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# Different Traditional Methods of Nux-Vomica Detoxification Have Therapeutic Rationales

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**Abstract**

**Background:** *Strychnos nux-vomica* is a well-known poisonous plant, which is detoxified by different methods in traditional medicines. Most of traditional medical schools introduce general detoxification methods that can be substituted with each other. The main goal of these methods is to reduce the total alkaloids. In contrast, Iranian traditional medicine (ITM) has suggested three non-general, non-substitutable methods, which have specific medical indications: 1, boiling the seeds in water until all the water evaporates (prescribed for diarrhea treatment); 2, soaking the seeds in cow's milk for seven days, followed by peeling the seeds (for addiction treatment); 3, soaking the seeds in cow's milk for seven days, peeling the seeds, and boiling them in milk (for arthralgia and paralysis).
**Objectives:** The aim of this study was to clarify the therapeutic rationale for each method.
**Materials and Methods:** Nux-vomica seeds were divided into four groups: three groups were detoxified via ITM methods, and one group was kept intact as a control. All samples were powdered, and their alkaloids and total phenolic compounds were determined by HPLC and spectrophotometery, respectively.
**Results:** The first ITM method reduced the strychnine level from 1.083 to 0.577 and brucine from 0.739 to 0.361. For the second and third ITM methods, the levels of strychnine reached 0.838 and 0.812, and the levels of brucine reached 0.522 and 0.568, respectively.
**Conclusions:** Clinically, these results are logical because the required dose of alkaloids for treatment of diarrhea is lesser than the needed doses to treat addiction, arthralgia, and paralysis.

*Keywords:* Iranian Traditional Medicine, Detoxification, Strychnine, Brucine, *Strychnos Nux-Vomica*

## 1. Background

*Strychnos nux-vomica* (*Loganiaceae* family) is a deciduous tree that is native to India and southeast Asia (1). It is a well-known poisonous plant in Iranian traditional medicine (ITM) (2, 3). The dried seed is the primary part of the plant used for medicinal purposes, and it is commonly known as Kachula or Azaraghi (2, 3). Because of its toxicity, ITM has restricted the oral prescription of this herb to certain severe and resistant diseases and conditions such as Zarab (a type of chronic and persistent diarrhea), cold neural ailments, especially paralysis and debility (due to conditions such as cerebro-vascular attack or multiple sclerosis), progressed phlegmatic joint problems (such as osteoarthritis), and addictions treatment (2-5). Traditionally, a lethal dose of the seeds is considered to be 4.6 grams, and therapeutic doses range from 0.5 to 1 gram (2). Due to its use of highly toxic ingredients, ITM has developed certain detoxification processes in which we assume the level of alkaloids in the seeds decreases.

The seeds mainly consist of two indol alkaloids: strychnine and brucine (1: 1) (6). Recent studies have shown that most of the therapeutic as well as the poisoning effects of nux-vomica can be attributed to strychnine (6). Strychnine is a deadly poison, with a human lethal dose being about 50 mg (equivalent to 4 grams of the seeds) (1). Despite previous usage as a rodenticide and a respiratory, circulatory, and digestive stimulant, strychnine is no longer used in any pharmaceutical products; notably, it is still used as a rodenticide (7). In the most severe cases, a patient with strychnine poisoning dies before reaching the hospital (8).

There are different detoxification processes in Unani, Ayurvedic, and Chinese traditional medicines (Table 1). Recent investigations have suggested that nearly all of these traditional processes have significantly reduced the alkaloid levels (9-13). These methods are general and can be substituted for one another. Because the main goal of these detoxification methods is to reduce alkaloids, some investigators have tried to introduce new detoxification methods that have greater power but come

