ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-8 | Issue-2 | May-Jun 2026 | **DOI: https://doi.org/10.36346/sarjps.2026.v08i02.002**

Original Research Article

Assessment of the Effects of Chia (*Salvia hispanica*) Seeds Extract on Gentamicin-Induced Kidney Damage in Male Rats

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Article History
Received: 15.04.2026
Accepted: 08.06.2026
Published: 11.06.2026

Abstract: ***Background*:** One of the major global health problem and oxidative stress has an important role in its pathogenesis is kidney injury. Researchers showing that chia seeds (*Salvia hispanica*) contain high levels of antioxidants, omega-3 fatty acids, and bioactive compounds that can works as nephroprotective agents. The aim of this study to evaluation of the protective effect of chia seed extract against gentamicin-induced nephrotoxicity in male tats. ***Methods*:** A total of forty male Wistar rats (200–250g) were assigned to five groups (n = 8). Group 1 (Control); Group 2 (Gentamicin, 100 mg/kg); Group 3 (Gentamicin + Low-dose chia extract,)200 mg/kg); Group 4 Gentamicin + Medium-dose chia extract,) 400 mg/kg) and Group 5 Gentamicin + High-dose-chia-extract,)600 mg kg [24]. Oral administration of treatments was done for 10 days. Biochemical parameters serum creatinine, blood urea nitrogen (BUN), uric acid also antioxidant markers (SOD, CAT, GPx and MDA) were determined. ***Results*:** An administration of gentamicin proved significantly higher serum creatinine (2.45±0.28 mg/dL), BUN (68.34±5.12 mg/dL), and uric acid levels compared to controls ($p< 0{\cdot}001$). The treatment with Chia extract was associated to a significant improvement in all these parameters, being more evident for the high-dose group (creatinine: 1.23±0.18 mg/dL; BUN: 35.67±3.89 mg/dL). Chia-treated groups showed strong restoration of activities of the antioxidant enzymes (SOD, CAT and GPx), compared to untreated counterparts, in association with decreased MDA levels. ***Conclusion*:** Chia seed extract protected the kidney from gentamicin-induced renal injury via antioxidant mechanisms and preservation of renal function. This result indicates the possible therapeutic usage of chia seeds in preventing drug-induced nephrotoxicity.

Keywords: Chia Seeds, Salvia Hispanica, Nephrotoxicity, Gentamicin, Oxidative Stress, Antioxidants, Kidney Function.

1. INTRODUCTION

The CKD affects millions of people around the globe and poses an enormous public health challenge with significant economic burden (Kovesdy, 2022). Background: Both acute kidney injury (AKI) and chronic kidney disease (CKD) are linked with increased morbidity, mortality, and burden on the healthcare system [1]. Drug-induced nephrotoxicity is one of the major causes of acute kidney injury, with aminoglycoside antibiotics being the most frequent nephrotoxic drugs used in routine clinical practice (Morales-Álvarez *et al*., 2020) and gentamicin being among the most common.

Gentamicin is known to cause nephrotoxicity in 10-25% of patients given therapeutic doses and is recognised as proximal tubular injury with impairment associated with decreased glomerular filtration rate1,2 and retention of nitrogenous waste products (Ozbek, 2012; Rahman *et al*., 2021). Gentamicin nephrotoxicity mechanisms include oxidative stress, mitochondrial dysfunction, inflammatory response and apoptosis (Al-Naimi *et al*., 2022). Reactive oxygen species (ROS) production induces lipid peroxidation, protein and DNA damage in kidney tissues, therefore affecting renal function (Adil *et al*., 2020).

Medicinal plants with natural antioxidant potential have been studied and among the most promising are compounds that can be effective against these disorders, and thus they attract attention as therapeutic strategies in kidney disease (Zhang *et al*., 2023). Chia seeds (Salvia hispanica L.) are a functional food and the most important native plant from Central and South America due to their nutritional value (Ullah *et al*., 2021). It is labelled as a superfood seeds because it rich in omega-3 fatty acids (α-linolenic acid), dietary fiber, proteins, vitamins, minerals and phenolic compounds like chlorogenic acids, caffeic acid and quercetin (Marinelli *et al*., 2020; Rodríguez-Martín *et al*., 2022).

