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The Protective Effect of Urtica dioica L. (Stinging Nettle) on Kidney Injuries Induced by Gentamicin

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Abstract

The excessive and widespread utilization of antibiotics in modern medical practices has sparked significant concerns regarding the diverse array of side effects and complications accompanying their usage. Gentamicin, among these antibiotics, is notably associated with a heightened risk of nephrotoxicity, particularly when administered in elevated doses or over prolonged treatment durations. In response to this escalating challenge, a surge of interest has emerged in exploring the potential of dietary plants as a prophylactic measure against kidney injuries and nephrotoxicity. Urtica dioica, commonly known as stinging nettle, emerges as a compelling botanical contender, distinguished by its rich composition of bioactive compounds such as flavonoids and phenolic acids renowned for their antioxidative and anti-inflammatory properties. The primary aim of the research endeavors to evaluate the impact of Stinging Nettle (Urtica dioica) on kidney injuries induced by Gentamicin, drawing insights from precedent investigations in the field. Empirical studies have underscored the capacity of Urtica extracts to mitigate oxidative stress, inflammation, and tissue damage in animal models subjected to gentamicin treatment, signaling a promising nephroprotective potential. Additionally, supplementation with Urtica has exhibited enhancements in kidney function markers, indicative of its efficacy in counterbalancing the deleterious effects of gentamicin on renal health. In summation, Urtica presents itself as a propitious adjunctive therapeutic avenue for alleviating the nephrotoxic repercussions of gentamicin and ameliorating renal function. The diverse spectrum of bioactive compounds inherent in Urtica not only holds promise for enhancing the safety and efficacy of antibiotic regimens but also for safeguarding renal health against their detrimental impacts.

Keywords: Stinging Nettle; Kidney; *Urtica;* antibiotic agent; gentamicin

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1. Introduction

The methodological investigation of natural resources for novel pharmacotherapeutic development has emerged as a cornerstone of global biomedical research. Naturally derived compounds currently constitute a substantial proportion of clinically approved pharmacotherapeutics, accounting for over 30% of prescribed agents, owing to their structural diversity and bio functional efficacy [1]. Concurrently, the escalating prevalence of antimicrobial-resistant infections has intensified the urgency for innovative therapeutic strategies, prompting a resurgence in phytochemical exploration [2]. Medicinal flora, with their ethnopharmacological relevance, have historically served dual roles as sustenance and therapeutic agents, while modern research underscores their potential as reservoirs for bioactive lead compounds [3]. Contemporary scientific inquiry has increasingly focused on plant-derived nutraceuticals and functional foods as prophylactic and adjunctive interventions against lifestyle-associated pathologies, including inflammatory disorders [4], dyslipidemia [5], and oncogenesis [6]. Botanicals abundant in nutraceutical constituents such as essential amino acids, vitamins, and polyphenolic compounds (e.g., phenolic acids, flavonoids) are garnering translational interest, with many standardized extracts commercialized in diverse formulations [7].

However, critical gaps persist in delineating bioactive phytoconstituents, elucidating their molecular mechanisms, and advancing formulation technologies to optimize bioavailability & therapeutic precision. Prioritizing phytochemical characterization, pharmacodynamic validation, and innovative drug delivery systems remain imperative to harness the full potential of plant-based therapeutics. Herbal medicine, also referred to as "phytomedicine," involves utilization of therapeutic plants, plant parts, or substances derived from plants to combat infections, diseases, or enhance overall health. For centuries, herbs have been integral to medicinal practices, offering various health benefits. Plants have long served as a valuable source of natural products, with increased emphasis on natural therapies in recent years, as evidenced by intensified research efforts [8]. Utilization of phytoalimurgic plants has surged in recent years due to their distinctive chemical composition and their positive impact on human health [9]. Emerging evidence underscores dual therapeutic capacity of botanical agents to exert anti-inflammatory effects while concurrently enhancing innate immune responses.

Notably, these phytotherapeutic compounds exhibit a favorable safety profile relative to allopathic pharmacotherapies, such as antibiotics, which are often associated with adverse iatrogenic sequelae [10]. Medicinal floras possess bioactive constituents that modulate homeostatic equilibrium & mitigate metabolic accumulation of xenobiotics, offering a distinct advantage over synthetic drugs due to their minimal toxicological liabilities. Analgesic phytochemicals including flavonoids, terpenoids, phenolic derivatives, alkaloids, and organic acids mediate their therapeutic effects via inhibition of cyclooxygenase enzymatic activity, thereby attenuating prostaglandin biosynthesis and associated inflammatory cascades [11]. Globally, the World Health Organization (WHO) catalogs over 21,000 plant species with ethnomedicinal applications, of which 2,500 are indigenous to India. Among these, 150 species are industrially valorized, solidifying India's status as preeminent producer of medicinal botanicals and a global epicenter of phytodiversity [8]. The Ayurvedic pharmacopeia, millennia-old system of medicine, enumerates flora with multimodal therapeutic properties, including immunomodulatory, nootropic, geroprotective, antimicrobial, antineoplastic, and adaptogenic activities.

Central to this tradition are *Rasayanas* a class of botanicals endowed with bidirectional immunomodulatory capacity, capable of non-specifically potentiating host defenses against pathogens and neoplasms or selectively modulating antigen-specific immune reactivity [10]. The overuse of antibiotics, such as gentamicin, has led to an increase in adverse effects, notably nephrotoxicity, which refers to kidney damage resulting in impaired function. Gentamicin, commonly used in clinical practice, is known to cause nephrotoxicity. Recently, there's been interest in natural remedies to counter gentamicin's nephrotoxic effects, with nettle (*Urtica dioica*) emerging as a potential solution. Nettle, a perennial plant with traditional medicinal uses like diuretic & urinary tract disorder treatment, contains bioactive substances such flavonoids and phenolic acids. Studies suggest nettle's anti-inflammatory and antioxidant qualities may protect against gentamicin-induced nephrotoxicity via oxidative stress reduction, free radical scavenging, and alleviating inflammation in renal tissue. The objectives of this research are to assess the impact of Nettle (Urtica dioica) phenols on nephrotoxicity caused by gentamicin and to determine the chemical makeup of Nettle (Urtica dioica).

1.1. Stinging Nettle (Urtica dioica) Botanical Characterization

Urtica dioica L., commonly known as stinging nettle, is a perennial herbaceous species belonging to the Urticaceae family and classified under genus Urtica (Table 1). The etymology of the genus name Urtica originates from the Latin verb urere, meaning "to burn," a reference to the plant's characteristic stinging trichomes. Similarly, the species epithet dioica, derived from the Greek term for "two houses," denotes its dioecious reproductive system, wherein male and female flowers are typically borne on separate individuals [12]. It is widespread in both temperate and tropical regions globally. Stinging nettle is predominantly distributed in Europe, North Africa, North America, and specific areas of Asia, Turkey, and New Zealand. In Nepal's highlands and mountains, it thrives in the wild [13]. It prefers rich, moist soil, especially near waterways, neglected yards, and along forest edges. It can also be found in fencerows and along railroad and highway right-of-ways.

The plants can reach heights of 4 to 6 feet, featuring fibrous, unbranched stems. Their leaves are green, slender, heart- or elm-shaped, serrated, and rough-textured, measuring 2 to 3 inches in length. These leaves grow at regular intervals along each stem (Figure 1A). During warm-weather months, clusters of inconspicuous yellow-green flowers appear where the leaves attach to the stem (Figure 1B) [14]. There are tiny, stinging hairs called trichomes all over leaves and stems (Figure 2A). Nettle is renowned for its capacity to inflict a sharp sting upon contact with these delicate hairs and bristles present on its foliage and stems, serving as a natural defense mechanism against plant predators. These hollow hairs feature a sharp-pointed, bulbous tip reminiscent of a hypodermic needle [15]. And bends upon contact to penetrate skin (Figure 2B). An inflammatory substance is released into the skin from a gland located at base of these hairs through broken bulb at tip [16]. Liquid found within nettle's hairs responsible for its stinging sensation, comprising serotonin (5-hydroxytryptamine), 1 in 500 to 1 in 2000 histamine, 1% acetylcholine, and trace amounts of leukotrienes & formic acid [17]. Leading to contact urticarial dermatitis in many people.

1.2. Phytochemistry of Stinging Nettle (Urtica dioica)

Phytochemicals, secondary metabolites synthesized by plants as adaptive responses to biotic stressors or metabolic processes, exhibit a diverse array of bioactive properties with therapeutic potential [18]. *Urtica dioica* (stinging nettle) is characterized by a complex phytochemical profile comprising approximately fifty identified compounds, spanning both lipophilic and hydrophilic fractions, with well-elucidated chemical structures. Although phytochemical screening has been conducted on a limited number of *Urtica* species globally, extant research reveals the presence of sterols, triterpenoids, coumarins, phenolic derivatives, lignans, ceramides, and fatty acids, variably distributed across distinct plant tissues [17]. The principal bioactive constituents of *Urtica dioica* include flavonoids, tannins, volatile organic compounds, polysaccharides, isolectins, sterols, terpenoids, proteins, vitamins, and essential minerals, underscoring its multifaceted pharmacological utility [19]. Given the extensive ethnomedicinal and nutraceutical applications of *Urtica dioica*, there is an imperative need for systematic phytochemical characterization and mechanistic exploration to optimize its therapeutic potential and facilitate evidence-based integration into clinical and dietary frameworks.

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1.3. Importance of Stinging Nettle (Urtica dioica)

Stinging nettle has long been used for beneficial purposes. Before its medicinal use, extracts of the plant were used for fabric weaving as early as the Bronze Age. Paper was made from nettle fibers. In a similar vein, people have traditionally eaten this plant.Particularly young leaves can be eaten raw; however, in order to prevent irritation, they should always be boiled. They can also be added to soups, tea, beer, and wine. Nettles are rich in important amino and fatty acids, vitamin C, carotenoids, iron, and other minerals. If someone wants to handle nettle plants for cooking, they should wear long sleeves and gloves to prevent direct skin contact [20]. The importance separated into:

1.3.1.Nutritional Importance

Because of their high levels of protein, amino acids, vitamins, minerals, and chlorophyll, nettles are referred to as "super food." You may significantly increase the number of nutrients in your diet that will help you feel stronger and more energized by simply eating nettles once or twice a week. You can consume the entire young plant, including the stems. Bigger stems should be cut off since they will become fibrous [21]. For generations, the plant has been utilized as a wholesome food source. The leaves and stalks are eaten as vegetables and in salads, and they are also consumed in tea prepared from the leaves, stalks, and roots. In remote highland regions of India and Nepal, people collect the tender shoots and leaves of this plant using bamboo or iron pincers and boil them to make a vegetable or soup. It is also boiled with other plants, such as millet, maize, or wheat flour, along with a little salt and chile, to make porridge. It has been utilized as a nutritional tonic and in numerous different recipes in recent years, including soups, infusions, decoctions, and dried leaves for herbal tea [22]. (**Table 3**) lists the nutritional makeup of Urtica dioica.

Influence of thermal processing on macronutrient, anti-nutrient, & elemental profiles of *L. peduncularis* and *Urtica dioica* leaves was systematically investigated. Cooking observed to reduce concentrations of crude fat, ash, carbohydrates, and ascorbic acid (vitamin C), while concurrently elevating tocopherol (vitamin E) levels. Although anti-nutritional factors, including phytates, cyanides, and saponins, exhibited a marginal increase in post-cooking, oxalate content demonstrated significant reduction. These findings revealed complex interrelationships both synergistic and antagonistic among nutrients, anti-nutrients, and trace elements within the leaf matrices. In a complementary study, an aqueous extract derived from the aerial parts of *Urtica dioica* L. (Urticaceae) was evaluated for its cardiovascular effects, demonstrating acute hypotensive activity. This suggests a direct pharmacological influence on vascular tone, further underscoring the therapeutic potential of *Urtica dioica* [23].