**Table 1.** Various Detoxification Methods of Nux-Vomica in Ayurvedic, Unani, Chinese, and Iranian Traditional Medicine

| Detoxification Factor | Method of Detoxification | Source |
|---|---|---|
| **Children's urine** | Submerging in urine from healthy children | Chinese traditional medicine |
| **Cow's ghee (clarified butter)** | Frying in cow's ghee (clarified butter) until brownish red | Ayurveda, Unani medicine |
| **Cow's milk** | Tying in a cotton cloth and placing it in a vessel of boiling cow's milk for three hours | Ayurveda |
| **Cow's milk** | Soaking the seeds for seven days in cow's milk and then peeling the seeds | Iranian traditional medicine, Unani medicine |
| **Cow's milk** | Soaking the seeds for seven days in cow's milk, peeling the seeds, and then boiling them in the milk | Iranian traditional medicine |
| **Cow's urine** | Soaking the seeds for seven days in cow's urine | Ayurveda |
| **Kanji (sour gruel)** | Dipping the seeds in Kanji | Ayurveda |
| **Licorice** | Treating via licorice decoction | Chinese traditional medicine |
| **Oil** | Frying in oil bath (about 240°C) for three minutes | Chinese traditional medicine |
| **Sand** | Parching in a sand bath (about 240°C) for three minutes | Ayurveda, Chinese traditional medicine |
| **Vinegar** | Treating with vinegar | Chinese traditional medicine |
| **Water** | Boiling the seeds in water until all of the water has evaporated | Iranian traditional medicine |
| **Yellow clay/cow's milk** | Burying the seeds in yellow clay for 10 days and then boiling them in cow's milk | Unani medicine |

at a lower cost. In these medical schools, by what method the levels of alkaloids are reduced is not of concern; the important issue is how much they are reduced (10, 12, 14).

Although ITM uses certain detoxification processes, these methods are not general and therefore cannot be substituted with one another. In ITM, the term Eslah, which literally means correction, is used for the detoxification of poisonous things including nux-vomica. Three Eslah methods have been presented in ITM texts for nux-vomica: I) boiling the seeds in water until all of the water evaporates; II) soaking the seeds for seven days in cow's milk and then peeling the seeds; III) soaking the seeds for seven days in cow's milk, peeling the seeds, and then boiling them in milk. Method I is only used for the preparation of anti-Zarab pills; method II is an anti-addiction formula; and method III is utilized for cold neural ailments and back and joint pain (4, 5).

## 2. Objectives

The aim of this study was to clarify the differences of these three detoxification methods on the basis of strychnine and brucine levels as well as total phenolic content. The results may illuminate the connection of each detoxification method to its corresponding diseases.

## 3. Materials and Methods

### 3.1. Plant Materials

Dried seeds of *Strychnos nux-vomica* were obtained from the Tehran botanical market and were identified by Hossein Hosseini, from the medicinal plants research center of Barij, Kashan, Iran. The seeds were divided into four groups. One group was kept as a control, and the other three were processed by traditional methods, as explained below:

I) The seeds were tied in a cotton cloth. The cloth was placed in a steel pot and water was poured over the seeds until they were covered. The pot was heated until all the water had evaporated.

II) The seeds were soaked in fresh cow's milk for seven days. The milk was changed every day. On the last day, the seeds were washed with water and peeled.

III) The seeds were soaked in fresh cow's milk for seven days. On day seven, the seeds were tied in a cotton cloth, boiled in fresh cow's milk, and then peeled.

### 3.2. Chemicals, Materials, and Solutions

The HPLC-grade methanol (MeOH), potassium dihydrogen phosphate, phosphoric acid, heptan-1-sulfonic acid sodium salt, acetonitrile (ACN), and water used in the experiment were from Merck. The gallic acid (GA), strychnine, and brucine used in the experiment were from Sigma-Aldrich. The chromatographic purity of the strychnine and brucine were simultaneously checked at several wavelengths by LC and UV spectroscopy. Stock standard solutions (500 $\mu g mL^{-1}$) of strychnine and brucine were prepared in methanol. These solutions were further diluted with methanol to furnish calibration

solutions at seven different concentrations in the range 0.1 - 200 μg/mL.

### 3.3. Sample Preparation

A powdered sample (0.25 g) was extracted with 20 mL of methanol, under reflux conditions, for 1 hour in a 50 mL round-bottomed flask on a magnetic stirrer. After cooling to room temperature, the solution was filtered into a 25 mL volumetric flask. The residue was washed with the same solvent, and the washings were added to the filtered extract. The combined filtrate was then adjusted to volume with methanol. This solution was used for chromatographic and spectrophotometric analyses.

