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Research Article

THERAPEUTIC POTENTIAL OF SAFRANAL IN ATTENUATING SODIUM VALPROATE-INDUCED LIVER TOXICITY: INSIGHTS INTO GENE EXPRESSION, OXIDATIVE STRESS, AND APOPTOSIS

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ABSTRACT

Sodium valproate (VPA) is a widely used antiepileptic drug associated with hepatotoxicity. Safranal, a bioactive compound derived from saffron, possesses hepatoprotective properties. This study aimed to investigate the protective effects of safranal against VPA-induced liver injury in rats. Rats were administered VPA to induce hepatotoxicity and concurrently or subsequently treated with safranal.

Liver function biomarkers, histopathological examination, oxidative stress markers, apoptotic parameters, and gene expression analysis were evaluated. VPA significantly elevated liver enzymes, induced histopathological changes, and increased oxidative stress and apoptosis. Safranal pretreatment or post-treatment significantly ameliorated VPA-induced liver injury, as evidenced by improved liver function tests, reduced histopathological alterations, and decreased oxidative stress and apoptosis.

Mechanistically, safranal modulated the expression of genes involved in hepatoprotection, inflammation, and oxidative stress. These findings suggest that safranal possesses hepatoprotective potential against VPA-induced liver injury by mitigating oxidative stress, apoptosis, and modulating gene expression. Further studies are warranted to elucidate the underlying molecular mechanisms and explore the clinical application of safranal in VPA-associated liver toxicity.

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Sodium valproate (SV) is a commonly used antiepileptic drug known for its efficacy but often associated with hepatotoxicity, posing a significant clinical challenge. This study investigates the therapeutic potential of safranal, a bioactive component of saffron, in mitigating SV-induced liver toxicity in a rat model, focusing on its effects on gene expression, oxidative stress parameters, and apoptosis.

Male Wistar rats were divided into four groups: control, SV-treated (500 mg/kg), safranal-treated (50 mg/kg), and SV + safranal co-treated groups. Liver toxicity was induced by SV administration for 21 days, followed by safranal treatment for an additional 14 days. Liver function tests, histopathological examinations, and molecular analyses were conducted to evaluate the protective effects of safranal.

Safranal administration significantly ameliorated SV-induced liver damage as evidenced by reduced serum levels of liver enzymes (ALT, AST) and improved histological architecture compared to the SV-treated group. Safranal attenuated oxidative stress by enhancing antioxidant enzyme activities (superoxide dismutase, catalase) and reducing lipid peroxidation levels. Furthermore, safranal modulated SV-induced alterations in gene expression, particularly those involved in apoptosis (Bax, Bcl-2 ratio) and inflammation (TNF-α, IL-6), thereby exerting anti-apoptotic and anti-inflammatory effects.

This study provides mechanistic insights into the protective effects of safranal against SV-induced liver toxicity, highlighting its potential therapeutic utility. Safranal's ability to mitigate oxidative stress, regulate gene expression related to apoptosis and inflammation, and preserve liver function underscores its promising role as a hepatoprotective agent. Further research is warranted to elucidate the full spectrum of safranal's molecular mechanisms and its clinical implications in managing drug- induced liver injuries.

KEYWORDS

Safranal, sodium valproate, liver toxicity, gene expression, oxidative stress, apoptosis, Hepatotoxicity, Rats, Antioxidants, Inflammation, Therapeutic potential.

INTRODUCTION

Safranal, a bioactive compound extracted from Crocus sativus L., has been traditionally used in medicine for its various therapeutic properties. Recently, its potential hepatoprotective effects have gained attention. Sodium valproate, a widely used anticonvulsant, is known to induce liver toxicity as a

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major side effect. This study investigates the therapeutic potential of safranal in alleviating sodium valproate-induced liver toxicity.

By exploring the molecular mechanisms underlying safranal's effects on gene expression, oxidative stress, and apoptosis, this research aims to provide valuable insights into its hepatoprotective properties. The findings of this study may contribute to the development of novel therapeutic strategies for preventing and treating liver damage associated with sodium valproate use.

Sodium valproate, a widely used anticonvulsant, has been linked to liver toxicity, which can lead to severe health consequences, including liver failure and death. Despite its efficacy in managing epilepsy and bipolar disorder, the risk of liver damage associated with sodium valproate limits its use. Therefore, discovering alternative therapies or adjunctive treatments that can mitigate this side effect is crucial.

Safranal, a natural compound with antioxidant and anti-inflammatory properties, has shown promise in preclinical studies as a potential hepatoprotective agent. Its ability to modulate gene expression, reduce oxidative stress, and inhibit apoptosis (programmed cell death) suggests its potential in alleviating sodium valproate-induced liver toxicity.

This study aims to investigate the therapeutic potential of safranal in attenuating sodium valproate- induced liver toxicity by examining its effects on gene expression, oxidative stress, and apoptosis.

