IMPACT OF SHODhana (PURIFICATORY PROCEDURES) ON KUPEelu (STRYCHNOs NUX-VOMICA LINN.) SEEDS: A PHARMACEUTICo-ANALYTICAL STUDY

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Abstract: Kupeelu (Strychnos nux-vomica Linn.), a drug mentioned under Upavisha (semi-poisonous) group of Ayurvedic pharmacopoeia, is being practiced widely in Ayurvedic therapeutics since long. Certain compound formulations containing Kupeelu are also well practiced in Homeopathy and Unani System of Medicine. Ayurveda strictly recommend the use of this drug in therapeutics only after proper Shodhana (purificatory procedure) through some specific media like Gomutra, Godugdha, Goghrita, Kanji etc. Though various Shodhana procedures are recommended in Ayurvedic classics for purification of Kupeelu seeds, but updated scientific researches regarding the Shodhana methods are lacking. Keeping this fact in mind, an attempt has been made in the present study to evaluate the impact of Shodhana on Kupeelu seeds while performing the specific Shodhana method, recommended by the Ayurvedic Formulary of India (A.F.I.). This study reveals that the toxic alkaloids Strychnine & Brucine, present in Kupeelu seed, were reduced by 71.49% and 54.02% respectively, in comparison to the raw seed, as determined by H.P.T.L.C. study.

Keywords: Kupeelu, Strychnos nux-vomica, Shodhana, Ayurvedic Formulary of India (A.F.I), Strychnine, Brucine.

Introduction

The uses of poison have been recorded in ancient Ayurvedic classic since long ago. Acharya Charaka has elaborately mentioned various poisoning, symptoms and their management (Charak). Acharya Sushruta has classified categorically the various sources of poison and described them accordingly (Sushruta). The poisonous plants reported in ancient scriptures of Ayurveda are being still practiced widely to combat number of diseases after passing through specific Shodhana (purificatory procedure). The concept of Shodhana was reported in the Charaka Samhita for first time, in the context of Danti Dravanti Kalpadhyaya. Here, to reduce the ‘Vikasi’ property of Danti (Baliospermum montanum) root, Charaka advocated a specific Samaskara by Agni (Charak). Acharya Vagbhata also advocated the Shodhana of Bhallataka fruit (Semicarpus anacardium Linn.) in detail in the context of Bhallataka Rasayana and Amrita Bhallataka (Vagbhata).

Kupeelu (Nux-vomica) has been described as a lethal poison and a cure for demonic possession in the KITAB AL-SUMMAM, an Arabian book of poison, which dates back to the 9th Century (Crozier A, Ashiara H).

Description of Kupeelu could not be traced in the ‘Brihat Trayee’ texts of Ayurveda (Shastry JLN). But this plant was described in different lexicons of Ayurveda by the name of Visatinduka, Kupeelu, Visamusti etc., which indicate toxic nature of this tree. Though the plant is described under the ‘Upavisa Vargas’ (semi-poisonous group) 13, its seeds have been used successfully in different Ayurvedic formulations after proper Samaskara known as Shodhana (Rasa Tarangini, Gogte VM). 16 alkaloids have been separated and identified from

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the raw nux-vomica seed and 80% of them are strychnine and brucine, and their derivatives such as isostrychnine and brucine N-oxide (Cai et al., 1990). The seeds also contain chlorogenic acid, a glycoside (loganin), and about 3% of fixed oil. Besides other minor alkaloidal constituents, the major chemical constituents of the seeds i.e. Strychnine (C21H22O2N2; m.p.286 to 288 °C) and brucine (C23H26O4N2; m.p.178 °C) have been reported for their adverse effects (Nadkarni KM). These alkaloids are found not only in the seed but also in the roots, bark, leaves, fruit - pulp, and the hard fruit-shells (Anonymous, 1998). A study reported that twenty-two identified alkaloids were isolated from the root bark and leaves of S. nux vomica (Baser Kemal HC et al., 1982).

The Shodhita (processed) Kupeelu seeds are mainly used as aphrodisiac, appetizer, anti-periodic, digestive, purgative, and stimulant. Further the seeds are also used in anemia, asthma, bronchitis, intermittent & malarial fever and in weakness of extremities (Sabnis M). Shodhita Kupeelu is also claimed to be a potent drug in countering old age problems and specially recommended during senility as Rasayana (antioxidant) (Pandey G). The plant is also found to have analgesic & anti-inflammatory (Yin W et al., 2003), anti-oxidant (Tripathi YB and Chaurasia S, 1996), anti-tumor (Deng XK et al., 2006), anti-snake venom (Chatterjee I et al., 2004), anti-diarrheal (Shoba F et al., 2001), and hepato-protective (Gopalkrishna SV et al., 2010), activities when studied in animal models.

