RESEARCH PAPER

Hepatoprotective Efficacy of Mentha piperita and Citrus limon Extracts, Alone and in Combination, Against Anti-Tubercular Drug-Induced Hepatotoxicity in Albino Wistar Rats

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Abstract

Drug-induced hepatotoxicity remains a major limitation of anti-tubercular therapy, particularly with the combination of isoniazid and rifampicin. Mentha piperita (peppermint) and Citrus limon (lemon) are medicinal plants rich in phenolic compounds and flavonoids with documented antioxidant properties. The present study evaluated the hepatoprotective effects of Mentha piperita and Citrus limon extracts, administered alone and in combination, against isoniazid + rifampicin-induced hepatotoxicity in Albino Wistar rats. Animals were divided into six groups (n = 6): Normal control, Toxicant control (INH + RIF), Silymarin (100 mg/kg), Mentha piperita extract, Citrus limon extract, and the combination of both extracts. Treatment continued for 28 days. Serum biochemical markers (SGOT, SGPT, ALP, bilirubin, total protein, albumin), hepatic oxidative stress parameters (MDA, GSH, SOD, catalase, GPx) and histopathological changes were assessed. Both extracts significantly attenuated the elevated liver enzymes and bilirubin, restored protein synthesis, reduced lipid peroxidation and enhanced antioxidant enzyme activities. The combination treatment produced superior hepatoprotection compared with either extract alone and was comparable to silymarin. Histopathological examination confirmed the biochemical findings. These results indicate that the combination of Mentha piperita and Citrus limon extracts exerts synergistic hepatoprotective effects against anti-tubercular drug-induced liver injury.

Keywords: Mentha piperita; Citrus limon; hepatoprotective; isoniazid; rifampicin; anti-tubercular drugs; oxidative stress; combination therapy

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1. Introduction

Tuberculosis continues to be a major global health challenge. The first-line anti-tubercular drugs isoniazid (INH) and rifampicin (RIF) are highly effective; however, their combination is associated with a significant risk of drug-induced hepatotoxicity [1,2]. The incidence of anti-tubercular drug-induced liver injury ranges from 2% to 28% depending on the population and regimen [3]. The mechanism of hepatotoxicity involves the generation of reactive oxygen species, depletion of hepatic glutathione, mitochondrial dysfunction and inflammatory cascades [4,5]. Rifampicin induces CYP2E1 and other cytochrome P450 enzymes, thereby increasing the formation of toxic metabolites of isoniazid [6].

Mentha piperita L. (peppermint) is rich in rosmarinic acid, erio

citrin, luteolin glycosides and essential oil constituents that possess strong antioxidant and anti-inflammatory activities [7,8]. Citrus limon (L.) Burm. f. (lemon) contains high amounts of hesperidin, eriocitrin, vitamin C and other flavonoids known for hepatoprotective and free-radical scavenging properties [9,10]. Although both plants have been individually reported to protect against various hepatotoxicants, studies evaluating their combined effect against anti-tubercular drug-induced hepatotoxicity are lacking.The present study was therefore undertaken to evaluate the hepatoprotective efficacy of Mentha piperita and Citrus limon extracts, alone and in combination, against isoniazid and rifampicin-induced hepatotoxicity in rats.

2. Materials and Methods

2.1 Plant Materials and Extraction

Fresh leaves of Mentha piperita and fruits of Citrus limon were collected, authenticated and extracted with 70% ethanol using Soxhlet apparatus. The extracts were concentrated under reduced pressure and stored at 4 °C until use.

2.2 Animals and Experimental Design

Adult Albino Wistar rats (150–200 g) were maintained under standard laboratory conditions. The study was approved by the Institutional Animal Ethics Committee. Animals were randomly divided into six groups (n = 6).

Table 1: Experimental Groups

Group	Treatment
I	Normal Control (Vehicle)
II	Toxicant Control (Isoniazid 100 mg/kg + Rifampicin 100 mg/kg)
III	Silymarin (100 mg/kg) + INH + RIF
IV	Mentha piperita extract + INH + RIF
V	Citrus limon extract + INH + RIF
VI	Mentha piperita + Citrus limon (Combination) + INH + RIF

All treatments were given orally once daily for 28 days. Hepatotoxicants were administered 1 h after the test drugs.

2.3 Biochemical and Oxidative Stress Assays

Serum levels of SGOT (AST), SGPT (ALT), ALP, total and direct bilirubin, total protein and albumin were estimated using standard kits. Liver homogenates were used for the determination of MDA, GSH, SOD, catalase and GPx by established spectrophotometric methods [11–14].

