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Protective effects of *Swertia longifolia* Boiss. and its active compound, swerchirin, on paracetamol-induced hepatotoxicity in mice

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Abstract

Aerial parts of *Swertia longifolia* Boiss. (Gentianaceae), which grows in the north of Iran, were screened for hepatoprotective activity against paracetamol (acetaminophen)-induced hepatotoxicity in Swiss mice. Pretreatment with total plant extract and swerchirin, the major component of the plant, significantly reduced the elevation of biochemical parameters, AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase), the enzymes that are increased by liver damage ($P < 0.001$). Our results indicated that total plant extract and swerchirin were hepatoprotective in the range of 6–50 mg kg^{-1} orally.

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Introduction

The liver is the main organ of metabolism that detoxifies pollutants and chemotherapeutic agents. Generally, the liver makes a non-polar molecule more polar so that it can either be excreted into the urine via the kidneys or be secreted into the faeces through bile. Thus, disorders associated with this organ are numerous and varied (Handa et al 1986). Nowadays liver disease is a worldwide problem. Conventional drugs used in treatment of liver disease are sometimes inadequate and can have serious adverse effects. In fact, a curative agent has not yet been found in modern medicine and drugs that are used only relieve symptoms. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety (Handa et al 1986; Ahmed & Khater 2001).

Swertia is a popular medicinal herb in Southeast Asia. It has been widely used in Ayurvedic and Unani medicine as an anthelmintic, febrifuge and stomach and liver tonic (Hase et al 1997). Some species such as *Swertia mileensis* and *Swertia mussoti* are especially efficacious for acute viral hepatitis and some preparations have been produced industrially in China (Zhou et al 1989). Several studies have been carried out in animals concerning the hepatoprotective activity of the genus and they have shown that the xanthone content of *Swertia* is the most responsible for its antihepatotoxic activity (Hase et al 1997; Karan et al 1999). There are some studies of the other effects of xanthones: it has been determined that swerchirin, a compound with xanthone structure, has hypoglycaemic properties (Bajpai et al 1991; Saxena et al 1991) and a protective effect on haematopoiesis (Ya et al 1999) in animal models. Therefore, xanthones seem to be important for purification and pharmacological effects. In Iran, there is one species from the *Swertia* genus, namely *Swertia longifolia* Boiss. This grows in the northern parts of Iran but no reports were available on the evaluation of this plant for possible hepatoprotective activity. In this publication, we report the hepatoprotective effects of aerial parts of *Swertia longifolia* Boiss. ethanol extract and swerchirin, the major component of the plant, on paracetamol (acetaminophen)-induced hepatotoxicity in mice.

Materials and Methods

Plant studies

Plant materials

Aerial parts of *Swertia longifolia* Boiss. (Gentianaceae) were collected in July 2001 from the north of Iran, Mazandaran province, Yush, Lavashm mountain, ca. 2900 m, Hajimehdipoor, No. 81007 (TARI) and authenticated by Dr Valiollah Mozaffarian, Research Institute of Forests and Rangelands, Tehran, Iran.

Extraction and isolation

Dried and milled aerial parts of plant (1 kg) were extracted in a soxhlet apparatus with ethanol for 12 h. The filtrate was evaporated under reduced pressure to obtain the dark green viscous mass (220 g). This mass was used to prepare ethanol extract solutions.

A part of the ethanol extract (200 g) was dissolved in petroleum ether (60–80°C) and the solution was extracted with aqueous 5% sodium hydroxide (4 × 100 mL). The alkaline aqueous layer was acidified with 10% hydrochloric acid and the acidic solution was successively extracted with chloroform (4 × 200 mL). On concentrating the combined chloroform extract under reduced pressure it yielded 1.5 g mass, which was then chromatographed over a silicagel column (4 × 60 cm) and eluted with toluene (1 L) and toluene–ethylacetate, 70:30 (2 L), respectively.

