



Therapeutic Effect of *Trigonella foenum-graecum* L Seeds Extract on Folic Acid-Induced Acute Kidney Injury

Ehab Massoud^{1,2} · Melyssia Samy Daniel³ · Attalla El-Kott^{4,5} · Sara Bayoumi Ali⁶ · Kareem Morsy^{4,6} · Ayman S. Mohamed⁶  · Sohair R. Fahmy⁶

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Abstract Acute kidney injury (AKI) is a serious clinical illness that produces renal failure quickly and has a high fatality rate. Fenugreek possesses anti-osteoporotic, anti-inflammatory, antipyretic, antidiabetic, and immunomodulatory activities. The current study aimed to evaluate the therapeutic efficacy of the *Trigonella foenum-graecum* L seeds extract (TFGE) against AKI induced by a high dose of folic acid. There were 18 male rats divided into three groups: control, folic acid, and folic acid + TFGE.

Our results revealed that *Trigonella foenum-graecum* decreased lipid peroxidation, improved antioxidant status, and prevented damage to the liver and kidney.

Hepatorenal toxicity induced by a dose of folic acid (250 mg kg⁻¹, i.p.) while TFGE (250 mg kg⁻¹) administered for seven consecutive days. The methanolic extract of TFG extract contains tannins, alkaloids, terpenoids, saponins, and phenols, while flavonoids were absent. High dose of folic acid caused increases in AST, ALT, creatinine, urea, uric acid, MDA and decreases in albumin, GSH, CAT levels. In contrast, the administration of TFGE suppresses hepatorenal toxicity and improved tissue functions and structure. TFGE, an abundant antioxidant source, has a renal protecting outcome. The current study demonstrated the therapeutic efficacy of TFGE on the liver

✉ Ayman S. Mohamed
ayman81125@cu.edu.eg

Ehab Massoud
ahmed_Moh14@yahoo.com

Melyssia Samy Daniel
melyssia.samy@msa.edu.eg

Attalla El-Kott
Rocket_hist32@hotmail.com

Sara Bayoumi Ali
sara.bayoumi@yahoo.com

Kareem Morsy
Kareemsaid156@yahoo.com

Sohair R. Fahmy
sohairfahmy@gmail.com

⁴ Biology Department, Faculty of Science, King Khalid University, Abha, Saudi Arabia

⁵ Zoology Department, Faculty of Science, Damanhour University, Damanhour, Egypt

⁶ Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

¹ Biology Department, Faculty of Science and Arts in Dahran Aljnoun, King Khalid University, Abha, Saudi Arabia

² Agriculture Research Centre, Soil, Water and Environment Research Institute, Giza, Egypt

³ Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA), 6th of October, Egypt

and kidney in the treatment of gentamycin-induced hepatotoxicity.

Keywords *Trigonella foenum graecum* · Folic acid · Toxicity · Oxidative stress · Natural product

Introduction

Acute kidney injury (AKI) is a serious clinical illness that produces renal failure quickly and has a high fatality rate [1]. Patients with partial AKI exhibit proteinuria that worsens with time and lower glomerular filtration rate (GFR) [2]. The worldwide burden of AKI-related death outweighs breast cancer, heart failure, or diabetes, and mortality rates have remained high for the past 50 years [3].

Drugs are responsible for around 20% of hospitalizations and extrinsic acute kidney injury [4]. Overdosing on folic acid (FA) causes AKI was reported in humans [5], which mimics the critical mechanisms of AKI in humans [6]. It is a nephrotoxic agent that causes fibrosis in the kidneys [7]. FA has significant nephrotoxic and hepatotoxic adverse effects, despite its favorable advantages. [8]. High FA levels are associated with adverse effects such as oxidative stress and non-alcoholic fatty liver disease [8].

Traditional treatments for renal illnesses may have both contraindications and side effects, causing or exacerbating nephrotoxicity [9]. Currently, there is no viable treatment to slow or stop AKI development [10]. However, some herbal medicines may have therapeutic efficacy in preventing and treating AKI [9]. Herbal medicine is gaining popularity due to its health benefits where compounds involved in pharmacodynamic action can be found in various plants [11, 12]. Fenugreek is a well-known nutritious herb that is grown all over the world. Fenugreek seeds and leaves have been shown to have nutritional and therapeutic value in many researches [13]. Fenugreek has been shown to have therapeutic benefits in a variety of conditions, including osteoporosis [14], inflammation [15], and diabetes [16]. Furthermore, some studies have suggested that fenugreek-based medicines have antioxidant properties [17]. Saponins, trigonelline alkaloids, trigocoumarin, phosphates, potassium, proteins (4-hydroxyisoleucine), choline, vitamin C, beta-carotene, and nicotinic acid are important phytochemical substances found in TFG [18]. Thus, the current study aimed to evaluate the therapeutic efficacy of the *Trigonella foenum-graecum* L seeds extract (TFGE) against AKI induced by a high dose of folic acid.

