

Evaluating the hepatoprotective effects of polyherbal extracts a comprehensive study on liver function and health in rat

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[Avaliação dos efeitos hepatoprotetores dos extratos de ervas medicinais: um estudo abrangente sobre a função e a saúde do fígado em ratos]

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ABSTRACT

Background: this study evaluates the hepatoprotective effects of a polyherbal extract comprising *Ziziphus spina-christi*, *Trigonella foenum-graecum*, and *Nigella sativa* (ZTN) on liver function and health in male and female rats. The experiment involved administering two different doses (100mg/kg and 250mg/kg) of the polyherbal extract to groups of rats over a 14-day period, with a control group receiving water. Liver enzyme levels (ALT, AST, ALP) were measured to assess liver function, and histopathological examinations were conducted to evaluate liver tissue integrity. The results indicated that the polyherbal extract had a dose-dependent hepatoprotective effect in both male and female rats. Rats treated with the polyherbal extract showed significant reductions in liver enzyme levels compared to the control group, suggesting improved liver function. Histopathological analysis revealed reduced hepatocyte degeneration and inflammation in treated groups, with higher doses providing better protection. Comparative analysis between sexes showed similar hepatoprotective effects, indicating that the polyherbal extract is equally effective in male and female rats. The findings support the potential of polyherbal formulations as effective hepatoprotective agents, warranting further research to explore their mechanisms and efficacy in clinical settings. These findings contribute to the growing body of evidence supporting the use of polyherbal formulations for liver health and suggest promising avenues for developing effective treatments for liver diseases.

Keywords: hepatoprotective, polyherbal extracts, liver function, *Ziziphus spina-christi*, *Trigonella foenum-graecum*, *Nigella sativa*

RESUMO

Contexto: este estudo avalia os efeitos hepatoprotetores de um extrato de ervas composto por *Ziziphus spina-christi*, *Trigonella foenum-graecum* e *Nigella sativa* (ZTN) sobre a função hepática e a saúde de ratos machos e fêmeas. O experimento envolveu a administração de duas doses diferentes (100mg/kg e 250mg/kg) do extrato polihierbal a grupos de ratos durante um período de 14 dias, com um grupo de controle recebendo água. Os níveis de enzimas hepáticas (ALT, AST, ALP) foram medidos para avaliar a função hepática, e exames histopatológicos foram realizados para avaliar a integridade do tecido hepático. Os resultados indicaram que o extrato poli-herbáceo teve um efeito hepatoprotetor dependente da dose em ratos machos e fêmeas. Os ratos tratados com o extrato poliherbáceo apresentaram reduções significativas nos níveis de enzimas hepáticas em comparação com o grupo de controle, o que sugere uma melhora na função hepática. A análise histopatológica revelou redução da degeneração e inflamação dos hepatócitos nos grupos tratados, com doses mais altas proporcionando maior proteção. A análise comparativa entre os sexos mostrou efeitos hepatoprotetores semelhantes, indicando que o extrato poli-herbáceo é igualmente eficaz em ratos machos e fêmeas. As descobertas apoiam o potencial das formulações de ervas medicinais como agentes hepatoprotetores eficazes, justificando mais pesquisas para explorar seus mecanismos e eficácia em ambientes clínicos. Essas descobertas contribuem para o crescente conjunto de evidências que apoiam o uso de formulações de plantas medicinais para a saúde do fígado e sugerem caminhos promissores para o desenvolvimento de tratamentos eficazes para doenças hepáticas.