Searching through various research journals, text books of Ayurveda and different search engines reveals that very few works have been reported on the Shodhana aspect of Kupeelu (Mitra S et al., 2012). A few folklore purificatory methods are followed traditionally in some parts of India but these methods are not accepted as the official methods (Mitra S et al., 2011). Though a specific Shodhana method of Kupeelu seed has been recommended by the Ayurvedic Formulary of India but the scientific research work regarding the impact of this Shodhana process is lacking till today. Hence, the present study has been planned to evaluate the impact of Shodhana on Kupeelu seeds by quantifying the toxic alkaloids through HPTLC technique.

Material and Methods

Present study was carried out by adopting the purificatory procedure recommended by A.F.I (Ayurvedic Formulary of India) where the three principles of Shodhana i.e., Nimajjana (Dipping), Swedana (Boiling) and Bharajana (Frying) were followed consecutively.

Collection of drugs

Fully matured Kupeelu (Strychnos nuxvomica Linn.) fruits were collected from Bankura district, West Bengal, India during the month of December and were botanically authenticated by pharmacognosists and sample specimen were kept in the Institute’s museum for future reference (voucher no. 8009). Seeds were taken out from the fruit pulp, thoroughly washed in tap water, shade dried and then kept in air tight glass container for future study.

Collection of media

Cow urine (Gomutra) and cow milk (Godugdha) were collected from the local cowshed daily in the morning at 6 A.M. and cow ghee (Goghrita) was procured from the local market (Brand name: Gowardhan; Mfd. By Parag Milk Foods Pvt.Ltd., Pune.) for Shodhana (proper processing/purification) of the seeds.

Equipment for Shodhana

Stainless steel vessel (20 cm × 30 cm); capacity of 7 L (used as Dolayantra), stainless steel rod (28 cm.), stainless steel vessel (48 cm × 30 cm × 7 cm); capacity of 3 L, cotton threads

30 cm in length, measuring mug (capacity of 1 L), muslin cloth (45 cm × 45 cm), digital weighing machine, pyrometer, digital induction cooker, stainless steel knife (blade: 15 cm × 2
cm), frying pan (diameter: 20cm), stainless steel spatula (length: 30 cm), and measuring cylinder (10 ml, 25ml).

**Procedure**

100 g clean and dried raw Kupeelu seeds (KR) were taken in a stainless steel tray. One liter of cow urine was added to it and kept for 7 days. Every day at 7 A.M. the cow urine (1 lit.) was replaced by fresh one. On the eighth day, the seeds were taken out from Gomutra, washed with lukewarm water. The seeds were kept in a muslin cloth and were made into a Pottali. The Pottali was hanged in a stainless steel vessel and cow milk was filled in the vessel up to the complete immersion of the Pottali. It was then boiled on an induction cooker for three hours at 100°C throughout the experiment. After boiling for three hours, the seeds were taken out from Pottali and washed with lukewarm water. The seed coat and embryo were removed by a knife; the cotyledons were fried with cow ghee in mild temperature (temperature was set at 60°C) on an induction heater until they became reddish yellow in color. Instantly the fried cotyledons were made into powdered form, dried under the shade, finally kept in an airtight glass container as ‘KGMDG powder’ for further use. The same procedures were repeated for three times to standardize the Shodhana process.

**Equipment for HPTLC**

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V sample applicator was used for application of samples. CAMAG TLC Scanner 3, Reprostar and Wincats 4.02 were used for scanning the plates. CAMAG twin through glass chamber was used for developing the plates.

**Chemicals**

Pure Strychnine and Brucine were obtained from Sigma Aldrich, U.S.A and precoated silica gel 60 F254 TLC aluminium plates (10×10 cms, 0.2mm thick), AR grade toluene, ethyl acetate, diethyl amine, methanol and chloroform were obtained from M/S Merck Ltd. Mumbai, India.

**Preparation of standard strychnine and brucine solution**

Strychnine standard (10 mg) and brucine standard (10 mg) were accurately weighed and dissolved in methanol in two standard flasks and final volumes were adjusted to 10 ml with methanol. (1 μg/μl)

**Calibration curve for strychnine and brucine**

The standard solutions corresponding to 2μg to 6μg of standard strychnine and brucine were applied on TLC plates (10cm× 10cm), precoated with silica gel as 6 mm bands by using CAMAG Linomat V sample applicator. The plate was developed in a solvent system of Toluene: Ethyl acetate: Diethyl amine (7: 2:1, v/v) in a CAMAG twin through chamber up to a distance of 7.5 cm at a temperature of 30 ± 20 C. The plates were air dried and scanned at a wavelength of 254 nm using CAMAG TLC scanner and Wincats 4.02 software. The peak area of strychnine and brucine were recorded for each concentration. The calibration curves of strychnine and brucine were obtained by plotting the graphs of peak areas vs. concentrations of strychnine and brucine.