Material and Methods

Material

Folic acid, methanol 100%, and dimethyl sulfoxide were purchased from local Sigma-Aldrich. All other chemicals and reagents were of the highest commercial grade.

Plant Material

Seeds of TFG were acquired from a local herbal store, in Giza, identified by Department of Botany, Cairo University. It is manually cleansed of all scums and crushed to achieve a fine powder. The methanolic extract was prepared according to Hossain et al. method [19]. 40 g of TFG powder was submerged in 100 ml of methanol and then incubated overnight at 25 °C in a rotary mixer. The solvent was filtered using filter paper (What-mann No.1) and then evaporated using a rotary evaporator at 450C (Bibby RE100, Staffordshire, UK). The methanol extract (5 gm) obtained was saved at – 20 °C till further use.

Phytochemical Qualitative Analysis

The plant extracts were assessed for the existence of the phytochemical activity by using the standard methods [20–27].

Ethical Consideration

This study's experimental protocols and procedures were approved by the October University for Modern Sciences and Arts, Faculty of Biotechnology, Institutional Animal Care and Use Committee (IACUC) (Egypt). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

Experimental Animals

The rats utilized were healthy male Wistar rats (*Rattus norvegicus*) weighing 150–170 g. Egypt's National Research Center (NRC) was used to procure the animals. For acclimatization, the rats were housed for one week prior to the start of the experiment. The animals were kept in polypropylene cages (six animals per cage) in a well-ventilated animal house at a temperature of 25 ± 1 °C and on a natural day-night cycle. The animals were given regular chow pellets and water to drink.

Induction of Acute Kidney Injury (AKI)

AKI was induced by intraperitoneal injection of a single dose of folic acid (250 mg/kg) dissolved in 0.3 M NaHCO₃ [20].

Study Design

Eighteen rats were split into three groups (each with six rats) as follows:

Group I (Control) After a single dose of NaHCO₃ (0.3 M, i.p), the rats received (5% DMSO, orally) daily for one week.

Group II (FA) After a single dose of folic acid (250 mg/kg, i.p) [21] dissolved in NaHCO₃, the rats received (5% DMSO, orally) daily for one week.

Group III (FA + (TFGE)) After a single dose of folic acid (250 mg/kg, i.p) dissolved in NaHCO₃, the rats received TFGE (250 mg/kg) [22] daily for one week.

Animal Handling

The rats were euthanized under deep anesthesia with sodium pentobarbital (50 mg/kg body weight). Blood was obtained by cardiac puncture in centrifuge tubes without anticoagulant and centrifuged for 15 min at 3000 rpm. The serum is kept at -80 °C until it is utilized in biochemical tests. The liver and kidney samples were rapidly removed, rinsed with physiological saline, and blotted using filter paper to eliminate any signs of blood.

Liver and Kidney Homogenate Preparation

In ice-cold 0.1 M Tris-HCl buffers (pH 7.4), liver and kidney tissues were weighted and homogenized (10% w/v). The homogenate was centrifuged for 15 min at 3000 rpm, and the supernatant was kept at -20 °C for biochemical analysis [23].

Histopathological Preparation

Sections of the liver and kidneys were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histological study under a light microscope [24].

Biochemical Assessment

The creatinine, urea, uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), and serum albumin are used according

to the manufacturer's instructions using Bio-diagnostic kits (Giza, Egypt).

MDA level is an index of lipid peroxidation, glutathione reduced (GSH), and catalase (CAT) were determined in the kidney homogenate supernatant according to the manufacturer's instructions using Biodiagnostic kits (Giza, Egypt).

Statistical Analysis

All values were reported as means with standard deviations (SEM). Within-group comparisons were assessed using one-way analysis of variance (ANOVA) with Duncan's post hoc test to compare group means, with $P < 0.05$ regarded statistically significant. The statistical analysis was performed using SPSS for Windows (version 15.0).

