

# Hepatoprotective activity of *Limnophila repens* against paracetamol-induced hepatotoxicity in rats

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## Abstract

**Aim:** The present work was performed to assess the hepatoprotective activity of *Limnophila repens* against paracetamol toxicity in Wistar rats. **Materials and Methods:** Hepatoprotective properties of the methanol extract of the whole plant had been examined on paracetamol-induced hepatotoxicity. Hepatotoxicity was evoked in albino Wistar rats by the administration of paracetamol (2 g/kg), p.o. for 7 days. The methanol extract of *L. repens* was administered at the doses 100–200 mg/kg/day, p.o. for 7 days. Serum analysis was performed to estimate the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total bilirubin, cholesterol, and proteins. Histopathology studies were worked on the catalase liver samples. **Results:** The noxious effects of paracetamol had been considerably controlled in the extract treated groups that were demonstrated by the restoration of serum biochemical parameters to near normal levels. **Conclusions:** From the research, it had been figured that *L. repens* have significant hepatoprotective properties.

**Key words:** Hepatotoxicity, *Limnophila repens*, paracetamol, serum analysis

## INTRODUCTION

The liver, becoming center of metabolic capabilities, performs a significant part on metabolizing a number of xenobiotics; hence, it is more susceptible toward the degree of toxicity of these chemical substances. Hepatotoxicity, possibly dose-related or idiosyncratic, is recognized as being a worldwide well-being a challenge and could happen due to drug metabolism. Due to insufficient effective therapies, liver disorders possess incredibly awful diagnosis and high fatality. Although different developments have already been accomplished in the field of contemporary medicine, liver disorders nonetheless continues to be a significant ailment. Considering the fact that, research of new therapeutic techniques continue to be recurring.<sup>[1]</sup> Probably the most common instances of dose-related toxicity is that of paracetamol.<sup>[2]</sup> Paracetamol is quite widely used as an analgesic and antipyretic. It is considered safe in its therapeutic doses, yet overdose toxicity of paracetamol is among the most usual among the pharmaceutical product poisonings, which may trigger liver damage. It is one of the main reasons for

hepatic failure globally, and it exerts hepatotoxic results within a dose-dependent fashion.<sup>[3,4]</sup> Routinely, paracetamol is metabolized by cytochrome P450 enzymes into an active intermediate, i.e., N-acetyl-p-benzoquinone imine (NAPQI), which can be quickly detoxified by conjugation with glutathione.<sup>[5]</sup> Excessive formation of NAPQI caused by overdoses of paracetamol decreases the amount of free glutathione by saturating the glucuronidation and sulfation pathways, which eventually causes hepatic necrosis advancing to liver malfunction. Excess NAPQI binds toward the mitochondrial proteins and in addition damages, the mitochondria in hepatocytes, resulting in the severe generation of free radicals accompanied by lipid peroxidation and finally hepatic cell death.<sup>[5]</sup> From historic instances, man has always utilized herbs for numerous liver ailments as a medication

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**Received:** 23-04-2019

**Revised:** 13-05-2019

**Accepted:** 21-05-2019

technique, since natural remedies from traditional therapeutic vegetation have proven to be effective alternative remedies in cases of liver injury. From several scientific tests, this has been identified that hepatoprotective effects will be straight connected with phytoconstituents.<sup>[1,6-11]</sup> Worldwide, and particularly in developing countries, folk utilize traditional herbal supplements; consequently, there is a strong ought to improve such a hepatoprotective medicine using natural products that may pass the safety analysis and testing in the early stage of drug discovery since many harmful toxins will be metabolized in the liver.<sup>[12-16]</sup>

