Hepatoprotective activity of Mentha arvensis in anti-tubercular drugs induced hepatotoxicity in rats

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Abstract

*Mentha arvensis* L. (Lamiaceae), usually known as Pudina, and traditionally used for various therapeutic purposes like food seasoner, household remedy, and industrial purposes etc. The present study appraised the hepatoprotective activity of the methanol extract of *Mentha arvensis* L. (MEMA) against by isoniazid and rifampicin (50 mg INH–100 mg RMP) induced liver impairment in rats. The dose of MEMA was 500 and 1000 mg/kg daily for 28days given by orally and Silymarin as a standard drug (100 mg/kg) was used. On basis of the biochemical estimation of liver function tests (SGPT, SGOT, ALP, total protein and total bilirubin), antioxidant assays of liver homogenate (lipid peroxidation, reduced glutathione content, superoxide dismutase and catalase activity) and histological study of liver tissue, the hepatoprotective activity were evaluated. The treated animals, the toxicity of INH were controlled prominently by restoration of the biochemical parameters as well as by the improvement of the antioxidant status as normal values. The results demonstrate that the methanol extract of *Mentha arvensis* has hepatoprotective activity against INH induced hepatotoxicity in rats.

**KEYWORDS:** Anti-tubercular drugs, hepatoprotective, antioxidant, Silymarin, *Mentha arvensis*.

INTRODUCTION

As per the report of World Health Organization (WHO), based on several publishing about codex of 91 countries (pharmacopeia) and medicinal plants may be approximately 20 thousand. Mentha species have essential oil, there are basic constituents such as menthol (33-60%), menthone (15-32%), isomenthone (2-8%), 1.8 sineol (eucalyptols) (5-13%), menthyl acetate (2-11%) menthofuran (1-7%), limonene (1-7%), β-myrcene (0.1-1.7%), β-caryophyllene (2-4%), pulegone (0.5-1.6%) carvone (1%) [1].

Mentha species have important therapeutic effects such as anti-inflammatory, analgesic, DNA damage protecting activity, antipyretic, anti-androgenic, antioxidant, antimicrobial, cytotoxic, antiviral, antiemetic, antibacterial, antiparasitic, antiallergic, sedative, anti-chlamydial, radioprotection, anti-cholinesterase, antispasmodic, acute toxicity effect, anti-mutagenic, cardiovascular effects and anti-tumour effects [2-6]. It is reported that plant based therapeutic agents like silymarin from Silybum marianum (milkthistle) are used worldwide as hepatoprotective [7].

Tuberculosis (TB), a disease which is caused by a bacillus (*Mycobacterium tuberculosis*). TB remains a severe health problem among certain world populations in the developing countries [8]. The most frequent adverse effects of anti-tubercular drugs are skin reactions, hepatitis and gastro-intestinal problèmes [9, 10]. The major deterrent to the prolonged use of both drugs is their potential to cause hepatotoxicity and further increased when the two drugs are used in combination [11, 12]. On the basis previous research, we have seen that *Mentha arvensis* proved as potential pharmacological effects in several experimental models. However, there are no reported any hepatoprotective effect in isoniazid (INH) and Rifampin (R) induced experimental model of hepatotoxic. Hence, this study is to propose the evaluate the hepatoprotective activity of *Mentha arvensis* in anti-tubercular drug induced hepatotoxicity in rats.

**MATERIALS AND METHODS:**

**Plant materials:**

The chemicals used in this research work were analytical grade. The leaves of *Mentha arvensis* were purchased from local market of Alwar. To remove dirt and soil, the freshly collected leaves of *Mentha arvensis* were washed with distilled water and dried in shade in a ventilated place at room temperature.
Preliminary phytochemical screening for presence of different phytoconstituents

The extracts of the plant material were submitted to preliminary phytochemical screening for the detection of various phytochemical constituents.

Extraction of plant material

500 gm coarse powdered of air dried leaves of *Mentha arvensis* loaded down in muslin cloth and submitted to Soxhlet Extractor for continuous Hot extraction with petroleum ether, chloroform, alcohol and distilled water, separately. Then the all extracts were filtered and filtrate was evaporated.

