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Hepatoprotective Activity of *Andrographis paniculata, Berberis aristata, Cichorium intybus* and *Rubia cordifolia* Root extracts Against Anti-TB Medication

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Abstract

Liver toxicity is a major adverse effect associated with the use of anti-tuberculosis (TB) medications, particularly isoniazid (INH) and rifampicin (RMP). This study investigates the hepatoprotective effects of natural compounds oleanolic acid, stigmasterol, and β-sitosterol obtained from *Andrographis paniculata, Berberis aristata, Cichorium intybus* and *Rubia cordifolia Root* extracts collectively referred to as ABCR compounds, against INH+RMP-induced hepatotoxicity. Chang liver cells and male Wistar albino rats were used as experimental models. The MTT assay assessed cell viability, and the in vivo study involved biochemical, histopathological, and antioxidant enzyme analyses. INH+RMP treatment significantly elevated serum levels of ALT, AST, ALP, and bilirubin, indicating liver damage. These alterations were markedly reduced in rats pre-treated with ABCR compounds and the standard hepatoprotective agent Silymarin. Histopathological examination revealed severe hepatocellular damavge in the INH+RMP group, while ABCR pre-treatment showed significant preservation of liver histology. Among the ABCR compounds, ABCR2 demonstrated the highest level of protection. The results suggest that ABCR compounds effectively mitigate INH+RMP-induced liver toxicity through their antioxidant and anti-inflammatory properties. Further clinical studies are warranted to explore their potential in preventing and managing drug-induced hepatotoxicity in TB patients.

Keywords: Hepatotoxicity, Anti-tuberculosis medication, Oleanolic acid, Stigmasterol, β-sitosterol

Introduction

Liver toxicity is a significant adverse effect of anti-tuberculosis (TB) medications, particularly isoniazid (INH) and rifampicin (RMP). These drugs are crucial for TB treatment but can lead to hepatotoxicity, resulting in elevated liver enzymes and histopathological changes. This study investigates the hepatoprotective effects of certain natural compounds (oleanolic acid, stigmasterol, and β-sitosterol), collectively referred to as ABCR compounds, derived from traditional medicinal plants.[1-2]

Materials and Methods

Before the commencement of the experiment, the rats were acclimatized to their environment for 3-5 days. The experimental groups received a methanolic

extract compared with Silymarin from milk thistle (Silybum marianum) at a dosage of 200 mg/kg per os (p.o.). The animals were randomly divided into six groups, with six rats in each group, and the treatments were administered for 14 days. The groups were as follows:

- **Group 1 (Control)**: Received 1 mL of normal saline per day.
- **Group 2 (Negative Control)**: Received 1 mL of saline per day for the first 14 days, followed by an acute dose of isoniazid (INH) and rifampicin (RMP) (50 mg/kg and 100 mg/kg body weight, respectively) administered intraperitoneally (i.p.) in normal saline for 5-6 hours after 14 days.
- **Group 3 (Positive Control)**: Received an oral suspension of Silymarin (200 mg/kg body weight per day) and the same toxin treatment as Group 2.**Groups 4, 5, and 6 (Treatment)**: Received an acute oral dose of ABCR2, ABCR4, and ABCR5 (Oleanolic acid, Stigmasterol, and β-sitosterol, respectively) at 3 mg/kg body weight per day (in oral suspension with Tween 80) and the same toxin treatment as Group 3.

Cell Culture

Preparation and Maintenance

Chang liver cells (normal human hepatocytes) were cultured to assess the cytotoxicity and protective effects of ABCR compounds. These cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented withserum albumin 1% antibiotic/antimycotic solution. The cells were incubated at 37°C in a humidified atmosphere with 5% CO2.[3-4]

Cell Viability Assay Using MTT

Procedure

The MTT assay was employed to evaluate cell viability. Chang cells were seeded at a density of 1 × 10^4 cells per well in 96-well plates and incubated overnight. The cells were then treated with various concentrations (10, 50, 100, 150, and 200 µg/mL) of the ABCR compounds (ABCR2, ABCR4, ABCR5 respectively) for 24 hours. After treatment, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for an additional 4 hours. The resulting formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader.[5-8]

Animals

Ethical Approval and Grouping

The animal studies were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee and as per approved CCSEA protocol. Male Wistar albino rats weighing 180-200 grams were used. The rats were randomly divided into six groups (n=6 per group):