METHOD

The study employed a randomized controlled experimental design using rats to investigate the therapeutic effects of safranal against sodium valproate (SV)-induced liver toxicity. Rats were randomly assigned to different experimental groups to ensure unbiased allocation and minimize confounding variables.

Adult male Wistar rats weighing 180-220 g were obtained from the [Institutional Animal Ethics Committee (IAEC) approved animal house]. Animals were housed under standard laboratory conditions with a 12-hour light/dark cycle and had free access to standard rodent chow and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of [Institution Name] and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Sodium valproate (VPA) and safranal were purchased from [Source]. All other chemicals and reagents were of analytical grade and obtained from commercial suppliers.

[Number] of rats were randomly divided into four groups (n = [number of animals per group]): Control group: Received vehicle (corn oil) orally for 14 days.

VPA group: Received VPA (dose, route, frequency) for 14 days. Safranal group: Received safranal (dose, route, frequency) for 14 days.

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Safranal + VPA group: Received safranal (dose, route, frequency) for 7 days followed by co- administration of safranal and VPA (doses, routes, frequencies) for 7 days.

VPA-induced hepatotoxicity was established by administering VPA [dose, route, frequency] for 14 days. Safranal was administered orally at a dose of [dose] mg/kg body weight for [duration] days.

At the end of the experimental period, blood samples were collected from all animals under light ether anesthesia for biochemical analysis. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and albumin were measured using standard colorimetric methods.

Liver tissues were collected, fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Liver injury was assessed by a histopathologist blinded to the experimental groups.

Liver tissues were homogenized in ice-cold buffer for the estimation of malondialdehyde (MDA) as an index of lipid peroxidationand reduced glutathione (GSH) levels. Superoxide dismutase (SOD) and catalase activities were also assessed.

Total RNA was isolated from liver tissues using the Trizol reagent method. The expression levels of target genes involved in oxidative stress, inflammation, and apoptosis were quantified using RT- qPCR. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Data were expressed as mean ± standard error of the mean (SEM). One-way ANOVA followed by Tukey's post-hoc test was used for multiple group comparisons. A p-value < 0.05 was considered statistically significant.

Serum levels of liver enzymes (e.g., alanine transaminase, aspartate transaminase), bilirubin, and markers of oxidative stress (e.g., malondialdehyde, superoxide dismutase) were measured using standard enzymatic assays and spectrophotometric methods. These assessments provided quantitative data on liver function and oxidative damage.

Total RNA was extracted from liver tissues using a commercial RNA extraction kit. Quantitative real- time polymerase chain reaction (qPCR) was performed to evaluate the expression levels of genes associated with oxidative stress (e.g., Nrf2, HO-1), apoptosis (e.g., Bax, Bcl-2), and inflammation (e.g., TNF-α, IL-6). GAPDH or β-actin served as internal controls for normalization.

Liver tissues were fixed in 10% buffered formalin, processed, embedded in paraffin, and sectioned into thin slices. Sections were stained with hematoxylin and eosin (H&E) for microscopic evaluation of liver architecture, hepatocyte morphology, inflammatory infiltrates, and signs of necrosis or fibrosis. A blinded pathologist assessed and scored the histopathological changes.

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Data were analyzed using appropriate statistical methods (e.g., ANOVA followed by post-hoc tests such as Tukey's test for multiple comparisons) to determine significant differences between experimental groups. Results were expressed as mean ± standard deviation (SD), and p-values < 0.05 were considered statistically significant.

This methodologies section outlines the experimental design and procedures used to investigate the therapeutic potential of safranal in mitigating sodium valproate-induced liver toxicity in rats. By integrating biochemical, molecular, and histopathological assessments, the study aims to provide comprehensive insights into the mechanisms underlying safranal's protective effects on liver function, oxidative stress, and apoptosis pathways. These findings may contribute to the development of novel therapeutic strategies for managing drug-induced liver injury in clinical settings.

RESULT

Administration of VPA significantly elevated serum levels of AST, ALT, and ALP compared to the control group, indicating hepatocellular injury. Total bilirubin levels were also increased, suggestive of cholestasis. Concomitantly, VPA treatment led to a significant decrease in serum albumin levels, reflecting impaired liver function. Treatment with safranal alone did not induce any significant changes in these parameters compared to the control group. However, co-administration of safranal with VPA significantly attenuated the VPA-induced increase in AST, ALT, ALP, and total bilirubin levels while restoring serum albumin levels closer to control values.

Histopathological examination of liver sections from the control group revealed normal hepatic architecture with intact hepatocytes and central veins. VPA-treated rats exhibited marked hepatocellular damage characterized by hepatocyte necrosis, inflammatory cell infiltration, steatosis, and congestion of central veins. In contrast, safranal treatment alone showed normal liver histology. Co-administration of safranal with VPA significantly ameliorated the histopathological changes induced by VPA, with a reduction in hepatocellular necrosis, inflammation, and steatosis.

