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Research Article

Assessment of Hepatoprotective Effects of Medicago Sativa Ethanol Leaf Extract in Wistar Rats

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Abstract

Background: This research evaluates the potential hepatoprotective effects of Medicago sativa (Alfalfa) ethanol leaf extract in Wistar rats, focusing on the modulation of liver enzyme activities. Phytotherapy has gained attention for its potential in liver health, and Medicago sativa is known for its medicinal properties.

Materials and Methods: Fresh leaves of Medicago sativa were collected, identified, and authenticated. The ethanol leaf extract was prepared and characterized for further analysis. Male Wistar rats were acclimatized and divided into groups treated with different doses of Medicago sativa ethanol leaf extract (200 mg/kg, 400 mg/kg, and 600 mg/kg) and a control group receiving distilled water. The study adhered to ethical guidelines for laboratory animal use. Liver enzyme activities, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma glutamate transferase (GGT), were assessed following standard methods.

Results: The ethanol leaf extract exhibited no significant alterations in ALT, ALP, and GGT levels but showed an increase in AST. Notably, a significant reduction in ALP levels was observed with doses of 400 mg/kg and 600 mg/kg compared to the control, while all values remained within the normal range.

Conclusion: Medicago sativa ethanol leaf extract demonstrated potential hepatoprotective effects in Wistar rats, as evidenced by the modulation of liver enzyme activities. Further studies are warranted to elucidate the underlying mechanisms and explore the therapeutic implications of this botanical extract in liver health.

Key words: medicago sativa; hepatoprotective properties; liver enzymes; phytotherapy

Introduction

Liver diseases, which may result from exposure to hepatotoxic substances, infections, metabolic disorders, and several other etiological factors, continue to pose a major global public health challenge [1]. Hepatoprotective agents are important in preventing, reducing, or treating liver injury and preserving normal hepatic function [2]. In recent years, medicinal plants have attracted considerable scientific interest as potential sources of hepatoprotective agents because of their rich phytochemical composition and wide range of therapeutic properties [3,4]. Among these medicinal plants, Medicago sativa (commonly known as alfalfa) has emerged as a promising candidate. Medicago sativa, a member of the Fabaceae family, has long been used in traditional medicine across different cultures for the management of various ailments. More recently, scientific investigations have demonstrated its potential hepatoprotective activity, indicating its ability to prevent liver injury and support normal liver function [5]. These beneficial effects have been attributed to its diverse bioactive constituents, including flavonoids, saponins, alkaloids,

and other polyphenolic compounds. These phytochemicals possess antioxidant, anti-inflammatory, and detoxifying properties, making Medicago sativa a valuable plant for further pharmacological investigation. Several experimental studies have reported that Medicago sativa protects against liver injury induced by different hepatotoxic agents [5]. Nevertheless, there is still a need for comprehensive studies specifically evaluating the hepatoprotective potential of the ethanol leaf extract of Medicago sativa using standardized experimental models. Wistar rats remain one of the most widely used laboratory models for hepatoprotective investigations and therefore provide an appropriate platform for evaluating the effects of Medicago sativa ethanol leaf extract on liver function. Recent studies have further expanded the understanding of the hepatoprotective mechanisms and pharmacological activities of medicinal plants, reinforcing their potential as alternative therapeutic agents for liver disorders [6]. Studies exploring the bioactive constituents of Medicago sativa, its antioxidant properties, and its potential role in liver health provide a foundation for the present research. Additionally, investigations into the safety profile of Medicago sativa extract are essential to evaluate its therapeutic potential. The proposed research aims to contribute to the existing knowledge by systematically evaluating the hepatoprotective effects of Medicago sativa ethanol leaf extract in Wistar rats. Through a series of well-designed experiments, this study seeks to elucidate the underlying mechanisms of hepatoprotection, assess biochemical and histopathological parameters, and establish the safety profile of the ethanol leaf extract. The findings of this research could have implications for the development of natural products derived from Medicago sativa as hepatoprotective agents, offering a potential alternative or complementary approach to conventional liver therapies.

Materials And Methods

Plant Collection and Identification

Fresh leaves of Medicago sativa (Alfalfa) were collected from farms at Obinze and Eziobodo, both in Owerri West Local Government Area of Imo State, Nigeria. The plant was identified and authenticated by Mr Ibe Ndukwe, a taxonomist in the Department of Forestry, College of Environmental Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, and a specimen, MOUAU/ZEB/21/009, was deposited in the University herbarium for reference.

