



Research Article

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SIGNIFICANCE OF STANDARD OPERATING PROCEDURE IN SAFETY PROFILE OF RASAUSHADHIES WITH SPECIAL REFERENCE TO KUPILU SHODHAN

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ABSTRACT

Kupilu is included in Upavisha Varga in Ayurveda classics by the name 'Vishtinduka'. It is toxic in nature due to presence of alkaloids namely Strychnine and Brucine but can produce miraculous therapeutic effects after specialised procedure mentioned in Ayurveda known as Shodhana (~ Detoxification). Raw Kupilu seeds are potent convulsant which after Shodhana are assumed to be nerve tonic, aphrodisiac, anthelmintic, purgative being useful in Asthma, Bronchitis, Hysteria, Nervous debility, Insomnia and many more signifying the importance of proper Shodhana process. More than six methods have been mentioned in classical text for Kupilu Shodhana. On the basis of previous studies, it has been found that Shodhana by all the three media viz. Gomutra, Godugdha and Goghrita collectively to be the best method for its toxicity reduction, as significant reduction in percentage of Strychnine and Brucine occurs when estimated by HPTLC before and after its processing.

Keywords: Kupilu, Strychnine, Brucine, Shodhana, Godugdha, Goghrita, Gomutra

INTRODUCTION

Ayurvedic formulations, believed to possess inbuilt safety profile have been traditionally used for thousands of years in India. Global resurgence of Ayurveda has necessitated its scientific validation in terms of both therapeutic efficacy and safety that ultimately depends on quality control of Ayurvedic pharmaceutical processes. Ayurveda has potential to convert a poisonous substance into therapeutically useful drug after undergoing through a number of processes like Shodhana, Marana etc. But if these methods are not followed strictly due to lack of proper SOP (Standard Operating Procedure), it may lead to a number of harmful effects, taking Kupilu (*Strychnos nux vomica*) as an example here. Kupilu is a poisonous drug mainly producing tetanus like convulsions and eventually death in large doses and mental derangement in lesser doses¹, due to presence of toxic alkaloids, but shows miraculous therapeutic effects after Shodhana as per Ayurveda. The word Strychnos was said to be derived from Greek word 'Strychon' usually translated as 'Nightshade' after Pliny and the word Nux vomica has been derived from Medieval Latin 'nux' for 'nut' and 'vomica' is Latin for 'lump' as the seed appears like a nut with a lump on it and feminine of 'vomicus' meaning an 'emetic'.² *Strychnos nux vomica* is an evergreen and medium sized deciduous tree, usually 15-20 m high and up to 70 cm diameter. Fruits are globose, 2.5-5 cm in diameter, orange red when ripe containing mainly discoid, compressed coins like seed in fleshy pulp. Dry seeds are very hard and odourless. They are disc shaped, 17-30 mm diameter, 3.5-5.3 mm thick and ash grey in colour, little depressed on one side and arched on the other, covered with numerous hairs radiating from centre to circumference.³ Alkaloids are the main bioactive principles in *Strychnos nux vomica*, responsible for its pharmacologic and toxic effects. Total alkaloids account

for 2.6-3.0 % out of which 1.25-1.5 % Strychnine and 1.7 % Brucine are the main alkaloids apart from others viz. Vamicine, Colubrine, Pseudo-strychnine, Navacine, Icajine etc.⁴ A total of 16 alkaloids have been separated and identified from crude nux vomica seeds, bark, roots, leaves, fruit pulp and hard fruit shells⁵ and 22 identified alkaloids from root bark and leaves.⁶ Among all alkaloids, Strychnine and Brucine are the potent toxins. Strychnine is a terrible tetanic poison, affecting the cerebrospinal system in a fatal dose being as low as ½ grain. Brucine is physiologically same as Strychnine, but in a markedly less degree, some considering it 1/16th, others 1/40-1/50th less energetic than Strychnine as a convulsant.⁷ Godugdha, Gomutra and Goghrita have been described for treating Vish- vikar in classics.⁸ Therefore, attempt has been made to compare the Vishahar (Detoxification) effect of these three with reference to Kupilu Shodhana. Ayurveda recommends purified nux vomica seeds in a number of formulations like Agnitundi vati, Navjeevan Ras, Vishtinduka vati etc, useful in various nervous and joint disorders.⁹ Standardization expression is used to describe all measures which are taken during the manufacturing process and quality control leading to a reproducible quality. But problem arises in selecting an appropriate method for preparation of a drug, as a number of methods have been described for the same. So, it is of utmost importance to set SOP's with regard to Ayurvedic formulations.