1. **Group I:** Normal control (vehicle only)
2. **Group II:** INH+RMP (induced toxicity control)
3. **Group III:** INH+RMP + Silymarin (25 mg/kg body weight, standard control)
4. **Group IV:** INH+RMP + ABCR2 (100 mg/kg body weight)
5. **Group V:** INH+RMP + ABCR4 (100 mg/kg body weight)
6. **Group VI:** INH+RMP + ABCR5 (100 mg/kg body weight)

Treatment Regimen

Rats in groups II to VI were administered INH (50 mg/kg) and RMP (100 mg/kg) orally for 14 days to induce hepatotoxicity. Concurrently, groups III to VI received their respective treatments (Silymarin or ABCR compounds) orally.[9]

Biochemical Analysis

Serum and Liver Homogenate Assays

Blood samples were collected from the retro-orbital plexus of rats under anesthesia on the 15th day. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. Liver tissues were excised, rinsed with ice-cold saline, and homogenized in phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 rpm for 20 minutes, and the supernatants were used for biochemical assays.[10]

Enzyme Activity Assays

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and levels of total protein, albumin, bilirubin, and creatinine were measured using commercial diagnostic kits. Liver homogenate was assayed for antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase (CAT).[11]

Histopathology

Liver tissues were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. Sections (5 µm thick) were stained with hematoxylin and eosin (H&E) for histopathological examination under a light microscope. The extent of hepatic damage and protective effects of treatments were assessed based on cellular integrity, presence of necrosis, and inflammation.[12]

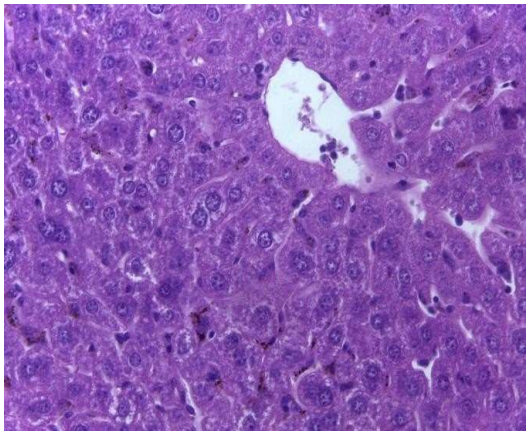


Fig 1: INH+RMP (group2) confirmed membrane disintegration with extreme vacuolation along with cytoplasmic rarefaction

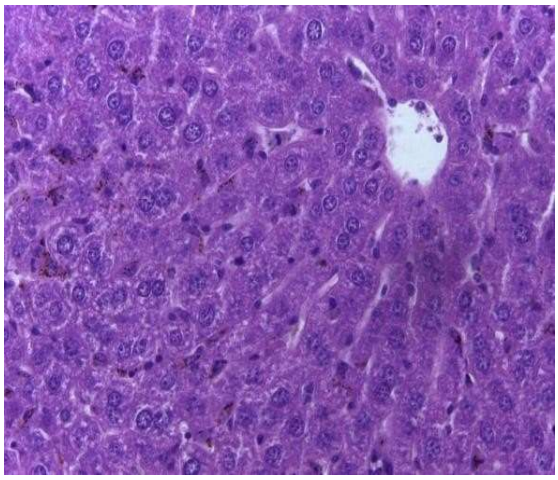


Figure 2: Formulation treated animal (group4) regulated the vacuolation and prevented the degradation of cell membrane

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test. A p-value < 0.05 was considered statistically significant.[13]

