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Inhibitory Effect of Tetrahydroswertianolin on Tumor Necrosis Factor-α-Dependent Hepatic Apoptosis in Mice

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ABSTRACT. We investigated the effect of tetrahydroswertianolin (THS), a hepatoprotective agent from *Swertia japonica*, on tumor necrosis factor-α (TNF-α)-dependent hepatic apoptosis induced by D-galactosamine (D-GalN) (700 mg/kg, i.p.) and lipopolysaccharide (LPS) (10 μg/kg, i.p.) in mice. Apoptotic symptoms were observed at the initial stage of liver damage. By 5 hr after intoxication, hepatic DNA fragmentation had risen to 2123%, with the value in untreated mice set at 100%, without a significant elevation of serum alanine transaminase (ALT) activity. There was a parallel increase in hepatocytes undergoing chromatin condensation and apoptotic body formation. By 8 hr after intoxication, serum ALT activity had risen to 3707 U/L. Pretreatment with THS (50 mg/kg, p.o.) at 18 and 2 hr before intoxication significantly reduced DNA fragmentation to 821% of that in untreated mice and prevented the emergence of chromatin condensation and apoptotic body formation. A significant and dose-dependent reduction in serum ALT activity at 8 hr also was observed with THS pretreatment. These effects of THS were different from those observed from pretreatment with glycyrrhizin (GCR), which is a clinically used hepatoprotective agent with membrane-stabilizing activity. GCR pretreatment (100 mg/kg, p.o.) did not inhibit hepatic DNA fragmentation (1588% of untreated mice), although this compound significantly protected against serum ALT elevation (1463 U/L). These data suggest that an inhibitory effect on the progression of hepatic apoptosis prior to liver injury may be involved in the hepatoprotective mechanisms of THS, whereas it appears that GCR affects the processes after apoptosis. In a separate experiment, we found that the concentration of serum TNF-α rose to 2016 pg/mL at 1 hr after intoxication of mice with D-GalN and LPS, but this increase was suppressed by THS pretreatment (10, 50, or 200 mg/kg, p.o.) to 716, 454, or 406 pg/mL, respectively. Further study with a reverse transcriptase-polymerase chain reaction method showed that THS blocked TNF-α production at the transcriptional level. Because TNF-α is a critical mediator to elicit apoptosis in this model, the property of suppressing TNF-α production may be of prime importance for THS inhibition of hepatic apoptosis. BIOCHEM PHARMACOL **57**;12:1431–1437, 1999.

KEY WORDS. tetrahydroswertianolin; apoptosis; tumor necrosis factor; DNA fragmentation; lipopolysaccharide; D-galactosamine

We previously demonstrated that an extract of the whole plant of *Swertia japonica* inhibits the elevation of serum transaminase in D-GalN§/LPS-treated mice; THS (see Fig. 1), a polyphenolic compound, is the major hepatoprotective constituent in this plant [1]. Administration of a subtoxic amount of LPS to D-GalN-treated mice has been reported to result in specific hepatic injury, without affecting other organs of the animal [2, 3]. The hepatic lesion induced in this model resembles those of human hepatitis at a point where they are mediated by inflammatory response [4–6]. Recently, Leist *et al.* [7] clearly showed that apoptotic symptoms, such as DNA fragmentation and apoptotic body formation, were induced in the livers of mice treated with D-GalN and LPS. It also has been reported that certain herbal medicines contain bioactive materials that interfere with signal transduction in programmed cell death *in vitro*. For instance, taxol or flavonoids induce apoptosis in various tumor cell lines [8, 9], whereas *Coptidis rhizoma* extract or its constituent, berberine, inhibits apoptosis induced by dexamethasone in primary cultured murine thymocytes [10].

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§ *Abbreviations:* D-GalN, D-galactosamine; LPS, lipopolysaccharide; THS, tetrahydroswertianolin; GCR, glycyrrhizin; TNF-α, tumor necrosis factor-α; mrTNF-α, mouse recombinant tumor necrosis factor-α; ALT, alanine transaminase; RT-PCR, reverse transcriptase-polymerase chain reaction; and GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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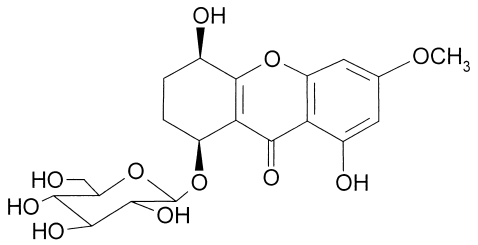

FIG. 1. Structure of tetrahydroswertianolin (THS).

