stress is mediated by an increase in ROS levels due to the depletion of tetrahydrobiopterin, which is a required cofactor for nitric oxide (NO) synthesis by nitric oxide synthases (NOS) **[13]**. The reduction of tetrahydrobiopterin results in the decoupling of nitric oxide synthase (NOS), leading to a cessation of NO synthesis and a concurrent escalation in ROS production **[2]**. Organ damage arises because of excessive free radical generation and a compromised endogenic antioxidant defense mechanism, which also results in cell membrane breakdown **[14]**. In such instances, MTX-induced oxidative stress disrupts the balance between pro-oxidants and antioxidants, favoring an increase in the pro-oxidative state. This heightened oxidative stress, compounded by exposure to ROS, results in

structural damage to cellular components **[15]**. MTX inhibits cytosolic nicotinamide adenine dinucleotide phosphate (NADP)-dependent dehydrogenases and NADP malic enzyme **[16]**. It is well-established that NADPH is utilized by glutathione reductase (GR) to sustain the reduced state of glutathione (GSH). Consequently, a decreased cellular GSH content may render the cells more susceptible to oxidative stress **[14]**.

Malondialdehyde (MDA) serves as a crucial marker for lipid peroxidation, a process induced by ROS and subsequently contributing to cellular damage. MTX has been shown to elevate MDA levels, indicative of increased lipid peroxidation and potential cell destruction **[16]**. Reduction in NADPH and GSH causes a reduction in glutathione peroxide activity that subsequently diminishes catalase activity. Continuous production of ROS by MTX diminishes the endogenous antioxidant system; causing damage to kidney tissues **[17]**.

4.2 Inflammation

After administration, MTX is actively transported into cells, including renal tubular cells. As MTX interferes with DNA synthesis, this halts cell division, causing cell stress and disrupting the normal turnover of renal epithelial cells. The impaired cell division contributes to cellular dysfunction and may result in cell death **[18]**. Under conditions of stress and cellular death, cells release molecules known as damage-associated molecular patterns (DAMPs), including high-mobility group box 1 (HMGB1) and heat shock proteins. These molecules act as danger signals and stimulate innate immune cells, such as macrophages and dendritic cells, to initiate inflammatory responses **[19]**. Activated immune cells and stressed tubular cells secrete pro-inflammatory cytokines like IL-1β, tumor necrosis factor-alpha (TNF-α), and IL-6. These cytokines play a central role in amplifying the inflammatory response by attracting additional immune cells and promoting further cytokine production **[20]**. Chemokines produced in response to MTX-induced cell stress and cytokine signaling attract more immune cells, such as neutrophils and monocytes, to the renal tissue. This leads to an influx of immune cells, which contributes to tissue damage through the release of ROS and the secretion of proteolytic enzymes **[21]**. MTX-induced cellular stress can activate the inflammasome, a multiprotein complex that plays a key role in the processing and release of pro-inflammatory cytokines, including IL-1β. Inflammasome activation further amplifies the inflammatory cascade **[22]**. The cumulative effects of a disrupted cell cycle, inflammatory cytokines, immune cell infiltration, and oxidative stress, collectively lead to structural damage within the renal tissue. This damage compromises renal function, leading to nephrotoxicity characterized by impaired filtration, electrolyte imbalance, and potential long-term kidney dysfunction **[23]**. In the context of MTX-induced inflammatory responses, it is noteworthy that multiple signaling cascades are implicated in the modulation of inflammatory responses including Toll-like receptor 4 (TLR4), nuclear factor-kappa B (NF-κB), and cyclooxygenase (COX), Janus kinase/signal transducers and activators of transcription (JAK/STAT), granulocyte colony-stimulating factor (G-CSF), as well as the mitogen-Activated Protein Kinase (MAPK) pathway. Other mechanisms contributing to the inflammatory effect of MTX are regulating Sirtuin 1(SIRT1) and peroxisome proliferator-activated receptor gamma (PPARγ). The following **Fig. 1**. illustrates the intricate cascade of events in the inflammation pathway, highlighting key molecular and cellular processes involved in the body's response to MTX injury.

