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USE OF *STRYCHNOS NUX-VOMICA* (AZRAQI) SEEDS IN UNANI SYSTEM OF MEDICINE: ROLE OF DETOXIFICATION

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Abstract

Some plants used in Unani system of medicine are toxic, even deadly poisonous. The drugs having such plants as their components are detoxified before they are dispensed to the patients. One such drug, capsule Hudar, has *Strychnos nux-vomica L.* (Azraqi) seeds as one of its components and is very effectively used to elevate blood pressure. Ancient manuscripts describe many methods of its detoxification. It has been found that the detoxification processes studied reduce the strychnine content, as determined either by using uv-vis spectrophotometer or HPLC, present in *Strychnos nux* vomica seeds which is responsible for *Strychnos nux vomica* toxicity. The decrease in strychnine amount was best when the seeds were immersed for detoxification in excess of water for 5 days, in milk for 2 days followed by their boiling in milk. Strychnine in small amounts has been reported to give subjective feeling of stimulation

Key words: Strychnos nux vomica; detoxification; Unani medicine

Introduction

An estimated 7,500 species of plants are used in the traditional medicines of India. The vast majority of these plants are lesser known local remedies, although many of these are poisonous in their natural states. Traditional healers using these toxic remedies have devised methods of "detoxification" (Shahnavaskhan, 1997)

Some drugs of the plant, animal and mineral origin used in Unani system of medicine have components that are naturally toxic in their properties. Such drugs, are therefore, detoxified in order to reduce/ overcome their toxicity. In Oriental medicine, *Strychnos nux-vomica L.* (Loganiaceae) seeds have been used in activating the channels, alleviating pain and reducing swelling (Bensky and Gamble, 1986). The pharmacological effects of this plant have also been known to increase spinal reflexes and stimulate respiratory and sensory centers of the cerebral cortex (Chung and Shin, 1989). *Strychnos nox-vomica* seeds contain a mixture of 13 alkaloids (Yang and Yan, 1993) but the main alkaloids are strychnine and brucine (Han, 1988). Larger doses of strychnine are known to be deadly poisonous with oral LD_{50} for humans between 0.7-2.1 mg/Kg (Jackson and Marsh, 1997). But in lower doses, it gives subjective feeling of stimulation (Samulesson, 1992). In Unani system of medicine, the *Strychnos nux-vomica* seeds (Azraqi) in combination with dried ginger powder have been used very effectively to elevate blood pressure but after their detoxification in the form of capsule Hudar. Different detoxification methods have been used to reduce the toxicity of Azraqi seeds.

Since the main constituents of Azraqi seeds responsible for the toxicity are alkaloid more especially strychnine, therefore, the effect of detoxification processes as mentioned in classical Unani texts on the contents of total alkaloids in general and strychnine in particular has been studied.

Material and methods

Azraqi seeds (voucher No. CRI/HYD-07-08) were provided by Central Research Institute of Unani Medicine, Hyderabad, Pharmacy (GMP Certified), India. Standard markers of Strychnine were procured from M/S Fluka, India. Solvents of HPLC grade were used throughout.

Methods of detoxification as given in classical Unani texts (Bakhsh , 1924)

I. Detoxification using water and cow milk.

Azaraqi seeds are immersed in excess of water for 5 days with change of water every day. They are then immersed in milk for 2 days, changing the milk everyday. The seeds are washed with water and boiled in milk till the seed coat become soft. The seed coat and the embryonic axis are removed and cotyledons powdered.

II. Detoxification using cow milk.

Azraqi Seeds are immersed in milk for 7 days with change of milk every day. Remaining process is the same as in No I above.

II. Detoxification using clarified butter.

Azraqi Seeds are roasted in Roughan-a- Zard (clarified butter from cow milk) till the colour of seed coat turns light reddish and seeds swell. The seed coat is removed and cotyledons powdered.

IV. Detoxification using yellow clay and Milk (Anonymous, 1981)

Azraqi seeds (70 grams) are buried in Peeli Matti (yellow clay) for 10 days. The clay is kept moist throughout. The seeds are then removed and washed. The seed coat is peeled off with the help of a sharp knife and the cotyledons are separated after removing the embryo. The healthy cotyledons are then washed with hot water. The cotyledons in a cloth bag are boiled in 2 litres of milk till it evaporates (the cloth bag should not touch the bottom of the container). The water washed cotyledons are then used subsequently.

