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Protective Effects of Syringin Isolated from *Kalopanax pictus* on Galactosamine Induced Hepatotoxicity

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Syringin, a phenylpropanoid monoglycoside, was isolated from the stem bark of *Kalopanax pictus* (Araliaceae). The isolated compound was tested for its hepato-protective activities *in vivo*, by using experimental hepatic intoxication in animals induced by intraperitoneal injection of galactosamine. Syringin recovered the blood biochemical parameters such as the serum enzyme activities and microsomal oxidative enzyme activities and inhibited the lipid peroxidation. These antihepatotoxic effects of syringin were dependent on the dose and duration of administration.

Keywords Syringin; *Kalopanax pictus*; Antihepatotoxic; Galactosamine; Microsomal enzyme system; Lipid peroxidation

Phenylpropanoid monoglycoside such as syringin and coniferin as well as phenylpropanoid diglucosides such as syringinoside, coniferinoside and magnolioside are found in natural sources.[1] Magnolioside was isolated from the stem bark of *Magnolia sieboldii* (Magnoliaceae).[2] Syringin, which is a precursor of natural aromatic organic compounds including lignan and lignin in some vascular plants has been isolated from the plants of the families Oleaceae, Araliaceae and Eucommiaceae.[3-5] The aglycone of syringin is syringenin or sinapyl alcohol and their derivatives are caffeic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid. These phenylpropanoids all contain aliphatic alcohols, which are often in the form of acids or esters. Interestingly, there is no report on the isolation of free syringenin from the natural sources. The phenoxy site is always in the form of glycoside or aliphatic ester.[6] It has been found that the phenylpropanoids, in general, show antioxidative activity,[7] and that the hydroxyl moiety and the double bond of side chain in caffeic acid play important roles in the antioxidative activity. Paik et al.[8] reported that caffeic acid oligomers such as *trans*-rosmarinic acid (dimer), lithospermate A (trimer), lithospermate B (tetramer) have strong antioxidative activities. Since chlorogenic acid and 1,5-dicaffeoylquinic acid possessing caffeoylquinic acid structures have strong antihepatotoxic effects,[9] syringin was expected to exert similar activities. However, the antihepatotoxic activities of the phenylpropanoid glycosides except those of phenylethanoid glycosides bearing caffeoyl moiety have not been reported. Therefore, we assayed the antihepatotoxic activity on the galactosamine (GalN)- induced liver toxicity. Syringin, from the stem bark of *Kalopanax pictus* was revealed to have on the antihepatotoxic activity and antioxidative activity *in vivo*.

MATERIALS AND METHODS

Materials Syringin used in this experiment was isolated

from the stem bark of *Kalopanax pictus*. The ethyl acetate fraction of a MeOH extract of dried plant material (4.8 kg) was chromatographed over silica gel by using the solvent system, $CHCl_3$: MeOH : H_2O (7 : 3 : 1, lower phase) to give colorless needles after recrystallization in MeOH (310 mg). The compound was identified as syringin (fig. 1) by the comparison of mp, and ^1H-, ^{13}C -NMR and MS spectra with those of literature (mp, 192 - 193°C). [10]

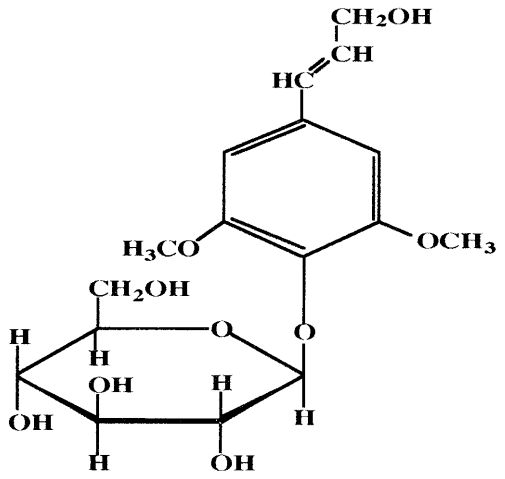

Fig. 1. Chemical Structure of Syringin from *Kalopanax pictus*

Animals and treatment - Adult male mice of ICR series weighing 18 - 22g were divided into 6 groups of 8 mice each after they were adjusted in the laboratory circumstances for a week. The hepatic intoxication was induced by the administration of GalN (400 mg/kg, i.p.). Each of the 6 groups was treated as tabulated in TABLE I.

Preparation of Enzyme Sources- Animals were decapitated 24 hr after the final treatment and the blood was collected from abdominal artery, which was used for the biochemical measurements.

