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Fenugreek's nephroprotective potential: sex-specific and dose-dependent effects on kidney function in healthy rats

[*Potencial nefroprotetor do feno-grego: efeitos específicos por sexo e dependentes da dose na função renal em ratos saudáveis*]

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ABSTRACT

This study investigates the dose- and sex-specific effects of *Trigonella foenum-graecum* (fenugreek) aqueous extract on renal function in healthy Wistar albino rats. Male and female rats were divided into control, 25mg/kg, and 50mg/kg treatment groups, and kidney function markers, including Blood Urea Nitrogen (BUN), uric acid, and creatinine, were assessed over a 21-day period. Histological analysis of renal tissues was also performed. In male rats, fenugreek extract significantly reduced BUN and creatinine levels, with the optimal effect observed at 25mg, indicating a dose-dependent response. Conversely, female rats exhibited increased BUN levels, while creatinine and kidney morphology remained unchanged. Histological analysis revealed preserved renal architecture across all doses in both sexes. These findings suggest fenugreek's renoprotective potential, particularly in male rats, while highlighting sex-based differences in response. Further studies are warranted to elucidate the underlying mechanisms and evaluate fenugreek's clinical relevance for kidney health.

Keywords: fenugreek (*Trigonella foenum-graecum*), renal function, Blood Urea Nitrogen (BUN), creatinine, uric acid, dose-dependent effects, sex-specific response, histological analysis, nephroprotection, kidney health.

RESUMO

Este estudo investiga os efeitos específicos da dose e do sexo do extrato aquoso de Trigonella foenum-graecum *(feno-grego) na função renal em ratos albinos Wistar saudáveis. Os ratos machos e fêmeas foram divididos em grupos controle, 25 mg/kg e 50mg/kg, e os marcadores da função renal, incluindo nitrogênio ureico no sangue (BUN), ácido úrico e creatinina, foram avaliados durante um período de 21 dias. Também foi realizada uma análise histológica dos tecidos renais. Em ratos machos, o extrato de feno-grego reduziu significativamente os níveis de BUN e creatinina, com o efeito ideal observado em 25 mg, indicando uma resposta dependente da dose. Por outro lado, as ratas apresentaram níveis aumentados de BUN, enquanto a creatinina e a morfologia renal permaneceram inalteradas. A análise histológica revelou arquitetura renal preservada em todas as doses em ambos os sexos. Esses achados sugerem o potencial renoprotetor do feno-grego, particularmente em ratos machos, ao mesmo tempo em que destacam diferenças na resposta com base no sexo. Estudos adicionais são necessários para elucidar os mecanismos subjacentes e avaliar a relevância clínica do feno-grego para a saúde renal.*

*Palavras-chave: feno-grego (*Trigonella foenum-graecum*), função renal, nitrogênio ureico no sangue (BUN), creatinina, ácido úrico, efeitos dependentes da dose, resposta específica por sexo, análise histológica, nefroproteção, saúde renal*

INTRODUCTION

Fenugreek (Trigonella foenum-graecum) has been recognized as a medicinal plant with extensive applications in traditional medicine (Sun *et al*., 2021), particularly in the treatment and management of metabolic and inflammatory disorders (Shadab *et al*., 2024; Singh *et al*., 2022; Almatroodi *et al*., 2021). The seeds and leaves of fenugreek contain a rich profile of bioactive compounds, including alkaloids, flavonoids, saponins, and amino acids (Gavahian *et al*., 2024; Salam *et al*., 2023), that contribute to its

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therapeutic potential. Emerging scientific evidence suggests that fenugreek exhibits significant effects on renal function, particularly by modulating the activity of key kidney enzymes.

Kidney enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase, play pivotal roles in maintaining renal oxidative balance and protecting against nephrotoxicity. The dysregulation of these enzymes is closely associated with the progression of renal disorders, including chronic kidney disease (CKD) and acute kidney injury (AKI). Fenugreek's antioxidative properties, attributed to its high content of polyphenols and flavonoids, have been hypothesized to restore enzyme activities and mitigate oxidative damage in renal tissues. Furthermore, its potential to modulate biomarkers of inflammation and oxidative stress underscores its promise as a nephroprotective agent.

