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# Hepatoprotective potential of vanillic acid against isoniazid-rifampicin-induced liver toxicity

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ABSTRACT

Liver toxicity induced by antitubercular drugs, such as isoniazid and rifampicin, poses a significant clinical challenge due to oxidative stress and hepatocellular damage. This study evaluated the hepatoprotective potential of vanillic acid in mitigating drug-induced liver injury in rats. Hepatotoxicity was induced by administering isoniazid and rifampicin, followed by treatment with vanillic acid at two different doses (50 mg/kg and 100 mg/kg). Silymarin, a well-known hepatoprotective agent, was used as a reference standard. Biochemical markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase, and bilirubin, were assessed to evaluate liver function and oxidative stress.

Results revealed significant elevation in AST, ALT, ALP, and bilirubin levels and a reduction in antioxidant enzymes (SOD and catalase) in the isoniazid and rifampicin-treated group, indicating severe liver damage. Co-administration of vanillic acid significantly reduced these elevated markers and restored antioxidant enzyme levels in a dose-dependent manner. The higher dose of vanillic acid (100 mg/kg) exhibited a more pronounced hepatoprotective effect, comparable to silymarin. These findings suggest that vanillic acid exerts its protective effects by enhancing antioxidant defense, reducing oxidative stress, and preserving liver cell integrity.

This study highlights the therapeutic potential of vanillic acid in preventing drug-induced liver toxicity and underscores its role as a promising candidate for hepatoprotection during antitubercular therapy. Further investigation into its molecular mechanisms and clinical applicability is warranted.

## 1. Introduction

Drug-induced liver injury (DILI) refers to hepatic damage caused by pharmaceuticals, illicit substances, or herbal supplements. The severity of DILI can range from mild elevations in liver enzymes to severe outcomes, including hepatitis or acute liver failure. Medications commonly implicated in hepatotoxicity include acetaminophen, antibiotics, statins, and certain herbal products (Mani et al., 2022). Clinical manifestations of DILI typically include jaundice, abdominal pain, nausea, vomiting, fatigue, and elevated levels of hepatic enzymes. Early recognition and discontinuation of the offending agent are critical to minimizing liver damage. Management strategies often involve supportive care, regular monitoring of liver function, and, in severe cases, liver transplantation. For individuals receiving potentially hepatotoxic medications, routine assessment of liver function via blood tests is crucial. Risk factors such as genetic predisposition, pre-existing liver conditions, and polypharmacy with hepatotoxic agents can increase the likelihood of developing DILI. Comprehensive monitoring, heightened awareness of symptoms, and timely medical intervention are essential for effective prevention and management. Isoniazid (INH) and rifampicin, which are key components of tuberculosis treatment, are notably associated with a high risk of DILI. The hepatotoxic effects of these drugs are primarily mediated through their metabolic processes in the liver. Isoniazid undergoes hepatic metabolism to produce reactive intermediates that can induce oxidative stress. These reactive species promote lipid peroxidation, resulting in damage to cellular membranes and hepatocyte injury. Furthermore, isoniazid depletes cellular antioxidant defenses, exacerbating oxidative damage (Tostmann et al., 2008). Comprehensive understanding of these mechanisms underscores the need for vigilant monitoring and risk mitigation strategies during therapy with these agents.

Rifampicin exacerbates hepatotoxicity primarily through the induction of cytochrome P450 enzymes, leading to accelerated drug metabolism and the increased generation of reactive metabolites. This process promotes the formation of free radicals, contributing to oxidative stress and hepatocellular damage (Su et al., 2021). Additionally,

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rifampicin impairs mitochondrial function, resulting in energy depletion and cellular dysfunction. When used in combination with isoniazid, the hepatotoxic effects are synergistically amplified, further intensifying oxidative stress and liver injury. Individual susceptibility to rifampicin-induced hepatotoxicity is influenced by genetic factors, including polymorphisms in genes involved in drug metabolism and detoxification pathways (Brewer, 2017). Despite advancements in modern medicine, no specific pharmacological agents are currently available to counteract rifampicin-induced liver damage. However, certain herbal remedies have demonstrated potential hepatoprotective effects. Natural compounds such as silymarin from milk thistle (*Silybummarianum*), curcumin from turmeric (*Curcuma longa*), and bioactive constituents of Schisandra (*Schisandrachinensis*) exhibit antioxidant and anti-inflammatory properties that support liver health (Jain, 1994). These agents help mitigate oxidative stress, reduce inflammation, and promote hepatocyte regeneration. For instance, silymarin has been extensively studied for its ability to enhance liver function and protect against toxin-induced damage through its free radical-scavenging and membrane-stabilizing effects. Another promising candidate is bearberry (*Arctostaphylosuva-ursi*), known for its robust antioxidant activity and potential organ-protective mechanisms. The molecular actions of bearberry involve the neutralization of reactive oxygen species and stabilization of cellular processes, highlighting its therapeutic promise in managing drug-induced hepatotoxicity (Ballet, 1997). Despite the availability of hepatoprotective agents such as silymarin and *N*-acetylcysteine, current treatments for drug-induced liver injury remain limited by inconsistent efficacy, poor bioavailability, and a lack of targeted molecular interventions. Many therapeutic options provide only symptomatic relief rather than directly mitigating oxidative stress and hepatocellular damage. Therefore, exploring novel hepatoprotective compounds with improved pharmacological properties remains a critical area of research.