Consequently, the regulation of apoptotic cell death may be involved in the wide range of bioactivities of herbal medicines, and it is plausible to assume that THS may affect the apoptotic process in this liver injury model.

The present study was designed to investigate the effect of THS on hepatic apoptosis. We first measured hepatic DNA fragmentation and apoptotic body formation induced by D-GalN and LPS in mice, and the effect of THS on hepatic apoptosis was compared with that of GCR, a well-documented hepatoprotective agent of herbal origin, which possesses hepatocyte-membrane stabilizing effects and a protective activity against necrotic damage of hepatocytes in various liver injury models [11, 12]. Recent studies have indicated that TNF-α from macrophages stimulated with LPS is a strong inducer of hepatic apoptosis, playing a central role in the pathology of D-GalN/LPS-induced inflammatory liver injury [7, 13, 14]. Our subsequent study was performed to investigate the effects of THS on TNF-α production in this model and on the hepatic apoptosis directly induced by injection of recombinant TNF-α into D-GalN-sensitized mice.

MATERIALS AND METHODS

Chemicals

D-GalN was obtained from Wako Pure Chemical Industries. LPS (*Escherichia coli* serotype 055:B5) was purchased from Difco Laboratories. mrTNF-α was obtained from the Genzyme Co. THS was isolated from a 70% ethanol/water extract of *Swertia japonica*, as described previously [1]. GCR was purchased from Nakalai Tesque Inc.

Animal Treatment

Male ddY mice (30–32 g), obtained from the Shizuoka Laboratory Animal Center, were maintained on a 12-hr light/dark cycle in a temperature- and humidity-controlled room. The animals were allowed free access to laboratory pellet chow (CE2; CLEA Japan Inc.) and water before the experiments. Liver injury was induced in 12-hr fasted mice by i.p. injection of D-GalN (700 mg/kg) and LPS (10 μg/kg), according to the method of Tiegs *et al.* [3]. In a separate experiment, liver injury was induced in D-GalN (700 mg/kg)-sensitized mice by i.v. injection of mrTNF-α (2.1 μg/kg) 15 min after sensitization. THS (10, 50, or 200 mg/kg) or GCR (100 mg/kg) was dissolved in aqueous 0.5% carboxymethylcellulose-sodium solution and administered orally at a volume of 0.2 mL/mouse, twice at 18 and 2 hr before intoxication. Control animals were given the same volume of vehicle orally. Mice were killed 5 or 8 hr after intoxication to collect liver and serum samples. Livers were washed with ice-cold buffer (10 mM EDTA in PBS, pH 7.4), and a portion of the lobe was frozen in liquid nitrogen and kept at −80° until assayed for DNA fragmentation. The study complied with the current ethical regulations of this University on animal research, and all the mice used received humane care.

Biochemical Analysis

ALT (EC 2.6.1.2) activity was measured by the Reflotron S system (Boehringer Mannheim) at 8 hr after intoxication. The serum TNF-α level was determined by an ELISA assay with hamster anti-mouse TNF-α monoclonal antibody (Genzyme) and biotin-conjugated rabbit anti-mouse TNF-α polyclonal antibody (Genzyme) at 1 hr after intoxication.

DNA Fragmentation

Hepatic DNA fragmentation was examined by electrophoresis and ELISA according to Leist *et al.* [7] with a few modifications. Briefly, the frozen liver (*ca.* 200 mg) was ground into powder, and an aliquot of ice-cold EDTA-PBS (pH 7.4) was added to make a 20% liver homogenate. The homogenate was centrifuged at 13,000 *g* for 20 min, and total DNA was prepared from an aliquot (200 μL) of supernatant by extraction with an equal volume of phenol/chloroform (1:1, v/v), precipitation in cold ethanol, and subsequent treatment with RNase (10 μg/mL). The purified DNA was analyzed by electrophoresis on a 1% agarose gel. The supernatant of 20% liver homogenate was diluted 300-fold and used for direct analysis of DNA fragmentation with an oligonucleosome-bound DNA ELISA kit (Boehringer Mannheim). DNA fragmentation was expressed as a relative percentage in comparison with that of untreated animals.