A solid (98 mg) was obtained from toluene fractions and recrystallized from ethanol to give yellow needles (72 mg). The mp, UV, IR, MS, ^{1}HNMR and ^{13}CNMR data of the compound was in good agreement with that described in the literature for 1,8-dihydroxy-3,5-dimethoxy xanthone (swerchirin) (Asthana et al 1991; Ya & Gen 1998) (Figure 1).

Pharmacological studies

Animals

Male Swiss mice, 20–25 g, were maintained under a 12-h light–dark cycle in a temperature and humidity controlled room. The mice were allowed free access to standard laboratory feed and water before the experiment. Liver injury was produced in the 12-h fasted mice. The animal Ethics Committee of the Tehran University of Medical Sciences, School of Medicine, Education Section of Basic Sciences approved all of experiments (210/20021, July 1, 2000).

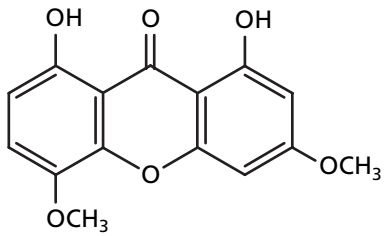

Figure 1 Structure of swerchirin.

Samples

Paracetamol was from Abooreihan Pharmaceutical Company (Analysis No: 98166-R) and was dissolved in 20% dimethyl sulfoxide (DMSO) in normal saline. The control solution was 20% DMSO in normal saline. Ethanol plant extract and swerchirin were dissolved as above.

In-vivo hepatoprotective studies

To study the plant's effect on the liver, ALT (alanine aminotransferase), AST (aspartate aminotransferase) and ALP (alkaline phosphatase) activity, which are the most indicative of liver function, were measured. The activity of the enzymes increased after liver injury (Karan et al 1999; Kew 2000; Ahmed & Khater 2001).

Preliminary experiments were performed on mice to find the effect of 20% DMSO in normal saline (NS) solution on liver enzymes. Two groups of 8 mice were selected. Group 1 was control that received NS solution, at first orally by gavage and after 1 h intraperitoneally. Group 2 was treated as group 1, except that NS solution was replaced by 20% DMSO (vehicle). After 4 h, blood samples were collected.

Hepatic injury in mice was induced by intraperitoneal administration of paracetamol (600 mg kg^{-1}). Total plant extract and swerchirin (3, 6, 12.5, 25, 50 mg kg^{-1}) were given orally before administration of paracetamol. Mice were divided into 4 groups of 8 each. Group 1 received 20% DMSO solution orally and after 1 h received intraperitoneal injection of 20% DMSO (control group). Group 2 was given 20% DMSO orally followed after 1 h by intraperitoneal administration of paracetamol. Group 3 was treated with plant extract or swerchirin orally followed after 1 h by intraperitoneal administration of 20% DMSO. Group 4 was treated similarly to group 2, except that plant extract or swerchirin was administered instead of 20% DMSO.

Assessment of liver function

Four hours after administration of paracetamol, the mice were killed by cervical dislocation then decapitatied and trunk blood was collected in tubes and centrifuged at 10000 rev min^{-1} for 20 min at room temperature to obtain serum. The activity of AST and ALT were measured according to the method described by Reitman & Frankel (1957). Briefly, 0.1 mL of serum was added to 0.5 mL of aspartic acid (AST substrate) or alanine (ALT substrate) and α-ketoglutaric acid mixture, followed by the addition of 0.5 mL of 2,4-dinitrophenylhydrazine and 5 mL of 0.4 M NaOH. The absorbance at 505 nm was measured immediately after mixing. ALT and AST activity was determined by extrapolating from standard curves. The estimation of ALP activity was carried out by the method of Bessey et al (1946) using *p*-nitrophenyl phosphate as substrate. Serum (50 μL) was mixed with the substrate and 0.02 M NaOH. *p*-Nitrophenoxide product was determined spectrophotometrically at 410 nm. ALP activity was determined by extrapolating from a standard curve.

Statistical analysis

The results are expressed as mean ± s.d. and all statistical comparisons were made by ANOVA followed by Tukey test. $P < 0.05$ was considered significant.

