Journal of Traditional Medicinen **11**, 134－141, 1994

Processing of Nux Vomica. III. Effects of seven different processings of nux vomica on chemical constituents and acute toxicity

Bao-Chang CAI[a] Hao-WU[a] Shigetoshi KADOTA[b] Masao HATTORI*[c] and Tsuneo NAMBA[b]

[a]*Nanjing College of Traditional Chinese Medicine, Departments* [b]*of Development of Drug Resources and* [c]*of Cell-Resources Engeneering, Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University*

(Received May 31, 1994. Accepted July 25, 1994.)

Abstract

For the purpose of evaluating various processings of nux vomica, we compared the chemical constituent and acute toxicity of the processed and unprocessed seeds of *Strychnos nux-vomica*. After treatment of the seeds with seven different methods, the contents of total alkaloids and most of the individual strychnos alkaloid were significantly lowered in the processed seeds, but the decreasing ratios varied depending on the processing methods used. Furthermore, the composition of alkaloids was also changed with the processing methods.

Acute toxicity of crude alkaloid fractions from all of the processed seeds decreased appreciably. Their LD_{50} values were in a narrow range of 2.18-2.57 mg/kg of mouse, while that of the unprocessed seeds was 1.21 mg/kg. However, no good correlations were observed between total alkaloid contents in the seeds and LD_{50} values and between the individual major alkaloid contents and LD_{50} values. This indicates that all of the processings examined reduce toxicity to a similar extent though they led significant variations in total and individual alkaloid contents and in their composition.

In view of convention, parching of the raw seeds in an sand bath, a method described in the Pharmacopeia of People's Republic of China, seems to be better, but other traditional processing methods examined in this experiment are worthwhile to investigate in view of therapeutic efficacy in traditional Chinese medicine.

Key words Acute toxicity, *Strychnos nux-vomica*, drug-processing, loganin, strychnos alkaloids.
Abbreviations EtOAc, ethyl acetate ; LD_{50}, 50 % lethal dose ; TLC, thin-layer chromatography.

Introduction

Nux Vomica is the dried seeds of *Strychnos nux-vomica* L., Loganiaceae, and medicinally used for promoting blood circulation, alleviating blood stasis, relieving pain, dissipating nodes and swelling masses and curing indigestion. The seeds are generally used after drug-processing because they are "cold" in nature of a drug, "bitter" in taste and poisonous, in terms of traditional Chinese medicine. In the Pharmacopeia of People's Republic of China, one processing method, in which the seeds are parched in a sand bath, is described. However, other methods, such as frying in an oil bath, treating with a licorice decoction, submerging in urine from healthy children, treating with vinegar *etc.*, have been traditionally carried out at various provinces, cities and anonymous regions in China.[1-3]

In our previous paper,[4] we reported a comparison of chemical constituents before and after processing of the seeds of *S. nux-vomica* using the sand bath and

*〒930-01 富山市杉谷2630 富山医科薬科大学和漢薬研究所 細胞資源工学部門 服部征雄
2630 Sugitani, Toyama 930-01, Japan

the frying-in-oil methods; on heat treatment, the contents of the major alkaloids such as strychnine (**1**) and brucine (**2**) decreased appreciably with increase in the amounts of isostrychnine (**5**), isobrucine (**6**), strychnine *N*-oxide (**7**) and brucine *N*-oxide (**8**). Furthermore, we reported that the latter two *N*-oxides were metabolized to **1** and **2** together with 16-hydroxy derivatives by human intestinal bacteria.[5]

In the present paper, we describe the effects of seven different processing methods on chemical constituents (12 alkaloids and loganin) and acute toxicity of the seeds of *S. nux-vomica*.

Materials and Methods

Materials: The seeds of *S. unx-vomica* were supplied by Nanjing Company of Chinese Herbal Drugs (Nanjing, China). The urine was collected at 8.00 AM from five male children in the kindergarten. Their health conditions had been previously examined by a medical doctor. The vinegar used in this experiment was purchased from a food store.

Chemicals: Strychnine (**1**), brucine (**2**), vomicine (**3**), icajine (**4**), isostrychnine (**5**), isobrucine (**6**), strychnine *N*-oxide (**7**), brucine *N*-oxide (**8**), novacine (**9**), pseudostrychnine (**10**), 16-hydroxy-α-colubrine (**11**), β-colubrine (**12**) and loganin (**13**) were isolated from the seeds of *S. nux-vomica*.[6,7] All reagents used were of analytical grade.