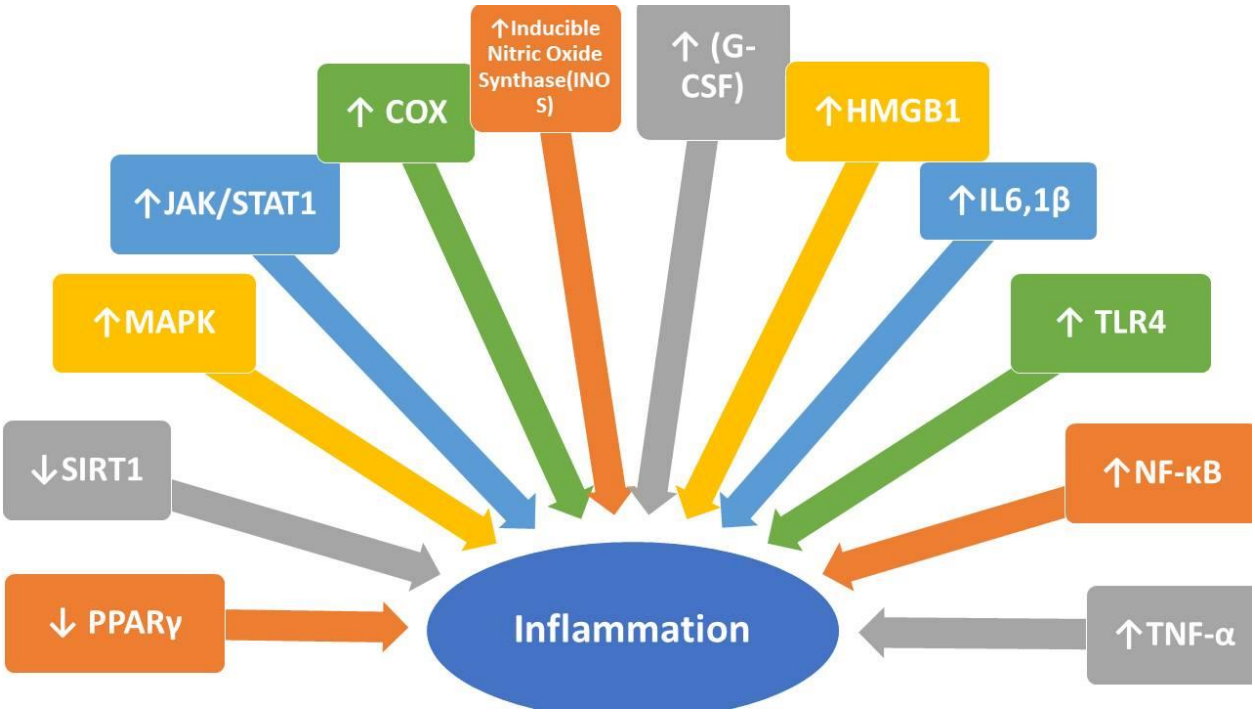

Fig. 1. The inflammatory mechanisms of MTX-induced kidney injury

4.3 Apoptosis

MTX-induced apoptosis in nephrotoxicity involves a series of cellular and molecular events that lead to programmed cell death within renal cells. One key regulator of apoptosis is the tumor suppressor protein, p53. In response to DNA damage, p53 is stabilized and activates downstream pathways that induce cell cycle arrest or apoptosis. Apoptosis can be initiated when the extent of damage is beyond repair **[24]**. The equilibrium between pro-apoptotic and anti-apoptotic members within the Bcl-2 protein family holds paramount importance in dictating the fate of a cell. MTX-induced stress has the potential to disrupt this equilibrium, tilting it towards pro-apoptotic proteins such as Bax and Bak. These proteins facilitate the permeabilization of the mitochondrial outer membrane, resulting in the release of cytochrome c into the cytoplasm **[25]**. The release of cytochrome c from the mitochondria initiates the formation of the apoptosome, a complex comprising cytochrome c, apoptotic protease-activating factor 1 (Apaf-1), and procaspase-9. This leads to the activation of caspase-9, an initiator caspase that sets off the caspase cascade **[25]**. Caspase-9, once activated, initiates the activation of downstream effector caspases, including caspase-3 and caspase-7. Effector caspases by proteolytic cleavage cleave various cellular substrates, including structural and functional proteins, resulting in cell dismantling and apoptotic body formation **[26]**. This process which is illustrated in **Fig. 2**, can lead to renal tissue damage and dysfunction, contributing to the overall nephrotoxic effects of MTX.

5. Assessment of MTX-induced kidney toxicity in animal models

Animal models are regarded as a fundamental component of scientific research, as they provide researchers with a means to study toxicity and explore the underlying mechanisms. These models are invaluable for the development of appropriate management strategies.

