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Effects of Picroliv, the active principle of *Picrorhiza kurroa*, on biochemical changes in rat liver poisoned by *Amanita phalloides*

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ABSTRACT The efficacy of Picroliv, a standardized iridoid glycoside fraction of *Picrorhiza kurroa*, was studied against the *Amanita phalloides*–induced biochemical changes in rat liver. *A phalloides* (50 mg · kg^{-1}) caused significant increases in the activities of hepatic 5′–nucleotidase, γ–glutamyl transpeptidase, acid ribonuclease, and succinate dehydrogenase, but a decrease in glucose–6–phosphatase. The level of cytochrome P–450 in microsomal fraction and content of glycogen in liver showed significant depressions. Picroliv (25 mg · kg^{-1} · d^{-1} × 10 d) provided significant restorations of all the biochemical changes caused by *A phalloides* except cytochrome P–450 and glycogen. These results demonstrated the protective effect of Picroliv against *A phalloides*–induced hepatotoxicity in rats.

KEY WORDS *Amanita*; *Picrorhiza kurroa*; Picroliv; liver; enzyme tests; cytochrome P–450; glycogen

Picroliv (Pic) is a standardized iridoid glycoside fraction isolated from roots and rhizomes of *Picrorhiza kurroa*. It contains 60% picroside I and kutkoside in a ratio of 1 : 1.5, the balance being minor constituents. Earlier studies have shown the hepato–protective efficacy of Pic against CCl_4–[1], galactosamine–[2], monocrotaline–[3], paracetamol–[4], and thioacetamide–[5] induced liver damages in rats, and *Plasmodium berghei* infection in *Mastomys*[6].

A phalloides (*AP*) is the most toxic mushroom causing 95% of total mushroom poisonings. It contains mainly 2 toxins : amanitin and phalloidin. Phalloidin is the major toxic agent and is known to act on plasma membrane of hepatocytes in rats[7]. However, the mortality of rats poisoned with *AP* was reduced by Pic[8]. In this communication we have reported the influence of Pic on biochemical changes poisoned by *AP* in rat liver.

MATERIALS AND METHODS

Adult ♂ rats (wt 130 ± *s* 15 g) of Sprague–Dawley strain, inbred in CDRI Animal House were used. The rats were fed *ad lib* standard pellet diet (Lipton, Bombay) and allowed free access to water.

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Induction of hepatotoxicity Rats were injected ip *AP* (50 mg · kg^{-1}), fasted overnight and decapitated after 10 d.

Treatment with Pic A freshly prepared aqueous solution of Pic was given intragastrically (ig) at a dose of 25 mg · kg^{-1} simultaneously with *AP* injection. Pic feeding was continued for 9 d further. Two groups of rats were used as control, one receiving normal saline with *AP* and the other an equal volume of normal saline only.

Before decapitation, blood was drawn from the retro-orbital plexus and kept for 30 min to obtain the serum. The liver was excised, washed with chilled NaCl 145 mmol · L^{-1} (solution), and 10% (wt / vol) homogenate was prepared in KCl 154 mmol · L^{-1}.

Biochemical estimations The activities of 5′-nucleotidase (EC 3.1.3.5), γ-glutamyl transpeptidase (EC 2.3.2.2), acid ribonuclease (EC 3.1.4.22), acid phosphatase (EC 3.1.3.2), succinic dehydrogenase (EC 1.3.99.1), glucose-6-phosphatase (EC 3.1.3.9), cytochrome P-450, and cytochrome b_5 in liver and GOT, GPT, and alkaline phosphatase in serum were assayed as described in Ref 4.

The levels of lipid peroxides, total lipids, phospholipids, cholesterol, DNA, RNA, total proteins, and liver glycogen and total serum protein and albumin were also measured as in Ref 4.

Statistical analyses The *AP*-treated group was compared with the control while the Pic-treated group was compared with *AP*-intoxicated group. One way analysis of variance was done among 3 groups. Individual significance of group means was done by Newman′s and Keul′s test for multiple comparison. *P* values were calculated by using *t* test. $P<0.01$ was considered significant.

RESULTS

Induction of hepatotoxicity by *AP* Pilot experiments were carried out at 2.5, 5.0 (iv) 25, 50 (ip) mg · kg^{-1}. The changes in rat liv and serum were studied after 1, 2, 10, an 20 d. The magnitude of changes was maxim on d 10 after 50 mg · kg^{-1}. The biochemic changes in liver poisoned by *AP* and their values are given in Tab 1.

