



## **Protective Effect of *Blumera lacera* in Rifampicin Induced Hepatotoxicity in Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Tuberculosis is one of the bacterial infection caused by mycobacterium tuberculosis strain and the treatment for it involves the use of anti-tubercular drugs. Rifampicin is one of the common drug that is used for tuberculosis but it potentiates liver toxicity due to metabolism. Objective: The present study was designed to Check the a hepatoprotective agent from a natural source. The natural source was chosen to minimize the side effects that occur due to conventional or synthetic drugs. The hepatoprotective activity of 70% ethanol extract of *Blumea lacera* leaves (EEBLL) was evaluated against Rifampicin induced hepatotoxicity in rats. Preliminary phytochemical screening, acute oral toxicity, *in vivo* antioxidant activity, physical and Biochemical parameters were evaluated for hepatoprotective activity. Preliminary phytochemical screening revealed that flavonoids, tannin and saponins are present in EEBLL. Acute oral toxicity was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines and selected doses of 200 and 400 mg/kg. In rifampicin induced hepatotoxicity in rats, the effect of EEBLL on the biochemical

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parameters (SGOT, SGPT, ALP, direct and total Bilirubin) was assessed. The increased levels of the above-mentioned biochemical indicators of hepatotoxicity were significantly normalized after the pretreatment with EE BLL (200 mg/ kg and 400mg/kg). The histopathological studies also supported above findings. It is concluded from this study that EE BLL possesses hepatoprotective properties which may be attributed to the polyphenolic compounds that are present in the plant. Further isolation and characterization are required to identify the potent compound as hepatoprotective.

**Keywords:** Hepatoprotective activity; *Blumea lacera*; rifampicin; biochemical parameters.

## 1. INTRODUCTION

Rifampicin is the first line drug to treat tuberculosis (TB) due to its efficacy, but its major adverse effects cause liver diseases [1]. Liver diseases include cirrhosis, viral hepatitis, hepatocellular carcinoma and alcohol-associated liver disease etc., are responsible for approximately 2 million deaths every year. Obesity, diabetes, viral hepatitis and drug-induced hepatotoxicity are also a major concern. Liver transplantation creates a financial burden. To overcome the major adverse effect of rifampicin and other drugs induced liver damage, available conventional or synthetic hepatoprotective drugs are sometimes insufficient and may induce adverse effects. In view to develop potent hepatoprotective agent from natural source against rifampicin induced hepatotoxicity in albino rats, the efficacy of *Blumea lacera* was investigated in rats.

*Blumea lacera* belongs to the family Asteraceae. It has terpine odour [2] and upto 0.9 m height with ash coloured [3]. As per the literature survey, it is astringent, expectorant, thermogenic, ophthalmic, antipyretic, digestive, febrifuge, anthelmintic, liver tonic and stimulant [4]. The aerial parts of plant were used to isolate the campesterol [5]. Recent studies have shown antimicrobial [4], analgesic [5] and antiulcer activity [6]. Our literature survey revealed that the hepatoprotective activity of EE BLL was not investigated; hence these activities have been investigated in the present study.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material and Preparation of EE BLL

The plant material was collected from the area of sudumbare, Pune and it was authenticated by Botanist Professor Dr. S. S. Deokule, Savtribhai Phule Pune University, Pune. Plant material was shade dried under dark condition and at room

temperature. It was pulverized and subjected to soxhlet extraction process using petroleum ether, chloroform and ethanol. Preliminary phytochemical analysis revealed the presence of saponins, steroids and flavonoids in Ethanol Extract of *Blumea lacera* leaves (EE BLL).

### 2.2 Animals

Mice weighted (18-25 g) and Wistar albino rats (150-220 g) were used.

### 2.3 Acute Toxicity Studies

Albino mice were used for the acute oral toxicity as per OECD Guideline no 420 given by CPCSEA [7].

### 2.4 Experimental Designs [8,9]

The animals were divided into five groups of six animals each.

Group I: Normal control group - The animals in this group received distilled water (1 ml/100 gm, p.o.) as vehicle.

Group II: Positive control (toxic) group - The animals in this group received rifampicin (1 gm/kg, p.o. in 5 % gum acacia) at day one.

Group III: Standard group - The animals in this group received rifampicin (1 gm/kg, p.o. in 5 % gum acacia) at day one, and after 30 min. Silymarin (100 mg/kg, p.o.) was administered.