Chromatography: GF_{254} silica gel thin layer plates were purchased from Qindao Sea Chemical Engineering Factory (Qindao, China). A CS-930 dual-wavelength chromatoscanner (Shimadzu, Kyoto, Japan) was used for quantitative analysis of strychnos alkaloids **1**-**12** and loganin (**13**) present in the processed and unprocessed seeds of *S. nux-vomica*.

Animal: ICR mice (body weight 18-22 g) were purchased from the Experimental Animal Center of Nanjing College of Traditional Chinese Medicine (Nanjing, China).

Processing of the seeds of S. nux-vomica: The following traditional drug-processings were carried out on a laboratory scale using the same batch of the raw seeds of *S. nux-vomica* and the processed seeds were investigated to determine the chemical constituents and acute toxicity in comparison with the unprocessed seeds (A) that were prepared by slicing the raw seeds and drying them at 80°C.

1) *Parching in a sand bath (processing B)*: Clean sand was put into an iron pan and heated up to 230°C when the temperature of the sand was measured 3 mm above the pan bottom. The seeds of *S. nux-vomica* were put into the pan, covered with the hot sand for a while, stirred continuously for 3 min and taken rapidly out when the seeds were swollen, burst and yellow in color.

2) *Processing with a licorice decoction (processing C)*: Sheets of licorice roots (*Glycyrrhiza* sp., 30 g) were boiled twice in water (300 ml and 180 ml, each) and the combined solutions were filtered. The seeds (300 g) of *S. nux-vomica* were put into the filtrate and boiled for 4 h, then they were taken out, cooled, sliced into 1.5 mm thick sheets. The sheets were then roasted into crisps below 80°C.

3) *Processing with urine and a sand bath (processing D)*: The seeds of *S. unx-vomica* (300 g) were put into fresh urine (300 ml) from healthy children and kept for 7 days, followed by treatment with processing B as described above.

4) *Processing with urine (processing E)*: The seeds of *S. nux-vomica* (300 g) were put into urine (300 ml) from healthy children. The bottle was sealed and kept at the cool place for 49 days, and the seeds were taken out, sliced into 1.5 mm thick sheets. The sheets were dried below 80°C.

5) *Frying in oil (processing F)*: Sesame oil (250 ml) was put into an iron pan and heated up to 230°C. The seeds of *S. nux-vomica* (300 g) were put into the hot oil, kept for 3 min and taken out when they became yellow. The oil on the surface of seeds was wiped off and the processed seeds were cooled for use.

6) *Processing with vinegar (processing G)*: The seeds of *S. nux-vomica* (300 g) were submerged in water (300 ml) for 2 days, taken out and sliced into 1.5 mm thick sheets while they were moistened. The sheets were submerged in vinegar (375 ml, containing 5 % acetic acid) for 5 days at room temperature, washed with water for 3 days and dried below 80°C.

7) *Processing with vinegar and a sand bath (processing H)*: The seeds of *S. nux-vomica* (300 g) were submerged in water (300 ml) for 2 days, sliced into 1.5 mm thick sheets. The sheets were put into vinegar (450 ml,

containing 5 % acetic acid), boiled for 10 min, submerged in vinegar for 12 h at room temperature, taken out, dried and then treated with processing B.

Preparation of crude alkaloid fractions : Sheets of the raw seeds of *S. nux-vomica* and processed ones obtained by different methods were dried at 80°C and ground into fine powder, which was passed through a sieve (type 20). Each powder (200 g) was put into a conical flasks with a plug, moistened with 15 % aqueous ammonia (20 ml) and kept for 2 h. The moistened powder was soaked in chloroform (600 ml) and the mixture was stirred thoroughly, kept at room temperature for 24 h with occasionally stirring. The solution was filtered and the residue was re-extracted with chloroform (600 ml) in a similar manner. The resulting residue was further re-extracted three times with chloroform (50 ml each). The combined solutions were concentrated *in vacuo* to a volume of 40 ml. The solution was extracted six times with 1 N HCl (50, 50, 35, 35, 20 and 20 ml, each). The combined acidic solutions were adjusted to pH 11-12 with 40 % NaOH and extracted with chloroform (100 ml×6). The combined chloroform solutions were evaporated *in vacuo* to give a crude alkaloid fraction.