Chia seeds have been shown to possess various biological activities over the years, such as antioxidant (Fernandes & Salas-Mellado, 2021), anti-inflammatory (Silva *et al*., 2018), cardioprotective and hepatoprotective effects respectively in recent investigations conducted in both laboratory and clinical trials (Silva *et al*., 2023). Chia seeds have a high concentration of phenolic compounds that are strong free radical scavengers and increase the endogenous antioxidant defensive systems in tissues (Timilsena *et al*., 2020). Additionally, high omega-3 operates in a fashion that plays a role in anti-inflammatory actions through alterations of eicosanoid synthesis and to diminished manufacture of pro-inflamed cytokines (Kulczyński *et al*., 2024).

Although several health benefits of chia seeds have been documented, the potential protective effect of chia seed against drug-induced nephrotoxicity (Sandoval-Oliveros *et al*., 2021). Based on the antioxidant and anti-inflammatory properties of components found in chia seeds, we hypothesized that an extract from chia seeds could protect against gentamicin-induced kidney injury. Accordingly, the present study aimed to assess the nephroprotective efficacy of chia seed extract on gentamicin-induced renal damage in male rats using different doses with respect to kidney function indices, oxidative stress parameters and histopathological investigation.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Gentamicin sulfate was obtained from Sigma-Aldrich (St. Louis, MO, USA). Randox Laboratories (UK) also provided us with commercial kits to measure serum levels of creatinine, blood urea nitrogen (BUN), and uric acid. Suppliers of superoxide dismutase (SOD) kit, catalase (CAT) kit, glutathione peroxidase (GPx) activity assay kit and malondialdehyde (MDA) kit: Biodiagnostic; Egypt. All other chemicals employed were of analytical grade.

2.2. Plant Material and Extract Preparation

Chia seeds (Salvia hispanica) were collected from the local market and identified by a botanist at the Department of Botany. The seeds were cleaned, dried and powdered in an electric grinder. Aqueous extract was prepared by stirring 100g of chia seed powder in 1000mL of distilled water at room temperature for 24 hours (stirred intermittently). Filter the mixture through Whatman No. 1 filter paper, and concentrate with a rotary evaporator at 40°C to obtain the concentrated extract, which was freeze-dried at low temperature (-20°C) until used. The extract had a yield of around 22% (w/w).

2.3. Experimental Animals

Forty adult male Wistar rats (200–250g) were purchased from the animal house of the Faculty of Medicine. Animals were maintained in polypropylene cages under standard laboratory conditions (temperature 22±2°C, humidity 55±5% and 12-hours light/dark cycle) fed ad libitum with standard pellet diet and water. The Journal of Immunology Research Experimental protocol were approved by Institutional Animal Ethics Committee and performed according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.4. Experimental Design

Rats acclimatized for one week and were then randomly distributed into five groups (n=8 each) as follows:
Group 1 (Control): Auditory delivery of normal saline (1 mL) for 10 days.
Group 2 (Gentamicin): Administered gentamicin (100 mg/kg body weight, intraperitoneally) once daily for a duration of 10 days.
Group 3 (GM + Low-dose CSE): Administered gentamicin (100 mg/kg, i.p.) plus chia seed extract (200 mg/kg, orally) for 10 days.
Group 4 (GM + Medium-dose CSE): Received gentamicin (100 mg/kg, i.p.) associated with chia seed extract (400 mg/kg, orally for 10 days.
Group 5 (GM + High dose CS): Received gentamicin (100 mg/kg, i.p.) plus chia seed extract (600 mg/kg, orally) for 10 days.

2.5. Sample Collection

One day after the last treatment, rats were anesthetized with ketamine (80 mg/kg) and xylazine (10mg/kg). Blood samples were collected via retro-orbital bleed and then stored at room temperature to clot for 30 mins. Each serum sample was separated by spinning at 3000 RPM for (15 min), denoting the amounts of each solution, storing 960 µL in a frozen

state (−80◦C) until days before analysis. After conducting cervical dislocation to sacrifice the rats, kidneys were weighed and quickly separated before being prepared for biochemical examination or histopathological analysis.

