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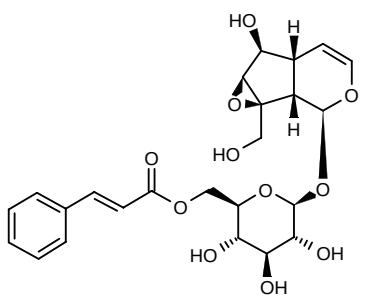
Picroliv

Hepatoprotectant
Immunomodulator

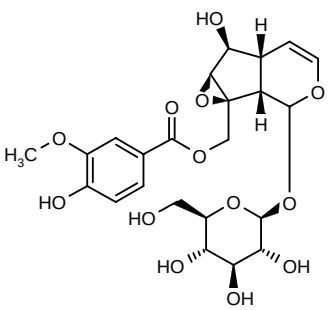
Standardized iridoid glycoside fraction obtained from roots and rhizomes of *Picrorhiza kurroa*. It contains kutkin (picroside I and kutkoside in a ratio 1:1.5), the balance being minor constituents

EN: 197629

Picroliv is a standardized iridoid glycoside fraction obtained from the roots and rhizomes of *Picrorhiza kurroa* Royle (Scrophulariaceae), containing at least 60% of a 1:1.5 mixture (w/w) of picroside I and kutkoside, the remainder (40%) being a mixture of iridoid and curcurbitacin glycosides and some unidentified substances (1-5). Picroliv-free extracts of *P. kurroa* were found to be devoid of hepatoprotective activity (6).



Picroside I



Kutkoside

Preparation

Roots and rhizomes of *P. kurroa* were collected, dried, powdered and extracted with alcohol by cold percolation. The extract was evaporated *in vacuo* below 50 °C. The extracts were dissolved in methanol:water (1:1 v/v) and extracted with chloroform. The chloroform phase was discarded and the aqueous-methanolic phase was extracted with ethyl acetate. The extracted aqueous-methanolic layer was then extracted with butanol. The ethyl acetate-soluble and butanol-soluble fractions were combined and evaporated to dryness in vacuo to give picroliv. On the basis of HPLC and TLC, picroliv contained about 60% of picroside I and kutkoside in a ratio of 1:1.5, the balance being minor constituents.

Description

Light yellowish brown amorphous powder with a characteristic odor and bitter taste.

Introduction

The liver has a pivotal role in the regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, the detoxification of a variety of drugs and xenobiotics occurs in the liver. The bile secreted by the liver has, among other things, an important role in digestion.

Liver diseases are among the most serious ailments and are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons, *etc.*), excess consumption of alcohol, infection and autoimmune disorders. Most hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage (7-10). Enhanced lipid peroxidation produced during liver microsomal metabolism of ethanol results in hepatitis and cirrhosis (11). The majority of cases of acute hepatitis are due to viruses. Hepatitis B infection often results in chronic liver disease and cirrhosis of the liver. Primary liver cancer has also been shown to be produced by these viruses. It has been reported that approximately 14-16 million people are infected with hepatitis virusus in Southeast Asia and about 6% of the total population in the region are carriers of the virus (12).

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Table I: Hepatoprotective activity of picroliv.

Toxin*	Dose/kg	Percent change (range)	Picroliv dose (mg/kg p.o.) x days	Percent protection with picroliv (range)	Ref.
Aflatoxin B	7 mg p.o.	36-600	25 x 7	80-100	67
Amanita toxin	50 mg p.o.	10-100	25 x 5	40-100	68, 69
Carbontetra chloride	0.7 ml x 6 i.p.	35-120	12 x 15	70-100	70, 71
d-Galactosamine	80 mg i.p.	35-890	12 x 7	50-100	72, 73
Ethylalcohol	20 ml (40%) x 21 p.o.	30-180	6 x 7	60-90	71, 74
Lanthanum chloride	4 mg i.v.	20-90	25 x 7	30-100	75
Monocrotaline	120 mg p.o.	15-105	12 x 12	70-95	76
Oxytetracycline	200 mg i.p.	40-100	12 x 7	80-100	30
Paracetamol	2 g p.o.	35-100	12 x 7	50-100	72, 77
Plasmodium berghei	5 x 10^3 parasitized RBC, i.p.	30-180	12 x 15	36-85	59, 60, 78
Rifampicin	50 mg x 5 i.p.	15-60	12 x 6	45-100	79, 80
Thioacetamide	100 mg s.c.	35-170	25 x 7	50-90	81

*All data from rat except *Plasmodium berghei* where mastomys was used.

