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Research Article

Hepatoprotective Effect of Picroliv Against Rifampicin-Induced Toxicity

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Picroliv, the active constituent of the plant *Picrorhiza Kurroa,* showed significant hepatoprotective as well as anticholestatic activity against rifampicin-induced hepatic damage. Rifampicin (50 mg/kg ip × 6 days) resulted in the reduction of bile flow as well as its contents (bile salts and bile acids) in the conscious rat and anesthetized guinea pig. Further, it also caused a decrease in the viability and rate of oxygen consumption in isolated rat hepatocytes. Picroliv treatment significantly reversed the altered parameters of bile and hepatocytes. The hepatoprotective drug silymarin on comparison was found to be less active than picroliv. Drug Dev. Res. 40:299–303, 1997.

Key words: picroliv; hepatoprotective; rifampicin; liver toxicity

INTRODUCTION

Picroliv was identified as an active hepatoprotective principle from the root and rhizome part of the plant *Picrorhiza kurroa* (Hindi: Kutki). It is a mixture of iridoid glycosides. Experimentally picroliv has shown marked liver protective activity against a wide variety of hepatotoxins [Dwivedi et al., 1991; Tripathi et al., 1991; Saraswat et al., 1993; Visen et al., 1991; Saksena et al., 1994; Dhawan, 1995]. Rifampicin is an antibiotic widely used in the treatment of tuberculosis and leprosy. Prolonged exposure to rifampicin treatment is reported to cause hepatitis, jaundice, and even death [Scheuer et al., 1974]. In the present study the activity of picroliv was assessed on the viability of rat hepatocytes (ex vivo) as well as on biliary flow (bile volume and bile contents) against rifampicin toxicity in the rat and guinea pig. The activity was compared with a standard drug, silymarin. The modulation of rifampicin toxicity by picroliv indicated that this agent could be given simultaneously to tuberculosis patients to protect the liver from rifampicin-induced toxicity.

MATERIALS AND METHODS

Animals

Adult Druckery rats (200–250 g) and guinea pigs (350–400 g) of either sex obtained from the Central Drug Research Institute (CDRI) animal house were used.

Hepatoprotective Agent

Picroliv was obtained from the Medicinal Chemistry Division of our institute. Silymarin was a gift from Dr. A. Bonati of Inverni della Beffa Milano, Italy. Both agents were used as fine aqueous suspensions.

Rifampicin (R-cin, Lupin Laboratory, Mumbai, India) was used to induce liver injury at a predetermined dose of 50 mg/kg (61 μmol/kg) ip for 6 days [Saksena et al., 1994].

Study on Bile Flow

The animals were divided into three major groups. Group I had normal animals, group II received rifampicin only, whereas the animals of group III were given different doses of picroliv simultaneously with rifampicin for 6 days. Six animals were used in each group and at each dose level. The study on bile flow was carried

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out 24 h post-treatment of the last dose of picroliv + rifampicin.

EXPERIMENTAL DESIGN

Conscious rat

The abdomen of each animal was opened with a midline incision under light ether anesthesia. The common bile duct was exposed and cannulated. A small cut was made on the back to take out the polyethylene tubing which was allowed to pass below the skin to outside the back. The abdominal opening was sutured. The animal was allowed to recover. The polyethylene tube was then passed through a flexible metallic spring to allow free movement of the animal. The outer end of the tubing was put into a graduated tube and bile was collected for 24 h in the conscious rat.

Anesthetized guinea pig

After 24 h of rifampicin injection the guinea pigs were anesthetized with urethane (0.6 ml of 25%/100 g ip). The abdomen was opened and the bile duct was cannulated with polyethylene tubing. The bile was collected for 5 h.

Estimation of Bile Contents

The method of Hawk et al. [1954] was followed for the estimation of bile salts. The method of Erwin et al. [1954] was followed for the estimation of bile acids.

Study on Isolated Hepatocytes

Rats were divided into three groups each consisting of six rats. The first group was normal. The second group received only rifampicin, while the third group received the hepatoprotective agent and rifampicin. Isolation of hepatocytes was carried out 24 h post-treatment with rifampicin. The method of Seglen [1975] was followed with slight modification [Visen et al., 1991] for the isolation of hepatocytes by continuously recirculating enzymic perfusion of the liver in situ.

Viability Test

Trypan blue exclusion test

One drop of hepatocyte stock suspension was mixed with three drops of trypan blue stain (0.2%). The unstained viable cells were counted under a microscope and were distinguishable from the stained damaged cells.

Oxygen uptake

The O_2 uptake in the isolated hepatocytes was determined by Gilsons oxygraph as described by Estrabrook [1967].

Statistical Analysis

Analysis of variance (ANOVA) followed by individual comparison by Student's *t*-test was utilized to determine the significance of mean (± s.d.) values of various groups of animals.