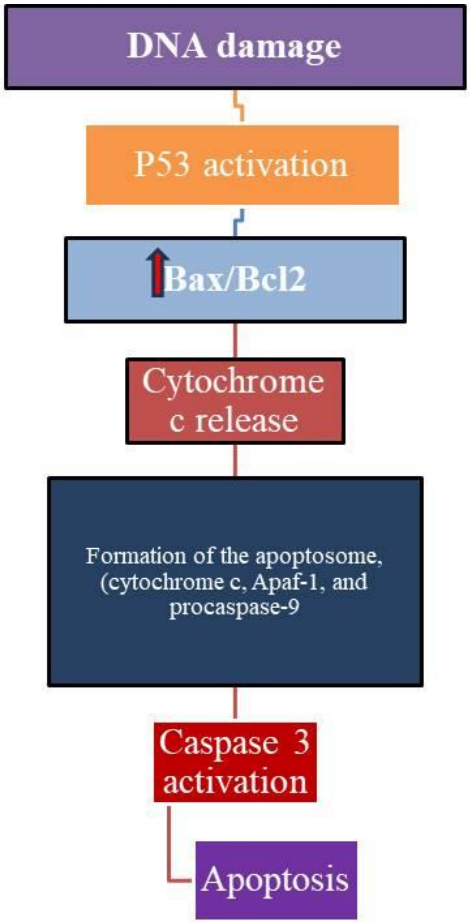

Fig. 2. The sequence of apoptotic events triggered by MTX in the kidney

5.1. Pathological Manifestation

In the assessment of AKI, detailed pathological changes manifest across various renal structures. Microscopic examination often reveals prominent tubular injury characterized by epithelial cell swelling, loss of brush border, and the presence of intraluminal debris **[27]**. Interstitial inflammation, marked by infiltrating immune cells, is a common feature, reflecting the inflammatory response to injury. Glomerular changes may include endothelial cell swelling and mesangial expansion **[28]**.

5.2 Laboratory testing

Serum creatinine, blood urea nitrogen, and urinary output should be measured to assess renal impairment, in acute kidney injury (AKI), urinary output decreases before creatinine concentrations increase and then followed by an increase in blood urea nitrogen **[29]**.

5.3. Urinary Protein

Urinary protein serves as a potential indicator of both acute and chronic renal injury resulting from nephrotoxic medications. Ordinarily, the glomeruli act as a barrier, preventing the passage of high-molecular-weight proteins from the bloodstream into the nephron's lumen. However, in pathological states, when nephron function is compromised, these high-molecular-weight proteins can be identified and detected in the urine **[30]**.

5.4. Biomarkers of Kidney Injury

Specific biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL) or kidney injury molecule-1 (KIM-1), can be measured to detect early signs of kidney injury in animal models. Moreover, N-Acetyl-β-d-glucosaminidase (NAG) is a lysosomal enzyme, found predominantly in proximal tubules so increased activity of this enzyme in the urine suggests injury to tubular cells **[31]**.

5.5. Glomerular Filtration Rate (GFR)

In animal models, GFR can be estimated using methods similar to those in humans, involving the clearance of markers such as inulin or creatinine **[32]**.

6. Promising phytotherapy for managing MTX nephrotoxicity in the last two years

Some herbs and natural compounds that have been studied for their potential nephroprotective properties include:

Apigenin (API): It is one of the most prevalent flavones, particularly in fruits and vegetables. It acts perfectly by augmenting the antioxidant defense system by reducing MDA levels and elevating superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities. Furthermore, it exerts a significant role in mitigating the inflammation induced by MTX through the reduction of proinflammatory markers such as iNOS, C-reactive protein (CRP), and G-CSF. This action aids the bone marrow in increasing the production of white blood cells. Moreover, it showed antiapoptotic effects by reducing caspase 3 expression **[33]**.

Capsaicin: This compound is responsible for the spicy sensation experienced when consuming chili peppers. Extensive research has explored its potential health advantages, including its anti-inflammatory and antioxidant properties. It exerts its antioxidant activity by reducing renal MDA, and NO, and significantly increases total antioxidant capacity (TAC) in kidneys. It also inhibits apoptosis by reducing the expression of caspase-3 in kidneys **[34]**.

