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Investigating the dose- and sex-specific impacts of fenugreek (trigonella foenum-graecum) extract on liver enzymes and histology in male and female rats

[*Investigação dos impactos específicos da dose e do sexo do extrato de feno-grego (trigonella foenum-graecum) nas enzimas hepáticas e na histologia em ratos machos e fêmeas*]

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ABSTRACT

The liver is a vital organ susceptible to damage from various stressors, necessitating exploration of protective agents. Fenugreek (*Trigonella foenum-graecum*), known for its bioactive compounds, has shown potential hepatoprotective effects. This study investigated the biochemical and histopathological impacts of aqueous fenugreek extract on liver function in male and female Swiss albino rats. Rats were divided into control, 25mg/kg, and 50mg/kg fenugreek extract groups and treated for 21 days. Liver enzyme levels (ALT, AST, ALP) were analyzed, and histopathological evaluations were performed. Histological analysis showed no structural abnormalities in liver tissues across all groups, indicating fenugreek does not induce morphological damage. Biochemical results revealed dose- and sex-specific effects. In male rats, ALP levels significantly increased at 50 mg, while no changes were observed at 25mg. Female rats exhibited a significant increase in ALT levels at 25mg, which diminished at 50 mg, and a marked increase in AST levels at 50mg, suggesting hepatic stress at higher doses. This study demonstrates that fenugreek extract, while safe structurally, may induce biochemical changes at higher doses, particularly in females. These findings emphasize the need for dose optimization and consideration of sex-based differences in therapeutic applications of fenugreek. Further research is recommended to explore underlying mechanisms.

Keywords: fenugreek, liver function, hepatoprotection, liver enzymes, sex-specific effects, dose optimization

RESUMO

O fígado é um órgão vital suscetível a danos causados por vários fatores de estresse, o que requer a investigação de agentes protetores. O feno-grego (Trigonella foenum-graecum), *conhecido por seus compostos bioativos, tem demonstrado potenciais efeitos hepatoprotetores. Este estudo investigou os impactos bioquímicos e histopatológicos do extrato aquoso de feno-grego na função hepática em ratos albinos suíços machos e fêmeas. Os ratos foram divididos em grupos controle, 25mg/kg e 50mg/kg de extrato de feno-grego e tratados por 21 dias. Os níveis de enzimas hepáticas (ALT, AST, ALP) foram analisados e avaliações histopatológicas foram realizadas. A análise histológica não mostrou anormalidades estruturais nos tecidos hepáticos em todos os grupos, indicando que o feno-grego não induz danos morfológicos. Os resultados bioquímicos revelaram efeitos específicos da dose e do sexo. Em ratos machos, os níveis de ALP aumentaram significativamente com 50mg, enquanto nenhuma alteração foi observada com 25mg. As ratas apresentaram um aumento significativo nos níveis de ALT com 25mg, que diminuiu com 50mg, e um aumento acentuado nos níveis de AST com 50mg, sugerindo estresse hepático em doses mais altas. Este estudo demonstra que o extrato de feno-grego, embora estruturalmente seguro, pode induzir alterações bioquímicas em doses mais elevadas, particularmente em fêmeas. Essas descobertas enfatizam a necessidade de otimização da dose e consideração das diferenças baseadas no sexo nas aplicações terapêuticas do feno-grego. Recomenda-se mais pesquisas para explorar os mecanismos subjacentes.*

Palavras-chave: feno-grego, função hepática, hepatoproteção, enzimas hepáticas, efeitos específicos por sexo, otimização da dose

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INTRODUCTION

The liver, a vital organ in metabolic regulation, detoxification, and synthesis of essential biomolecules, is frequently exposed to various endogenous and exogenous stressors (Alamri, 2018;). These stressors, including oxidative stress, toxins, and high-fat diets, can lead to hepatic dysfunction and contribute to chronic conditions such as non-alcoholic fatty liver disease (NAFLD), hepatitis, and cirrhosis. Given the rising prevalence of liver diseases worldwide, the exploration of preventive and therapeutic strategies remains a significant area of scientific investigation (Smirne *et al*., 2022; Mantena *et al*., 2008; Yang *et al*., 2019; Svegliati-Baroni *et al*., 2019; Sharma *et al*., 2022; Pierantonelli and Svegliati-Baroni, 2022).

