



Research Article

EVALUATION OF HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *PHYSALIS IXOCARPA* AGAINST RIFAMPICIN-ISONIAZID INDUCED HEPATOTOXICITY IN WISTAR RATS

N. Delhiraj^{1*}, M. K. Jaganathan², A. R. Vijayakumar³

¹Department of Pharmaceutical Chemistry, School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

²Department of Biotechnology, School of Bioengineering, Faculty of Engineering and Technology, SRM Institute of Science and Technology, Tamil Nadu, India

³Department of Pharmacology, JIPMER Karaikal, Puducherry, India

*Corresponding Author Email: pharmaraj1981@gmail.com

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ABSTRACT

The liver is the primary organ that metabolizes the majority of the drug. Toxicity caused by these drugs to the liver is called hepatotoxicity. Hepatotoxicity is a major concern in tuberculosis therapy, especially Rifampicin - Isoniazid (R-H). Studies showed that these drugs induce oxidative stress in the liver. This study attempts to determine whether the ethanolic extract of *Physalis ixocarpa* (EEPI) protects against R-H induced hepatotoxicity in adult male Wistar rats. Adult male Wistar rats were divided into five groups (each group n=6 animals). Group I, control treated with normal saline (5ml/kg, b/w, p.o.). Group II, Hepatotoxicity induced by combination of R-H (each 50mg/kg, i.p.) administered up to 14 days. Group III and IV, EEPI (100 mg/kg & 200 mg/kg, b/w) were administered orally one hour before the R-H inducing agent up to 14 days. Group V, Silymarin (25 mg /kg, b/w., p.o.) was served as standard. After 14th days animals were allowed fast overnight and blood was collected through orbital puncture and animal was sacrificed then liver tissue was collected for biochemical analysis and histopathological studies. Our results show a significant reduction in the level of alkaline phosphate (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin. Treatment with EEPI also showed a significant increase in the activity of antioxidant enzymes and decreased levels of malondialdehyde (MDA) in the liver. EEPI also reduced the macrovesicular steatosis and ballooning caused by the R-H. The present study demonstrates that administration of ethanolic extract of *Physalis ixocarpa* ameliorating hepatoprotective activity as evidenced by the biochemical and histopathological parameters.

Keywords: Hepatoprotective activity, *Physalis ixocarpa*, Rifampicin, Isoniazid, Antioxidant activity.

INTRODUCTION

Rifampicin (R) and isoniazid (H) are the component of first-line anti-tuberculosis treatment (ATT), which is causing severe liver injury using long terms. Around 6.9% of tuberculosis patients were reported that drug induces liver damage due to ATT. In approximately 5.7-22% of people affect acute liver failure during the ATT.^{1,2} Acetyl hydrazine is one of the metabolites of isoniazid, which is responsible for liver toxicity. Toxicity develops within hours if treated with high doses of isoniazid.³⁻⁵ Isoniazid induced liver injury may be mediated by the adaptive immune system.^{6,7} Rifampicin is an enzyme inducer, and isoniazid is an enzyme inhibitor, in combination with both used in the beneficial effect in ATT from 2 years to 6 months and used in individually produce the specific side effects. The combination of R-H may produce many metabolic and morphological changes in the liver because the liver is one of the major detoxifying sites for ATT and other drugs.⁸

Herbal medicine is a major component of traditional medicine and a common element in ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine.⁹ According to the WHO, 80% of the world population currently uses herbal medicines in their primary health care for the treatment of heart disease, cancer, diabetes, blood pressure, pain, asthma, and other pathological problems. Substances derived

from the plant's origin remain the basis for a large proportion of the commercial medications used today. Recently, major pharmaceutical industries are conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value for the betterment of avoiding the side effects to humanity.¹⁰

The liver is a crucial organ that regulates a wide variety of high-volume biochemical reactions requiring highly specialized tissues, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions, and it's reached to systemic circulation into the entire body. Liver malfunctioning leads to cause severe side effects.¹¹ Despite the phenomenal growth of the allopathic system of medicine, the synthetic drug to prevent or cure the hepatic damage due to various hepatotoxin and produces unwanted side effects. Treatment of hepatotoxicity with plant sources has been mentioned in the ancient indigenous systems of medicine in many countries. Even today, rural folks and aboriginal tribes all over the world, including India, are using many plants in the treatment of drug-induced liver damage.