Measurement of TNF-α mRNA Level by RT-PCR

Mice were killed 40 min after intoxication. Liver specimens were collected and frozen in liquid nitrogen. Total RNA was extracted from the liver with RNAzol (Cinna/Biotecx). First-strand cDNA was synthesized using each total RNA sample for the template. Total RNA (2 μg) was incubated at 37° for 60 min with a mixture of 3.5 mM $MgCl_2$, RT (200 U), 1x RT buffer, 0.5 mM dNTP, RNase inhibitor (40 U), and oligo-dT primer (10 pmol) in a volume of 40 μL. The reaction mixture was incubated at 95° for 5 min to denature and to inactivate the RT. One microliter of RT product was subjected to a reaction mixture containing sense and antisense primers (10 pmol), 1.1 mM $MgCl_2$, 1x PCR buffer, 0.1 mM dNTP, and *Taq* DNA polymerase (2.5 U) for PCR amplification. Primers used were sense 25-mer,

TABLE 1. Effects of THS and GCR on hepatic DNA fragmentation induced by D-GalN and LPS in mice

Treatment	Dose (mg/kg)	DNA fragmentation	
		OD_{405}	(% of Untreated)
Untreated		0.046 ± 0.004*	100 ± 8
Control		0.976 ± 0.088†	2123 ± 94
GCR	100	0.731 ± 0.104†	1588 ± 225
THS	50	0.377 ± 0.115*	821 ± 234

Liver damage was induced in mice by i.p. injection of D-GalN (700 mg/kg) and LPS (10 μg/kg). THS or GCR was administered orally, twice, at 18 and 2 hr before intoxication. Liver samples were taken at 5 hr after intoxication, and hepatic DNA fragmentation was quantified by ELISA with fragmented oligonucleosome-bound polyclonal antibody. The ELISA reaction finally yields blue products in proportion to the amount of fragmented DNA, which have a maximal absorbance at 405 nm. DNA fragmentation is expressed also as the relative ratio (%) in comparison with untreated value. Results are the means ± SEM of 3 (untreated) or 10 mice.

*,†Values not sharing a common superscript differed significantly ($P < 0.05$) when analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test.

5′-CACCACGCTCTTCTGTCTACTGAAC-3′; antisense 25-mer, 5′-CCGGACTGCGTGATGTCTAAGTACT-3′. PCR amplification was scheduled: denaturation, annealing, and elongation at 95°, 62°, and 72° for 1, 1, and 2 min, respectively, for 35 cycles.

Histology

A lobe of the liver was removed at 5 hr after D-GalN/LPS intoxication. It was fixed in 10% neutralized formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

Statistical Analysis

Data are presented as means ± SEM. Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer test. A difference was considered significant when $P < 0.05$.

RESULTS

In our preliminary study, the ELISA assay with oligonucleosome-bound polyclonal antibody demonstrated that hepatic DNA fragmentation rose to 1079 ± 256% (N = 6) of untreated mice (set as 100%) at 5 hr after intoxication with D-GalN (700 mg/kg) and LPS (10 μg/kg); serum ALT activity remained unchanged (64 ± 9.5 U/L). Serum ALT activity increased drastically, reaching 4671 ± 815 U/L by 8 hr after intoxication. Based on these results, we assessed the effects of THS on hepatic DNA fragmentation at 5 hr after intoxication, when it was possible to selectively document an effect on apoptosis.