2.6. Biochemical Analyses

Serum levels of creatinine, blood urea nitrogen (BUN) and uric acid were recorded according to the manufacturers' instructions using commercial kits. Fifty mg of kidney was homogenized in 10% w/v ice-cold phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C to obtain supernatants for the determination of oxidative stress markers. Measurement of superoxide dismutase (SOD) activity was performed according to the method based on inhibition of pyrogallol autoxidation. Catalase (CAT) activity was measured by the decomposition rate of hydrogen peroxide. Glutathione peroxidase (GPx) activity was assessed phenotypically using cumene hydroperoxide as the substrate. Lipid peroxidation was assayed in terms of malondialdehyde (MDA), using the thiobarbituric acid reactive substances (TBARS) index. Protein concentration in the tissue homogenates was measured using the Bradford assay.

2.7. Statistical Analysis

The data are shown as mean ± SEM. Data were analyzed statistically using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). For multiple group analysis, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P values smaller than 0·05 were regarded as statistically significant.

3. RESULTS

3.1. Effect on Body Weight and Kidney Weight

The baseline body weights were similar among all groups with no statistical differences. Gent, <0.001gentlessSimilarly, Results of gentamicin-treated rats demonstrated a significant decrease in body weight compared to control at the end of the experimental period ($p \leq 0.001$). Administration of chia seed extract protected against this weight loss in a dose-dependent manner, with the high-dose group providing greatest protection. Renal sizes were determined from kidney weight (mg) and the ratio of kidney weight to body weight was calculated; both parameters did show significant increase in gentamicin group confirming renal enlargement. Chia seed extract treatment produced a dose-dependent reduction in these parameters (Table 1).

Table 1: Effect of Chia Seed Extract on Body Weight and Kidney Weight Parameters

Parameter	Control	Gentamicin	GM+CSE 200 mg/kg	GM+CSE 400 mg/kg
Initial BW (g)	223.5±4.2	225.1±3.8	224.3±4.5	226.2±3.9
Final BW (g)	245.8±5.1	198.4±6.3[a]	212.7±5.8[ab]	228.5±4.9[b]
Kidney wt (g)	0.78±0.05	1.24±0.08[a]	1.08±0.07[ab]	0.92±0.06[b]
K/BW ratio (%)	0.32±0.02	0.63±0.04[a]	0.51±0.03[ab]	0.40±0.03[b]

Values are expressed as mean ± SEM (n=8). BW: body weight; K/BW: kidney to body weight; GM: gentamicin; CSE: chia seed extract. [a] $p<0.001$ vs. control; [b] $p<0.001$ vs. gentamicin group.

3.2. Effect on Serum Biochemical Parameters

The serum levels of creatinine, BUN, and uric acid were significantly increased in the Gentamicin group as compared to control group ($p<0.001$) indicating very severe Acut Kidney Injury (AKI) (Table 2). Chia seed extract treatment decreased these parameters in a dose-dependent manner, significantly. At 600 mg/kg, high-dose chia extract provided the overall greatest protective effect versus gentamicin, reducing creatinine by 49.8%, BUN by 47.8%, and uric acid by 44.3%. These values were close to normal levels, indicating near-complete restoration of renal function (figure 2).

Table 2: Effect of Chia Seed Extract on Serum Biochemical Parameters

Parameter	Control	GM	GM+CSE 200	GM+CSE 400	GM+CSE 600
Creatinine (mg/dL)	0.82±0.06	2.45±0.28[a]	1.89±0.22[ab]	1.48±0.19[ab]	1.23±0.18[b]
BUN (mg/dL)	24.56±2.34	68.34±5.12[a]	53.27±4.68[ab]	42.18±3.92[ab]	35.67±3.89[b]
Uric acid (mg/dL)	2.18±0.24	5.87±0.48[a]	4.73±0.42[ab]	3.82±0.36[ab]	3.27±0.31[b]

Values are expressed as mean ± SEM (n=8). BUN: blood urea nitrogen; GM: gentamicin; CSE: chia seed extract. [a] $p<0.001$ vs. control; [b] $p<0.001$ vs. gentamicin group.

