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EFFECT OF GLYCYRRHETINIC ACID ON PYRIDINE TOXICITY IN MOUSE

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ABSTRACT: *The present work was undertaken to investigate the effect of glycyrrhetinic acid on pyridine toxicity. When glycyrrhetinic acid was pretreated, pyridine-induced CNS depression and mortality were decreased. A striking enhancement of serum transaminase activities after pyridine administration was markedly decreased by glycyrrhetinic acid pretreatment. It was also observed that the hepatic microsomal aniline hydroxylase activity, which is concerned with pyridine metabolism, was significantly increased in glycyrrhetinic acid pretreated mice. These results indicated that the inducing effects of glycyrrhetinic acid on the pyridine metabolizing enzymes are believed to be a possible detoxication mechanism for the pyridine toxicity in mice.*

Keywords: *Glycyrrhetinic acid, Pyridine, Aniline hydroxylase*

INTRODUCTION

Licorice is a widely distributed plant used in Europe and Asia as a popular remedy for several thousand years. Its importance as a medicinal agent was recognized thousand years ago for the treatment of ulcer and intoxication.

Recently, a number of investigators have performed chemical and pharmacological studies on Glycyrrhizae Radix, the pharmacologically active components such as glycyrrhizin (Serrano, 1946; Beaton and Spring, 1955), liquiritin (Shinoda and Ueeda, 1934) and herniarine (Reiners, 1964) have been isolated from the plant. It is reported that glycyrrhetinic acid, obtained from hydrolysis of glycyrrhizin (aglycone) (Kuboki, 1954; Beaton, 1955), one of the constitutents of licorice, exerts anti-inflammatory (Finney and Somers, 1958; Kraus, 1960; Capass *et al.*, 1983), antitussive (Anderson and Smith, 1961) and adrenal corticoid hormone like action (Molhuysen, 1950).

However, despite of extensive studies, the detoxication mechanism of licorice is still obscure. Therefore, the present work was undertaken to investigate the effect of glycyrrhetinic acid on pyridine intoxication including CNS depresseion, mortality and pyridine metabolizing enzyme activity in liver.

MATERIALS AND METHODS

Materials

NADP, glucose 6-phosphate, bovine serum album in were obtained from Sigma Chemical Co. and glycyrrhetinic acid, p-aminophenol from Wako Chemical Co. and nicotinamide from Nakarai Chemical Co. The other reagents used were of reagent grade.

Experimental Animals

Male mice weighing 25g were used for all studies. Mice were given glycyrrhetinic acid (10mg/kg) intraperitoneally once daily for 5 days. Control mice were given 0.9% sodium chloride solution intraperitoneally. All experimental animals were allowed free access to food and water but deprived of food for the 16 hr prior to sacrifice.

CNS Depression

Mice were given pyridine (0.43g/kg) intraperitoneally 24 hr after the last dose of glycyrrhetinic acid. Control mice were given pyridine alone. The animals were observed for CNS depression as evidence by the loss of the righting reflex.

Acute Toxicity

Mice were treated pyridne (0.87g/kg) intraperitoneally 24 hr after the last dose of glycyrrhetinic acid. Control mice were given pyridine alone. Mice were observed any death within 24hr.

Hepatotoxicity

The animals were injected intraperitoneally 60 min after glycyrrhetinic acid administration for 3 days. The degree of the liver damage produced by pyridine was expressed as the serum transaminase activity observed in the experimental animals.

Preparation of Liver Microsomal Fraction

The animals were killed by exsanguination from the abdominal vein. The liver was exhaustively perfused with cold 0.15M NaCl solution through the protal vein until it was uniformly paled and quickly removed. The liver was homogenized in cold 0.1M Na^+/K^+ phosphate buffer (containing 1.15% KCl, pH 7.4) by three passes of a motor-driven teflon pastle in a glass homogenizing vessel. The homogenate (20% W/V) was sequential centrifuged at $600\times g$, $10{,}000\times g$ and $105{,}000\times g$. Then the $105{,}000\times g$ pellet was used as the microsomal fraction.