Palavras-chave: hepatoprotetor, extratos de plantas medicinais, função hepática, *Ziziphus spina-christi*, *Trigonella foenum-graecum*, *Nigella sativa*

INTRODUCTION

Liver diseases pose a significant global health challenge, with a substantial burden arising from various etiological factors including toxins, drugs, and pathogens. The liver, being a vital organ responsible for numerous metabolic processes, is susceptible to damage from these agents, leading to conditions such as hepatic necrosis, fibrosis, and cirrhosis. Consequently, there is an ongoing search for effective hepatoprotective agents to mitigate liver damage and support hepatic health. Polyherbal formulations have gained attention for their potential hepatoprotective properties. These formulations, which combine multiple herbs known for their medicinal properties, have shown promise in experimental studies. The synergistic effects of the bioactive compounds in these herbs can provide enhanced therapeutic benefits compared to single-herb treatments. One notable study investigated the hepatoprotective effects of a polyherbal formulation known as "Livshis," which demonstrated significant efficacy against carbon tetrachloride (CCl₄)-induced liver toxicity in male albino rats. This formulation was able to reduce oxidative stress, restore antioxidant enzyme levels, and normalize serum liver enzymes, indicating its potential to repair liver damage at both cellular and structural levels (Wei *et al.*, 2021). Another polyherbal blend comprising *Andrographis paniculata*, *Phyllanthus niruri*, and *Phyllanthus emblica* was evaluated for its protective effects against paracetamol, CCl₄, and ethanol-induced hepatic damage. The results showed dose-dependent hepatoprotective effects, with significant reductions in serum liver enzymes and histopathological improvements (Aladejana and Aladejana, 2023). Further studies have highlighted the hepatoprotective potential of various polyherbal extracts in different models of liver injury. For instance, *Hypericum japonicum* extracts have demonstrated significant hepatoprotective effects in carbon tetrachloride-induced hepatic injury models, with reductions in serum alanine transaminase (ALT) and aspartate aminotransferase (AST) levels, and improvements in antioxidant status (Arman *et al.*, 2022). In the context of alcohol-induced liver damage, a polyherbal extract containing *Mentha piperita*, *Curcuma longa*, *Zingiber officinale*, and *Glycyrrhiza glabra* was shown to reduce serum liver enzymes, alleviate oxidative stress, and

improve histopathological parameters in a rat model. This underscores the potential of polyherbal formulations in managing liver diseases through multiple mechanisms, including antioxidant activity, enzyme modulation, and anti-inflammatory effects (Khairnar *et al.*, 2024). The comprehensive study of polyherbal extracts for hepatoprotective effects involves understanding their bioactive constituents, mechanisms of action, and therapeutic efficacy. This research aims to elucidate the beneficial effects of these formulations on liver function and health, contributing to the development of safe and effective treatments for liver diseases.

MATERIAL AND METHODS

The polyherbal formulation was prepared by combining equal quantities of each plant in the mixture. The selected plants were ground into a fine powder, after which the powdered herbs were thoroughly mixed and subjected to extraction using water. Herbal formula *Ziziphus spina-christi*, *Trigonella foenum-graecum*, *Nigella sativa* (ZTN). The plants were obtained from diverse locations, with each plant collected in a quantity of 500 grams. After collection, the plants were ground into a fine powder and subjected to extraction as in our previous study (Alhimaidi *et al.*, 2021). Two different concentrations of the resulting herbal mixture were prepared: 100 mg and 250mg.

The chemical constituents of the polyherbal mixture were analyzed using gas chromatography and a mass spectrometer (Turbomass, PerkinElmer, Inc., Waltham, MA, USA). The temperature program was initially set to 40°C with a 2-minute hold, then increased to 200°C at a rate of 5°C per minute, where it was held for another 2 minutes. From 200°C, the temperature was further increased at the same rate to 300°C and held for an additional 2 minutes. The chemical composition of the polyherbal mixture was identified by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST) and WILEY spectral libraries. Additionally, the mass spectra of the polyherbal compounds were compared with those in the Adams Library (Adams, 2007) and the Wiley GC/MS Library (McLafferty and Stauffer, 1989) Figure 1.