Results

Phytochemical Analyses of Fenugreek Seed Extract (TFGE)

The compounds present in the methanolic extract of TFG are recorded in Table 1. The extract contains tannins, alkaloids, terpenoids, saponins, and phenols, while flavonoids were absent.

Effect of TFGE on Kidney Function Markers

A high dose of FA caused a significant increase ($P < 0.05$) in creatinine, urea, and uric concentrations compared to the control group, while the treatment with TFGE decreased the levels of the aforementioned parameters significantly ($P < 0.05$) compared with the FA group (Table 2).

Effect of TFGE on Liver Function Markers

The FA group showed a significant increase ($P < 0.05$) in AST, ALT, while albumin decreased significantly

Table 1 Results of phytochemical analysis

Test	Ethanolic extract
Alkaloids	+
Saponins	+ +
Steroidsor Terpenoids	+
Tannins	+ +
Flavonoids	-
Phenols	+

+ = Indicates positive result, + + = Indicates more positive reaction towards the test, -- = Indicates negative result

Table 2 Comparison between kidney function parameters among all studied groups

Parameters	Control	FA	TFGE
Urea (mg/dl)	25.46 ± 0.60 ^a	48.22 ± 1.34 ^c	35 ± 0.90 ^b
Creatinine (mg/dl)	0.45 ± 0.04 ^a	1.01 ± 0.04 ^c	0.75 ± 0.90 ^b
Uric acid (mg/dl)	1.64 ± 0.05 ^a	4.16 ± 0.15 ^c	2.53 ± 0.13 ^b

All values are represented as mean ± SE (*n* = 6)

Values with different superscript letters are significantly different (*P* < 0.05)

Table 3 Comparison between liver function parameters among all studied groups

Parameters	Control	FA	TFGE
ALT(U/ml)	71.61 ± 2.92 ^a	98.31 ± 4.66 ^c	83.12 ± 3.74 ^b
AST (U/ml)	140.22 ± 6.98 ^a	200.84 ± 8.49 ^c	170.97 ± 7.39 ^b
Albumin (g/dl)	2.42 ± 0.15 ^c	1.35 ± 0.25 ^a	2.24 ± 0.34 ^b

All values are represented as mean ± SE (*n* = 6)

Values with different superscript letters are significantly different (*P* < 0.05)

Table 4 Comparison between oxidant-antioxidant parameters among all studied groups

Parameters	Organ	Control	FA	TFGE
MDA (nmol/g.tissue)	Kidney	4.67 ± 0.83 ^a	8.78 ± 1.15 ^b	5.61 ± 0.389246 ^a
	Liver	6.13 ± 0.31 ^a	10.05 ± 1.58 ^c	8.48 ± 0.94 ^b
GSH (mg/g.tissue)	Kidney	10.37 ± 0.40 ^c	6.76 ± 0.42 ^a	8.25 ± 0.49 ^b
	Liver	12.82 ± 1.00 ^c	7.64 ± 0.68 ^a	9.64 ± 0.15 ^b
CAT (U/g.tissue)	Kidney	0.13 ± 0.01 ^c	0.567 ± 0.01 ^a	0.97 ± 0.02 ^b
	Liver	1.02 ± 0.34 ^c	0.61 ± 0.05 ^a	0.80 ± 0.04 ^b

All values are represented as mean ± SE (*n* = 6)

Values with different superscript letters are significantly different (*P* < 0.05)

compared to the control group. However, treatment with TFGE restored the levels of these parameters near the normal value (Table 3).

Effect of TFGE on Oxidative Stress Markers

The level of malondialdehyde (MDA) was significantly increased (*P* < 0.05), whereas GSH and CAT levels decreased significantly after injection with a high dose of FA. On the other hand, TFGE administered decreased MDA level significantly (*P* < 0.05) and increased GSH and CAT levels (Table 4).

Histopathological Effects

Microscopic examination of the kidney of control groups showed normal histoarchitecture of the tissue where

glomeruli appear as dense tufts of capillaries enclosed in the outer layer of Bowman capsules. Numerous renal tubules were observed (Fig. 1). On the other hand, the histopathological study of kidney FA-treated rats showed severe degeneration in the glomerulus with deformed renal tissue architecture (Fig. 1). In contrast, the treatment with TFGE caused marked regeneration in the glomerulus and Bowman's capsule with standard renal tissue architecture (Fig. 1).