Enzyme Assay

Aniline hydroxylase activity was measured by the amount of *p*-aminophenol formed according to Imai and Sato method (1966). The standard assay mixture (4ml) contained 0.1M Na^+/K^+ phosphate buffer (pH 7.5), 5 μ moles aniline, 0.5 μ mole NADP, 10 μ moles glucose 6-phosphate, 50 μ moles nicotinamide, 25 μ moles $MgCl_2$ and enzyme solution. Enzyme activity was defined as a *p*-aminophenol n moles per mg protein per hr at 37°C. Serum transaminase (ALT, AST) activities were estimated according to the procedure described Reitman and Frankel (1957). A unit of transaminase was expressed as the Karmen unit (1955) per ml of serum. Protein was determined by the method of Lowry *et al.* (1951) and bovine serum albumin as a standard.

RESULTS

Effect of Glycyrrhetinc Acid on Pyridine-induced CNS Depression

Table 1 shows the effect of glycyrrhetinic acid on the CNS depression in pyridine treated mice. The CNS depression time was diminished by the pretrearment of glycyrrhetinic acid about 35%.

Effect of Glycyrrhetinic Acid on Acute Pyridine Toxicity

The influence of glycyrrhetinic acid on acute pyridine toxicity in mice is shown in table 2. When the pyridine was administered after glycyrrhetinic acid pretreatment, the mortality decreased 45% in glycyrrhetinic acid pretreatment group compared to 60% in the control group given pyridine alone.

Effect of Glycyrrhetinic Acid on the Liver Microsomal Aniline Hydroxylase Activity

Table 3 shows the effect of glycyrrhetinic acid on the liver microsomal aniline hydroxylase

Table 1. Effect of glycyrrhetinic acid on the CNS depression in pyridine-treated mice.

Treatment	CNS depression (min)
Control	42±6.6
Glycyrrhetinic acid	31±4.3

Pyridine (0.43g/kg) was injected intraperitoneally to mouse for 1 day after glycyrrhetinic acid (10mg/kg) treatment once per day for 5 days. The assay procedure was described in the text. The values were means of 10 experiments.

Table 2. Effect of glycyrrhetinic acid on the mortality in pyridine-treated mice.

Treatment	Mortality
Control	60%
Glycyrrhetinic acid	45%

Mice were given glycyrrhetinic acid(10mg/kg) intraperitoneally once per day for 5 days.
Pyridine (0.87g/kg) was injected intraperitoneally one day. The assay procedure were described in the text. 20 animals were used in each group.

Table 3. Changes of hepatic aniline hydroxylase activity in glycyrrhetinic acid-treated animals.

Duration (day)	aniline hydroxylase activity**
0	1.39±0.06
1	1.45±0.08
3	1.60±0.10
5	1.77±0.12*

Glycyrrhetinic acid (10mg/kg) was injected i.p. in a various time course before sacrifice.
The procedure was described in the text. The values are means±S.E. of 5 animals. *;$p<0.05$
**;p-aminophenol formed n moles/mg protein/hr

activity in male mice. The liver aniline hydroxylase activities were increased by the lapse of administration times. In this condition, aniline hydroxylase activities were significantly increased when glycyrrhetinic acid was treated for 5 days.

Effect of Glycyrrhetinic Acid on the Liver Aliline Hydroxylase Activity *in vitro*.

The glycyrrhetinic acid effect on the microsomal aniline hydroxylase activity was demonstrated in vitro system and the results shown in Fig. 1. The activities of mice liver aniline hydroxylase in the presence of glycyrrhetinic acid was not activated.

Effect of Glycyrrhetinic Acid on the Serum Transaminase Activity

As shown in Fig. 2, the serum level of transaminase activities were increased about 2 fold in pyridine treated group, while the activities were not changed in glycyrrhetinic acid pretreated group.