Group IV: EE BLL group - The animals in this group received rifampicin (1 gm/kg, p.o. in 5 % gum acacia) at day one, after 30 min. EE BLL (200 mg/kg p.o.) was administered

Group V: EE BLL Group - The animals in this group received rifampicin (1 gm/kg, p.o. in 5 % gum acacia) at day one, after 30 min. EE BLL (400 mg/kg p.o.) was administered.

On the 2<sup>nd</sup> day, blood was withdrawn from all the animals using retro-orbital plexus method and centrifuged the blood at 2000 rpm for 10 minutes.

Collected serum was used to analyze the SGPT [10], SGOT [11], ALP [12], Bilirubin (total and direct) [13] parameters. The liver was isolated from all the animals and subjected for estimation of physical parameters (wet liver weight, liver volume), *in vivo* Antioxidant parameters (GSH and LPO) and histopathological investigations.

#### 2.4.1 *In vivo* tissue GSH estimation [14]

Liver tissue sample was added in ice cold trichloroacetic acid (1gm tissue in 10 ml 10% TCA) and homogenized using an ultra trux tissue homogenizer. The homogenized mixture was centrifuged at 3000 rpm for 10 minutes. Collected supernatant (0.5 ml) was added in 2ml of (0.3M) disodium hydrogen phosphate solution. The absorbance of solution was measured at 412 nm after addition of 0.2 ml dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium acetate) (Fig. 3).

#### 2.4.2 *In vivo* lipid peroxidation estimation [15]

The biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0  $\mu$ mol of lipid phosphate) of liver tissue was prepared. The 1.0 ml of prepared biological sample was mixed with 2.0 ml TCA-TBA-HCL solution and heated for 1 hour. The solution was centrifuged at 1000 rpm for 10 minutes and supernatant was collected to determine the absorbance at 535 nm (Fig. 4).

### 2.5 Statistical Analysis

Results were expressed as mean  $\pm$  SEM (n=6). For statistical analysis, Graph Pad Instat

Software was used using one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test. For statistical significance P value less than 0.05 was considered to be ideal.

## 3. RESULTS AND DISCUSSION

Administered Rifampicin is metabolized to active compound diacetyl rifampicin. It binds to RNA polymerase and thereby inhibit the synthesis of RNA. This causes cirrhosis and acute hepatic failure. Which is evidenced by the increased level of some serum enzymes [8,16].

In the rifampicin induced hepatotoxicity rats, liver cells were damaged as indicated by increased levels of SGOT and SGPT in serum [17]. Alkaline phosphatase (ALP) is the prototype of these enzymes that are released unequally [18] and reflected in the pathological phenomenon in biliary flow [19]. Serum bilirubin content was also elevated in the Rifampicin induced liver toxicity in rats.

The rifampicin treated animals showed significant ( $p < 0.01$ ) increase in the level of SGPT, SGOT, ALP, Bilirubin, wet liver weight, liver volume, liver peroxide content (LPO) and a significantly ( $p < 0.01$ ) decreased tissue glutathione (GSH), when compared with animals in the normal group. EE BLL treated animals showed significant ( $p < 0.01$ ) recovery in liver architecture in all the parameters (Table 1). Histopathological observations also supported these findings (Fig. 5).

**Table 1. Effect of EE BLL on biochemical markers in rifampicin induced hepatotoxicity**

Treatment	Biochemical parameters (Mean $\pm$ SEM)				
	SGOT IU/L	SGPT IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl
Normal control (1ml dist. water p.o.)	90 $\pm$ 5.10	59 $\pm$ 5.26	116.5 $\pm$ 13.576	0.62 $\pm$ 0.026	0.19 $\pm$ 0.019
Rifampicin control (intoxicated) (1ml dist. water p.o.+1gm/kg p.o.)	619 $\pm$ 29.57	693 $\pm$ 79.38	680.17 $\pm$ 24.89	2.56 $\pm$ 0.216	1.485 $\pm$ 0.06
Rifampicin + Silymarin (1 gm/kg p.o. + 100 mg/kg, p.o.)	145 $\pm$ 6.875***	110 $\pm$ 7.30***	190.16 $\pm$ 7.29***	0.728 $\pm$ 0.037***	0.2 $\pm$ 0.09***
Rifampicin + EE BLL (1 gm/kg p.o.+ 200mg/kg p.o.)	389.83 $\pm$ 16.93***	154 $\pm$ 10.47***	430.67 $\pm$ 25.5***	0.635 $\pm$ 0.04***	0.177 $\pm$ 0.01***
Rifampicin + EE BLL (1 gm/kg p.o.+ 400mg/kg p.o.)	375.67 $\pm$ 22.32***	219 $\pm$ 33.34***	563.17 $\pm$ 10.94**	0.727 $\pm$ 0.053***	0.208 $\pm$ 0.02***

