EFFECT OF PURIFICATION (SODHANA) ON THE ACUTE TOXICITY OF SEEDS OF NUX-VOMICA

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Summary

The Sodhana (Detoxification/Purification) process occupies an important part in the traditional ayurvedic system of medicine in India. The sodhana processes are used to remove visha or toxic substances from various medicinal substances. The sodhana process performed in three stages for nux vomica seeds. In the present investigation, *Strychnos nux vomica* Linn. seeds are detoxified by subjected to sodhana process by keeping in cow's urine for 7 nights and they are subjected to swedana (seeds are boiled in cow's milk for 3 hours). Then the seed coat and embryo are removed. Further roasted with cow's ghee and powdered. The purpose of sodhana is to reduce toxicity by reducing the toxic alkaloids, toxic glycosides by treatment with cow's urine.Then seeds are boiled with cow's milk and roasted with cow's ghee to eliminate irritant trichomes on the surface of seeds so that the powder drug can be swallowed safely and also easy to make the seed powder. In the present study, the impact of the sodhana on the acute toxicity of the seeds is verified and it is found that the LD_{50} of unprocessed nux vomica seed powder is 256mg/kg and sodhana processed seed powder LD_{50} is 2600mg/kg. The acute toxicity of the sodhana processed product is significantly reduced (about $1/10^{th}$) when compared to unprocessed seed powder LD_{50}. It would be worthwhile to determine the major alkaloids strychnine and brucine content in the unprocessed and sodhana processed seed powder and screening the biological activities in order to understand the significance of the sodhana process of the seeds of nux vomica for its safety and efficacy as Ayurvedic medicinal preparation.

Keywords: *Strychnos nux vomica*, Sodhana, Detoxification, Acute toxicity, Lethal dose 50 (LD_{50}).

Introduction

In recent years, traditional Indian Ayurvedic system of medicine is gaining increasing popularity worldwide. Analysis of components in the herbs is essential for the discovery and development of new drugs of natural origin as well as for herbal standardization, toxicological and biological investigations. There are indications that most of the herbs possess mild side effects and some plants are reported to be toxic. In Rasa-shastra of Ayurvedic system of medicine, the crude drugs such as aconite, nux vomica, croton, kavera etc., are listed as toxic substances and are used in the clinical practice only after subjecting them to proper detoxification (sodhana) process. Sodhana is a term used for various processes including simple washing to swedana (boiling in cow's milk or any other liquid substances in dola yanthra for prescribed period) or combinations of various sodhana processes. Since seeds of nux vomica are included in group of Upavisha (sub-toxic), they have to be used only after subjecting them to sodhana process[1]. In ancient literature seeds of nux vomica are used for treating nervous diseases, arthritis, tumor, snake bite, diabetes, obesity, diuretic, ulcers, aphrodiasic, appetizer etc.,[2,3]. In modern literature it was found that the brucine and brucine-N-oxides of seeds possess anti-inflammatory & analgesic properties[4], antitumor[5], antidiarrhoeal[6], anti snake venom[7]. The seeds of nux vomica contain various alkaloids like strychnine, brucine, strychnine-N-oxides, brucine-N-oxides, pseudostrychine, colubrine, novacine, vomicine, other indole alkaloids and glycosides like loganin etc., are responsible for the pharmacological activities of seeds of the plant[8]. Strychnine and brucine are extremely toxic alkaloids which exist in the seeds of *Strychnos nux vomica* L. and other species of genus *Strychnos*, which are frequently used as important ingredients in traditional Chinese herbal medicines to treat nervous diseases, vomiting, arthritic and traumatic pains[9]. At low doses, *Strychnos* alkaloids exhibit high pharmacological activities. However, higher doses of strychnine are known to be deadly poisonous and sometimes can cause violent muscular convulsions[10]. However its toxicity is limiting the use of nux vomica. Therefore several ancient scriptures including Ayurveda and Chinese system of medicine prescribes various detoxification processes. There are reports that the alkaloid content of the seeds of nux vomica is reduced significantly after subjecting to detoxification process as explained by Chinese system of medicine [11, 12, 13] . However there are no such reports regarding the impact of the sodhana process on the phytochemical and toxicological properties of the seeds of nux vomica. Keeping this in view the present study is designed to evaluate the impact of the sodhana on the acute toxicity of the seeds of nux vomica.

Materials and methods

Plant material

The seeds of *Strychnos nux vomica* were collected from Yucca enterprises, Mumbai and identified and authenticated. A Herbarium specimen (SCSCOP/P.COG/12/04-05) was preserved in the college.