Vanillic acid, a phenolic compound derived from various plants, exhibits potent antioxidant, anti-inflammatory, and hepatoprotective properties. Its therapeutic potential has been extensively studied in experimental models of liver injury. Vanillic acid demonstrates hepatoprotective effects against CCl4-induced liver damage by reducing oxidative stress and enhancing antioxidant defense systems (Itoh et al., 2010). The co-synthesis of silver nanoparticles (AgNPs) with Vanillic acid further enhances its protective activity against CCl4-induced hepatotoxicity in male rats (Alamri et al., 2022). Additionally, vanillic acid mitigates liver fibrosis by inhibiting autophagy in hepatic stellate cells via the MIF/CD74 signaling pathway, reducing extracellular matrix deposition and preserving liver architecture (Qin et al., 2023). It also exerts a chemopreventive effect against diethylnitrosamine- and 1, 2-dimethylhydrazine-induced hepatocarcinogenesis by modulating oxidative stress, apoptosis, and pro-inflammatory cytokine levels (Punvittayagul et al., 2021). The pharmacokinetics and tissue distribution of vanillic acid were previously investigated using ultra-performance liquid chromatography–high resolution mass spectrometry, confirming its systemic absorption and bioavailability *in vivo* (Huayu et al., 2023). Given the hepatotoxic risks associated with isoniazid and rifampicin, this study aims to evaluate the hepatoprotective potential of vanillic acid in mitigating drug-induced liver injury. By assessing biochemical markers of liver function and oxidative stress, this research seeks to establish vanillic acid as a potential therapeutic agent for preventing liver toxicity during antitubercular therapy.

## 2. Experimental

### 2.1. Animals

Wistar rats (180–200 g) of either sex were housed in polypropylene cages, maintained under standardized conditions (12 h light/dark cycles, 28 ± 2 °C) were used in the study. Animals were provided with standard pellet food and had free access to drinking water. All the animal study protocols were duly approved by the Institutional Animal Ethics Committee.

### 2.2. Chemicals

Arbutin was purchased from Central Drug House, Mumbai, India. All the other chemicals used in the study were of analytical grade.

### 2.3. Selection of dose

As per studies performed by Sindhu et al. (2015),Vanillic acid was used in the dose of 50 and 100 mg/kg of body weight.

### 2.4. Animal group and dosing

Animals were divided into 5 groups with six animals in each
Group I Normal Control
Group II Isoniazid (100 mg/kg) + Rifampicin (100 mg/kg)
Group III Isoniazid (100 mg/kg) + Rifampicin (100 mg/kg) + Silymarin (100 mg/kg)
Group IV Isoniazid (100 mg/kg) + Rifampicin (100 mg/kg) + Vanillic acid (50 mg/kg)
Group V Isoniazid (100 mg/kg) + Rifampicin (100 mg/kg) + Vanillic acid (100 mg/kg)

On the 21st day, blood was withdrawn by a tail vein for the estimation of biochemical parameters.

### 2.5. Biochemical analysis

Blood samples were collected into the epindrop tubes and centrifuged for 10 min at 7000 rpm using micro-centrifuge to separate the serum. The levels of serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic-pyruvic transaminase (SGPT/ALT) serum alkaline phosphatase (SALP) were estimated using commercial kits (Span Diagnostics, India).

