Protective Effects of *Acanthopanax senticosus* Harms from Hokkaido and Its Components on Gastric Ulcer in Restrained Cold Water Stressed Rats

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The aim of this study is to investigate the pharmacological effect of the stem bark of *Acanthopanax senticosus* Harms from Hokkaido (Japanese name: Ezoukogi) in place of the root bark as a restorative tonic on the stress-induced gastric ulcer. In the test, the extract of the stem bark of *A. senticosus* prepared with hot water was dissolved in water and used for the assay of the protective effect of gastric ulcer (erosion) on stressed rats that were restrained on cold water. The result from a single oral administration of the stem bark of *A. senticosus*-extract (50, 100 and 500 mg/kg, per day) dissolved in 1 ml distilled water did not show any protective effect on gastric ulcer, but the protective effect was observed in a dose-dependent manner from the oral administration of the extract (50, 100 and 500 mg/kg, per day) for 2 weeks. Pre-administration of the stem bark of *A. senticosus*-extract in a dose of 500 mg/kg showed the most potent inhibition without affecting either body or adrenal glands weights. Among ether, chloroform, n-butanol and aqueous residue extracts from the stem bark of *A. senticosus*-extract, the n-butanol extract used for oral administration for 2 weeks showed an obvious inhibition of 61.1% on gastric ulcer, compared with the control group which was treated with distilled water in the same way.

Chlorogenic acid and siringaresinol di-o-β-D-glucoside, as the major components of the n-butanol extract, showed a significantly inhibitory effect on gastric ulcer, at 21.4% and 51.3%, respectively. We suggested that the protective effect of the stem bark of *A. senticosus* on gastric ulcer may be partially due to those of chlorogenic acid and siringaresinol di-o-β-D-glucoside.

**Key words** *Acanthopanax senticosus*; gastric ulcer; restrained cold water stress; siringaresinol di-o-β-D-glucoside; chlorogenic acid; n-butanol extract

The root bark of *Acanthopanax senticosus* Harms (Araliaceae) has been used as agents for the regulation of blood pressure, as tonics, for mental and emotional stability, and to treat rheumatismal arthralgia in China, 2-5 and as agents for anti-hypertension, for the functional promotion of the adrenal cortex or gonad, for anti-stress, for effectively prolonging life, and for improving the effect of blood cells destroyed by X-ray irradiation in the U.S.S.R. 3-7 In addition, *A. senticosus* has been well-known as an adaptogenic medicine 8 and as a Bu-Yang-Yaw 9 in traditional Chinese medicine. The aqueous extract of the root bark of *A. senticosus* has been known to protect mice from stress-induced decreases in sex- and learning-behaviors and in rectal temperature, and from the stress-induced enlargement of the adrenal gland. 10-12 However, the aqueous extract of the stem bark of *Acanthopanax senticosus* Harms has previously been reported to prolong the swimming time in the forced swimming test. 13, 14 In this study, we examined the effect of the stem bark of *A. senticosus* Harms from Hokkaido, Japan (Japanese name: Ezoukogi) on the stress-induced gastric ulcer, and analyzed the major components of the stem bark of *A. senticosus* which possessed potential effects on the inhibition of gastric ulcers.

**MATERIALS AND METHODS**

**Animals** Male Wistar rats, 6 weeks old, were purchased from the Hokkaido Experimental Animal Center (Hokkaido, Japan). In the single-administration group, rats were subjected to preliminary housing and handling for 4 weeks, and in the two-weeks-administration group, rats were subjected to preliminary housing and handling for 2 weeks and pretreatment for 2 weeks. At 10 weeks of age, rats were used throughout the experiment. Animals were fed with a standard diet (MF, Oriental Yeast Manufacturing Co., Ltd., Tokyo) and water ad libitum. They were kept in a room maintained at 25±3°C by a 12-h light/12-h dark lighting cycle (lights on at 08:10).

**Sample Preparations** Powdered stem bark of *Acanthopanax senticosus* Harms which was extracted with hot water by Yakuhan Pharmaceutical Manufacturing Co., Ltd., (Hokkaido, Japan) was dissolved with distilled water and used as a material for administration. The powdered stem bark of *A. senticosus* (ASH, 500 g) was extracted with hot methanol four times. The methanol solution was evaporated to a small volume under reduced pressure, then filtrated after water was added. The filtrate was successively extracted with ether, chloroform and n-butanol to give an ether extract (11.42 g), chloroform extract (6.58 g), n-butanol extract (90.78 g), and an aqueous residue extract (119.16 g). Then, each sample was dissolved with distilled water and used as a material for administration.

Chlorogenic acid (CHA, 986 mg) and siringaresinol di-o-β-D-glucoside (SYG, 705 mg) were isolated from n-butanol extract (50 g), respectively, by the procedure described in the previous paper. 13 Then, the components were identified by comparing their spectral and chromatographic properties with those of authentic samples.
in ASH extract were quantitatively determined by high-performance liquid chromatography (HPLC). The major components were shown to be CHA (1473 mg/100 g) and SYG (1156 mg/100 g).15,16

**Administration of Samples** 1. ASH (50, 100 and 500 mg/kg/d) dissolved in distilled water in a volume of 1 ml was administered orally through a probe once 1 h just before starting the restraint stress in the water (Fig. 1). The same volume of distilled water was administered to the control group in the same way.