Fenugreek (Trigonella foenum-graecum), an annual herb from the Fabaceae family, has garnered attention in recent years for its potential health benefits. Widely used in traditional medicine and as a dietary spice, fenugreek seeds are a rich source of bioactive compounds, including saponins, flavonoids, alkaloids, and dietary fiber (Khatoon *et al*., 2024; Kachhwaha *et al*., 2024; Ahmed, 2020; Ali, 2019). These phytochemicals have been associated with a variety of pharmacological properties, such as anti-inflammatory, antioxidant, hypoglycemic, and hypolipidemic effects (Pradeep and Srinivasan, 2017; El Bairi *et al*., 2017) Emerging evidence suggests that fenugreek may exert protective and therapeutic effects on liver health. Its bioactive components have demonstrated the ability to mitigate oxidative stress, modulate lipid metabolism, and reduce hepatic inflammation in preclinical models (Lam *et al*., 2016; Gupta*et al*., 2021; Sharma *et al*., 2023). These mechanisms are particularly relevant to liver conditions characterized by oxidative damage, dyslipidemia, and chronic inflammation. However, while preclinical findings are promising, there remains a gap in understanding the precise molecular pathways through which fenugreek influences liver physiology and pathology, as well as its potential efficacy and safety in clinical settings. This study aims to investigate the effects of fenugreek on liver health, focusing on its biochemical and histopathological impacts. By combining in vitro, in vivo, and clinical approaches, we seek to elucidate the therapeutic potential of fenugreek and contribute to the growing body of knowledge on its medicinal properties. The findings of this research may inform the development of fenugreek-based interventions for liver diseases, offering a natural and accessible solution to a global health challenge.

MATERIALS AND METHODS

The aqueous extract of fenugreek was created by grinding the plant into a fine powder, thoroughly mixing it, and then extracting it with water. The plants were acquired at well-known marketplaces, where a total of 250 grams was collected. After collection, the plant was pulverized into a fine powder and underwent extraction as detailed in our prior research (Alhimaidi *et al*., 2022). Two distinct concentrations of the resultant aqueous extract of fenugreek were formulated: 50mg and 25mg.

Male Swiss albino rats (250–320g) were procured from the animal facility of the Department of Zoology at King Saud University, College of Science. The animals were kept under regular circumstances during the trial period. The rats were provided with regular pellets. Water was supplied ad libitum. The rats were categorized into three groups, each including six males. The initial group, designated as a control, was administered water. The second group got 25mg of aqueous extract of fenugreek, whilst the third group was administered 50mg/kg of aqueous extract of fenugreek for a duration of 21 days.

Liver enzymes from rat blood samples were examined to assess the impact of aqueous fenugreek extract. Upon conclusion of the experiment, blood samples from both female and male rats were obtained in non-heparinized glass tubes for serum extraction. The serum was subsequently isolated using centrifugation at 3000 rpm for 15 minutes. The enzymatic activity of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were quantified utilizing diagnostic kits with the BioSystem equipment BTS-350.