Acute toxicity test : A crude alkaloid fraction (20 mg) was dissolved in 1 N HCl (1 ml), adjusted to pH 6.0-6.5 with 1 N NaOH and diluted with distilled water to a concentration of 0.08 mg/ml. The maximal and minimal lethal doses were preliminarily estimated with four groups of ICR mice (two male and two female mice for each group) by the method of Sun.[8] The dosages for determination of LD_{50} were then calculated according to the ratio of dose for each group (r value) and intraperitoneally administered to five dosage groups (10 mice each, with a 1 : 1 ratio of male and female ; 0.2 ml/20 g body weight). The animals were kept in a humidity of 62 % at 23°C. The number of dead mice were recorded 3 days after administration of the alkaloid fraction. The 50 % lethal dose (LD_{50}) and the 95 % confidence limit were calculated by the Blis method.

Quantitative analysis of alkaloids and loganin : For determination of strychnos alkaloids, powder of the processed or the unprocessed seeds of *S. nux-vomica* (2.00 g) was put into an iodometric bottle, moistened with 10 % aqueous ammonia (2.5 ml), kept for 1 h and submerged in chloroform (80 ml). The mixture was well dispersed with an ultrasonicator for 20 min and kept for 3 days at room temperature, then filtered. The residue was re-extracted further three times with chloroform (10 ml each). The combined chloroform solutions were evaporated *in vacuo* to give a residue, which was transferred into a 5 ml volumetric flask and adjusted with chloroform to an exact volume. Portions (10 μl each) of the solution were applied to three thin layer plates. The plates were separately developed 16 cm in distance with three solvent systems : A, n-hexane-EtOAc-MeOH-Et_2NH (8 : 6 : 0.3 : 1.5) for determination of strychnine (**1**), brucine (**2**), isostrychnine (**5**), pseudostrychnine (**10**) and 16-hydroxy-α-colubrine (**11**) ; B, EtOAc-MeOH-aqueous NH_3 (20 : 0.7 : 0.1) for determination of icajine (**4**), vomicine (**3**), novacine (**9**) and β-colubrine (**12**) ; C, EtOH-Et_2O-Et_2NH (8 : 2 : 0.3) for determination of strychnine N-oxide (**7**) and brucine N-oxide (**8**). The intensity of spots on the plates was measured with a TLC-scanner (λ_S=260 nm, λ_R=360 nm) and the quantity of the respective alkaloids was determined using standard lines which were prepered with authentic samples.

For determination of loganin (**13**), powder of the processed or the unprocessed seeds of *S. nux-vomica* (1.00 g) was put into methanol (15 ml) in an iodometric flask. The mixture was well dispersed with an ultrasonicator for 20 min and kept for 3 days at room temperature, then filtered. The residue was re-extracted three times with methanol (10 ml each). The combined methanolic solutions were evaporated *in vacuo* to give a residue, which was transferred into a 2 ml volumetric flask and diluted with methanol to an exact volume. A portion (5 μl) of it was applied to a TLC plate, which was developed with solvent system D, n-hexane-EtOAc-formic acid-H_2O (8 : 4 : 2 : 0.4). The spots of iridoid glucosides on the TLC plate were visualized by spraying with 20 % H_2SO_4 followed by heating at 90°C. Loganin (**13**) was quantitatively analyzed with a TLC-scanner at 490 nm using a standard line prepared with an authentic sample.

Stability experiment : A portion (8 μl) of each standard solution was applied to TLC plates and developed with solvent systems A, B and C for alkaloids and with solvent system D for loganin (**13**). The

spots on the plates were measured at intervals for 16 h with a TLC scanner as mentioned above.

Recovery experiment : An exactly weighed authentic sample was added to a solution containing an extract of the seeds of *S. nux-vomica*. A portion of the resulting solution was taken out and quantitatively analyzed by TLC-densitometry. The net amount of an added compound was calculated from a standard line (see Table II).

