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"Evaluation of ayurvedic detoxification process (Shodhana) of Strychnos nux vomica linn seeds w. s. r. to its LD50".

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ABSTRACT

In Ayurveda a very useful procedure of Shodhana Sanskar i.e. detoxification process is mentioned. It helps not only to remove impurities but also is useful to enhance required/favourable properties of the poison and avoid the harmful ones, by which we can confidently state the safety of the drug prepared from the vishadravyas (poisonous drug). Here the attempts were made to study the changes in phytochemical properties and LD50 values of Strychnos nux vomica seed powder due to detoxification process (shodhana sanskar). LD50 is frequently used as a general indication of substance acute toxicity. As the signs and symptoms of toxicity of nux vomica seed is seen immediately after consumption. Hence, acute toxicity study has been chosen.

Objectives: To evaluate the effects of shodhana process i.e. detoxification process on toxicity level of Strychnos nux vomica seeds.

Material and methods: Detoxification (Shodhana) of Strychnos nux vomica seeds was done by method mentioned in Ayurvedic text Rasatarangini. The phytochemical analysis was performed for both before and after detoxification (shodhana) samples. Also, acute toxicity study i.e. LD 50 study was performed for both samples by following OECD Guidelines for Testing Chemicals.

Results: Strychnine value was reduced by 1.02% in after detoxification sample.

Before detoxification (shodhana) process LD50 value is found to be less than 300 mg/kg but more than 50 mg/kg body weight in albino mice by oral route. After detoxification (shodhana) process LD50 value is greater than 2000 mg/kg body weight in albino mice by oral route.

Conclusion: Detoxification process (Shodhana) affects phyto-chemical properties of Strychnos nux vomica seed powder. After the process of detoxification, the quantity of main phytochemical constituents strychnine and brucine are found to be reduced on HPTLC.

Detoxification (Shodhana) process increases the value of LD50 of nux vomica seed powder. i.e. it reduces the toxicity of Strychnos nux vomica seed powder. .

INTRODUCTION

Visha is a substance which when enters in the body produces disturbances in all the functions of humor (dosha), tissues (dhatu), waste materials(mala). Also degrades the health of human beings or animals resulting in destruction of life.1

Ayurveda mentions categorization of poisonous substances into highly toxic (visha) and lesser toxic (upavisha). This literature also mentions the wide use of these poisonous substances in medicinal preparation also.

Ayurveda believes in the concept that even a poison can become an excellent remedy if it is used in proper way and proper technique. On the other hand, even, a medicine can prove fatal if it is not prepared or used properly.2 Being poisonous drugs they need to be detoxified before used as medicine. Various processes of purification especially for all poisonous substances are mentioned in Ayurvedic texts called as shodhana process i.e. detoxification process.

Acharya Sadanand Sharma has categorized nux vomica seed (kupeelu beeja) in lesser toxic 11 drugs (upavishas) in his treatise Rasatarangini.3 Seed contains strychnine which is a spinal neurotoxic poison.4 If nux vomica seed is to be used in medicine or consumed in unprocessed form it produces many harmful effects on human body. So, it is necessary to subject Strychnos nux vomica seed to detoxification (shodhana) process before using as medicine.

The effort has been made to study experimentally whether detoxification (shodhana) reduces toxicity of nux vomica seed or not? and to what extent? Hence, effort was made to study the effect of detoxification (shodhana) on LD_{50} of nux vomica seed.

The dose amount of poisonous or toxic substance required to kill 50% of tested population is the LD50 of drug[5]. LD50 figures are frequently used as a general indication of substance acute toxicity[6]. The LD50 is usually expressed as the mass of substance administered per unit mass of test subject, such as mg or grams of substance per kg of body mass[7].

As the signs and symptoms of toxicity of nux vomica seeds are seen immediately after consumption[8], hence acute toxicity study has been chosen.