Results and Discussion

Paracetamol (*N*-acetyl-*p*-aminophenol, acetaminophen), a widely used analgesic and antipyretic drug, is known to cause hepatotoxicity in experimental animals and man at high doses (Prescott et al 1971; Mitchell 1988; Kuma & Rex 1991; Eriksson et al 1992; Thompsen et al 1995) and can produce hepatic necrosis. It is metabolized in the liver primarily to glucuronide and sulfate conjugates. Paracetamol toxicity is due to formation of toxic metabolite (*N*-acetyl-*p*-benzoquinoneimine, NAPQI) when a part of it is metabolized by a specific isoenzyme of cytochrome P-450 (Handa & Sharma 1990). A physiologically important protective mechanism involving glutathione is available to curtail the progression of cellular damage (Potter et al 1974), but when paracetamol is used in high doses glutathione storage is not enough for conjugating NAPQI. Therefore, NAPQI alkylates proteins present in biomembranes of microsomes and mitochondria, which produces hepatic necrosis (Aldridge 1981). The most indicative factors for liver injury are liver enzymes, such as transaminases and alkaline phosphatase that increase significantly after liver damage (Karan et al 1999; Kew 2000; Ahmed & Khater 2001).

Paracetamol-induced hepatotoxicity in rodents is a widely used animal model to assess the hepatoprotective activity of new compounds (Slater 1965; McLean 1975; Handa et al 1986; Plaa & Hewitt 1989).

Comparison of the control group and vehicle group, 20% DMSO, showed no difference between the groups (data not shown). This indicates that 20% DMSO in NS solution does not change liver enzyme activity; therefore, this solution was used for the rest of experiments as a vehicle for samples.

Mice treated with paracetamol alone developed significant hepatocellular damage as was evident from a significant increase in the serum levels of AST, ALT and ALP when compared with control ($P < 0.001$) (Tables 1 and 2). The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of liver (Chenoweth & Hake 1962), because these are cytoplasmic in location and are released into the circulation after cellular damage (Kew 2000).

Pretreatment of mice with ethanol extract of *Swertia longifolia* Boiss. or swerchirin at doses of 6, 12.5, 25 and

Table 1 Effect of different dosages of ethanol extract of *Swertia longifolia* Boiss. in paracetamol-induced hepatotoxicity in mice

Group	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})
Control	230.2 ± 11.0	176.0 ± 14.7	86.2 ± 8.8
Paracetamol (600 mg kg^{-1})	500.4 ± 21.8†	685.9 ± 38.2†	140.1 ± 7.4†
Extract (3 mg kg^{-1})	231.2 ± 13.6	174.8 ± 16.4	85.3 ± 10.8
Extract (6 mg kg^{-1})	228.3 ± 14.7	170.6 ± 14.4	88.2 ± 5.9
Extract (12.5 mg kg^{-1})	239.2 ± 17.3	179.7 ± 13.9	91.2 ± 5.9
Extract (25 mg kg^{-1})	235.3 ± 15.0	185.5 ± 18.1	87.5 ± 14.4
Extract (50 mg kg^{-1})	240.2 ± 13.6	186.9 ± 17.8	89.6 ± 12.7
Paracetamol + extract (3 mg kg^{-1})	420.9 ± 23.2	590.2 ± 44.7	145.8 ± 13.9
Paracetamol + extract (6 mg kg^{-1})	212.5 ± 13.0*	146.9 ± 17.8*	87.1 ± 16.1*
Paracetamol + extract (12.5 mg kg^{-1})	225.4 ± 9.3*	166.3 ± 22.0*	94.7 ± 17.5*
Paracetamol + extract (25 mg kg^{-1})	235.1 ± 10.8*	224.5 ± 22.0*	100.4 ± 11.6*
Paracetamol + extract (50 mg kg^{-1})	238.3 ± 8.2*	273.0 ± 23.2*	108.0 ± 10.2*

Values are mean ± s.d. of 8 samples. †$P < 0.001$, compared with control; *$P < 0.001$, compared with paracetamol alone.

