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Hepatoprotective activity of methanolic root extract *Mahonia leschenaultii* of in wistar rats

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Abstract
The ethanolic extract of *Mahonia leschenaultii* root was screened for hepatoprotective in hepatotoxic Albino rats induced via isoniazid and rifampicin. The degree of protection was measured by estimating biochemical parameters such as serum total protein, serum glutamate pyruvate transaminases (ALT/SGPT), serum glutamate oxaloacetate transaminases (AST/SGOT) and alkaline phosphatase (ALP). The ethanolic extract (100 mg/kg, 200mg/kg, 400mg/kg) exhibited significant hepatoprotection in isoniazid and rifampicin in toxicated rats in a dose dependant manner. The hepatoprotective effects of the extract were comparable with the standard drug silymarin 75mg/kg body weight, orally.

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Keywords: *Mahonia leschenaultii*, Hepatoprotective activity, isoniazid, rifampicin, Silymarin

Introduction

The Liver is a key organ in the human body, regulating homeostasis and is a frequent target for a number of toxicants [1]. In spite of tremendous scientific advancement in the field of hepatology during recent years, liver problems are on the rise. Regrettably there are only a few drugs with serious side effects available for the treatment of liver ailments [2]. In view of the undesirable side effects of synthetic agents, there is growing focus towards the therapeutic evaluation of medicinal plants using systemic research methodology.

The genus *Mahonia* belongs to the Berberidaceae family. Around 70 species of the *Mahonia* are distributed worldwide, and have pharmacologically potential bioactive compounds. It is reported that leaves, roots, stems, and barks of *Mahonia* spp. have potential antibacterial, antifungal, anticancer, and anti-inflammatory effects, irrespective of curing dermatologica! Disorders [3, 4]. *Mahonia* (M.) *leschenaultii* is used by the ethnic tribes, especially Toda of Nilgiris for their medical practices. Stem bark paste of *M. leschenaultii* is used not only in postnatal treatment for women, fever, cold, and jaundice but also to arrest other complications of Nilgiri tribe Todas, India. In spite of berberine (a major component), other compounds such as 3', 8, 8-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1', 4, 4'-tetrone, canadine, oxoberberine, oxyberberine, 1-diphenylmethylsilyloxy-4-methoxybenzene, and griseofulvin were isolated from bark extract of this plant [5]. In view of the vast medicinal properties of *M. leschenaultii*, the present context aimed to explore the anticancer effect of methanol root extract of *M. leschenaultii* (MEML) and its isolated propitious compound berberine on Dalton's ascitic lymphoma (DAL) induced cancer in mice.

Experimental

Plant material

The roots of *Mahonia leschenaultii* collected from the surrounding areas of Ranga Reddy district, Telangana, India during the month of June and authenticated by Botanical survey of India (BSI), Hyderabad, Telangana. The authentication certificate number is No. BSI/HYD/6/24/2022-06/TECH/2114. Soon after collection the leaves were cleaned, dried in shade and crushed to a coarse powder, stored in an air tight plastic container, until further use.

Preparation of extract

The roots (MLRT) were subjected to shade dry and powdered. The powder (1Kg) was extracted three times by cold percolation method with 3 L methanol at room temperature for 72 h, and then the extract was filtered. The extraction was repeated three times. The filtrates were concentrated under reduced pressure at 40 ºC and stored in a refrigerator at 2–8 ºC for use in subsequent experiments. The percent extractive yield of the hexane, ethyl acetate, methanol and ethanol of leaves, stem and root were weighed and noted.

Preliminary phytochemical screening

On preliminary phytochemical screening using the reported method [6], the methanolic extract of the *Mahonia leschenaultia* root showed positive tests for tannin, Saponins, flavonoids and steroids.

Animals

Hepatoprotective activity was carried out on adult male albino Wistar rats (150-250 g), supplied by the animal house facility of our instituttion (Registration no.---------). The rats were maintained in a 12 h light/dark cycle at 25 ± 2°C. They were allowed free access to a standard pellet diet (Amrut Laboratory Rat Feed, Navamaharashtra, Pune, India) and water *ad libitum*. The study was approved by the ethics committee CPCSEA and ethical norms were strictly followed during all experimental procedures.