OBJECTIVES:

To evaluate the effects of shodhana process i.e. detoxification process on toxicity level of Strychnos nux vomica seeds with the help of phytochemical analysis

To study the effect of detoxification on (in vivo) LD50 of nux vomica seed powder.

MATERIALS AND METHODS:

Collection of samples: -

crude sample of nux vomica seeds (kupeelu beeja) was collected from authorized source.

Authentification: -

Morphological identification of the Strychnos nux vomica seeds (kupeelu beeja) was done at Department of botany in authorized institute.

Detoxification/ shodhana process: - Detoxification process/shodhana process described in Ayurvedic rasagrantha Rasatarangini was followed for detoxification.[9]

Table (a)

Ingredients	unprocessed nux vomica seed, cow milk, hot water.
Equipments	Dolayantra/swing-apparatus, cotton cloth, weighing machine, Gas cylinder, steel pots.

Procedure: -

Unprocessed nux vomica seeds were boiled in cow milk (godugdha) by dolayantra/ swing-apparatus process for 3 hrs.(1 yaama as described in ayurvedic literature). After boiling the seeds were removed from milk and thoroughly washed with hot water. Then covering and embryo were removed from each seed. After drying seeds were made into powder.[9]

Phytochemical analysis study:

The phytochemical analysis was done at FDA and AGMARK approved Laboratory. Following tests were done for both the samples i.e. processed and unprocessed nux vomica seed powder:

moisture content

total Ash

acid insoluble Ash

alcohol soluble extractives

water-soluble extractives

Quantity of alkaloid strychnine

HPTLC (High performance thin layer chromatography) was done for both samples of nux vomica seed powder.

Toxicity study: -

Acute toxicity study of both samples (before and after detoxification) of nux vomica seed powder was carried out in authorized centre.

Table (b) Materials –

Animal species used	- Albino mice.
No. of animals	-1 for each dose for preliminary range finding study -4 for each sample for main LD50 study.
Avg. wt of animals	25gm
Sex of animals	female
Period of fasting	overnight
Dosing	oral route.
Samples	2, in fine powder form. before process after process

Procedure for experimental studies OECD guidelines for testing chemicals[10] are followed.

Both samples of nux vomica seed powder (before and after process) were converted into suspensions by thorough mixing of very fine powder in water separately.

Samples were freshly prepared for each group (before and after process) in different concentrations (increasing order) and then administered.

Doses were given to animals by gavage according to their body weight by applying following formula[11].

$$\text{Amount of drug mg/kg} = \frac{\text{Volume of the drug solution} \times \text{strength in mg/ml of the drug solution}}{\text{Body weight of the animal (kg)}}$$

Animals were deprived of feed overnight before and 2 hrs after administration of the sample. Water was allowed ad libitum.

First of all, preliminary range finding study was done.

a] Preliminary range finding study: -

One female albino mouse per dose was assigned to the treatment with unprocessed of nux vomica seed powder as follows: Table (c)

Group	No. of mice	Dose(mg/kg)
I	1	5
Ii	1	50
Iii	1	300
Iv	1	2000

Same dose protocol was applied for treatment with processed sample of nux vomica seed. Mice were observed for mortality for 7 days after dosing. For both samples mortality was observed up to 2000mg /kg of body weight dose.

b] LD_{50} in albino mice: -

From the data obtained in the preliminary range finding study, mice were assigned to the doses as follows: - Table (d)

Group	No. of mice	Dose (mg/kg)
I (vehicle control group)	4	0.00
II (before process)	4	300
III (after process)	4	2000

Mice were observed for mortality for 14 days after dosing.

Observations

Observations of this study were done in two main stages as follows:

a) Organoleptic and Phytochemical analytical observations.

b) Median lethal dose observations.

a) Organoleptic and Phytochemical analytical observations.