As shown in Table 1, at the 5-hr time point, the ELISA assay showed that hepatic DNA fragmentation rose to 2123% of the untreated value without a significant increase in serum ALT activity (185 ± U/L). Electrophoresis of total hepatic DNA in mice intoxicated with D-GalN and

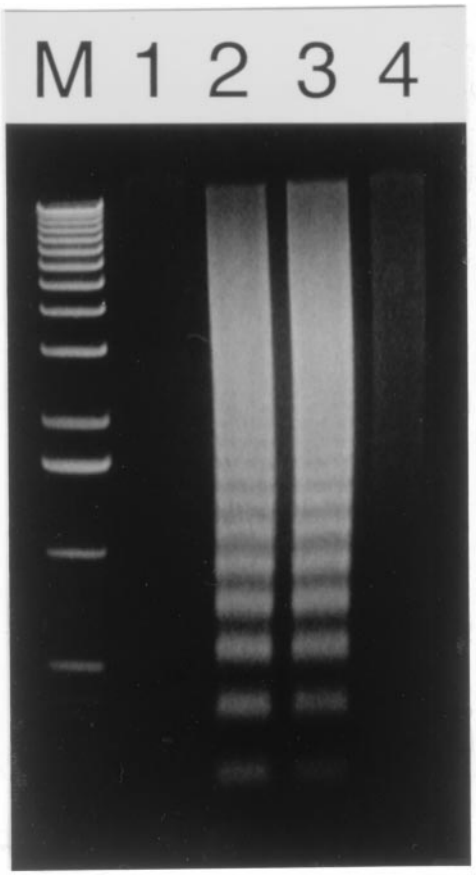

FIG. 2. Agarose gel electrophoresis of total DNA from livers of mice treated with D-GalN and LPS. Liver samples were taken at 5 hr after intoxication. Total DNA was prepared from the livers with a phenol/chloroform method and analyzed by electrophoresis on 1% agarose gel. THS and GCR were administered orally, twice, at 18 and 2 hr before intoxication. The lanes of the gel show samples from mice treated as follows: (1) untreated, (2) D-GalN (700 mg/kg)/LPS (10 μg/kg), (3) D-GalN/LPS + GCR (100 mg/kg), and (4) D-GalN/LPS + THS (50 mg/kg). M: 1-kb ladder marker.

LPS further demonstrated a typical ladder pattern fragmented at the nucleosome unit (Fig. 2). Pretreatment with THS (50 mg/kg) at 18 and 2 hr before intoxication significantly inhibited DNA fragmentation to the level of 821% of untreated mice (Table 1). The attenuation of DNA fragmentation by THS was observed also by electrophoresis of total hepatic DNA (Fig. 2). To confirm the effect of THS on hepatic apoptosis, we performed a histopathological examination of the mouse livers. At 5 hr after D-GalN/LPS intoxication, histological sections of liver specimens showed the emergence of large numbers of nuclei with condensed chromatin and apoptotic bodies, which were the indices of apoptotic cell death (Fig. 3B). Apoptotic bodies and nuclei with chromatin condensation were found throughout the organ. Administration of THS reduced the emergence of both chromatin condensation and apoptotic bodies (Fig. 3D). On the other hand, pretreatment with GCR (100 mg/kg), used as a reference compound, inhibited neither hepatic DNA fragmentation,