Method of estimation of total alkaloids (Anonymous 1987)

Accurately weighed (about 10-20 gm) drug is extracted repeatedly with 0.5N H_2SO_4 till complete extraction of alkaloids is achieved (as tested using Dragendroff's reagent). The acid extract is made alkaline by adding excess of dilute NH_4OH solution. The alkaloids are extracted completely by shaking the alkaline solution with a mixture of chloroform and ether (2:1) in a separating funnel, The chloroform /ether extracts are extracted with 20, 15, 10, 10 and 10 ml of 0.5 N H_2SO_4. The combined acid extracts are filtered into a separating funnel and made alkaline with dilute ammonia solution. The alkaloids in the solution are extracted with successive portions of 20, 15, 10 and 05 ml of chloroform, to ensure their complete extraction. The combined chloroform extract is washed with two 10-ml portions of water. The washings are extracted again with two 10 ml portions of chloroform. The chloroform washing is added to the main chloroform extract and filtered in a tarred 100ml conical flask. The chloroform is distilled off the chloroform on a water bath till a few ml are left. The solvent is removed completely in a vacuum desiccator. 5 ml of alcohol (90%) is added to the residue and the solvent removed. The step is repeated once again. The residue is dried under vacuum till a constant weight is achieved and weighed as total alkaloids. The residue obtained is a mixture of strychnine and brucine and other alkaloids in minor amounts

Separation of strychnine from brucine

The process for separating strychnine from brucine is based on exploiting the property of brucine that it is nitrated with HNO_3 more easily and quickly as compared to strychnine. A fixed amount of alkaloid mixture is dissolved in 10 ml of dilute H_2S0_4, filtered and washed with 50 ml of water. Filtrate is taken into a flask and 5-ml of concentrated HNO_3 is added to it. The addition of the HNO_3 causes the solution to attain a brilliant crimson color. After 15 mins the liquid is transferred to a separating funnel and at once rendered alkaline with NaOH solution. The strychnine is extracted with three portions of chloroform. After distilling off the chloroform a little ethyl alcohol is added and the mixture evaporated to dryness.

Determination of Strychnine
Spectrophotometeric method (Keye and Hoff, 1952)

Efforts have been made to find out a quick and accurate method of strychnine estimation. After an extensive literature survey, a Spectrophotometeric method of estimation of strychnine in biological samples has been chosen which was modified as per our requirements and standardized in different Azaraqi seed samples

a) Determination of absorption spectra.

It is fairly well established that many of the alkaloids have characteristic absorption bands in the ultraviolet region of the spectrum. Absorption spectra recorded in the UV visible spectrophotometer using strychnine standard is used to find out λ max (Figure1)

b) A fixed amount of extracted strychnine is dissolved in water (made up to 5ml) and then made slightly alkaline with one drop of concentrated ammonia. 25 ml of chloroform is taken in a separating funnel. The alkaline strychnine solution is added to it, gently shaken for about 5 minutes and extracted. The Chloroform layer is allowed to settle & then separated. The chloroform extract is filtered through filter paper to remove excess water droplets and 20 ml of filtrate is collected and then extracted with 20ml of 0.5N sulfuric acid. The acidic aqueous layer is allowed to stand and finally separated into centrifuge tube. Excess chloroform drops are removed by centrifugation. The extract is read at 255nm in a spectrophotometer using 0.5 N sulfuric acid as blank. Standard graph of Strychnine was prepared using standards strychnine dissolved in 0.5 N sulfuric acid solution at 255 nm (λmax). There was a linear relationship between the amount of the strychnine and the corresponding optical density (Figure 2).

HPLC Method (Choi et al., 2004)

The strychnine in different samples of Azaraqi was also estimated using HPLC. Using this technique it was possible to detect the amount of strychnine as low as 1ng. Pulverized plant material (100mg) is extracted with methanol (50ml) at 40^0C for 30min in an ultrasonic apparatus and filtered. The methanolic extract is evaporated to dryness. The residue is dissolved in water (10ml) and washed thrice with chloroform (10ml each). The aqueous phase is adjusted to a pH of 9.5 using NH_4OH (28%) and

extracted thrice with 10ml methanol. The final solution is filtered using a 0.45 µm nylon membrane filter and diluted. Five microlitres of sample solution is injected through the automatic sample injector. Separation is performed on a RP-18 column (150mm×2.1mm, particle size µm) at 40^{o}C. The flow rate of mobile phase i.e. acetonitrile is 0.2ml/min. The quality of strychnine is calculated directly by comparing the peak area with that of the standard marker.

Results and Discussion

It is well documented that most of the alkaloids have their characteristic absorption maxima in the ultra violet region of the spectrum; strychnine also absorbs maximally at 255 nm (Figure 1) and shows a linear relationship between the amount of strychnine and the corresponding absorption at the wave length (Figure 2).

Tables 1-3 contain the results of total alkaloids and strychnine content in Azraqi seeds, raw, detoxified and Capsule Hudar. The results indicate that there was a general reduction in total alkaloid and strychnine content in all the detoxified seeds, though, in varied amounts irrespective of the method of detoxification used. Capsule Hudar also contained strychnine that was lesser in amount as compared to raw Azraqi seeds and ginger powder taken together. The results on the measurement of strychnine were similar in pattern when obtained either using HPLC or UV absorption measurements though the HPLC method was more powerful.