Studies have explored fenugreek's impact on renal health in various experimental models. For instance, a study by Mbarki *et al*. (2017) demonstrated that fenugreek supplementation ameliorated oxidative stress-induced renal damage in rats by enhancing the activities of antioxidant enzymes and reducing lipid peroxidation levels. Similarly, Abd-Elrahman (2019) reported that fenugreek extract mitigated cisplatin-induced nephrotoxicity by modulating kidney enzyme expression and reducing pro-inflammatory cytokine levels. These findings align with the broader pharmacological profile of fenugreek, emphasizing its ability to target multiple pathways involved in renal pathology.

Given the rising prevalence of kidney-related disorders globally, the exploration of plant-based therapeutic strategies has gained momentum. Fenugreek's accessibility, safety profile, and multifunctional properties position it as a compelling candidate for further research and potential integration into nephrology therapeutics. Future investigations, particularly well-designed clinical trials, are essential to validate its efficacy and elucidate the molecular mechanisms underlying its protective effects on kidney enzymes and renal function.

MATERIALS AND METHODS

Adult male and female Wistar albino rats, weighing 250–320 grams, were obtained from the animal facility of the Zoology Department, College of Science, King Saud University (KSU). The rats were randomly housed in polypropylene cages, three per cage, and maintained under standard laboratory conditions. Prior to the experiment, the animals underwent a one-week acclimatization period in a well-ventilated environment with a controlled temperature of 25 ± 2 ºC and a regular 12-hour light/dark cycle. They were provided with a standard diet and had unrestricted access to water. All experimental procedures adhered to the ethical guidelines approved by the Institutional Animal Care and Use Committee of KSU (Approval No: KSU-SE-24-3).

The aqueous extract of fenugreek was prepared by finely grinding the plant into a powder, followed by thorough mixing and extraction with water. The plant material, totaling 250 grams, was sourced from well-local marketplaces. After collection, the plant was pulverized into a fine powder and subjected to extraction following the method outlined in our previous study (Alhimaidi *et al*., 2022). Two concentrations of the resulting aqueous extract were prepared: 50mg and 25mg.

Male and female Swiss albino rats (250–320 g) were obtained from the animal facility of the Department of Zoology, College of Science, King Saud University. The animals were maintained under standard conditions throughout the experimental period, with access to regular pellet feed and water provided ad libitum. The rats were divided into three groups male and female separated, each consisting of six males and six females. The first group, serving as the control, received water. The second group was administered 25mg/kg of aqueous fenugreek extract, while the third group received 50mg/kg of the same extract. The treatments were conducted over a 21-day period.

At the conclusion of the 21-day treatment period, all animals were fasted overnight and euthanized by exsanguination. Anesthesia was administered via intraperitoneal injection of pentobarbital at a dose of 45mg/kg body weight. Blood samples were collected, allowed to clot for 1 hour at 4°C,

and centrifuged at 3000 × g for 10 minutes to obtain serum. The kidneys were promptly excised, perfused with cold isotonic saline solution, and fixed in 10% formalin for histological analysis.

The kidney enzymes in rat blood samples were analyzed to assess the dose- and sex-specific effects of fenugreek extract. At the conclusion of the experiment, blood samples were collected from both male and female rats in non-heparinized glass tubes for serum extraction. The serum was separated by centrifugation at 3000 rpm for 15 minutes. Enzymatic activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatinine levels were measured using diagnostic kits with a BioSystem BTS-350 instrument. Kidney samples from all treated and control rats were collected for histological evaluation. Each rat was euthanized in a CO_2 chamber, and the kidneys were excised and preserved in 10% neutral formalin for 48 hours as small cubes (approximately 0.5cm) for fixation. The samples were then dehydrated through a graded series of alcohol concentrations (30%, 50%, 70%, and 100%) (Ammari *et al*., 2025a, b).