Selection of seeds

The dry seeds were first dropped in a beaker containing water. The seeds which float on the surface of water or found broken, black in colour are rejected and the seeds which are found settled at the bottom of the beaker are selected for purification after drying in air.

Detoxification (Sodhana) process

Sodhana (detoxification) of nux-vomica seeds is performed as per the method described in Ayurvedic Rasashastra. The Rasashastra describes stepwise procedure for detoxification (sodhana) of nux-vomica and it was adopted in the present study[1].

Step 1: The clean and dried seeds (1kg) are kept in cow's urine for 7 nights. The urine is changed every day.

Step 2: The seeds after 1st step are collected and subjected to swedana (swedana process was done by keeping the seeds after 1st step in muslin cloth with banana leaf and tied. It is completely dipped in cow's milk and boiled on low flame) for 3 hours using dolayantra (shown in fig. 1).

Step 3: The above seeds are collected and washed with water. The seed coat and embryo removed. The seeds are roasted with cow's ghee in low flame on iron pan. The seeds become dark brown and crispy. Then the seeds are immediately powdered. The nux vomica which is unprocessed form (UNV) and the seeds detoxified by sodhana process (PNV) were subjected to phytochemical and acute toxicity studies.

Preliminary phytochemical screening

The UNV and PNV seed powders were extracted with 70% alcohol and screened for the phytoconstituents.

Detection of major alkaloids

The major alkaloids strychnine and brucine were identified by thin layer chromatography (TLC) [14]. All the samples were defatted with petroleum ether. The defatted (2g) sample was mixed with 10% ammonia and extracted with 25 ml methanol for 1 hour. The methanol extract was concentrated to 5 ml and used as test sample. Silica gel G is used as stationary phase while toluene, ethyl acetate and diethylamine (7:2:1) were used as solvent system. All the extracts along with pure markers (1% strychnine in methanol and 1% brucine in methanol) were run in thin

layer chromatography chamber for 30 minutes. The developed chromatogram is dried and first detected at UV-254nm in UV chamber. Then they were detected by spraying with dragendorff's reagent. The Rf values of pure markers and test samples were compared for the presence of major strychnine and brucine alkaloids in the test samples.

Animals

Adult, normal Swiss albino mice (18-22g) of both sexes were used for the experiment. They were housed in well ventilated room under standard husbandry conditions, fed with standard rodent pellet diet (Lipton India Ltd., Mumbai, India) and with tap water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee (reg. no. 157/99/CPCSEA).

Acute toxicity study

Acute toxicity was carried out in Swiss albino mice. The animals were divided into 14 groups (5 groups for UNV, 9 groups for PNV) of 10 animals. The animals were fasted for 4 hours with free access to water throughout study and treated with UNV with varying doses of 200 to 300mg/kg and PNV with varying doses of 2000 to 2800mg/kg orally. The animals were observed for 72 hours for mortality [15, 16]. For the determination of acute toxicity, the percent mortality values are converted to probit values by reading the corresponding probit units from the probit table. A correction factor is applied to 0 and 100 per cent mortality group. The dose is converted to log dose. The data from Table 1 & 2 revealed the dose corresponding to percentage of mortality of animals in UNV and PNV respectively. The LD_{50} value for UNV and PNV were determined by plotting the probit values against log doses and read LD_{50} value as the dose that correspond to probit 5 (Fig. 2 & 3).

Results

The phytochemical investigation showed the presence of alkaloids, glycosides, tannins, proteins and fixed oils in all the extracts. The presence of strychnine and brucine alkaloids was confirmed by chemical tests. The presence of major alkaloids strychnine and brucine were identified on TLC by comparing the Rf values of standard chemical markers with test extracts. The strychnine and brucine spot diameter is less in PNV when compared with UNV. In the present study, it is found that the LD_{50} of unprocessed nux-vomica seed powder is 256mg/kg and sodhana processed seed powder LD_{50} is 2600mg/kg.

Table 1: Acute toxicity studies of UNV in mice.

Group	Dose mg/kg, oral	Log dose	Dead/Total	% Dead	Corrected %	Probit
1	200	2.30	0/10	0	2.5*	3.04
2	225	2.35	1/10	10%	10	3.72
3	250	2.39	2/10	20%	20	4.16
4	275	2.43	6/10	60%	60	5.25
5	300	2.47	10/10	100%	97.5*	6.96

***corrected for 0% dead =100(0.25/n) and 100% dead =100(n - 0.25/n).**

Table 2: Acute toxicity studies of PNV in mice.

