HEPATOPROTECTIVE ACTIVITY OF MAHONIA LESCHENAULTII TAKEDA. (ROOT WOOD AND ROOT BARK) ON COUNTRY MADE-LIQUOR (CML) INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT:
The hepatoprotective potential of Mahonia leschenaultii Takeda. (root wood and root bark) extract was evaluated in Country-Made Liquor (CML)-induced hepatotoxicity in Wister rats. The rats were made hepatotoxic by administering (CML) (3 ml/100g body weight/day in two divided doses) and corn oil (1 ml/100g body weight /day in a single dose) orally for 21 days. The administration of root wood and root bark of Mahonia leschenaultii Takeda. extract (200 mg/kg body weight) mixed with tween 80 (0.5% w/w) orally from day 15 to day 21 along with CML. The different biochemical parameters tested are ALT, AST, ALP, LDH, TGL, total protein, total cholesterol, Albumin, total bilirubin and direct bilirubin. The results were compared with standard drugs i.e. Silymarin (70 mg/kg body weight) orally. The histopathological study was carried out and histopathological changes were observed and compared. The study showed that the extract of root wood and root bark of Mahonia leschenaultii Takeda. is definitely a hepatoprotective agent and help in reduction of liver damage.

Key words: Hepatoprotective, Mahonia leschenaultii Takeda. Country-Made Liquor; Root wood, and Root bark.

INTRODUCTION
Liver is a major organ system involved in the metabolism of various drugs, xenobiotics and toxins. During the metabolism, free radicals are generated and may cause liver damage.

Alcoholic liver diseases, common consequence of prolonged and heavy alcohol intake are a leading health problem after cardiovascular disease and cancer. Alcohol in the form of Country-Made Liquor (CML) containing 28.50% v/v ethanol content is consumed for thousands of year by the people is a common cause for Reactive Oxygen Species (ROS) insult in the liver 1. Despite the claim that small amount of alcohol consumption may be beneficial for preventing and reducing the rate of coronary heart diseases and ischemic heart stoke, it should also noted that alcohol is toxic to almost every organ of the body especially to the liver 2.

In the absence of reliable liver protective drugs in allopathic medical practices, naturally occurring compounds have been found to have major role in the management of various liver diseases. Numerous medicinal plants and their formulation are used for liver disorder in ethno medical practices and in traditional systems of medicine in India 3. However a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued.

Mahonia leschenaultii Takeda. Belonging to the family berberidaceae 4, 5 are abundant in Nilgiris district of Tamil Nadu. The plant is reported to contain alkaloids like oxyacanthine, berberine, neprotine, palmatine and jatrorrhizine 6. The plant was reported that a group of people called Todas, use the past of the stem bark of the plant for woman in postnatal treatment 7. It is reported that 50% ethanolic extracts of the aerial parts have effect on respiration and have diuretic activity 8.

The literature survey reveal that much work was not done on the plant and based on this and by careful study of the above points, I felt to do extensive study on the plant, which is native to the Nilgiris. Hence the present study deals with the hepatoprotective study of Mahonia leschenaultii Takeda. root wood and root bark.

MATERIAL AND METHODS
Collection and Treatment
The plant Mahonia leschenaultii Takeda. is found in various parts of Nilgiris, such as Ootacamund, Pykara and Kotagiri. For my work, the plant was collected from Doddabetta region of Ootacamund by peeling the root in the month of June and was identified, confirmed and authenticated by botanist Dr. S. Rajan, MSc, DPM, DCA, PhD, Field Botanist, Survey of Medicinal Plants & Collection Unit. (Central Council for Research in Homoeopathy), Department of AYUSH, Ministry of
Preparation of Drug Material

The root wood and root bark of the plant was extracted successfully with methanol. The solvent is purified by standard procedure. The dried powder drug was loosely packed into the soxhlet apparatus and then extracted with methanol for 6 hours. The extract was concentrated by using condensation apparatus and then kept in the desiccators. The root wood and root bark extracts were prepared in tween 80 5% w/w. The extract of root wood and root bark solution was prepared at dose level of 200 mg/kg body weight.