### 2.6. Marker enzymes

#### 2.6.1. Superoxide dismutase assay

Superoxide dismutase (SOD) activity in liver homogenate was determined (Masayasu and Hiroshi, 1979). The method was based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan colour development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan colour development). The liver homogenate was centrifuged to 10000 rpm for 15 min at 4$^{o}$C. To 0.25 ml of supernatant, 0.5 ml of triscacodylic buffer, 0.1 ml of 16 % Triton x- 100 and 0.25 ml NBT were added. The reaction was started by the addition of 0.01 ml diluted pyrogallol. Incubation was maintained for 5 min at 37$^{o}$C. The reaction was stopped by the addition of 0.3 ml of 2 M formic acid. The formazan colour developed was determined spectrophotometrically at wavelength 430 nm. Enzymatic activity was expressed as ug/gm of tissue.

#### 2.6.2. Catalase activity

The catalase activity was measured according to the method of Sinha (Sinha, 1972). 0.1 ml of liver homogenate was mixed with 1.0 ml of 0.01 M phosphate buffer (pH 7.4) and incubated with 0.4 ml of 0.2 M $H_2O_2$ at 37 °C accurately for 1.0 min and the reaction was stopped with 2.0 ml of 5 % potassium dichromate (1:3 with glacial acetic acid). Further, the samples were incubated in a boiling water bath for 15 min. Tubes were centrifuged at 5000 rpm for 15 min and the supernatant was used to quantify the amount of $H_2O_2$ to calculate catalase activity at 570 nm.

### 2.7. MDA estimation

The lipid peroxidation in the liver was assayed by measurement of malondialdehyde (MDA) formation (Ohkawa et al., 1978).

### 2.8. Determination of total bilirubin

The determination of total bilirubin was performed according to the standard principles and procedures of the kit manufacturer manual (Tulip Diagnostics, India).

### 2.9. Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out by using One way ANOVA followed by Dunnett's test and $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered significant.

## 3. Results

### 3.1. AST

The impact of various treatments on AST levels was evaluated, and significant alterations among the groups were observed. Hepatotoxicity was induced in the group treated with isoniazid and rifampicin, as indicated by a pronounced elevation in AST levels compared to the normal control. Co-administration of silymarin with isoniazid and rifampicin resulted in a substantial reduction in AST levels, demonstrating its hepatoprotective potential. Similarly, treatment with vanillic acid mitigated the increase in AST levels, with the higher dose showing a more pronounced effect. The protective action of vanillic acid was found to be dose-dependent, further supporting its role in reducing drug-induced liver damage (Fig. 1). Both silymarin and vanillic acid effectively reduced AST levels, highlighting their ability to preserve hepatic integrity under toxic conditions.

### 3.2. ALT

The effects of various treatments on ALT levels were assessed, and significant differences among the groups were observed. Hepatotoxicity was induced in the group treated with isoniazid and rifampicin, as evidenced by a substantial elevation in ALT levels compared to the normal control. The co-administration of silymarin with isoniazid and rifampicin resulted in a marked reduction in ALT levels, demonstrating its hepatoprotective efficacy. Treatment with vanillic acid also led to a notable attenuation of ALT elevation, with greater hepatoprotection observed at the higher dose. These findings suggest that vanillic acid, similar to silymarin, mitigates liver damage induced by isoniazid and rifampicin. The dose-dependent effect of vanillic acid further highlights its potential as a protective agent against drug-induced hepatotoxicity (Fig. 2). Overall, ALT levels were effectively reduced by both silymarin and vanillic acid, indicating their roles in preserving hepatic function under toxic conditions.

### 3.3. ALP

The effects of different treatments on ALP levels were analyzed, and significant variations among the groups were noted. Hepatotoxicity was induced in the group treated with isoniazid and rifampicin, as evidenced by a marked elevation in ALP levels compared to the normal control. Co-treatment with silymarin significantly reduced ALP levels, demonstrating its hepatoprotective properties. Treatment with vanillic acid also resulted in a reduction of ALP levels, with the higher dose showing a more pronounced protective effect than the lower dose. The dose-dependent efficacy of vanillic acid in lowering ALP levels highlights its potential as a hepatoprotective agent (Fig. 3). Both silymarin and vanillic acid were effective in mitigating the ALP elevation induced by isoniazid and rifampicin, indicating their roles in maintaining hepatic function.