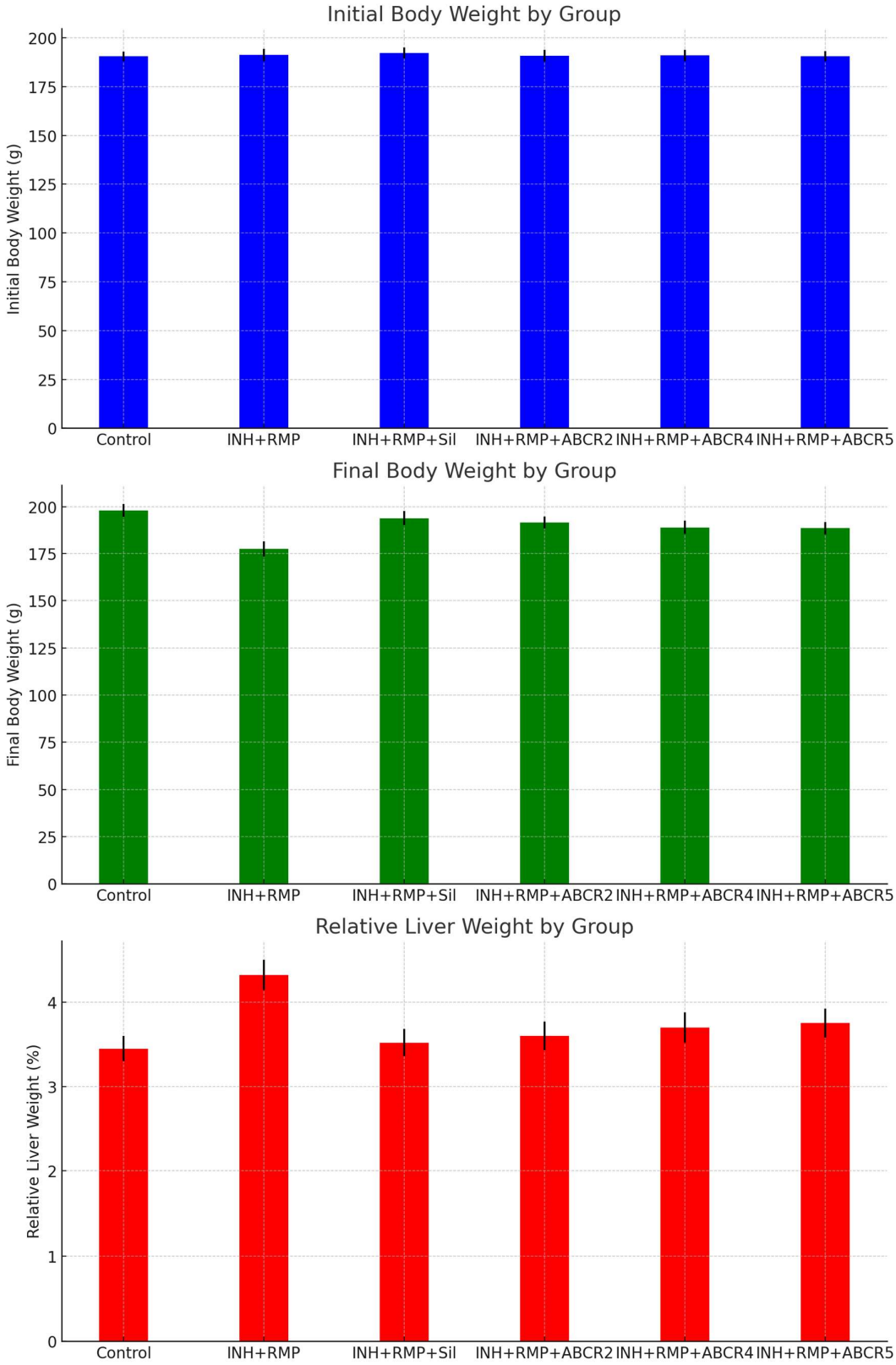
Results

Effect on Body and Relative Organ Weights

The body weights and relative liver weights of the rats were measured to assess the overall impact of the treatments. Rats treated with INH+RMP showed a significant reduction in body weight and an increase in relative liver weight, indicating liver damage. Pre-treatment with ABCR compounds and Silymarin mitigated these effects, with ABCR2 showing the most substantial protective effect.[14]

Group	Initial Body Weight (g)	Final Body Weight (g)	Relative Liver Weight (%)
Control	190.5 ± 2.5	198.2 ± 3.4	3.45 ± 0.15
INH+RMP	191.2 ± 3.1	177.5 ± 4.2*	4.32 ± 0.18*
INH+RMP+Sil	192.3 ± 2.8	194.1 ± 3.7#	3.52 ± 0.16#
INH+RMP+ABCR2	190.8 ± 3.0	191.9 ± 3.2#	3.60 ± 0.17#
INH+RMP+ABCR4	191.0 ± 2.9	189.2 ± 3.5#	3.70 ± 0.18#
INH+RMP+ABCR5	190.6 ± 2.7	188.8 ± 3.3#	3.75 ± 0.17#

- Values significantly different from control group ($p < 0.05$)