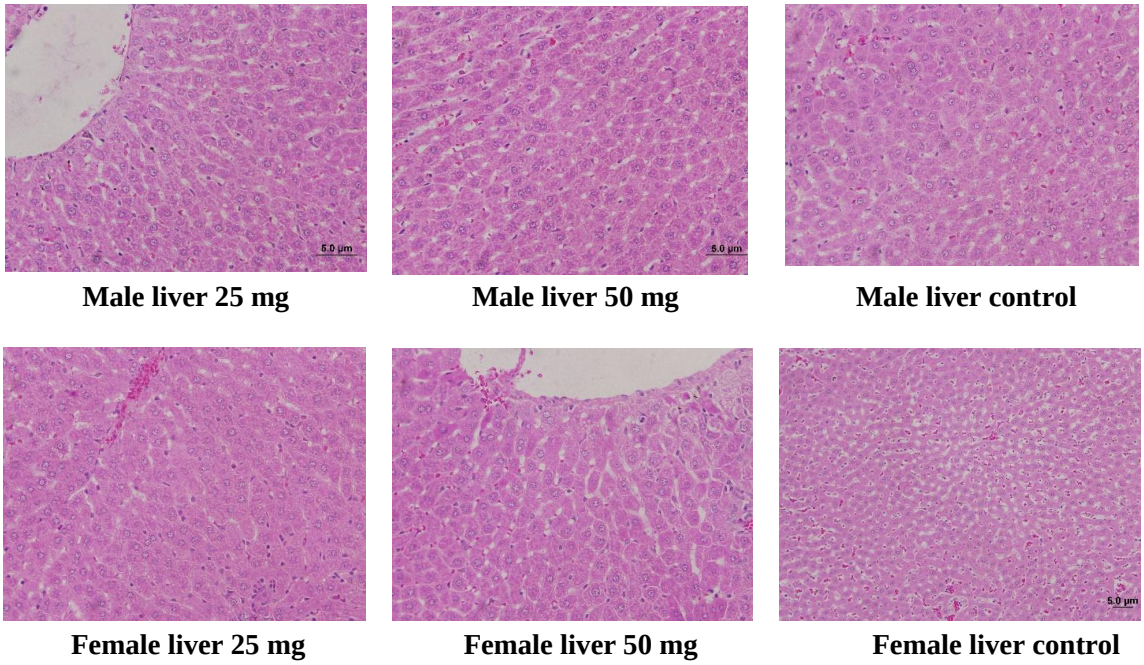
Liver excisions were promptly washed in physiological saline and subsequently fixed in 70% phosphate-buffered formalin. Following fixation, the samples were embedded in paraffin

and longitudinally sectioned into 5 µm thick slices. The sections were subsequently deparaffinized, dried, and stained with hematoxylin and eosin (Sigma). The liver microstructures were analyzed utilizing an Olympus BH2 microscope (DP71, Olympus, Tokyo, Japan).

The experimental data were analyzed utilizing GraphPad Prism software (version 10.1.1).

RESULTS

In figures 1 The histological examination of liver tissues treated with fenugreek extract at doses of 25 mg and 50 mg in both male and female samples reveals no significant alterations compared to the control groups. In male liver tissues, the overall architecture, including hepatic cords, sinusoids, and cellular integrity, appears preserved across both treatment doses, indicating no observable structural damage or histological abnormalities. Similarly, in female liver tissues, the morphology remains intact, with no apparent disruptions in the organization or structure of the liver cells and surrounding tissues at either dosage level. These findings suggest that fenugreek extract does not induce histological changes or damage in liver tissues at the doses tested, maintaining normal hepatic structure in both male and female samples.



Figures 1. Histological Assessment of Liver Tissues Following Fenugreek Extract Administration in Male and Female Samples.