The present study was planned with following aims and objectives-

- Compilation of various Kupilu Shodhana methods from classical text.
- Assessing the role of media and process involved in Shodhana on modern scientific parameters.

- Showing the need of SOP in Kupilu Shodhana to maintain its efficacy and safety.
- Evaluating the best method for Kupilu Shodhana amongst Godugdha, Gomutra and Goghrita.

MATERIALS AND METHODS

More than six media viz. Gomutra, Godugdha, Goghrita, Kanji, Eranda taila and Ardraka swarasa has been mentioned in classics regarding Kupilu Shodhana. Both general and specific Shodhana has been mentioned as given below-

General Shodhana

Small (gram size) pieces of seeds are soaked in Gomutra for three days with daily Gomutra changing and are then dried in sunlight.¹⁰

Specific Shodhana-

- (a) Seeds are fried in Goghrita on low flame until their skin gets separated and are pulverized thereafter.¹¹
- (b) Swedana is done in Godugdha by Dolayantra method for three hours. Then outer covering is scrapped with knife and are dried in sunlight.¹¹
- (c) Seeds are soaked in Gomutra for seven days with daily Gomutra changing. After this, Swedana is done in Godugdha by Dolayantra method after separating

the skin. At last, seeds are fried in Goghrita and pulverized immediately.¹² This is the official method of Kupilu Shodhana.

- (d) Seeds are soaked in Kanji for three days. Then their skin is separated and dried in sunlight before powdering.¹¹
- (e) Seeds are dipped in Ardraka swarasa for 20 days with daily stirring by a glass rod. Then the seeds are washed with lukewarm water, the outer seed coat and embryo are removed, cotyledons are dried and pulverized.¹³
- (f) Eranda taila¹⁴ is also mentioned for Kupilu Shodhana.

HPTLC estimation of Strychnine and Brucine

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V sample applicator was used. Pure Strychnine and Brucine were obtained from Sigma Aldrich, U.S.A. in which methanolic extracts of all the individual samples was spotted with standard solutions of Strychnine and Brucine. Mobile phase used was Toluene: Ethyl acetate: Diethyl amine (7:2:1, v/v). Peak areas were noted and their quantities were calculated by comparing the areas of standard solutions from calibration curve.¹⁶

Table 1: Comparison of three different media for Kupilu Shodhana

Nux vomica seeds	Sample 1 ¹⁵		Sample 2 ¹⁶		Sample 3 ¹⁷	
	Strychnine	Brucine	Strychnine	Brucine	Strychnine	Brucine
Crude form	1.92 %	0.93 %	1.44 %	0.66 %	0.63 %	0.77 %
Gomutra purified seeds	0.96 %	0.19 %	0.449 %	0.314 %	0.74 %	0.23 %
Godugdha purified seeds	0.46 %	0.345 %	0.289 %	0.201 %	0.52 %	0.68 %
Goghrita fried seeds after Gomutra purification	0.068 %	0.012 %	0.131 %	0.079 %	0.62 %	0.57 %

RESULTS AND DISCUSSION

Shodhana of Kupilu by three different media Gomutra, Godugdha and Goghrita is compared by evaluating percentage loss in Strychnine and Brucine before and after procedure as depicted in the Table 1:

Methods mentioned for Kupilu Shodhana signifies the importance of two aspects namely-