Results and Discussion

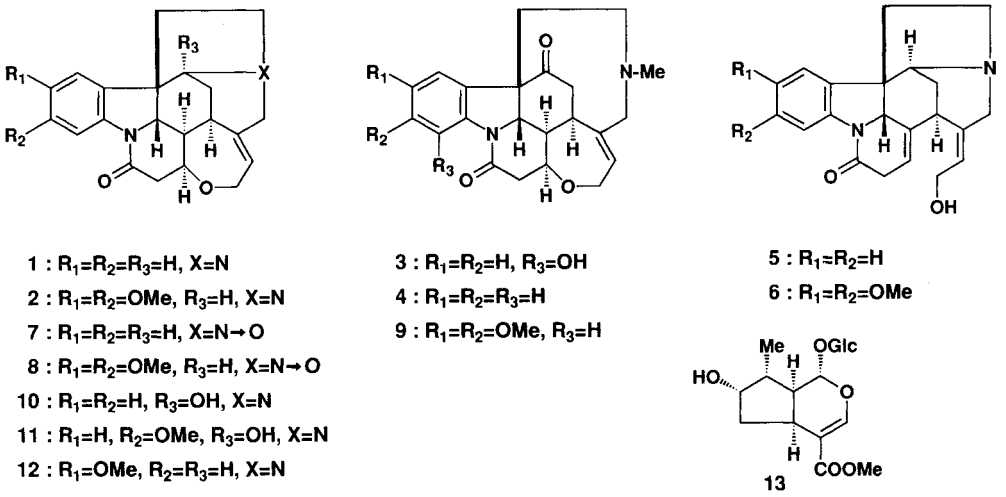
Analysis of strychnos alkaloids and loganin by TLC densitometry

For comparing the alkaloid contents in the processed and unprocessed seeds of *S. nux-vomica*, the raw seeds were treated with the following seven methods : parching in a sand bath (processing B), processing with a licorice decoction (processing C), processing with urine and a sand bath (processing D), processing with urine (processing E), frying in oil (processing F), processing with vinegar (processing G), processing with vinegar and a sand bath (processing H).

The processed and unprocessed seeds of *S. nux-vomica* contain various alkaloids together with iridoid glucosides.[5, 6] These compounds were used in this experiment as indices to the change in chemical constituents before and after drug-processing. However, various attempts to determine all of strychnos alkaloids (**1**-**12**, see Fig. 1) and a major iridoid glucoside, loganin (**13**), at once by thin layer chromatography (TLC)-densitometry with an appropriate solvent system were unsuccessful because some of these compounds had quite different chromatographic behaviour. Therefore, the contents of individual alkaloid (**1**-**12**) and loganin (**13**) were separately analyzed by TLC using four different solvent systems. The spots of alkaloids on the TLC plate were stable in intensity within 2 h and that of loganin (**13**) was relatively stable within 1 h. As reported in previous paper,[5] calibration curves of these compounds showed good linearity between peak - height and concentration (Table I), and the accuracy of the respective equations of regression lines was satisfactory as shown by the recovery experiments (Table II). In addition, the results were demonstrated with coefficients of variation (0.08-4.58 %, Table III).

Effects of various processings of nux vomica on the total alkaloid content

Table IV shows the changes in strychnos alkaloid (**1** - **12**) and loganin (**13**) contents before and after processing. The major alkaloids, such as strychnine (**1**) and brucine (**2**) decreased appreciably depending on the methods used. When compared to the respective alkaloid contents of unprocessed seeds (A, control), the strychnine (**1**) content was not so much changed by parching in a sand bath (processing B ; 89 % of con-



1 : R_1=R_2=R_3=H, X=N
2 : R_1=R_2=OMe, R_3=H, X=N
7 : R_1=R_2=R_3=H, X=N→O
8 : R_1=R_2=OMe, R_3=H, X=N→O
10 : R_1=R_2=H, R_3=OH, X=N
11 : R_1=H, R_2=OMe, R_3=OH, X=N
12 : R_1=OMe, R_2=R_3=H, X=N

3 : R_1=R_2=H, R_3=OH
4 : R_1=R_2=R_3=H
9 : R_1=R_2=OMe, R_3=H

5 : R_1=R_2=H
6 : R_1=R_2=OMe

13

Fig. 1 Structures of strychnos alkaloids and loganin.

Table I Regression equations and their correlation factors for quantitative analysis of strychnos alkaloids and loganin.