RESULTS

Figure 1 illustrates the effects of fenugreek extract on kidney weight in male rats. The control group exhibited significantly higher kidney weights compared to both the 25mg and 50mg treatment groups, with statistical significance at $p<0.01$ for both comparisons. However, no significant difference was observed between the 25mg and 50mg groups, suggesting a plateau effect where increasing the dose beyond 25mg does not result in further reductions in kidney weight.

These findings suggest that fenugreek extract reduces kidney weight in male rats in a dose-dependent manner, with the effect reaching its maximum at the 25mg dose. The absence of additional reduction at the 50mg dose indicates that higher doses do not amplify the observed effect. This dose-dependent response may reflect physiological changes in the kidneys associated with fenugreek extract administration. Further studies are warranted to elucidate the mechanisms underlying this response.

Figure 1 also depicts the effects of fenugreek extract on kidney weight in female rats across three groups: control, 25mg, and 50mg doses. Unlike in male rats, no statistically significant differences were observed between the control group and the 25mg or 50mg groups, as indicated by the "ns" (not significant) labels. However, a small but statistically significant reduction in kidney weight was noted between the 25mg and 50mg groups ($p<0.05$), suggesting a slight dose-dependent effect.

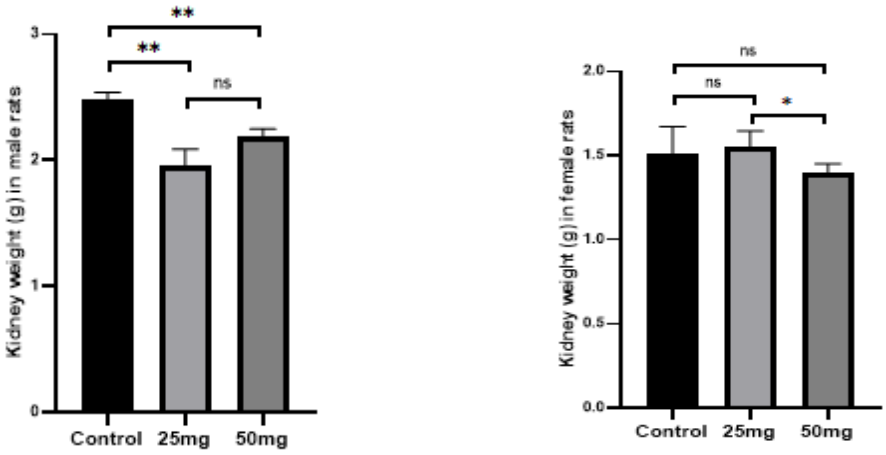
These findings indicate that fenugreek extract has a minimal impact on kidney weight in female rats. The observed reduction in kidney weight at the 50mg dose compared to 25mg suggests a minor dose-dependent effect, but the absence of significant differences between the control and treatment groups implies that fenugreek extract does not substantially alter kidney weight in female rats under the experimental conditions. Further research is needed to determine whether these subtle changes are biologically meaningful or simply reflect normal variability.

The control group exhibits normal renal histology, characterized by intact glomeruli and well-defined tubular structures, with no signs of structural disruption or damage. This observation serves as the baseline for comparison.

In the 25mg treatment group, slight alterations in renal tissue are observed when compared to the control group, potentially indicating mild physiological changes induced by fenugreek extract. However, the overall integrity of the renal tissue remains intact, with no significant disruption to the glomeruli or tubules. This suggests that fenugreek extract at 25mg exerts minimal impact on kidney morphology.

Similarly, the 50 mg treatment group does not display any significant deviations from the 25mg group or the control group. The renal architecture remains well-preserved, with intact glomeruli and organized tubular structures. This indicates that increasing the dose beyond 25mg does not result in additional morphological alterations in male kidney tissue.

The control group for female rats also reveals normal renal histological architecture, with healthy, well-defined glomeruli and organized tubular structures. This establishes the baseline morphology for female kidney tissue.