1.3.2. Industrial Importance

Stinging nettle is occasionally used in cosmetics and is utilized as a source of textile bast fibers [24]. The fiber is robust and can be used to construct nets, fishing lines, and rope. In China, nettle clothing dating back two millennia was discovered to be in perfect condition [21]. Chlorophyll, a component used in foods and medications as a green coloring (E140), is extracted from the plants for commercial use [25].

1.3.3. Pharmacological Importance

Stinging nettle is utilized medicinally in both its herbs and root form and has been utilized as a natural medicine due to its therapeutic qualities. Though in distinct ways. The plant is generally advised to be used as a nutritional tonic, as an adjuvant treatment for rheumatic disorders, and, more recently, to cure allergies with the fresh, freeze-dried leaves. Root is utilized to lessen benign prostate hyperplasia-related problems. Clinical, in vitro, and animal studies have all helped to clarify the mechanism of action and offer evidence in favor of some of these conventional applications. Research on stinging nettle herbs has shifted from clinical to pharmacological since then. Fresh leaf topical treatment has also been utilized historically and clinically [15]. *Urtica dioica* (stinging nettle) has been employed for centuries in traditional medicine systems for management of diverse pathologies, including rheumatism, eczema, urinary tract infections, nephrolithiasis, & benign prostatic hyperplasia in its early stages. Additionally, its ethnomedicinal applications extend to treatment of epistaxis, arthritis, anemia, allergic rhinitis, and dermatological conditions. The plant has also been utilized as a diuretic, astringent, and hematinic agent, with historical use cases encompassing gout, sciatica, neuralgia, hemorrhoids, and alopecia [26].

1.3.3.1. Anti-proliferative Effect

The gland responsible for excreting urine from the bladder, known as the prostate, is a part of the male reproductive system. Because of its anti-inflammatory and diuretic qualities, nettle has shown to be a viable method of preventing prostate growth in males, however prostate enlargement is still a significant aspect to take into account [27]. Men's biggest concerns as they age are prostate enlargement and prostate cancer, and stinging nettle has been found to be a useful remedy for reducing prostate growth. The ability of stinging nettles to stop or slow the invasion of cells, especially malignant ones, into nearby tissues is one of its traits. Numerous studies have demonstrated that nettle roots disrupt several pathways involved in the development of benign prostatic hyperplasia. The anti-proliferative effects of UDA and methanolic alcohol root extracts on prostate cancer cells have been demonstrated in both in vitro and in vivo experiments.

Lignans, which are derived from root extract, inhibit the proliferative activity of androgens on prostate tissues by preventing them from binding to the prostate's membrane receptors and their transporter protein, SHBG (Sex Hormone Binding Globulin) [28], [29]. Because the root extract inhibits aromatase, less estrogen is produced and androgens are converted to estrogens [30]. The effect of Urtica dioica root to inhibit 5α-reductase has been investigated through in vitro research on testosterone-induced BPH. Among the treatments used for BPH research include petroleum ether, ethanolic extracts (10, 20, and 50 mg/kg) and isolated β-sitosterol (10 and 20 mg/kg). Urtica dioica shows potential for managing benign prostatic hyperplasia (BPH), as evidenced by a decrease in weekly urine output, reduction in the prostate/body weight ratio, lower serum testosterone levels, and a decrease in prostate-specific antigen levels [31].

1.3.3.2. *Anti-diabetic Effect*

Diabetes mellitus (DM) is a metabolic disorder characterized by dysregulated blood glucose homeostasis, frequently accompanied by hypertriglyceridemia and diminished high-density lipoprotein (HDL) levels. The condition is defined by chronic hyperglycemia, a pathological elevation in blood glucose concentrations, which manifests clinically as polyuria, polyphagia, and polydipsia [32]. Beyond its systemic effects, DM exerts profound functional and structural alterations on the central nervous system, underscoring its multifaceted pathophysiological impact. Aqueous nettle leaf extracts have been shown in an in vivo investigation to exhibit anti-diabetic properties [33]. The hypoglycemic effects were tested in diabetic mice. These outcomes are the result of reduced intestinal absorption of glucose [34]. Numerous investigations have revealed that nettle increases the release of insulin, which lowers blood sugar. Examining both healthy and sick rats after intraperitoneal administration of aqueous extract verified this conclusion [35].

Recent investigations have demonstrated the antidiabetic efficacy of zinc oxide (ZnO) nanoparticles synthesized from *Urtica dioica* leaf aqueous extract in an alloxan-induced diabetic rat model. Administered at a dosage of 8 mg/kg body weight/day via intraperitoneal injection, the treatment significantly reduced fasting blood glucose, total cholesterol, and serum triglyceride levels, while concurrently elevating high-density lipoprotein (HDL) and insulin concentrations [36]. These findings underscore the therapeutic potential of *U. dioica*-derived ZnO nanoparticles in modulating glycemic and lipidemic profiles in diabetic conditions. In two recent controlled trials, stinging nettle treatment for type 2 diabetes mellitus participants was investigated. In one study, nettle extract at a dose of 100 mg/kg per day reduced inflammatory biomarkers but did not increase insulin sensitivity [37]. In the second research, participants who needed insulin administered 500 mg of nettle leaf extract once every eight hours for three months. Hemoglobin A1c levels, 2-hour postprandial glucose, and fasting blood glucose were all markedly reduced by this treatment [38].

1.3.3.3. *Anti-Inflammatory, Analgesic Effect*

Herbal remedies demonstrate anti-inflammatory properties by affecting different cellular signaling pathways or endogenous enzymes crucial in the inflammatory process. Extracts from Urtica dioica, for instance, target inflammatory pathways such as cyclooxygenase (COX)-1 and COX-2 to hinder the production of inflammatory prostaglandins. Additionally, they inhibit mast cell tryptase, thus preventing degranulation and further inflammation [39]. In an *in vitro* study by [40], the ethanol extract of *Urtica dioica* demonstrated potent anti-inflammatory activity by suppressing the release of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) in lipopolysaccharide (LPS)-stimulated human whole blood. At a concentration of 5 mg/mL, the extract significantly reduced TNF-α and IL-1β levels by 50.8% and 99.7%, respectively, following 24 hours of LPS exposure. Complementing these findings, [32] conducted an *in vivo* anti-inflammatory assessment of *Urtica dioica* using solvent extracts (hexane, chloroform, ethyl acetate, methanol, and aqueous) administered at 200 mg/kg body weight to Wistar rats. Carrageenan-induced rat paw edema was employed as the inflammatory model, with indomethacin serving as the reference anti-inflammatory agent. The hexane extract exhibited the most pronounced anti-inflammatory efficacy, reducing paw edema volume by 46.51% at three hours post-administration, comparable to the 53.48% reduction achieved with indomethacin. In contrast, methanol, aqueous, and ethyl acetate extracts showed negligible inhibition of edema volume, highlighting the solvent-dependent variability in bioactive efficacy.

1.3.3.4. *Antioxidant Effect*

Antioxidants are becoming more widely acknowledged as therapeutic and preventive medicines that target and neutralize reactive oxygen species, also known as free radicals, to prevent their negative effects. Free radicals are implicated in the aging process and have been connected to emergence of diseases like diabetes, cancer, cardiovascular disease, autoimmune disorders, and neurological diseases [41]. The antioxidant qualities of medicinal herbs have been the subject of much research since they are essential in halting lipid peroxidation and shielding cells from the harmful effects of reactive oxygen species (ROS). Plants serve as a rich natural source of antioxidants, with nettles being identified for their particularly high antioxidant properties. This discovery suggests potential applications in the pharmaceutical industry, where nettle could be utilized as a natural antioxidant supplement [42]. Because of their redox characteristics, phenols may have antioxidant effects. Because phenolics contain hydroxyl groups, total phenolic content can be used for quick screening of antioxidant activity. The abundance of free OH groups is associated with the antioxidant capacity of plant compounds known as flavonoids [43]. According to [44], fresh nettle leaves have higher levels of soluble peroxidase activity along with trace amounts of proteins, carotenoids, and chlorophyll.

The in vivo study demonstrated that polyphenols bind to blood lipids, thereby reducing lipid peroxidation [45]. These chemicals' mechanisms of antioxidant activity usually include the ability to bind metal ions, transfer hydrogen atoms, and transfer electrons. Transfer of a hydrogen atom is generally preferred over electron transfer due to the higher energy requirement for the electron transfer process. Additionally, the presence of free hydroxyl groups enables polyphenolic compounds to interact with transition metals and chelate them [46]. Antioxidant capacity of *Urtica dioica* (UD) systematically evaluated using cupric ion-reducing antioxidant capacity (CUPRAC) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, complemented by high-performance liquid chromatography (HPLC) analysis, with comparative assessments involving parsley and celery leaves. Antioxidant activity was measured for a 70% methanolic UD extract, its hydrolysate, and the hydrolysate of dried plant material. Among these, UD extract demonstrated most pronounced antioxidant activity in CUPRAC assay [47].

In a separate investigation, the antioxidant efficacy of methanolic and ethanolic root extracts of UD was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The methanolic root extract exhibited significantly higher antioxidant activity compared to its ethanolic counterpart, underscoring the solvent-dependent variability in bioactive potential [48]. *Urtica dioica* (stinging nettle) extracts demonstrate significant reactive oxygen species (ROS) scavenging activity, as evidenced by spectrophotometric assays quantifying antiradical efficacy against nitric oxide (NO•), hydroxyl (OH•), and superoxide ($O_2^{•-}$) radicals. Empirical studies further reveal that methanolic and ethanolic leaf extracts exhibit potent antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, underscoring their redox-modulatory potential [49-50]. Beyond antioxidant mechanisms, *Urtica dioica* demonstrates therapeutic promise in dermatological applications. Its bioactive constituents accelerate tissue repair, attenuate dermal imperfections (e.g., scars, blemishes), and mitigate bacterial colonization, rendering it efficacious in acne management. Furthermore, its antioxidative properties confer anti-aging benefits, including the reduction of epidermal wrinkles and hyperpigmentation, likely through inhibition of oxidative stress-mediated collagen degradation.

1.3.3.5. Cardioprotective Effect

Cardioprotection encompasses all methods and practices that contribute to maintaining heart health by reducing or even avoiding myocardial injury [51]. At doses of 20 and 150 mg/kg/day, respectively, U. dioica water and petroleum extract raised the blood lipid level in rats. decreased blood cholesterol levels and LDL/HDL lipoprotein ratios after 30 days. Conversely, U. dioica ethanol extract decreased LDL and cholesterol levels at doses of 100 and 300 mg/kg [52-53]. In a Langendorff-perfused rat heart, aqueous preparations of U. dioica (1 and 2 g/L) increased left ventricular pressure and decreased heart rate. It also strengthened the ischemia-reperfusion resistance of the isolated rat heart [54]. An *in vivo* investigation evaluated the dose-dependent hypotensive effects of *Urtica dioica* in anesthetized rat models. Intravenous administration of the extract at 0.1, 1, and 10 mg/kg elicited significant reductions in mean arterial pressure (MAP). Baseline MAP was recorded at 96.5 ± 0.5 mmHg. Post-administration, the 0.1 mg/kg dose reduced MAP to 79.5 ± 0.5 mmHg, while 1 mg/kg and 10 mg/kg doses further lowered MAP to 65.5 ± 5.5 mmHg and 55.5 ± 9.5 mmHg, respectively. Following the transient hypotensive peak (observed 2–3 minutes post-administration), MAP gradually normalized to baseline levels, indicating a reversible pharmacological effect [55].