Compound	Range of concentration (μg/spot)	Equation of regression line	Correlation coefficient
Strychnine (**1**)	4.6−23.0	$Y=46379.7+39941.8X$	$r=0.9939$
Brucine (**2**)	4.5−22.5	$Y=2126.1+41742.0X$	$r=0.9934$
Vomicine (**3**)	1.5−24.7	$Y=-1502.2+6462.4X$	$r=0.9811$
Icajine (**4**)	1.0−17.1	$Y=6971.9+83311.4X$	$r=0.9976$
Isostrychnine (**5**)	2.5−12.5	$Y=3406.4+22156.3X$	$r=0.9813$
Isobrucine (**6**)	2.7−13.4	$Y=1078.4+10509.9X$	$r=0.9880$
Strychnine *N*-oxide (**7**)	2.1−18.7	$Y=-1863.8+65317.1X$	$r=0.9975$
Brucine *N*-oxide (**8**)	2.5−22.5	$Y=5998.9+41606.7X$	$r=0.9922$
Novacine (**9**)	8.5−40.2	$Y=6343.1+6025.1X$	$r=0.9820$
Pseudostrychnine (**10**)	2.0−12.0	$Y=3049.3+7536.4X$	$r=0.9880$
16-Hydroxy-α-colubrine (**11**)	1.1−5.3	$Y=6343.4+1166.2X$	$r=0.9940$
β-Colubrine (**12**)	10.6−50.6	$Y=2059.3+1043.7X$	$r=0.9920$
Loganin (**13**)	3.2−28.8	$Y=18767.8+10872.1X$	$r=0.9953$

X and Y represent the peak height and the added amount of alkaloid (μg) or log anin (μg).

Table II Accuracy of the analysis of strychnos alkaloids and loganin by TLC-densitometry (n=3).

Compound	Added amount (μg)	Found (μg)	Recovery (%)
Strychnine (**1**)	9.14	9.10	99.6
Brucine (**2**)	9.00	9.02	100.2
Vomicine (**3**)	5.84	5.77	98.8
Icajine (**4**)	4.02	3.96	98.6
Isostrychnine (**5**)	10.02	9.84	98.8
Isobrucine (**6**)	13.40	13.32	99.4
Strychnine *N*-oxide (**7**)	8.30	8.14	98.1
Brucine *N*-oxide (**8**)	9.98	10.10	101.2
Novacine (**9**)	2.96	3.03	102.9
Pseudostrychnine (**10**)	1.61	1.58	97.7
16-Hydroxy-α-colubrine (**11**)	2.50	2.47	98.8
β-Colubrine (**12**)	5.32	5.28	99.4
Loganin (**13**)	9.60	9.40	97.8

trol) but greatly changed by treating with vinegar (processing G ; 23 % of control) (Fig. 2). Other methods resulted in decrease of strychnine (**1**) but the contents were in a range of 50-60 % of control. On the other hand, the brucine (**2**) content was similar among the seeds processed by the methods of processings B, C, E, F and H (approximately 60 % of control), except processings D and G. In processing D, the brucine (**2**) content in the seeds was changed a little (84 % of control), while the strychnine (**1**) content was moderate. In processing G, the contents of **1** and **2** were the lowest among the seven processed seeds. In accordance with the previous observation,[4] the contents of isostrychnine (**5**), isobrucine (**6**), strychnine *N*-oxide (**7**) and brucine *N*-oxide (**8**) in the seeds were increased by processings B and F, compared to those of the raw seeds. This is due to that **1** and **2** were subjected to thermal cleavage and oxidation reactions to give **5**-**8**.[4]

Loganin (**13**) also decreased in content depending

Table III Coefficients of variation (C.V.) in precision tests for determination of strychnos alkaloids and loganin.