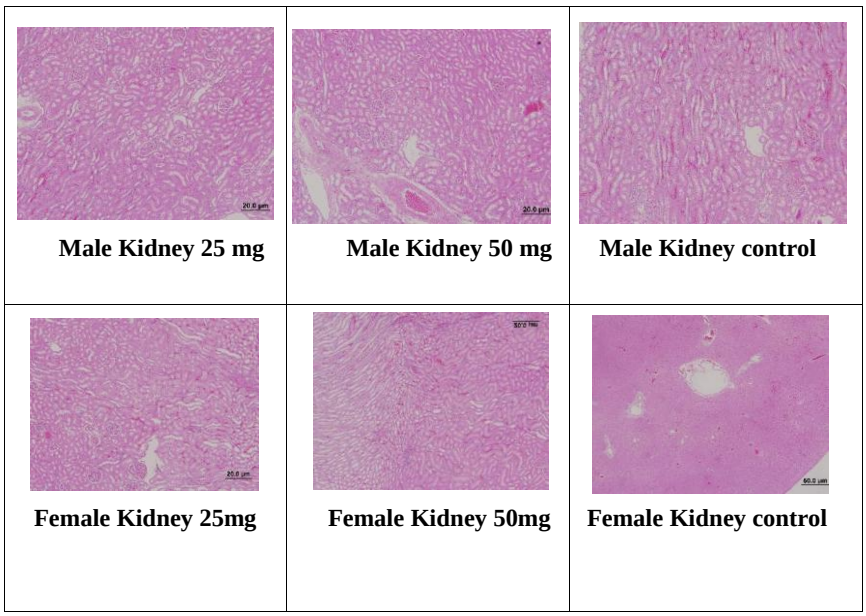


Figures 1. Effects of fenugreek extract on kidney weight in male and female rats.

In the 25mg treatment group, the kidney tissue maintains its structural integrity, with no observable deviations from the control group. The glomeruli and tubular structures appear normal, indicating that fenugreek extract at this dose does not induce discernible changes in female kidney morphology under the experimental conditions.

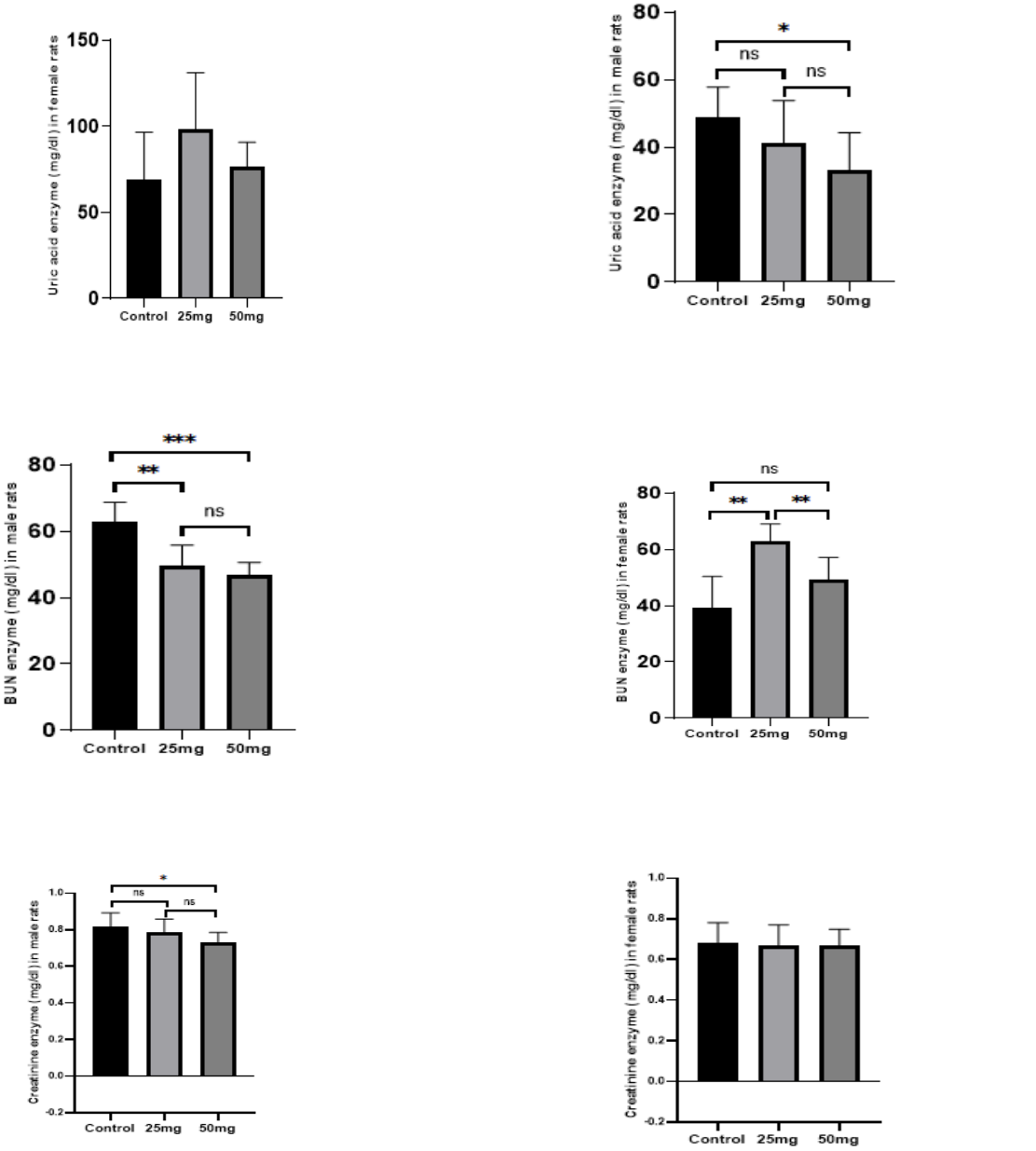
Similarly, the 50mg treatment group exhibits no notable differences in renal architecture compared to the control or 25mg groups. The kidney tissue retains its intact structure, with no evidence of damage or disruption, suggesting that fenugreek extract at 50 mg also does not significantly impact renal morphology in female rats.