1.3.3.6. Aids in Women's Health

Women's health can be greatly enhanced by drinking nettle leaf tea. Nettle is a popular herb for women's health since it has historically been used to enhance milk production. Compared to men, women are more prone to have UTIs. Stinging nettle acts as a diuretic, helping women eliminate more toxins associated with UTIs [56]. Stinging nettle's astringent properties can alleviate menstrual cramps and bloating, while its high mineral content explains its historical use as a nutritional tea supplement for pregnant women. It acts as a tonic for hormonal fluctuations, benefiting women approaching menopause. Its coagulating effects are helpful for managing excessive bleeding and aiding in labor, and nettle tea is traditionally used for women experiencing intense labor pains. Moreover, it promotes milk production and eases nursing for newborns. However, due to its impact on the menstrual cycle and potential to induce uterine contractions, pregnant women should avoid using this herb to prevent miscarriage [27].

Endometriosis, a pathological disorder characterized by the ectopic implantation of endometrial tissue outside the uterine cavity, is associated with chronic pelvic pain and inflammation. While certain phytotherapeutic agents may accelerate lesion regression, others risk potentiating disease progression. In a surgically induced endometriosis rat model, oral administration of a methanolic extract derived from aerial parts of *Urtica dioica* (100 mg/kg body weight/day) demonstrated significant anti-endometriotic efficacy. Treatment attenuated ectopic implant proliferation and adhesion formation, concomitant with reductions in peritoneal concentrations of tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6)—key mediators of angiogenesis and inflammation. The Histopathological analyses corroborated these findings, revealing the diminished lesion viability and the stromal remodeling, as reported by [57].

1.4. **Toxicity of *Urtica dioica***

Touching the Urtica plant frequently causes skin redness and irritation, notwithstanding the occasional reports of perspiration and stomach pain [35]. Cases of hypersensitivity have been documented in patients with renal conditions. Additionally, Urtica aerial parts can enhance urine flow, so it's crucial to inform healthcare providers about any diabetes or kidney issues [55]. Although nettle's role as an abortifacient and its effect on menstrual cycles suggested by traditional medicine, rats given 250 mg/kg of nettle orally did not exhibit any antifertility effects. However, it should be avoided during pregnancy and lactation because there is no proof that it has antifertility effects [58]. Although Urtica preparations are used postpartum to treat anemia and promote lactation, there's no scientific backing for their safety and effectiveness for babies or nursing moms. For rheumatoid arthritis, Urtica dioica used as an anti-inflammatory, but caution is advised in cases of acute arthritis to avoid potential drug interactions [59]. The non-toxic nature of Urtica dioica-derived fixed oil demonstrated by fact that it was entirely non-lethal even at concentrations as high as 12.8 mL/kg [60].

Rats given an aqueous extract or infusion of the roots intravenously have been shown to have LD50s of 1.721 g/kg and 1.929 g/kg b.w., respectively, whereas rats given an oral root infusion can tolerate up to 1.310 g/kg body weight [61]. Approximately 2 kg rabbits were given 50 ml of a 50% ethanol extract orally for 10 days as part of another toxicity research. After a few days of treatment, this resulted in mortality, a 40% drop in body weight, and sporadic diarrhea. Purulent blisters were found near the injection site during autopsy, and the rabbits' central excitatory behavior and increased breathing were noted before they died [62]. The hydrosoluble components of nettle, which are eliminated by boiling and are believed to possess a pyran-coumarin structure, have been associated with its toxicity. However, adverse effects from nettle root treatment are highly unlikely. Studies using standardized nettle extract have shown minimal mortality in mice, even at 50% doses [28-29].

1.5. Definition of Gentamicin

Gentamicin, a broad-spectrum aminoglycoside antibiotic first isolated in 1963, remains a critical therapeutic agent for infections caused by multidrug-resistant gram-negative pathogens [63]. Its bactericidal activity targets aerobic gram-negative bacteria via oxygen-dependent transmembrane transport, rendering it ineffective against anaerobic organisms due to their hypoxic metabolic milieu. Clinically, gentamicin is employed in the management of bacterial septicemia, meningitis, urinary tract infections, peritonitis, and soft tissue infections, with its application guided by culture susceptibility data or epidemiological evidence of pathogen prevalence. Pharmacodynamic profile of aminoglycosides, including gentamicin, characterized by concentration-dependent bactericidal efficacy, wherein higher peak serum concentrations correlate with enhanced microbial eradication. A distinctive feature of this class is postantibiotic effect (PAE), wherein transient suppression of bacterial regrowth persists for hours after drug concentrations fall below minimum inhibitory concentration (MIC).

This property is amplified by optimized dosing regimens that prioritize high peak concentrations. Per U.S. Food and Drug Administration (FDA) guidelines, gentamicin therapy should ideally be informed by culture and susceptibility testing. However, empirical use is permissible in scenarios with established pathogen susceptibility profiles. Clinical decision-making incorporates patient-specific variables, including age, symptomatology, and regional antibiotic resistance patterns, to maximize therapeutic precision and mitigate resistance development [64]. Owing to limited oral bioavailability, gentamicin is administered parenterally (intramuscular or intravenous), topically, or ophthalmically. Its spectrum of activity encompasses aerobic and facultative gram-negative bacteria, including Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Serratia* spp., *Enterobacter* spp.), *Pseudomonas aeruginosa*, and select strains of *Neisseria*, *Moraxella*, and *Haemophilus*. Resistance, though documented, remains relatively uncommon due to the conserved aerobic metabolic pathways targeted by aminoglycosides.

1.6. Formulations and Clinical Applications

Gentamicin demonstrates versatile clinical utility across multiple formulations, each tailored to specific therapeutic contexts. Ophthalmic preparations, including 0.3% (w/v) solutions and ointments, are indicated for bacterial conjunctivitis and keratitis. Topical applications utilize 0.1% (w/v) creams or ointments to manage cutaneous and subcutaneous infections secondary to burns, abrasions, or traumatic wounds. For systemic infections, parenteral administration employs weight-based intramuscular or intravenous dosing regimens, optimized through pharmacokinetic-pharmacodynamic principles to maximize efficacy and minimize toxicity. Collectively, these applications underscore gentamicin's enduring relevance in antimicrobial stewardship, strategically balancing therapeutic potency with judicious use to mitigate resistance proliferation while addressing diverse infectious pathologies.

1.7. Adverse Effects of Gentamicin

The inner ear contains large quantities of gentamicin. Damage could result in problems with hearing and, particularly, the vestibular system. High-pitched tinnitus is frequently the initial sign of cochlear damage, and it might persist for a few weeks after the gentamicin is stopped. About two-thirds of patients on aminoglycosides, such as gentamicin, may have a high-frequency hearing loss, yet very few report any hearing problems. During the first two weeks, nausea, vomiting, balance issues, and vertigo are symptoms of vestibular toxicity. A chronic phase is characterized by ataxia and frequently leaves some residual dysfunctions. It can last for around two months. Also, the renal cortex contains large quantities of gentamicin. That can harm renal proximal tubular cells because it tends to accumulate there. Therefore, gentamicin use may result in mild proteinuria and a decrease in glomerular filtration rate, which occurred in 14% of gentamicin users. Renal damage and its aftereffects are frequently reversible if proximal tubular cells possess the ability to regenerate. A prolonged period of gentamicin serum levels over the toxicity threshold is implied by the divided-doses strategy, which raises the risk of ototoxicity and nephrotoxicity. One uncommon but potentially harmful side effect of practically all aminoglycosides is neuromuscular inhibition. Concurrent illnesses like (myasthenia gravis) or drugs like (vecuronium) that affect the neuromuscular junction are recognized risk factors. The blockage most likely stems from aminoglycoside-mediated decrease of acetylcholine presynaptic release and interference with acetylcholine postsynaptic receptor activity. This toxicity can be addressed with intravenous calcium therapy [64].

1.8. Previous Studies

First Study: An experimental investigation conducted in Iraq utilizing a male rabbit model demonstrated the nephroprotective efficacy of orally administered Urtica dioica (stinging nettle) ethanol extract against gentamicin-induced renal toxicity. The extract preserved intracellular homeostatic pathways critical to renal function while enhancing the urinary excretion of gentamicin, thereby attenuating drug accumulation and subsequent nephrotoxicity. These findings highlight the extract's capacity to maintain redox equilibrium and promote detoxification mechanisms [65].

Second Study: A preclinical trial in Iran evaluated the synergistic effects of methanolic *Urtica dioica* (UD) leaf extract in mitigating gentamicin-induced acute kidney injury in rats. Co-administration of UD extract significantly ameliorated renal pathology by suppressing oxidative stress biomarkers, lipid peroxidation cascades, and reactive oxygen species (ROS) generation. Histopathological and biochemical analyses attributed this renoprotection to the extract's dual antioxidative and anti-inflammatory properties, suggesting its utility as an adjunctive therapy in attenuating antibiotic-associated nephrotoxicity [66].

Third Study: A recent Egyptian study investigated the dose-dependent nephroprotective effects of *Urtica dioica* leaf and seed powders (2.5% and 5% concentrations) in gentamicin-exposed male rats. The intervention normalized renal function biomarkers, including serum uric acid, creatinine, and urea levels, while modulating lipid profiles (triglycerides, total cholesterol, LDL-c, VLDL-c, and HDL-c). Additionally, the treatment stabilized serum glucose concentrations and reduced hepatic enzyme activities (ALP, AST, ALT), underscoring the plant's multifactorial therapeutic potential in mitigating systemic metabolic dysregulation induced by gentamicin [67].

2. Material and Methods

2.1. First Study (Impact of nettle (Urtica dioica) extract on male rabbits' nephrotoxicity caused by gentamicin) Material and Methods

[65] Collected Urtica dioica (Nettle) from Kurdistan region / Iraqi. The plant was washed, dried at room temperature, and crushed. Ethanolic extraction using a Soxhlet apparatus was performed, with 10 g of leaf powder and 50 mL of 95% ethanol. To create a 200 mg/mL concentration for the investigation, the dried ethanolic extract was dissolved in dimethylsulfoxide [68]; [69]. An experimental chore comprising twenty rabbits was stratified into four groups (n = 5 per group) and subjected to a 10-day intervention protocol: Group 1 (Control): Administered normal saline (1 mL/kg/day); Group 2 (GM): Treated with gentamicin (100 mg/kg/day); Group 3 (UD): Received ethanol extract of *Urtica dioica* (100 mg/kg/day); Group 4 (GM + UD): Co-administered gentamicin (100 mg/kg/day) and *Urtica dioica* extract (100 mg/kg/day). Following the intervention period, blood samples were obtained via marginal ear vein puncture, subjected to centrifugation, and analyzed for renal function biomarkers. Serum concentrations of blood urea nitrogen (BUN) and creatinine were quantified using spectrophotometric analysis (LISA-200 autoanalyzer) to assess nephrotoxic outcomes. Then sacrificed the rabbits, and excised their kidneys for tissue homogenate, placed in chilled phosphate buffer, blotted, and weighed. Homogenized 1 g of kidney in same buffer to make 10% homogenate [69-70]. Histopathological evaluation of renal tissue performed to investigate nephrotoxic alterations.

Kidney samples were fixed in 10% neutral buffered formalin, dehydrated through an ascending ethanol gradient, embedded in paraffin wax, and sectioned into 4–6 µm slices. Tissue sections were stained with hematoxylin and eosin (H&E) for microscopic assessment of structural pathology, following established protocols [71]. Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay. This method involves the formation of a red chromophore through reaction of MDA with thiobarbituric acid, with absorbance measured spectrophotometrically at 532 nm [72]. Reduced glutathione (GSH) concentrations were determined via Ellman's method, wherein 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) reacts with free thiol groups to yield a yellow derivative, quantified at 412 nm [69]. Total phenolic content in *Urtica dioica* leaves was assessed using the Folin-Ciocalteu assay, with results standardized to gallic acid equivalents (GAE). The analysis revealed a phenolic content of 128.9 mg GAE per mg dry weight, highlighting the plant's significant antioxidant capacity [70-73]. Statistical analyses were conducted using one-way ANOVA, with data expressed as mean ± standard error (SE). Post-hoc Duncan's multiple range test identified significant intergroup differences at a threshold of $P < 0.05$ [74].