There are several hepatoprotective agents available such as silymarin (Milk Thistle), N-acetylcysteine, garlic, antioxidants, ursodeoxycholic acid, and S-adenosine-L- methionine used in the treatment of drug-induced liver damage.¹ *Physalis ixocarpa*

possesses an active ingredient such as alkaloids, glycosides, flavonoids, phytosterol, tannins, and saponins. Traditionally, *Physalis ixocarpa* plant is claimed to treat inflammations, diuretic, abdominal troubles, appetite stimulant, and cytoprotective action.¹² Till yet, there are no scientific pharmacological studies reported that this plant used in the treatment of drug-induced liver damage. Therefore, we attempt to evaluate the hepatoprotective effect of ethanol extract of *Physalis ixocarpa* (EEPI) against by rifampicin-isoniazid (R-H) induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Chemicals

The biochemical assay used for the kits purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India. Rifampicin and isoniazid at analytical grade were purchased from Sigma-Aldrich, India. All other chemicals and reagents are purchased from the analytical grade to the relevant sources.

Plant collection and authentication

The leaves of *Physalis ixocarpa* were collected from the surrounding of the campus, which was authenticated by Plant Taxonomist Prof. P. Jayaraman, Ph.D., Plant Anatomy Research Centre (PARC), Chennai. A specimen was (Dept. P.Cognosy/012/2010) deposited in the Department of Pharmacognosy in the same college for future reference.

Preparation of extract

The fresh leaves were shade dried, powdered, and extracted successively with ethanol (96% v/v) in a Soxhlet extractor for 18–20 hrs. The extract was concentrated to dryness under reduced pressure using a rotary flask evaporator, and the percentage of yield of EEPI was 5.3% w/v.

Phytochemical screening

The alcoholic extracts obtained were subjected to preliminary phytochemicals screening to identify the chemical constituents.¹³

Animals

Adult Wistar male rats weighing 150–250 g was used in the study. Animals were kept in standard laboratory conditions maintained in a well-ventilated room, under light and dark cycles, and are housed at ambient temperature ($25 \pm 2^\circ\text{C}$) and relative humidity ($55 \pm 5\%$). Animals had access to standard pellet diet with water *ad libitum* throughout the experimental period. All the experimental protocols were followed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. All the experimental procedures were approved by the standing Institutional Animal Ethics Committee (IACE), and the approval number is IAEC/137/2010.

Acute toxicity study

Acute oral toxicity study of alcoholic extract of *Physalis ixocarpa* was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines 423.¹⁴

Experimental design

Hepatotoxicity was induced by the administration of a combination of rifampicin-isoniazid (R-H) (50 mg/kg each, i.p.) for 14 days in Wistar rats.¹⁵ The animals were divided into five groups, and each group consists of six animals (n=6).

Group I: Animals received Normal Saline (NaCl, 0.9%), 5 ml/kg, p.o.

Group II: Animal received R-H, each 50 mg/kg, i.p.

Group III: Animal received R-H, each 50 mg/kg, i.p. + EEPI100 mg/kg, p.o.

Group IV: Animal received R-H, each 50 mg/kg, i.p. + EEPI200 mg/kg, p.o.

Group V: Animal received R-H, each 50 mg/kg, i.p. + Silymarin 25 mg/kg, p.o.

All the animals were treated with respective drugs and extract for up to 14 days. After the administration of respective drugs on the 14th day, animals were allowed to overnight fasting. On the next day, blood was collected from retro-orbital puncture by using the sterile polished microcapillary tubes and used the serum for estimation of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were performed by using available commercial assay kits as per the manufacturer's protocol. The estimation of albumin, total proteins and total bilirubin as per the standard protocol of kits (Agappe Diagnostics Ltd., India). Then animals were sacrificed, and liver tissues were collected for enzymatic and histopathological studies.

Preparation of rat liver homogenate to biochemical assay

Tissue homogenate was prepared in a ratio of 1 g of wet tissue to 10 times (w/v) 0.05M-ice-cold phosphate buffer (pH 7.4) and homogenized by using a Teflon homogenizer. After homogenization of liver tissues allowed to centrifugation for 10 min and collect the supernatant for determination of lipid peroxidation level estimated as thiobarbituric acid reactive substance (TBARS),¹⁶ superoxide dismutase,¹⁷ catalase¹⁸ and reduced glutathione content.¹⁹

Histopathological study of liver

After sacrifice, the animal liver portion was collected for histopathological examination, and it was fixed in 10% buffered neutral formalin, dehydrated, and embedded in paraffin. The specimens were stained with hematoxylin and eosin and were examined under a light microscope.

Statistical analysis

All the data were expressed as mean $\pm$ SEM. Statistical significance was tested by using one-way ANOVA and the statistical comparisons among the groups were performed with Dunnett's-"t" test using SPSS version 20.0. Differences were considered statistically significant at $p < 0.05$.