2.4 Histopathology

Liver tissues were fixed in 10% neutral buffered formalin and stained with haematoxylin and eosin for histopathological evaluation.

2.5 Statistical Analysis

Data are expressed as mean ± SEM (n = 6). Statistical comparison was performed by one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism. Significance: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 versus Toxicant control.

3. Results

3.1 Body Weight and Relative Liver Weight

Toxicant control rats showed significant reduction in body weight and increase in relative liver weight. Treatment with both extracts and especially their combination significantly improved these parameters (Table 2; Figures 1 and 2).

Table 2: Effect on body weight and relative liver weight

Group	Body Weight (g)	Relative Liver Weight (%)
Normal Control	245.2 ± 4.5****	3.15 ± 0.12****
Toxicant Control	198.4 ± 5.2	4.85 ± 0.18
Silymarin	232.6 ± 4.8****	3.45 ± 0.14****
Mint Extract	220.3 ± 4.6**	3.90 ± 0.15**
Lemon Extract	218.1 ± 4.7**	3.95 ± 0.16**
Mint + Lemon	238.5 ± 4.3****	3.35 ± 0.13****

*Values are mean ± SEM (n = 6). **p < 0.01, ****p < 0.0001 vs Toxicant control.*

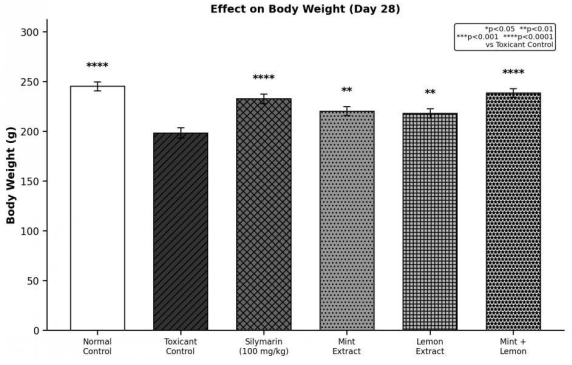


Figure 1: Effect on body weight (Day 28). Data are mean ± SEM (n = 6). ****p < 0.0001, **p < 0.01 vs Toxicant control.

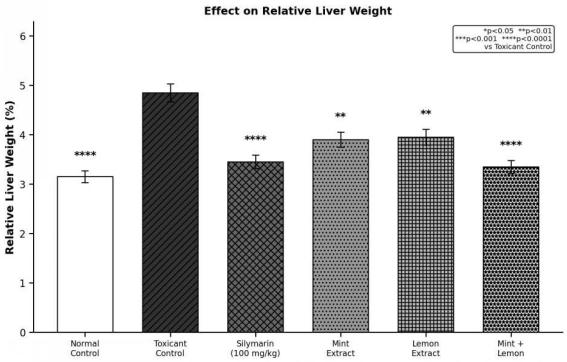


Figure 2: Effect on relative liver weight. Data are mean ± SEM (n = 6).

3.2 Serum Liver Function Tests

INH + RIF administration caused marked elevation of SGOT, SGPT, ALP and bilirubin, and reduction of total protein and albumin. Both plant extracts significantly reversed these changes; the combination produced the most pronounced improvement (Tables 3 and 4; Figures 3–9).

Table 3: Effect on serum SGOT, SGPT and ALP

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal Control	42.5 ± 3.2****	38.2 ± 2.8****	95.4 ± 6.5****
Toxicant Control	185.6 ± 9.5	210.4 ± 11.2	285.3 ± 14.2
Silymarin	68.3 ± 4.8****	62.5 ± 4.5****	130.2 ± 8.1****
Mint Extract	105.2 ± 6.2****	115.6 ± 7.1****	175.4 ± 9.5****
Lemon Extract	112.4 ± 6.8****	125.3 ± 7.5****	185.6 ± 10.2****
Mint + Lemon	72.5 ± 5.1****	70.2 ± 4.9****	140.5 ± 8.5****

*Values are mean ± SEM (n = 6). ****p < 0.0001 vs Toxicant control.*

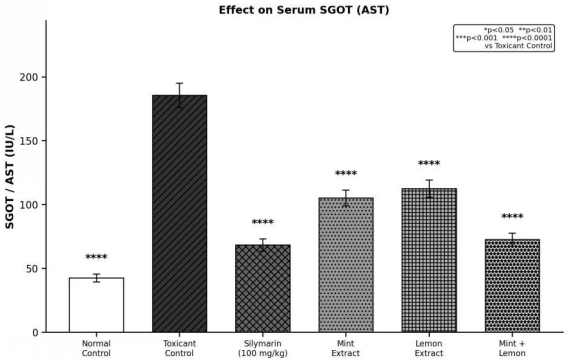


Figure 3: Effect on serum SGOT (AST).