Despite the tremendous advances made in medicine, no effective hepatoprotective agent is yet available. Plant-derived drugs are known to play a vital role in the management of liver diseases. A number of plants are described in the Ayurveda (the ancient system of medicine in India whereby patients were treated with plants) for use in the treatment of liver disorders. *Picrorhiza kurroa* constitutes a major ingredient of many such Indian herbal preparations (13-15). It is a mild hairy perennial herb growing wild in the Alpine Himalayan region from Kashmir to Sikkim at an altitude of 2700-5000 meters.

Several experimental studies have shown that the extract of *P. kurroa* protects rats against carbon tetrachloride-induced liver damage (16) and has a cholerectic effect in dogs (17). Clinical studies have also been done with the extract in patients suffering from jaundice (18-20). All these studies have demonstrated the hepatoprotective effects of this plant.

Pharmacological Actions

The hepatoprotective activity of picroliv has been studied in rats with hepatic damage induced by various agents, *i.e*., galactosamine hydrochloride, paracetamol, thioacetamide, carbon tetrachloride, lanthanum chloride, monocrotaline, ethyl alcohol and *Plasmodium berghei* infection. The activity was assessed by changes in various serum and tissue biochemical parameters used as indicators of liver function, *i.e.*, total protein, cholesterol, phospholipids, acid and alkaline phosphatase, GOT, GPT, lactate dehydrogenase, albumin, triglycerides, lipoproteins, glutamate dehydrogenase, bilirubin, DNA, RNA, glycogen, total lipid, lipid peroxidase, succinate dehydrogenase, glucose-6-phosphatase, acid ribunuclease, 5'-nucleotidase, gamma-glutamyltranspeptidase, super-oxidase dismutase, cytochrome P-450, *etc.* The effect was also assessed by histopathological examination of the liver.

Picroliv (3-12 mg/kg p.o. for 1-2 weeks) showed potent, dose-dependent hepatoprotective/antihepatotoxic activity as indicated by significant reversal (30-100%) of the altered parameters. Histopathological changes were also normalized in picroliv-treated animals (Table I).

In most of these studies, silymarin, the active principle of *Silybum marianum* (21), was used as a standard for comparison. Picroliv was more active than silymarin in the majority of experiments (22).

Studies were conducted on isolated hepatocytes *ex vivo* or *in vitro*. Hepatocytes were isolated from rat liver (23) and the effects of picroliv on viability (trypan blue exclusion test), oxygen uptake, selected biochemical parameters such as transaminases and bilirubin, and metabolic activity, as judged by the rate of RNA/DNA synthesis, were examined. Picroliv was devoid of any effect on hepatocytes per se, but could prevent or reverse the effects of toxins such as thioacetamide (24), galactosamine (25), paracetamol (26), rifampicin (27), alcohol (28, 29) and oxytetracycline (30).

Picroliv showed significant curative activity *in vitro* in primary cultured rat hepatocytes against toxicity induced by thioacetamide, galactosamine and carbon tetrachloride. Activity was assessed by determining the change in hepatocyte viability, rate of oxygen uptake and other biochemical parameters. The toxic agents produced 40-62% inhibition of cell viability and altered biochemical parameters (GOT, GPT and alkaline phosphatase) after 24 h of incubation at 37 °C. Incubation of damaged hepatocytes with picroliv (1-100 μg/ml) resulted in a concentration-dependent restoration of altered viability and biochemical parameters (31).

Picroliv showed dose-dependent (1.5-12 mg/kg p.o. x 7 days) choleretic effects in conscious rats and anesthetized guinea pigs and cats (25). It also possessed marked anticholestatic effects against paracetamol- (32), carbon tetrachloride- (33), galactosamine- (25), ethinylestradiol- and thioacetamide-induced cholestasis (34). It almost completely antagonized the reduced volume of bile and bile contents (bile salts and bile acids) at the

doses of 6 and 12 mg. In these studies, picroliv was again more active than silymarin.

Studies conducted in partially hepatectomized rats (35) using several biochemical and histological parameters assessed the effect of picroliv on liver regeneration. Picroliv facilitated liver regeneration following partial hepatectomy, the most marked effects being an increase in mitotic index and RNA and DNA synthesis during the early phase of regeneration (36, 37). An increase in protein and nucleic acid synthesis was also observed in liver homogenates of normal rats treated with picroliv for 7 days. The drug could also reverse the inhibitory effect of cycloheximidine on protein synthesis. The FoF1, ATPase activity of liver mitochondria was also increased (38).