2. ASH (50, 100 and 500 mg/kg/d) dissolved in distilled water in a volume of 1 ml was administered orally within the 08:10—09:30 time frame once a day for 14 consecutive days and once 1 h just before starting the restraint stress in the water (on the 14th day after beginning the administration) (Fig. 1). The same volume of distilled water was administered to the control group in the same way.

3. Each of ether extract, chloroform extract, n-butanol extract and aqueous residue extract was administered orally within the 08:10—09:30 time frame once a day for 14 consecutive days and once 1 h just before starting the restraint stress in the water (on the 14th day after beginning the administration) (Fig. 1). The dose of these extracts corresponded to that contained in ASH. That is, the n-butanol extract was established as a dose of 100 mg/kg, and each dose of the remaining extracts was calculated as follows: ether extract, 13 mg/kg; chloroform extract, 7 mg/kg; and aqueous residue extract, 131 mg/kg. The same volume of distilled water was administered to the control group in the same way.

4. CHA or SYG (50 mg/kg, respectively) dissolved in distilled water in a volume of 1 ml was administered orally within the 08:10—09:30 time frame once a day for 14 consecutive days and once 1 h just before starting the restraint stress in the water (on the 14th day after beginning the administration) (Fig. 1). The same volume of distilled water was administered to the control group in the same way. The doses of CHA or SYG were determined in the manner described in the pharmacological reports on the root bark of ASH.10,11

**Measurements of the Body Weight and Adrenal Glands** The body weights of rats were measured before the oral administration of ASH. The measurements were carried out three times between 06:30—08:00 and then the means were calculated for 14 d. The wet weight of the adrenal glands in the rat which was treated with each sample was assessed as the specific gravity of these per 100 g body weight before or after the restraint stress in the water.

**Restraint Stress in the Water** One hour after the last administration of ASH (on the 14th day after the first administration), the rats were exposed to a restrained cold water stress as described previously.17–20 At 10 weeks of age, rats were put in individual small restraining wire net cages (17 cm(H) × 7 cm(L) × 4 cm(W)), and immersed into the water to the chest. The temperature of the water was kept constant at 23 ± 0.5 °C. After 7 h of the restraint stress in the water, rats were removed from the cages and decapitated.

**Fixation of Stomachs** After 7 h of the restraint stress in the water, the animals were decapitated. Stomachs were removed, fixed with 1% formalin and opened along the greater curvature following the fixation for 30 min.

**Inhibitory Rate (%) of Gastric Ulcer** The inhibitory effects concerning the means of the gastric ulcer index were calculated using the following formula:

\[
\text{Inhibition of gastric ulcer (%) = } \frac{\text{total erosion number of control} - \text{total erosion number of each sample}}{\text{total erosion number of control}} \times 100
\]

**Statistical Analysis** The mean and SE of the data were determined and presented in the Table and Figures. The significance of difference between the values was analyzed by unpaired Student's t-test or Dunnett's post-hoc procedure test after performing an F-test (two groups) or Bartlett test (more than two groups), respectively. p values
of less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

The results from the non-stressed and stressed rats showed that the weight of rat body and adrenal glands was not affected by ASH or by the ether, chloroform, n-butanol, or aqueous residue extracts from ASH or the major components of the n-butanol extract which were used in oral administration for 2 weeks (Table 1(A) (B) (C)). A single administration of ASH in doses of 50, 100 and 500 mg/kg/d, respectively, did not show any inhibition of the gastric ulcer (Fig. 2(A)). However, significant and dose-dependent inhibition of the gastric ulcer was observed from the 2-week-consecutive administration of ASH. The strongest inhibition resulted from the administration of 500 mg/kg/d and reached 59.7% compared with the control group, which was treated with distilled water (Fig. 2(B)). In the 2-week-administration group, the inhibition by different extracts from ASH was also examined, and only the n-butanol extract showed significant inhibition, 61.1%, of gastric ulcer formation, as shown in Fig. 2(C).

Table 1. Influences of ASH, and of Each Extract from ASH and the Major Components in the n-Butanol Extract on the Weight of Rat Body and Adrenal Glands

<table>
<thead>
<tr>
<th>Group</th>
<th>Stress</th>
<th>Body weight (g)</th>
<th>Adrenals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>(A) Control</td>
<td>-</td>
<td>187.0±3.6</td>
<td>234.5±5.0</td>
</tr>
<tr>
<td>ASH</td>
<td>-</td>
<td>185.0±3.3</td>
<td>236.0±5.0</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ASH</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(B) Control</td>
<td>-</td>
<td>201.8±2.7</td>
<td>249.1±3.6</td>
</tr>
<tr>
<td>EtO</td>
<td>-</td>
<td>198.2±2.1</td>
<td>241.9±3.9</td>
</tr>
<tr>
<td>CHCl</td>
<td>-</td>
<td>211.6±2.0</td>
<td>267.0±5.8</td>
</tr>
<tr>
<td>BuOH</td>
<td>-</td>
<td>199.8±1.9</td>
<td>248.2±3.7</td>
</tr>
<tr>
<td>H2O</td>
<td>-</td>
<td>204.5±2.4</td>
<td>250.4±3.7</td>
</tr>
<tr>
<td>(C) Control</td>
<td>-</td>
<td>176.7±2.1</td>
<td>230.8±3.6</td>
</tr>
<tr>
<td>CHA</td>
<td>-</td>
<td>193.2±2.9</td>
<td>245.0±4.2</td>
</tr>
<tr>
<td>SYG</td>
<td>-</td>
<td>179.7±2.6</td>
<td>235.7±3.7</td>
</tr>
</tbody>
</table>