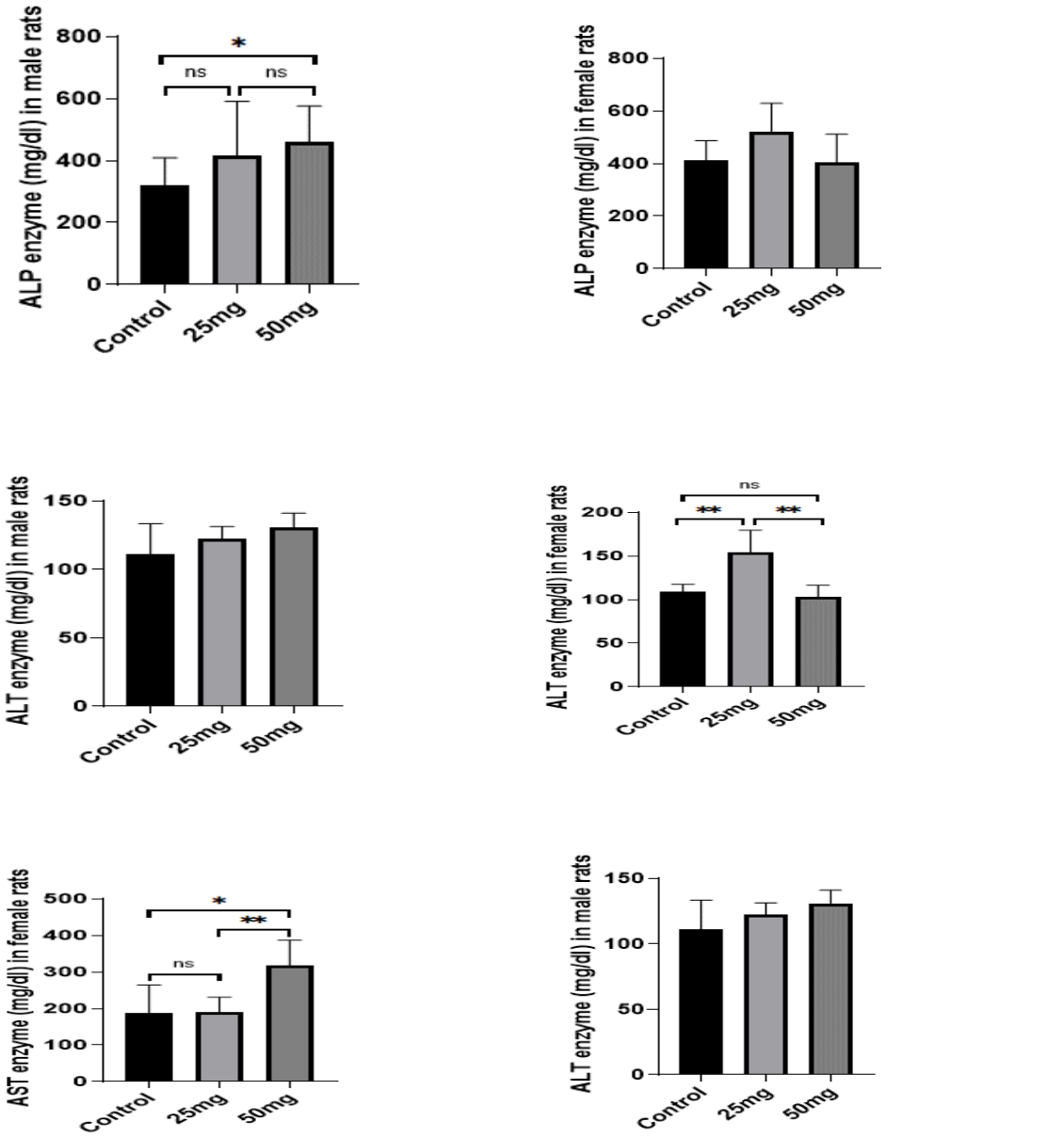
In figure 2 For ALP enzyme levels, male rats showed no statistically significant difference between the control and the 25mg treatment group. However, a statistically significant increase in ALP levels was observed in the 50mg treatment group compared to the control, suggesting that the higher dose affects liver function. In female rats, no significant differences in ALP levels were observed between the control and the treatment groups (25 mg or 50mg), indicating that fenugreek extract at these dosages does not affect liver enzyme activity.

Regarding ALT enzyme levels, male rats exhibited no statistically significant differences between the control group and either the 25mg or 50mg treatment groups, suggesting no effect of fenugreek extract on this enzyme. In contrast, female rats showed a statistically significant increase in ALT levels in the 25mg treatment group compared to the control group. However, no significant difference was observed between the 25mg and 50mg groups, with ALT levels in the 50mg group being similar to the control,

suggesting a dose-dependent effect that diminishes at the higher dose.