RESULTS

Anticholestatic Effect

Conscious rat

Rifampicin reduced the flow of bile by 30%. This was accompanied by reduction in bile salts (28%), cholic acid (28%), and deoxycholic acid (27%). Complete protection of reduced values was obtained by the 12 mg/kg dose of picroliv. A low to moderate level of protection (42–51%) was observed at 3 and 6 mg/kg.

Silymarin also showed significant protection (82–86%) when used at the 20 mg/kg dose. Lower doses of silymarin (6 and 12 mg/kg) showed lesser activity (17–48%). The results are summarized in Table 1.

Anesthetized guinea pig

Rifampicin decreased the bile flow (32%). This effect was accompanied by a decrease in bile salts, cholic acid, and deoxycholic acid (26%). Complete protection of the inhibitory effect of rifampicin was observed at the 12 mg/kg dose of picroliv in all of the parameters. The 6 mg/kg dose also showed a significant reversal of bile flow (44%), bile salts (56%), and cholic acid (48%). However, on deoxycholic acid the effect was much less.

Silymarin also exhibited a dose-dependent (6–20 mg/kg) reversal of the effect of rifampicin on bile flow (28–89%), bile salts (31–77%), cholic acid (33–89%), and deoxycholic acid (22–77%), but a significant effect was observed at the higher doses (Table 2).

Effect on Isolated Hepatocytes

Trypan blue exclusion test

Rifampicin (50 mg/kg × 6 days) treatment resulted in a 50–52% reduction in the viable hepatocytes. Treatment with picroliv resulted in 93% reversal at 12 mg/kg. Significant (65%) reversal was also observed at 6 mg/kg. The lower dose of 3 mg/kg produced only 32% protection. Silymarin in comparison gave dose-related protection of 23–84% at relatively higher doses (6–20 mg/kg). The data are summarized in Table 3.

Oxygen consumption

Rifampicin reduced the O_2 uptake rate by 50%. Significant protection of 63% and 86% was noticed with 6 and 12 mg/kg doses of picroliv, while a dose of 3 mg/kg gave only 28% protection (Table 3).

Silymarin exhibited 27–82% protective activity at 6–20 mg/kg doses (Table 3).

The ED_{50} values of both picroliv and silymarin were calculated for different parameters and are given in Table 4.

TABLE 1. Anticholestatic Effect of Picroliv and Silymarin Against Rifampicin (50 mg/kg × 6 days)-Induced Cholestasis in the Conscious Rat*

Parameter	Group I: normal	Group II: rifampicin	Group III					
			Picroliv (mg/kg) + rifampicin			Silymarin (mg/kg) + rifampicin		
			3	6	12	6	12	20
Bile flow (ml/100 g/h)	0.146 ± 0.003	0.101 ± 0.004	0.110 ± 0.003	0.120 ± 0.002**	0.148 ± 0.006**	0.112 ± 0.004*	0.122 ± 0.001**	0.140 ± 0.01**
Bile salts (mg/ml)	3.89 ± 0.07	2.80 ± 0.04	3.04 ± 0.03	3.28 ± 0.05**	3.92 ± 0.02**	3.10 ± 0.06**	3.32 ± 0.05**	3.70 ± 0.03**
Bile acids (µg/ml)								
Cholic acid	1.62 ± 0.08	1.17 ± 0.06	1.29 ± 0.02**	1.40 ± 0.07**	1.63 ± 0.09**	1.25 ± 0.03**	1.37 ± 0.01**	1.55 ± 0.02**
Deoxycholic acid	1.53 ± 0.05	1.11 ± 0.01	1.21 ± 0.03	1.34 ± 0.04**	1.53 ± 0.07**	1.19 ± 0.05	1.31 ± 0.06**	1.46 ± 0.05**

*Values are means ± s.d. of 6 animals in each group and each dose level. Group I compared with group II ($P < 0.001$) in all values.
**Group II compared with group III ($P < 0.001$).

TABLE 2. Anticholestatic Effect of Picroliv and Silymarin Against Rifampicin (50 mg/kg × 6 days)-Induced Cholestasis in the Anesthetized Guinea Pig*

Parameter	Group I: normal	Group II: rifampicin	Group III					
			Picroliv (mg/kg) + rifampicin			Silymarin (mg/kg) + rifampicin		
			3	6	12	6	12	20
Bile flow (ml/100 g/h)	0.56 ± 0.001	0.38 ± 0.03	0.40 ± 0.05	0.46 ± 0.01**	0.57 ± 0.003**	0.43 ± 0.07**	0.47 ± 0.02**	0.54 ± 0.03**
Bile salts (mg/ml)	1.60 ± 0.03	1.16 ± 0.04	1.29 ± 0.06	1.48 ± 0.03**	1.62 ± 0.07**	1.36 ± 0.03**	1.43 ± 0.04**	1.52 ± 0.01**
Bile acids (µg/ml)								
Cholic acid	2.04 ± 0.05	1.47 ± 0.07	1.62 ± 0.03	1.80 ± 0.06**	2.08 ± 0.03**	1.73 ± 0.03**	1.83 ± 0.07**	1.99 ± 0.02**
Deoxycholic acid	1.02 ± 0.03	0.75 ± 0.03	0.78 ± 0.04	0.90 ± 0.03**	1.04 ± 0.02**	0.81 ± 0.01	0.89 ± 0.03**	0.96 ± 0.05**