Significance \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , <sup>ns</sup> $P > 0.05$ , compared to rifampicin treatment

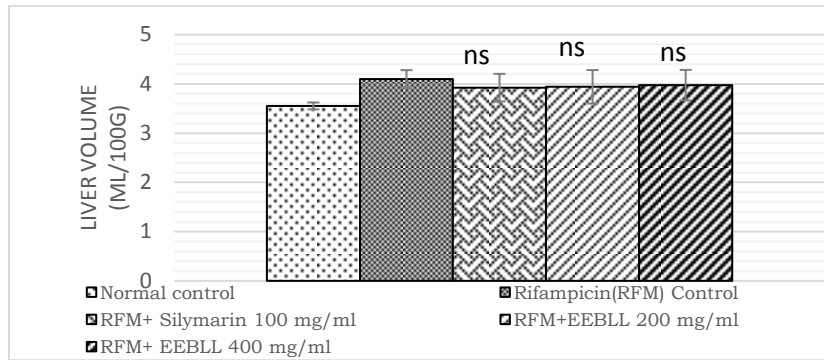


Fig. 1. Effect of EEELL on liver volume level in rifampicin induced hepatotoxicity

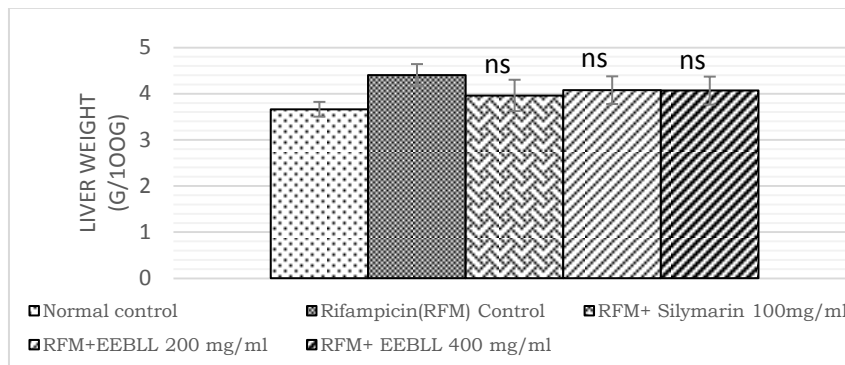


Fig. 2. Effect of EEELL on liver weight level in rifampicin induced hepatotoxicity in rat

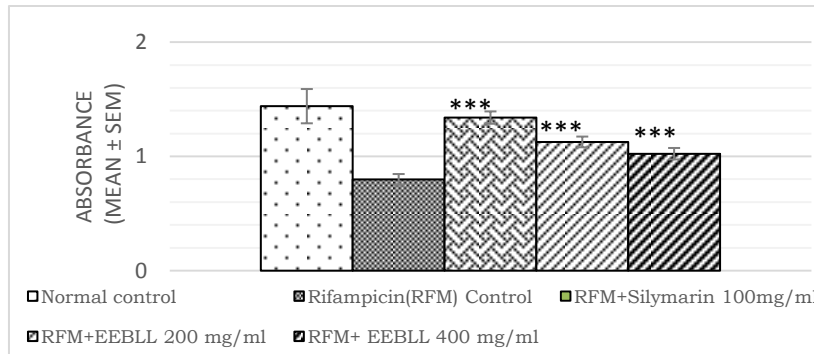


Fig. 3. Effect of EEELL on tissue GSH level in rifampicin induced hepatotoxicity in rat