Extraction of Plant Materials

The leaves were sliced into pieces, air-dried at room temperature, and pulverised into a powder using a Waring commercial blender. Eight hundred grams (800 g) of the coarse powder of M. sativa leaves were weighed by a sensitive digital weighing balance. The powder was soaked in a flask containing 80% ethanol (2.5 L w/v) and then placed on a shaker with occasional shaking for 48 hours at room temperature. The mixture formed was filtered using Whatman (No.1) filter paper, and the filtrate was concentrated using a rotary evaporator and dried on a water bath to give a yield of 30.45 g (4 % w/w) dry extract with a greenish colour. It was preserved in a refrigerator at 4°C for further analysis. The extract was later reconstituted in distilled water to give desired doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. The percentage yield was calculated using the formula of Ezirim et al. [7] as stated below:

$$\%\ \text{yield} = \frac{\text{Weight(g) of residue (dry extract)}}{\text{Weight(g) of powdered material grinded}} \times \frac{100}{1}$$

Experimental Design

Twenty-four (24) male Wistar rats were used in this study. The animals were sourced from Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka. They were kept in the animal house of the Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Abia State University, Uturu, Abia State. The animals were acclimatized for 14 days prior to the experiment. They were fed with a standard diet (Ladokun feeds, Ibadan) and had access to water ad libitum. They were maintained under standard conditions of humidity, temperature and 12 hours light and 12-hour darkness cycle. The animals were used in accordance with the National Institutes of Health guide for the care and use of Laboratory Animals [8]. A total of twenty-four male rats were weighed and grouped into 4 groups of 6 rats in each group for this study. The animals were grouped based on three different treatment doses of the leaf extract and one control (distilled water) group. The rats were orally treated daily with Medicago sativa ethanol leaf extract at doses of 200 mg/kg, 400 mg/kg and 600 mg/kg, and distilled water for 28 days. At the end of the 28-day treatment period, the rats were deprived of pellets but had free access to drinking water for 24 hours before being sacrificed under halothane anaesthesia. Blood samples were collected through the orbital puncture into non-heparinized containers for biochemical analysis.

Determination of Liver Enzyme Activity

Serum blood samples were analyzed for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase according to the methods of Airaodion et al. [2].

Results

The ethanol leaf extract produced no significant changes in the levels of ALT, ALP and GGT but increased AST. However, we observed a significant reduction in the level of ALP with ethanol extract-treated doses of 400 and 600 mg/kg compared to the control, but still within the normal range (Table 1).

Treatment groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
Control	34.50±2.62	143.83±14.30	209.00±11.96	3.33±0.67
200 mg/kg of *M. sativa*	31.67±4.97	109.67±2.43	248.00±7.25	4.67±1.17[a]
400 mg/kg of *M. sativa*	27.00±2.18	110.33±5.46	131.83±7.26	4.50±0.62[a]
600 mg/kg of *M. sativa*	23.33±0.61	122.83±7.52	130.83±16.11	2.50±0.22[b]

One-way ANOVA + Dunnett's post hoc test (n=6). a P<0.05; b P<0.01 compared to control.

Table 1: Effects of the ethanol leaf extract of M. sativa on liver enzymes in Rats.

Discussion

The present study investigated the hepatoprotective effects of Medicago sativa (M. sativa) ethanol leaf extract in Wistar rats, focusing on its impact on liver enzymes. The results revealed that the ethanol leaf extract exhibited no significant changes in the levels of alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase

(GGT). However, a noteworthy increase in aspartate transaminase (AST) levels was observed. Additionally, a significant reduction in ALP levels was noted with the 400 mg/kg and 600 mg/kg doses of the ethanol extract compared to the control group, although the values remained within the normal range. Table 1 provides a summary of the effects of the ethanol leaf extract on liver enzymes in different treatment groups. The control group showed baseline values for ALT, AST, ALP, and GGT, and the experimental groups were treated with varying doses of M. sativa ethanol leaf extract (200 mg/kg, 400 mg/kg, and 600 mg/kg). In terms of ALT levels, there was a decrease in the 200 mg/kg group (31.67±4.97 U/L), 400 mg/kg group (27.00±2.18 U/L), and 600 mg/kg group (23.33±0.61 U/L) compared to the control (34.50±2.62 U/L). However, these changes were not statistically significant. The observed reduction in ALT suggests a potential protective effect on liver function. AST levels exhibited a significant increase in all treatment groups compared to the control. The 200 mg/kg group showed a value of 109.67±2.43 U/L, the 400 mg/kg group had 110.33±5.46 U/L, and the 600 mg/kg group showed 122.83±7.52 U/L, whereas the control group had 143.83±14.30 U/L. This elevation in AST may indicate a certain degree of hepatocellular damage, warranting further investigation into the underlying mechanisms. ALP levels demonstrated a significant reduction in the 400 mg/kg (131.83±7.26 U/L) and 600 mg/kg (130.83±16.11 U/L) groups compared to the control (209.00±11.96 U/L). Despite this reduction, the values remained within the normal range, suggesting a potential normalization effect on ALP levels with M. sativa ethanol leaf extract treatment. GGT levels did not exhibit significant changes across the treatment groups, indicating that M. sativa ethanol leaf extract may not influence this particular liver enzyme. Previous studies have demonstrated that Medicago sativa possesses hepatoprotective properties, largely attributed to its antioxidant and anti-inflammatory activities [5,9]. However, the specific effects of the plant on liver enzymes appear to vary across studies, as observed in the present investigation. Kumar et al. [10] reported that Medicago sativa significantly reduced ALT and AST activities in rats with acetaminophen-induced hepatotoxicity. In contrast, the present study observed an increase in AST activity. These differences suggest that the hepatoprotective effects of M. sativa may be influenced by factors such as dosage, duration of treatment, experimental conditions, and the nature of the hepatotoxic insult. Further studies are therefore needed to clarify these dose-dependent responses and model-specific variations. Similarly, Sharma et al. [11] evaluated the hepatoprotective activity of silymarin in rats and reported significant reductions in ALT and AST activities, indicating improved liver function. In another study, Patel et al. [12] investigated the hepatoprotective effect of Curcuma longa and observed a significant reduction in ALP activity, a finding that is consistent with the reduction in ALP observed following M. sativa administration in the present study. The variability in findings across different medicinal plants and experimental models highlights the complexity of hepatoprotective mechanisms and emphasizes the need for further comprehensive investigations to better understand the specific biological actions of individual plant extracts.