The liver homogenate was prepared from macerated liver with phosphate buffered saline (pH 7.5). This homogenate was centrifuged (600 g, 10 min) to remove non-macerated portion, and the supernatant was centrifuged again (105,000 g, 1 hr), to prepare a cytosolic fraction and a microsomal fraction which were was used as enzyme sources as described below.

Measurement of Enzyme Activities in Blood- The aminotransferase (AST, ALT) activities were measured by Reitman and Frankel's method with kit reagent (Asan Pharm. Co.),[11] sorbitol dehydrogenase (SDH)- activity, by Gerlach's method (kit reagent, Asan Pharm. Co.)[12], γ -glutamyltransferase (γ -GT)- activity by Szasa's method (kit reagent, Asan Pharm. Co.)[13], alkaline phosphatase (ALP)-activity, by Kind and King's method (kit reagent, Asan Pharm. Co.)[14] and Lactate dehydrogenase (LDH)- activity, by Berga and Boida's method (kit reagent, Asan Pharm. Co.).[15]

Measurement of Liver Enzyme Activity- The activities of liver enzyme systems were measured by the following methods: Microsomal cytochrome P-450 (Cyt P-450)- Omura and Sato's method[16]; aminopyrine N-demethylase (AD); alanine hydroxylase (AH)- Bidlack's method[17].

Measurement of Malondialdehyde- The liver obtained from each group was homogenized. 17.5%-trichloroacetic acid (TCA, 1 ml) and thiobarbituric acid (1 ml) were added to each homogenate (1 ml). Then the mixtures were boiled for 15 min and cooled, to which 1 ml of 70%-TCA was added. The solution was incubated for 20 min at 37 ℃ and centrifuged (2,500 rpm, 30 min). Finally, the absorbance of the supernatants was measured at 535 nm for the determination of malondialdehyde produced (MDA).

RESULTS AND DISCUSSION

Changes of Biochemical Values by Administration of Syringin- The effects on the blood biochemical parameters, the activities of serum aspartic acid transferases (AST), serum alanine transferase (ALT)

TABLE I. Animal Grouping and Treatment

Group	No. of animal	Treatment
Normal	8	-
Control	8	galactosamine only 400 mg/kg (i.p.)
Syringin A	8	syringin 5 mg/kg once a day(i.p.) 3 days+galactosamine 400 mg/kg (i.p.) final day
Syringin B	8	syringin 10 mg/kg once a day(i.p.) 3 days +galactosamine 400 mg/kg (i.p.) final day
Syringin C	8	syringin 5 mg/kg once a day(i.p.) 5 days +galactosamine 400 mg/kg (i.p.) final day
Syringin D	8	syringin 10 mg/kg once a day(i.p.) 5 days +galactosamine 400 mg/kg (i.p.) final day

TABLE II. Effect of Syringin on Serum Biochemical Parameters in Galactosamine Induced Liver Intoxication

Group	Dose	Duration	Activities		
			AST*	ALT**	SDH***
Normal	-	-	62.7 ± 5.37^{a}	30.6 ± 1.97^{a}	20.3 ± 1.46^{a}
Control	-	-	116.4 ± 7.90^{b}	63.9 ± 2.36^{b}	56.6 ± 3.80^{b}
Syringin-A	5mg/kg	3 days	$107.6 \pm 8.27^{b,c}$	$57.6 \pm 3.46^{b,c}$	$51.6 \pm 4.26^{b,c}$
Syringin-B	10mg/kg	3 days	98.6 ± 5.27^{c}	52.9 ± 2.46^{c}	48.6 ± 2.37^{c}
Syringin-C	5mg/kg	5 days	83.6 ± 4.24^{d}	49.4 ± 9.51^{c}	39.4 ± 2.58^{d}
Syringin-D	10mg/kg	5 days	78.4 ± 3.96^{d}	40.6 ± 4.70^{d}	33.9 ± 3.26^{d}

The assay procedure was described in the experimental methods.
Values repersent mean ± S.D.(n=8) AST*, ALT** unit: Karmen unit/ml SDH*** unit: Sigma unit/ml
Values having the same superscript are not significantly different from each other ($p < 0.05$) by Duncan's new multiple range test.

and sorbitol dehydrogenase (SDH) are shown in TABLE II. The activities of serum aminotransferase (ALT and AST) in the GalN administrated control group were two times those of normal group. Pretreatment with syringin inhibited these increases and the result implies that the antihepatotoxic effect of syringin on GalN induced hepatic intoxication is dependent on the dose and duration of administration. Apparently, prolonged syringin administration (group D) is more effective. The inhibition rates were 71% for AST and for SDH 64%, which suggested that syringin can protect liver from GalN induced damage.