The liver of control rats is formed of the classic hepatic lobules. Blood sinusoids (S) were seen separating the cords of the liver cells and lined by flattened endothelial cells and Von Kupffer cells (K) (Fig. 1). The histology of liver sections obtained from FA-treated rats showed the loss of hepatic lobular architecture morphological alterations, necrosis (N), and apoptosis (A) (Fig. 1). Liver sections of TFGE-treated rats showed moderate to mild degenerated

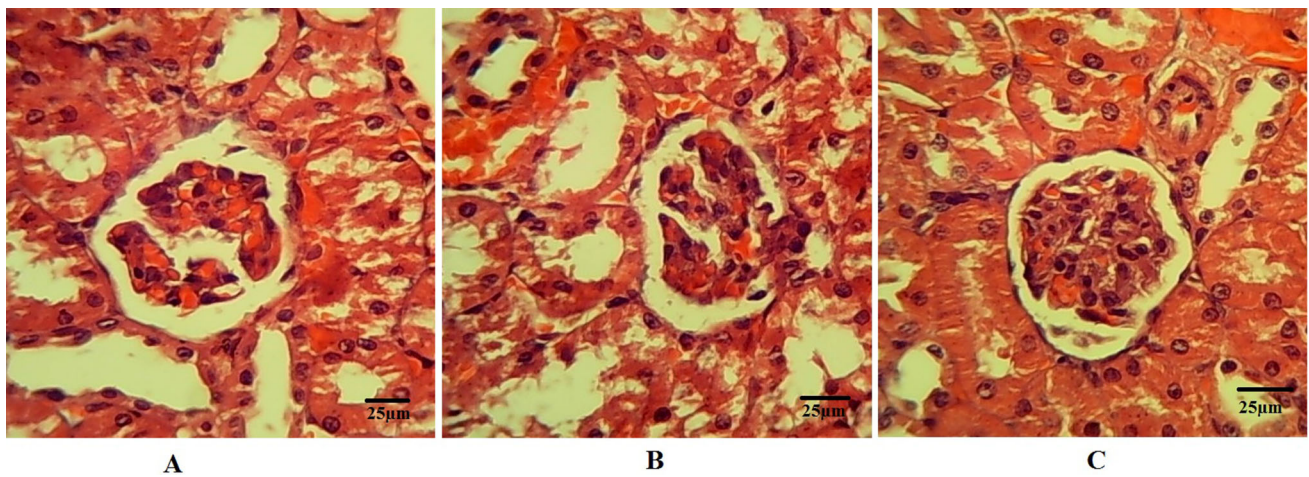


Fig. 1 Histopathological examination of the kidney (400X H&E). **A** Control group, **B** FA group and **C** TFGE group