Animals

A total of 30 Wistar rats of either sex weighing 200-250 g were used for the present investigation. They were procured from National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab and were housed in clean polycrystalline cages and maintained under standard laboratory condition (temperature 22 ± 2 °C relative humidity 55-65%, with dark/light 12/12h.). They were allowed free access to standard pellet died and water. The Animals were acclimatized to laboratory condition for one week prior to experiment. All experimental procedures described were approved by Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of control and Supervision of Experiment on Animal (CPCSEA).

Treatment Schedule

The rats were divided into 5 groups, with 6 animals in each group and were treated as under:-

**Group I**, normal healthy control group, received food and water.

**Group II**, pathogenic hepatotoxic, received Country-made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally for 21 days to induce hepatotoxicity.

**Group III**, treated group, received Country- made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally for 21 days followed by single dose (200 mg/ kg body weight) treatment of *Mahonia leschenaultii* Takeda.of root wood extract from day 15 to day 21.

**Group IV**, treated group, received Country- made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally for 21 days followed by single dose (200mg/ kg body weight) treatment of *Mahonia leschenaultii* Takeda. of root bark extract from day 15 to day 21.

**Group V**, standard group, received Country- made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally for 21 days followed by single dose (70 mg/ kg body weight) treatment of silymarin (standard drug) from day 15 to day 21.

Blood Collection

Blood samples were collected after the experimental period of 21 days. All the animals were anesthetized with diethyl ether. Blood were collected by retro-orbital plexus, kept for 30 minutes without disturbance, and then centrifuged for 15 - 20 minutes at 2000 rpm to separate the serum and was used for various biochemical estimation like Alanine aminotransferase (ALAT)10, Aspartate aminotransferase (ASAT), Alkaline phosphatase (ALP)11, Lactate dehydrogenase (LDH), Triglycerides (TGL), Total proteins (TP), Total cholesterol (TC), Albumin, Total bilirubin (TB) and Direct bilirubin (DB).

Histopathological Studies of Liver

The liver were excised quickly and kept in 10% buffer formalin. The material was processed by standard method.12, 13 paraffin block were made and sections were cut. The section were stained with haematoxylin and mounted on the glass slides. The histopathological changes were observed and compared.14

Histopathological study of liver of control group showed normal hepatocellular architecture (Group-I). Liver challenged with Country- made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally showed disarrangement of normal hepatic cells with massive interlobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (Group-II).

The treated rats 200 mg/kg body weight of root wood and root bark extract of *Mahonia leschenaultii* Takeda exhibited significant protection against Country- made Liquor (CML) (3 ml/kg body weight) intoxication (Group-III & Group-IV). The standard group treated with silymarin 70 mg/kg body weight exhibited significant protection against Country- made Liquor (CML) (3 ml/kg body weight) intoxication (Group-V).

Statistical Analysis

The data were expressed as mean ± SEM and analyzed using the one-way t-test analysis of the variance. A probability level of P < 0.05 was chosen as criterion of statistical significance.

RESULT AND DISCUSSION

The serum ALAT, ASAT, ALP, LDH, TGL, TP, TC, Albumin, TB and DB level in rats fed with 5% tween 80 (Group-I) along with were found stable throughout the experimental period (as shown in the table no. 1). Administration of Country- made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally for resulted in significant (P< 0.05) rise in the
ALAT, ASAT, ALP, LDH, TGL, TP, TC, Albumin, TB and DB level in rats, as compared to Group-I (Table 1). Oral administration of Mahonia leschenaultii Takeda. (root wood and root bark 200mg/kg body weight) extract and Silymarin (70mg/kg body weight) the rats attenuated the Country- made Liquor (CML) (3 ml/kg body weight) induced rise in serum ALAT, ASAT, ALP, LDH, TGL, TP, TC, Albumin, TB and DB level (Table 1). Histopathological changes were also studied in the liver and observed that the control group showed normal hepatocellular architecture (Group-I). Liver challenged with Country- made Liquor (CML) (3 ml/100gm body weight/day) in two divided dose and corn oil (1ml/100gm body weight/day in a single dose) orally showed disarrangement of normal hepatic cells with massive interlobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (Group-II). The treated rats 200 mg/kg body weight of root wood and root bark extract of Mahonia leschenaultii Takeda, exhibited significant protection against Country- made Liquor (CML) (3 ml/100gm body weight) intoxication (Group-III & IV). The standard group treated with silymarin 70 mg/kg body weight exhibited significant protection against Country- made Liquor (CML) (3 ml/kg body weight) intoxication (Group-V). The treated group is compared with the standard group and is near to comparable (Fig 1).