Animal

Adult Wistar rats (male) weighing 160 ± 20 g were procured from the Alwar Pharmacy College, Alwar. For the housing of animals, separate polypropylene cages were acclimatized at a temperature- and humidity-controlled room at an ambient temperature of 25° ±2 °C with 12 h light/dark cycle before and during the experimental instigation. Animals were kept on a standard pellet diet ((Lipton, Calcutta, India) and water ad libitum. Administration of extract was done orally, and that for toxicant was done by intraperitoneal (i.p) route in the morning session throughout the study period. All investigational proceedings concerned with animals were conducted as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The study protocol was approved by the Ethical Institutional Animal Ethical Committee.

**In vivo study of plant extract**

The rats were randomly distributed into 6 groups, each group containing 5 rats. In this study hepatotoxicity is induced by isoniazid and rifampicin (50 mg INH–100 mg RMP). Drugs treatment was given as per the schedule given below. Isoniazid and rifampicin were administered per oral after the half an hour from administration of test drugs in each group for 28th days and with draw blood from rat tail vein under ether anesthesia for liver function test.

### Table 1: Experimental Schedule

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drug Treatment</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>CMC (5ml/kg p.o.) once a day for 28th days</td>
</tr>
<tr>
<td>II</td>
<td>INH+R</td>
<td>50 mg/kg +100mg/kg each, p.o daily for 28th days</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (S)+INH+R</td>
<td>100 mg/kg(S) p.o and 50 mg/kg(INH+R) each, p.o. daily for 28th days</td>
</tr>
<tr>
<td>IV</td>
<td>MA1+INH+R</td>
<td>500 mg/kg (MA1) p.o. daily and 50 mg/kg(INH+R) each for 28th days</td>
</tr>
<tr>
<td>V</td>
<td>MA2+INH+R</td>
<td>1000 mg/kg p.o daily and 50 mg/kg(INH+R) each for 28th days</td>
</tr>
<tr>
<td>VI</td>
<td>MA2 PERSE</td>
<td>1000 mg/kg only test drug p.o daily for 28th days</td>
</tr>
</tbody>
</table>

CMC-Carboxy methyl cellulose, INH-Isoniazid, R- Ripampicin, MA2. Higher dose of *Mentha arvensis* extract, MA1- Lower dose of *Mentha arvensis* extract.

### Parameters to be assessed

**Liver function test in serum**

Biochemical estimation

Estimation of SGOT (serum), Estimation of SGPT (serum) [13], Estimation of alkaline phosphatase (serum) [14], Estimation of total bilirubin (serum)[15], Serum was analyzed for the following parameters as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP) and total bilirubin (TB).

Biochemical estimation (in Liver tissue)

Thiobarbituric Acid Reactive Substances [16], Glutathione [17], Superoxide Dismutase [18], Catalase [19], Protein [20]

### Statistical analysis

The statistical data was executed by using GRAPHPAD INSTAT 3 software (Graph Pad Software Inc, San Diego, CA). Results got from experiments were expressed as arithmetic mean±SEM. The comparison between all groups was performed by one-way analysis of variance (ANOVA), and the effects in treatment groups were compared with toxic control or control group by the Dunnet multiple comparison test. *p<0.05 was considered to be significant [*p<0.05; **<50.01].

**RESULTS:**

**Phytochemical screening**

Phytochemical screening (Table-1) of the extract showed small quantities of alkaloids, saponins, flavonoids and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins.

### Table 1: Preliminary phytochemical evaluation of the leaves of *Mentha arvensis*

<table>
<thead>
<tr>
<th>Test for</th>
<th>Pet. Ether Extract</th>
<th>Chloroform Extract</th>
<th>Methanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fixed oils and fats</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Proteins and amino acids - - + -
Gum and Mucilage - - - -
Phenolic Compounds - - - -
Volatile oils - - - -

Present (+); Absent (-)