### 3.4. SOD

The effects of various treatments on SOD levels were assessed, and significant differences among the groups were observed. A notable decrease in SOD levels was induced in the group treated with isoniazid and rifampicin, reflecting impaired antioxidant defense mechanisms compared to the normal control. Co-administration of silymarin with isoniazid and rifampicin significantly restored SOD levels, demonstrating its antioxidant and protective properties. Treatment with vanillic acid also led to an improvement in SOD levels, with the higher dose exhibiting a more pronounced effect. The dose-dependent enhancement of SOD activity by vanillic acid highlights its potential in mitigating oxidative stress associated with drug-induced hepatotoxicity. Both silymarin and vanillic acid effectively restored antioxidant enzyme levels, underscoring their roles in preserving hepatic

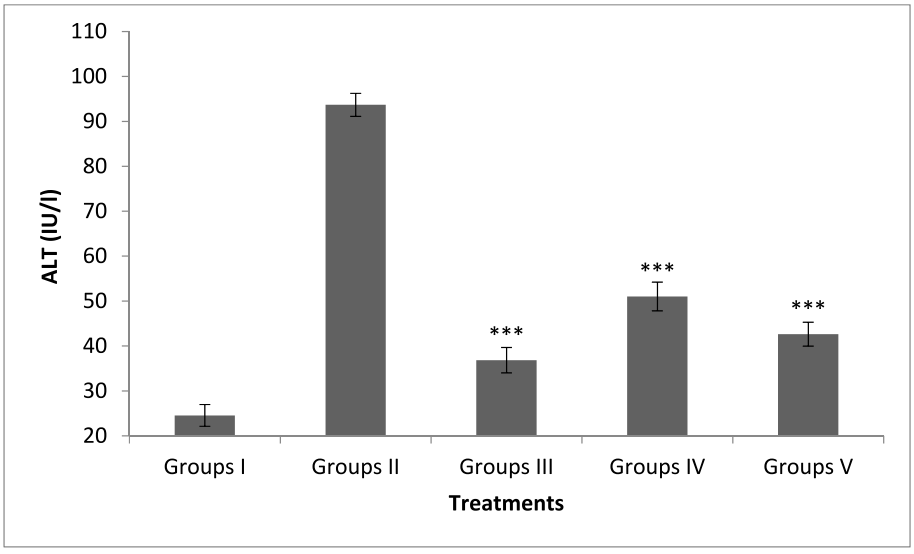


**Fig. 1.** Effect of Vanillic acid administration on AST levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *P < 0.05; **P < 0.01; ***P < 0.001.

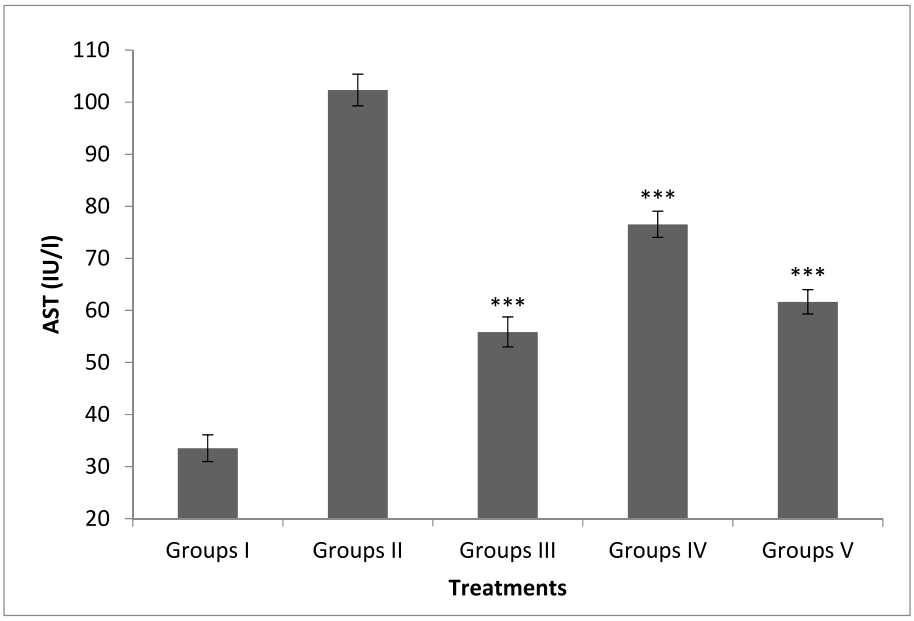


**Fig. 2.** Effect of Vanillic acidadministration on ALT levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *P < 0.05; **P < 0.01; ***P < 0.001.