Following table shows organoleptic observations of nux vomica seed powder: Table (e)

	Before process	After process
Appearance	Soft powder	Soft powder
Colour	Dark, blackish brown	Grayish brown
Odour	odourless	odourless
Taste	Not done	Not done

Observations of phytochemical analysis**: -**

Following table shows phytochemical analytical data of before and after detoxification (shodhana) samples of nux vomica seed powder. Table (f)

Tests	**nux vomica seed powder**	
	Before process	**After process**
1) Moisture content/Loss on drying	3.2%	4.0%
2) Total Ash	0.88%	0.51%
3) Acid insoluble ash	0.16%	0.04%
4) Alcohol soluble extractives	6.22%	6.55%
5) Water soluble extractives	14.22%	8.42%
6) Quantity of alkaloid strychnine	1.26%	0.24%

HPTLC of nux vomica seed powder chromatogram (figure 1,2 &3) and RF values (table no.1) shows the decrease in amount of the chemical constituents of nux vomica seed powder due to detoxification process.

b] Observations of median lethal dose study: -

1) Preliminary range finding study, for before and after detoxification (shodhana) process samples

Before process sample. Table (g)

Group	**Dose(mg/kg)**	**No.of animals dosed**	**Effect on animals**
i	5	1	-
ii	50	1	-
iii	300	1	Died
iv	2000	1	Died

After process sample. Table (h)

Group	Dose(mg/ks)	No. of animals dosed	Effect on animals
i	5	1	-
ii	50	1	-
iii	300	1	-
iv	2000	1	-

2) LD_{50} in albino mice. Table (i)

Group	Dose (mg/kg)	No. of animals died No. of animals dosed	Mortality%
I (Control)	0.00	0/4	0%
II (Before process)	300	3/4	75%
III (After process)	2000	0/4	0%

RESULTS:

From the observations results can be drawn as follows –

Due to detoxification process chemical constituents (mainly strychnine and brucine) decrease in amount

LD50 values of nux vomica seed powder before and after (shodhana) detoxification process are as follows.

Before detoxification (shodhana) process – LD50 value is less than 300 mg/kg but more than 50 mg/kg. body weight in albino mice by oral route.

After detoxification (shodhana) process – LD50 value is greater than 2000 mg/kg body weight in albino mice by oral route.

DISCUSSION

Poisonous drugs (Vish and upvisha) have the toxic effects due to the harmful contents. But many properties of these drugs are useful to treat diseases. As stated above after detoxification (shodhana) the poisonous drug (visha) gets detoxified, hence can be used as medicine i.e. because of detoxification(shodhana) the harmful effects can be minimized or avoided and the useful can be used. Detoxification (shodhana) causes the useful changes in the drug for which the detailed processes are described in Ayurvedic texts. But all of them are not quantified in relative parameters.

Invaluable herbal drugs are to be used in very judicious manner. It is also necessary to confirm the safety and efficacy of such herbal drugs. Toxicity of the substance can be tested experimentally by acute, subacute and chronic toxicity study according to poison. LD_{50} is the method to test the acute toxicity. LD_{50} is the dose amount of a poisonous substance required to kill 50% of tested population. It is useful to decide extent of toxicity of poisonous substance. The acute toxicity aims at establishing the therapeutic index i.e. the ratio between pharmacologically effective dose and lethal dose on the same strain and species (LD50/ED50). Greater the index safer the compound and vice versa. This study was aimed to evaluate the effect of shodhana sanskara i. e. detoxification process on nux vomica seed with the help of phytochemical tests and experimental method i.e. LD50 studies.