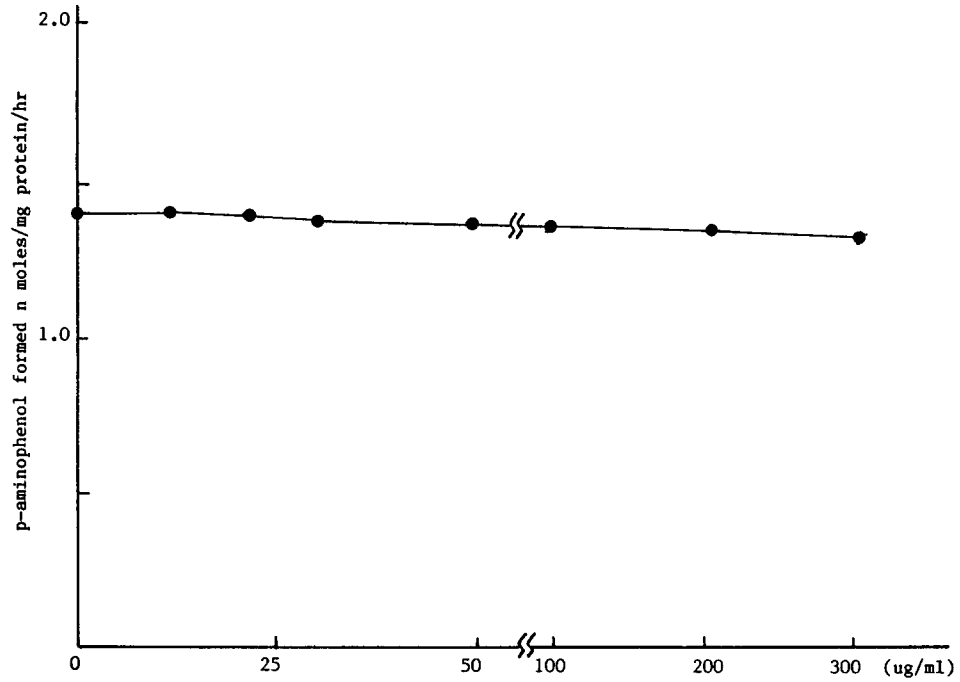

Fig. 1. Changes on the hepatic aniline hydroxylase activity in various concentration of glycyrrhetinic acid *in vitro*. Each value is the mean of 3 experiments.

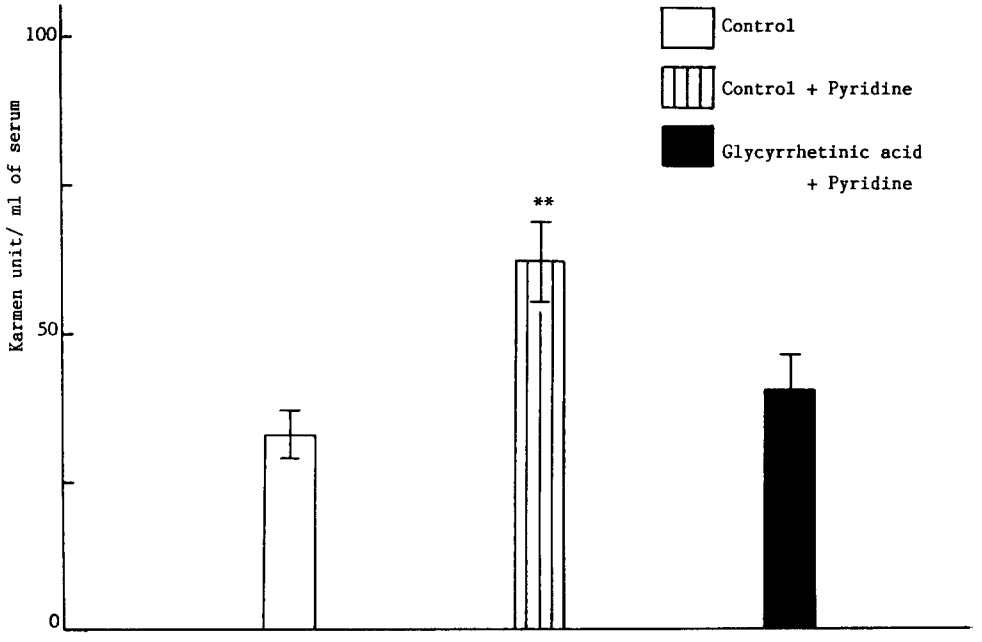

Fig. 2-A. Effect of glycyrrhetinic acid on the serum alanine transaminase level in pyridine-treated mice. Glycyrrhetinic acid (10mg/kg) was injected intraperitoneally once per day for 5 days and pyridine was injected intraperitoneally once per day for 3 days. The assay procedure was described in the text. The values are mean $\pm$S.E. of 10 experiments.**; $p<0.01$