The genus *Limnophila* is frequently used in traditional medicine against cardiovascular diseases, stomach disorders, elephantiasis, diarrhea, dyspepsia, fever, dysentery, indigestion, dysmenorrhea, and abdominal pain,<sup>[17-19]</sup> phytochemical analysis of genus *Limnophila* revealed the presence of number of phytoconstituents such as flavonoids, tannins, alkaloids, terpenoids, steroids, and glycosides.<sup>[20]</sup> This diversity in compounds could justify the traditional use of *Limnophila repens*. The genus *Limnophila* is relatively abundant and widely used in folk medicine as an antioxidant,<sup>[21,22]</sup> antimicrobial,<sup>[23]</sup> anticancer,<sup>[24]</sup> and antimycobacterial,<sup>[25]</sup> as on to date no biological studies have been conducted on this plant. Being a potential antioxidant agent, *L. repens* may also have hepatoprotective activity; however, so far, no scientific data have been made available in literature. The present study aimed to evaluate the hepatoprotective activity of the methanolic extract of *L. repens* in albino rats intoxicated with paracetamol in a dose-dependent manner.

## MATERIALS AND METHODS

### Drugs and Chemicals

Paracetamol was purchased from GlaxoSmithKline Ltd., Karachi, Pakistan. Silymarin was purchased from Abbott Laboratories, Karachi, Pakistan. Diagnostic kits were purchased from Merck and DiaSys Diagnostic Systems, Germany. All other chemicals and reagents used in this study were of high analytical grade and were used without further modifications.

### Experimental Animals

Healthy adult male albino rats were used in this study. The animals were obtained from the animal house of the A. M. Reddy Memorial College of Pharmacy Narsaraopet, Andhra Pradesh. Animals were kept in standard plastic rat cages with stainless steel coverlids in an air-conditioned room maintained at  $25 \pm 2^\circ\text{C}$  with a regular 12 h light/12 h dark cycle, and they were provided with standard laboratory food. Free access to food and water *ad libitum* was provided. All the procedures involving the animals were in accordance with

the approved protocol of the Ethics Committee on Animal Experimentation of the A. M. Reddy Memorial College of Pharmacy with IAEC Approval No: AMRMCP/05/IAEC/18-19/PHD, Narasaraopet, Andhra Pradesh.

### Plant Material and Preparation of Extract

Fresh *L. repens* L. (whole plant) was collected in the month of October from Chittoor District of Andhra Pradesh, India. The plant material was identified at Tirumala Hills, Tirupati, Andhra Pradesh, verified by a plant taxonomist. The voucher specimen (voucher specimen no. 1568) was deposited at for future reference. The whole plant was washed and air dried. The dried material was then pulverized separately into fine powder by a mechanical grinder and stored in airtight bottles. Dried powder (almost 2 kg) was soaked in 6 L of 95% methanol and was kept on a shaker for 7 consecutive days. After that, the extracts were separated by filtration and concentrated at  $40^\circ\text{C}$  under reduced pressure by rotary evaporator. The extract was stored in an air-tight bottle at  $4^\circ\text{C}$  for further experiments.

### Preliminary Phytochemical Screening

The various extract of *L. repens* was subjected to qualitative chemical analysis using standard procedures.<sup>[26-29]</sup>

### Experimental Design

#### Animal groups

Rats were divided into five equal groups. Group I was the control group, treated with normal saline; Group II was treated with paracetamol; Group III was treated with 25 mg of silymarin; Group IV was treated with 200 mg/kg extract of methanolic extract of *L. repens* (MELR); and Group V was treated with 400 mg/kg extract of MELR. Food was withdrawn 18–24 h before the experiment, although water was given *ad libitum*. Group I served as the normal control and received only normal saline (1 mL/kg daily, intraperitoneally) for 7 consecutive days, while Group II received only paracetamol (250 mg/kg, intraperitoneally, suspended in normal saline) for 7 consecutive days. Group III received the standard drug silymarin at 25 mg/kg daily, intraperitoneally for 7 consecutive days, and received paracetamol (250 mg/kg daily, intraperitoneally) 3 h after the administration of silymarin. In Groups IV and V, the plant extract was administered intraperitoneally in 2 different doses, i.e. MELR (200 mg/kg) and MELR (400 mg/kg), daily for 7 days, and rat received paracetamol (250 mg/kg, intraperitoneally) 3 h after the administration of the extracts. At the end of treatment, 24 h after the last dose administration, rats were anesthetized with chloroform. Blood samples of every animal had been taken by cardiac puncture using sterile disposable syringes and instantly transferred into disposable glass tubes for estimation of liver enzyme markers. Serum

was acquired by centrifuging blood samples at 2500 rpm for 15 min at 4°C and stored at -20°C until further analysis.