During the course of this study it was observed that, during detoxification (shodhana) process, fumes evaporating from swing apparatus (dolayantra) caused eye irritation and had nauseating smell. These side effects can be avoided by doing the procedure of detoxification (shodhana) in open space. It was found that before and after detoxification (shodhana) samples differed in their phytochemical properties. Before starting the detoxification (shodhana) process (as shown in table f) moisture content (3.2%), total ash value (0.88%) acid insoluble ash (0.16%), quantity of strychnine alkaloid is (1.26%) were ruled out for nux vomica seed powder. After detoxification (shodhana) the values were ruled out. From table f we can see that, total ash value was decreased by 0.37%. Acid insoluble ash was decreased by 0.12%. The alkaloid strychnine was decreased by 1.02%. From all observations we can summarize that the reduction in toxicity is due to loss in quantity of chemical constituents during detoxification (shodhana) process. The toxic contents may have been dissolved in the milk while boiling. During detoxification (shodhana) the poisonous content may be reduced/removed due to heating treatment.

The covering and embryo were removed after washing, which results in reduction of poisonous content in the detoxified sample.

For oral LD50 study, the dosage was administered for both the samples by mixing the powder with water. The LD50 value for before detoxification (shodhana) sample of nux vomica seed powder was found to be greater than 50 mg/kg and lesser than 300 mg/kg of body weight by oral route in albino mice. While LD50 value for after detoxification (shodhana) sample of nux vomica seed powder was found to be greater than 2000 mg/kg of body weight by oral route in albino mice. i.e. The LD50 value was found to be increased. This change in the value of LD50 of nux vomica seed powder is due to the phytochemical changes due to detoxification process.

CONCLUSION

So, the study can be concluded as follows –

Detoxification process (Shodhana samskara) affects phyto-chemical properties of nux vomica seed powder. Some proportion of organic materials may have been dissolved in the milk while boiling. After the process of detoxification, the quantity of main elements strychnine and brucine are found to be reduced.

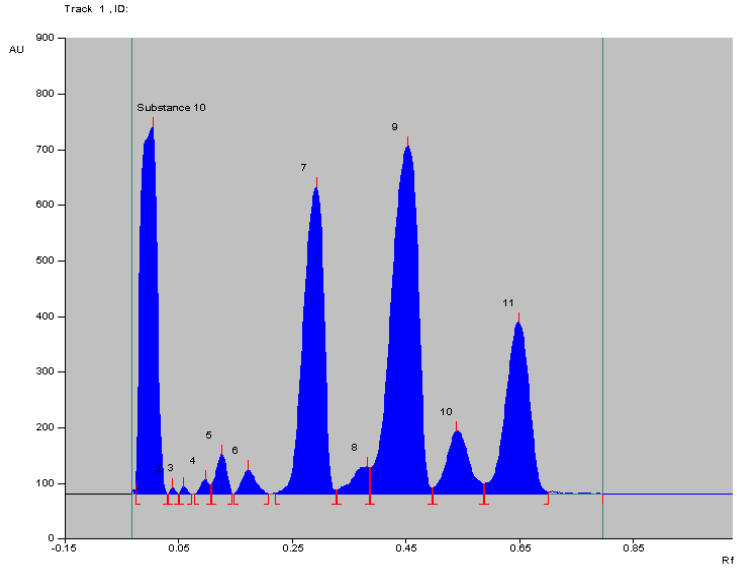
Detoxification / Shodhana process increases the value of LD_{50} of nux vomica seed powder. i.e. it reduces the toxicity of nux vomica seed powder.

Further studies can be done for the target organ toxicity.

The effect of nux vomica seed powder after detoxification (shodhana) on spinal cord and brain by histopathological studies can be evaluated.

The present study is not sufficient, those may be remaining some lacuna in the study. Hence the further studies in this field are needed..