In another study in picroliv-fed, partially hepatectomized rats, the rate of recovery in lipid peroxidase, cytochrome P-450, DNA, RNA, glycogen, total proteins and acid ribonuclease was faster. The results indicated that picroliv stimulated regeneration due to accelerated removal of lipid peroxides accumulated as a result of hepatectomy, which may lead to more rapid repair of damage to the cellular membrane. In addition, picroliv helps restore hepatic glycogen and total proteins, which may also result in an acceleration of the reparative process (39, 40).

Viral hepatitis is one of the most common diseases of the liver and the antiviral activity of picroliv has also been studied. Hepatitis B surface antigen (HBsAg)-positive serum samples from patients with acute and chronic liver disease, as well as healthy carriers, were incubated with picroliv and a promising anti-HBsAg-like effect was observed (41).

Picroliv could also prevent or clear viremia in ducks infected with a related virus and protected mice against other unrelated viruses such as encephalomyocarditis virus (unpublished observations).

Picrorhiza kurroa also constitutes a major ingredient in many Ayurvedic preparations prescribed for the treatment of bronchial asthma. Several experimental and clinical studies have been reported on the antiasthmatic and antiallergic activity of *P. kurroa* (42-48). Picroliv (6-25 mg/kg p.o.) inhibited passive cutaneous anaphylaxis (PCA) in mice (82%) and rats (85%) and protected mast cells from degranulation (60-80%) in a concentration-dependent manner. Its effect was also studied in sensitized guinea pig ileum preparation *in vitro* and in normal guinea pigs *in vivo*. Inhibition of the Schultz-Dale reaction in sensitized guinea pig ileum was seen, but *in vivo* bronchospasm induced by histamine could not be antagonized or prevented by picroliv, indicating the absence of a direct postsynaptic histamine receptor-blocking effect. It was further confirmed that picroliv is devoid of antihistaminic or smooth muscle relaxant activity. These results were comparable to those for the clinically used antiallergic drug disodium cromoglycate (DSCG, 50 mg/kg i.p.). It was concluded that picroliv also possesses antiallergic activity which may be due to suppression of chemical mediator release from mast cells and immunomodulatory activity (49).

Picrorhiza kurroa extracts have been shown to modulate certain cell-mediated and humoral immune responses (50) and to stimulate the production of macrophage inhibition factor by sensitized human lymphocytes (51, 52). In a clinical study with *P. kurroa* in 25 patients suffering from bronchial asthma along with other diseases, *i.e.*, ankylosing and cervical spondylitis, osteoarthritis, psoriasis, leukoderma, *etc.*, symptom relief for up to several months was achieved after the treatment. The only common characteristic of all these cases of unknown etiology appeared to be an immunological factor. Therefore, it was concluded that *P. kurroa* has significant immunomodulating activity (43).

The effect of picroliv was then studied on specific and nonspecific immune responses in Balb/c mice and hamsters. Picroliv pretreatment produced a significant increase in hemagglutinating antibody titer, plaque-forming cells and delayed-type hypersensitivity responses to sheep red blood cells in mice. It also facilitated nonspecific immune responses, *i.e.*, it activated macrophages, increased [^{3}H]-thymidine uptake by lymphocytes in Balb/c mice and protected against *Leishmania donovani* promastigote challenge in hamsters (53).

Picroliv has also been found to be a potent inhibitor of hepatocarcinogenesis induced by *N*-nitrosodiethylamine (NEDA) in male Wistar rats. Animals in the carcinogen-administered group had a 100% incidence of tumors and the liver weight was increased.Carcinogen-induced elevation of γ-glutamyltranspeptidase levels in liver and serum were reduced to normal by simultaneous administration of picroliv (40 and 200 mg/kg). Moreover, elevated levels of bilirubin, alkaline phosphatase, GPT and peroxides in serum were reduced to the levels of normal rats by picroliv. Similar effects were also seen on glutathione and glutathione-*S*-transferase levels in liver. Picroliv was found to increase the life span of tumor-bearing animals (54).

Picroliv is also able to lower serum lipids (total, VLDL and LDL cholesterol), associated with an increase in plasma lecithin-cholesterol acyltransferase and lipolytic activity, as well as HDL cholesterol, in both normal and hyperlipidemic animals. In the latter case, elevated levels of serum triglycerides and phospholipids were also reduced towards normal. Cholesterol biosynthesis in the liver was inhibited and the fecal excretion of bile acids was increased by picroliv (55).