Table 1: Effect of EEPI on serum AST, ALT, ALP, Total protein and Total bilirubin levels against Rifampicin-Isoniazid induced hepatotoxicity in rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dL)	TOTAL BILIRUBIN (mg/dL)
I. Control (NS, 5ml/ kg, p.o.)	18.33 ± 2.51	31.16 ± 1.99	42.66 ± 1.74	3.05 ± 0.38	0.61 ± 0.07
II. R-H (50mg/ kg each, i.p.)	53.67 ± 2.82a [@]	45.50 ± 3.77a [@]	119.33 ± 5.02a [@]	0.77 ± 0.05a [#]	2.78 ± 0.4a [@]
III. EEPI (100mg/ kg, p.o.)	48.50 ± 2.51b [*]	41.00 ± 3.62b [*]	60.66 ± 4.29b [@]	2.65 ± 0.32b [@]	0.93 ± 0.08b [#]
IV. EEPI (200mg/ kg, p.o.)	42.67 ± 4.12b [*]	39.16 ± 3.57b [*]	54.00 ± 4.43b [@]	2.92 ± 0.14b [@]	0.64 ± 0.10b [#]
V. Silymarin (25mg/ kg, p.o.)	34.33 ± 3.32b [@]	36.16 ± 2.19b [#]	49.50 ± 3.18b [@]	2.71 ± 0.33b [@]	0.65 ± 0.08b [@]

Comparisons were made between: a - Group I versus II, b - Group II versus III, IV & V.

The values are expressed as mean ± SEM (n=6). The p value <0.05 was considered significant (*p <0.05; [#]p<0.01; [@]p<0.001).

Table 2: Effect of EEPI on serum SOD, CAT and GSH and TBARS levels against Rifampicin-Isoniazid induced hepatotoxicity in rats.

Groups	SOD (Units/mg protein min)	CAT (μm of H ₂ O ₂ consumed /min/mg protein)	GSH (μg of GSH consumed/ g of wet tissues)	TBARS (nm of MDA/ g of wet tissues)
I. Control (NS, 5ml/ kg, p.o.)	13.85 ± 1.46	63.09 ± 4.64	75.84 ± 5.06	67.65 ± 2.97
II. R-H (50mg/ kg each, i.p.)	3.75 ± 0.82a [@]	35.83 ± 6.70a [#]	27.66 ± 2.98a [@]	172.06 ± 4.90a [@]
III. EEPI (100mg/ kg, p.o.)	7.29 ± 0.75b [#]	51.97 ± 3.17b [#]	58.63 ± 2.11b [@]	127.51 ± 7.94b [#]
IV. EEPI (200mg/ kg, p.o.)	11.70 ± 1.03b [@]	54.54 ± 3.12b [#]	67.85 ± 3.02b [@]	100.90 ± 3.44b [@]
V. Silymarin (25mg/ kg, p.o.)	12.69 ± 1.53b [@]	59.49 ± 3.32b [#]	74.09 ± 3.06b [@]	87.71 ± 4.21b [@]

Comparisons were made between: a - Group I versus II, b - Group II versus III, IV & V.

The values are expressed as mean ± SEM (n=6). The p value <0.05 was considered significant (*p <0.05; [#]p<0.01; [@]p<0.001)