The histological analysis reveals that fenugreek extract has a minimal impact on renal tissue in both male and female rats. In male rats, slight alterations were observed at the 25mg dose, but these changes were not exacerbated at the 50mg dose. In female rats, no significant morphological changes were noted at either dose Fig. 2.



Figures 2. Histological evaluation of kidney tissues in male and female rats following fenugreek extract administration.

Figure 3 demonstrates the impact of fenugreek extract on BUN levels in male rats. The control group exhibited significantly higher BUN levels compared to both the 25mg and 50mg treatment groups. Administration of 25mg of fenugreek extract resulted in a notable reduction in BUN levels, which was statistically significant ($p<0.01$). Furthermore, the 50mg dose led to an even greater reduction compared to the control group, with highly significant differences ($p<0.001$). Interestingly, no significant difference was observed between the 25mg and 50mg groups, suggesting a plateau effect where increasing the dose beyond 25mg does not provide additional benefits. These results suggest that fenugreek extract has a dose-dependent effect in reducing BUN levels up to 25mg, with the extract demonstrating therapeutic potential for improving kidney function in male rats.



Figures 3. Evaluation of the renal protective effects of fenugreek extract on bun, uric acid, and creatinine levels in male and female rats

Figure 3 also illustrates the effect of fenugreek extract on BUN levels in female rats. Unlike the male rats, the control group exhibited lower BUN levels than both treatment groups. Administration of 25mg of fenugreek extract led to a statistically significant increase in BUN levels compared to the control group ($p<0.01$), a trend that was also observed at the 50mg dose ($p < 0.01$). However, no significant difference was observed between the 25 mg and 50 mg groups, indicating a plateau effect. These findings highlight a stark contrast to the results observed in male rats, where BUN levels decreased with fenugreek extract administration. This sex-specific response to fenugreek extract underscores the need for further investigation into its underlying mechanisms.

The effects of fenugreek extract on uric acid levels in male rats are also depicted in Figure 3. The control group displayed higher uric acid levels compared to the treatment groups. Administration of 25mg of fenugreek extract led to a statistically significant reduction in uric acid levels ($p<0.05$). However, no significant differences were observed between the 25mg and 50mg groups, or between the 50mg group and the control. These findings suggest a mild, dose-limited impact of fenugreek extract on uric acid levels, with 25mg being sufficient to achieve the observed reduction.