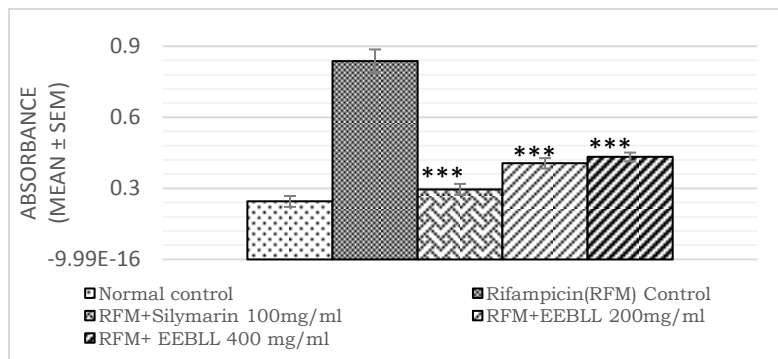
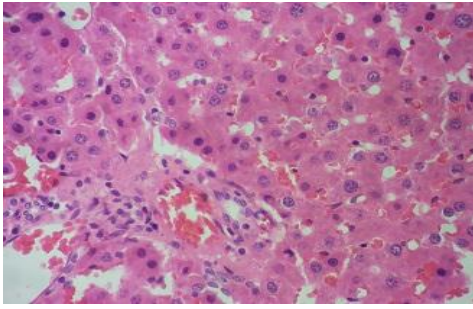
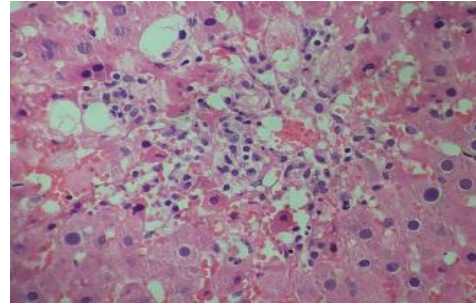


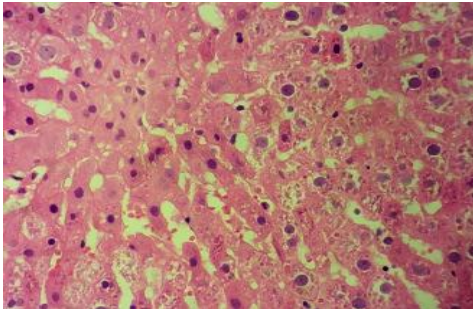
Fig. 4. Effect of EEELL on tissue LPO level in rifampicin induced hepatotoxicity in rat



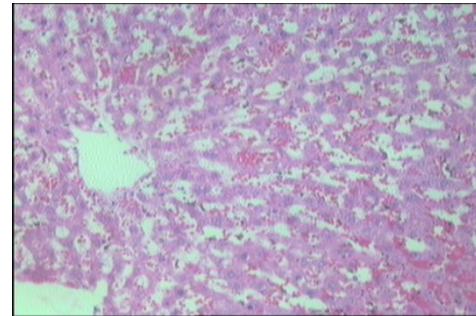
**Group I (Normal control) Normal hepatic globular structure. Normal central vein and portal tract**



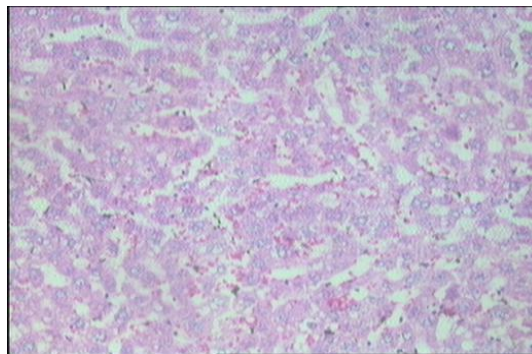
**Group II (RFM control 1 g/kg p.o.) Severe necrosis of hepatocytes. Disturbed liver architecture. Infiltration of inflammatory cells. Congestion of sinusoids**



**Group III (RFM + Std drug silymarin 100 mg/kg) Normal liver architecture. Minimal degeneration of hepatocytes and sinusoidal congestion**



**Group IV (RFM + EEPLL 200 mg/kg p.o.) Mild sinusoidal congestion and NAD (not other abnormality detected)**



**Group V (EEPLL 400 mg/kg, p.o.) Minimal sinusoidal congestion and NAD (not other abnormality detected)**

**Fig. 5. Histopathological studies of liver architecture (H & E 40X)**

In the rifampicin induced hepatotoxicity in rats, EEPLL exhibited hepatoprotective properties that may be due to inhibition of the active metabolite formation leading to a reduction in the drug metabolizing enzymes. The EEPLL showed significant ( $p < 0.01$ ) reduction in the above mentioned biochemical markers, which suggest

the stability of the biliary functions and a sign of regeneration process of liver.

#### **4. CONCLUSION**

It can be concluded that administrated EEPLL exhibited hepatoprotective property in rifampicin

induced liver damage in rats. This may be due to the presence of steroids, flavonoids, saponins and antioxidant properties in the EEBLL. Isolation, characterization, formulation and screening of bioactive compounds are planned and projected for further studies.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Protocol for Animal activity was approved from the institutional animal ethical committee (1554/PO/a/11/CPCSEA) for preclinical screening.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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