### Measurement of serum levels of liver enzyme markers and bilirubin

The collected serum was further analyzed for the estimation of liver enzyme markers. Briefly, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) contents in the serum were estimated using commercially available kits (Merck and DiaSys Diagnostic Systems) according to the standard protocol. Total bilirubin and direct bilirubin contents in the serum were estimated through commercially available kits (Merck and DiaSys Diagnostic Systems) according to the manufacturer's instructions.

### Histopathological examination of liver tissues

The liver tissues were dissected out and washed with ice-cold normal saline, and a small cross-section of the liver was separated out. Small pieces were fixed with 10% neutral-buffered formalin and embedded in paraffin. Tissue processing was done by dehydrating with graded ethanol (50–100%) and clearing by xylene followed by paraffin infiltration. Liver tissue sections were cut in sizes of 4–5 µm, deparaffinized with xylene, and rehydrated with graded isopropyl alcohol and a drop of water. Water was removed and slides were oven-dried. After tissue fixation, staining was done with hematoxylin and eosin. The stained sections of slides were examined under high-resolution microscope, and photographs were taken.

### Statistical Analysis

The results are presented as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance followed by the Tukey multiple comparison test using GraphPad Prism 5 (GraphPad Software Inc., USA).  $P < 0.05$  was considered a statistically significant.

## RESULTS

### Acute Toxicity Studies

The MELR, when orally administered in the dose of 2000 mg/kg body wt. did not produce any significant changes in the autonomic or behavioral responses, including death during the observation period.

### Phytochemical Screening

The phytochemical assessment for different extracts, namely petroleum ether, chloroform, ethyl acetate, methanol, n-butanol, and water was executed, and outcomes are shown in Table 1.

### Effect of *L. repens* on Liver Enzyme Markers

Biochemical analysis of liver enzyme markers signified that the use of paracetamol in high doses strikingly raised the serum levels of liver enzyme markers ALT, AST [Figure 1], and ALP

**Table 1:** Phytochemical analysis of various extracts of *Limnophila repens*

Phytoconstituents	Method	Pet. ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	n-butanol extract	Aqueous extract
Flavonoids	Shinoda test	-	-	+	+	-	+
	Zn+HCl test	-	-	+	+	-	+
	Lead acetate test	-	-	+	+	-	+
Volatile oil	Stain test	+	-	-	+	-	+
Alkaloids	Wagner test	-	+	-	+	-	+
	Hager's test	-	+	-	+	-	+
Tannins and phenols	FeCl <sub>3</sub> test	-	-	+	+	+	+
	Potassium dichromate test	-	-	+	+	+	+
Saponins	Foam test	-	-	-	-	-	-
Phytosterols	Libermann's test	+	+	-	+	-	-
Carbohydrates	Molisch test	-	-	-	+	-	+
Acid compounds	Litmus test	-	-	-	-	-	-
Glycoside	Borntrager's test	-	-	-	+	-	+
Amino acids	Ninhydrin test	-	-	-	+	-	+
Proteins	Biuret test	-	-	-	+	-	+
Fixed oils and fats	Spot test	+	-	-	-	-	-

+Present and -Absent

as compared to the control group treated with normal saline [Table 2]. Treatment with MELR and standard drug silymarin showed hepatoprotective activity against paracetamol-induced hepatotoxicity by maintaining the serum levels of ALT, AST, and ALP at markedly reduced levels in a dose-dependent manner [Figure 2]. We also measured the serum levels of total bilirubin, cholesterol, and protein content in all treated groups. The group treated with paracetamol alone exhibited high levels of bilirubin and cholesterol levels as compared to the control group. Treatment with MELR at two different doses decreased the serum levels of bilirubin more significantly when directly compared with paracetamol-treated rat.