Acute toxicity study

To evaluate the toxicity of *M. leschenaultii* root methanol extract, the acute toxicity study was performed based on Irwin test [7]. Five groups of fasted healthy rats (six per group) were orally administered the plant extract at a dose of 1–5 g/kg bw. Each of the rats in control group was treated with vehicle alone (distilled water). The rats in the test and control groups were allowed to access to food and water and observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 14 days for any physical signs of toxicity, such as palpitation, gasping, writhing and decreased respiratory rate or mortality. For further studies, the concentrations of plant extract were fixed at 200 and 400 mg/kg bw and berberine as 10 and 20 mg/kg bw.

Induction of hepatotoxicity

Seven days after acclimatization, the rats were divided randomly into five groups of six animals each and treated for four weeks i.e. 28 days[41]. Rifampicin and isoniazid solution were prepared separately in sterile distilled water. Rats were treated with isoniazid (100 mg/kg bw, orally) and co-administered with rifampicin (100 mg/kg, orally) for 28 days [42, 43] in order to study the effect of methanolic root extract (200 and 400 mg/kg bw) of *Mahonia leschenaultii* in rats, were used orally. Silymarin (75 mg/kg bw orally) was used as a standard drug in this study [44].

Treatment Protocol

Seven days after acclimatization, rats were divided into seven groups having six animals each and treated for four weeks i.e. 28 days [8].

Group 1: Normal control, received 10ml/kg bw with normal saline.

Group 2: Toxic control group received rifampicin and isoniazid 100 mg/kg bw administered orally.

Group 3: Extract (MEML) treatment groups received 200 mg/kg bw administered orally. Group 4: Extract (MEML) treatment groups received 400 mg/kg bw administered orally.

Group: Standard group received silymarin 75 mg/kg bw administered orally.

Groups G3 and G4 was given two doses of *M. leschenaultii* root methanol extract, one hour prior to the administration of rifampicin and isoniazid. Weights of these rats were monitored sequentially in control and experimental animals for a period of 28 days.

Biochemical Analysis

Preparation of serum from blood

Rats were sacrificed 1h after administration of drug on day-28. The blood was collected by retro-orbital artery puncture. The blood sample of each animal was allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600 g for 15 min and analyzed for various biochemical parameters including total protein [9], serum glutamate pyruvate transaminases (ALT/SGPT) [10, 11], serum glutamate oxaloacetate transaminases (AST/SGOT) [10, 11] and alkaline phosphatase (ALP) [11].

Histopathological analysis

Tissues of liver were collected for histopathological analysis. After fixation, the tissues were processed routinely and embedded in paraffin. Sections stained with hematoxylin-eosin (HE) were evaluated qualitative and quantitatively. Qualitative analyses of the liver were performed using an Olympus BX50 microscope (10 and 40x) in order to verify alterations in the tissues, as well as presence of cells from the parenchymal polymorphonuclear inflammatory infiltrate (PMNII). The inflammatory infiltrate in the liver was quantified using twenty fields taken randomly (total area traveled equal to 1.5 x 106 μm2, 40x). The morphometric analysis of the inflammatory infiltrate in this organ was performed from digital images obtained using the micro-camera Leica DFC340FX associated to the microscope Leica DM5000 B (Germany), and all the images were analyzed using the image processing software, Leica Qwin.

Organs weight

Euthanization of animals was done by exsanguinations with anesthesia on the 14th day after treatment. The liver, kidneys, lung and brain were quickly removed soon after the animals' death, cleaned with saline and their wet weight was determined. The relative organ weight of each animal was calculated relating the absolute organ weight and body weight of the animal on the day of sacrifice [12]. (Table 1).

Table: 1 Relative organ weight (g/100 g body weight) of Wister rats treated with *Mahonia leschenaultii* methanolic root extract and berberine in isoniazid and rifampicin (INH + RIF) induced hepatotoxicity study