Compound	C.V. %
Strychnine (**1**)	2.18
Brucine (**2**)	2.67
Vomicine (**3**)	2.31
Icajine (**4**)	3.53
Isostrychnine (**5**)	2.42
Isobrucine (**6**)	2.64
Strychnine *N*-oxide (**7**)	2.52
Brucine *N*-oxide (**8**)	3.73
Novacine (**9**)	2.67
Pseudostrychnine (**10**)	4.58
16-Hydroxy-α-colubrine (**11**)	3.33
β-Colubrine (**12**)	2.38
Loganin (**13**)	0.08

Coefficient of variation (c.v.) given, in parentheses, is expressed by the following equation :

$$\text{C.V. } (\%) = \frac{\text{standard deviation (S.D.)}}{\text{mean value}} \times 100$$

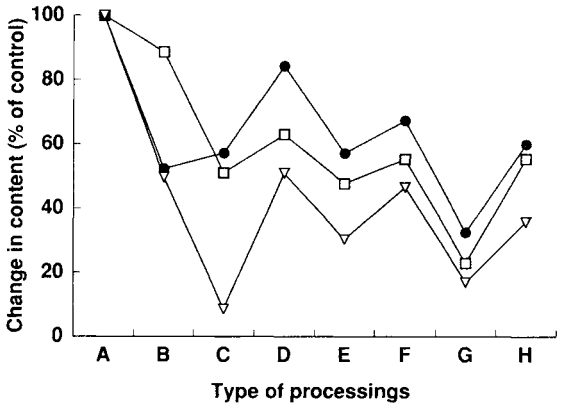


Fig. 2 Changes in major alkaloids (**1** and **2**) and loganin (**13**) contents after processing of the seeds of *S. nux-vomica*. The changes in content of the respective compounds are indicated by percentages of control (the unprocessed seeds). (□), strychine (**1**) ; (●), brucine (**2**) ; (▽), loganin (**13**).

Table IV Alkaloid and loganin contents in the processed and unprocessed seeds of *S. nux-vomica*.

	Alkaloid and loganin contents (%)							
Compound	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Strychnine (**1**)	1.905	1.688	0.972	1.200	0.908	1.053	0.436	1.053
Brucine (**2**)	1.009	0.528	0.577	0.850	0.576	0.679	0.327	0.604
Vomicine (**3**)	0.009	0.002	0.004	0.002	faint	0.002	0.005	0.005
Icajine (**4**)	0.002	faint	faint	faint	faint	faint	faint	faint
Isostrychnine (**5**)	0.005	0.010	0.007	0.011	0.001	0.005	0.001	0.005
Isobrucine (**6**)	faint	0.012	0.005	0.004	faint	0.002	faint	0.004
Strychnine *N*-oxide (**7**)	0.010	0.035	0.008	0.010	0.004	0.037	0.006	0.007
Brucine *N*-oxide (**8**)	0.009	0.039	0.008	0.007	0.001	0.045	0.004	0.005
Novacine (**9**)	0.218	0.067	0.185	0.058	0.080	0.092	0.128	0.063
Pseudostrychnine (**10**)	0.214	0.177	0.190	0.121	0.105	0.126	0.174	0.097
16-Hydroxy-α-colubrine (**11**)	0.235	0.101	0.143	0.083	0.122	0.146	0.136	0.057
β-Colubrine (**12**)	0.004	0.002	0.002	0.002	0.002	0.030	0.002	0.002
Loganin (**13**)	1.560	0.775	0.134	0.794	0.472	0.726	0.264	0.556

The content of each component in the processed and unprocessed seeds is indicated as a percentage (wt %). Each value is the mean of three determinations.

on the processings. By treatment with a licorice decoction followed by roasting (processing C), the loganin content in the seeds became *ca.* 9 % of control (Fig. 2).

Fig. 3 shows the sum of alkaloids **1** - **12** (total alkaloid content) in the processed and unprocessed samples. After treatment of the seeds by processings B-H, the total alkaloid contents decreased to 34-74 % of control. The highest total alkaloid content was observed in the seeds treated with processing B, while the lowest one was in the seeds treated with processing G. The decreases in total alkaloid content may be due to the elimination of a part of the alkaloids by dissolving in an acidic medium (processings G and H) and binding with any substances present in the licorice decoction (processing C) and urine (processing E).

As regards the chemical composition, alkaloids

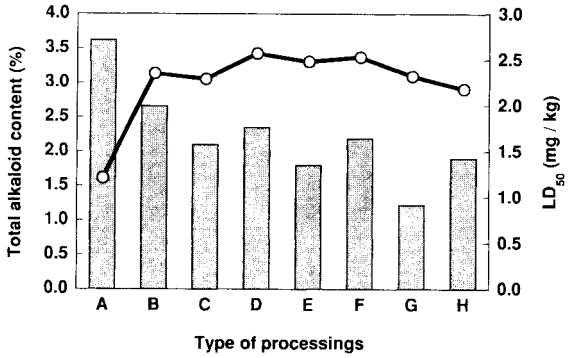


Fig. 3 Total alkaloid contents and LD_{50} of the processed and unprocessed seeds of *S. nux-vomica*. The bar graph represents total alkaloid contents (wt %) in the processed and unprocessed seeds and small circles in the line graph show LD_{50} values of the crude alkaloid fractions obtained from the respective seeds, in mice.