Group	Dose mg/kg, oral	Log dose	Dead/ Total	% Dead	Corrected %	Probit
1	2000	3.30	0/10	0%	2.5*	3.04
2	2100	3.32	1/10	10%	10	3.72
3	2200	3.34	2/10	20%	20	4.16
4	2300	3.36	2/10	20%	20	4.32
5	2400	3.38	3/10	30%	30	4.48
6	2500	3.39	4/10	40%	40	4.75
7	2600	3.41	5/10	50%	50	5.00
8	2700	3.43	6/10	60%	60	5.25
9	2800	3.44	10/10	100%	97.5*	6.96

***corrected for 0% dead =100(0.25/n) and 100% dead =100(n - 0.25/n).**

Fig.1: Detoxification (Sodhana) of nux vomica seeds by swedana process (Boiling with milk) in Dolayantra (vessel used for boiling the drug with milk)

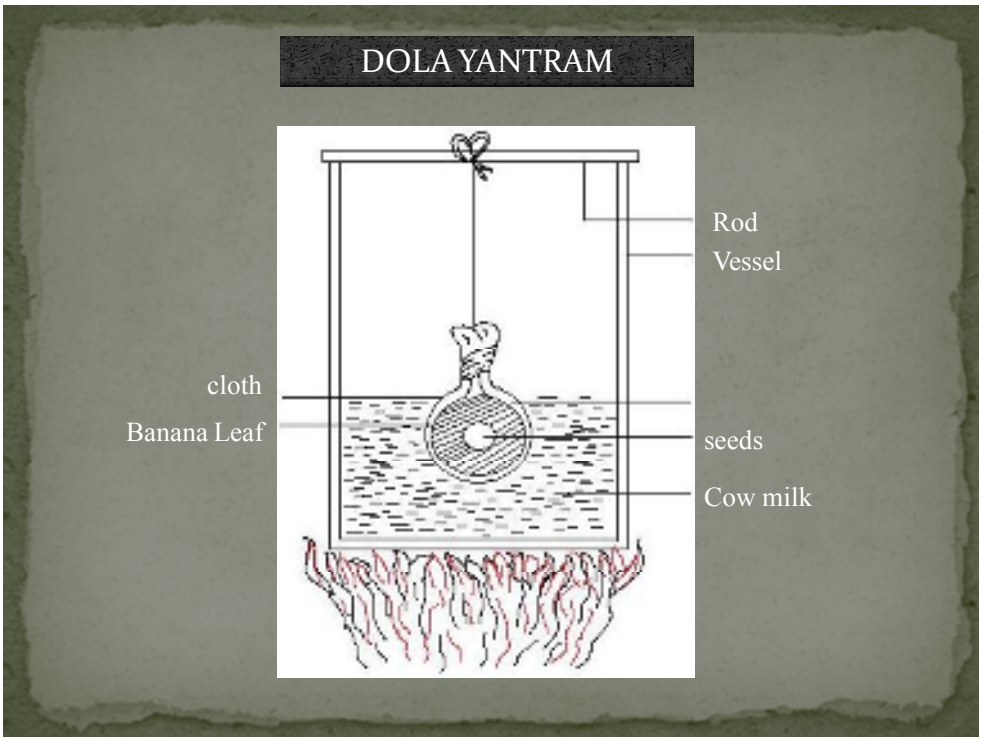


Fig 2: Determination of LD_{50} of UNV

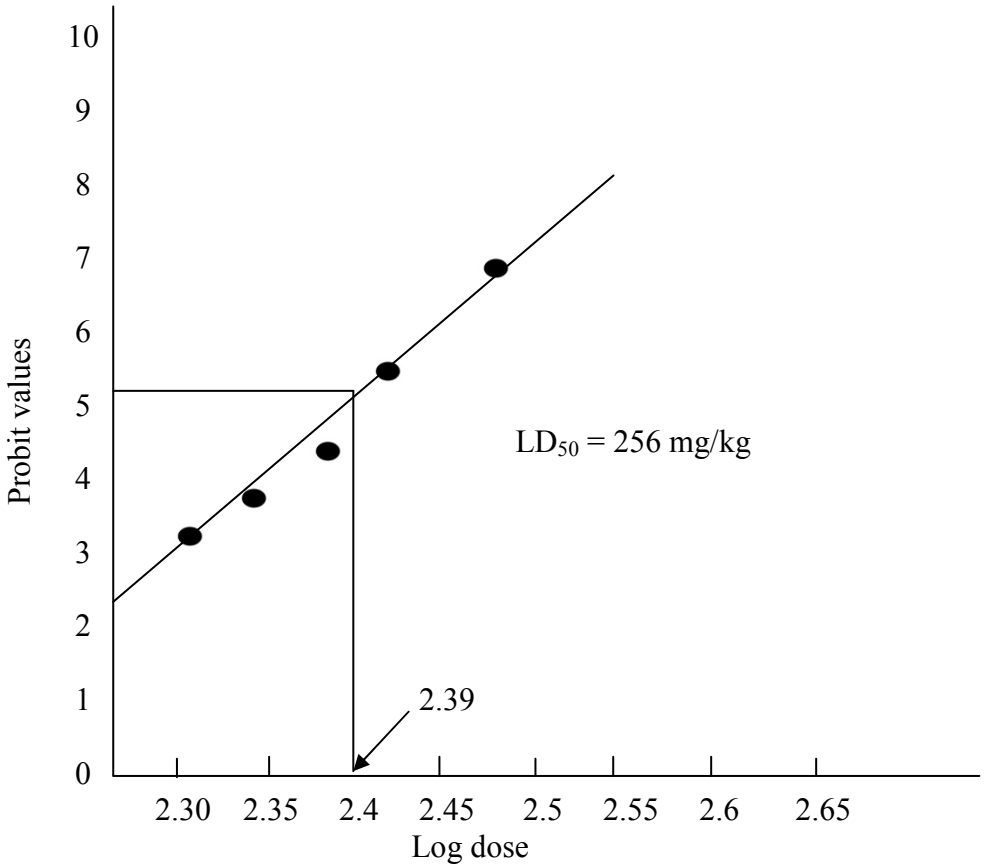
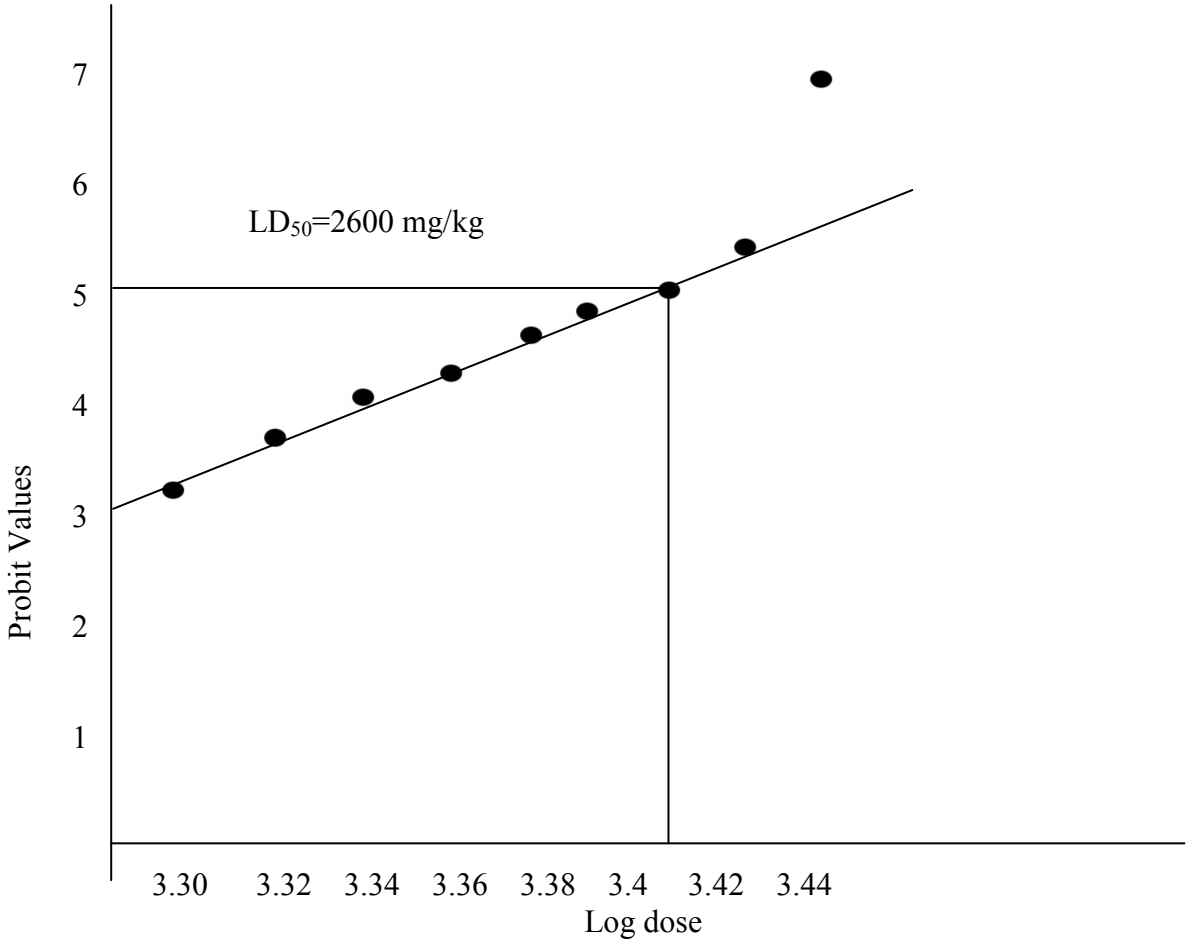