Table 2: Effect of Mentha arvensis on TBAR and Antioxidant enzymes

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment</th>
<th>TBARs (nmol of MDA/ mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>SOD (units/mg protein)</th>
<th>CATALASE (nmol of H2O2/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.314±0.021</td>
<td>3.019±0.237</td>
<td>2.349±0.317</td>
<td>4.45±0.763</td>
</tr>
<tr>
<td>II</td>
<td>INH+R</td>
<td>1.917±0.051##</td>
<td>0.798±0.156##</td>
<td>0.701±0.217##</td>
<td>1.32±0.288##</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin(S)+INH+R</td>
<td>0.413±0.038**</td>
<td>2.831±0.309**</td>
<td>2.042±0.401**</td>
<td>4.21±0.892**</td>
</tr>
<tr>
<td>IV</td>
<td>MA1+INH+R</td>
<td>0.719±0.012*</td>
<td>1.452±0.312*</td>
<td>1.071±0.211</td>
<td>3.069±0.921</td>
</tr>
<tr>
<td>V</td>
<td>MA2+INH+R</td>
<td>0.39±0.081**</td>
<td>2.621±0.287**</td>
<td>2.008±0.097**</td>
<td>4.031±1.321**</td>
</tr>
<tr>
<td>VI</td>
<td>MA2 PERSE</td>
<td>0.348±0.078</td>
<td>2.881±0.213</td>
<td>2.412±0.305</td>
<td>4.37±1.932</td>
</tr>
</tbody>
</table>

Result are expressed as MEAN ±SEM, ## P<0.01; Toxic control group Vs Normal control group, ** P<0.01; Treated group Vs Toxic control group, * P<0.05; Treated group Vs Toxic control group

Table 3: Effects of Mentha arvensis on liver profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Treatment</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>TOTAL BILIRUBIN (mg/100ml)</th>
<th>TOTAL PROTEIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>45.73±7.342</td>
<td>21.42±3.146</td>
<td>11.543±1.243</td>
<td>1.76±0.762</td>
<td>9.762±2.617</td>
</tr>
<tr>
<td>II</td>
<td>INH+R</td>
<td>108.52±6.31#</td>
<td>75.03±2.072#</td>
<td>57.782±3.671#</td>
<td>4.21±0.971##</td>
<td>4.43±1.421##</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (S)+INH+R</td>
<td>59.04±6.31**</td>
<td>38.29±1.873*</td>
<td>13.419±0.987*</td>
<td>1.91±0.432**</td>
<td>8.761±2.108**</td>
</tr>
<tr>
<td>IV</td>
<td>MA1+INH+R</td>
<td>88.77±9.12*</td>
<td>52.5±4.756*</td>
<td>36.672±1.21</td>
<td>2.98±0.512*</td>
<td>5.672±1.091</td>
</tr>
<tr>
<td>V</td>
<td>MA2+INH+R</td>
<td>62.32±2.11**</td>
<td>35.61±3.315*</td>
<td>14.781±1.671*</td>
<td>2.22±0.871**</td>
<td>8.001±2.812**</td>
</tr>
<tr>
<td>VI</td>
<td>MA2 PERSE</td>
<td>49.58±9.004</td>
<td>25.32±2.182</td>
<td>12.415±2.098</td>
<td>1.87±0.671</td>
<td>9.01±1.918</td>
</tr>
</tbody>
</table>

Result were expressed as MEAN ±SEM, ## P<0.01; Toxic control group Vs Normal control group, ** P<0.01; Treated group Vs Toxic control group, * P<0.05; Treated group Vs Toxic control group

Effect of Mentha arvensis of TBARS in liver tissue

The treated Group with INH+R exhibited significant (P<0.01) enhance in the levels of TBARS in the liver tissue compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R -induced rats significantly (P<0.01) reduced the levels of TBARS in the liver tissue as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R -induced rats significantly (P<0.01) decreased the levels of TBARS in the liver tissue as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R -induced rats significantly (P<0.05) reduced the levels of TBARS in the liver tissue as compared to group treated with INH+R.

Pre-treatment with only MIA2 rats non-significantly (P>0.05) decreased the levels of TBARS in the liver tissue as compared to group treated with normal saline (Table 2).

Effect of Mentha arvensis of SOD in liver tissue

Group treated with INH+R exhibited significant (P<0.01) reduce in the levels of SOD in the liver tissue compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R -induced rats significantly (P<0.01) enhanced the levels of SOD in the liver tissue as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R -induced rats significantly (P<0.01) increased the levels of SOD in the liver tissue as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R -induced rats significantly (P<0.01) increased the levels of SOD in the liver tissue as compared to group treated with INH+R.
nonsignificantly \( (P>0.05) \) increased the levels of SOD in the liver tissue as compared to group treated with INH+R. Pre-treatment with only MIA2 rats nonsignificantly \( (P>0.05) \) increased the levels of SOD in the liver tissue as compared to group treated with normal saline(Table 2).