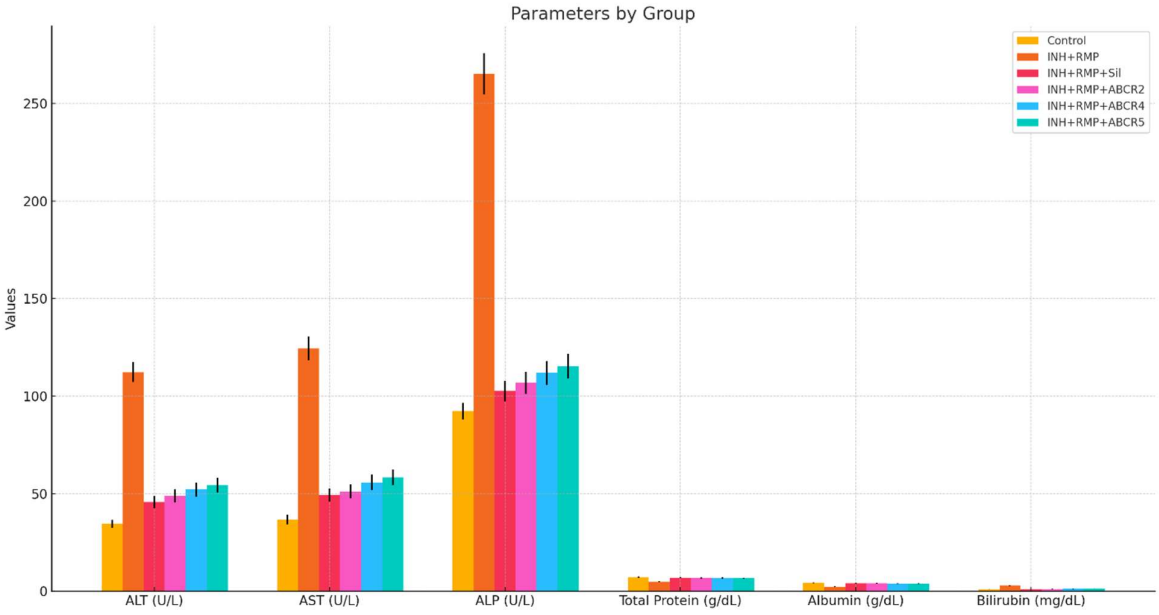
Serum Biochemical Parameters

Serum biochemical parameters were measured to assess liver function. Elevated levels of ALT, AST, ALP, and bilirubin in the INH+RMP group indicated liver damage. Pre-treatment with

ABCR compounds and Silymarin significantly reduced these levels, suggesting a protective effect. Among the ABCR compounds, ABCR2 exhibited the most substantial hepatoprotective effect.[15]

Parameter	Control	INH+RMP	INH+RMP+Sil	INH+RMP+ABCR2	INH+RMP+ABCR4	INH+RMP+ABCR5
ALT (U/L)	34.5 ± 2.1	112.3 ± 5.2*	45.7 ± 3.1#	48.9 ± 3.3#	52.1 ± 3.7#	54.3 ± 3.8#
AST (U/L)	36.7 ± 2.5	124.5 ± 6.1*	49.4 ± 3.4#	51.2 ± 3.5#	55.8 ± 3.9#	58.4 ± 4.0#
ALP (U/L)	92.3 ± 4.3	265.2 ± 10.7*	102.5 ± 5.2#	106.8 ± 5.7#	111.9 ± 6.1#	115.3 ± 6.3#
Total Protein (g/dL)	7.1 ± 0.3	4.8 ± 0.2*	6.9 ± 0.3#	6.8 ± 0.3#	6.7 ± 0.3#	6.6 ± 0.3#
Albumin (g/dL)	4.2 ± 0.2	2.3 ± 0.1*	4.1 ± 0.2#	4.0 ± 0.2#	3.9 ± 0.2#	3.8 ± 0.2#
Bilirubin (mg/dL)	0.9 ± 0.1	2.8 ± 0.2*	1.0 ± 0.1#	1.1 ± 0.1#	1.2 ± 0.1#	1.3 ± 0.1#

- Values significantly different from control group ($p < 0.05$)



Antioxidant Enzyme Activities

Antioxidant enzyme activities in the liver homogenate were measured to evaluate the oxidative stress status. The INH+RMP group showed a significant decrease in SOD and catalase activities, indicating increased oxidative stress. Pre-treatment with ABCR compounds and Silymarin significantly restored these enzyme activities, suggesting an enhancement of antioxidant defense mechanisms. ABCR2 was particularly effective in restoring antioxidant enzyme levels.[16]