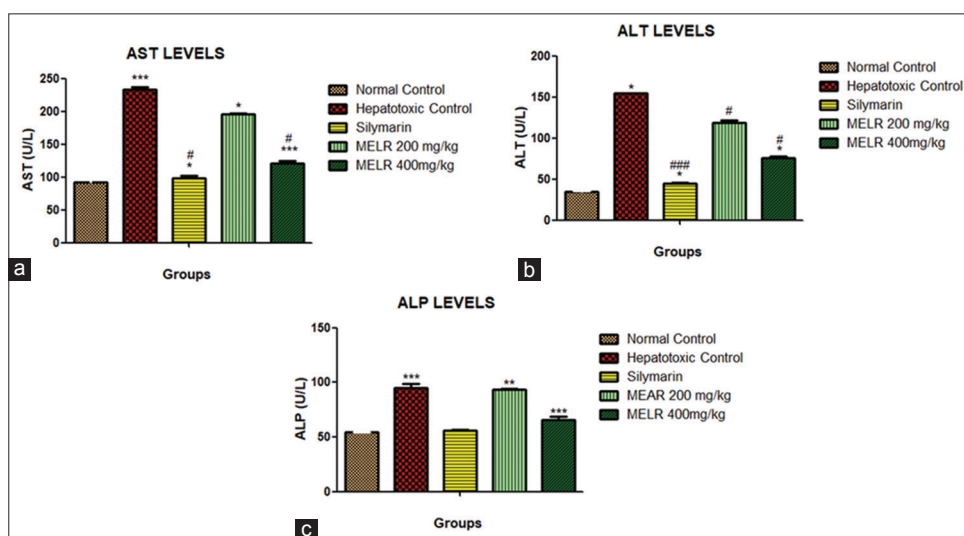
### Histopathological Examination of the Liver

Histopathological analysis revealed that Group I, treated with normal saline, showed normal sections of liver tissues [Figure 3a], whereas the liver sections of paracetamol-treated rat lost their normal architecture. Severe congestion of blood vessels along with hepatic cell necrosis, vacuolization, eosinophils, macrophages, plasma cells infiltration, degeneration of hepatocytes nuclei, and fibrosis is shown in Figure 3b. Silymarin treatment followed by paracetamol administration displayed the normal structure of hepatocytes, mild infiltration, and vacuolization [Figure 3c]. Treatment with *L. repens* extract (MELR-200) followed by paracetamol

**Table 2:** Effects of pretreatment with *Limnophila repens* methanolic extract on the serum levels of AST, ALT, ALP, bilirubin, cholesterol, and total proteins in PCM induced hepatotoxicity in rat. All values expressed as mean±SEM;  $n=5$  rat in each group, by one-way ANOVA followed by Tukey's Multiple Comparison Test

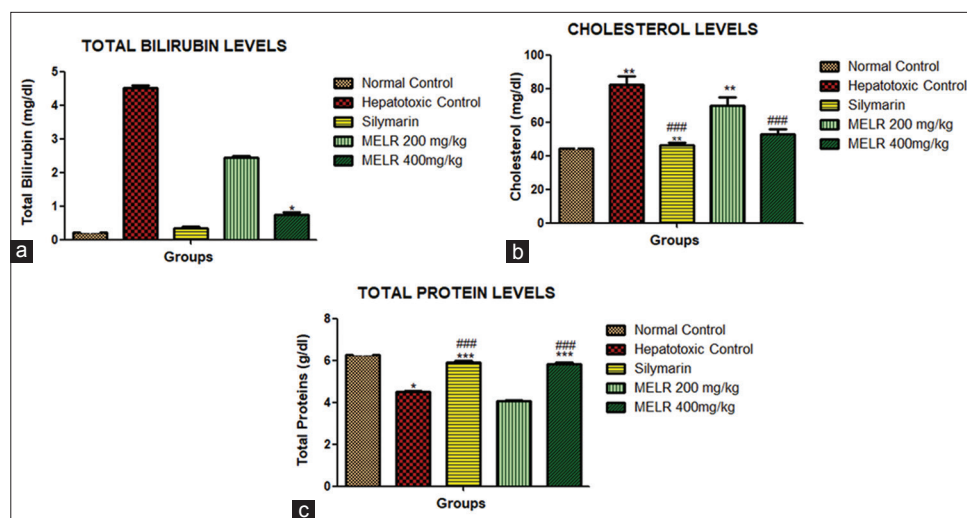
Treatment groups and liver-specific variables	I (Normal control: 0.5% Tween 80 1 ml/kg b.wt.)	II (Hepatotoxic control: 0.5% Tween 80 1 ml/kg b.wt.+PCM (2 g/Kg b.wt.))	III (Silymarin 25 mg/kg b.wt.+PCM 2 g/kg b. wt.)	IV (MELR 200 mg/kg b.wt.+PCM 2 g/kg. b.wt.)	V (MELR 400 mg/kg b.wt.+PCM 2 g/kg. b.wt.)
AST (U/L)	92.65±2.56	232.78±2.32	98.25±4.85	196±1.56	121.25±3.15
ALT (U/L)	35.08±0.33	154.22±0.93	44.58±1.22	118.3±2.89	75.65±2.15
ALP (U/L)	54.29±0.28	95.28±3.12	56.25±0.83	93.22±0.78	65.52±3.41
Total bilirubin (mg/dl)	0.22±0.03	4.52±0.06	0.35±0.05	2.43±0.07	0.74±0.08
Cholesterol (mg/dl)	44.26±3.92	82.52±4.58	46.58±1.25	69.65±5.25	52.67±3.28
Total proteins (g/dl)	6.27±0.02	4.52±0.03	5.92±0.06	4.08±0.03	5.82±0.08

ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, PCM: Paracetamol, MELR: Methanol extract of *Limnophila repens*

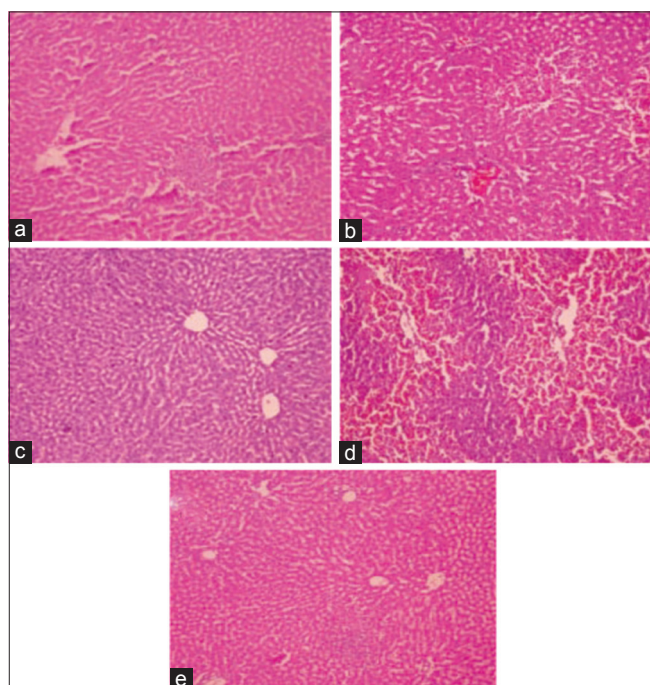


**Figure 1:** Serum enzymes indices of liver toxicity in rats intoxicated with paracetamol and administered methanolic extract of *Limnophila repens* (200 mg/Kg and 400 mg/Kg) and silymarin (25 mg/Kg). All values expressed as the mean ± standard error of the mean; the  $n = 5$  rat in each group, by one-way ANOVA followed by Tukey's multiple comparison test. (a) Aspartate aminotransferase activity in all groups. \*\*\* $P < 0.001$  versus control, \* $P < 0.05$  versus control, # $P < 0.05$  versus hepatotoxicity control (b) alanine aminotransferase activity in all groups. \* $P < 0.05$  versus control, ### $P < 0.001$  versus hepatotoxicity control; # $P < 0.05$  versus hepatotoxicity control (c) alkaline phosphatase activity in all groups \*\*\* $P < 0.001$  versus control; \*\* $P < 0.01$  versus control