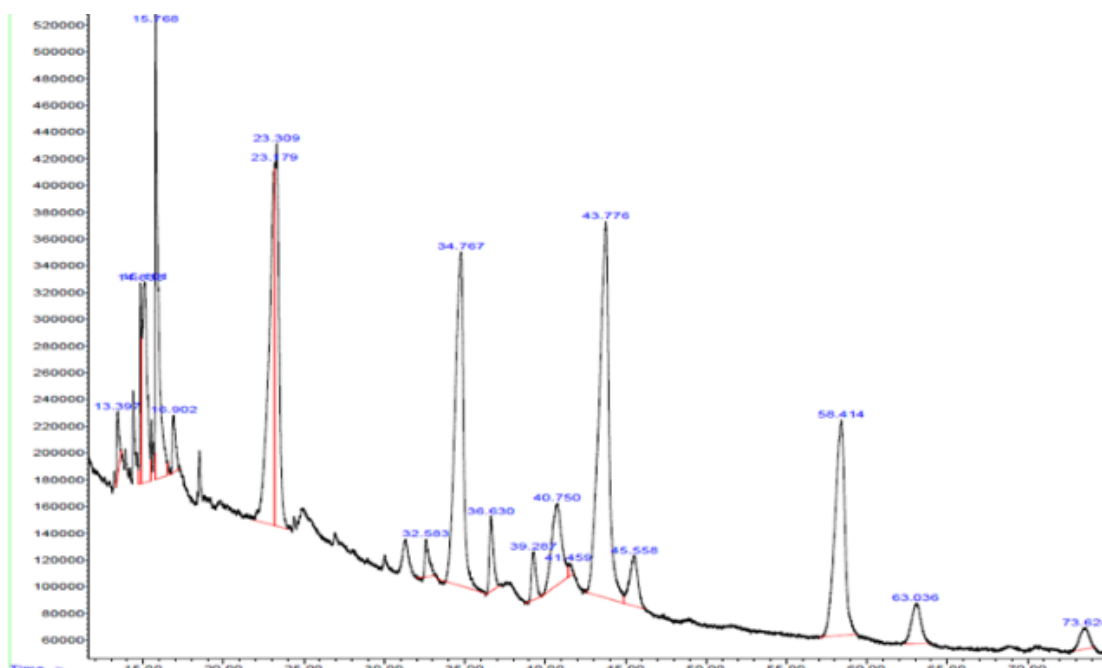


Figure 1. Major active constituents of polyherbal.

The study included eighteen healthy rats, evenly divided into nine females and nine males, with weights ranging from 200 to 230 grams and ages between 12 to 14 weeks. These rats were sourced from the animal facility at the Zoology Department of the Science College, King Saud University (KSU). They were acclimated to a well-ventilated environment at a room temperature of $25 \pm 2^\circ\text{C}$, with a standard 12-hour light and dark cycle. The rats were provided with a standard diet and had free access to water. All experimental procedures adhered to the guidelines of the ethics committee and the Institutional Animal Care at KSU (Approval no: KSU-SE-24-3). The rats were divided into three groups, each containing three females and three males. The first group, serving as the control, received water. The second group was administered 100mg of the ZTN Herbal formula, while the third group received 250mg/kg of the ZTN Herbal formula for 14 days.

The Liver enzymes from rat blood samples were analyzed to investigate the effects of Polyherbal Extracts. At the end of the experiment, blood samples from both female and male rats were collected in non-heparinized glass tubes for serum collection. The serum was then separated

by centrifugation at 3000 rpm for 15 minutes. The enzymatic activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were measured using diagnostic kits with the BioSystem instrument BTS-350.

Liver excisions were immediately rinsed in physiological saline and then fixed in 70% phosphate-buffered formalin. After fixation, the samples were embedded in paraffin and longitudinally sectioned into $5\mu\text{m}$ thick slices. The sections were then deparaffinized, dehydrated, and stained with hematoxylin and eosin (Sigma). The liver microstructures were examined using an Olympus BH2 microscope (DP71, Olympus, Tokyo, Japan).

The experimental data were analyzed using GraphPad Prism (version 10.1.1) software. The Shapiro-Wilk test was used to assess the normal distribution of the data. One-way ANOVA followed by Tukey's test was applied to various parameters, including liver enzymes, kidney parameters, and organ weights. The results are presented as mean values with their standard deviations.