2.2. Second Study (Investigating the ameliorative effects of cotreatment with methanolic leaf extract of Urtica dioica on gentamicin-induced acute kidney injury in rats) Material and Methods

[66] Collected Urtica dioica leaves from the mountains of Kurdistan / Iran. The plant leaves were washed, dried at room temperature, and crushed. Powdered plant (500g) was extracted with methanol by soaking for 72 hrs filtered, filtrate evaporated at 60°C. Then the extract was incubated at 60°C for 24 hrs to remove methanol, yielding dry plant extract. The experimental protocol utilized thirty-two male albino Wistar rats (200–250 g), housed under standard conditions with ad libitum access to water and rodent chow. Subjects were randomized into four cohorts (n = 8 per group) for 8-day intervention: Group 1 (Control): Intraperitoneal administration of 0.9% sodium chloride (0.5 mL/day); Group 2 (GM): Intraperitoneal gentamicin (100 mg/kg/day); Group 3 (UD): Intraperitoneal saline (0.5 mL/day) co-administered with oral methanolic *Urtica dioica* (UD) extract (200 mg/kg/day); Group 4 (GM + UD): Intraperitoneal gentamicin (100 mg/kg/day) combined with oral UD extract (200 mg/kg/day). Following the intervention period, 24-hour urine samples were collected via metabolic cages. Systolic blood pressure (SBP) was quantified using non-invasive tail-cuff plethysmography [75].

Renal hemodynamic parameters were subsequently assessed by isolating the left renal artery and vein, with blood flow measured over a 30-minute interval using a transonic flowmeter interfaced with a PowerLab data acquisition system (ADInstruments), following established protocols [76]. The MDA levels, an indicator of lipid peroxidation, were measured in kidney tissue homogenized in phosphate buffer (1/10 w/v). The homogenate was mixed with (200 µl of sodium dodecyl sulfate SDS), thiobarbituric acid, acetic acid, and water, heated at 95°C for 1 hr, and centrifuged. The supernatant absorbance was measured at 532 nm using a spectrophotometer to quantify MDA [77]. The ferric reducing antioxidant power (FRAP) assay was employed to quantify total antioxidant activity, based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ), forming a chromogenic blue complex with absorbance measured spectrophotometrically at 593 nm. The FRAP reagent consisted of 10 mM TPTZ, 20 mM ferric chloride, and 300 mM acetate buffer (pH 3.6). Tissue homogenates were mixed with the reagent, incubated at 37°C for 4 minutes, and analyzed to determine FRAP values, expressed as millimoles per gram of renal tissue [78].

For histopathological evaluation, renal specimens were fixed in 10% neutral buffered formalin, paraffin-embedded, and sectioned into 5-µm slices. Sections were mounted on glass slides, stained with hematoxylin and eosin (H&E), and examined microscopically for structural anomalies, including glomerular hypercellularity, tubular epithelial necrosis, intraluminal proteinaceous casts, Bowman's capsule dilatation, and vacuolar degeneration. Histopathological scoring was stratified as follows: Grade 0 (no pathology), Grade 1 (<25% involvement), Grade 2 (25–50%), Grade 3 (50–75%), and Grade 4 (>75% parenchymal injury) [79]. Renal functional parameters, including creatinine clearance rate and absolute/relative sodium/potassium excretion, calculated using standardized formulae to assess glomerular filtration efficiency and tubular reabsorptive capacity. Then statistical analysis performed using SPSS software. Data were reported as mean±standard error of mean (S.E.M.). One-way ANOVA, Kruskal-Wallis, and Tukey's test were used for data analysis. Probability values ≤0.05 were considered statistically significant.

2.3. Third Study (Protective Effect of Nettle (Urtica dioica), Leaves and Seeds on Kidney Disorder in Gentamicin-Induced Rats) Material and Methods

[67] Used 48 albino rats weighing around 150±10 g. The rats were divided into eight equal groups. The first group served as the negative control (-ve), while the following seven groups received intraperitoneal injections of gentamicin at a dose of 10mg/kg body weight for 10 consecutive days to induce renal dysfunction. The male rats with gentamicin-induced renal disease were then orally supplied varied doses of nettle leaves, seeds, and their mixtures as powder (2.5% and 5%). At the end of the experiment, biochemical analysis conducted, encompassing the assessment of renal function parameters such as uric acid, creatinine, and urea serum levels. Moreover, lipid fractions including triglycerides, total cholesterol, LDL-c, VLDL-c, and HDL-c levels, along with serum glucose levels and liver enzyme activities (ALP, AST, and ALT), were examined.

3. Results and discussion

3.1. Results

3.1.1. First Study (Impact of nettle (Urtica dioica) extract on male rabbits' nephrotoxicity caused by gentamicin) Results

[65] Found that the group treated with gentamicin (G2) exhibited elevated levels of serum blood urea nitrogen compared to (G1) and (G3). And G4 (combination treatment group) showed values similar to normal control (Table 5). The nettle extract treatment group (G3) demonstrated a significant increase ($P<0.05$) in serum creatinine levels. Additionally, this treatment led to a reduction in malondialdehyde (MDA) levels and a significant increase in the gentamicin treatment group (G2) (Table 6).

Control Group Histopathology: Renal sections from the control group exhibited preserved histological architecture, characterized by intact renal corpuscles with well-defined glomeruli enclosed within Bowman's capsules. Tubular structures, including proximal and distal convoluted tubules and loops of Henle, displayed cuboidal epithelial lining with normal luminal patency. Interstitial tissue demonstrated no evidence of degenerative alterations or hemorrhagic foci (Figures 3A, 3B).

Gentamicin-Treated Group Histopathology: Histopathological analysis of the gentamicin-treated cohort revealed significant glomerulotubular injury. Renal corpuscles exhibited glomerular swelling, dilation of Bowman's space, and disruption of the urinary pole. Tubular epithelia displayed cytoplasmic vacuolization, attenuation of brush borders, and loss of cellular definition, particularly in collecting ducts and loops of Henle. Hyaline droplet inclusions were observed in proximal and distal convoluted tubules, with intraluminal hyaline cast formation. Focal interstitial hemorrhage was noted (Figures 4A, 4B).

Nettle Extract-Treated Group Histopathology: Sections from the *Urtica dioica* (nettle) extract-treated group demonstrated near-normal renal morphology. Glomeruli retained intact capillary loops, while tubules exhibited cuboidal epithelial cells with preserved brush borders and luminal integrity. No proteinaceous casts or interstitial inflammatory infiltrates were observed (Figure 5).

Combination Therapy Group Histopathology: Renal tissue from the gentamicin-nettle co-treatment group showed near-complete architectural preservation, with minimal focal hydropic degeneration in tubular epithelia. These findings suggest a marked attenuation of gentamicin-induced nephrotoxicity by *Urtica dioica* extract, indicative of its renoprotective efficacy (Figure 6).

3.1.2. Second Study (Investigating the ameliorative effects of cotreatment with methanolic leaf extract of Urtica dioica on gentamicin-induced acute kidney injury in rats) Results

A study by [66] evaluated the hemodynamic effects of *Urtica dioica* extract on systolic blood pressure (SBP) and renal blood flow (RBF) in experimental cohorts. The findings revealed that co-treatment with *Urtica dioica* and gentamicin (Group 4) did not induce statistically significant alterations in SBP relative to the saline-treated control group (Group 1) (Figure 7A). In contrast, gentamicin monotherapy (Group 2) significantly diminished RBF compared to Group 1. Notably, concomitant administration of *Urtica dioica* with gentamicin (Group 4) ameliorated this effect, restoring RBF to levels significantly higher than those observed in Group 2 (Figure 7B). Intergroup analysis confirmed no significant differentials in SBP across all cohorts, underscoring the specificity of *Urtica dioica*'s renoprotective hemodynamic modulation. This study investigated the renal effects of *Urtica dioica* extract, with a focus on urinary sodium ($UNaV_0$) and potassium (UKV_0) excretion rates, fractional excretion of sodium (FENa) and potassium (FEK), and creatinine clearance (CCr). Comparative analysis revealed distinct outcomes across experimental groups. Group 2 (G2) exhibited a marked elevation in plasma creatinine and blood urea nitrogen (BUN) levels relative to Group 1 (G1), alongside reduced urinary creatinine and BUN concentrations a pattern indicative of renal impairment.

Notably, Gentamicin (GM) administration in Group 3 (G3) resulted in a significant decline in creatinine clearance compared to the control cohort (G1), with statistically significant disparities observed between G3 and G1. In contrast, concurrent therapeutic intervention in Group 4 (G4) demonstrated a restorative effect, evidenced by a substantial increase in creatinine clearance, suggesting potential nephroprotective benefits of *Urtica dioica* extract under gentamicin-induced renal stress. Analysis of the data revealed distinct patterns in electrolyte excretion across experimental groups. Group 2 (G2) demonstrated a marked elevation in fractional sodium excretion (FENa) relative to Group 1 (G1), whereas Group 3 (G3) showed no statistically significant deviation from G1 in this parameter. Notably, concurrent therapeutic intervention in Group 4 (G4) resulted in a pronounced attenuation of FENa levels compared to G2, suggesting moderating effect. Similarly, fractional potassium excretion (FEK) significantly heightened in G2 compared to G1, while G3 again exhibited no significant divergence from baseline values. In contrast, G4 displayed a substantial reduction in FEK relative to G2, further underscoring potential regulatory influence of co-administration regimen.

However, absolute urinary sodium ($UNaV_0$) and potassium (UKV_0) excretion rates remained consistent across all cohorts, with no intergroup disparities reaching statistical significance (Table 7). This study evaluated the influence of *Urtica dioica* (UD) extract on urinary biomarkers, including creatinine ([Cr]u), potassium ($[K^+]$ u), sodium ($[Na^+]$u), urea ([BUN]u) concentrations, and osmolality (Osmol_u). Comparative analysis revealed distinct intergroup variations. Group 2 (G2) exhibited a marked elevation in urinary sodium concentration ($[Na^+]$u) relative to

Group 1 (G1), whereas Group 3 (G3) showed no statistically significant divergence from G1 in this parameter. Conversely, urinary potassium concentration ($[K^+]u$) in G2 was significantly diminished compared to G1, while Group 4 (G4) demonstrated a pronounced increase in $[K^+]u$ relative to G2. Urinary creatinine levels ([Cr]u) in G2 were substantially reduced compared to G1, though no significant alteration was observed in G3. Notably, G4 displayed a further decline in [Cr]u compared to G2. Similarly, urinary urea concentration ([BUN]u) was significantly suppressed in G2 relative to G1, but co-administration in G4 elevated [BUN]u above G2 levels. Concurrent treatment with UD extract and gentamicin (GM) also induced a statistically significant rise in urinary osmolality compared to G2, indicative of enhanced renal concentrating capacity (Table 8).

This investigation further assessed the systemic impact of *Urtica dioica* (UD) extract on plasma biomarkers, including creatinine ([Cr]p), potassium ($[K^+]p$), sodium ($[Na^+]p$), urea ([BUN]p) concentrations, and osmolality (Osmolp). Group 2 (G2) demonstrated a marked elevation in plasma creatinine and [BUN]p levels relative to the control cohort (G1), consistent with renal compromise. In contrast, UD extract monotherapy (G3) elicited no statistically significant alterations in these parameters compared to G1 baseline values. Notably, concurrent administration of UD extract in Group 4 (G4) attenuated the gentamicin (GM)-induced elevations in [BUN]p and [Cr]p, suggesting a nephroprotective effect against GM-associated toxicity. Plasma sodium concentrations ($[Na^+]p$) remained stable across all experimental groups, with no intergroup disparities reaching significance. Similarly, $[K^+]p$ levels exhibited no notable variation between G2, G3, and G1, nor between G4 and G2. Osmolality (Osmolp) likewise showed no significant differentials among cohorts, further underscoring the selective renal modulation observed (Table 9). This investigation evaluated the influence of *Urtica dioica* (UD) extract on oxidative stress parameters in renal tissue, specifically malondialdehyde (MDA), a lipid peroxidation marker, and ferric reducing antioxidant power (FRAP), an indicator of antioxidant capacity.