Fig 3: Determination of LD_{50} of PNV



Discussions

The strychnine and brucine contents were decreased in PNV than UNV. This is due to diffusion of these alkaloids during processing with cow urine and cow's milk. The acute toxicity of the sodhana processed products is significantly reduced ($1/10^{th}$) when compared to unprocessed seed powder. It may be attributed to elimination of major alkaloids strychnine and brucine during detoxification of seeds of *S. nux vomica* L. Further it was planned to estimate the amount of strychnine and brucine content before and after sodhana process in order to establish its safety, efficacy and influence on various biological activities.

Conclusion

Since the LD_{50} value of PNV is reduced to $1/10^{th}$ when compared with UNV. Thus, the toxicity of the processed sample is decreased significantly after sodhana process indicating that the safety and therapeutic window of the processed sample is increased after purification.

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References

1. Rasatarangani, Commentary by Acharya Haridutta Shastri, Stanza, Sunder Lal Jain Motilal Banarasidas, Varanasi, 1954; 152-164,178-202,676-680.

2. Nadakarni AK, The Indian Materia Medica, Popular prakashan, Mumbai, Vol.2, 1976; 1175-1180.

3. Kirtikar KR, Basu BD, Indian medicinal plants, 2nd ed., Periodical experts book agency, New Delhi, Vol. 2, M/s Bishen singh Mahendrapal singh, Dehradun, 1975;2:1645-1647.

4. Wu Yin., Tian-Shan Wang, Fang-Zhou Yin and Bao-Chang Cai, Analgesic and anti-inflammatory properties of brucine and brucine-N-oxide extracted from seeds of *Strychnos nux-vomica*, Journal of Ethnopharmacology 2003; 88:205-214.

5. Xu-Kun Deng, Wu Yin, Wei-Dong Li, Fang-Zhou Yin, Xiao-Yu Lu et al, The anti-tumor effects of alkaloids from the seeds of *Strychnos nux-vomica* on HepG2 cells and its possible mechanism, Journal of Ethnopharmacology 2006, 106:179-186.

6. Gricilda Shoba F. and Molly Thomas, Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhea, Journal of Ethnopharmacology 2001; 76:73-76.

7. Chatterjee I, Chakravarty AK, Gomes A, Antisnake venom activity of ethanolic seed extracts of *Strychnos nux-vomica* Linn., Indian Journal of Experimental Biology 2004;42: 468-75.

8. Trease GE and W.C. Evans, Pharmacognosy, 15th ed., W.B. Sauders Company, 2002: 378.

9. The Committee of the Pharmacopoeia of the Ministry of Health of the Peoples of China, Pharmacopoeia of the Peoples Republic of China, vol.1, Chemical Industry Press, Beijing, 2000:38.

10. Bao-Chang Cai, Masao Hattori and Tsuneo Namba, Processing of Nux-vomica II. Changes in alkaloid composition of the seeds *of Strychnos nux vomica* on traditional drug processing, Chem Pharm Bulletin 1990;38:1295-1298.

11.M.Grieve, A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-Lore of Herbs, Grasses, Fungi Shrubs & Trees with their Modern Scientific Uses, 3rd ed., Tiger Books International, London, 1992:592.

12.Cai BC, Yang WX, Zhu WU, Lu JC and Ye DJ, Effect of processing on the extraction of alkaloids from *Strychnos nux-vomica* L, Journal of Chinese Herbal Medicine1993;18:23,62.

13. Pharmacopoeia of the People´s Republic of China (English ed.), The Pharmacopeia Commission of RPC, Beijing 1995: 38-39, Part 1.

14.Wagner H, Blandt S, Zgainski EM, Translated by T.A. Scott, Plant drug analysis, A Thin Layer Chromatography Atlas, Wagner, Springer-Verlag, New York, Inc., 2nd ed. 1996:2,3,25.

15. Kulkarni SK, Hand book of Experimental Pharmacology, Vallabh Prakashan, Delhi, 2007:168-171.

16. Miller, LC and Trainter, ML, Proc.Soc., Exptl. Biol. Med.57 : 261,1944.

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