**Effect of Mentha arvensis of GSH in liver tissue**

Group treated with INH+R exhibited significant \( (P<0.01) \) reduce in the levels of GSH in the liver tissue compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R -induced rats significantly \( (P<0.01) \) enhanced the levels of GSH in the liver tissue as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R - induced rats significantly \( (P<0.01) \) enhanced the levels of GSH in the liver tissue as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R -induced rats significantly \( (P<0.05) \) increased the levels of GSH in the liver tissue as compared to group treated with INH+R. Pre-treatment with only MIA2 rats nonsignificantly \( (P>0.05) \) changed the levels of GSH in the liver tissue as compared to group treated with normal saline(Table 2).

**Effect of Mentha arvensis of CATALASE in liver tissue**

Group treated with INH+R exhibited significant \( (P<0.01) \) reduce in the levels of CATALASE in the liver tissue compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R - induced rats significantly \( (P<0.01) \) enhanced the levels of CATALASE in the liver tissue as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R -induced rats significantly \( (P<0.01) \) increased the levels of CATALASE in the liver tissue as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R - induced rats nonsignificantly \( (P>0.05) \) increased the levels of CATALASE in the liver tissue as compared to group treated with INH+R. Pre-treatment with only MIA2 rats nonsignificantly \( (P>0.05) \) enhanced the levels of CATALASE in the liver tissue as compared to group treated with normal saline(Table 2).

**Effect of Mentha arvensis of SGOT in Serum**

Group treated with INH+R exhibited significant \( (P<0.01) \) enhance in the levels of total protein as compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R - induced rats significantly \( (P<0.01) \) reduced the levels of total protein in serum as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R - induced rats significantly \( (P<0.01) \) reduced the levels of total protein in serum as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R - induced rats nonsignificantly \( (P>0.05) \) reduced the levels of total protein in serum as compared to group treated with INH+R. Pre-treatment with only MIA2 rats nonsignificantly \( (P>0.05) \) changed the levels of total protein in serum as compared to group treated with normal saline(Table 3).

**Effect of Mentha arvensis of Total bilirubin in Serum**

Group treated with INH+R exhibited significant \( (P<0.01) \) enhance in the levels of total bilirubin as compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R - induced rats significantly \( (P<0.01) \) reduced the levels of total bilirubin in serum as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R - induced rats significantly \( (P<0.01) \) reduced the levels of total bilirubin in serum as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R - induced rats nonsignificantly \( (P>0.05) \) reduced the levels of total bilirubin in serum as compared to group treated with INH+R. Pre-treatment with only MIA2 rats nonsignificantly \( (P>0.05) \) changed the levels of total bilirubin in serum as compared to group treated with normal saline(Table 3).

**Effect of Mentha arvensis of SGPT in Serum**

Group treated with INH+R exhibited significant \( (P<0.01) \) enhance in the levels of SGPT as compared to group treated with Normal Saline. Pre-treatment with MIA2 in INH+R - induced rats significantly \( (P<0.01) \) reduced the levels of SGPT in serum as compared to group treated with INH+R. Pre-treatment with Silymarin in INH+R - induced rats significantly \( (P<0.01) \) reduced the levels of SGPT in serum as compared to group treated with INH+R. Pre-treatment with MIA1 in INH+R - induced rats significantly \( (P<0.05) \) reduced the levels of SGPT in serum as compared to group treated with INH+R. Pre-treatment with only MIA2 rats nonsignificantly \( (P>0.05) \) changed the levels of SGPT in serum as compared to group treated with normal saline(Table 3).

**DISCUSSION**

Tuberculosis still a serious health problem among certain world populations in the developing countries [8]. With the coming of anti-tubercular agents, there has been a graded improvement in the history of the disease. Among the anti- tubercular agents, isoniazid (INH) and rifampin (RMP) are the first-line agents and a backbone of combination therapy along with second-line drugs, pyrazinamide, ethambutol and streptomycin. Anti-tubercular agents- induced hepatotoxicity causes significant morbidity and mortality. The major deterrent to the prolonged use of both drugs is their possible to cause hepatotoxicity and further increased when the two drugs
are used in combination [11, 12]. The past studies have proposed a possible role of enhanced formation of toxic INH metabolites (acetylisoniazid, acetylhydrazine, hydrazine and their reactive products) by RMP and the resultant oxidative injury in the hepatotoxicity caused by combination drugs [13].