For female rats, the results of fenugreek extract administration on uric acid levels, as shown in Figure 3, indicate no statistically significant differences among the control, 25mg, and 50 mg groups. While the 25mg dose exhibited a slight numerical increase in uric acid levels, and the 50 mg dose appeared to return closer to the baseline, these variations were not statistically significant and are likely due to natural variability. This contrasts with the mild reduction observed in male rats, suggesting potential sex-specific differences in the response to fenugreek extract.

Figure 3 highlights the impact of fenugreek extract on creatinine levels in male rats. The control group exhibited slightly higher creatinine levels compared to the treatment groups. A statistically significant reduction was observed between the control group and the 50mg treatment group ($p<0.05$). However, no significant differences were found between the control and 25mg group, or between the 25mg and 50mg groups. These results suggest a modest effect of fenugreek extract on reducing creatinine levels, with the effect becoming more apparent at the 50mg dose. However, the overall impact on kidney function, as measured by creatinine levels, appears limited.

Lastly, Figure 3 illustrates the effects of fenugreek extract on creatinine levels in female rats. The results show consistent creatinine levels across all groups, with no significant differences observed among the control, 25mg, and 50mg groups. This lack of variation suggests that fenugreek extract does not influence creatinine levels in female rats under the experimental conditions. These findings indicate that fenugreek extract may not significantly affect kidney function in female rats, as measured by creatinine levels.

The results of our study demonstrate that fenugreek extract has a dose- and sex-specific impact on kidney function markers in rats. In male rats, fenugreek extract appears to have a protective effect by reducing BUN and uric acid levels, and to a lesser extent, creatinine levels. Conversely, in female rats, fenugreek extract increases BUN levels and has no significant impact on uric acid or creatinine levels. These findings highlight potential sex-based differences in response to fenugreek extract, emphasizing the need for further research to explore these differences and the mechanisms driving them.

DISCUSSION

The study by Inegbedion *et al*. (2022) similarly demonstrates the kidney-protective effects of fenugreek seed aqueous extract, with improvements noted in BUN and creatinine levels in albino rats. our research aligns with these findings, particularly in showing reduced BUN and creatinine levels in male rats treated with fenugreek extract, highlighting its therapeutic potential. However, our study offers additional insights into sex-specific and dose-dependent responses, which were not explored in Inegbedion *et al*. (2022) work. For example, while male rats in our study exhibited significant reductions in BUN levels, female rats showed increased BUN, suggesting a contrasting response.

Moreover, our findings revealed effect at the 25 mg dose, where higher doses did not yield further benefits, whereas Inegbedion *et al*. (2022) reported consistent improvements with increasing doses. The inclusion of uric acid and kidney weight in our research also adds depth, highlighting fenugreek's minimal impact on these parameters in female rats while confirming its mild effect in males. These nuanced observations provide a broader understanding of fenugreek's renal effects, emphasizing the importance of considering sex and dose in its therapeutic application.

The study by Uslu *et al*. (2019) investigates the hepatoprotective and nephroprotective effects of fenugreek seed extract against sodium nitrite-induced toxicity in rats, emphasizing its antioxidant and protective properties. Similar to our research, Uslu *et al*. (2019) demonstrate the renoprotective effects of fenugreek, particularly through its ability to reduce Blood Urea Nitrogen (BUN) and creatinine levels, indicating improved kidney function.

However, while our study focuses on the direct effects of fenugreek extract in healthy rats, Uslu *et al*. (2019) examine its protective role under chemically induced toxicity. This difference in experimental context highlights a key distinction: our research reveals dose- and sex-specific responses to fenugreek in normal physiological conditions, with significant reductions in BUN levels in male rats and contrasting increases in females. Such sex-specific findings were not addressed in Uslu *et al*. (2019) study, as their analysis does not stratify by gender or examine the influence of dosage beyond its protective role.