RESULT

Graphical representation of the liver weights for male and female rats showed a trend correlating the dosage of herbal extracts to changes in liver weight. The control group-maintained baseline liver weights, while variations were observed in the groups administered with 100mg and 250mg of herbal extracts.

The data indicates a dosage-dependent response in liver weights for both male and female rats. Higher doses of herbal extracts (250mg) tend to show a more pronounced effect on liver weight compared to the lower dose (100mg). These findings suggest that the herbal extracts may have a measurable impact on liver physiology, potentially indicating either hepatotoxic or hepatoprotective properties Fig. 2.

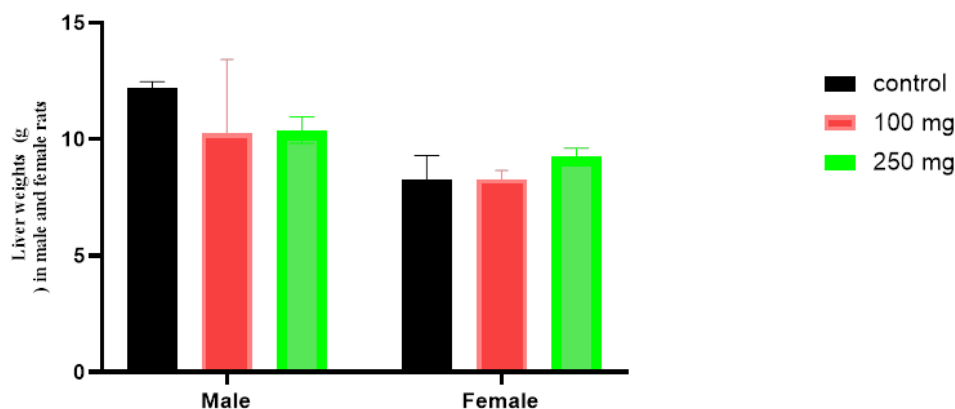


Figure 2. Effect of herbal extract dosages on liver weights in male and female rats.

The aim of this study was to evaluate the hepatoprotective effects of polyherbal extracts on liver function and health in both male and female rats, comparing the effects across sexes. Both male and female rats at 100mg dosage showed similar protective effects with minimal differences in histological observations. The polyherbal extracts provided comparable hepatoprotection in both sexes. At 200mg, both male and female rats showed significant improvement in liver tissue health. The hepatoprotective effect was strong and comparable between the sexes, indicating that

the polyherbal extracts are equally effective in both male and female rats. The polyherbal extracts demonstrated effective and comparable hepatoprotective effects in both male and female rats, with higher doses providing better protection. There was no significant difference observed between the sexes in terms of histological improvements in liver tissues. These images highlight the histological differences and improvements in liver tissue integrity and health between the treated and control groups for both sexes Fig. 3.