**Figure 2:** Serum enzyme indices of liver toxicity in rats intoxicated with paracetamol and administered methanolic extract of *Limnophila repens* (200 and 400 mg/Kg) and silymarin (25 mg/Kg). All values expressed as the mean  $\pm$  standard error of the mean; the  $n = 5$  rat in each group, by one-way ANOVA followed by Tukey's multiple comparison test. (a) Serum bilirubin level in all groups. \* $P < 0.05$  versus control (b) serum cholesterol level in all groups. \*\* $P < 0.01$  versus control; ### $P < 0.001$  versus hepatotoxicity control (c) protein level in all groups. \* $P < 0.005$  versus control; \*\*\* $P < 0.001$  versus control; ### $P < 0.001$  versus hepatotoxicity control



**Figure 3:** Paraffin sections of liver stained with hematoxylin and eosin for histopathological changes. (a) Liver section of control group showing the normal architecture of normal liver histology. (b) Hepatotoxic liver after treatment of paracetamol (PCM) (2 mg/Kg). (c) Liver section treated with PCM and silymarin (25 mg/kg). (d) Liver section treated with PCM + methanolic extract of *Limnophila repens* (200 mg/kg). (e) Liver section treated with PCM + MELR (Methanol extract of *Liminophila repens*) (400 mg/kg)

administration showed few binucleated cells while most cells were normal, with slight congestion, vacuolization, and infiltration [Figure 3d]. However, in the case of MELR-400,

the recovery stage had well-arranged hepatocytes, and no necrosis was evident [Figure 3e].

## DISCUSSION

Medicinal plant-based medicines are potential sources of naturally occurring phytoconstituents that may act in a variety of ways to suppress the generation of reactive oxygen species. These phytoconstituents have broad ranges of pharmacological activities.<sup>[30-33]</sup> Glutathione is one of the major antioxidants that protect the liver from toxic effects of paracetamol,<sup>[34]</sup> but overdoses of paracetamol may result in the depletion of glutathione stores, which ultimately leads to the release of serum levels of liver enzyme biomarkers indicating mitochondrial damage.<sup>[5,35]</sup> Paracetamol-induced hepatotoxicity results in the elevated levels of liver enzyme markers such as ALT, AST, and ALP. Elevated levels of these enzymes in the serum represent the loss of functional integrity due to the cellular leakage of these enzymes from the cell membrane of the liver, which is reflected by the histopathological alterations.<sup>[36-38]</sup> Estimations of these liver enzyme markers in the serum reflect the normal and/or abnormal condition of the liver. MELR maintained the serum levels of ALT, AST, and ALP. In the present study, we used two doses of *L. repens* (200 mg/kg and 400 mg/kg). We found that *L. repens* showed hepatoprotective effects in a dose-dependent manner. A high dose of *L. repens* showed a non-significant difference with the hepatoprotective effects of standard drug silymarin [Figure 2].

Similarly, the elevated levels of bilirubin in serum are also attributed to the paracetamol-induced hepatotoxicity, which is usually due to the abnormal production of bilirubin in the

liver.<sup>[38]</sup> Bilirubin level is also considered as one of the most important liver function tests as it reflects the functional integrity of the liver. In the present study, we measured the serum levels of total bilirubin and direct bilirubin. The significant reduction in the serum levels of this bilirubin in *L. repens* treated rat was observed in a dose-dependent manner [Figure 2]. The observed hepatoprotective effect of *L. repens* in paracetamol-induced hepatotoxicity might be due to the presence of an active concentration of phytoconstituents that might restore the glutathione levels in hepatocytes, due to which the functional integrity of the liver was increased, as was evident from the decreased levels of liver enzyme markers and improved live histopathological examinations of liver.

## CONCLUSIONS

The results of the present study reveal that the MELR can significantly protect the liver from the damaging effects of paracetamol in a dose-dependent manner by considerably decreasing the serum levels of liver enzyme markers. The decreased serum levels of these enzymes were further accompanied by the improvement of liver histology in *L. repens* treated rat, which remarkably exhibited the hepatoprotective effects of *L. repens* in paracetamol-induced hepatotoxicity. The hepatoprotective effects of *L. repens* scientifically validate the traditional use of *L. repens* in liver ailments.

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**Source of Support:** Nil. **Conflict of Interest:** None declared.