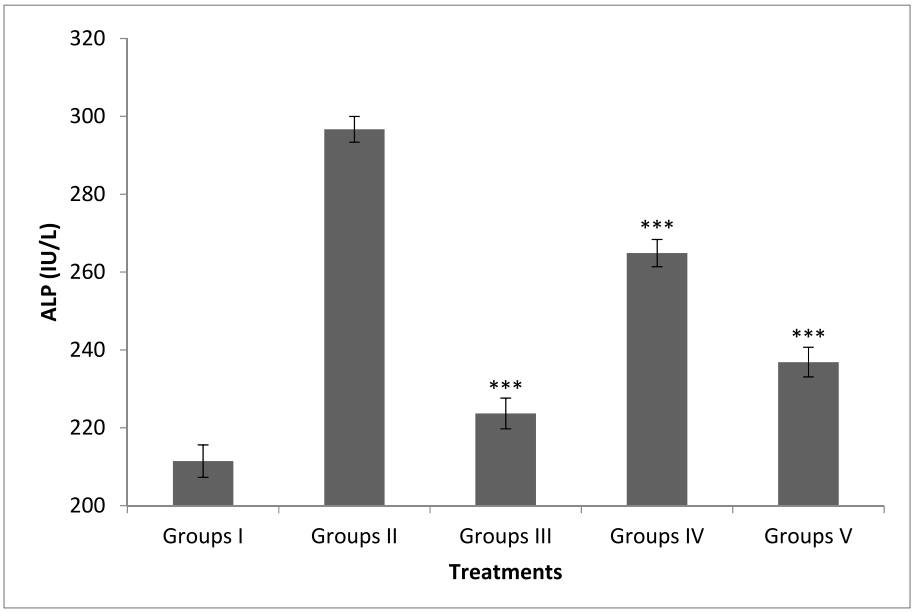


**Fig. 3.** Effect of Vanillic acidadministration on ALP levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *P < 0.05; **P < 0.01; ***P < 0.001.

antioxidant defences (Fig. 4). These findings suggest that vanillic acid, akin to silymarin, may serve as a promising agent for combating oxidative stress-induced liver damage.

### 3.5. Catalase

The effects of various treatments on catalase levels were evaluated, and significant variations among the groups were observed. A reduction in catalase levels was induced in the group treated with isoniazid and rifampicin, indicating impaired enzymatic antioxidant activity compared to the normal control. Co-administration of silymarin with isoniazid and rifampicin significantly improved catalase levels, demonstrating its antioxidative and protective effects. Treatment with vanillic acid also led to an enhancement of catalase activity, with the higher dose producing a more substantial effect than the lower dose. The dose-dependent restoration of catalase levels by vanillic acid highlights its role in counteracting oxidative stress induced by drug toxicity. Both silymarin and vanillic acid were effective in improving catalase levels, indicating their potential to preserve hepatic antioxidant defences (Fig. 5). These findings support vanillic acid as a promising agent for mitigating oxidative damage associated with hepatotoxicity.

### 3.6. Bilirubin

The effects of different treatments on bilirubin levels were analyzed, and significant variations among the groups were noted. Elevated

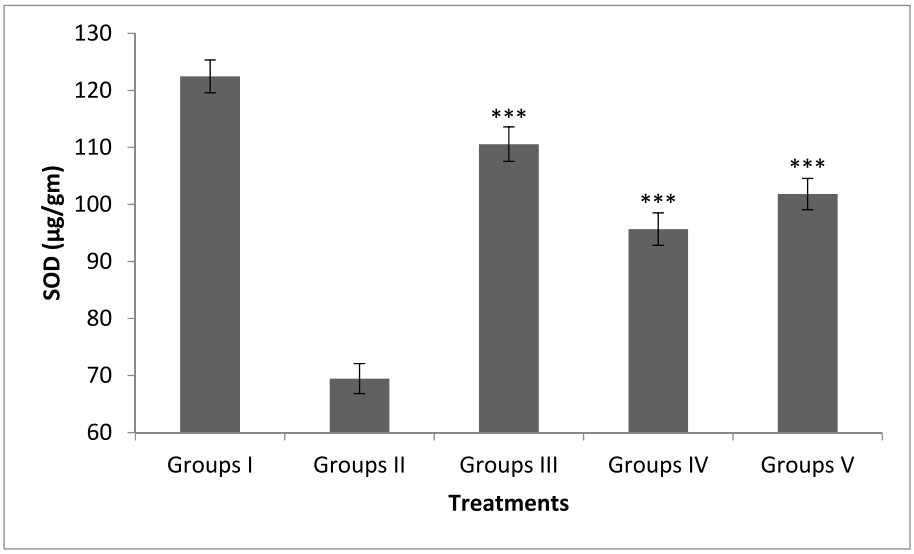


**Fig. 4.** Effect of Vanillic acidadministration on SODlevels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *P < 0.05; **P < 0.01; ***P < 0.001.