Naringin (NG): NG is a flavonoid compound commonly found in citrus fruits such as grapefruits and oranges. NG may influence various pathways related to oxidative stress. NG keeps the oxidative balance by reducing MDA and NO and restoring antioxidant defenses by elevation of GSH, GPx, CAT, SOD, and GR activities. In the development of MTX-induced nephrotoxicity, there exists a reciprocal relationship between inflammatory reactions and oxidative stress. Therefore, NG protects against this nephrotoxicity by diminishing the levels of proinflammatory mediators, specifically TNF-α and IL-6. NG could also influence signaling pathways involved

in apoptosis and tissue damage, by lowering the level of caspase-3 activity **[35]**.

Paeonol: It is a natural phenolic compound found in the root bark of Paeonia suffruticosa, exerts an influence on the expression of the renal efflux transporter known as p-glycoprotein (P-gp), which plays a pivotal role in the elimination of MTX. The mechanistic impact of phenol on the modulation of oxidative stress markers is notable, as it leads to a substantial increase in GSH levels and SOD activity. Additionally, it results in a noteworthy decrease in the levels of MDA and NO. Also, it affects inflammatory markers, including a reduction in the expression of NF-κB, IL-1β, and TLR4, which is a receptor involved in the immune response. Moreover, it induced a reduction in caspases-3 level as a marker

for apoptosis **[36]**.

Mangiferin (MF): MF is a natural compound found in mangoes and several other

plants, and it has been investigated for its potential protective effects against various diseases, including nephrotoxicity induced by MTX. MF treatment significantly counteracted MTX-induced inflammation by reducing the pro-inflammatory markers; NFκB, -IL1-ß, TNF-α, and COX-2. It also hampered MTX-induced oxidative stress through upregulating expression of the nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), hemoxygenase-1(HO-1), GSH, while reducing MDA level, and nitrosative stress (NO and iNOS) levels in the kidney. All of these parameters are affected by the increased renal expression of PPARγ which is a protein that regulates gene expression and is involved in various cellular processes, including inflammation and metabolism **[37]**.

Apocynin (APC): APC is a natural compound that is derived from the root of the plant *Picrorhiza kurroa* and has been investigated for its potential therapeutic effects, particularly its antioxidant and anti-inflammatory properties in an experimental model of MTX-induced nephrotoxicity. APC could repress inflammatory signals by prohibiting NF-κB and TLR4. In addition, it demonstrated inhibitory activity against several kinases that play key roles in promoting inflammatory responses, including the Jak/STAT pathway, as well as the p38-MAPK pathway. Another mechanism contributing to the anti-inflammatory effects is the upregulation of PPAR-γ. Besides, APC could also enhance cell survival through upregulating SIRT1 which is a NAD-dependent deacetylase that plays critical roles in modulating cellular stress response and metabolism through regulating various transcription factors such as forkhead box O3 (FOXO3) which is a transcription factor that regulates genes related to stress resistance and longevity **[38]**.

Dioscin: It is a natural compound found in various plants, including several species of wild yam (Dioscorea spp.); it is classified as a steroidal saponin. Dioscin significantly weakened MTX-induced kidney damage by reducing the expression of a specific microRNA; miRNA, miRNA-145-5p which directly targets SIRT5 that has important roles in metabolism, detoxification, oxidative stress, and apoptosis, It also boosted the antioxidant defense system by upregulating NrF2 thus elevating the expression of the downstream antioxidant genes; HO-1, SOD1, glutathione-S-transferase (GST), GSH cysteine ligase catalytic subunit (GCLC) and NAD(P) Hquinone dehydrogenase (NQO1). This effect was associated with downregulating kelch-like ECH-associated protein 1(Keap1) which acts as the negative regulator of NrF2 transcription factor **[39]**.

Rosmarinic Acid: It is a phenolic compound known for its antioxidant and anti-inflammatory properties. These properties make it potentially useful for counteracting the oxidative stress and inflammation that can contribute to organ damage caused by MTX. It mainly reinforces the antioxidant system by increasing GSH, GPx, and CAT activities and lowering the level of MDA. It also enhances blood flow to the kidneys, helping to maintain proper renal function **[40]**.