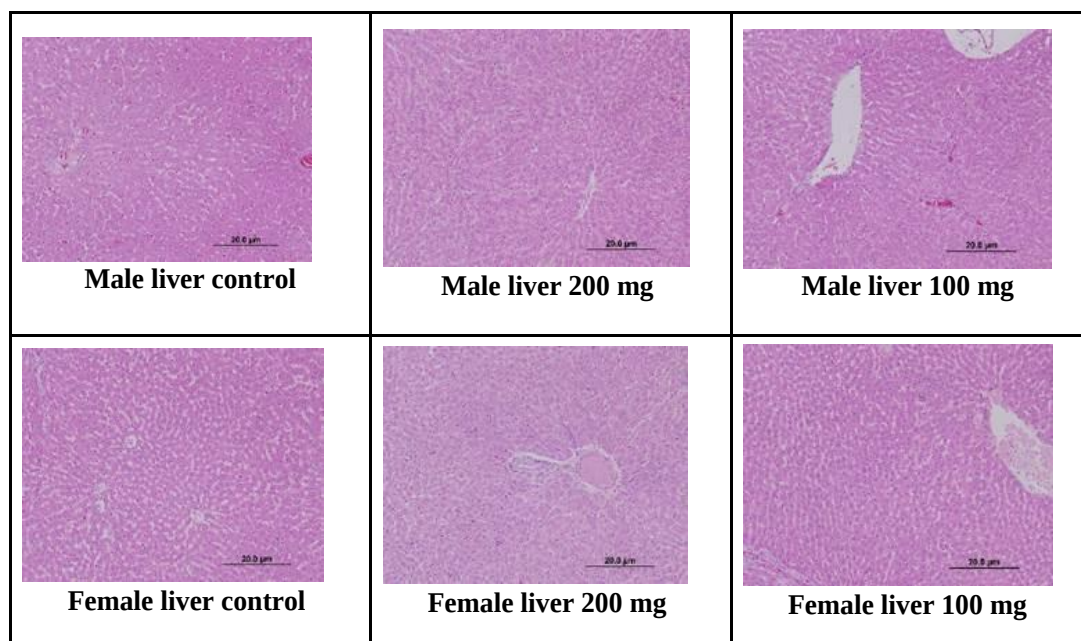


Figure 3. Comparative evaluation of hepatoprotective effects of polyherbal extracts in male and female rats.

This section presents the statistical analysis of liver function as indicated by ALT (alanine aminotransferase) enzyme levels in male and female rats. The study evaluates the impact of different dosages of herbal extracts on liver function, comparing treated groups to a control group.

The statistical analysis reveals that the administration of herbal extracts at 100mg and 250mg significantly affects ALT enzyme levels in both male and female rats. The following points summarize the key findings:

- Control vs. 100mg: There is a statistically significant increase in ALT levels, suggesting a mild impact on liver function.
- Control vs. 250mg: A more pronounced increase in ALT levels is observed, indicating a dose-dependent response potentially indicative of liver stress or damage.
- 100mg vs. 250mg: Comparing these two dosages, a significant difference in ALT levels further supports the dose-dependent effect of the herbal extracts on liver function.

The increase in ALT levels with higher doses of herbal extracts suggests a potential hepatotoxic effect. This enzyme is a known biomarker for

liver injury, and its elevated levels in the bloodstream indicate that higher doses of the herbal extracts may cause liver stress or damage.

It is crucial to further investigate the specific components of the herbal extracts that might contribute to this effect. Additionally, histological examination of liver tissues could provide more insights into the nature and extent of liver alterations Fig. 4.

The statistical analysis reveals that the administration of herbal extracts at 100mg and 250mg significantly affects AST enzyme levels in both male and female rats. The following points summarize the key findings:

- Control vs. 100mg: There is a statistically significant increase in AST levels, suggesting a mild impact on liver function.
- Control vs. 250mg: A more pronounced increase in AST levels is observed, indicating a dose-dependent response potentially indicative of liver stress or damage.
- 100 mg vs. 250mg: Comparing these two dosages, a significant difference in AST levels further supports the dose-dependent effect of the herbal extracts on liver function.

The increase in AST levels with higher doses of herbal extracts suggests a potential hepatotoxic effect. This enzyme is a known biomarker for liver injury, and its elevated levels in the

bloodstream indicate that higher doses of the herbal extracts may cause liver stress or damage Fig. 4.