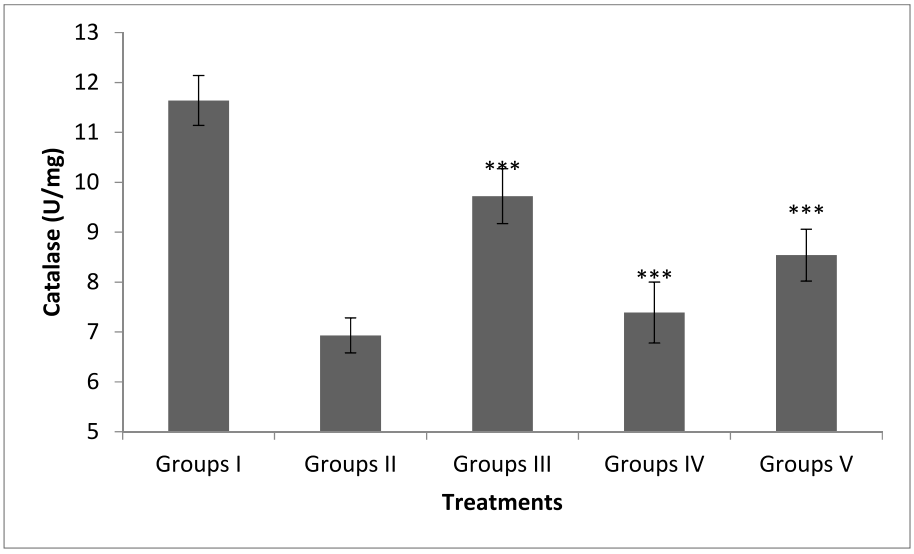


**Fig. 5.** Effect of Vanillic acidadministration on Catalase levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *P < 0.05; **P < 0.01; ***P < 0.001.

bilirubin levels were induced in the group treated with isoniazid and rifampicin, reflecting impaired hepatic function compared to the normal control. Co-administration of silymarin with isoniazid and rifampicin resulted in a marked reduction in bilirubin levels, indicating its hepatoprotective efficacy. Treatment with vanillic acid also reduced bilirubin levels, with a more pronounced effect observed at the higher dose. The dose-dependent decrease in bilirubin levels with vanillic acid underscores its potential in mitigating drug-induced liver dysfunction (Fig. 6). Both silymarin and vanillic acid effectively improved bilirubin levels, highlighting their roles in preserving liver function under toxic conditions.

## 4. Discussion

The liver, a vital organ responsible for various metabolic, detoxification, and biosynthetic functions, is often susceptible to damage caused by drugs and toxins (Corless and Middleton, 1983). The present study aimed to evaluate the hepatoprotective effect of vanillic acid against isoniazid and rifampicin-induced toxicity in rats. By investigating biochemical markers of liver function, oxidative stress, and antioxidant defense systems, the study sought to determine the efficacy of vanillic acid in mitigating liver damage caused by these commonly used antitubercular drugs.

Aspartate aminotransferase (AST) is an important enzyme found in the liver, heart, muscle, and other tissues. It is released into the bloodstream when liver cells are damaged, making it a reliable biomarker for assessing liver injury (Ndrepepa, 2021). In the present study, significant elevation in AST levels was observed in the group treated with isoniazid and rifampicin, indicating hepatotoxicity induced by these drugs. The elevated AST levels suggest liver cell membrane damage, leading to the leakage of the enzyme into the circulation. Co-administration of vanillic acid, particularly at higher doses, led to a noticeable reduction in AST levels, highlighting its hepatoprotective properties. This decrease in AST suggests that vanillic acid may mitigate liver cell damage by enhancing antioxidant defense, reducing inflammation, or promoting cell membrane stability.