Organs	Group 1	Group 2 (100 mg/kg)	Group 3 (200 mg/kg)	Group 4 (400 mg/kg)	Group 5 (75 mg/kg)
Heart	0.340 ± 0.005	0.289±0.023	0.320 ±0.025	0.328 ± 0.015	0.335 ± 0.005
Liver	2.866 ± 0.037	2.005±0.002	2.819 ± 0.177	2.923 ± 0.064	3.215 ± 0.032
Lungs	0.535 ± 0.046	0.436 ± 0.054	0.512 ± 0.018	0.564 ± 0.053	0.607 ± 0.041
Spleen	0.246 ± 0.012	0.196 ± 0.032	0.221 ± 0.043	0.235 ± 0.052	0.241 ± 0.012
Thymus	0.235 ± 0.051	0.155 ± 0.003	0.206 ± 0.024	0.217 ± 0.010	0.233 ± 0.046
Kidney	0.390 ± 0.05	0.329 ± 0.015	0.407 ± 0.062	0.463 ± 0.054	0.489 ± 0.078
Adrenal	0.010 ± 0.001	0.004 ± 0.001	0.007 ± 0.000	0.009 ± 0.001*	0.009 ± 0.000*

Values are expressed as mean ± SEM (n = 6), *****P < 0.05: G1 - Normal control, G2 - Toxic control (Isoniazid INH and Rifampicin RIF) (100+100) mg/kg bw, G3 - MEML 200 mg/kg bw + (INH+RIF), G4 - MEML 400 mg/kg bw + (INH+RIF), G5 - silymarin 75 mg/kg bw +(INH+RIF)

Statistical analysis

The results of biochemical parameters are reported as mean ± SEM. The statistical significance was determined by means of a one-way analysis of variance (ANOVA) followed by Dunnet's t-test [13]. A p-value of < 0.05 was considered as being statistically significant.

Results

Biochemical analysis

Total protein and total albumin levels.

The results showed that rifampicin (RIF) and isoniazid (INH) caused significantly decreased (p<0.05) the level of serum total protein and total albumin in rats of toxic control group (G2) as compared to the normal control (G1) due to rifampicin (RIF) and isoniazid (INH), but these levels were significantly increased (p<0.01) in rats of G3, G4 and G5 treated with silymarin and *M. leschenaultii* root methanol extract (MEML) at two different doses of 200 and 400 mg/kg bw (Table 2).

Serum AST levels

Results showed significantly increased (p<0.01) level of serum AST in rats of toxic control groups (G2) as compared to normal control (G1) due to rifampicin (RIF) and isoniazid (INH), but these levels were significantly reduced (p<0.01) in rats of G5, G3 and G4, treated with silymarin and *M. leschenaultii* root methanol extract (MEML) at two different doses of 200 and 400 mg/kg (Table 2).

Serum ALT levels

It was found a significantly increased (p<0.01) level of serum ALT in rats of toxic control groups (G2) as compared to normal control (G1) due to rifampicin (RIF) and isoniazid (INH), whereas these levels were significantly reduced (p<0.01) in rats of G5, G3 and G4 treated with silymarin and *M. leschenaultii* root methanol extract (MEML) at two different doses of 200 and 400 mg/kg bw respectively (Table 2).

Serum ALP levels

It was observed that rifampicin (RIF) and isoniazid (INH) caused significantly increased (p<0.01) level of serum ALP in rats of toxic control group (G2) as compared to normal control (G1) due to rifampicin (RIF) and isoniazid (INH), whereas these levels were significantly reduced (p<0.01) in rats of G5, G3 and G4 treated with silymarin and *M. leschenaultia* root methanol extract (MEML) at two different dose s of 200 and 400 mg/kg bw respectively (Table 2).

Table 2: Effect of *Mahonia leschenaultii* root extract and berberine on serum enzymes in isoniazid and rifampicin-induced hepatotoxicity study

Group	Total protein(g/dl)	Total albumin (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 1	9.26 ± 0.68	6.22 ± 0.63	152.15 ± 6.48	82.45 ± 3.75	124.40 ± 3.15
Group 2	5.29 ± 0.22*****a	3.40 ± 0.25*****a	255.30 ± 8.75*****a	165.25 ± 5.65*****a	267.40 ± 6.25*****a
Group 3	7.48 ± 0.58*****b	6.40 ± 0.40*****b	220.15 ± 7.35*****b	110.15 ± 4.36*****b	245.45 ± 5.42*****b
Group 4	7.85 ± 0.46*****b	7.16 ± 0.48*****b	216.20 ± 7.15*****b	100.65 ± 4.05*****b	225.45 ± 5.05*****b
Group 5	8.90 ± 0.62*****b	6.45 ± 0.42*****b	184.20 ± 6.40*****b	89.25 ± 3.86*****b	212.90 ± 5.30*****b