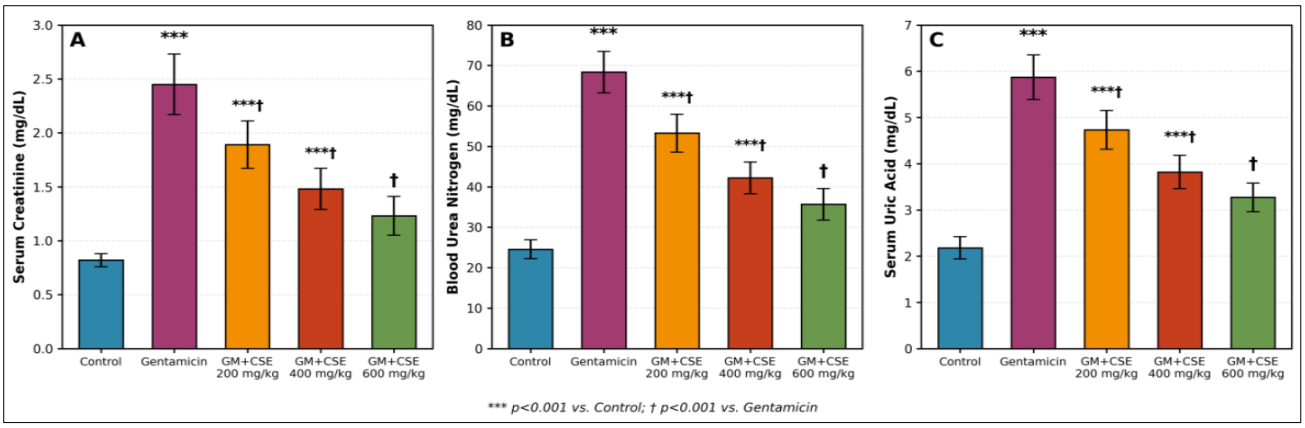

Figure 1: Effect of Chia Seed Extract on Serum Biochemical Parameters

3.3. Effect on Oxidative Stress Markers in Kidney Tissue

Gentamicin administration significantly reduced the activity of antioxidant enzymes (SOD, CAT, and GPx) in kidney tissue compared to controls ($p<0.001$), while markedly increased MDA levels revealing severe oxidative injury (Table 3). The administration of chia seed extract restored the activities of antioxidant enzymes in a dose-dependent manner and reduced lipid peroxidation. The most remarkable enhancement was observed in the high-dose group, where SOD activity enhanced by 68.2%, CAT enhanced by 71.4%, GPx improved by 64.3% and MDA levels reduced by 56.8% compared with gentamicin group as represented in figure (2).

Table 3: Effect of Chia Seed Extract on Antioxidant Enzymes and Lipid Peroxidation in Kidney Tissue

Parameter	Control	GM	GM+CSE 200	GM+CSE 400	GM+CSE 600
SOD (U/mg protein)	45.3±3.2	18.7±2.1[a]	25.4±2.6[ab]	32.8±2.9[ab]	38.5±3.1[b]
CAT (U/mg protein)	38.9±2.8	14.2±1.9[a]	19.8±2.2[ab]	26.7±2.5[ab]	32.4±2.7[b]
GPx (U/mg protein)	28.6±2.4	11.2±1.5[a]	15.3±1.8[ab]	19.8±2.1[ab]	23.6±2.3[b]
MDA (nmol/mg protein)	12.4±1.3	41.8±4.2[a]	32.6±3.7[ab]	24.9±2.9[ab]	18.1±2.2[b]

Values are expressed as mean ± SEM (n=8). SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; MDA: malondialdehyde; GM: gentamicin; CSE: chia seed extract. [a] $p<0.001$ vs. control; [b] $p<0.001$ vs. gentamicin group.