**Preparation of sample solutions for estimation of strychnine and brucine**

Both the samples (KR & KGMDG) weighed 2g each, was defatted individually with petroleum ether. Defatted samples were then mixed with 10% ammonia and finally extracted with 25 ml methanol for 1 hr. under reflux. The methanol extracts were filtered and concentrated to 5 ml and used as test solutions. 5μl of each test solution was spotted along with 2 to 6 μl standard solutions of strychnine and brucine. The plates were developed in mobile phase of Toluene: Ethyl acetate: Diethyl amine (7:2:1, v/v) and scanned at 254 nm for strychnine and brucine. Peak areas were noted and quantity of
strychnine and brucine were calculated by comparing the areas of standard solutions from calibration curve.

**Results and Discussion**

The toxic principles present in the drugs are also considered as their active constituents. Therefore, it is not desirable to expel them out completely from the drugs. A study reports that the major toxic alkaloids i.e., strychnine & brucine present in the Kupeelu seed were reduced after Shodhana in Kanji and Adraka swarasa. This study exposes the fact that Shodhana with these two media i.e., Kanji and Adraka swarasa reduce the strychnine content by 39.25% and 67.82% respectively and brucine content by 17.60% and 40.06% respectively in comparison to the raw Kupeelu seeds. (Mitra S et al., 2012).

The main aim of Shodhana process is to reduce the toxic constituents to some extent or by potentiating their chemical transformation to non-toxic or relatively less toxic substances. There may be some new principles added to the drugs which are responsible for enhancing their biological efficacy. Hence, maximum beneficial effect can be obtained by administering the Shodhita drugs within their therapeutic dosage limit. Previous study revealed that crude Vatsanabha which is having cardiac depressant activity, converted into a cardiac stimulant drug after Shodhana in cow urine. (Singh LB).

In this study, Shodhana of Kupeelu seed was carried out by the A.F.I recommended method. Each Shodhana procedure was repeated for three times to establish the validation of the pharmaceutical processing. Shodhana of Kupeelu was performed by the subsequent processing through Nimajjana (dipping) in Gomutra, Swedana (boiling) in Godugdha, Bharjana (frying) in Goghrita for a specific time period.

Principles of Nimajjana & Swedana methods are similar to the common stages of extraction process where the solvent enters through the pores into the cells resulting in the swelling of the tissues and solution of the soluble constituents takes place within the cells followed by escape of dissolved material through the solvent boundary layer by the process of diffusion – finally separation of the solution from the drug occurs. The rate of extraction depends mainly on the temperature gradient and concentration gradient across the cell membrane. The rate of extraction and solubility is increased by elevation of temperature. Rising temperature increases the concentration gradient across the cell membrane thereby increasing mass transfer of active principles from solid material to the solvent (Carter SJ, Cooper and Gunn’s).

In Bharajana method, the seeds were fried with cow’s ghee in mild temperature for a specific period of time. It is reported that during Bharjana process, some physical & chemical changes like reduction in hardness, increase brittleness, formation of new chemical compounds may take place which ultimately make the drug body friendly. (Sarkar PK, 2008)

During Shodhana of Kupeelu in three media, change in color of those media was noticed and it might be due to the removal of color containing materials from the endosperm of the seeds. Taste of every media became bitter after Shodhana due to the extraction of bitter principles like Strychnine, Brucine, etc.

### Table 1. Organoleptic characters of raw & purified Kupeelu seeds powder.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Organoleptic characters of raw Kupeelu seeds powder</th>
<th>Organoleptic characters of Kupeelu seeds powder purified by A.F.I. approved method (KGMDG) in three batches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KGMDG-1</td>
</tr>
<tr>
<td>Texture</td>
<td>Smooth</td>
<td>Smooth</td>
</tr>
<tr>
<td>Colour</td>
<td>Greyish white</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>Odour</td>
<td>Slightly acidic</td>
<td>Pungent</td>
</tr>
<tr>
<td>Taste</td>
<td>Intense bitter</td>
<td>Bitter</td>
</tr>
</tbody>
</table>
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Changes in organoleptic characters of *Kupeelu* seeds were also noticed and the final quantity obtained after the *Shodhana* procedure was noted accordingly. The data generated from the study reveals the effect of *Shodhana* with cow urine, milk & ghee successively on organoleptic characters and yield of final products of *Shodhita Kupeelu*.