Troxerutin: It is a flavonoid compound that is commonly used in medicine for its potential therapeutic properties. It is a derivative of the natural bioflavonoid rutin, often found in various plants and fruits. it attenuates the inflammation produced by MTX by down-regulating the protein expression of HMGB1 which is a receptor of advanced glycation of end product (RAGE). Furthermore, it suppressed nuclear NF-κB signaling while also diminishing the expression of its downstream genes, including COX-2 and TNF-α. Additionally, troxerutin elicited activation of the autophagy flux, as indicated by the up-regulation of renal Beclin 1 and the activation of the adenosine

monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin complex (mTOR) pathway. Remarkably, troxerutin effectively countered renal apoptotic alterations, as demonstrated by a reduction in caspase-3 activity, decreased Bax expression, and a lowered Bax/Bcl-2 ratio. Likewise, it also augmented cell survival as shown by upregulating proliferating cell nuclear antigen (PCNA). Moreover, troxerutin exhibited a protective effect against oxidative damage, as indicated by the reduction in NADPH Oxidase 1 (NOX-1) and lipid peroxide levels. It also restored antioxidant levels, including GSH, GPx, and SOD, while activating the Nrf2/ HO-1 pathway **[41] Table 1**.

Table 1. Experimental models of reno-protective phytochmical approaches against MTX induced renal injury

Protective agent	Model	mechanism of nephroprotection
Apigenin	Animals: mice Experimental design: • Induction: MTX (20 mg/kg.i.p) single dose was given on the fourth day. • Treatment: The API group was treated with API (3 mg/kg i.p) for 7 days. Model Duration: 7 days **[33]**.	✓ Anti-oxidant:↓MDA, ↑SOD, ↑ CAT, ↑GPx. ✓ Anti-inflammatory:↓ iNOS, ↓ CRP, ↓ G-CSF. ✓ Anti-apoptotic:↓caspase 3
Capsaicin	Animals: male Sprague-Dawley rats Experimental design: • Induction: MTX (20 mg/kg) ip daily for 7 days, along with CMC administered orally • Treatment: capsaicin (10 mg/kg/day, p.o.) Model Duration: 7 days **[34]**.	✓ Anti-oxidant: ↓ MDA, ↓ NO, ↑TAC ✓ Anti-apoptotic:↓caspase-3
Naringin	Animals: rats Experimental design: • Induction: single IP dose of MTX (20 mg/kg) on the fourth day. • Treatment: naringin (IP daily dose; 20 mg/kg). Model duration:10 days **[35]**.	✓ Anti-oxidant: ↓MDA,↓NO, ↑GSH, ↑GPx, ↑CAT, ↑SOD, ↑ GR ✓ Anti-inflammatory: ↓TNF-α, ↓IL-6 ✓ Anti-apoptotic:↓caspase-3
Phenol	Animals: rats Experimental design:	✓ Anti-oxidant: ↓ MDA, ↓NO, ↑GSH, ↑SOD ✓ Anti-inflammatory: ↓NF-κB, ↓IL-1β,

	• Induction: MTX as a single i.p dose of 20 mg/kg on the fifth day of the experiment. • Treatment: paeonol administered in a 0.5% CMC, as a single daily oral dose of 100 mg/kg/day. Model duration:10 days **[36]**.	↓TLR4 ✓ Anti-apoptotic:↓caspase-3
Mangiferin	Animals: male Wistar albino rats Experimental design: • Induction: MTX was injected i.p. with a single dose (20 mg/kg) on day 6. • Treatment: Daily oral doses of MF (10, 20, or 40 mg/kg). Model duration:10 days **[37]**.	✓ Anti-inflammatory: ↓NFκB, ↓IL1-ß, ↓TNF-α, ↓COX, ↑ PPARγ ✓ Anti-oxidant: ↑NFE2, ↑Nrf2, ↑HO-1, ↑ GSH, ↓MDA, ↓iNOS
Apocynin	Animals: mice Experimental design: • Induction: single i.p. dose of 20 mg/kg MTX at the end of the 5th day • Treatment: a daily oral dose of APC (100 mg/kg) for 10 days. Model duration:10 days **[38]**.	✓ Anti-inflammatory: ↓NF-κB, ↓TLR4, ↑ PPARγ ✓ ↓ (Jak/STAT) pathway, ✓ ↓p38-MAPK pathway. ✓ ↑ SIRT1
Dioscin	Animals: rats Experimental design: • Induction: (MTX) at a dose of 20 mg/kg On the 7th day • Treatment: intragastrically at doses of 15, 30, and 60 mg/kg once a day for consecutive 7 days. Model duration:10 days **[39]**.	✓ Anti-oxidant: ↑Nrf2, ↑HO-1, ↑SOD, ↑GST, ↑GCLC, ↓KEAP1 ✓ ↑SIRT5
Rosmarinic acid	Animals: rats Experimental design: • Induction: MTX was administered at a dose of 20 mg/kg.	✓ Anti-oxidant:↑GSH, ↑GPx, ↑CAT, ↓MDA