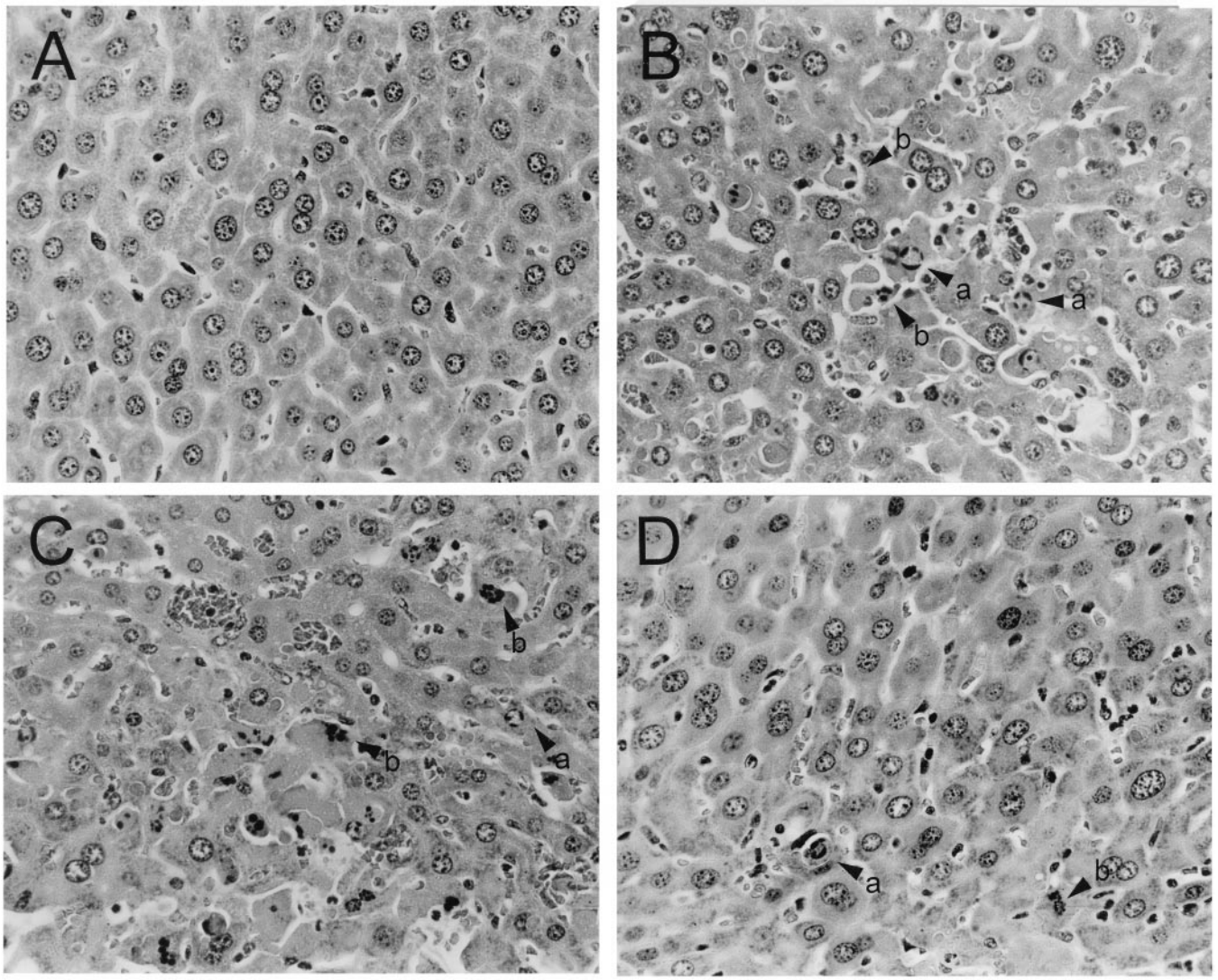

FIG. 3. Histopathology of livers of mice treated with D-GalN and LPS. Liver specimens were taken at 5 hr after intoxication to prepare the microscopic sections. The liver sections were stained with hematoxylin and eosin. THS and GCR were administered orally, twice, at 18 and 2 hr before intoxication. (A) untreated, (B) D-GalN (700 mg/kg)/LPS (10 μg/kg), (C) D-GalN/LPS + GCR (100 mg/kg), and (D) D-GalN/LPS + THS (50 mg/kg). The emergence of nuclei with condensed chromatin (a) and apoptotic bodies (b) was observed in the livers of mice intoxicated with D-GalN and LPS.

assessed by the ELISA reaction (1588% of untreated) (Table 1) and electrophoresis (Fig. 2), nor the emergence of apoptotic bodies and chromatin condensation, assessed by histological examination (Fig. 3C).

Eight hours after intoxication, serum ALT activity had risen to 3707 U/L (Table 2), while hepatic DNA fragmentation remained at the increased level of 1761 ± 148% of untreated animals. Pretreatment with THS (10, 50, and 200 mg/kg) significantly attenuated these pathological events in a dose-dependent manner; serum ALT activity was reduced to 1148, 614, and 414 U/L, respectively (Table 2) and DNA fragmentation to 1293 ± 235, 791 ± 191, and 674 ± 175% of untreated values, respectively. Pretreatment with GCR (100 mg/kg) also significantly reduced the elevation of serum ALT activity to 1463 U/L (Table 2); however, this compound did not attenuate hepatic DNA

TABLE 2. Protective effects of THS and GCR on serum ALT elevation induced by D-GalN and LPS in mice

Treatment	Dose (mg/kg)	ALT (U/L)
Untreated		45 ± 2*
Control		3707 ± 631†
GCR	100	1463 ± 592*
THS	10	1148 ± 349*
	50	614 ± 283*
	200	414 ± 185*

Liver damage was induced in mice by i.p. injection of D-GalN (700 mg/kg) and LPS (10 μg/kg). Serum samples were taken at 8 hr after intoxication. THS or GCR was administered orally, twice, at 18 and 2 hr before intoxication. Results are the means ± SEM of 3 (untreated) or 8 mice.