Each sample, dissolved in distilled water in a volume of 1 ml, was orally given every day for 2 weeks in the following doses: the aqueous extract of ASH from Hokkaido extracted with hot water (ASH, 500 mg/kg/d), the different extracts from the ASH: ether extract (EtO, 13 mg/kg/d), chloroform extract (CHCl, 7 mg/kg/d), n-butanol extract (BuOH, 100 mg/kg/d), aqueous residue extract (H2O, 131 mg/kg/d) and the major components: chlorogenic acid (CHA, 50 mg/kg/d) and syringaresinol di-O-ß-D-glucoside (SYG, 50 mg/kg/d), as described in the Materials and Methods. The same volume of distilled water was administered to each control group in the same way. The body weights of rats were measured between 06:30–08:00 before the oral administration of each sample every day for 2 weeks. The relative weight (%) of the adrenal glands in rats which were treated with each sample indicates the proportion of organ weight per 100 g body weight before or after the stress (water temperature: 23±0.5°C, immersion time: 7h, restriction from bilateral directions). Each value represents the mean±S.E. of 5 to 14 samples.

Fig. 2. Protective Effects of ASH and Each Extract from ASH on Gastric Ulcer in Restrained Cold Water Stressed Rats

In the single-administration group, the aqueous extract of ASH from Hokkaido extracted with hot water was orally administered once 1 h just before starting the stress. In the 2-week-administration group, the aqueous extract of ASH extracted with hot water and of each extract from ASH was orally administered in the same way as described in Table 1. The same volume of distilled water was administered to each control group in the same way. The rat was subjected to the stress for 7 h. Each numerical value at the top of the columns shows the inhibition percent (%) to the generated gastric ulcer as compared to each control group which had been treated with distilled water. Each value represents the mean±S.E. of 5 to 14 samples. (A) a) p<0.05 vs. control, (B) p<0.01 vs. control, (C) c) p<0.01 vs. control, d) p<0.05 vs. EtO, e) p<0.01 vs. CHCl, f) p<0.01 vs. H2O.
The ether extract showed 14.9% inhibition, and no significant inhibition was statistically observed from the extracts other than the n-butanol extract (Fig. 2(C)). Thus, these results suggest that the active components might be contained in the n-butanol extract.

We analyzed whether CHA and SYG, which are known to be major components of the n-butanol extract, were able to induce an inhibitory effect on stress-induced gastric ulcers. In the 2-week-administration group, SYG showed a 51.3% inhibition, more potent than that of CHA, 21.4%, but both inhibitions were statistically significant (p < 0.05), as shown in Fig. 3. In a previous study, Takeda showed that SYG prolonged the swimming time in the forced swimming test but CHA did not. Thus, our results were not in agreement with the former data because we found that CHA also possesses an inhibitory effect on stress-induced gastric ulcers.

Taken together, we conclude that the stem bark of ASH from Hokkaido, Japan and the n-butanol extract from ASH show obvious inhibition on gastric ulcers (erosion) induced by stress, and the n-butanol extract contains potential components which are able to inhibit the gastric ulcer. This effect may be partially due to the SYG and CHA as major components in the n-butanol extract. To correctly assess the effect of the major components of the n-butanol extract on gastric ulcer, it is still necessary to clarify the protective effects by which other components in the n-butanol extract. In addition, the mechanism that the stress-induced gastric ulcer was inhibited by ASH and its active components will be continuously investigated.

The experiments reported herein were conducted according to the principles set forth in the Guides to the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council [DHEW publication (NIH) 85-23].

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REFERENCES AND NOTES

1) Present address: Department of Biochemistry, Faculty of Medicine, Mie University, 2-174 Edobashi, Tsu, Mie 514, Japan.


16) Quantitative determination of the constituents of the extract of the stem bark of A. senticosus by HPLC (conditions: apparatus, ERC-8710 (Erma); column, ERC-OAD (3 μm) × 6 mm × 100 mm; eluent, CH₃CN–H₂O–HCOOH (15:85:1); flow rate, 1.0 ml/min; detection, UV at 270 and 345 nm; room temperature) was carried out by Yakuhan Pharmaceutical Co., Ltd. The results obtained (mg/100g) are as follows: isofraxidin, 55; syringaresinol-di-O-β-glucoside, 1156; syringin, 454; chlorogenic acid, 1473; isofraxidin-7-O-β-glucoside, 109.


