Short Research Communication

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Hepatoprotective activity of kutkin—the iridoid glycoside mixture of *Picrorhiza kurrooa*

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The alcoholic extract of the root and rhizome of *P. kurrooa* exhibited hepatoprotective activity in rat and mastomys. The active principle was identified as kutkin and the kutkin-free fractions of the extract were found to be devoid of any activity. Kutkin showed significant hepatoprotective activity in hepatic damage induced by galactosamine (in rats) and *Plasmodium berghei* (in mastomys) as assessed by changes in several serum and liver biochemical parameters.

Picrorhiza kurrooa forms an ingredient of many Indian herbal preparations used for the treatment of liver ailments[1]. Extracts of the plant have been reported to possess hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatic damage[2]. It also exhibits choleretic activity in dogs[3]. Iridoid glycosides from the plant exhibit hepatoprotective properties against CCl_4 induced damage in mice[4]. Singh and Rastogi[5], from this Institute, reported the presence of iridoid glycosides picroside I and kutkoside (Fig.) in the alcoholic extract of the roots and named the mixture as kutkin. Kutkin also contains a small amount of other minor glycosides of *P. kurrooa*.

In the last two years, more intensive studies have been initiated on a few selected plants under the ICMR Centre for Advanced Pharmacological Research on Traditional Remedies set up at the Central Drug Research Institute (CDRI), Lucknow. Most of these plants are also undergoing clinical trials in different parts of India simultaneously. *P. kurrooa* is one such plant[6] selected for advanced study at this Centre. A systematic study is in progress, therefore, to confirm the hepatoprotective activity of the plant and to identify the active constituents. In this communication, we report the results obtained with the alcoholic extract of *P. kurrooa* and kutkin in some models

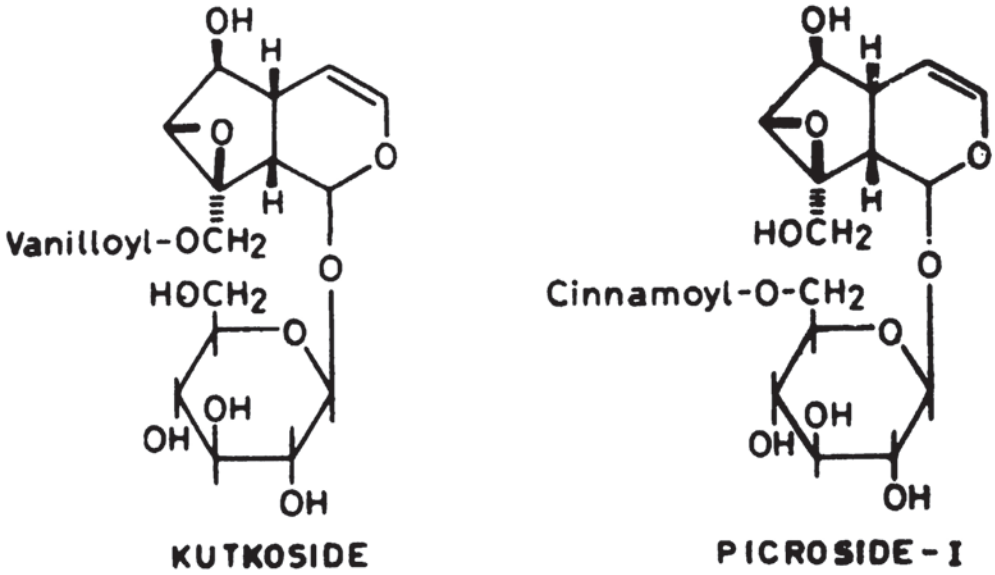

Fig. Chemical structure of major iridoid glycosides of *P. kurrooa*.

of hepatic damage in rodents.

P. kurrooa was collected from Garhwal (U.P.), identified pharmacognistically and the roots and rhizomes extracted with 95 per cent alcohol. To obtain kutkin the alcoholic extractive was macerated with 10 per cent aqueous acetone. The aqueous acetone soluble portion was evaporated and the residue was again dissolved in acetone. Partial precipitation was carried out with ether. The acetone-ether solution was evaporated. The process was repeated 8 times. The residue from the final acetone-ether solution contained mostly kutkin. In order to obtain kutkin free fractions the alcoholic extractive was macerated with 10 per cent aqueous acetone and the residue (A) was separated. The solution was evaporated and chromatographed over silica gel. All the fractions eluted before kutkin were mixed together to give pre-kutkin fraction F002. Fractions eluted after kutkin were pooled together and mixed with residue A. This formed post-kutkin fraction F003.

Adult albino rats (Sprague-Dawley) weighing 120-150 g and mastomys (*Mastomys natalensis*), weighing 50-60 g of either sex bred at the CDRI animal house were used. In each experiment the animals were divided into 3 groups each containing 8-10 animals. Group I received no treatment, group II received the hepatotoxic agent while the third group animals were treated with the test drug and the hepatotoxic agent.