	• Treatment: 100,200 mg/kg RA. **[40].**	
Troxerutin	Animals: mice Experimental design: • Induction: On the 19th day, received a single i.p. injection of MTX (20 mg/kg; 1 mL/kg) • Treatment: Troxerutin (150 mg/kg/day; suspended in CMC vehicle for 21 days by oral gavage. Model Duration: 21 days **[41]**.	✓ Anti-inflammatory: ↓NFκB, ↓HMGB1, ↓TNF-α, ↓COX ✓ Stimulation of the autophagic process: ↑beclin1, ↑AMPK/mTOR ✓ Anti-apoptotic: ↓caspase3, ↓BAX, ↑Bcl2 ✓ ↑ PCNA ✓ Anti-oxidant:↑GSH, ↑GPx, ↑SOD, ↑Nrf2, ↑HO-1, ↓NOX

Conclusion

Numerous experimental models have consistently demonstrated that MTX leads to renal damage, with adverse implications for human health. The potential underlying mechanisms encompass oxidative stress, apoptosis, and inflammation. Presently, there is no dedicated treatment for MTX-induced renal toxicity. Nonetheless, animal studies have identified several promising phytochemical approaches that could potentially mitigate MTX-induced renal toxicity without compromising MTX anti-cancer efficacy. This review offers a comprehensive analysis of recent research on MTX-induced nephrotoxicity, specifically focusing on oxidative stress, inflammation, and apoptosis as key mechanisms. The emphasis on recent literature ensures an up-to-date understanding of this field. A unique aspect of the review is its in-depth discussion of promising phytochemical protective strategies, highlighting molecular mechanisms behind nephroprotective effects. By delving into recent experimental studies on natural compounds, the review provides practical insights into potential therapeutic interventions. Importantly, the review goes beyond mechanistic discussions by integrating information on kidney toxicity assessment in animal models, bridging the gap between mechanistic insights and translational aspects of nephrotoxicity research.

Abbreviations

AICAR, 5-Aminoimidazole-4-carboxamide ribonucleotide; AKI, Acute Kidney Injury; AMPK, Adenosine Monophosphate-Activated Protein Kinase; Apaf-1, Apoptotic Protease-Activating Factor; CAT, Catalase; CMC, Carboxymethyl cellulose; COX-2, Cyclooxygenase-2; CRP, C-Reactive Protein; DAMPS, Damage-Associated Molecular Patterns; DNA, Deoxyribonucleic Acid; FDA, Food and drug administration; FOXO3, Forkhead Box O3; GCLC, Glutamate-Cysteine Ligase Catalytic Subunit; G-CSF, granulocyte-colony stimulating factor; GFR, Glomerular filtration rate; GPx, Glutathione Peroxidase; GSH, Glutathione; GST, Glutathione-S-Transferase; HMGB1, High-Mobility Group Box 1; HO-1, Heme Oxygenase-1; IL-1β, Interleukin-1 beta; IL6, Interleukin 6; INOS, Inducible Nitric Oxide Synthase; Jak/STAT, Janus kinase/signal transducers and activators of transcription; Keap1, Kelch Like ECH-Associated Protein 1; KIM, Kidney Injury Molecule; MAPK, Mitogen-Activated Protein Kinase; MDA, Malondialdehyde; miRNA, MicroRNA; mTOR, Mammalian Target of Rapamycin; MTX,

Methotrexate; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; NAG, N-Acetyl-β-D-Glucosaminidase; NF-κB, Nuclear Factor-kappa B; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NO, Nitric Oxide; NOX-1, NADPH Oxidase 1; NQO1, NAD(P)H Quinone Dehydrogenase 1; Nrf2, Nuclear Factor Erythroid 2-related Factor 2; PCNA, Proliferating Cell Nuclear Antigen; P-gp, P-Glycoprotein; PPARγ, Peroxisome Proliferator-Activated Receptor Gamma; RAGE, Receptor for Advanced Glycation End Products; ROS, Reactive Oxygen Species; SIRT1, Sirtuin 1; SOD, Superoxide Dismutase; TAC, Total antioxidant capacity; TLR4,Toll-Like Receptor 4; TNF-α, Tumor Necrosis Factor-alpha.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

The authors declare that no competing interests exist

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Authors' contributions

The manuscript was drafted and written Asmaa Algendy. All authors have read and approved the final manuscript.

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