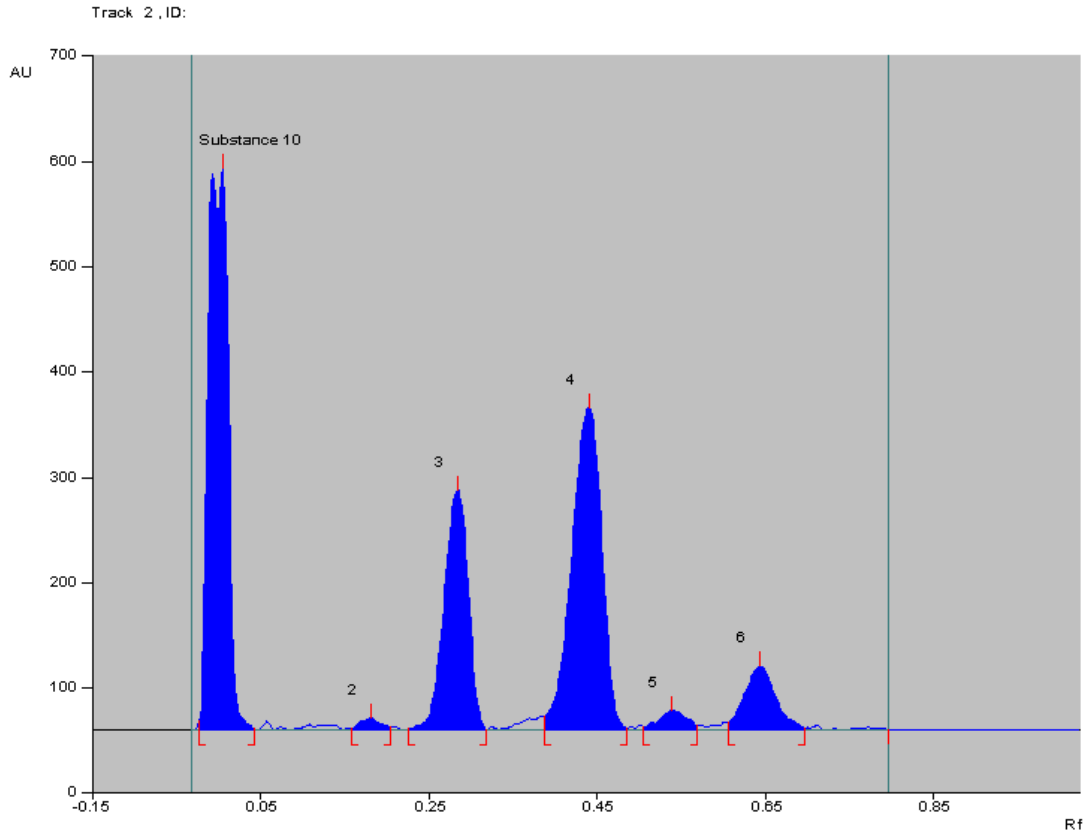
Figure 1: **Chromatogarm For Nux vomica powder extract before purification**



Peak	Start position	Start Height	Max.position	Max Height	%Max	End position	End height	Area	Area %	Assigned substance
1	-0.02 Rf	16.3 AU	0.01 Rf	659.7 AU	26.67%	0.03 Rf	0.6 AU	14475.6 AU	20.90%	Substance 10
2	0.04 Rf	0.5 AU	0.04 Rf	11.1 AU	0.45%	0.05 Rf	0.0 AU	69.3 AU	0.10%	unknown *
3	0.05 Rf	1.2 AU	0.06 Rf	12.7 AU	0.51%	0.07 Rf	0.0 AU	99.7 AU	0.14%	unknown *
4	0.08 Rf	0.1 AU	0.10 Rf	25.7 AU	1.04%	0.11 Rf	15.6 AU	291.5 AU	0.42%	unknown *
5	0.11 Rf	16.0 AU	0.13 Rf	71.6 AU	2.90%	0.15 Rf	1.2 AU	1013.7 AU	1.46%	unknown *
6	0.15 Rf	0.1 AU	0.17 Rf	43.2 AU	1.75%	0.21 Rf	0.9 AU	806.4 AU	1.16%	unknown *
7	0.22 Rf	2.0	0.30 Rf	551.7	22.31%	0.33 Rf	7.0	15158.3	21.88%	unknown *