*,†Values not sharing a common superscript differed significantly ($P < 0.05$) when analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test.

TABLE 3. Suppressive effects of THS on serum TNF-α elevation induced by D-GalN and LPS in mice

Treatment	Dose (mg/kg)	TNF-α (pg/mL)
Untreated		ND
Control		2016 ± 310*
THS	10	716 ± 163†
	50	454 ± 127†
	200	406 ± 91†

Liver damage was induced in mice by i.p. injection of D-GalN (700 mg/kg) and LPS (10 μg/kg). Serum samples were taken at 1 hr after intoxication. THS was administered orally, twice, at 18 and 2 hr before intoxication. Results are the means ± SEM of 3 (untreated), 8 (control), or 6 (drug-tested). ND: not detected (< 50 pg/mL).

*,†Values not sharing a common superscript differed significantly ($P < 0.05$) when analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test.

TABLE 4. Effect of THS on hepatic DNA fragmentation induced by mrTNF-α in D-GalN-sensitized mice

Treatment	DNA fragmentation	
	OD_{405}	(%)
Untreated	0.041 ± 0.002*	100 ± 5
D-GalN	0.031 ± 0.007*	76 ± 18
mrTNF-α	0.046 ± 0.004*	111 ± 10
D-GalN/mrTNF-α	0.668 ± 0.142†	1617 ± 344
THS + D-GalN/mrTNF-α	0.382 ± 0.130*†	925 ± 315

Liver damage was induced in D-GalN (700 mg/kg, i.p.)-sensitized mice by i.v. injection of mrTNF-α (2.1 μg/kg) at 15 min after sensitization. THS was administered orally, twice, at 18 and 2 hr before intoxication. Liver samples were taken at 5 hr after intoxication, and hepatic DNA fragmentation was quantified by ELISA with fragmented oligonucleosome-bound polyclonal antibody. The ELISA reaction finally yields blue products in proportion to the amount of fragmented DNA, which have a maximal absorbance at 405 nm. DNA fragmentation is expressed also as the relative percentage in comparison with the untreated value. Results are the means ± SEM of 3 (untreated, D-GalN, or mrTNF-α group) or 7 mice.

*,†Values not sharing a common superscript differed significantly ($P < 0.05$) when analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test.

fragmentation significantly (1414 ± 276% of untreated) at 8 hr.

We next studied whether THS affected TNF-α production. In our preliminary tests, the serum TNF-α level rose to 1538 ± 161 pg/mL (N = 8) at 1 hr after D-GalN/LPS intoxication and then declined to 686 ± 181 pg/mL at 2 hr and 124 ± 71 pg/mL at 4 hr. Serum TNF-α was not detected (<50 pg/mL) in untreated mice. Accordingly, in a further experiment, serum TNF-α level was measured at 1 hr after intoxication. Pretreatment with THS at doses of 10, 50, and 200 mg/kg significantly inhibited the increase in serum TNF-α activity by 64.5, 77.5, and 79.9% (Table 3).

Further study was designed to investigate whether THS affected TNF-α gene expression in the liver region. This was estimated by RT-PCR analysis of the TNF-α mRNA level. RT-PCR, using specific primers for TNF-α, amplified a predicted 546-bp sequence based on the reported sequence for cloned cDNA from mice [15]. To identify the PCR products, they were cut with the restriction enzyme *Hae*III, and three fragments of the expected sizes were found. As shown in Fig. 4, D-GalN/LPS obviously caused an induction of TNF-α mRNA in the livers of mice at 40 min after intoxication, whereas the TNF-α mRNA level was much lower when THS (50 or 200 mg/kg) was administered before intoxication. In all treated groups, GAPDH, which served as an internal standard, was expressed equally (Fig. 4).