All values are expressed as Mean ± SEM (n = 6), *(a) values were significantly different from normal control at p<0.01, *(b) Values were significantly different from toxic control at p<0.01. G1 - Normal control, G2 - (INH+RIF), G3 – MEML 200 mg/kg bw + (INH+RIF), G4 - MEML 400 mg/kg bw + (INH + RIF), G5- silymarin 50 mg/kg bw + (INH + RIF)

Histopathological analysis

Histopathological analysis revealed that there was no alterations of the organs from control animals, as well as the liver of treated animals at two doses of the MEML root extract. The liver of control animals presented normal (Figure 1 A). The liver histological analysis of normal control showed the structure of liver with sheets of hepatocytes separated by sinusoids cartial vein and portal tract appear normal while toxic control (Isoniazid and Rifampicin) showed hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, Kuffe cell proliferation, hepatocyte diffuse necrosis and mononuclear infiltrate (Figure 1 B) whereas standard control group showed mild hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, no Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate (Figure 1 C). Likewise, treatment groups of methanolic root extract of *M. leschenaultii* (200 or 400 mg/kg bw) showed moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate (Figure 1 D) on dose-dependent manner.

Nevertheless, the liver parenchyma had a similar effect as the controls. The administration of 400 mg/kg bw of extract, the liver presented an intense inflammatory process besides the alterations found at lower doses (Figure 1 E). Histological changes in the liver can sustain the biochemical changes

observed for AST and ALT in animals that received the extract and berberine in the two evaluated doses.

Histopathological study

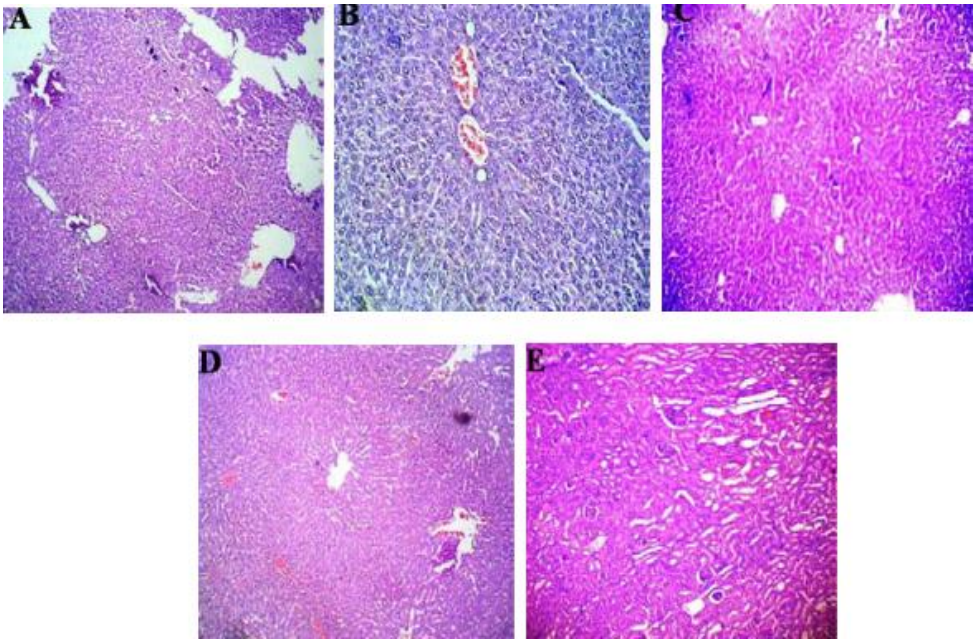

Fig 1: A: G1- Normal control B: G2- Toxic control (Isoniazid INH and Rifampicin RIF) (100+100) mg/kg bw C: G7- Standard control silymarin 75 mg/kg bw + (INH+RIF) D: G3- MEML 200 mg/kg bw + (INH+RIF) E: G4- MEML 400 mg/kg bw +(INH+RIF)

Discussion

In our study, we observed the hepatoprotective effect of methanolic root extract (MEML) of *M. leschenaultii* on rifampicin (RIF) and isoniazid (INH) induced hepatotoxicity in rats. G2 rats which received rifampicin (RIF) and isoniazid (INH) showed significant ($P< 0.01$) elevated levels of serum AST (15.64%), ALT (117.97%), ALP (90.55%) and significant decrease level total protein (47.80%) and total albumin (48.64%) when compared to normal control group (G1). Elevated levels of these parameters in serum are presumptive markers of hepatotoxic lesions in the liver.