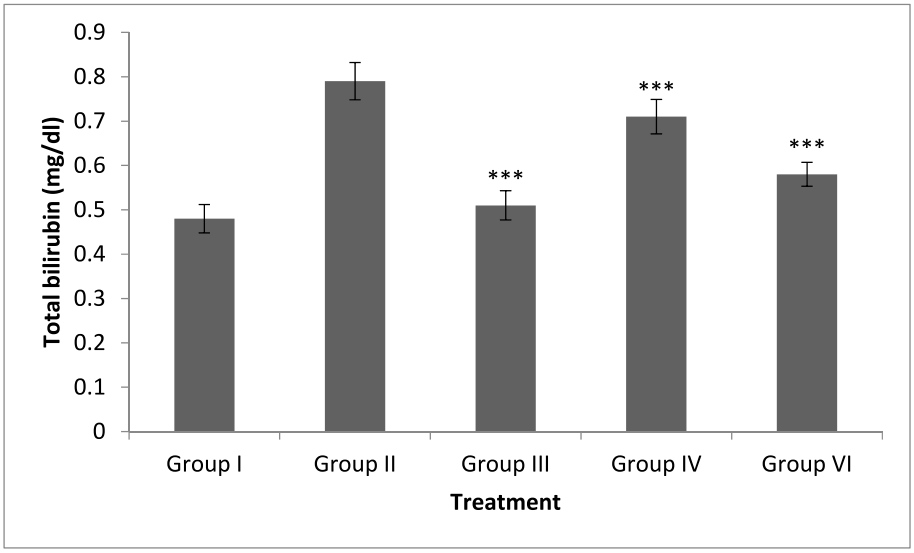


**Fig. 6.** Effect of Vanillic acidadministration on Bilirubin levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Significance at *P < 0.05; **P < 0.01; ***P < 0.001.

Alanine aminotransferase (ALT) is a key enzyme found primarily in the liver, and its elevation in the bloodstream is a well-established marker of hepatocellular injury. In the present study, a significant increase in ALT levels was observed in the group treated with isoniazid and rifampicin, reflecting hepatic damage caused by these antitubercular drugs. The elevation in ALT levels indicates the disruption of liver cell integrity, leading to the release of the enzyme into circulation (Senior, 2012). Co-administration of vanillic acid resulted in a dose-dependent reduction in ALT levels, indicating its protective effect on liver cells. The observed decrease in ALT suggests that vanillic acid may alleviate oxidative stress, reduce inflammatory responses, and stabilize liver cell membranes, thereby preventing cellular damage.

Alkaline phosphatase (ALP) is an enzyme found in various tissues, including the liver, bone, and bile ducts, and its elevated levels often indicate cholestatic liver injury or hepatobiliary dysfunction. In the present study, a marked increase in ALP levels was observed in the group treated with isoniazid and rifampicin, suggesting hepatic dysfunction and possible bile duct damage (Poupon, 2015). The elevation in ALP levels reflects impaired bile secretion or obstruction, which can occur due to drug-induced liver injury. Treatment with vanillic acid led to a reduction in ALP levels, with the higher dose exhibiting a more pronounced effect. This reduction suggests that vanillic acid may protect against hepatobiliary dysfunction by improving bile flow, reducing oxidative stress, or restoring normal hepatic architecture.

Superoxide dismutase (SOD) is a critical antioxidant enzyme that plays a vital role in protecting cells from oxidative stress by catalyzing the conversion of superoxide radicals into less harmful molecules. In the present study, a significant reduction in SOD levels was observed in the group treated with isoniazid and rifampicin, indicating an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, leading to oxidative damage in liver cells (Saxena et al., 2022). Co-administration of vanillic acid resulted in a dose-dependent restoration of SOD levels, suggesting its antioxidant properties and ability to combat oxidative stress. The higher dose of vanillic acid provided a more pronounced enhancement in SOD activity, reflecting its potent protective effect. By increasing SOD levels, vanillic acid may reduce ROS-induced damage to liver cells, thereby preserving hepatic function and mitigating liver injury. These findings highlight the therapeutic potential of vanillic acid in protecting the liver from oxidative damage caused by drug-induced hepatotoxicity.

Catalase is an essential antioxidant enzyme that plays a crucial role in the detoxification of hydrogen peroxide, a reactive oxygen species (ROS) that can cause cellular damage if not efficiently removed. In the present study, a notable decrease in catalase levels was observed in the group treated with isoniazid and rifampicin, suggesting an impaired antioxidant defense mechanism in response to drug-induced oxidative stress (Gebicka and Krych-Madej, 2019). The decrease in catalase activity points to an inability of the liver cells to effectively neutralize oxidative damage, which may contribute to hepatotoxicity. Treatment with vanillic acid, particularly at higher doses, led to a significant increase in catalase levels, indicating its potential to enhance the liver's antioxidant capacity. By restoring catalase activity, vanillic acid may help mitigate oxidative damage, reduce inflammation, and protect liver cells from injury.