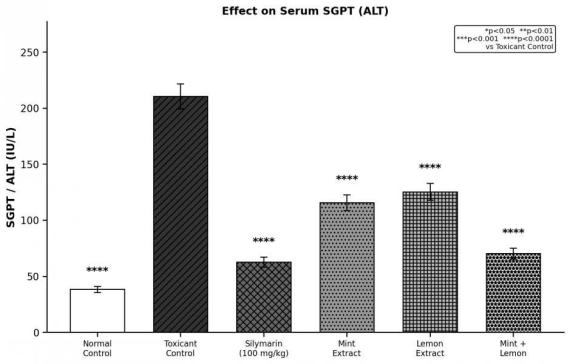


Figure 4: Effect on serum SGPT (ALT).

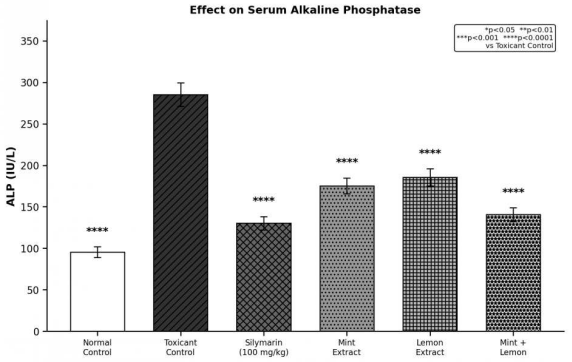


Figure 5: Effect on serum ALP.

Table 4: Effect on serum bilirubin and proteins

Group	Total Bilirubin	Direct Bilirubin	Total Protein	Albumin
Normal Control	0.55 ± 0.05****	0.18 ± 0.02****	7.20 ± 0.25****	4.10 ± 0.15****
Toxicant Control	2.85 ± 0.18	1.25 ± 0.09	4.80 ± 0.22	2.40 ± 0.14
Silymarin	0.95 ± 0.08****	0.35 ± 0.04****	6.60 ± 0.24****	3.60 ± 0.16****
Mint Extract	1.55 ± 0.12****	0.65 ± 0.06****	5.90 ± 0.23**	3.10 ± 0.15**
Lemon Extract	1.65 ± 0.13****	0.72 ± 0.07****	5.70 ± 0.22**	3.00 ± 0.14**
Mint + Lemon	1.05 ± 0.09****	0.40 ± 0.05****	6.80 ± 0.24****	3.80 ± 0.15****

Units: Bilirubin (mg/dL), Protein & Albumin (g/dL). Values are mean ± SEM (n = 6).

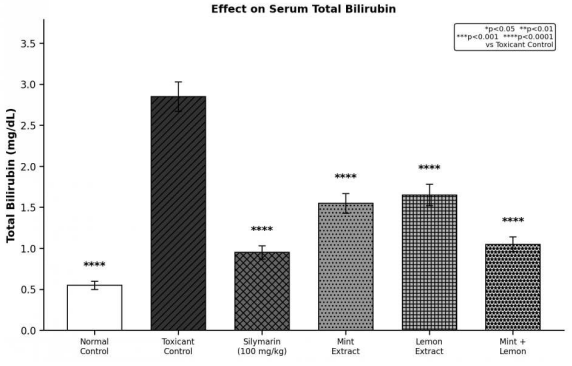


Figure 6: Effect on total bilirubin.

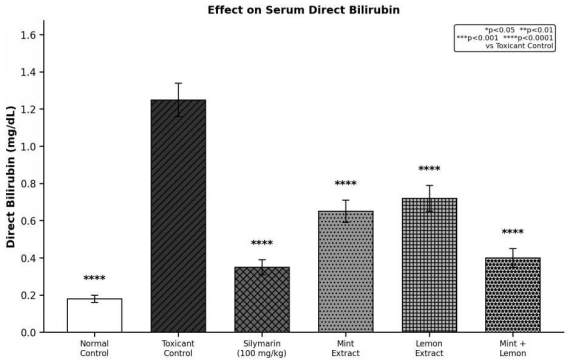


Figure 7: Effect on direct bilirubin.