The effects of syringin on the activities of serum γ - glutamyltransferase (γ - GT), alkaline phosphatase (ALP) and lactic acid dehydrogenase (LDH) are shown in TABLE III. Similar trends were observed. i.e. increase of γ - GT, ALP and LDH enzyme activities due to administration of GalN were inhibited by syringin by 73% for γ - GT, by 81% for ALP and by 76% for LDH, and the order of the potency of this effect was group D > C > B > A, as for the effects on the AST, ALT and SDH activities. The inhibitory effect of syringin on the LDH increase was significant. Thus, the antihepatotoxic effects of syringin on γ - GT, ALP and LDH were also dependent on the dose and duration of administration.

Effect on Hepatic Lipid Peroxidation- To evaluate the inhibitory effect of syringin on GalN- induced lipid peroxidation, the serum malondialdehyde (MDA) concentrations in each group were determined. (fig. 2.) The MDA concentration in GalN injected control group was 73.2 ± 3.46 nmole. In the group administered with 10mg/kg of syringin for 5 days, the malondialdehyde production in the liver tissue was lower by 57% and

TABLE III. Effect of Syringin on Serum Biochemical Parameters in Galactosamine Induced Liver Intoxication

Group	Dose	Duration	Activities		
			γ -GT*	LDH**	ALP***
Normal	-	-	27.6 ± 3.69^{a}	25.4 ± 3.97^{a}	50.1 ± 2.36^{a}
Control	-	-	74.3 ± 4.20^{b}	52.7 ± 5.27^{b}	89.6 ± 5.36^{b}
Syringin-A	5mg/kg	3 days	63.5 ± 4.37^{c}	43.7 ± 3.26^{c}	75.3 ± 3.27^{c}
Syringin-B	10mg/kg	3 days	60.9 ± 3.95^{c}	$40.6 \pm 4.76^{c,d}$	70.6 ± 4.23^{c}
Syringin-C	5mg/kg	5 days	47.3 ± 3.26^{d}	$33.6 \pm 2.67^{c,d}$	62.3 ± 3.29^{d}
Syringin-D	10mg/kg	5 days	40.4 ± 2.27^{d}	$30.7 \pm 3.26^{a,d}$	59.7 ± 2.46^{d}

The assay procedure was described in the experimental methods.
Values repersent mean ± S.D.(n=8).
γ -GT* (γ -glutamyltransferas) unit: mU/ml, LDH** (lactate dehydrogenase) unit: Wroblewski unit
ALP*** (alkaline phosphatate) unit: K-A unit.
Values having the same superscript are not significantly different from each other ($p < 0.05$) by Duncan's new multiple range test.

in the group administered with 5 mg/kg for 3 days, by 15%. Thus, we found that syringin was a potent antioxidative agent to prevent the hepatic intoxication, and that the antioxidative activity depended on the administered dose. From the above observations, it was considered that the antioxidative activity of syringin in mice was responsible for the antihepatotoxic activity.

Effect on Microsomal Oxidative Enzyme System- GalN, one of amino sugars, is well known to induce liver-tissue damage resulted from RNA biosynthesis together with lipid accumulation by the inhibitory activity on the metabolic pathway of D-galactose followed by the change of carbohydrate composition and the Ca^{++} concentration of the constituents of cell membranes.[18] In acute intoxication by GalN, liver necrosis occurs, and in chronic GalN intoxication, liver cirrhosis and cellular tumor develop. In the GalN metabolic pathway, injected GalN is oxidized by the microsomal oxidative system to be excreted in urine as a glutathion conjugate.[19-22] The effect of Syringin on the cytochrome P-450 (Cyt P-450), aminopyrine N-demethylase (AD) and aniline hydroxylase (AH) are shown in TABLE IV. GalN lowered the enzyme activities of cytochrome P-450, AD and AH by approximately 40%, 52% and 41%, respectively, of those of normal group. By increases of doses and administration duration of syringin, these enzyme activities were considerably elevated. Statistically significant differences were not observed between the activities of the group which was treated with 10 mg/kg for 5 days, and those of normal group. Cytochrome P-450 is a mixed-functional oxidize system which oxidizes and detoxifies foreign substances in the Phase I, the step of oxidation and non synthesis of introduced drugs in the liver. These enzyme systems can be classified into two groups such as hydroxylase(AH) and demethylase(AD). Thus, a large dose of syringin (10 mg/kg) considerably normalize the activity of the microsomal metabolic enzyme system and induced the excretion of the metabolites from foreign substances, in a relatively higher rate.