Additionally, Uslu *et al*. (2019) emphasize fenugreek's antioxidant potential as a mechanism of protection, demonstrated through histopathological improvements in kidney tissues exposed to sodium nitrite. our results also highlight fenugreek's minimal impact on kidney morphology in healthy rats, with no significant structural changes observed in male or female kidneys across doses. However, the context of toxicity in Uslu *et al*. (2019) study provides a unique lens for evaluating fenugreek's efficacy under stress conditions, whereas our study elucidates its baseline effects on kidney function markers, such as uric acid and kidney weight.

In summary, Uslu *et al*. (2019) reinforce the nephroprotective potential of fenugreek, especially under toxicological stress, while our study adds depth by exploring its nuanced effects in healthy conditions, emphasizing the importance of dose and sex. Together, these findings underscore fenugreek's versatility as a therapeutic agent for renal health in both protective and baseline scenarios.

The study by Hadi *et al*. (2020) demonstrates fenugreek's potential to improve kidney function in patients with type 2 diabetes mellitus, evidenced by reductions in serum creatinine and improvements in BUN levels. These findings align with our research, which also shows fenugreek's ability to lower BUN and creatinine levels, particularly in male rats. However, while Hadi *et al*. (2020) focused on a clinical population with diabetes, our study provides novel insights into sex- and dose-specific effects in healthy rats. For example, our findings revealed contrasting responses, with male rats showing reduced BUN levels and female rats exhibiting increases. Additionally, our research identified a plateau effect at the 25 mg dose, a phenomenon not addressed in Hadi *et al*. (2020) study.

Hadi *et al*. (2020) attribute fenugreek's benefits to its anti-inflammatory and antioxidant properties, mechanisms that may also underlie the improvements observed in our study, albeit under different conditions. Together, these studies highlight fenugreek's therapeutic versatility, suggesting benefits for both diseased and healthy kidneys while emphasizing the importance of considering sex and dose in its application.

The study by Massoud *et al*. (2022) investigates the therapeutic effects of Trigonella foenum-graecum (fenugreek) seed extract on acute kidney injury induced by folic acid in rats. Their findings demonstrate that fenugreek extract mitigates kidney damage by improving renal function markers such as Blood Urea Nitrogen (BUN), creatinine, and oxidative stress parameters. This aligns with our study, which also highlights fenugreek's ability to modulate kidney function, albeit in healthy rats under normal conditions.

Massoud *et al*. (2022) emphasize fenugreek's role in reducing kidney damage through its antioxidant and anti-inflammatory properties. Similarly, our study suggests fenugreek's renoprotective potential, as evidenced by reductions in BUN and creatinine levels, particularly in male rats. However, while their work focuses on fenugreek's reparative effects in a pathological model, our research adds valuable insights into its baseline effects in healthy kidneys, including sex- and dose-specific responses.

Notably, our findings revealed a plateau effect at the 25mg dose, whereas Massoud *et al*. (2022) primarily explored therapeutic doses in a damaged renal context, making direct comparisons challenging. Furthermore, our study highlights sex-specific differences, with male rats benefiting more prominently from fenugreek extract than females, a factor not addressed in Massoud *et al*. (2022) research.

In conclusion, both studies reinforce fenugreek's nephroprotective and therapeutic potential. While Massoud *et al*. focus on its curative effects in kidney injury, our research expands understanding by exploring its dose-dependent and sex-specific effects in healthy conditions. Together, these studies provide a comprehensive perspective on fenugreek's applications for kidney health.

CONCLUSION

This study demonstrates the renoprotective effects of *Trigonella foenum-graecum* (fenugreek) aqueous extract, highlighting dose- and sex-specific responses in healthy rats. Male rats exhibited significant reductions in Blood Urea Nitrogen (BUN) and creatinine levels, with an optimal effect observed at 25mg. In contrast, female rats showed increased BUN levels, with creatinine and kidney morphology remaining unchanged. Histological analysis revealed preserved renal architecture across all doses in both sexes. These findings suggest fenugreek's potential in improving kidney function, particularly in males, while emphasizing the importance of sex-specific considerations. Further research is needed to explore the underlying mechanisms and clinical applications.

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