Table 2 Effect of different dosages of swerchirin in paracetamol-induced hepatotoxicity in mice

Group	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})
Control	212.7 ± 15.0	142.0 ± 11.9	70.1 ± 6.8
Paracetamol (600 mg kg^{-1})	678.9 ± 32.0†	783.3 ± 41.9†	147.7 ± 10.8†
Swerchirin (3 mg kg^{-1})	211.7 ± 17.8	143.1 ± 13.3	78.3 ± 13.9
Swerchirin (6 mg kg^{-1})	214.9 ± 16.7	148.3 ± 9.9	82.5 ± 16.4
Swerchirin (12.5 mg kg^{-1})	217.5 ± 20.9	146.7 ± 11.6	79.2 ± 10.8
Swerchirin (25 mg kg^{-1})	217.3 ± 11.9	156.2 ± 10.8	77.7 ± 15.0
Swerchirin (50 mg kg^{-1})	230.2 ± 15.8	160.3 ± 15.0	75.3 ± 5.9
Paracetamol + swerchirin (3 mg kg^{-1})	654.3 ± 28.9	740.2 ± 48.4	138.3 ± 14.4
Paracetamol + swerchirin (6 mg kg^{-1})	329.3 ± 20.1*	156.6 ± 16.4*	83.4 ± 5.9*
Paracetamol + swerchirin (12.5 mg kg^{-1})	368.1 ± 26.0*	172.3 ± 12.2*	92.8 ± 11.0*
Paracetamol + swerchirin (25 mg kg^{-1})	381.0 ± 23.5*	241.3 ± 17.5*	98.5 ± 6.5*
Paracetamol + swerchirin (50 mg kg^{-1})	398.3 ± 23.8*	258.5 ± 17.5*	104.2 ± 5.9*

Values are mean ± s.d. †$P < 0.001$, compared with control; *$P < 0.001$, compared with paracetamol alone.

50 mg kg^{-1} markedly reduced the elevation of serum levels of these hepatospecific enzymes ($P < 0.001$) (Tables 1 and 2). The hepatoprotective effects of ethanol extract of *Swertia longifolia* Boiss. and swerchirin diminished by increasing dose. A dose of 50 mg kg^{-1} was determined to be less effective and a dose of 6 mg kg^{-1} was found to have the highest efficacy and potency. A dose of less than 6 mg kg^{-1} was investigated as well and it was found not to be effective. At a dose of 6 mg kg^{-1}, the ethanol extract of the plant reduced increases in ALT, AST and ALP levels (78.6%, 57.5% and 37.8%, respectively) and swerchirin also reduced the elevation of serum enzymes (80.0%, 51.5% and 43.5%, respectively). Therefore, not only the ethanol extract of *Swertia longifolia* Boiss. but also the pure compound was more effective at reducing the ALT level compared with AST and ALP levels. This may be because ALT is the more specific marker of hepatocellular injury (Kew 2000).

Our results indicate that the minimum effective dose of both total extract and swerchirin is 6 mg kg^{-1}. It is concluded that swerchirin, with other components of the plant, probably have hepatoprotective effects.

When total plant extract or swerchirin are used orally alone, they do not change the enzyme activity compared with the control group at dose of 3–50 mg kg^{-1} ($P > 0.05$).

References

Ahmed, M. B., Khater, M. R. (2001) Evaluation of the protective potential of *Ambrosia maritima* extract on acetaminophen-induced liver damage. *J. Ethnopharmacol*. **75**: 169–174

Aldridge, W. N. (1981) Mechanism of toxicity: new concepts are required in toxicology. *Trends Pharm. Sci*. **2**: 228–231

Asthana, R. K., Sharma, N. K., Kulshreshtha, D. K., Chatterjee, S. K. (1991) A xanthone from *Swertia chirayita*. *Phytochemistry* **30**: 1037–1039

Bajpai, M. B., Asthana, R. K., Sharma, N. K., Chatterjee, S. K., Mukherjee, S. (1991) Hypoglycemic effect of swerchirin from the hexane fraction of *Swertia chirayita*. *Planta Med*. **57**: 102–104