1. Role of media
2. Type of Process involved

Role of Media

Studies has confirmed the presence of alkaloids in milk used for Kupilu Shodhana signifying the importance of role of media in the same.¹⁸ Media has a role in either breaking down or destroying the chemical constituents that are not required. Heat treatment or constant boiling of a drug in a particular media for a specific period of time has a role in modification of the chemical constituents.¹⁹

Process involved

Different processes involved in Kupilu Shodhana with modern co-relation is given below-

Nimajjan or Sthapana Process is in accordance with modern Maceration method of extraction whereby

impurities or toxic substances from the drug gets dissolved in the solvent or media used for its extraction rendering the drug non-toxic.

Swedana Process is in accordance with Percolation method of extraction where toxic principles gets dissolved in media used for boiling.

Bharjana Process- Cai *et al* reported significant decrease of Strychnine and Brucine in heat treated seeds with significant increase in amount of novel alkaloids such as Isostrychnine, Isobrucine, Strychnine-N oxide and Brucine-N oxide.⁵

Studies has shown that cotyledon portion contain higher percentage of Strychnine than present in seed coat for the entire sample as estimated by Modified Bharjana Process (1958) except for milk purified seeds which shows that purification with Godugdha markedly reduces the toxicity of crude nux vomica than that treated with Ghrita.¹⁵ Analysis of given data proved the official method of Shodhana i.e. with Gomutra, Godugdha and Goghrita to be the best one as far as the amount of toxic alkaloids is concerned.

Evidences supporting Pharmacological action of Kupilu

Researchers are going on in modern medicine to evaluate the therapeutic effect of nux vomica. Various studies are as follows-

Alcoholic seed extract has shown good lipid peroxidation effect in rat liver.²⁰

Crude extract has been reported to exhibit an inhibitory effect on rRNA tumour virus, protein kinase and HIV-I protease.²¹⁻²³

Brucine and Brucine-N oxide has been reported for its analgesic and anti-inflammatory properties.²⁴

Anti-microbial activity

Gnanavel *et al* reported n-butanol extract of nux vomica showed good antibacterial activity against gram +ve bacteria and antifungal activity against *Aspergillus terreus* and *flavus*.²⁵

Anti-convulsant activity

Katiyar *et al* reported that seeds processed in milk exhibit marked inhibition of PTZ induced convulsions and maximal potentiating of hypnosis with safest LD50.²⁶

Anti-tumour effect

Deng *et al* reported Brucine, Strychnine and Isostrychnine showed inhibitory effect against Hep G2 cell proliferation.²⁷

Anti-nociceptive effect

Cai *et al* reported crude alkaloid fraction of sand processed seeds of nux vomica showed marked antinociceptive effect in all analgesic tests performed.²⁸

Anti-diarrhoeal effect

Methanolic extract showed good results against castor oil induced diarrhoea in mice.²⁹

Anti-snake venom activity

Chatterjee *et al* reported that seed extract potentiated polyvalent snake venom antiserum action in experimental animals.³⁰

Immunomodulatory effect

Nux vomica has been proved to show immunomodulatory effect also.³¹

Therefore, each and every step in Shodhana should be done carefully with respect to time, temperature and purity of media for maximum extraction of toxic alkaloids from crude nux vomica seeds.

CONCLUSION

Both pros and cons are associated with any scientific intervention, so is the case with drugs. Drugs are the most common medical interventions, used primarily to relieve suffering but it has been recognized long before that drugs themselves can prove fatal, as the saying rightly goes "Drugs are double edged weapons". Toxic effects reported in preparations containing Kupilu can be attributed to short cut methods employed for its

Shodhana, as a result of which amount of toxic alkaloids does not get reduced up to acceptable level. So, it is the need of hour to set SOP's with regard to Ayurvedic preparations. Here, it can be concluded that official method of Kupilu Shodhana is the best one to decrease the toxicity on the one hand and to increase the therapeutic efficacy on the other, thereby supporting the ancient claims of Ayurveda regarding Shodhana process.

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