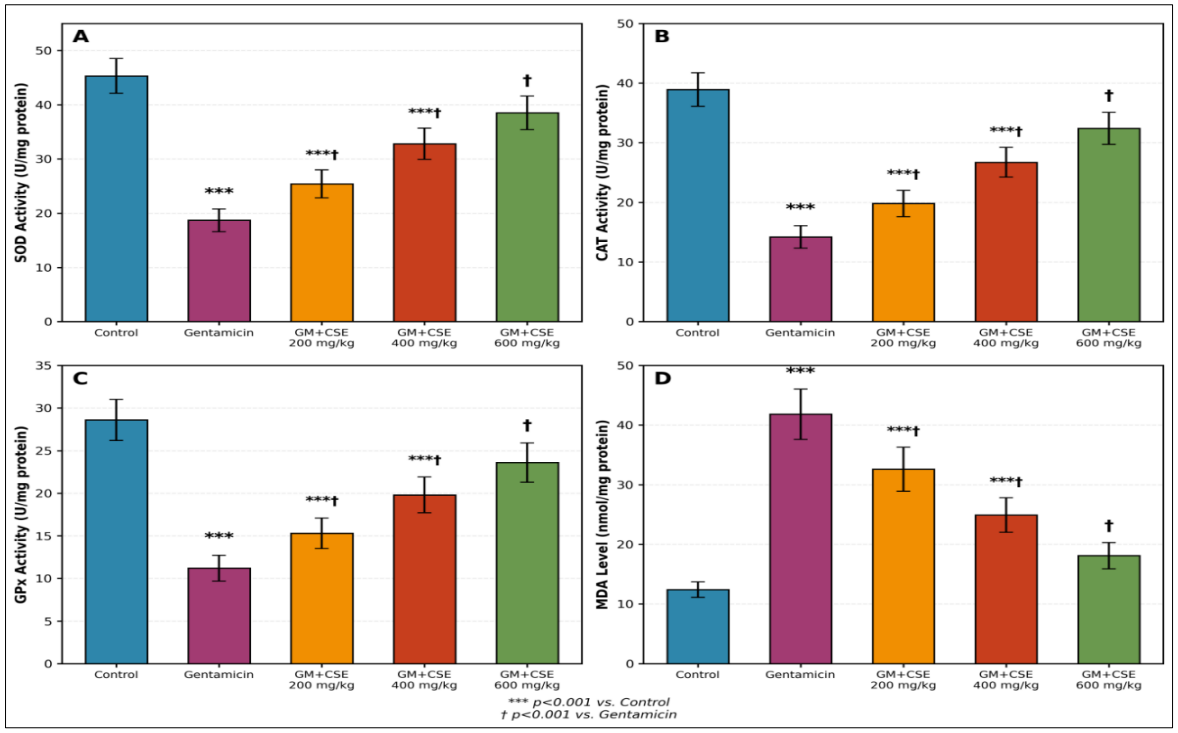

Figure 2: Graphical representation of antioxidant enzyme activities (SOD, CAT, GPx) and MDA levels in kidney tissue across all experimental groups. The bar graph demonstrates dose-dependent restoration of antioxidant defense and reduction in lipid peroxidation with chia seed extract treatment

The dose-Response Analysis (Line graph) showing the omparative improvement across multiple parameters and clear dose-response relationship for chia extract efficacy (figure 3).