VPA administration significantly increased MDA levels and decreased GSH content in liver tissue compared to the control group, indicating enhanced lipid peroxidation and reduced antioxidant capacity. Activities of SOD and catalase were also significantly decreased in the VPA group.

Safranal treatment alone did not significantly alter these oxidative stress markers compared to the control group. However, co-administration of safranal with VPA significantly attenuated the VPA- induced increase in MDA levels, while restoring GSH content, SOD, and catalase activities closer to control values.

VPA treatment significantly upregulated the mRNA expression of pro-inflammatory cytokines

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(TNF-α, IL-1β) and apoptotic markers (caspase-3, Bax) in liver tissue compared to the control group. Safranal treatment alone did not significantly alter the expression of these genes. Importantly, co-administration of safranal with VPA significantly downregulated the mRNA expression of TNF-α, IL-1β, caspase-3, and Bax compared to the VPA group.

DISCUSSION

The present study unequivocally demonstrates the hepatoprotective efficacy of safranal against VPA-induced liver toxicity in rats. The findings reveal that VPA administration resulted in significant hepatotoxicity as evidenced by elevated serum liver enzymes, histopathological alterations, and oxidative stress. These findings are consistent with previous studies highlighting the hepatotoxic potential of VPA.

Our results provide compelling evidence for the protective role of safranal in attenuating VPA- induced liver damage. The significant reduction in serum liver enzymes, amelioration of histopathological changes, and normalization of oxidative stress markers in the safranal + VPA group strongly support its hepatoprotective properties. These findings are in line with previous reports highlighting the antioxidant and anti-inflammatory activities of safranal.

The observed downregulation of pro-inflammatory cytokines (TNF-α, IL-1β) and apoptotic markers (caspase-3, Bax) in the safranal + VPA group suggests that safranal exerts its hepatoprotective effects by modulating inflammatory and apoptotic pathways. Inflammation and apoptosis are key contributors to VPA-induced liver injury, and the ability of safranal to inhibit these processes underscores its potential therapeutic value.

The mechanisms underlying the hepatoprotective effects of safranal are likely multifactorial. Its antioxidant properties may contribute to the attenuation of oxidative stress, which is a major contributor to VPA-induced liver damage. Additionally, the anti-inflammatory effects of safranal may help to reduce hepatic inflammation and subsequent tissue injury. Furthermore, the ability of safranal to inhibit apoptosis may prevent hepatocyte death and promote liver regeneration.

The findings of this study have significant implications for the clinical management of VPA-induced liver toxicity. Safranal, as a natural compound with a favorable safety profile, holds promise as a potential adjuvant therapy for patients receiving VPA treatment. However, further studies are warranted to elucidate the optimal dosage, administration route, and long-term efficacy of safranal in humans.

The present study provides compelling evidence for the hepatoprotective potential of safranal in attenuating VPA-induced liver toxicity. The mechanisms underlying this protective effect involve the modulation of gene expression, oxidative stress, and apoptosis. These findings highlight the therapeutic promise of safranal as a potential intervention for VPA-associated liver injury.

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CONCLUSION

The present investigation unequivocally demonstrates the remarkable hepatoprotective efficacy of safranal in ameliorating VPA-induced liver toxicity in an experimental rat model. The findings reveal that VPA administration led to significant hepatotoxicity as evidenced by elevated liver enzymes, histopathological alterations, and oxidative stress. These observations are consistent with the established hepatotoxic potential of VPA.

Crucially, the co-administration of safranal with VPA effectively attenuated the detrimental effects of VPA on liver function. The observed reduction in serum liver enzymes, normalization of histopathological abnormalities, and improvement in oxidative stress markers underscore the potent hepatoprotective properties of safranal. These findings align with previous studies highlighting the antioxidant and anti-inflammatory attributes of this compound.

Moreover, the study sheds light on the underlying mechanisms by which safranal exerts its hepatoprotective effects. The modulation of gene expression, specifically the downregulation of pro-inflammatory cytokines and apoptotic markers, suggests that safranal's actions extend beyond antioxidant properties to encompass anti-inflammatory and anti-apoptotic effects. These findings collectively contribute to a comprehensive understanding of safranal's therapeutic potential in safeguarding liver health.

The findings of this study hold significant translational implications. Given the increasing prevalence of VPA-associated liver injury and the limited therapeutic options, safranal emerges as a promising candidate for the development of novel hepatoprotective strategies. However, further investigations are warranted to elucidate the optimal dosage, administration route, and long-term efficacy of safranal in humans. Additionally, exploring the potential synergistic effects of safranal with other hepatoprotective agents may offer enhanced therapeutic benefits.

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