Co-administration of silymarin and low & high dose of (200 & 400 mg/kg bw) of methanolic root extract (MEML) of *M. leschenaultii* with rifampicin (RIF) and iIsoniazid (INH) in G3, G4, and G7 animals maintained the levels of AST, ALT, ALP, and serum total protein (36%, 43%, and 50% respectively) and total albumin towards normal as compared to G2 rats. This was most likely due to the antioxidant effect of methanolic root extract *M. leschenaultii* (MEML) constituents.

Changes in organ weight have long been accepted as indicators of test induced changes, which are often associated with treatment related effects. Animal body weight is also an important factor to evaluate the toxicity of a substance. The reduction in body weight and internal organ weight can be a simple and sensitive index of toxicity after exposure to a toxic substance. In the present work, the extract of *M. leschenaultii* did not induce significant changes in the relative weight of the organs of mice (heart, liver, lung and kidney). Water consumption as well as food intake in all groups that received 200 or 400 mg/kg bw of root extract respectively was similar to that of the control group. The determinations of water and food consumption are important parameters to evaluate the safety of a product with potential therapeutic activity.

The oral treatment of rats with the crude methanolic root extract of *M. leschenaultii* and berberine, in general did not induce significant modifications of the biochemical profile when compared to the control group. However, a decrease in AST, ALT and ALP serum blood levels were observed in the animals that received the two doses (200 or 400 mg/kg bw) of extract in comparison to the control group (Table 2).

The measurement of serum AST, ALT and ALP levels serves as a means for the indirect assessment of the condition of the liver. The level of these enzymes significantly increased in serum, when animals were administered with isoniazid and rifampicin. The pre-treatment of the animals with methanolic root extract of *M. leschenaultii* and berberine, with respect to intoxication with isoniazid and rifampicin controlled the AST, ALT and ALP levels when compared with the toxic group.

Histopathological observations after isoniazid and rifampicin administration showed severe damage in hepatocytes, which basically supported the alterations observed in biochemical analysis. Hepatocellular necrosis, infiltration of periportal mononuclear cell of liver cells were characteristic alterations occurred due to acetaminophen intoxication. Treatment of methanolic root extract of *M. leschenaultii* and berberine decreased focal necrosis, vacuolation and reduced the lymphocytic infiltration in the liver and presented regenerative effects. This can be considered as an expression of the functional improvement of hepatocytes, which might be due to accelerated regeneration of hepatic cells or slight damage of cells.

On morphological examination in G3, low dose methanolic root extract of *M. leschenaultii* (MEML) showed partial recovery in some liver. While in G4 high dose methanolic root extract of *M. leschenaultii* (MEML) as well as the berberine treated groups G5 and G6 showed a significant recovery towards normal, shows hepatoprotection after a both dose of methanolic root extract of *M. leschenaultii* (MEML) as well as the berberine in experimentally drug induced hepatitis (DIH) in rats. In this study alkaloids in

methanolic root extract of *M. leschenaultii* (MEML) as well as the berberine might have a role in the recovery in rifampicin (RIF) and isoniazid (INH) induced hepatotoxicity in rats.

Histology of liver section of a normal control animal exhibited a normal hepatic cells each with well-defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein. Histological changes in the liver can sustain the biochemical changes observed for ALP, AST and ALT in animals that received the extract and berberine in the two evaluated doses. Morphometric analysis of the liver showed that the extract does not present hepatotoxic effect by time and dose dependent manner.

Conclusion

The results of serum biochemical markers and histopathological studies in methanol extract treated group support the hepatoprotective effect and provide evidence for the traditional use of *M. leschenaultii* root for treatment of liver disorders. The larger doses produced a remarkable hepatoprotective activity, which was comparable to silymarin. The presence of natural antioxidants in the methanol extract may explain the observed hepatoprotective activities. These suggest that synergy created between the antioxidant activity and intrinsic protective effects of the plant extract underlie attenuation of liver injury.

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