### 3.4. Chromatography

LC was performed with Knauer platin blue (Well-Chrom, Germany) equipment, including a platin blue pump and PDA detector, with simultaneous detection at four wavelengths, and an injector with a 20 μL loop. Compounds were separated at ambient temperature on a Eurospher 100, C18 (25 × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of acetonitrile and a buffer (1:1 mixture of 0.01 M sodium 1-heptanesulfonate monohydrate and 0.02 M $KH_2PO_4$, pH = 2.8, adjusted by 10% $H_3PO_4$). A gradient solvent system was used with 10, 25, and 30% of acetonitrile for 5, 5, and 15 minutes, respectively. The column effluent was simultaneously monitored, resulting in use of 254 nm for detection. Spectral data of the analytes, in the range 220 - 380 nm, were collected by the UV detector throughout the run. Quantifications of strychnine and brucine were performed by the external standard method.

### 3.5. Total Phenolic Content

Total phenolic contents (TPC) in the crude and detoxified seeds of *Strychnos nux-vomica* were determined by a spectrophotometer (Perkin-Elmer Lambda EZ-210 US/VIS), with a dual-beam. First, 0.2 mL of the sample solution were transferred into a 5 mL volumetric flask and swirled with 2 mL of distilled water. Next, 0.25 mL of Folin's reagent were added and swirled. After two minutes, 0.5 mL of 20% (w/v) sodium carbonate solution were added and mixed. Distilled water was then added until the volume reached 5 mL exactly. Finally, the solution was mixed thoroughly and allowed to stand at ambient temperature for two hours. (until the characteristic blue color had developed). Absorbance of the reaction mixture was measured at 760 nm. Quantification of TPC was based on a standard curve, which was generated with GA at 760 nm using the following Equation:

$$\mathrm{Abs} = 0.0042C + 0.0326$$

Where Abs is absorbance and C is the concentration (μg/mL) of GA. All tests were conducted in triplicate and averaged. The results were expressed as the percentage of TPC in the sample and as gallic acid equivalents (GAE).

## 4. Results

The results showed that ITM detoxification methods reduced both strychnine and brucine of the seeds (Table 2). Among the methods, boiling the seeds in water (method I) reduced strychnine and brucine to half their original amount; therefore, this method reduced the toxicity more efficiently. Based on the fact that ITM utilizes method I for preparation of the anti-Zarab drug, it seems that lower doses of alkaloids suffice to defeat diarrhea. The results also confirmed that soaking the seeds in cow's milk (method II) and soaking them and boiling them in cow's milk (method III) work similarly. Both methods II and III reduced the level of alkaloids to a lesser degree than method I. These methods prepare nux-vomica for defeating addiction, cold neural ailments, and back and joint pain. Hence, based on ITM notifications, higher amounts of strychnine and brucine are needed for treatment of these ailments.

Total phenolic assay showed that, in comparison with a crude drug, methods I and III have almost no effect on phenolic content. Surprisingly, method II elevated the level of total phenolic compounds (Table 2).

**Table 2.** Percentage of Strychnine, Brucine, Total Phenolic, Total Ashes, and Acid Insoluble Ashes in Differently Processed Samples of Nux-Vomica Seeds

| Samples | Strychnine, %w/w | Brucine, %w/w | Total Phenolic, %w/w GAE | Total A %w/w | Acid Insoluble ashes, %w/w |
|---|---|---|---|---|---|
| **Crude drug** | 1.083 | 0.739 | 0.48 | 0.91 | 0.25 |
| **Detoxified I**[a] | 0.577 | 0.361 | 0.40 | 0.91 | 0.25 |
| **Detoxified II**[b] | 0.838 | 0.522 | 0.59 | 0.97 | 0.2 |
| **Detoxified III**[c] | 0.812 | 0.568 | 0.39 | 1.03 | 0.14 |

[a]Detoxified I, Nux-vomica seeds whichthat have beenwere detoxified via boiling them in water (method I of detoxification).
[b]Detoxified II, Nux-vomica seeds whichthat have beenwere detoxified via soaking them in cow's milk for seven days (method II of detoxification).
[c]Detoxified III, Nux-vomica seeds whichthat have beenwere detoxified via soaking and boiling them in cow's milk (method III of detoxification).

## 5. Discussion

Iranian traditional medicine (ITM) has been established through clinical trial and error. During a span of more than 1000 years, Iranian physicians have examined different methods for Eslah (detoxification) of nux-vomica. From those examinations, they have specified three different detoxification methods for three different ailments. In this study, we attempted to find the probable reasons for each method.