For AST enzyme levels in female rats, there was no significant difference between the control and 25 mg groups. However, a statistically significant increase in AST levels was observed in the 50 mg group compared to both the control and the 25mg groups, indicating a marked effect of the higher dose on liver enzyme activity.

Overall, the findings suggest that while fenugreek extract has minimal effects on most liver enzymes in male rats, it significantly affects certain enzymes, particularly ALT and AST, in female rats at specific doses, with the 50mg dose showing a more pronounced impact.



Figures.2. Impact of Fenugreek Extract Dosages on Liver Enzyme Levels (ALT, AST, and ALP) in male and female rats.

DISCUSSION

The study by Okorie *et al*. (2024) investigated the hepatic effects of prolonged oral administration of aqueous extracts of fenugreek seeds using pathological techniques. Their results provide an important point of comparison with our study, particularly regarding the effect of fenugreek extract on liver enzyme activity in male and female rats.

A key observation in Okorie *et al*. (2024) research was the dose-dependent effect of fenugreek extract on liver enzyme levels. They reported significant increases in liver enzymes, particularly ALT and AST, at higher doses of the extract. This is consistent with our findings, as a 50mg dose of fenugreek extract resulted in a significant increase in AST levels in female rats. However, our study also identified a unique pattern in ALT levels, with a 25mg dose causing a significant increase in female rats, but this effect was diminished at the higher dose of 50mg. This observation suggests a possible regulatory or adaptive mechanism that may differ from the linear dose-dependent relationship observed by Okorie *et al*., 2024.

Interestingly, Okorie *et al*. (2024) did not confirm significant changes in ALP levels, whereas my study observed a significant increase in ALP levels in male mice at the 50 mg dose. This discrepancy may be due to differences in experimental design, such as the duration of fenugreek extract administration, the method of extract preparation, or the specific pathology techniques used. It highlights the need for further research to determine the conditions under which ALP levels are affected by fenugreek extract.

The study conducted by Abdrabouh (2019) examined liver disorders induced by exposure to gasoline fumes in male rats and evaluated the potential protective role of fenugreek seed supplementation. This research provides a complementary perspective to our study, particularly in exploring the broader effects of fenugreek on liver function under stress conditions.

Abdrabouh reported that fenugreek seed supplementation significantly mitigated the adverse effects of gasoline fumes on liver function, as evidenced by reductions in ALT, AST, and ALP levels compared to rats exposed to gasoline fumes without fenugreek supplementation. These findings underscore fenugreek's hepatoprotective properties, particularly its ability to restore liver enzyme levels toward normalcy in a toxic environment.

In comparison to our study, the focus of Abdrabouh's work on protective rather than direct effects of fenugreek presents an interesting contrast. our results indicate that fenugreek extract can, at higher doses, induce significant changes in liver enzymes, such as the increase in AST levels in female rats at 50mg and the increase in ALP levels in male rats at 50mg. These findings suggest that while fenugreek may protect the liver under certain stress conditions, higher doses in otherwise healthy systems might exert hepatic stress, particularly in females. This dual role—protective under toxicity but potentially stress-inducing at high doses—aligns with the broader understanding of many plant-based compounds that exhibit dose-dependent effects.

The study by Baset *et al*. (2020) investigated the anti-diabetic effects of fenugreek (Trigonella foenum-graecum) in animal models, comparing the outcomes of oral and intraperitoneal administration. The findings offer valuable insights into the mode of administration's role in fenugreek's biological effects, particularly on metabolic and hepatic functions.

In their study, both oral and intraperitoneal administration of fenugreek significantly improved glycemic control and reduced liver enzyme levels (ALT, AST, and ALP) in diabetic rats. These effects suggest that fenugreek exhibits hepatoprotective properties in diabetic models, regardless of the route of administration. The study also highlighted that the intraperitoneal route had a more pronounced effect, possibly due to the bypassing of first-pass metabolism, leading to higher bioavailability of the active compounds.