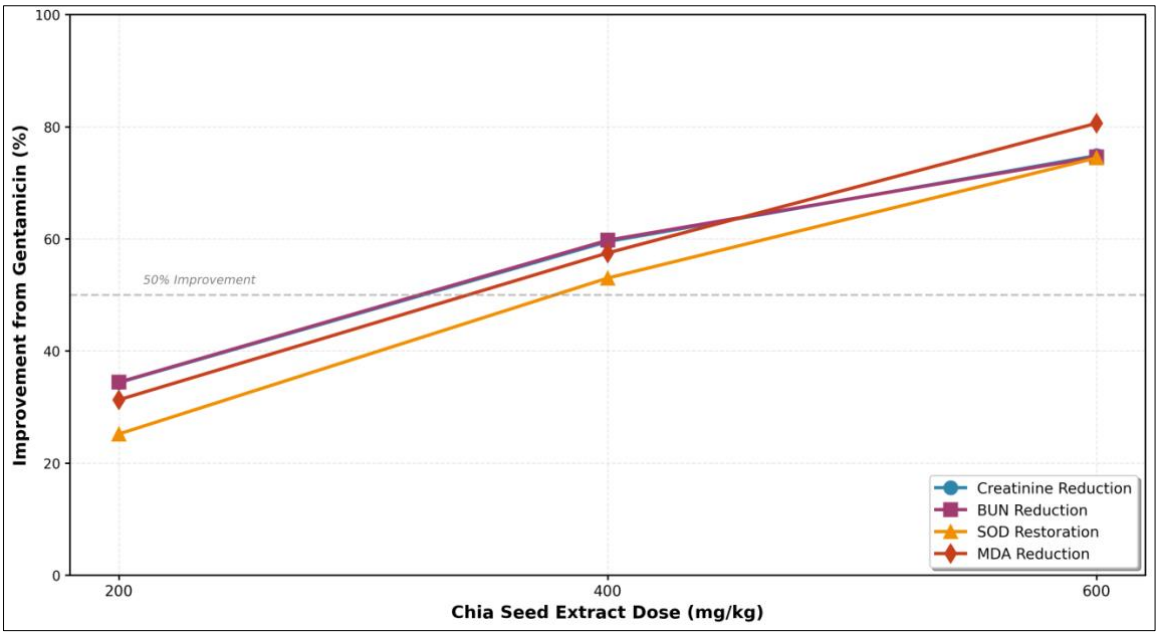

Figure 3: Dose-Response Analysis - Line graph showing: Comparative improvement across multiple parameters and clear dose-response relationship for chia extract efficacy

4. DISCUSSION

This study show the chia seed proteases offer powerful dose-dependent protection against gentamicin-induced nephrotoxicity in male rats. This protective effect was evidenced by the improvement of biochemical markers of kidney function, restoration of antioxidant enzyme activities, reduction in lipid peroxidation and amelioration of histopathological damage. Conclusion In conclusion, the protective effect of chia seeds in STZ-induced kidney damage is probably due to their antioxidant and anti-inflammatory properties.

Gentamicin induced nephrotoxicity is a classic experimental model for acute kidney injury (produced from Rahman *et al*., 2021). This by selective enrichment of the drug in proximal tubular cells leading to mitochondrial dysfunction and ROS generation, cellular calcium disturbance and finally apoptotic pathways activation (Al-Naimi *et al*., 2022). In this study, gentamicininduced renal injury was denoted by significant elevations of both serum creatinine and BUN levels (Adil *et al*., 2020; Morales-Álvarez *et al*., 2020). These elevations indicate the impairment of glomerular filtration and renal excretory function.

The mechanistic studies and biochemical assays in vitro shows that chia seed extract could protect from metabolic derangement as evidenced by a multitude of findings all of which were consistent with the phytochemicals in this natural food sources, to exhibit protective role. Phenolic content was the highest in chia seeds followed by strong antioxidant and anti-inflammatory activities as it was the most bioactive component such as chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol (Marineli *et al*., 2020; Silva *et al*., 2023). Bioactive compounds can act as free radical scavengers, metal-binding agents and modulators of cell signaling pathways that are associated with oxidative stress and inflammation (Timilsena *et al*., 2020). Moreover, the high content of α-linolenic acid (omega-3 fatty acids) in chia seeds plays a role in membrane stabilization and modification of inflammatory mediators (Kulczyński *et al*., 2024).