AP caused significant augumentation i the activities of 5′-nucleotidase an γ-glutamyl transpeptidase. The increase i the activity of 5′-nucleotidase was relative slight (18%) but that of γ-glutam transpeptidase was much higher (60%). About 50% elevation in the activities of ac ribonuclease and succinic dehydrogenase wa observed. The activity of glucose-6- phosphatase in liver and content o cytochrome P-450 in the microsomal fractio of liver cells were decreased significantly, th degree of reduction being 43% and 78% respectively. Other hepatic enzymes ac phosphatase [55 ± 3 nmol of *p*-nitrophenol formed / (min · mg protein)] and cytochrom b_5 [0.31 ± 0.02 nmol / (mg microsomal pro tein)] remained unaffected. The hepati glycogen was found to be almost completel exhausted. However, other chemic constituents of liver like total lipids (1 ± 4 mg · g^{-1}), phospholipids (19.5 ± 0 mg · g^{-1}), cholesterol (9.7 ± 0.3 mg · g^{-1}) lipid peroxides (117.5 ± 26.0 nmol of malon dialdehyde formed · g^{-1}), DNA (2.6 ± 0 mg · g^{-1}), RNA (4.38 ± 0.37 mg · g^{-1}), an proteins (164 ± 5 mg · g^{-1}) remained unaffe ed. Serum parameters did not show any sig nificant change following *AP* exposure. Th levels of cytochrome b_5, DNA, RNA, protei total lipids, phospholipids, cholesterol, an lipid peroxides in liver and GOT, GPT, alk line phosphatase, bilirubin, protein, an albumin in serum were not altered at any o the doses of *AP* during 1-20 d.

Hepatoprotection by Pic Pic 25 m · kg^{-1} for 10 d, significantly reversed the in crease in the activities of 5′-nucleotida

Tab 1. Effects of Picroliv on biochemical indices in liver poisoned by *Amanita phalloides* and Q values with significances. $n=6$ rats. $\bar{x} \pm s$. $^{***}P<0.01$ *vs* Control. $^{+}P>0.05$, $^{+++}P<0.01$ *vs A phalloides*. $^{\S}P>0.05$, $^{\S\S\S}P<0.01$ *vs A phalloides* + Picroliv. (% of protection in parentheses)

	Control	*A phalloides*	*A phalloides* + Picroliv
5′-Nucleotidase, nmol P_i released / (min · mg protein)	38 ± 2 $Q=0.94^{\S}$	45 ± 1 $Q=11.79^{***}$	39 ± 1 (86%) $Q=10.85^{+++}$
γ-Glutamyl transpeptidase, ng *p*-nitroaniline released / (min · mg protein)	338 ± 46 $Q=7.35^{\S\S\S}$	540 ± 40 $Q=12.25^{***}$	456 ± 33 (42%) $Q=4.90^{+++}$
Acid ribonuclease, change in absorbance / (min · mg protein)	0.043 ± 0.009 $Q=3.43^{\S\S\S}$	0.065 ± 0.004 $Q=9.33^{***}$	0.052 ± 0.002 (62%) $Q=5.90^{+++}$
Succinate dehydrogenase, change in absorbance / (min · mg protein)	0.0081 ± 0.0009 $Q=0.66^{\S}$	0.0123 ± 0.0016 $Q=6.94^{***}$	0.0086 ± 0.0007 (88%) $Q=6.12^{+++}$
Glucose-6-phosphatase, nmol of P_i released / (min · mg protein)	82 ± 6 $Q=0.46^{\S}$	47 ± 8 $Q=13.56^{***}$	81 ± 3 (97%) $Q=13.10^{+++}$
Cytochrome P-450, pmol / (mg microsomal protein)	386 ± 68 $Q=9.93^{\S\S\S}$	86 ± 61 $Q=11.92^{***}$	144 ± 56 $Q=1.98^{+}$
Glycogen, $mg \cdot g^{-1}$	78 ± 11 $Q=25.18^{\S\S\S}$	1.97 ± 0.25 $Q=26.55^{***}$	5.9 ± 4.3 $Q=1.37^{+}$

(68%), γ-glutamyl transpeptidase (42%), acid ribonuclease (62%), and succinic dehydrogenase (88%). Almost complete restoration of the reduced activity of glucose-6-phosphatase was also noted. However, the decreased content of cytochrome P-450 and glycogen in liver remained unaffected by Pic.

DISCUSSION

Rats are quite refractory to α-amanitin and they develop phalloidin-like toxicity when poisoned with whole mushroom[9]. Phalloidin acts at the membrane by binding to actin-like protein, thus causing damage of membrane integrity[10]. Our results also showed that *AP* increased the activities of enzymes 5′-nucleotidase and γ-glutamyl transpeptidase as consequence of damage to plasma membrane of liver cells. Significant protection in both of these enzymes was also observed after Pic administration. We have earlier reported that Pic provided significant restoration in the biochemical changes induced by *D*-galactosamine[2]. In this case the structural and functional impairment in membrane is perhaps one of the mechanisms involved. Histological studies[11] showed that phalloidin acts primarily on the membranes of liver lysosomes leading to release of lysosomal enzymes. Our results also showed increased activitity of acid ribonuclease following administration of *AP*. Its reversal by Pic indicated that the damage of lysosomal membrane caused by *AP* is repaired by Pic administrtion. The alterations in the activities of succinic dehydrogenase, glucose-6-phosphatase and cytochrome P-450 by *AP* is indicative of altered mitochondrial, microsomal, and drug metabolizing functions of the liver. The activities of succinic dehydrogenase and glucose-6-phosphatase have significantly been restored by Pic, thus demonstrated its effectiveness against the

mitochondrial and microsomal damages. The drastic decrease in the levels of cytochrome P–450 and glycogen in liver remained unaffected with Pic, and a longer exposure of Pic might be required. Experiments are proceeding to elucidate the exact mechanism of action of Pic in antagonising the effects of *AP* and other toxic agents on liver.

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