When comparing these results to our study, several key differences emerge. My findings show that fenugreek extract at a high dose (50mg) induced significant increases in AST levels in female rats and ALP levels in male rats in otherwise healthy systems. These observations contrast with the hepatoprotective effects

reported by Baset *et al*. (2020) in diabetic rats, suggesting that fenugreek's impact on liver enzymes might depend on the physiological or pathological state of the organism.

Additionally, Baset *et al*. (2020) emphasized the role of administration routes in determining fenugreek's efficacy. Our study used oral administration exclusively, which may limit the comparison with the more pronounced effects seen in the intraperitoneal group in their study. The differences in outcomes highlight the importance of considering how fenugreek is delivered when assessing its biological effects.

In conclusion, the study by Baset *et al*. (2020) underscores fenugreek's potential as a hepatoprotective agent in diabetic conditions, with variations in efficacy based on the administration route. Our study, focusing on healthy systems, highlights the dose- and sex-specific effects of fenugreek, suggesting that its impact on liver function is context-dependent. Together, these findings provide a nuanced understanding of fenugreek's dual role as both a therapeutic agent in diseased states and a potential stressor at high doses in healthy systems.

The study by Sakr and Abo-El-Yazid (2012) explored the protective effects of fenugreek seed extract against adriamycin-induced hepatotoxicity and oxidative stress in albino rats. Their findings provide critical insights into fenugreek's antioxidant and hepatoprotective properties, particularly in mitigating chemically induced liver damage.

Sakr and Abo-El-Yazid (2012) reported that adriamycin, a chemotherapeutic agent, significantly elevated liver enzyme levels (ALT, AST, and ALP), indicating hepatotoxicity. Administration of fenugreek seed extract mitigated these increases, effectively normalizing the enzyme levels and reducing oxidative stress markers such as lipid peroxidation. They attributed these effects to fenugreek's antioxidant compounds, which scavenge free radicals and protect hepatic tissues from oxidative damage.

The hepatoprotective effects observed in Sakr and Abo-El-Yazid (2012) study contrast with the findings from our research, which showed that high doses (50mg) of fenugreek extract induced significant increases in AST levels in female rats and ALP levels in male rats. These results suggest that fenugreek's effects on liver function might depend on the physiological context. In the presence of hepatotoxic stress (e.g., adriamycin exposure), fenugreek's antioxidant properties play a restorative role, protecting and normalizing liver enzyme activity. In contrast, in otherwise healthy systems, as tested in our study, fenugreek at high doses may exert hepatic stress.

A notable similarity lies in the potential dose-dependent effects of fenugreek. Sakr and Abo-El-Yazid (2012) did not specify a threshold dose for hepatoprotection, but our findings align with the general principle that fenugreek's efficacy and safety depend on appropriate dosing. In our study, a 25mg dose had no significant adverse effects, while the higher 50mg dose induced detectable changes in liver enzyme levels, particularly in female rats.

The study by Sakr and Abo-El-Yazid (2012) emphasizes fenugreek's therapeutic potential in countering oxidative stress and liver damage caused by external agents like adriamycin. This aligns with findings in other studies (bdrabouh, 2019) that highlight fenugreek's hepatoprotective role under stress conditions. However, our study adds to the literature by demonstrating that fenugreek may have dose- and sex-specific effects in healthy systems, with high doses potentially inducing stress rather than protection.

Sakr and Abo-El-Yazid (2012) work highlights the importance of oxidative stress as a key factor in hepatotoxicity and showcases fenugreek's role as a protective agent. In contrast, our findings suggest caution when administering fenugreek in healthy systems, as higher doses might lead to unintended hepatic effects. Together, these studies suggest that fenugreek's dual role—as a hepatoprotective agent in stressed systems and a potential stressor at high doses in healthy individuals—depends on dosage, context, and underlying physiological conditions.

CONCLUSION

While fenugreek extract holds promise as a therapeutic agent, this study underscores the critical importance of dose optimization and consideration of sex-based physiological

differences. Further research is necessary to delineate the mechanisms underlying these effects and to establish safe and effective dosing guidelines for diverse populations.

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