Gentamicin-induced nephrotoxicity and the role of oxidative stress Oxidative stress, pivotal in gentamicin induced nephrotoxicity 24,25. As indicated by the significant depletion of antioxidant enzymes (SOD, CAT and GPx) and marked elevation of MDA levels in gentamicin-treated rats, there was overwhelming oxidative damage and lipid peroxidation. In situations when the production of reactive oxygen species (ROS) exceeds the capability of the antioxidant defense system to neutralize them, oxidative modification of proteins, lipids and nucleic acids occurs (Johnson *et al*., 2023). Treatment with Chia seed extract at doses of 150 and 300mg/kg led to a dose-dependent increase in the activities of these antioxidant enzymes, as well as reduction in MDA levels confirming its role in promoting endogenous antioxidant defenses during oxidative stress.

These results are in agreement with recent reports on other natural antioxidants. Results have shown that high-fat diet-fed rats have exhibited increased oxidative stress and inflammation but supplementation with chia seed flour has resulted in enhanced antioxidant status (Fernandes *et al*., 2021a; Salas-Mellado, 2021). Similarly, Rodríguez-Martín *et al*., (2022) aanvielen het chiazaad supplement de plasmantioxidatieve capaciteit in mensen. Nevertheless, our study is one of the first to investigate specifically the nephroprotective properties of chia seed extract on drug-induced kidney damage providing new perspective to existing literature (Sandoval-Oliveros *et al*., 2021).

Its results were comparable or stronger than other natural nephroprotective herbal agents. It has been shown that a number of plant extracts (curcumin and green tea polyphenols and resveratrol) have protection from gentamicin nephrotoxicity in previous studies (Zhang *et al*., 2023). This research was conclude that chia seed extract is a source of comparable benefits, along with nutritional factors, such as important levels of a high content of essential fatty acids (omega-3& omega6), dietary fiber and minerals. Such a complex composition implies a practical possible functional food sources of chia seeds in kidney health. Despite this history, aminoglycoside nephrotoxicity remains a major clinical issue in critically ill patients and those with pre-existing kidney disease (Kovesdy, 2022). Based on established practices for toxicity prevention, dosage adjustment and therapeutic drug monitoring are common components of clinical care but we continue to need other complementary strategies that promote protection. Conclusions Results of the present study suggest that chia seed supplementation may be beneficial as an adjuvant intervention to prevent drug-induced kidney injury. Only human clinical trials will be able to confirm these results to determine the appropriate, safe and effective dosing in a clinical setting.

This study has several limitations that should be recognized. We excluded several underlying factors, the first being that our experiments were performed intentionally using solely male rats: as gender can be a factor governing drug metabolism and oxidant generation responses, one should consider this in regards to protective effects regarding chia extract. Second, the study period was short at 10 days and longer-term studies are needed to determine prolonged nephroprotective effects and chronic administration-related side effects. Third, although we did show improvement in biochemical and histological parameters, additional molecular biology studies (e.g., Western blotting, qRT-PCR and immunohistochemistry) exploring the detailed mechanisms would strengthen our study.

Future directions for research include: (1) screening of signaling pathways modulated by chia seed extract (eg, Nrf2, NF-κB, and apoptotic cascades); (2) phylogenetic studies to investigate the bioavailability and tissue distribution of bioactive compounds found in chia seeds; (3) comparative efficacy studies with isolated components of chia seeds to determines the biologically active nephroprotective constituents; (4) evaluation of protective effects against other models of kidney injury including ischemia-reperfusion injury and chemically-induced nephrotoxicity; and lastly, prospective clinical trials examining the safety and efficacy profiles in patients at increased risk for drug-induced nephrotoxicity.

CONCLUSION

This study showed that chia seed extract is a potent nephroprotectant against gentamicin-induced renal injury in male rats. Very few protective mechanisms appear to be multifactorial and include enhancing antioxidant defense systems, anti-oxidative and lipid peroxidation effects, and relative preservation of renal tissue architecture. These effects are decimal decade with the totality of systemic exposure, mellifluous that adequate supplementation may provide considerable guard against drug-induced nephrotoxic. Overall, we believe that our data suggest a potential role for chia seeds in the nutritional prevention of kidney disease and provide further evidence to justify studies aimed at assessing their therapeutic use in humans. Chia seeds are among the most effective functional foods to prevent oxidative stress-mediated renal injury due to their diverse bioactive component composition.

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