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. Extracts of the plant have been reported to possess hepatoprotective activity against carbon tetrachloride (CCl₄) induced hepatic damage. It also exhibits choleretic activity in dogs. Iridoid glycosides from the plant exhibit hepatoprotective properties against CCl₄ induced damage in mice. Singh and Rastogi, 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 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.
Hepatoprotective activity of kutkin

![Chemical structures of Vanillyl-O-CH₂ and Cinnamoyl-O-CH₂](image)

**KUTKOSIDE**

**PICROSIDE-I**

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

of hepatic damage in rodents.

*P. kurrooa* was collected from Garhwal (U.P.), identified pharmacognostically 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 CCl₄ (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 CCl₄ 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 CCl₄ 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 1).

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 in comparison with the crude alcoholic extract (Table 1), 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).
Hepatoprotective activity of kutkin

Table II. Effect of kutkin (6 mg/kg, po) and kutkin-free fractions (F002, F003) in a dose of 1 mg/kg, po x 7 days on galactosamine induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Per cent protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kutkin</td>
<td>F002</td>
</tr>
<tr>
<td>Glutamate oxaloacetate transaminase</td>
<td>48</td>
</tr>
<tr>
<td>Glutamate pyruvate transaminase</td>
<td>27</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>70</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>30</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>28</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>95</td>
</tr>
</tbody>
</table>

A. Serum:

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. 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.

References


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