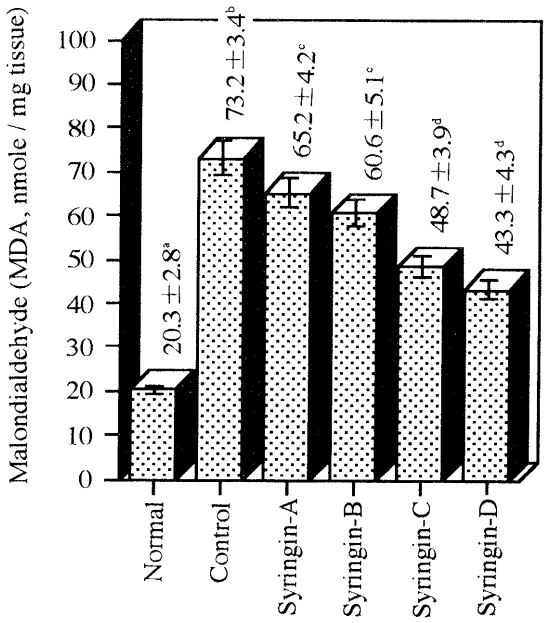

Fig. 1. Effects of Syringin on Lipid Peroxidation in Galactosamine Induced Hepatic Intoxication.
Values having the same superscript are not significantly different from each other ($p < 0.05$) by Duncan's new multiple range test.

TABLE IV. Effect of Syringin on the Hepatic Microsomal Oxidation System in Galactosamine-induced Intoxication

Group	Dose	Duration	Activities		
			Cytochrome P-450*	AD**	AH***
Normal	-	-	0.42 ± 0.043^{a}	6.74 ± 0.47^{a}	0.86 ± 0.096^{a}
Control	-	-	0.17 ± 0.023^{b}	3.52 ± 0.53^{b}	0.35 ± 0.027^{b}
Syringin-A	5mg/kg	3 days	$0.23 \pm 0.036^{b,c}$	4.15 ± 0.25^{b}	$0.43 \pm 0.041^{b,c}$
Syringin-B	10mg/kg	3 days	$0.29 \pm 0.052^{c,d}$	4.23 ± 0.63^{b}	$0.48 \pm 0.052^{c,d}$
Syringin-C	5mg/kg	5 days	$0.35 \pm 0.042^{a,d}$	5.75 ± 0.49^{c}	$0.59 \pm 0.073^{d,c}$
Syringin-D	10mg/kg	5 days	0.38 ± 0.053^{a}	$6.01 \pm 0.72^{a,c}$	0.68 ± 0.082^{c}

The assay procedure was described in the experimental methods.
Values repersent means ± S.D.(n=8).
Cytochrome P-450* unit: nmole/mg protein
AD** (aminopyrine N-demethylase) unit: formaldehyde nmole/mg protein/min
AH*** (aniline hydroxylase) unit: *p*-aminophenol nmole/mg protein/min
Values having the same superscript are not significantly different from each other ($p < 0.05$) by Duncan's new multiple range test.

As regards the biological activities of syringenin derivatives, the antioxidative activity of sinapine[23] and the prostaglandin biosynthesis- inhibitory activity of sinapyl aldehyde have been reported. About syringin, only its hypotensive activity has been reported previously. By our search on the antihepatotoxic activity of syringin, it was clearly shown that it is a potent antihepatotoxic agent. Besides, it was expected that recovery of the enzyme activities of the microsomal enzyme system such as cytochrome P-450, together with the inhibition of lipid peroxidation might improve metabolism of hepatotoxic substances, and normalize the impaired hepatic functions.

CONCLUSION

Syringin, a phenylpropanoid glycoside isolated from the stem bark of *Kalopanax pictus* was found to inhibit GalN-induced liver damage when assayed by the serum biochemical parameters. This effect of syringin was dose and duration dependent. Its antihepatotoxic activity was considered, in part, to be due to its anti-oxidative activity on lipid peroxidation. Syringin was expected to accelerate the metabolism and excretion of the foreign hepatotoxic substance, GalN by stabilizing the microsomal metabolic enzyme system.

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