*Values are means ± s.d. of 6 animals in each group and each dose level. Group I compared with group II ($P < 0.001$) in all values.
**Group II compared with group III ($P < 0.001$).

TABLE 3. Effect of Picroliv and Silymarin on the Percent Viability and Oxygen Consumption of Isolated Rat Hepatocytes Against Rifampicin (50 mg/kg ip × 6 days) – Induced Hepatotoxicity (Ex vivo)*

Group	Viability (%)	Oxygen consumption (μl/h/mg protein)
Normal (group I)	97.4 ± 4.21	4.89 ± 0.09
Rifampicin (group II)	48.7 ± 2.79	2.43 ± 0.04
Picroliv (mg/kg po ×6 days) + rifampicin (group III)		
3	64.2 ± 3.37**	3.12 ± 0.03**
6	80.4 ± 4.32**	3.98 ± 0.07**
12	94.2 ± 3.17**	4.56 ± 0.04**
Normal (group I)	98.6 ± 3.21	4.54 ± 0.06
Rifampicin (group II)	47.2 ± 2.12	2.31 ± 0.03
Silymarin (mg/kg po × 6 days) + rifampicin (group III)		
6	58.2 ± 2.19	2.92 ± 0.09**
12	75.6 ± 4.16**	3.48 ± 0.06**
20	90.6 ± 3.12**	4.17 ± 0.02**

*Values are means ± s.d. of 6 animals in each group and each dose level. Group II compared with group I ($P < 0.001$ in all values).
**Group III compared with group II ($P < 0.001$).

DISCUSSION

Rifampicin is used for the treatment of tuberculosis and due to longer use its hepatotoxicity is well established in humans [Scheuer et al., 1974]. Experimentally rifampicin-induced hepatotoxicity has been reported by us in the rat at a dose of 50 mg/kg × 6 days as evidenced by significant changes in biochemical serum and liver markers [Saksena et al., 1994]. In the present study it has also produced a marked cholestatic effect as observed by the reduction in the volume of bile and its contents (bile salts and bile acids) in the conscious rat and anesthetized guinea pig. Altered values in bile flow were also reported by Back et al. [1979] following rifampicin treatment. This may be due to alteration in the transport mechanism from sinusoids to hepatocytes [Capelle et al., 1972]. Simultaneous treatment with picroliv showed significant anticholestatic activity by producing an increase in the bile flow and its contents (bile salts and bile acids). Rifampicin injection might inhibit the synthesis of bile acids, bile salts, and their conjugation with protein, thereby causing cholestasis. Picroliv reverses the altered values toward normal. It may have a possible role in inducing the Bile Salt Dependent Fraction (BSDF), thereby increasing synthesis of bile salts and bile acids and enhancing the conjugation with protein.

Following rifampicin injection, the viability of hepatocytes and rate of oxygen uptake by the hepatocyte were reduced considerably. This may be due to the disruption of the permeability of plasma membrane due to influx of Ca^{++} from the outer medium. Picroliv treatment increases the viability of hepatocytes, suggesting the stabilizing action on hepatic cell membrane by promoting the repair of injured tissues. Picroliv thus showed significant and dose-dependent activity as described earlier in other hepatotoxic models [Visen et al., 1991; Shukla et al., 1992; Dwivedi et al., 1991]. As evidenced by the ED_{50} values, picroliv showed a higher hepatoprotective activity than silymarin. The present study thus proves the hepatoprotective effect of picroliv against rifampicin toxicity, which indicates the simultaneous use of it during the treatment of tuberculosis in order to protect against rifampicin-induced liver damage.

TABLE 4. ED_{50} Values of Picroliv and Silymarin in Rifampicin Model

	ED_{50} values (mg/kg)			
	Guinea pig		Rat	
Parameter	Picroliv	Silymarin	Picroliv	Silymarin
Anticholestatic				
Bile flow	6.8	11.0	6.3	11.8
Bile salt	5.8	11.8	6.2	12.6
Cholic acid	6.2	10.2	6.5	12.6
Deoxycholic acid	5.8	13.0	6.0	12.8
Isolated hepatocytes (ex vivo)				
Viability	—	—	5.8	11.8
Oxygen uptake	—	—	6.4	12.0

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