ITM utilizes method I for preparation of the anti-Zarab drug. Because the treatment of Zarab (diarrhea) requires lower doses of alkaloids, ITM utilizes the most powerful detoxification method (i.e. method I) to reduce the toxicity and prepare a safe drug therapy. Recent investigations have vindicated this powerful detoxification method of nux-vomica. These studies indicate that *Strychnos nux-vomica* extract is very effective in the treatment of diarrhea; however, it is toxic to rats (15). Detoxification can place this herb on the first lines of diarrhea treatment.

ITM detoxifies nux-vomica via method II for addiction treatment. According to our results, this method provides higher levels of alkaloids. ITM has utilized this type of detoxified nux-vomica on people attempting to withdraw from opium. These people are faced with two kinds of dependence: psychological and physical. Psychological dependence is one of the main causes of failure in the treatment of addiction because it encourages the user to reuse a particular substance with enjoyable mental affects (16). Nux-vomica, being a neuro-stimulant and nootropic substance, can act as a substitute (17, 18). According to ITM, this substitution occurs through a gradual reduction in opium simultaneous to gradual increases in nux-vomica doses (19).

Neuro-stimulant activity of nux-vomica is related to alkaloids, especially strychnine. Pharmacological investigations have shown that strychnine acts through the blockade of glycine and acetylcholine receptors (17, 18). Although lower doses act as an energizer, doping in sporting competitions present a case in point; higher doses work as a neurotoxin, which leads to persistent muscle spasm and death (20). Thus, reducing the dose of alkaloids in the herb, such as what happens in ITM, plays an important role in observing the therapeutic effects.

Method III detoxifies nux-vomica for cold neural ailments and joint pain. This reduces the level of strychnine and brucine to the same degree as method II. Nux-vomica likely acts through its neuro-stimulant and nootropic activities to defeat neural ailments. Regarding back and joint pain, recent investigations have uncovered the anti-inflammatory and anti-nociceptive activities of nux-vomica (21-23). Cai et al. (1996) revealed that crude alkaloid fractions of nux-vomica have anti-nociceptive effects, and detoxification processes increase this biological activity (21). Moreover, Yin et al. (2003) have suggested that brucine and brucine N-oxide have pain modulation and anti-inflammatory effects through both the central and peripheral mechanisms (22). Within the same line of thought, Mitra et al. (2011) showed, via animal studies, that the detoxification process does not reduce the anti-inflammatory effects (23). These study results were in accordance with the need to detoxify nux-vomica and the effectiveness of this herb for arthralgia and inflammation.

In spite of the similarity in alkaloid levels, seeds that were detoxified via method III, in comparison with that of method II, contained fewer phenolic compounds. Regarding the role of inflammation in joint problems and some neural ailments as well as the anti-inflammatory effects of the phenolic compound (24, 25), using method II instead of method III may be more preferable for these kinds of ailments. Moreover, method III requires one additional step. Thus, based on our results, we suggest substitution of method II with method III when formulating nux-vomica for joints and neurological ailments.

Overall, detoxification via method I is simple and can be done at a low cost. The main material used in method I is boiling water. Therefore, utilizing method I in industrial processes to produce anti-diarrheal drugs from nux-vomica seems to be executable and cost-effective. Conversely, methods II and III are time-consuming and expensive due to multiple steps being involved and the use of cow's milk in both procedures. Therefore, these methods should be optimized if intended for industrial use.

### 5.1. Conclusions

The main goal of nux-vomica detoxification in Indian and Chinese traditional medicine is to reduce the level of alkaloids without consideration for therapeutic indications. Iranian traditional medicine has, vice versa, specified its detoxification methods for special ailments. In this article, we aimed to clarify the rationale of each ITM detoxification method. We found that the levels of alkaloids in two of the three methods were different from each other, and this difference had a therapeutic rationale. Thus, substituting these methods with each other should be avoided or, if it is inevitable, should be done with caution.

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## Footnotes

**Authors' Contribution:**Study concept and design: Mohammad Mahdi Ahmadian-Attari; acquisition of data: Leila Mohammad Taghizadeh Kashani, Alireza Hatami and Alireza Safaei; analysis and interpretation of data: Mohammad Mahdi Ahmadian-Attari and Meysam Shirzad; drafting of the manuscript: Mohammad Mahdi

Ahmadian-Attari and Meysam Shirzad; critical revision of the manuscript: Mohammad Mahdi Ahmadian-Attari and Meysam Shirzad; study supervision: Mohammad Mahdi Ahmadian-Attari.

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