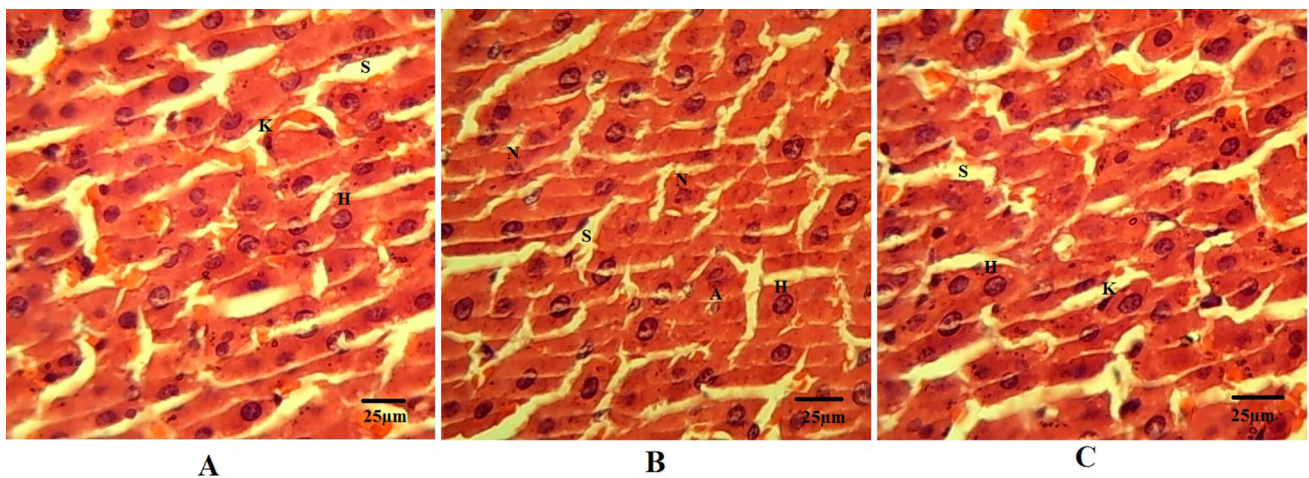


Fig. 2 Histopathological examination of the liver (400X H&E). **A** Control group, **B** FA group and **C** TFGE group

changes in hepatocytes and a clear improvement in the hepatic architecture (Fig. 2).

Results and Discussion

Acute kidney injury (AKI) is defined as a rapid and reversible loss in renal function [25]. Experimental folic acid (FA)-induced AKI is equivalent to human AKI and has been used to investigate the pathogenic pathway of human AKI and the therapeutic efficacy of various medications in the treatment of AKI [26]. In conjunction with the present study, a high dose of FA-induced AKI is established by elevation in serum creatinine, urea, uric acid, widespread tubular necrosis, and brush border loss [27]. FA-induced renal tubule destruction is a popular model of AKI, which is characterized by severe

inflammation [28]. Furthermore, apoptosis is another essential mechanism in FA-induced AKI [29].

When viewed in conjunction with the findings of Uslu et al. [30], results from this study show that TFGE therapy resulted in considerable renal repair, as seen by a considerable reduction in kidney function indicators. Improvement in kidney function may relate to fenugreek's ability to prevent renal stone formation. Furthermore, the current study's histopathological findings confirmed the favorable effects of TFGE on kidney structure, suggesting that TFGE has antinephrotoxic action, preventing renal damage induced by FA.

The liver is an essential organ involved in folate metabolism. Hepatotoxicity has been linked to high-dose folic acid (FA) ingestion. In agreement with the current study result, Christensen et al. and Elrouby et al. reported that large dosages of FA induce liver toxicity in rats which

confirmed by elevation of aminotransferase enzymes and depletion in albumin level. The liver injury could be linked to the hyperhomocysteinemia state that develops after high FA administration. Treatment of rats with TFGE, on the other hand, restores liver function markers to near-normal levels and improves histology of the liver. In agreement with the current study, Tewari et al. reported that dietary supplementation with fenugreek seed improves hepatic enzymes. Furthermore, fenugreek seed extracts have been shown to have phenolic-associated protective properties against cypermethrin-induced hepatic damage in rats.

It has been shown that oxidative stress, which occurs when large dosages of FA are administered, has a role in kidney and liver damage. At the cellular or systemic level, oxidative stress is defined as a disruption of redox homeostasis, due to an excess of reactive species or a decrease in antioxidant system capacity. The observed decrease in levels of glutathione (GSH) and catalase (GAT), and increased malondialdehyde (MDA) concentrations could all be contributing to the increased lipid peroxidation, which noticed in the current study after high dose of FA injection. MDA is a highly reactive metabolic product that results from a chain reaction involving lipid peroxide. The rise in MDA levels seen in this study might be attributed to the buildup of homocysteine as a result of high FA dosages, which self-oxidize to form reactive oxygen species (ROS). GSH is an antioxidant enzyme that scavenges ROS and overcomes oxidative stress in order to keep the cell's metabolic pathways running smoothly. Antioxidant enzymes like catalase (CAT) have a critical function in preventing oxidative stress in kidney and liver disorders. The decrease of CAT activity is related to oxidative stress in the pathogenesis of FA-induced AKI. On the other hand, TFGE improves rats' antioxidant status and inhibits lipid peroxidation. Furthermore, fenugreek seeds have been shown to have potent free radical scavenging properties.

Conclusion

The current study adds to the growing number of prior research on herbal medicine therapeutic items, confirming that TFGE had a beneficial impact on rats' FA-induced kidney and liver damage. TFGE reduced lipid peroxidation and enhanced antioxidant state, preventing hepatorenal toxicity.

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Author's Contribution EM and ASM developed the theoretical formalism, MTE, SBA performed the analytic calculations, AE and KM data collection and interpretation, SB and SEF drafting the article.

Data Availability The derived data generated in this research will be shared on reasonable request to the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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