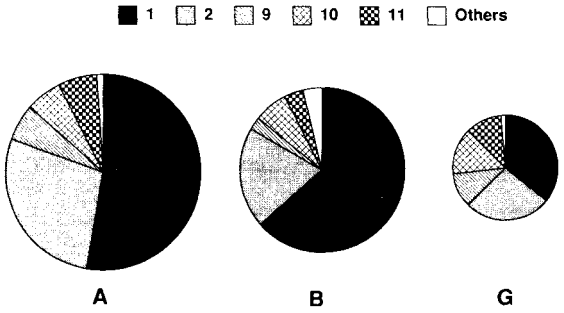


Fig. 4 Compositions of strychnos alkaloids in the processed and unprocessed seeds of *S. nux-vomica*. A, B, and G indicate the composition of alkaloids in the unprocessed seeds and the two processed seeds which were treated with processings B and G. **1**, strychnine ; **2**, brucine ; **9**, novacine ; **10**, pseudostrychnine ; **11**, 16-hydroxy-α-colubrine.

from the unprocessed seeds consisted of 53 % of strychnine (**1**), 28 % of brucine (**2**) and others (Fig. 4). After processings C, D, E, F and H, the major alkaloid composition was not significantly changed, when compared to those of the unprocessed seeds. On the contrary, the composition was remarkably changed by processings B and G. Strychnine (**1**) and brucine (**2**) were 63 % and 20 % in the former, but 36 % and 27 % in the latter.

Effects of processings of nux vomica on acute toxicity

Table V Yields of crude alkaloid fractions from the processed and unprocessed seeds of *Strychnos nux-vomica*.

Sample	Powder used (g)	Yield (g)
A	200.00	4.82
B	200.00	4.44
C	200.00	2.30
D	200.00	4.08
E	200.00	1.99
F	200.00	4.22
G	200.00	2.23
H	200.00	1.68

A, unprocessed ; **B**, parching in a sand bath ; **C**, processed with a licorice solution ; **D**, processed with urine and a sand bath ; **E**, processed with urine ; **F**, frying in an oil bath ; **G**, processed with vinegar ; **H**, processed with vinegar and a sand bath.

Table VI LD_{50} of crude alkaloid fractions obtained from the processed and unprocessed seeds of *S. nux-vomica*.

Sample	Ratio of dosages in different groups (r)	LD_{50} (95 % confidence limit) (μg/kg)
A	0.70	1.21 (1.17−1.24)
B	0.75	2.35 (2.20−2.50)
C	0.80	2.29 (2.02−2.83)
D	0.75	2.57 (2.41−2.73)
E	0.75	2.48 (2.12−2.64)
F	0.80	2.53 (2.08−3.08)
G	0.70	2.32 (1.93−2.72)
H	0.80	2.18 (1.91−2.49)

A, unprocessed ; **B**, parching in a sand bath ; **C** processed with a licorice solution ; **D**, processed with urine and a sand bath ; **E**, processed with urine ; **F**, frying in an oil bath ; **G**, processed with vinegar ; **H**, processed with vinegar and a sand bath.

Table VI shows a comparison of the acute toxicity of the processed and unprocessed seeds of *S. nux-vomica* in mice. The unprocessed seeds were quite toxic with LD_{50} of 1.21 mg/kg of mouse, while the processed ones were less toxic with LD_{50} of 2.18-2.57 mg/kg, approximately two-fold higher than the value of the unprocessed seeds. Although the total alkaloid contents in the processed seeds appreciably varied from processing to processing, a little difference in LD_{50} value was observed among them as shown in Fig. 2 (the respective LD_{50} values are represented as small circles). No linear correlations were observed between total alkaloid contents in the seeds and LD_{50} values

and between the individual alkaloid contents and LD_{50} values (data not shown). This indicates that the toxicity of the seeds is not simply represented by the contents of strychnine (**1**) and/or brucine (**2**), or of total strychnos alkaloids **1**-**12**.