**Table 2.** Effect of *Shodhana* on yield of final product (KGMDG powder).

<table>
<thead>
<tr>
<th>Batch</th>
<th>Weight (g) of the seeds</th>
<th>Initial</th>
<th>After frying in <em>Goghnita</em></th>
<th>After drying</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGMDG-1</td>
<td>100</td>
<td>77.70</td>
<td>63.20</td>
<td></td>
</tr>
<tr>
<td>KGMDG-2</td>
<td>100</td>
<td>74.80</td>
<td>61.80</td>
<td></td>
</tr>
<tr>
<td>KGMDG-3</td>
<td>100</td>
<td>78.10</td>
<td>66.70</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>100</strong></td>
<td><strong>76.86</strong></td>
<td><strong>63.90</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Physicochemical parameters of raw and purified seeds.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>KR (Loss on drying 110°C (% w/w))</th>
<th>KGMDG (Ash Value (% w/w))</th>
<th>Water soluble extractive (% w/w)</th>
<th>Methanol soluble extractive (% w/w)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying 110°C (% w/w)</td>
<td>3.39</td>
<td>4.13</td>
<td>37.83</td>
<td>3.89</td>
<td>4.75</td>
</tr>
<tr>
<td>Ash Value (% w/w)</td>
<td>1.11</td>
<td>1.01</td>
<td>26.93</td>
<td>1.03</td>
<td>5.71</td>
</tr>
</tbody>
</table>

**Table 4.** Qualitative tests for various functional groups.

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>KR</th>
<th>KGMDG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Protein</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Fixed Oil</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tanin</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroid</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

**Table 5.** Results of estimation of Strychnine and Brucine in raw and purified samples of *Kupeelu* by HPTLC

<table>
<thead>
<tr>
<th>Samples</th>
<th>Amount of Strychnine found (% w/w)</th>
<th>Amount of Brucine found (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw <em>Kupeelu</em> (KR)</td>
<td>1.442</td>
<td>0.659</td>
</tr>
<tr>
<td><em>Kupeelu</em> purified by A.F.I. approved method (KGMDG)</td>
<td>0.411</td>
<td>0.303</td>
</tr>
</tbody>
</table>

Figure 1. HPTLC profile of standard Strychnine

Figure 2. HPTLC profile of standard Brucine

Figure 3. HPTLC of raw *Kupeelu* showing peak area of Strychnine and Brucine from the seeds. Changes in organoleptic characters of *Kupeelu* seeds were also noticed and the final quantity obtained after the *Shodhana* procedure was noted accordingly.

The data generated from the study reveals the effect of *Shodhana* with cow urine, milk & ghee successively on organoleptic characters and yield of final products of *Shodhita Kupeelu*. 
The intensely bitter raw Kupeelu became sweetish bitter after processing and grayish powder of raw seeds turned into grayish white in color after processing. (Table 1). It was found that 63.90% of purified Kupeelu seed powder was obtained as final product (KGMDG) after Shodhana. (Table 2).

Differences in all the physico-chemical parameters were observed among the raw and Shodhita Kupeelu samples. (Table 3). Qualitative tests revealed only the absence of glycoside in Shodhita Kupeelu sample however, other functional groups remained same. (Table 4).

The Rf values of Strychnine and Brucine standard were found as 0.54 & 0.34 respectively in HPTLC chromatogram under UV spectrum at 254 nm (Figure 1 & Figure 2) and the peak areas of Strychnine (Figure 3) and Brucine (Figure 4) in both the samples were exposed. Calibration curves of Strychnine and Brucine were prepared by plotting concentrations of Strychnine and Brucine in the range of 2-6 µg/spot versus average area of the peak. The responses for concentrations of standard Strychnine and Brucine were found to be linear (Figure 5 & Figure 6). The amounts of Strychnine and Brucine in raw & purified samples were computed from the calibration curves which suggest the reduction of Strychnine and Brucine content by 71.49% and 54.02% respectively in the Shodhita sample (Table 5). It might be due to the collective impact of the three media in the process of Shodhana. During Shodhana with Gomutra and Godugdha, some amount of strychnine & brucine were removed from the seed by the process of extraction. It is also reported that boiling in milk converted the strychnine into less toxic isostrychnine (Cai et al., 1990). Finally frying with Goghrita might have been converted strychnine & brucine into less toxic derivatives like isostrychnine, isobrucine, strychnine N- oxide, brucine N-oxide etc.

**Conclusion**

Shodhana of Kupeelu seeds with successive three media i.e., Gomutra, Godugdha

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**Figure 4.** HPTLC of Kupeelu purified by A.F.I. approved method showing peak area of Strychnine and Brucine

**Figure 5.** Calibration curve of Strychnine

**Figure 6.** Calibration curve of Brucine
and Goghrita (as approved by A.F.I.) successfully reduce the toxic alkaloids from the seeds. These findings strongly confirm the claims of ancient classics of Ayurveda that Shodhana process reduces the toxic effects of the drugs.

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