Finally, we examined the effect of THS on mrTNF-α-induced hepatic apoptosis in D-GalN-sensitized mice to clarify whether THS affected the function of TNF-α to induce hepatic apoptosis. As shown in Table 4, neither D-GalN nor mrTNF-α alone caused an increase in hepatic DNA fragmentation, whereas intoxication with D-GalN and mrTNF-α induced hepatic DNA fragmentation, up to 1617% of the untreated group without a significant increase in serum ALT activity (48.1 ± 5.8 U/L) at 5 hr. This result resembled that observed at 5 hr after intoxication with D-GalN and LPS (Table 1). The level of hepatic DNA fragmentation in the group pretreated with THS (50 mg/kg) was 925% that of the untreated group; there were no significant differences between the control and THS groups (Table 4).

DISCUSSION

In the present study, we observed apoptotic symptoms in the livers of mice intoxicated with D-GalN (700 mg/kg) and

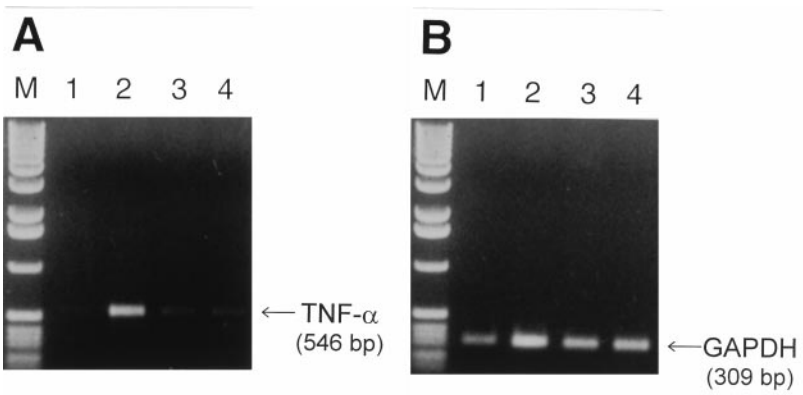

FIG. 4. Expression of TNF-α mRNA in livers of mice treated with D-GalN and LPS. Liver samples were taken at 40 min after intoxication. Total mRNA was prepared from the livers with RNAzol and analyzed with an RT-PCR method under the conditions described in Materials and Methods. THS was administered orally, twice, at 18 and 2 hr before intoxication. The lanes of the gels show samples from mice treated as follows: (1) untreated, (2) D-GalN (700 mg/kg)/LPS (10 μg/kg), (3) D-GalN/LPS + THS (50 mg/kg), and (4) D-GalN/LPS + THS (200 mg/kg). M: 1-kb ladder marker.

LPS (10 μg/kg). Electrophoresis of total hepatic DNA showed a typical ladder pattern fragmented at the nucleosome unit. Histological sections of the liver specimens exhibited the emergence of numerous apoptotic bodies and nuclei with chromatin condensation. A time-course study of hepatic DNA fragmentation and serum ALT levels showed that these pathological events appeared at 5 and 8 hr after intoxication, respectively. These observations coincide with previous reports that hepatocyte apoptosis always precedes membrane disintegration [7].