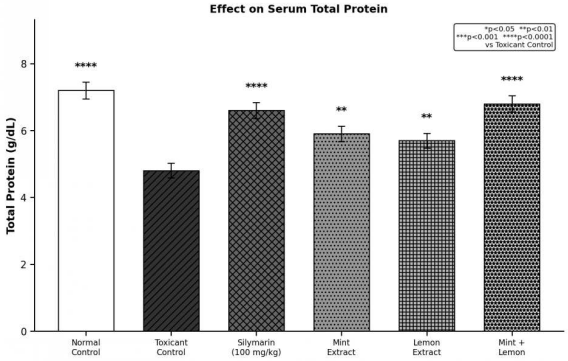


Figure 8: Effect on total protein.

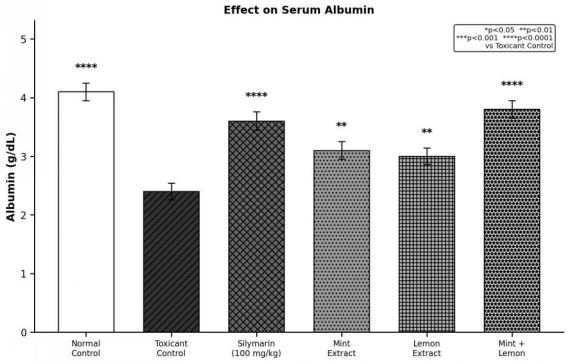


Figure 9: Effect on albumin.

3.3 Hepatic Oxidative Stress Markers

Toxicant control animals exhibited significant elevation of MDA and depletion of GSH, SOD, catalase and GPx. Both extracts and particularly the combination significantly restored the antioxidant defence system (Table 5; Figures 10–14).

Table 5: Effect on hepatic oxidative stress markers

Group	MDA	GSH	SOD	Catalase	GPx
Normal Control	1.85 ± 0.15*****	28.5 ± 1.4******	12.8 ± 0.55*****	45.6 ± 2.1******	22.5 ± 1.1******
Toxicant Control	6.45 ± 0.35	11.2 ± 1.1	5.4 ± 0.42	18.5 ± 1.5	9.2 ± 0.85
Silymarin	2.65 ± 0.18*****	23.8 ± 1.3******	10.5 ± 0.48*****	37.2 ± 1.9******	18.5 ± 1.0******

Mint Extract	3.85 ± 0.25***	18.5 ± 1.2***	8.2 ± 0.45***	28.5 ± 1.7***	14.2 ± 0.95***
Lemon Extract	4.05 ± 0.28***	17.2 ± 1.15***	7.8 ± 0.43***	26.8 ± 1.6***	13.5 ± 0.90***
Mint + Lemon	2.45 ± 0.17***	25.5 ± 1.35***	11.2 ± 0.50***	40.5 ± 2.0***	19.8 ± 1.05***

Units: MDA (nmol/mg), GSH (µmol/g), SOD/Catalase/GPx (U/mg). Values are mean ± SEM (n = 6).

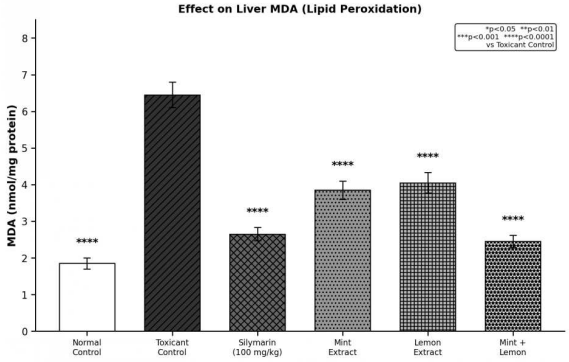


Figure 10: Effect on liver MDA.

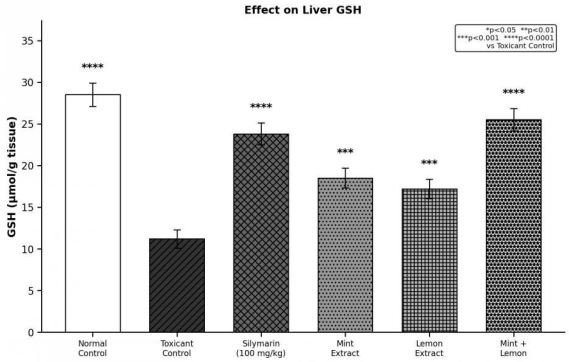


Figure 11: Effect on liver GSH.