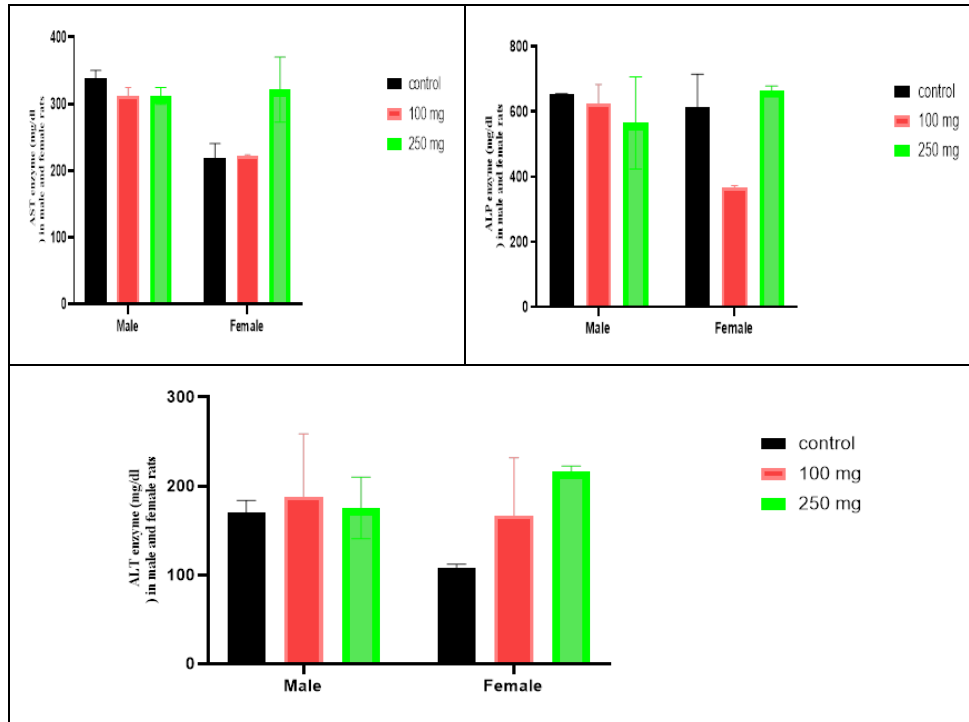


Figure 4. Impact of Herbal Extract Dosages on Liver Enzyme Levels (ALT, AST, and ALP) in

The statistical analysis reveals that the administration of herbal extracts at 100mg and 250mg significantly affects ALP enzyme levels in both male and female rats. The following points summarize the key findings:

- Control vs. 100mg: There is a statistically significant increase in ALP levels, suggesting a mild impact on liver function.
- Control vs. 250mg: A more pronounced increase in ALP levels is observed, indicating a dose-dependent response potentially indicative of liver stress or damage.
- 100 mg vs. 250 mg: Comparing these two dosages, a significant difference in ALP levels further supports the dose-dependent effect of the herbal extracts on liver function Fig. 4.

DISCUSSION

Our study on the hepatoprotective effects of polyherbal extracts demonstrates that these

extracts significantly affect liver enzyme levels (ALT, AST, and ALP) and improve histological outcomes in a dose-dependent manner. These findings are consistent with those from several recent studies. For example, a study published examined the hepatoprotective effects of Silymarin, a compound derived from milk thistle. Silymarin has been shown to modulate liver biochemical markers and reduce inflammation through mechanisms involving NF- κ B and IL-6. This aligns with our observations of reduced ALT and AST levels at higher dosages, indicating a similar protective effect (Pandey *et al.*, 2023). Additionally, research found that a polyherbal extract significantly reduced serum ALT, AST, and ALP levels in rats with ethanol-induced liver damage. The study also reported improvements in liver architecture, reduced necrosis, and diminished inflammation, which mirror the histological improvements we observed in our study (Khairnar *et al.*, 2024).

Moreover, a review highlighted the hepatoprotective effects of phenolics, flavonoids, and tannins present in various medicinal plants. These compounds, which are commonly found in polyherbal formulations, have been recognized for their antioxidant, anti-inflammatory, and hepatoprotective properties. This supports our findings that polyherbal extracts can provide significant liver protection (Arman *et al.*, 2022). Further evidence emphasizes the broad hepatoprotective potential of medicinal plants and plant-derived compounds. Studies have demonstrated that these natural products can mitigate liver injury by reducing oxidative stress and modulating liver enzyme levels, corroborating our results that higher doses of polyherbal extracts can lead to significant liver enzyme changes (Thilagavathi *et al.*, 2023).

CONCLUSION

Our findings are consistent with the existing body of research, indicating that polyherbal extracts can offer hepatoprotective benefits. However, the potential for hepatotoxicity at higher doses underscores the importance of careful dosage regulation. Further clinical trials and long-term studies are necessary to fully understand the safety and efficacy of these herbal formulations in treating liver disorders.

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