THS (50 mg/kg) exerted significant protection against DNA fragmentation at 5 hr after intoxication (Table 1, Fig. 2). The protective effect of THS against hepatic apoptosis was further confirmed by histological examination. Pretreatment with THS (50 mg/kg) clearly reduced the emergence of apoptotic bodies and chromatin condensation in livers of mice at 5 hr (Fig. 3). Also, THS exhibited a protective effect against liver injury characterized by an acute increase in serum ALT at 8 hr (Table 2). The inhibitory effect (84.5%) of THS (50 mg/kg) on serum ALT elevation at 8 hr paralleled that (64.4%) on apoptosis at 5 hr. Infiltrating neutrophils are thought to be relevant to the pathogenesis of inflammation, leading to liver cell necrosis at a late stage in this model [16, 17]. Leist *et al.* [7] suggested that in this model hepatocyte apoptosis may act as an initial step of liver damage, and, subsequently, neutrophils are activated by dying hepatocytes that are not removed swiftly enough under this pathological condition. This idea was proved recently by a study with the caspase inhibitor Z-VAD, which allowed the selective blockage of apoptosis. Z-VAD treatment not only prevented caspase activation and apoptosis but also suppressed neutrophil transmigration and liver cell necrosis [13]. This indicates that a large number of hepatocytes undergoing apoptotic cell death can represent a stimulus for primed neutrophils in sinusoids to transmigrate and activate, leading to massive hepatocyte necrosis. Therefore, prevention of apoptosis will protect to some extent against liver injury. The present data clearly show that THS inhibited hepatic apoptosis as well as liver injury (Tables 1 and 2 and Figs. 2 and 3), indicating that the suppression of hepatic apoptosis is involved in the mechanisms by which THS protects mice from liver injury. These effects of THS were different from those observed after pretreatment with GCR, a well-documented hepatoprotective agent of herbal origin [11, 12, 18]. In our previous study, we observed that administration of GCR reduced the mortality of mice after intoxication with D-GalN (700 mg/kg) and a high dose (50 μg/kg) of LPS; 80% of mice in the control group died within 48 hr after intoxication, whereas no mice in the GCR-pretreated group died (unpublished data). The present data show that GCR exerted 61.3% protection against the elevation of the serum ALT level (Table 2). Thus GCR possesses hepatoprotective activity against liver injury induced by D-GalN and LPS. However, this compound did not attenuate DNA fragmentation significantly (26.3% protection) (Table 1 and Fig. 2) or apoptotic body formation (Fig. 3). This suggests that GCR may affect the liver injury processes downstream of apoptosis; however, further studies are necessary to clarify the detailed hepatoprotective mechanisms of GCR.

TNF-α is a critical and terminal mediator to elicit pathological events, i.e. hepatocyte apoptosis, adherent molecule expression, and neutrophil accumulation, in this model [7, 13, 19–22]. This is supported by the fact that D-GalN-sensitized mice intoxicated with TNF-α, instead of LPS, also develop a similar hepatitis as well as hepatocyte apoptosis, and passive immunization against TNF-α can protect mice from the development of liver injury [3, 5]. In a separate study, we found that THS significantly prevented the serum TNF-α elevation that occurred as an early pathological event after D-GalN/LPS intoxication. The inhibitory effect (64.5 to 77.9%) of THS on serum TNF-α elevation was observed at a dose range (10–200 mg/kg) similar to that observed with the inhibitory effect (64.8% at a dose of 50 mg/kg) on hepatic apoptosis (Tables 1 and 3). On the other hand, pretreatment with THS did not attenuate mrTNF-α-induced hepatic apoptosis significantly in D-GalN-sensitized mice (Table 4). These data suggest that protection by THS against hepatic apoptosis induced by D-GalN and LPS may be due predominantly to the inhibition of TNF-α production. TNF-α is responsible for not only induction of apoptosis but also initial inflammatory responses such as expression of adherent molecules and sequestration of neutrophils in sinusoids [13, 21]. The inhibition of TNF-α by THS, therefore, may contribute to the suppression of the initial inflammatory response as well.

Several investigators have reported that the increase in the serum TNF-α level during endotoxemia is associated with the induction of TNF-α-specific mRNA transcripts in liver, which was mainly expressed in hepatic resident macrophages (Kupffer cells) [23–25]. In these studies on LPS-induced cytokine release with a sepsis model, a high dose of LPS (*ca.* 2 mg/kg) was employed, whereas in the present liver injury model an LPS dose as low as 10 μg/kg was used. There has been no report on TNF-α gene expression in this model. The present data demonstrated for the first time that serum TNF-α elevation in the D-GalN/LPS model proceeded from its gene expression in the region of the liver. Interestingly, pretreatment with THS (50 mg/kg) suppressed hepatic TNF-α gene expression. This means that THS may inhibit TNF-α production at the transcriptional level.

THS is a polyphenolic compound isolated from Swertiae Herba, which is used to cure viral hepatitis [1]. There are a number of reports on the hepatoprotective effects of polyphenols, such as flavonoids or lignans, derived from medicinal herbs on various experimental liver injuries induced by xenobiotics or immunological stimuli. The hepatoprotective mechanisms of these naturally derived polyphenols have been reported to proceed mainly from their antioxidant properties, inhibitory effects of the arachidonic acid cascade, or immunosuppressive activity [26–30]. The present data indicate that the inhibition of hepatocyte

apoptosis may be one of the important mechanisms by which polyphenols exert hepatoprotective effects.

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