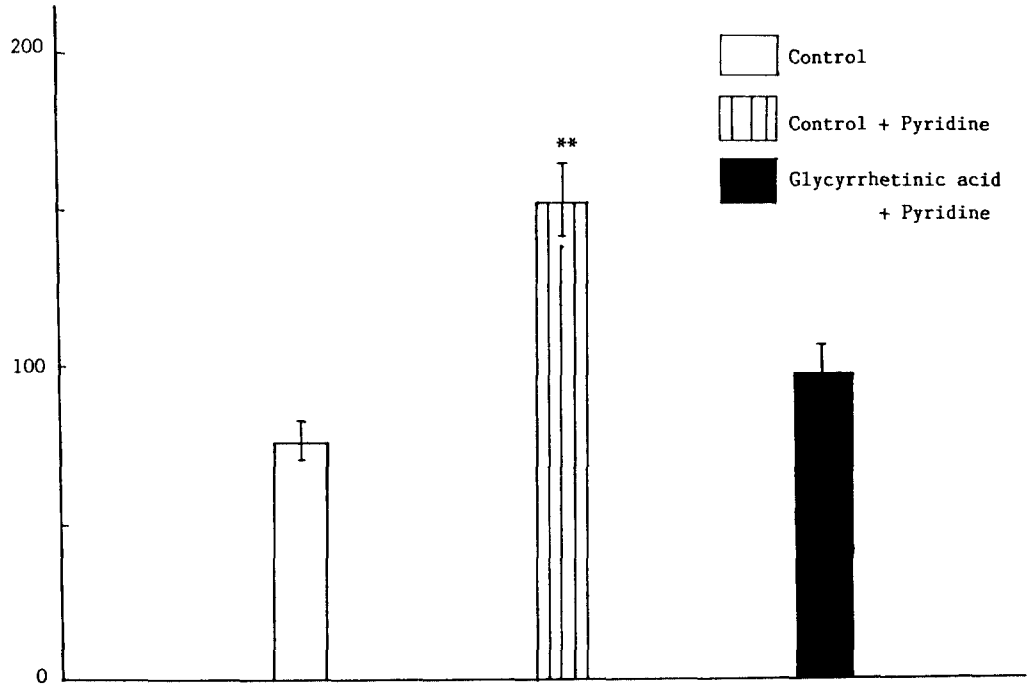

Fig. 2–B. Effect of glycyrrhetinic acid on the serum aspartate transaminase level in pyridine–treated mice. Glycyrrhetinic acid (10mg/kg) was injected intraperitoneally once per day for 5 days and pyridine was injected intraperitoneally once per day for 3 days. The assay procedure was described in the text. The values are mean±S.E. of 10 experiments.**; $p<0.01$

DISCUSSION

It was reported that components of licorice have the antidotal action against various toxic substances (strychnine, caffeine, chloral hydrate, urethane, acethylcholine, barbital, picrotoxin) (Sakaguchi, 1956; Sakaguchi, 1957) and may raised excretion or metabolism of bilirubin (Ichioka, 1968). However, the action mechanism of licorice on detoxication of the endogenous and exogenous compounds has not been characterized previously in detail or within the context of an appropriate toxicity model. Thus, we have examined the defence mechanism of glycyrrhetinic acid on pyridine toxicity in experimental model.

Pyridine is widely used as a solvent in the chemical industries. Therefore, human is frequently exposured to pyridine. When pyridine was given acutely and chronically, it was induced nausea, vomitting, CNS depression, hepatic and renal damage (Weiss, 1980). It is generally accepted that pyridine is metabolized by liver microsomal aniline hydroxylase, phase I enzyme in mice (Shoeman and Chaplin, 1969; Sesame and Gillette, 1964).

Our results demonstrate that glycyrrhetinic acid, aglycone of glycyrrhizin, reduced the pyridine induced CNS depression, mortality and serum transaminase activities in mice. Aniline hydroxylase activity was significantly increased when glycyrrhetinic acid was treated for 5 days. It is considered not to be direct action. Therefore, these results strongly suggested that glycyrrhetinic acid may prevent the CNS depression, mortality and hepatotoxicity by increment of hepatic microsomal aniline hydroxylase, pyridine metabolizing enzyme activity.

These findings led us to conclude that the glycyrrhetinic acid may regulate the hepatic microsomal aniline hydroxylase activity to prevent pyridine–induced toxicity in mice. In this studies, it is indicated that detoxication mechanism of licorice on the various toxic compounds is associated with glycyrrhetinic acid, one of the components of Glycyrrhiza glabra, which induced liver microsomal drug metabolizing enzyme.

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