		AU		AU			AU	AU		
8	0.33 Rf	7.1 AU	0.39 Rf	48.7 AU	1.97%	0.39 Rf	47.7 AU	1160.7 AU	1.68%	unknown *
9	0.39 Rf	47.1 AU	0.46 Rf	625.3 AU	25.28%	0.50 Rf	10.5 AU	22755.1 AU	32.85%	**Strychnine**
10	0.50 Rf	10.6 AU	0.54 Rf	114.1 AU	4.61%	0.59 Rf	18.9 AU	3526.1 AU	5.09%	unknown *
11	0.59 Rf	18.9 AU	0.65 Rf	309.3 AU	12.51%	0.70 Rf	3.6 AU	9914.8 AU	14.31%	**Brucine**

Figure 2 : Chromatogarm For Nux vomica powder extract after purification



Peak	Start position	Start Height	Max. position	Max Height	%Max	End position	End height	Area	Area %	Assigned substance
1	-0.02 Rf	9.2 AU	0.01 Rf	533.5 AU	26.04%	0.05 Rf	0.8 AU	10028.2 AU	38.25 %	Substance 10*
2	0.16 Rf	0.8 AU	0.18 Rf	11.1 AU	0.96 %	0.21 Rf	2.7 AU	214.4 AU	0.82%	unknown *
3	0.23 Rf	0.9 AU	0.29 Rf	227.9 AU	19.67%	0.32 Rf	0.5 AU	5127.6 AU	19.56 %	unknown *
4	0.39 Rf	12.5 AU	0.44 Rf	306.6 AU	16.46%	0.49 Rf	1.9 AU	8595.1 AU	32.78 %	**Strychnine**
5	0.51 Rf	3.1 AU	0.54 Rf	18.9 AU	1.64 %	0.57 Rf	3.8 AU	485.3 AU	1.85%	unknown *
6	0.61 Rf	7.6 AU	0.65 Rf	60.6 AU	5.23 %	0.70 Rf	1.8 AU	1769.0 AU	6.75%	**Brucine**

Figure 3: Overlapping Chromatogram

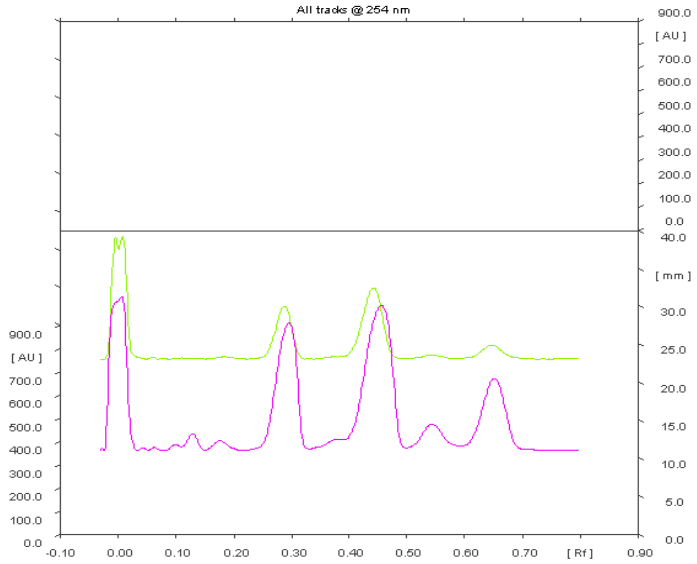


Table no. 1 : On spraying with Dragendorff's reagent followed by 5% methanolic sulphuric acid, it showed spots of RF values as follows

Spots	Before shodhana	After shodhana
1	0.01RF	0.01 RF
2	0.04RF	-
3	0.06RF	-
4	0.10 RF	-
5	0.13 RF	-
6	0.17 RF	0.18 RF
7	0.30 RF	0.29 RF
8	0.39 RF	-
9	0.46 RF	0.44 RF
10	0.54 RF	0.54 RF
11	0.65 RF	0.65 RF

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