Picroliv is devoid of any other significant effects (CNS, cardiovascular or antiinflammatory). In view of its immunomodulatory actions, it was also tested for efficacy against filarial and leishmanial infections, but was found to be ineffective.

Picroliv has shown the ability to protect against isoproterenol- and coronary artery ligation-induced ischemia (56).

Mechanism of Action

Studies are under way to elucidate the basic mechanisms involved in the pharmacological actions of picroliv.

Picroliv antagonizes paracetamol-induced decreases in LDL receptor cell-surface expression and increases in conjugated dienes in hepatocytes (57). It is also a potent scavenger of superoxide anions (58). In rats infected with *P. berghei*, picroliv reduced the increased levels of lipid peroxidation products in the liver and brain and normalized glutathione metabolism (59-61). Thus, its hepatoprotective effect appears to result from a combination of membrane-stabilizing, hypolipidemic and antioxidant properties. These properties may also be responsible for the effects on the immune system.

Toxicity

The LD_{50} by the intraperitoneal route in mice was 2026.9 mg/kg. When given orally, no mortality was observed up to a dose of 2500 mg/kg in mice or rats.

Three different doses (15, 30 and 60 mg/kg) of picroliv were administered orally to rats for 90 consecutive days. The hematology, biochemistry and histopathology were within normal limits. In another study, 20 (10 male and 10 female) healthy adult rhesus monkeys were administered picroliv orally at doses of 7.5, 15.0 and 30.0 mg/kg/day for 90 days. Daily examination, food intake, monthly body weight, hematology and blood biochemistry data, as well as gross and microscopic observations, did not reveal any abnormalities (62).

Picroliv has also been found to be devoid of mutagenic or teratogenic potential in two species of laboratory animals (63).

Clinical Studies

In a 2-part phase I study, single and multiple doses of picroliv were given orally for 4 weeks. The principal aim of the trial was to determine the safety of picroliv upon acute administration of gradually escalating doses (20-300 mg) in healthy volunteers. Clinical monitoring up to 24 h revealed stable vital parameters. Pre- and postdrug electrocardiogram and laboratory parameters were within normal limits. Subsequently, a multiple-dose study was undertaken in which a low (100 mg) and a high dose (200 mg) of picroliv was administered orally for 4 weeks. In the low-dose group no side effects were observed and the drug was well tolerated. Out of 10 cases in the high-dose group, 1 complained of heaviness in the abdomen and another developed mild constipation. However, the drug could be continued in both cases for 4 weeks. There were no significant changes in vital signs, electrocardiogram, hematological or biochemical parameters in either the low- or high-dose group (64).

A phase II clinical study of picroliv was carried out in 20 male and female patients, aged 15-65 years, suffering from acute viral hepatitis of less than 15 days' duration, with serum bilirubin of more than 5 mg% and SGPT of more than 300 U/l. In this double-blind study, patients received either picroliv (100 mg) or matching placebo twice daily for 4 weeks. Clinical and organ function tests were repeated weekly, and basal HBsAg status, if positive, was repeated on day 28. The composite score for symptomatology was comparable in both groups on day 0. However, after 1 week, a significant difference in symptom score (reduction) was observed in the picroliv-treated group ($p < 0.05$). Analysis of individual symptoms and signs revealed that in the picroliv-treated group, anorexia, weakness, nausea and vomiting, body ache and icterus were significantly improved by day 7. In the placebo group, these symptoms persisted until day 28. A reduction in bilirubin was also seen. The early recovery in the picroliv group was significant compared to the placebo group ($p < 0.05$).

HBsAg status conversion occurred in 2 out of 4 patients in the picroliv group who were positive on day 0, while there was no change in 2 HBsAg-positive cases on placebo. This study highlights the ability of picroliv to accelerate recovery in patients with viral hepatitis (65).

In a recently concluded clinical study, 14 patients with acute viral hepatitis were treated with picroliv (100 mg twice daily for 4 weeks). Response was judged by improvement in clinical symptoms/signs, as well as in liver function tests (serum bilirubin, alkaline phosphatase, proteins and SGPT).

There was marked improvement in the signs and symptoms of patients at the end of the 4-week trial. Progressive improvement in liver function tests was also seen, with serum bilirubin, SGPT and alkaline phosphatase decreasing from initial values after 4 weeks of picroliv therapy. No drug-related side effects were observed (66).

Further multicenter clinical trials are in progress.

Manufacturer

Central Drug Research Institute (IN).

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