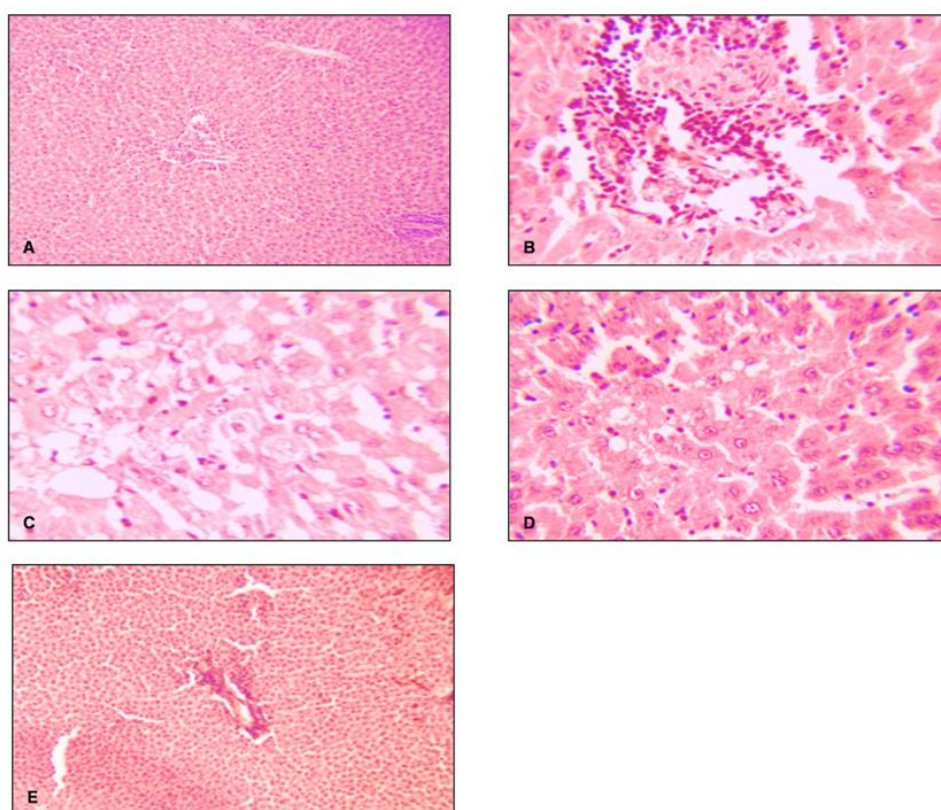


Figure 1: *Physalis ixocarpa* ameliorate rifampicin-isoniazid induced hepatic injury in rat model: Photomicrograph of rat liver cross section was showing

(A) Hepatocytes with normal lobular architecture. (B) Hepatocytic necrosis and inflammation seen in centrilobular region. (C) Moderate inflammation with moderate portal triaditis and their lobular architecture was normal in EEPI (100mg/kg) treated rat. (D) Minimum inflammation with mild portal triaditis and their lobular architecture was normal in EEPI (200mg/kg) treated rat. (E) Minimum inflammation with normal portal triaditis and normal lobular architecture seen in Silymarin treated rat.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the plant extract of *Physalis ixocarpa* reveals the presence of alkaloids, carbohydrates, phytosterols, glycosides, saponins, tannins, and flavonoids.

Acute toxicity study

There was no mortality sign for up to 24hrs and the behavioural changes, and any sign of toxicity was observed to all animals. EEPI extract was safe up to 2000 mg/kg body weight of animals. Therefore, the present study was carried out at doses of 100 and 200 mg/kg body weight of Wistar rats.

Hepatoprotective activity of EEPI

The level of aspartate transaminase, alanine transaminase, alkaline phosphate and total bilirubin were significantly increased in rifampicin-isoniazid (R-H) treated rats while a significantly decrease levels are seen in the EEPI (100 mg/kg and 200 mg/kg) and silymarin treated rats ($p < 0.001$) when compared to the R-H treated animals. Total protein was significantly decreased in the R-H treated animals while a significant ($p < 0.001$) increase in the levels observed in both doses EEPI and standard silymarin treated animals ($p < 0.001$) present in Table 1.

Lipid peroxidation level was significantly increased in the R-H treated animals when compared with control animals. The plant extracts both doses (100 mg/kg and 200 mg/kg) ($p < 0.01$; $p < 0.001$), and silymarin treated animals showed a significant ($p < 0.001$) decrease in the MDA levels when compared to R-H only treated animals. Superoxide dismutase, catalase, and reduced glutathione levels were significantly decreased in the R-H treated animals when compared with the control group. Both doses of extract and standard silymarin treated animals were showed significantly ($p < 0.001$) increased the levels of the enzyme when compared to the R-H treated animals present in Table 2.

Histopathological study

The extent of hepatic damage was assessed by conventional histological evaluation along with the levels of various biochemical parameters in circulation. The animals in the R-H group showed severe hepatotoxicity evidenced by profound macrovesicular steatosis, centrilobular necrosis, prominent ballooning marked sinusoidal dilation, and fibrosis as compared to the typical hepatic architecture of the control group animals. EEPI higher dose and silymarin treated animals were showed the appearance of mild macrovesicular steatosis and few ballooning than low dose treatment animals. Both the EEPI treated group's decreased R-H induced hepatic damage, which is indicating the protection of structural integrity of hepatocyte cell membrane or the regeneration of damaged liver cells showed in Figure 1.