**Figure 1:** Histopathology of liver from control and experimental groups of animals, circle mark indicated that damage of liver cell. (a) Liver section of normal control Group I, (b) liver incision of antitubercular drug (INH+R)-treated Group II, (c) liver section of (INH+R) and Mentha arvensis extract(500 mg/kg)-treated Group IV, (d) liver section of (INH+R) and Mentha arvensis extract (1000 mg/kg)treated Group V, and (e) liver section of rats treated (INH+R)- 50mg and 100 mg/kg of silymarin Group III.

The efforts have been built in the past to investigate the hepatoprotective agents of some herbal preparations, synthetic compounds or natural antioxidants against the toxicity of these drugs. Silymarin, an extract from the seeds of *S. marianum*, has been shown to protect rat liver against toxic effects of antitubercular drugs [7]. It has reported that *Mentha arvensis* Linn. has been used as antiseptic, carminative, headache, stomachic, and a refrigerant. Therapeutically, this plant has been proved to be useful in various conditions: Anti-ulcerogenic Effect, inflammatory bowel syndrome. However, there is no data is available on the effectiveness of *Mentha arvensis* in the liver diseases and in particular hepatic injury induced by antitubercular drugs. The present study is designed to evaluate the hepatoprotective and antioxidant potential of *Mentha arvensis* against liver injury caused by INH and RMP combination in rats in order to understand the mechanism of hepatoprotective action of *Mentha arvensis*.

Our study hepatotoxicity was induced in rats by intraperitoneal administration of anti-tubercular drugs combination of INH and R. The stage of liver injury was apparent from about two fold increase in serum marker enzymes, which was in line with the observations reported in previous studies where this model has been used to clarify the mechanism of hepatotoxicity produced by anti-tubercular drugs. Various mechanisms have been used to explain the toxicity of these drugs. Recent studies have advised that the toxic metabolites of INH, mainly CYP 2E1-mediated reactive oxidative products of hydrazine and acetylhydrazine, play a essential role in liver injury. The use of INH and R in combination leads to enhance the risk of hepatotoxicity in both clinical and experimental studies. Rifampin alone also may also raise hepatocellular dysfunction. But earlier its mechanism of hepatotoxicity was unknown. Later on, it was described that rifampin particularly, activates cytochrome P450 (CYP3A4) via hepatocyte xeno sensing pregnane X receptor (PXR) which leads to increased metabolism of isoniazid and thus formation of toxic metabolites. Rifampin, a potent inducer of xenobiotic metabolising enzymes and increase the metabolism of isoniazid by a hydrolase and CYP 2E1 to reactive toxic metabolites. Pretreated with the extract of *Mentha arvensis* protect the liver tissue by significant reducing the liver markers enzyme in the serum.

Pre-administration of *M. Arvensis* with (INH-R) combination in our research resulted in marked protection against the INH-R induced hepatotoxicity. This was further supported by restoration of liver marker enzymes by *M. Arvensis* treatment. Moreover, the ethanol extract of *Mentha arvensis* Linn. reported as hepatoprotective and antioxidant activity against carbon tetrachloride induced hepatic damage in rats. In fact, Pre-administration of *M. Arvensis* with (INH-R) combination preserved the hepatocytes cell by restoring antioxidant activity and liver marker enzymes.

**CONCLUSION:**

On the basis of results, The results of the our histopathological study (Figure 8) suggest that the fundamental mechanisms of hepatoprotective action of *Mentha Arvensis* and its phytochemical constituents regard suppression of oxidative stress, reinforcement of cellular antioxidant defenses, along with restoration of liver cell membrane stabilization by reducing liver marker enzymes in INH- R induced hepatotoxicity. *Mentha Arvensis* may be used as a hepatoprotective herbal drug, especially in the patients receiving anti-tubercular therapy which are at a risk of

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hepatotoxicity. Still further research may be expressed out with multiple doses of Mentha Arvensis in various experimental models of hepatotoxicity with a longer duration to confirm our hypothesis.

REFERENCES:


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Abbreviations used: None