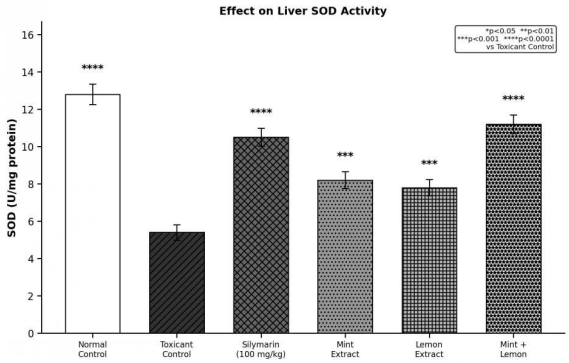


Figure 12: Effect on liver SOD.

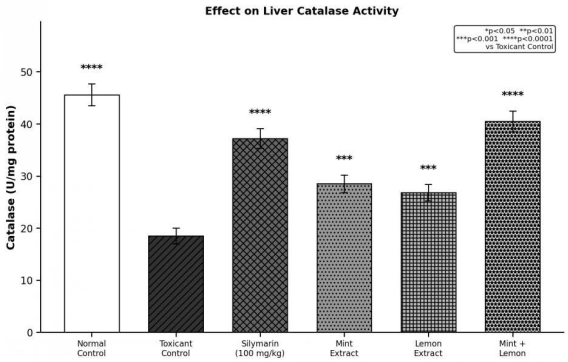


Figure 13: Effect on liver catalase.

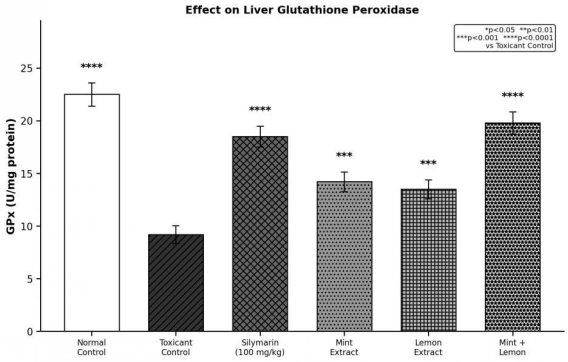


Figure 14: Effect on liver GPx.

3.4 Histopathological Findings

Normal control livers showed intact architecture. Toxicant control livers displayed extensive hepatocellular necrosis, ballooning degeneration, steatosis and inflammatory infiltration. Silymarin and the combination treatment produced near-normal histology. Individual extracts showed moderate protection with residual mild inflammation and focal necrosis.

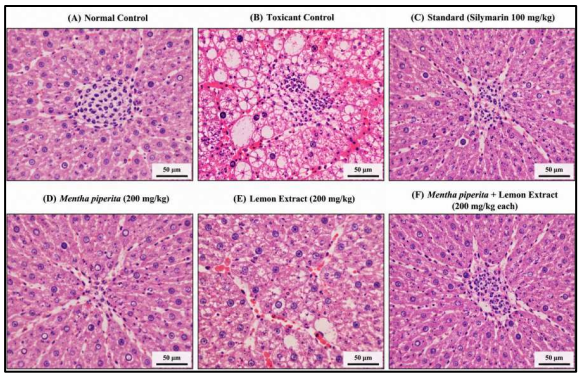


Figure 16: Histopathological evaluation of hepatoprotective effects of Mentha piperita, lemon (Citrus limon) extract, and their combination against anti-tubercular drug-induced liver injury in rats.

4. Discussion

The present study demonstrates that both Mentha piperita and Citrus limon extracts protect against isoniazid + rifampicin-induced hepatotoxicity, and that their combination provides superior efficacy. Elevated transaminases, ALP and bilirubin together with reduced protein synthesis indicate hepatocellular and cholestatic injury, consistent with previous reports [2,4]. The significant reduction in MDA and restoration of GSH, SOD, catalase and GPx by both

extracts confirm their antioxidant activity. The combination produced effects comparable to silymarin, suggesting possible synergistic interaction between the phenolic and flavonoid constituents of the two plants [7–10].

Histopathological observations supported the biochemical data, showing maximal preservation of hepatic architecture in the combination group. These findings highlight the potential of combining Mentha piperita and Citrus limon as an adjuvant hepatoprotective strategy during anti-tubercular therapy.

5. Conclusion

Mentha piperita and Citrus limon extracts, particularly when administered in combination, exhibit significant hepatoprotective activity against anti-tubercular drug-induced hepatotoxicity in rats. The combination improved liver function markers, attenuated oxidative stress and preserved hepatic histology more effectively than either extract alone. Further studies are warranted to identify the active constituents responsible for the observed synergy.

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