DISCUSSION

Antitubercular therapy (ATT) drugs are one of the most typical groups underlying idiosyncratic liver damage in worldwide. ATT drugs induced liver damage mainly mediated via the production of its toxic metabolites in the metabolic pathway, which covalently binds to the liver macromolecular cells and causes liver cell injury. The rate of liver damage is much higher in developing countries like India (approx. 8% to 30%) than

compared to that in advanced countries.²⁰ Rifampicin is well known synergistically increases the effect of isoniazid by hydrolase and CYP2E1 mediated pathways. Besides, rifampicin increases the metabolism of isoniazid through CYP450 and pregnane X receptor (PXR).²¹ Exposures to a combination of R-H is one of the best and widely recognized models for investigating different hepatoprotective effects in natural products.²²

The structural homologies of rat CYP450 enzymes are similar in humans; therefore, the present study we have chosen the rats as an experimental model for hepatoprotective activity. The female rats are less susceptible to chemical-induced liver damage than male rats; this is a reason for the present study we used in male rats.²³ Rifampicin and isoniazid are bactericidal activity against *Mycobacterium tuberculosis*. Rifampicin inhibits the messenger RNA synthesis. Isoniazid is a prodrug inhibits the biosynthesis of mycolic acid, which is an essential component of the cell wall of the bacteria. The administration of R-H caused a significant elevation in the estimation of AST (appr. 2.9-fold increase), ALT (appr. 1.5-fold increase), and ALP (appr. 2.8-fold increase) due to rat liver damage release the enzymes into circulation. The reversal of an increase in serum enzymes level in ATT drugs induced liver damage by the EEPI may be due to the prevention of the leakage of intracellular enzymes by its membrane-stabilizing activity.²⁴

The EEPI both doses significantly decreased the levels of AST, ALT, and ALP, which indicate the plant possess the ability to prevent the intracellular enzymes leakage. The serum bilirubin level also increased by approximately 4.6-fold, which indicates the membrane damage in the liver. The serum albumin level was decreased together with a rise in serum globulin levels due to the administration of ATT drugs caused severity and impairment of liver function.²⁵ The extract treated animals showed a significant decrease in the serum bilirubin levels and an increase in the level of total protein (appr. 3.8-fold) when compared to the R-H treated animals.

The standard silymarin treated animals showed the decreased levels of AST, ALT and ALP and the levels of bilirubin and also increase the levels of total protein when compared to the drug-induced liver damage group animals. This clearly indicates that, silymarin has protective effects on the toxic effect induced by ATT drugs.²⁶

R-H causes the cytotoxic effects due to the peroxidation of the endogenous lipids.²⁵ ATT drugs cause the oxidative damage and produce the highly reactive oxygen species, which as stimulators of lipid peroxidation and source for injury to the cell membrane.²⁷ Metabolites of isoniazid, which play an essential role in liver injury by lipid peroxidation and DNA strand breaks.^{28,29}

The combination of R-H induced liver damage is intervened through oxidative damage to the hepatocyte, which caused the leakage of enzymes into vascular compartments and an increase in the malondialdehyde content which indicated the increased the level of lipid peroxidation.²⁵ The results of the present study showed a significant increase in the liver lipid peroxidation levels (appr. 2.5-fold) together with a significant decrease in GSH (non-enzymatic free radical scavenger) (appr. 2.7-fold) and superoxide dismutase (appr. 3.7-fold) and catalase (appr. 1.7-fold) (enzymatic free radical scavenger) levels were demonstrated in a combination of ATT drugs treated rats. The EEPI both doses treated rats showed decreased the levels of hepatic lipid peroxidation together with increase the levels of SOD, CAT and

GSH which is indicated the plant possess the antioxidant defence system against drugs induced liver damage.

R-H caused severe centrilobular necrosis, hepatocyte ballooning and infiltration of inflammatory tissues and which may increase in the relative liver weight in rats.³⁰ The treatment of EEPI was showed significantly improved liver histology. These effects due to the plant possess the antioxidant potentials of flavonoids and other phenolic compounds, which may play the role in reducing the R-H induced free radicals in the animal liver.

The biological compounds with its antioxidant properties may play a role in protection of the cells and tissues against deleterious effects of reactive oxygen species and other free radicals.³¹ The plant *Physalis ixocarpa* possess the antioxidant constituents which play the major role against oxidative stress and other free radicals damage to the liver induced by ATT drugs.

CONCLUSION

In the present study, we have made an attempt to evaluate the hepatoprotective activity of ethanol extract of *Physalis ixocarpa* (EEPI) by examining the prevention of Rifampicin-Isoniazid (R-H) induced hepatotoxicity in Wistar rats. From all biochemical and histopathological findings, we would conclude that the plant *Physalis ixocarpa* has significant hepatoprotective activity against in ATT drugs. The present findings provide scientific evidence to the ethnomedicinal use of *Physalis ixocarpa* genetic resources by the tribal people in treating hepatotoxicity. In the future, the detailed investigations may need to explore the molecular mechanism and genetic profile of the *Physalis ixocarpa*.

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