Comparative analysis revealed that Group 2 (G2), the GM-treated cohort, exhibited a marked elevation in renal MDA levels relative to the control group (G1), indicative of oxidative injury. In contrast, UD extract monotherapy (G3) showed no statistically significant divergence from baseline MDA values (G1). Notably, concurrent UD and GM administration in Group 4 (G4) attenuated this oxidative stress response, evidenced by a pronounced reduction in MDA levels compared to G2 (Figure 8B). Similarly, FRAP levels in renal tissue were significantly suppressed in G2 compared to G1, reflecting diminished antioxidant activity. However, co-treatment in G4 restored antioxidant capacity, with FRAP levels rising significantly above those observed in G2 (Figure 8B). Histopathological assessment revealed profound renal injury in Group 2 (G2), characterized by moderate to severe (Grade 3–4) manifestations, including tubular epithelial necrosis, intraluminal protein cast formation, pronounced renal tubular vacuolization, marked expansion of Bowman's capsule space, reduced erythrocyte density within glomerular capillaries, and cellular dispersion into tubular lumina. Concurrent administration of *Urtica dioica* (UD) extract with gentamicin (GM) in Group 4 (G4) attenuated histopathological injury relative to G2, as evidenced by significant reductions in lesion severity.

Specifically, UD co-treatment mitigated tubular necrosis (Grade 1), minimized Bowman's space dilation (Grade 1), suppressed protein cast deposition (Grade 1), reduced luminal cellular dispersion (Grade 1), and attenuated epithelial vacuolization (Grade 2), while concurrently restoring glomerular erythrocyte counts to near-baseline levels (Grade 1). Renal architecture in both the UD monotherapy group (G3) and the untreated control cohort (G1) remained histologically unremarkable (Grade 0), aligning with normative tissue integrity (Table 10; Figure 9). Figure 9: Representative photomicrographs of renal histopathology across experimental cohorts. (A) Control group (G1) demonstrating preserved glomerulotubular architecture with intact Bowman's space (BS) and absence of pathological alterations. (B) Gentamicin-treated cohort (G2) exhibiting marked tubular epithelial necrosis (N), intraluminal proteinaceous casts (C), and vacuolar degeneration (V). (C) *Urtica dioica* (UD) monotherapy group (G3) retaining normative glomerular and tubular morphology, comparable to G1. (D) Concurrent UD-gentamicin treatment (G4) displaying attenuated tubulointerstitial injury, including diminished necrosis (N), reduced luminal casts (C), & partial restoration of glomerular erythrocyte (RBC) density. Histopathological scoring criteria: necrosis (N), intratubular casts (C), vacuolization (V), Bowman's space dilatation (BS), cellular detritus (D).

3.1.3. Third Study (Protective Effect of Nettle (Urtica dioica), Leaves and Seeds on Kidney Disorder in Gentamicin-Induced Rats) Results

[67] Identified that optimal protection and enhancement of renal functions, liver functions, lipid fractions, and glucose levels were achieved with 5.0% mixture of nettle leaves and seeds powder. Use of nettle leaves and seeds, either individually or in combination as a powdered supplement, in routine beverages, showcases significant nutraceutical therapeutic benefits for addressing renal disorders.

3.2. Discussion

The diagnosis of acute kidney injury (AKI) and gentamicin (GM)-induced nephrotoxicity was substantiated through functional markers, including diminished creatinine clearance (CCr) and urinary urea excretion rates; these findings were corroborated by histopathological evidence of renal parenchymal injury, such as tubular necrosis and glomerular structural compromise. Creatinine, a metabolic byproduct predominantly excreted via glomerular filtration, functions as a biomarker for renal excretory capacity. Elevated serum creatinine concentrations are indicative of diminished renal clearance efficiency or compromised kidney function [80]; [81]. The clearance rate of creatinine serves as a surrogate marker for glomerular filtration rate (GFR), reflecting functional integrity of renal filtration mechanisms. In this study, gentamicin administration induced marked elevation in serum creatinine levels compared to untreated controls and alternative therapeutic cohorts, which exhibited no statistically significant deviations from baseline [65]. Nephrotoxic effects of gentamicin were further evidenced by a reduction in GFR, attributable to pathomechanistic processes such as mesangial cellular contraction, electrostatic charge neutralization across the glomerular filtration barrier, and apoptosis of mesangial cells [66].

Blood urea nitrogen (BUN) is a clinically validated biomarker for evaluating renal excretory capacity, frequently employed in the diagnosis and monitoring of nephropathies. In this experimental framework, gentamicin-treated subjects exhibited a marked elevation in BUN concentrations relative to baseline and comparator cohorts, whereas *Urtica dioica* (UD)-supplemented groups maintained values within normative physiological ranges [82-83]. The nephrotoxic sequelae of gentamicin were further characterized by concurrent increases in plasma creatinine and BUN, reflecting its dual disruption of glomerulotubular homeostasis. These biochemical perturbations elevated BUN and hypercreatininemia correlate strongly with deteriorating renal functional integrity, underscoring their utility as indices of compromised nephron activity. Elevations in serum creatinine and blood urea nitrogen (BUN) levels correlated closely with gentamicin-induced nephrotoxic pathology, while the attenuation of these biomarkers in *Urtica dioica*-supplemented cohorts underscored its renoprotective efficacy against drug-mediated renal injury. The kidneys exhibit marked vulnerability to oxidative insults due to their high abundance of polyunsaturated fatty acids (PUFAs) within membrane phospholipids, rendering lipid bilayers susceptible to peroxidative cascades.

Figure 1. Images of stinging nettle (Urtica dioica). A: Non-flowering juvenile plant. (Image provided by Bill Schultz.) B: Fully developed flowering plant.

Figure 2. Close-up images of stinging nettle (Urtica dioica). A: Observe the plentiful, minute stinging hairs covering the leaves and stems. B: Trichome of stinging nettle

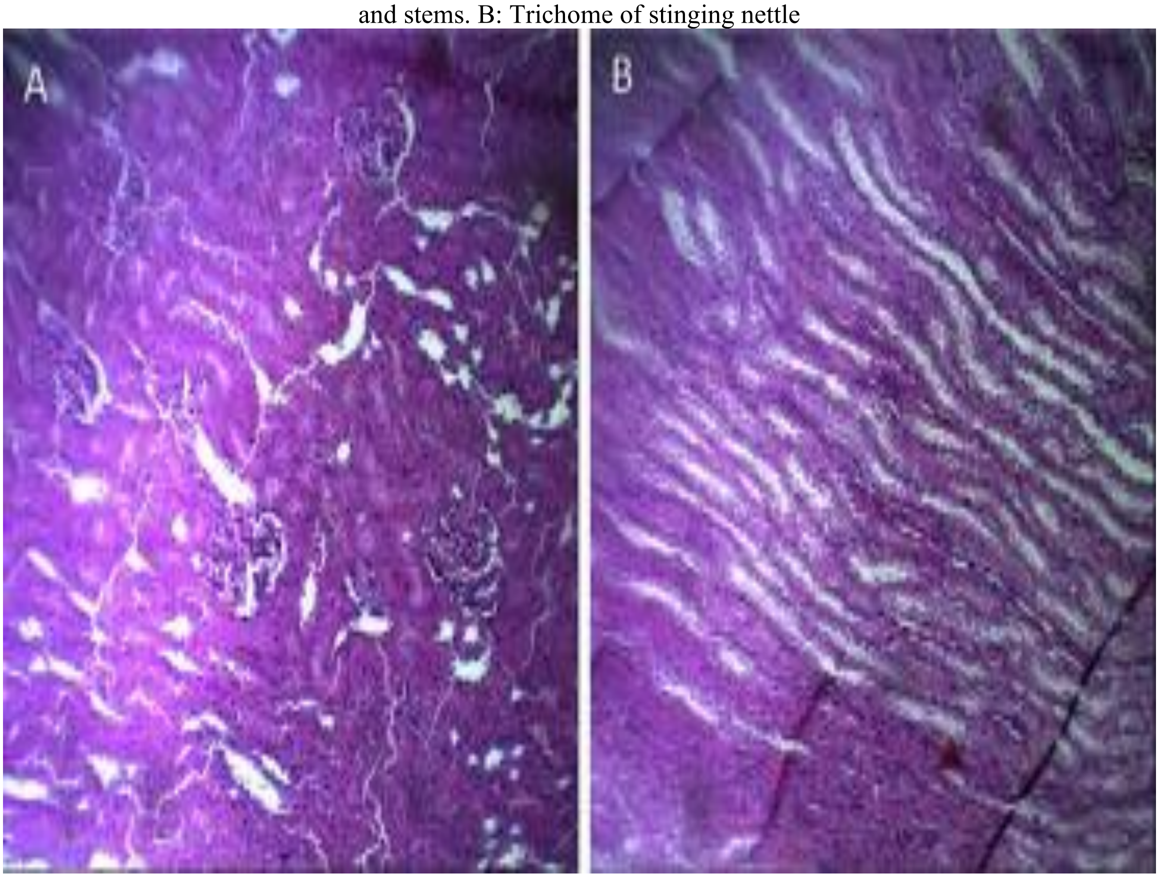

Figure 3. Renal histoarchitecture in the control group. **(A)** Histologically unremarkable renal corpuscles with intact glomeruli (G) and collecting tubules (CT). **(B)** Preserved parenchymal architecture, including cuboidal epithelial lining of proximal (PCT) and distal convoluted tubules (DCT), with no pathological alterations.

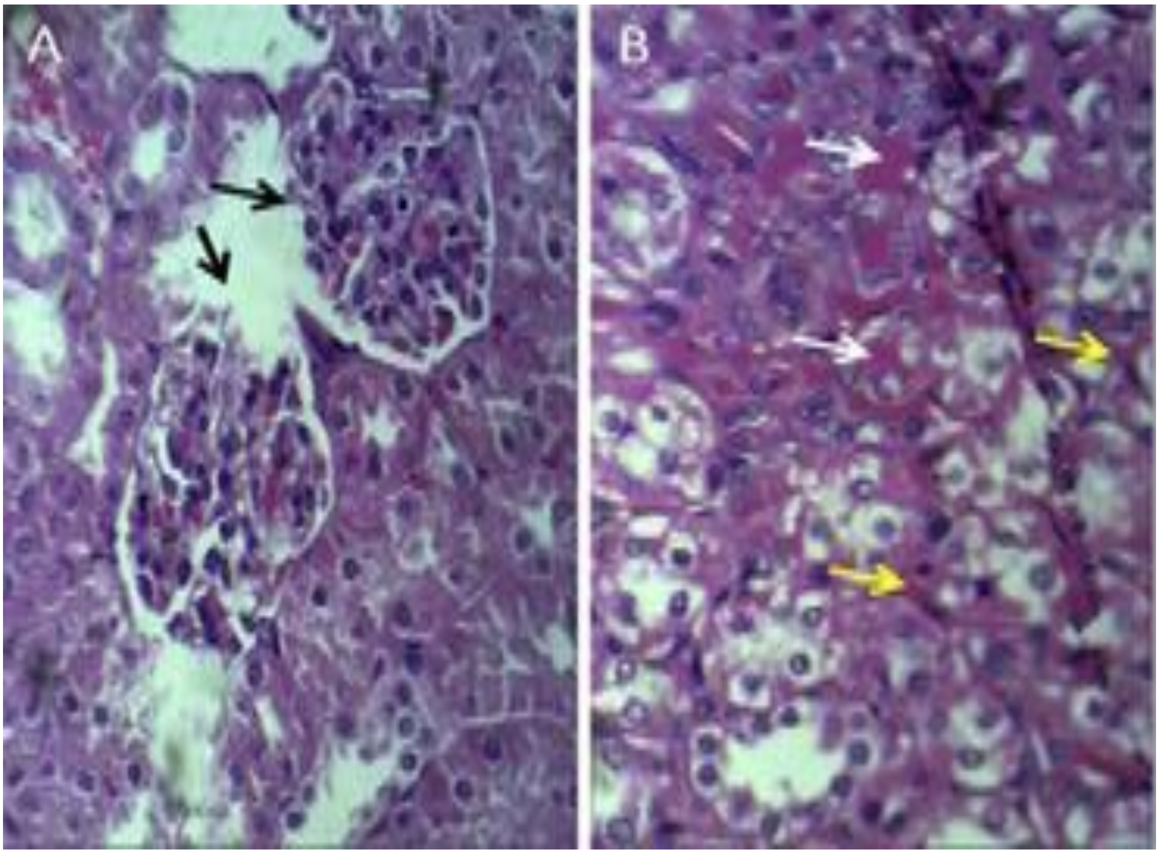

Figure 4. Gentamicin-induced nephropathology. **(A)** Mild glomerular swelling (arrowheads) and tubular hydropic degeneration, resulting in Bowman's space dilatation (black arrow) and urinary pole disruption. **(B)** Cytoplasmic vacuolization in tubular epithelia (white arrow), intraluminal hyaline cast deposition (asterisk), and interstitial hemorrhage (yellow arrow).