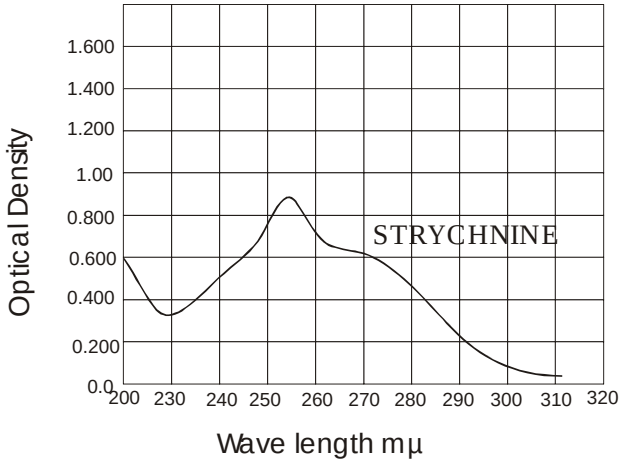

Figure 1: Absorption spectrum of strychnine

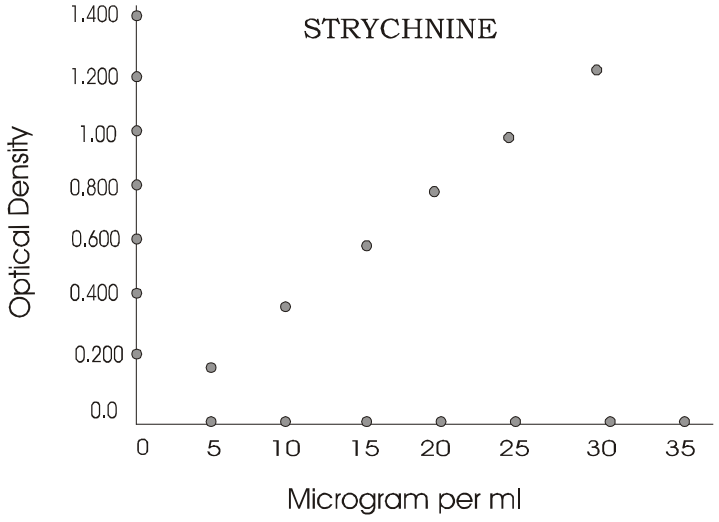

Figure 2: Standard graph of strychnine

Table 1: Total alkaloid contents in Capsule Hudar and its constituents.

Sample	Total alkaloids (%)
1. Sample I (crude Azraqi seeds)	3.32
2. Dried Ginger	0.24
3. Capsule Hudar	
(Detoxified Azraqi seeds+Ginger powder 1:1 W/W)	1.74
(yellow clay and milk)	
4. Sample II (detoxification using water & milk	1.08
5. Sample III (using milk)	0.93
6. Sample IV (using clarified butter)	2.79
7. Sample V (Yellow clay & milk)	1.24

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Table 2: Strychnine contents in Capsule Hudar and Azraqi as determined using UV-Visible spectrophotometer

Sample	Strychnine (%)
1. Sample I (crude Azraqi seeds)	1.21
2. Capsule Hudar	
(Detoxified Azraqi seeds+Ginger powder 1:1 W/W)	0.79
(yellow clay and milk)	
3. Sample II(using water & milk)	0.52
4. Sample III (using milk)	0.47
5. Sample IV (using clarified butter)	1.06
6. Sample V (using yellow clay and milk)	0.54

Table 3: Contents of strychnine in capsule Hudar and Azaraqi as estimated by HPLC

Sample	**strychnine (%)**
1. Sample I (crude Azraqi seeds)	1.37
2. Capsule Hudar	
(Detoxified Azraqi seeds+Ginger powder 1:1 W/W)	0.82
(yellow clay and milk)	
3. Sample II(using water & milk)	0.58
4. Sample III (using milk)	0.51
5. Sample IV (using clarified butter)	1.13
6. Sample V (using yellow clay and milk)	0.58

Detoxification of Azraqi seeds employing different methods as mentioned in National Unani Formulary and Mistahul Khazain resulted in decrease in the strychnine content. Reduction in strychnine content was highest when the seeds were detoxified with cow milk and water and lowest when detoxification was done by their roasting in clarified butter.

The cell wall of *strychnine nux vomica* seeds is non- lignified but contains appreciable amount of complex carbohydrates. Therefore, on coming in contact with water, it swells and facilitates leaching strychnine out of the seed cotyledons. This results in a decrease in strychnine content in Azaraqi seeds when they are immersed in excess of water for 5 days and in milk for 2 days (unpublished results). Boiling the seeds in milk further reduces strychnine content by converting it to isostrychnine (Cai et al., 1990). Though larger doses of strychnine are known to be lethal (Jackson and Marsh, 1997), but in lower doses it gives subjective feeling of stimulation (Samulesson, 1992).

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