Drug-processing is the process of treating a variety of natural products to meet the therapeutic, dispensing and pharmaceutical requirements before they are administered or made into various preparations. The purposes of drug-processing are (1) to eliminate or reduce the toxicity, drastic actions and side effects of some crude drugs, (2) to increase the efficacy of crude drugs, (3) to change the property and functions of crude drugs in terms of traditional medicine so as to meet the therapeutic demand, (4) to facilitate decoction, preparation and preservation and (5) to eliminate impurities, non-medicinal part and various bad tastes.

As regards the seeds of *S. nux-vomica*, the processing has been mainly carried out for reducing their toxicity. In the present experiments, all of the traditionally used processing methods lowered the toxic alkaloid content in varying degrees and reduced the toxicity of crude alkaloid fractions from the seeds to the similar extent in mice. However, some methods, such as processings D (treatment with urine followed by parching in a sand bath), E (treatment with urine), F (frying in oil), and H (treatment with vinegar followed by parching in a sand bath), were time-consuming and trouble-some. In processing G (treatment with vinegar), large amounts of the characteristic constituents tended to be lost. Therefore, processing B (parching in a sand bath) seems to be more convenient and adequate in view of reducing toxicity, but it may be desirable to evaluate the processing in view of the therapeutic efficacy in traditional Chinese medicine.

和文抄録

種々の馬銭子の修治法の可否を科学的に評価するため，修治前後の種子に含まれる化学成分や急性毒性を検討した。7種の伝統的修治法はともに，含まれる多くのアルカロイド及び総アルカロイド含有量を減少させた。しかも，その減少の度合いは修治法により異なっていた。さらに個々のアルカロイド組成も修治法により差異があった。修治した種子から得た粗アルカロイド画分のマウスに対する急性毒性は顕著に減少した。これらの画分のLD_{50}値は2.18-2.57 mg/kgの狭い範囲内にあり，修治しない種子の同画分のLD_{50}=1.25 mg/kgより，2倍程度大きな値を示した。しかし，種子中の総アルカロイド含量，あるいはそれぞれの主アルカロイドとLD_{50}値との間に明確な相関関係は認められなかった。このことは今回試みた全ての修治法は馬銭子中のアルカロイド含有量，さらに組成を種々の割合で変化させるが，総じてその毒性は同程度に減弱させることを示している。

修治法の簡便さの観点からは現在中国薬典に記載されている，馬銭子を砂と共に炒る方法が良いが，薬効との観点から他の修治法も検討してみることも必要である。

References

1) Jangsu college of new medicine, *"Dictionary of Chinese Materia Medica"*, Shanghai People's Publishing House, Shanghai, pp. 291-293, 1977.
2) Sha, D.: Studies on the relation of alkaloids in Semen Strychni and its processing. *Journal of Chinese Medicinal Materials* **14**, 24-26, 1989.
3) Yan, L.: Two traditional processing methods of Semen Strychni. *He Nan Journal of Chinese Materia Medica* **3**, 17-20, 1985.
4) Cai, B., Hattori, M. and Namba, T.: Processing of nux vomica. II. Changes in alkaloid composition of the seeds of *Strychnos nux-vomica* on traditional drug-processing. *Chem. Pharm. Bull.* **38**, 1295-1298, 1990.
5) El-Mekkawy, S., Meselhy, M. R., Kawata, Y., Kadota, S., Hattori, M. and Namba, T.: Metabolism of strychnine *N*-oxide and brucine *N*-oxide by human intestinal bacteria. *Planta Med.* **59**, 347-350, 1992.
6) Cai, B., Yang, X., Hattori, M. and Namba, T.: Processing of nux vomica. I. Four new alkaloids from the processed seeds of *Strychnos nux-vomica*. *Shoyakugaku Zasshi* **44**, 42-46, 1990.
7) Sun, R.: *"Quantity Method of Pharmacology"*, People's Health Publishing House, Beijing, pp. 50-58, 1987.
8) Cai, B., Wu, H., Hattori, M. and Namba, T.: Analysis of spectral data for 13C NMR of sixteen strychnos alkaloids. *Acta Pharm. Sinica* **29**, 44-48, 1994.