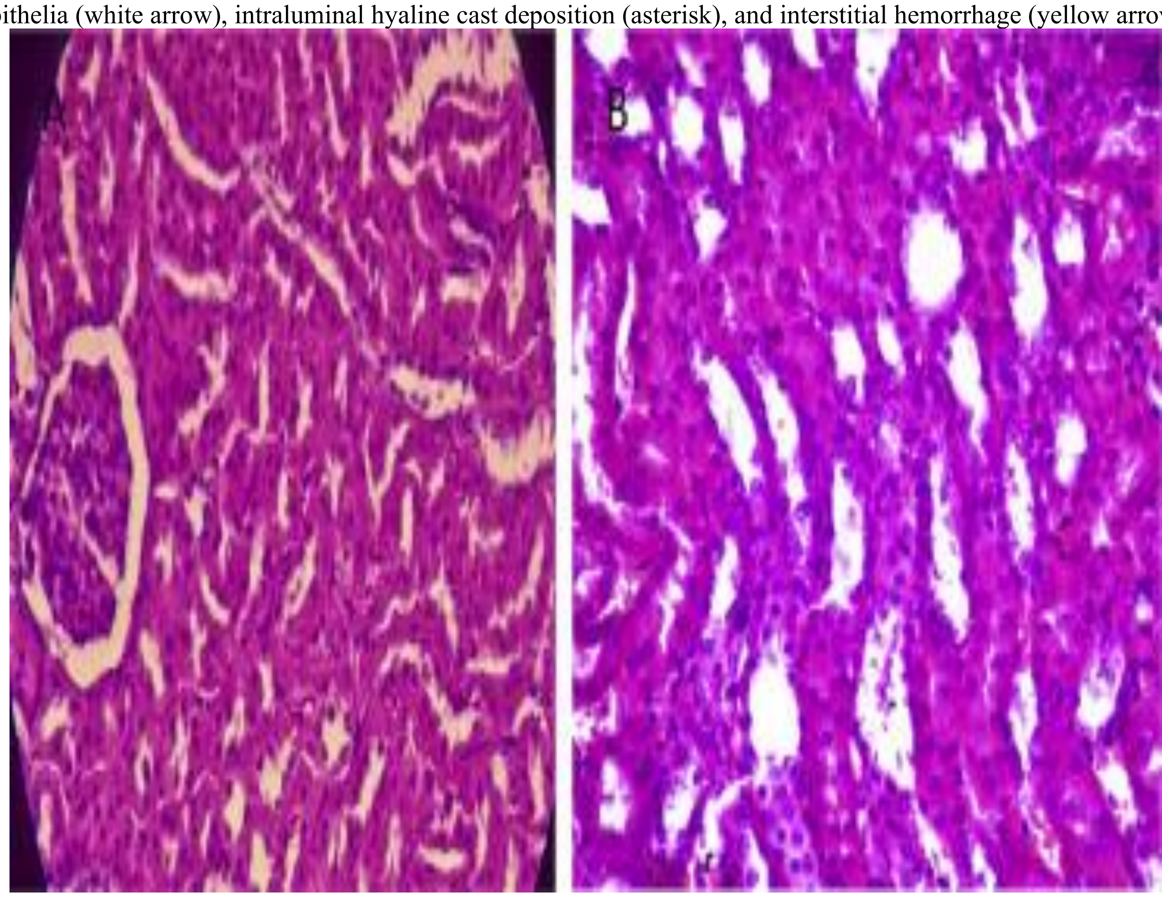

Figure 5. Renal section from the Urtica dioica-treated cohort (G3). Normocellular glomeruli (G) and intact renal tubules with distinct brush borders (BB). Note the absence of inflammatory infiltrates or proteinaceous casts in interstitial spaces.

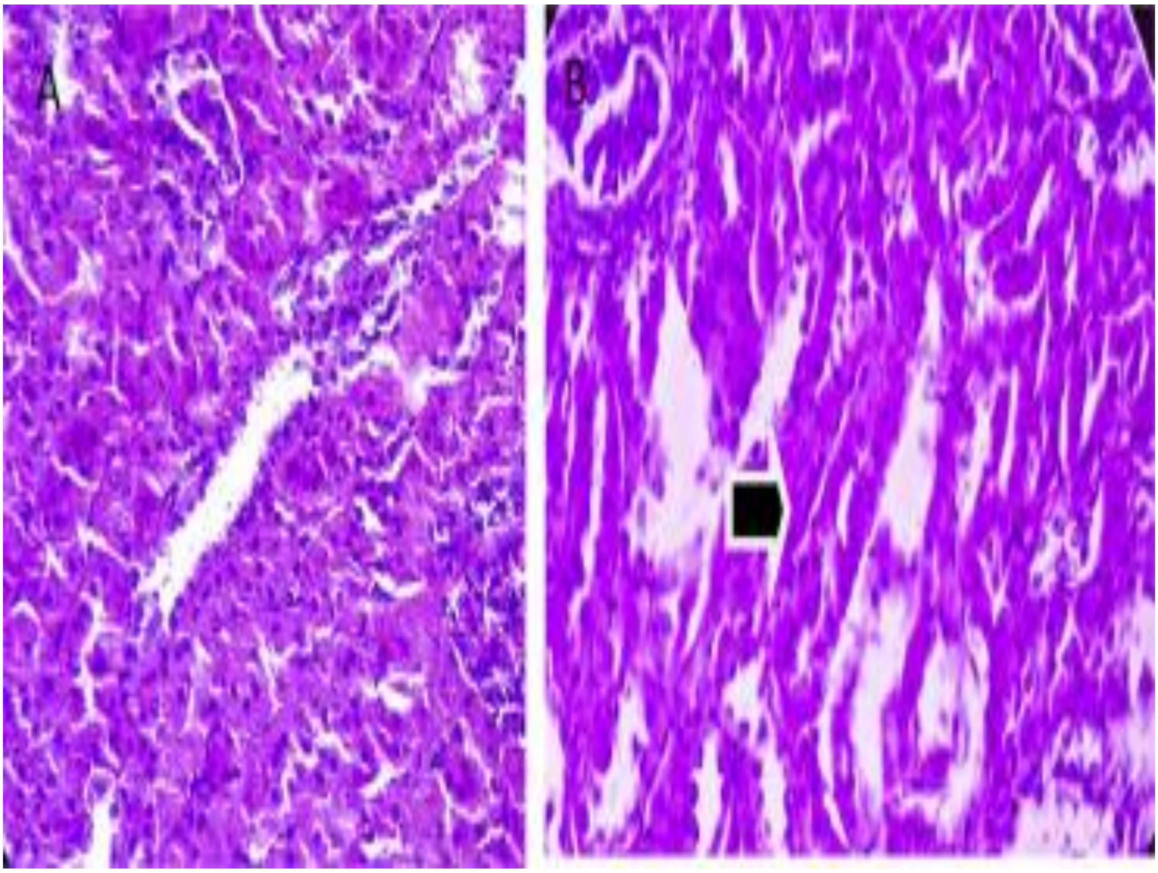

Figure 6. Renal histology in the gentamicin-Urtica dioica co-treatment group (G4). Near-complete architectural preservation comparable to controls, with minimal focal hydropic degeneration of tubular epithelia (black arrows).

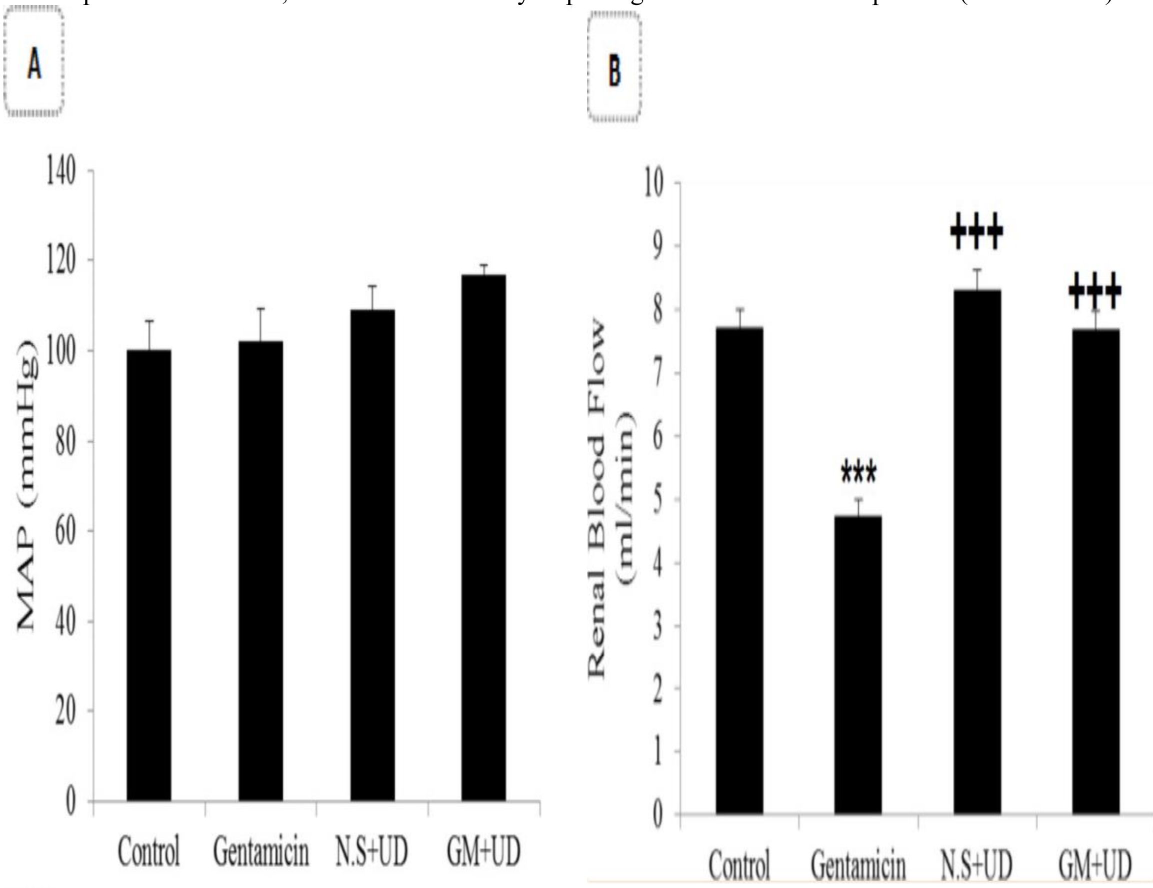

Figure 7. Comparison of A) Renal blood flow and B) Systolic blood pressure among the groups.