Bessey, O. A., Lowry, O. H., Brock, M. J. (1946) Method for the rapid determination of alkaline phosphatase with 5 cubic millimetres serum. *J. Biol. Chem*. **164**: 321–329

Chenoweth, M. B., Hake, C. L. (1962) The smaller halogenated aliphatic hydrocarbons. *Annu. Rev. Pharmacol*. **2**: 363–398

Eriksson, L., Broome, U., Kahn, M., Lindholm, M. (1992) Hepatotoxicity due to repeated intake of low doses of paracetamol. *J. Int. Med*. **231**: 567–570

Handa, S. S., Sharma, A. (1990) Hepatoprotective activity of andrographolide against galactosamine and paracetamol intoxication in rats. *Indian J. Med. Res*. **92**: 284–292

Handa, S. S., Sharma, A., Chakraborthi, K. K. (1986) Natural products and plants as liver protecting agents. *Fitoterapia* **LVII 5**: 307–351

Hase, K., Li, J., Basnet, P., Xiong, Q., Takamura, S., Namba, T., Kadota, S. (1997) Hepatoprotective principles of *Swertia japonica* MAKINO on D-Galactosamine/Lipopolysaccharide-induced liver injury in mice. *Chem. Pharm. Bull*. **45**: 1823–1827

Karan, M., Vasisht, K., Handa, S. S. (1999) Antihepatotoxic activity of *Swertia chirata* on paracetamol and galactosamine-induced hepatotoxicity in rats. *Phytother. Res*. **13**: 95–101

Kew, M. C. (2000) Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet* **355**: 591–592

Kuma, S., Rex, D. (1991) Failure of physicians to recognize acetaminophen hepatotoxicity in chronic alcoholics. *Arch. Intern. Med*. **151**: 1189–1191

McLean, A. (1975) Models of liver diseases. In: Karger, B. A. (ed.) *New trends in the therapy of liver diseases*. S. Karger, Basel, pp 2–12

Mitchell, J. (1988) Acetaminophen toxicity. *N. Engl. J. Med*. **319**: 1601–1602

Plaa, G. L., Hewitt, W. R. (1989) Detection and evaluation of chemically induced liver injury. In: Hayes, A. W. (ed.) *Principles and methods of toxicology*. 2nd edn, Raven Press, New York, pp 599–628

Potter, W. Z., Thorgeirsson, S. S., Jollow D. J., Mitchell, J. R. (1974) Acetaminophen-induced hepatic necrosis V. Correlation of hepatic necrosis, covalent binding and glutathione depletion in hamsters. *Pharmacology* **12**: 129–143

Prescott, L. F., Roscoe, P., Wright, N., Brown, S. S. (1971) Plasma paracetamol half life and hepatic necrosis in patients with paracetamol overdose. *Lancet* **1**: 519–522

Reitman, S., Frankel, A. S. (1957) A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol*. **28**: 56–63

Saxena, A. M., Bajpai, M. B., Mukherjee, S. K. (1991) Swerchirin induced blood sugar lowering of streptozotocin treated hyperglycemic rats. *Indian J. Exp. Biol*. **29**: 674–675

Slater, T. F. (1965) Biochemical studies on liver injury. In: *Biochemical mechanism of liver injury*. Academic Press, London, pp 1–44

Thompsen, M., Loft, S., Roberts, D., Poulsen, H. (1995) Cytochrome P450 2E1 inhibition by propylene glycol prevents acetaminophen hepatotoxicity in mice without cytochrome P450 1A2 inhibition. *Pharmacol. Toxicol*. **76**: 395–399

Ya, B. Q., Gen, X. P. (1998) Four xanthones from *Swertia calycina* Franch. *Pharm. Pharmacol. Commun*. **4**: 595–596

Ya, B. Q., Nian, L. C, Li, C., Gen, X. P. (1999) Protective effect of swerchirin on hematopoiesis in 60Co-irradiated mice. *Phytomedicine* **6**: 85–88

Zhou, H. M., Liu, Y. L., Blaskó, G., Cordell, G. A. (1989) Swertiabisxanthone-I from *Swertia macrosperma*. *Phytochemistry* **28**: 3569–3571