![Group-I](image1)
![Group-II](image2)
![Group-III](image3)
![Group-IV](image4)
![Group-V](image5)

**Figure 1: Histopathological Changes in the Liver**
Figure 2: Graphical representation of different biochemical parameters in serum of rats intoxicated with CML.
Table 1: Effect of Methanolic Extract of Root Wood, Root Bark of *Mahonia leschenaultii* Takeda. and Silymarin on Biochemical Parameters in Serum of Rats Intoxicated with Country-Made Liquor (CML).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>ALAT (U/L)</th>
<th>ASAT (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (U/L)</th>
<th>TGL (mg/dl)</th>
<th>Total Protein (mg/dl)</th>
<th>Total Cholestrol (mg/dl)</th>
<th>Albium (g/dl)</th>
<th>Total Direct bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 80</td>
<td>30.991 ± 0.734</td>
<td>82.683 ± 1.027</td>
<td>114.723 ± 1.800</td>
<td>224.981 ± 1.060</td>
<td>4.693 ± 0.854</td>
<td>6.184 ± 0.249</td>
<td>97.583 ± 0.480</td>
<td>5.415 ± 0.018</td>
<td>0.723 ± 0.242</td>
<td>0.242 ± 0.021</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Hepatotoxicant</td>
<td>97.425 ± 1.210(^a)</td>
<td>310.166 ± 1.520(^a)</td>
<td>275.283 ± 0.739(^a)</td>
<td>331.388 ± 0.537(^a)</td>
<td>32.425 ± 0.551(^a)</td>
<td>3.136 ± 0.446(^a)</td>
<td>45.625 ± 0.982(^a)</td>
<td>2.866 ± 0.156(^a)</td>
<td>2.645 ± 0.402(^a)</td>
<td>0.637 ± 0.034(^a)</td>
</tr>
<tr>
<td>CML(3ml/100g)</td>
<td>59.450 ± 0.500(^b)</td>
<td>155.575 ± 0.527(^b)</td>
<td>175.608 ± 0.398(^b)</td>
<td>269.816 ± 0.943(^b)</td>
<td>37.975 ± 0.570(^b)</td>
<td>4.318 ± 0.363(^b)</td>
<td>70.416 ± 1.022(^b)</td>
<td>3.976 ± 0.099(^b)</td>
<td>1.408 ± 0.131(^b)</td>
<td>0.476 ± 0.029(^b)</td>
</tr>
<tr>
<td>+ Root wood</td>
<td>49.641 ± 0.606(^b)</td>
<td>145.366 ± 0.382(^b)</td>
<td>135.150 ± 0.370(^b)</td>
<td>265.783 ± 0.685(^b)</td>
<td>44.816 ± 0.966(^b)</td>
<td>5.530 ± 0.403(^b)</td>
<td>77.050 ± 0.264(^b)</td>
<td>4.963 ± 0.166(^b)</td>
<td>0.992 ± 0.083(^b)</td>
<td>0.394 ± 0.041(^b)</td>
</tr>
<tr>
<td>+ Root bark</td>
<td>38.981 ± 0.494(^b)</td>
<td>92.710 ± 1.551(^b)</td>
<td>121.758 ± 1.083(^b)</td>
<td>132.541 ± 0.824(^b)</td>
<td>46.478 ± 0.334(^b)</td>
<td>5.760 ± 0.432(^b)</td>
<td>90.910 ± 0.600(^b)</td>
<td>5.433 ± 0.769(^b)</td>
<td>0.765 ± 0.033(^b)</td>
<td>0.282 ± 0.009(^b)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 6 animals
\( ^a \) denotes statistical significance in comparison to hepatotoxicant group with normal group at \( p < 0.05 \)
\( ^b \) denotes statistical significance in comparison to treated with hepatotoxicant group at \( p < 0.05 \)

The present study suggests that *Mahonia leschenaultii* Takeda. (root wood and root bark) prevents hepatotoxicity by reducing hepatic injury and exhibiting membrane stabilizing. Our result could be the first major step for research on hepatoprotective potential of *Mahonia leschenaultii* Takeda. (root wood and root bark) in liver injury.

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