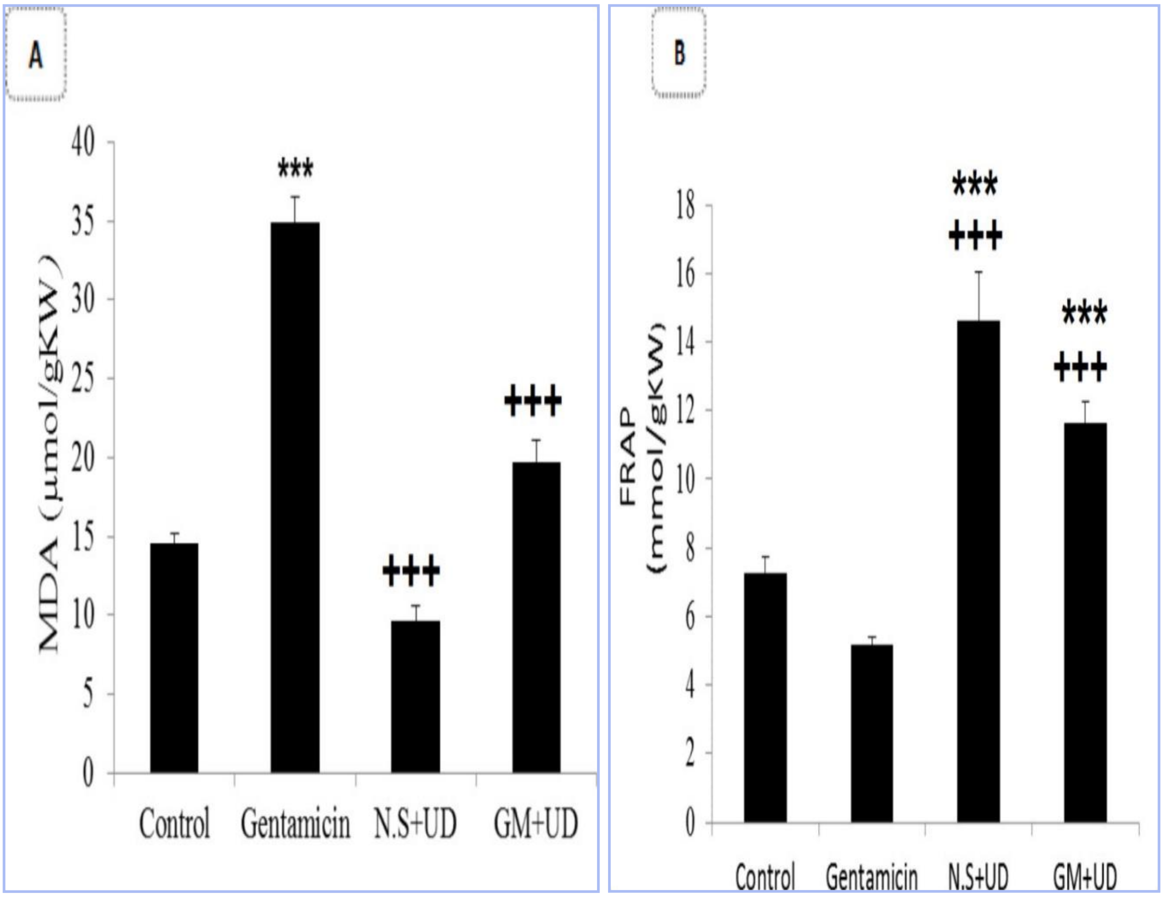

Figure 8. Quantitative representation of oxidative stress biomarkers in renal tissue: Panel A

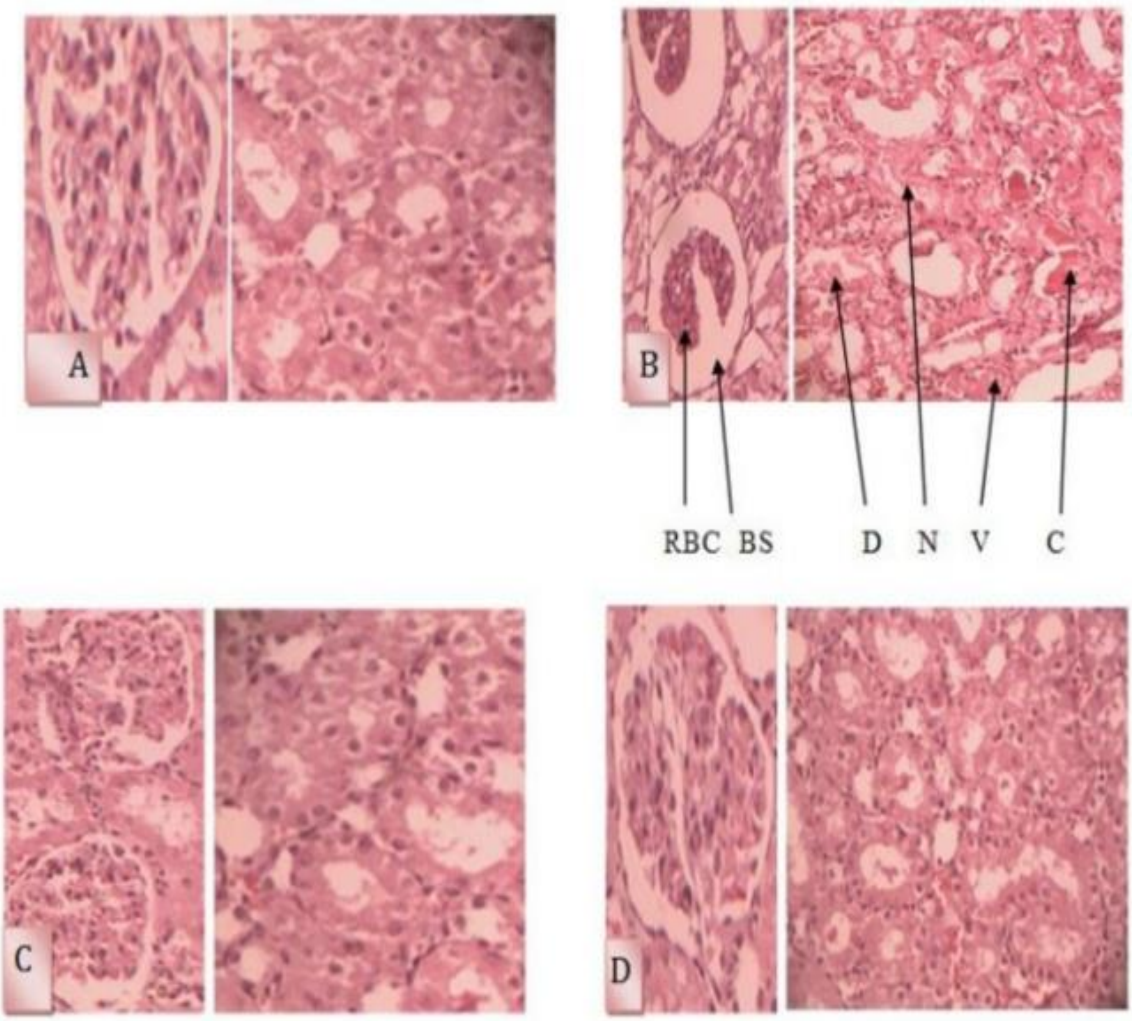

Figure 9. Comparative histopathological assessment of renal tissue across experimental cohorts, highlighting variations in glomerular and tubular architecture, cellular integrity, and structural preservation.

Table 1: TAXONOMY of Urtica dioica [12].

Kingdom	-Plantae – Plants
Subkingdom	-Tracheobionta - Vascular plants
Superdivision	-Spermatophyta - Seed plants
Division	-Magnoliophyta - Flowering plants
Class	-Magnoliopsida– Dicotyledons
Subclass	-Hamamelidae
Order	-Urticales
Family	-Urticaceae - Nettle family
Genus	-Urtica L.
Species	-Urtica dioica L., - stinging nettle P5

Table 2. Phytochemistry of Urtica dioica L. [15-60-84].

Chemical constituents	Main compounds
Phenolics	Phenyl propanes derived from shikimic acid, including caffeic acid, and a range of esters of this acid such as chlorogenic acid and caffeoylmalic acid (up to 1.8%).
Flavonoids	Patuletin, isorhamnetin, kaempferol, quercetin, isoquercitrin, astragalin, rutin, along with 3-rutinosides and 3-glycoside.
Fatty acids	20.2% of linoleic acid, 1.1% of stearic acid, 3.6% of oleic acid, and 12.4% of linolenic acid, 6.8% of palmitic acid.
Essential oil	Free alcohols (2%), ketones (38.5%), and esters (14.7%), characterized as 2-methyl-2-hepten-2-one, acetophenone, ethylketone, with traces of nitrogen-containing compounds, aldehydes, & phenols.
Carotenes	B-carotene (2.95-8 mg/100 g in fresh plants), 20.2 mg/100 g in dry young plants, lutoxanthin (10.3%), lutein epoxide (13.1%), and violaxanthin (14.7%), hydroxy-carotene (0.9%).
Vitamins	Ascorbic acid (36-269 mg%), VitaminB2-lactoflavin-1.5 mg/100g in dried, 0.25 mg in the fresh leaves, panthotenic acid, Vitamin-K1 0.64g/100g
Minerals	Silicates in a relatively big quantity (1-4% SiO_2) magnesium, phosphorus, calcium, iron, potassium and sodium are also present.
Amino acids	Alanine, glutamic acid, leucine, proline, phenylalanine, tyrosine, GABA, and valine.
Organic acids	Formic acid, succinic acid, citric acid, acetic acid.

Table 3. (Table 4): Nutritional makeup of U. dioica L. [85-86].

Serving size	100 g
Vitamins	Daily Value (%)
Vitamin B1 (Thiamin) 0.0 mg	1%
Vitamin B3 (Niacin) 0.4 mg	2%
Choline, total 17.4 mg	3%
Vitamin B6 0.1 mg	8%
Vitamin B2 (Riboflavin) 0.2 mg	12%
Vitamin A 2011.0 IU	67%
Vitamin K 498.6 µg	416%
Minerals	
Selenium 0.3 µg	1%
Zinc 0.3 mg	2%
Phosphorus 71.0 mg	7%
Copper 0.1 mg	8%
Potassium 334.0 mg	9%
Iron 1.6 mg	9%
Magnesium 57.0 mg	14%
Manganese 0.8 mg	34%
Calcium 481.0 mg	37%

Calories	
Carbohydrates 7 g	2%
Fiber 7 g	24%
Protein 2.4 g	5%

Table 5. Bioactive constituents identified in the foliar, root, and seed tissues of Urtica dioica (stinging nettle) [87] [88].

Parts	Bioactive compounds
Leaves and root	Vitamins (vitamin A, C, K, and B vitamins), minerals (calcium, iron, magnesium, phosphorus, potassium and sodium), fats (linoleic acid, linolenic acid, palmitic acid, stearic acid and oleic acid), amino acids (all of the essential amino acids), polyphenols (kaempferol, quercetin, caffeic acid, coumarins and other flavonoids), pigments (beta-carotene, lutein, luteoxanthin and other carotenoids)
Seed	Vitamins (vitamin A, B, C, E and K), minerals (iron, silicon, calcium, magnesium, manganese, phosphorus, potassium), beta-carotene, folic acid, essential fatty acids

Table 6. Effect of gentamicin and ethanol extract of nettle on serum creatinine and blood urea nitrogen levels (mg/dL).

Groups	Treatments	Serum creatinine	Blood urea nitrogen
G1	Control	1.44 ± 0.38a	12.70 ± 0.43a
G2	Gentamicin	3.80 ± 0.14b	17.44 ± 0.48b
G3	Nettle	1.80 ± 0.22a	13.74 ± 0.33a
G4	Gentamicin and nettle	1.62 ± 0.25a	12.70 ± 0.27a

Values expressed as mean ±SE, values in the same column with different letters mean significant differences.