Parameter	Control	INH+RMP	INH+RMP+Sil	INH+RMP+ABCR2	INH+RMP+ABCR4	INH+RMP+ABCR5
SOD (U/mg protein)	4.5 ± 0.3	1.8 ± 0.2*	4.3 ± 0.3#	4.2 ± 0.3#	4.1 ± 0.3#	4.0 ± 0.3#
Catalase (U/mg protein)	5.3 ± 0.4	2.0 ± 0.2*	5.1 ± 0.4#	5.0 ± 0.4#	4.9 ± 0.4#	4.8 ± 0.4#

- Values significantly different from control group ($p < 0.05$)

Values significantly different from INH+RMP group ($p < 0.05$)

Histopathological Examination

Findings

Histopathological examination revealed that the liver sections from the INH+RMP group exhibited severe hepatocellular damage, including necrosis, inflammation, and fatty degeneration. In contrast, liver sections from the control group displayed normal cellular architecture with no signs of damage.

Rats pre-treated with Silymarin showed marked reduction in hepatocellular damage, demonstrating near-normal histology. Similarly, liver tissues from rats pre-treated with ABCR compounds (ABCR2, ABCR4, ABCR5) showed significant preservation of cellular integrity, with reduced necrosis and inflammation. Among the ABCR compounds, ABCR2 provided the highest level of histological protection, similar to the effect of Silymarin.[17]

Discussion

Hepatoprotective Mechanisms

Biochemical and Histological Correlations

The hepatoprotective effects of ABCR compounds can be attributed to their ability to reduce oxidative stress and enhance antioxidant defense mechanisms. The reduction in ALT, AST, ALP, and bilirubin levels suggests a protective effect on hepatocyte membranes and overall liver function. The preservation of liver histology further supports these biochemical findings.

Potential of ABCR Compounds

Oleanolic acid, stigmasterol, and β-sitosterol have been previously reported to possess anti-inflammatory, antioxidant, and hepatoprotective properties. This study confirms their efficacy in protecting against INH+RMP-induced hepatotoxicity, highlighting their potential as therapeutic agents in managing drug-induced liver injury.

Key Findings:

1. **Cell Viability and In Vivo Studies:**
    - The MTT assay showed that ABCR compounds significantly enhanced cell viability in Chang liver cells exposed to INH+RMP.
    - In vivo studies revealed that INH+RMP treatment caused a significant increase in serum levels of liver enzymes (ALT, AST, ALP) and bilirubin, indicating liver damage. These adverse effects were notably mitigated in rats pre-treated with ABCR compounds and Silymarin, a standard hepatoprotective agent.

2. **Histopathological Examination:**
    - Severe hepatocellular damage, including necrosis and inflammation, was observed in the INH+RMP group.
    - Pre-treatment with ABCR compounds preserved liver histology, with ABCR2 demonstrating the highest level of protection, comparable to Silymarin.
3. **Biochemical and Antioxidant Enzyme Analyses:**
    - ABCR pre-treatment resulted in significant reductions in ALT, AST, ALP, and bilirubin levels, indicating hepatoprotection.
    - Antioxidant enzyme activities (SOD, catalase) were significantly restored in the liver homogenates of ABCR-treated rats, suggesting reduced oxidative stress.

Apart from the hepatoprotective, the combination provides a wide-uses like treatment of diarrhea, hemorrhoids, gynecological disorders, osteoporosis, diabetes, eye and ear infections, wound healing, jaundice, skin diseases, etc.

Conclusion

The present study demonstrates the hepatoprotective potential of ABCR compounds—oleanolic acid, stigmasterol, and β-sitosterol—derived from Andrographis paniculata, Berberis aristata, Cichorium intybus, and Rubia cordifolia root extracts. These compounds were investigated against isoniazid (INH) and rifampicin (RMP)-induced hepatotoxicity in Chang liver cells and male Wistar albino rats.

Mechanisms of Hepatoprotection: The hepatoprotective effects of ABCR compounds are likely due to their antioxidant and anti-inflammatory properties. The observed reduction in liver enzyme levels and preservation of liver histology underscore the ability of these compounds to protect hepatocytes from INH+RMP-induced oxidative damage.

Clinical Implications: The findings of this study suggest that ABCR compounds hold significant potential in preventing and managing drug-induced hepatotoxicity in TB patients. Given the efficacy observed in this study, further clinical trials are warranted to explore their therapeutic utility in a clinical setting.

Additional Benefits: Besides their hepatoprotective properties, the ABCR compounds also offer benefits for treating a variety of conditions, including diarrhea, hemorrhoids, gynecological disorders, osteoporosis, diabetes, eye and ear infections, wound healing, jaundice, and skin diseases.

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