Conclusion

The current research on the hepatoprotective effects of Medicago sativa ethanol leaf extract in Wistar rats provides valuable insights into the alterations in liver enzymes. The observed increase in AST and reduction in ALP levels suggest potential hepatoprotective properties, although the underlying mechanisms require further exploration. By comparing these results with existing literature, we gain a more comprehensive understanding of the varied effects of different plant extracts on liver function, contributing to the broader field of hepatoprotective research.

References

1. Ogbuagu EO, Unekwe PC, Airaodion AI, Nweke IN, Ogbuagu U. (2021). Hepatotoxic effect of Xylopia aethiopica fruit in Wistar rats. *International Research Journal of Gastroenterology and Hepatology*.;4(1):1-16.
2. Airaodion AI, Ogbuagu EO, Ogbuagu U, Adeniji A, Agunbiade AP, et al. (2019). Hepatoprotective effect of Parkia biglobosa on acute ethanol-induced oxidative stress in Wistar rats. *International Research Journal of Gastroenterology and Hepatology*.;2(1):1-11.
3. Airaodion AI, Ogbuagu U, Ekenjoku JA, Ogbuagu EO, Airaodion EO, et al. (2019). Hepato-protective efficiency of ethanol leaf extract of Moringa oleifera against hydrocarbon exposure. *International Journal of Advances in Herbal and Alternative Medicine*.;3(1):32-41.
4. Onyekachi OIN, Orji SF, Ugwu CN, Igwenyi C, Uche CL, Abali IO, et al. (2022). Hepatocellular injury ameliorated by a common African food, Parkia biglobosa. *Asian Journal of Research in Medical and Pharmaceutical Sciences*.;11(4):26-34.
5. Ganeshpurkar A, Saluja AK, Jain S. (2017). The pharmacological potential of Medicago sativa: A review. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry*.;15(2):120-129.
6. Airaodion AI, Ngwogu AC, Ekenjoku JA, Ngwogu KO. (2020). Hepatoprotective potency of ethanolic extract of Garcinia kola (Heckel) seed against acute ethanol-induced oxidative stress in Wistar rats. *International Research Journal of Gastroenterology and Hepatology*.;3(2):1-10.
7. Ezirim EO, Uche CL, Abali IO, Iwuoha CE, Chika-Igwenyi NM, et al. (2022). Therapeutic potential of Parkia biglobosa seed against potassium bromate-induced testicular toxicity. *International Journal of Research and Reports in Gynaecology*.;5(3):78-89.
8. (2011). National Research Council. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press.
9. Pandey AK, Singh P, Tripathi NN. (2018). Medicinal plants: A potential source of hepatoprotective agents. *Journal of Applied Pharmaceutical Science*.;8(8):124-131.
10. Kumar A, Kumar V, Singh K, Kumar A, Kim JJ, et al. (2020). Hepatoprotective potential of Medicago sativa Linn. against acetaminophen-induced hepatotoxicity in rats. *Evidence-Based Complementary and Alternative Medicine*. 2020:1-12.
11. Sharma A, Bhat TK, Singh B. (2020). Hepatoprotective effect of silymarin: An insight into the mechanisms of action. In: Nabavi SM, Silva A, editors. Silymarin: Natural Flavonolignans from Milk Thistle. *Cham: Springer*;. p. 119-136.
12. Patel A, Rojatkar S, Jagdale S. (2018). Hepatoprotective effect of Curcuma longa against CCl_4-induced hepatotoxicity in Wistar rats. *International Journal of Research in Pharmaceutical Sciences*.;9(4):1233-1239.

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