Table 7. Effect of gentamicin and ethanol extract of nettle on renal tissue MDA and GSH levels in experimental animal model.

Groups	Treatment	MDA nmol/g tissue	GSH µmol/g tissue
G1	Control	210.00 ± 0.81a	18.00 ± 0.25a
G2	Gentamicin	410.00 ± 0.98c	16.52 ± 0.89a
G3	Nettle	239.00 ± 0.29b	25.43 ± 0.83b
G4	Gentamicin and nettle	230.00 ± 0.95b	35.23 ± 0.98c

Values expressed as mean ±SE, values in the same column with different letters mean significant differences, value with (c) letter is the most significant group at $P < 0.05$.

Table 7. Comparative analysis of renal functional parameters, including creatinine clearance (CCr), sodium excretion metrics (absolute [$UNaV_0$] and fractional [FENa]), and potassium excretion indices (absolute [UKV_0] and fractional [FEK]).

Groups	FEK%	FENa%	Uk V (mmol/min/kg)	UNa V (mmol/min/kg)	Ccr (ml/min/kg)
Control	34.63±2.49	0.43±0.03	1.99±0.16	0.77±0.05	1.29±0.03
Gentamicin	139.00±7.71	5.06±0.42	2.65±0.11	2.78±0.22	0.39±0.01
NS+UD	36.24±3.66	0.43±0.05	2.16±0.27	0.97±0.14	1.44±0.06
Gentamicin + UD (*Urtica dioica*)	53.52±7.53	0.73±0.10	2.98±0.38	1.43±0.18	1.35±0.09

Table 8. Comparison of urinary concentrations of sodium ([Na]u), potassium ([K]u), creatinine ([Cr]u), and urea ([BUN]u), and osmolality (Osmolu) among the groups.

Groups	[Na]u (µmol/ml)	[K]u (µmol/ml)	[Cr]u (mg/dl)	[BUN]u (mg/dl)	Osmolu (mOsm/kgH2O)
Control	55.7±2.9	140.5±2.1	54.3±1.7	140.6±16.7	1290.0±81.3
Gentamicin	81.3±1.8	77.8±3.0	27.5±1.3	39.2±5.8	553.0±48.8
UD+NS	65.2±3.9	143.1±1.2	58.5±1.5	120.2±11.9	1212.9±47.4
Gentamicin + UD (*Urtica dioica*)	67.3±1.0	139.2±1.6	58.0±2.2	127.2±11.1	1218.7±56.4

Table 9. Systemic biomarker analysis across experimental cohorts, evaluating plasma concentrations of sodium ([Na^+]p), potassium ([K^+]p), creatinine ([Cr]p), and urea ([BUN]p), alongside osmolality (Osmolp).

Groups	[Na]p (µmol/ml)	[K]p (µmol/ml)	[Cr]p (mg/dl)	[BUN]p (mg/dl)	Osmolp (mOsm/kgH2O)
Control	140.7±1.3	4.45±0.1	0.6±0.02	20.5±1.0	287.0±3.1
Gentamicin	138.5±0.5	4.8±0.1	2.3±0.1	92.0±2.4	292.6±3.7
UD+NS	145.0±1.2	4.5±0.1	0.6±0.03	21.2±1.2	286.6±2.4
Gentamicin + UD (*Urtica dioica*)	140.0±0.9	4.6±0.1	0.9±0.1	27.9±1.3	291.7±3.3

Table 10. Systematic evaluation of histopathological indices, including tubular necrosis severity, intraluminal protein cast formation, luminal cellular dispersion, renal epithelial vacuolation, glomerular erythrocyte density reduction, Bowman's capsule dilatation, and composite glomerular injury scores.

Groups	Necrosis	Formation of protein casts	Cell scattering	vacuolization	Reduced number of red blood cells	Increased Bowman's space	Total glomerular injury
Control	0	0	0	0	0	0	0
Gentamicin	4	3	3	4	3	4	4
UD+NS	0	0	0	0	0	0	0
Gentamicin+UD	1	1	1	2	1	1	1

Oxidative degradation of phospholipid membranes in subcellular compartments such as the endoplasmic reticulum rich in PUFAs initiates a cascade of lipid peroxidation byproducts, including malondialdehyde (MDA), which disrupt membrane integrity and impair cellular homeostasis. Aminoglycosides, exemplified by gentamicin, exert nephrotoxicity predominantly via ROS overgeneration and depletion of endogenous antioxidant defenses, exacerbating oxidative stress within renal parenchyma [89-91]. Gentamicin elicits a state of oxidative stress via the overproduction of reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2). These reactive intermediates induce oxidative modification of critical biomolecules such as nucleic acids, proteins, and lipid membranes, initiating pathophysiological cascades that compromise renal cellular integrity. The resultant oxidative damage precipitates glomerular dysfunction, ischemic renal injury, perfusion deficits, and progressive tubular degeneration, culminating in acute kidney injury (AKI), as documented by [92-93].

Glutathione peroxidase (GPx) and malondialdehyde (MDA) are pivotal biomarkers of oxidative stress, reflecting systemic free radical activity and lipid peroxidation dynamics. Tissue-specific concentrations of these markers, particularly in renal parenchyma, are regarded as more diagnostically robust than serum levels due to their direct association with localized oxidative injury. In gentamicin-treated animal models, renal GPx activity exhibited marked depletion, concurrent with elevated MDA accumulation findings indicative of antioxidant exhaustion and heightened peroxidative damage under sustained oxidative stress [94]; [91]. Elevated renal MDA levels in gentamicin-exposed cohorts further corroborated structural nephron injury, implicating lipid peroxidation and free radical-mediated cytotoxicity as central mechanisms of drug-induced nephrotoxicity. Reactive oxygen species (ROS) overproduction, a hallmark of aminoglycoside toxicity, exacerbates proximal tubular epithelial necrosis and disrupts glomerular hemodynamics. This is mechanistically linked to mesangial cell contraction, diminished filtration surface area, and subsequent reductions in glomerular filtration rate (GFR), culminating in functional renal impairment [95].

Histopathological assessment serves as a gold-standard diagnostic modality for evaluating gentamicin-induced nephrotoxicity, which is mechanistically linked to reactive oxygen species (ROS) overproduction, membrane lipid peroxidation, and structural protein denaturation. Microscopic analysis of renal tissue in gentamicin-treated cohorts revealed pronounced tubular epithelial necrosis, intraluminal protein cast deposition, cytoplasmic vacuolization, vascular congestion, Bowman's capsule dilatation, and diminished glomerular erythrocyte density morphological hallmarks of oxidative and ischemic renal injury. The preferential accumulation of gentamicin within proximal tubular epithelia, exceeding serum concentrations, exacerbates its nephrotoxic potential through direct cellular insult [96-97]. Notably, phytotherapeutic coadministration of *Urtica dioica* (UD) extract with gentamicin attenuated histopathological lesions, suggesting viable nephroprotective strategy against tubulointerstitial, vascular, and glomerular compromise. Histomorphological analyses corroborated renoprotective efficacy of UD, demonstrating preserved glomerulotubular integrity and mitigation of structural anomalies in treated cohorts, as evidenced by [82-98].

The neuroprotective efficacy of *Urtica dioica* (UD) extract has been mechanistically linked to its redox-modulatory capacity, particularly its antioxidant activity, as demonstrated by [99]. UD's bioactive phytochemical profile, enriched with polyphenolic constituents such as caffeic acid, malic acid, nicotinamide, and adenine, confers multifunctional properties, including free radical scavenging, anti-inflammatory activity, & attenuation of oxidative stress [12]. These polyphenols, ubiquitous in UD, neutralize reactive oxygen species (ROS) including singlet oxygen, hydroxyl radicals, and superoxide anions thereby mitigating oxidative cellular damage [94]. Ethanol-derived UD extract, with a phenolic content quantified at 128.9 gallic acid equivalents (GAE), exemplifies a potent natural antioxidant source. Empirical evidence further correlates diets abundant in polyphenols and flavonoids with enhanced longevity, underscoring their therapeutic potential [98-100]. Similarly, vitamin E exerts nephroprotective effects against gentamicin-induced renal injury, primarily via its robust antioxidative activity. This fat-soluble antioxidant preserves intracellular redox homeostasis, stabilizes membrane integrity, and enhances renal clearance of gentamicin, thereby attenuating drug-induced nephrotoxicity [81-93].

Empirical evidence indicates that antioxidant agents such as resveratrol augment glomerular filtration rate (GFR) by suppressing mesangial cell contractility, thereby enhancing creatinine clearance via free radical scavenging [101]. In parallel, *Urtica dioica* (UD) extract administration attenuated gentamicin-induced oxidative stress, as evidenced by reduced plasma blood urea nitrogen (BUN) and creatinine (Cr) levels in experimental cohorts. Concurrent administration of UD extract with gentamicin also ameliorated renal electrolyte dysregulation, underscoring its redox-modulatory capacity to neutralize reactive oxygen species (ROS) and preserve tissue integrity. Furthermore, UD extract demonstrated therapeutic efficacy in mitigating gentamicin-induced plasma electrolyte imbalances, suggesting multifactorial renoprotection [66]. [65] Corroborated these findings, revealing that UD ethanol extract exhibits robust antioxidant activity, likely mediated by its high phenolic content. These polyphenolic constituents serve as cytoprotective agents, counteracting nephrotoxic pathways instigated by gentamicin. [66] Further demonstrated that UD co-administration attenuates histopathological and biochemical markers of gentamicin-induced renal injury. Divergent outcomes may arise from variations in therapeutic protocols, such as the abbreviated eight-day intervention period. The nephroprotective efficacy of UD is hypothesized to stem from synergistic antioxidant and anti-inflammatory mechanisms intrinsic to its phytochemical profile.

4. Conclusion

Urtica dioica, commonly referred to as stinging nettle, is a phytochemically rich botanical species traditionally used in ethnomedicine and now integrated into modern therapeutic practices as a herbal and nutraceutical supplement. It has demonstrated efficacy in managing conditions such as arthritis, hay fever, and urinary tract infections. Emerging evidence highlights its promising role in mitigating drug-induced nephrotoxicity, particularly associated with aminoglycosides like gentamicin. Proposed mechanisms for its renoprotective effects include diuretic activity that supports toxin elimination and prevents renal accumulation; antioxidant properties, attributed to flavonoids and phenolic acids, that neutralize free radicals (ROS), reduce lipid peroxidation, and alleviate oxidative stress, key drivers of renal damage; and anti-inflammatory effects that preserve glomerular and tubular integrity.

For patients undergoing gentamicin therapy, Urtica dioica can be incorporated into dietary regimens through fresh consumption, juicing, or addition to salads and smoothies, offering dual benefits of hydration support due to its high-water content and electrolyte balance via potassium enrichment, enhances diuretic efficacy. However, preclinical studies advise caution for patients with chronic comorbidities or polypharmacy, necessitating multidisciplinary medical consultation prior to dietary modifications to avoid herb-drug interactions. Despite these promising findings, critical gaps remain, including establishing clinically optimal dosing protocols, elucidating precise molecular mechanisms underlying its renal benefits, and conducting long-term clinical trials to validate safety and efficacy. Thus, Urtica dioica emerges as a compelling candidate for adjunctive nephroprotective therapy, requiring collaborative efforts among nutritionists, physicians, and pharmacists to ensure safe therapeutic integration.

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Data Availability Statement

All data underpinning the empirical findings and conclusions presented in this study are comprehensively detailed within the manuscript. In adherence to principles of methodological transparency and reproducibility, the datasets referenced herein are integrated into the article's analytical framework, ensuring accessibility for scholarly verification and further inquiry.

Declaration of Competing Interest

All authors declare no conflicts of interest in this paper.

Author Contributions

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