Bilirubin is a bile pigment produced during the breakdown of hemoglobin, and its levels in the blood are commonly used as indicators of liver function. Elevated bilirubin levels suggest impaired hepatic processing or excretion, often due to liver cell damage, bile duct obstruction, or dysfunction in the bilirubin conjugation process. In the present study, a significant increase in bilirubin levels was observed in the group treated with isoniazid and rifampicin, indicating drug-induced liver injury and compromised hepatic function (Guerra Ruiz et al., 2021). Co-treatment with vanillic acid resulted in a decrease in bilirubin levels, suggesting its potential to protect liver function by improving the liver's ability to process and excrete bilirubin. The higher dose of vanillic acid showed a more pronounced effect in lowering bilirubin levels, highlighting its dose-dependent hepatoprotective properties.

Some phytochemicals like Silymarin primarily exerts its effects by stabilizing hepatocyte membranes, reducing lipid peroxidation, and enhancing protein synthesis, making it a widely used hepatoprotective agent (Basiglio et al., 2009). Curcumin's hepatoprotection is largely attributed to its ability to inhibit NF-κBsignaling, thereby reducing inflammation and oxidative stress (Zhong et al., 2016). Resveratrol, on the other hand, activates SIRT1, which improves mitochondrial function and enhances cellular resistance to oxidative damage (Xu et al., 2012). This study specifically focused on vanillic acid's antioxidant-driven hepatoprotection, as evidenced by its impact on biochemical markers of liver function. These findings suggest that vanillic acid may help prevent liver damage and restore normal bilirubin metabolism, making it a promising candidate for mitigating drug-induced liver toxicity. Although vanillic acid demonstrates moderate bioavailability, its rapid metabolism and clearance may limit its systemic effects. In contrast, curcumin and resveratrol suffer from poor bioavailability, requiring advanced delivery strategies for improved absorption. While vanillic

acid's hepatoprotective efficacy has been demonstrated in models of drug-induced liver injury, further studies are needed to compare its effectiveness against these well-established natural compounds in clinical settings.

This study shows that vanillic acid has hepatoprotective effects, as evidenced by improvements in biochemical markers (e.g., ALT, AST, ALP, SOD, catalase, and bilirubin). However, the molecular mechanisms behind its action need further investigation. The antioxidant effects of vanillic acid may be linked to the activation of the Nrf2/ARE pathway. This pathway controls the expression of genes that protect cells, such as heme oxygenase-1 (HO-1) and glutathione peroxidase (GPx), which in turn helps lower oxidative stress. Furthermore, vanillic acid might inhibit the NF-κB signaling pathway, which could reduce inflammation by lowering levels of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. Additionally, vanillic acid could have anti-apoptotic effects by modulating Bcl-2 family proteins, thereby preventing mitochondrial dysfunction and the apoptosis of hepatocytes. As seen in earlier investigations, its effect on the regulation of autophagy could additionally aid in maintaining the structure of the liver. Future investigations that clarify these molecular mechanisms would improve the understanding of vanillic acid's potential as a treatment to prevent drug-induced hepatotoxicity.

## 5. Conclusion

In conclusion, the results of this study demonstrate that vanillic acid exhibits significant hepatoprotective effects against isoniazid and rifampicin-induced liver toxicity in rats. The administration of vanillic acid led to a marked reduction in liver injury, as evidenced by the improvement in various biochemical markers such as AST, ALT, ALP, SOD, catalase, and bilirubin levels. The dose-dependent restoration of antioxidant enzyme activities, along with a reduction in liver enzyme leakage and bilirubin accumulation, suggests that vanillic acid effectively mitigates oxidative stress and liver dysfunction. These findings highlight the potential of vanillic acid as a therapeutic agent in preventing or alleviating drug-induced liver injury, offering a promising alternative to support liver health during antitubercular treatment. Further studies are warranted to explore its molecular mechanisms and evaluate its long-term safety and efficacy for clinical application in hepatotoxicity management.

## Perspective

Vanillic acid has been extensively studied for its antioxidant properties, and its potential applications in oxidative stress-related diseases have been increasingly recognized. Its protective effects have been demonstrated not only in hepatotoxicity but also in neurodegenerative disorders, cardiovascular diseases, and metabolic syndromes. Recent studies have indicated its role in mitigating inflammation and oxidative damage in various pathological conditions (Wahnou et al., 2024a, 2024b). Further investigations are required to explore its mechanisms in these diseases, optimize its bioavailability, and assess its therapeutic potential in clinical settings. Expanding its applications could provide novel treatment strategies for multiple oxidative stress-mediated disorders.

## CRediT authorship contribution statement

**Mohd Islam Ansari:** Methodology, Investigation. **Nazneen Dubey:** Writing – original draft, Software. **Aditya Ganeshpurkar:** Visualization, Project administration, Investigation.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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