Hepatic damage was induced in rats by ip administration of CCl_4 (0.7 ml/kg) thrice a week for 2 wk or a single dose of galactosamine hydrochloride (800 mg/kg). In both cases, animals were sacrificed 24 h later, serum and liver tissue being collected for biochemical analysis. The mastomys were inoculated with about half a million *Plasmodium berghei* parasitized RBC ip. The animals were sacrificed 15 days later when a parasitaemia of 10-12 per cent had been obtained and their serum sample and liver specimens were collected. All

biochemical tests were performed with standard procedures.

The rats were pretreated with the alcoholic extract of *P. kurrooa* in a dose of 20 mg/kg, po × 15 days in CCl_4 model, mastomys were given 12.5 mg/kg, po daily for 15 days from the day of inoculation with the *P. berghei* parasitized RBC. Kutkin-free fractions 50 mg/kg, po × 7 days and kutkin 6 mg/kg, po × 7 days were tested against galactosamine model. In addition, kutkin 6 mg/kg, po × 15 days was also tested in *P. berghei* model.

The alcoholic extract of *P. kurrooa* showed a significant hepatoprotective activity in CCl_4 and *P. berghei* induced hepatic damage as indicated by the lowering of the liver and serum enzymes and other parameters which were increased due to the toxic agents (Table I).

The results with kutkin in the *P. berghei* model and in the galactosamine model are given in Tables I and II respectively. Galactosamine model was chosen as it required a shorter period of drug administration and because the biochemical changes induced by galactosamine evince a greater similarity to those observed in human viral hepatitis. In both the models, a significant protection has been observed. In the *P. berghei* model higher protection was obtained n comparison with the crude alcoholic exkact (Table I), although a smaller dose of kutkin was employed. Further, this dose provided 100 per cent protection against changes in liver glucose-6-phosphatase, nucleotidase and glutamate pyruvate transaminase.

Due to availability in limited quantities, kutkin-free fractions of the alcoholic extract (F002 and F003) were tested in the galactosamine model only (in a dose of 50 mg/kg, po for 7 days). Galactosamine induced changes in serum GPT, acid and alkaline-phosphatase were significantly (37, 180 and 91% respectively) aggravated by F002 and in alkaline phosphatase only (110%) by F003. No alteration was seen in other serum parameters or liver lipids (Table II).

Table I. Hepatoprotective activity of alcoholic extract of *P. kurrooa* (20 mg/kg, po and 12.5 mg/kg, po respectively daily for 15 days) against CCl_4 and *P. berghei* induced hepatic damage and of kutkin 6 mg/kg, po × 15 days in *P. berghei* model (in mastomys)

Parameter	Per cent protection		
	CCl_4 model	*P. berghei* model	
	Alc ext	Alc ext	Kutkin
A. Serum:			
Glutamate pyruvate transaminase	31	29	27
Glutamate oxaloacetate transaminase	41	33	57
Alkaline phosphatase	53	33	65
Lipoprotein-X	—	55	26
Bilirubin	78	87	68
Triglyceride	32	58	85
Cholesterol	53	15	39
B. Liver:			
Total lipids	50	69	32
Phospholipid	71	70	53
Cholesterol	—	43	—
Lipid peroxides	12	64	28
Superoxide dismutase	—	30	44
Acid phosphatase	—	40	100
Acid ribonuclease	—	37	15
N'-Nucleotidase	100	—	100
Succinate dehydrogenase	100	—	100
Glucose-6-phosphatase	100	—	100

Table II. Effect of kutkin (6 mg/kg, po) and kutkin-free fractions (F002, F003) in a dose of a mg/kg, po × 7 days on galactosamine induced hepatic damage in rats

Parameter	Per cent protection		
	Kutkin	F002	F003
A. Serum:			
Glutamate oxaloacetate transaminase	48	—37	— 3
Glutamate pyruvate transaminase	27	—13	—11
Acid phosphatase	70	—180	4
Alkaline phosphatase	30	—91	—110
Glutamate dehydrogenase	28	—5	0
Bilirubin	95	0	0
B. Liver:			
Total lipids	66	0	0

(—) indicates increased hepatotoxicity

In conclusion, it may be stated that *P. kurrooa* extract demonstrated significant hepatoprotective effect which appeared to be due to a mixture of iridoid glycosides, kutkin. The situation seems similar to another drug *Commiphora mukul* in which several steroids are responsible for hypolipidaemic activity and a standardised fraction found most useful clinically[7]. The kutkin-free extracts were not only devoid of activity but seemed to aggravate galactosamine toxicity and therefore should be avoided in treatment of liver disorders. Preliminary experiments suggest that pure glycosides picroside I and kutkoside are not more active than kutkin (unpublished data of CDRI, Lucknow). It appears thus advisable to develop kutkin as a hepatoprotective agent. Further studies are in progress to elucidate the mechanism of hepatoprotective effect of kutkin as well as the spectrum of its biological activities.

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