

KUPILU – VARIOUS SHODHANA PROCEDURES WITH HPLC ANALYSIS

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ABSTRACT

Kupilu is a poisonous drug used as medicine in *Ayurvedic* formulations, after its *shodhana* procedure. Many procedures are explained in *Ayurvedic* classics for *Shodhana* of a toxic drug to reduce its toxic effects. This study consists of 8 different types of *shodhana* procedure. HPLC analysis was done for each sample to find out percentage decrease in Strychnine and Brucine. The study was conducted to understand the role of different *Shodhana* procedures in changing its chemical constituents. The media selected for *Shodhana* were cows milk, cows dung, cows urine, cows ghee and *kanji*. Total eight samples were taken for the study. All the eight *shodhana* procedures brought about significant changes in Strychnine and Brucine. The most remarkable change occurred in the cows milk *shodhita* sample followed by cows urine +cows milk +cows ghee sample showing remarkable changes.

KEYWORDS: Kupilu, Strychnine, Brucine, Shodhana.**INTRODUCTION**

Kupilu means *Strychnos nux-vomica*, is derived from a Greek word *Strychnos*, means poisonous and *nux-vomica* means having vomiting effects.^[1] *Kupilu* is a poisonous drug of vegetable origin, included in *upavisha varga*.^[2] Its *rasa* is *katu, tikta, kashaya; vipaka* is *katu; virya* is *ushna* and its having *laghu, ruksha* and *tikshna guna*.^[3] Literature claims that though it's a poisonous drug, it can be used for therapeutic purpose after its *shodhana* which gives not only *gunantar dhanam* but also *guna utkarsha*^[4] effect. Its *matra* for the therapeutic purpose is $\frac{1}{4}$ ratti-1 ratti^[5] (25- 125 mg). *Kupilu* is used in many *kalpas* like *laxmi vilas ras, agnitundi ras, navajeevan ras* etc.^[6] It's properties are *medohara, krumihara, swasahara, gulm hara, arshohara, mushika visha hara, vishtambi, rochana, agni krut, grahi, kushta hara, prameha jit, (kaiyyadev nighantu) mada krutaa, pittasra nashanam (bhavaprakash) kanthaamyahara (raj nighantu)*. Its antidote is *paribhadra (erithrina variegata) Katu, Tikta ras, Ushna virya, Laghu*.^[7] It is described in *surasadi gana* of *sushruta* as *vishmushtika* and *amradi phala varga* of *bhavaprakash*.

According to previous work done, about 18 alkaloids are identified from the seeds. The seed usually contain about 1.23 % Gram of Strychnine and about 1.55 % Gram of Brucine. Minor related alkaloids include α -colubrine, β -colubrine icajine, 3-methoxyicajine, strychnine-N-

oxide, isostrychnine-N-oxide, loganine, iso Brucine, methoxy strychnine, vomicine, novacaine, iso Brucine-N-oxide, 16-Hydroxy- α -colubrine, isostrychnine, protostrychnine, 2-Hydroxy-3-, N-oxystychnine, 44-Brucine-N-oxide diaboline.^[8]

The re- investigation in seeds resulted in isolation of two colorless monoquaternary bisindole alkaloids named as 4-N-hydroxy methyl strychnidin-17-acetic acid and 10, 11-dimethoxy-4-N- hydroxy methyl strychnidin -17-acetic acid. Isolation of colored monoquaternary bisindole alkaloids named strychnochrysin from the roots was done in one study.^[9] Strychnine has action on anterior horn cells of spinal cord and its fatal dose is 15-50mg and fatal period is 1-2 hours.^[10] Research has been done on its analgesics, anti inflammatory activity^[11], anti oxidant activity^[12], anti convulsant activity^[13], anti tumor activity^[14], anti snake venom activity^[15], anti diarrheal activity^[16], hepatoprotective activity^[17], hepatoprotective and anticholestatic activities^[18], immunomodulatory effect^[19], anti amnesic activity.^[20]

Many *shodhana* procedures have been described for *Kupilu* in literature. *Panchagavya* has been mentioned for *shodhana* of *upavisha* by *Rasa Chandanshu*. *Kanji, errand tailam, adrak swaras* are also been used for *shodhana* purpose. Though *panchyagavya* has been mentioned, till date no research work has been published on the *shodhana* process of *kupilu* using cowdung. One

of the practitioners *Vaidya Degvekarji* used *kupilu* for treating *amavata* in dose of 1 *masha* three times a day for months together. He used cowdung for *kupilu shodhana* followed by their *bharjan* with *cows ghee* and here came the need of research in this spectrum.

In this study *kupilu shodhana* has been done using eight different methods. This study is also an attempt to find out which *shodhana* procedure is ideal in reducing the toxicity of seeds with the help of High Performance Layer Chromatography analysis. This study also gives information regarding *shodhana* of *kupilu* using cowdung for the first time.

AIMS AND OBJECTIVES

To perform *shodhana* of *kupilu* by eight different methods along with its High Performance Layer Chromatography analysis.

To find out best method of *Kupilu shodhana*.

To show the efficacy and safety of *kupilu shodhana* using cow dung.

MATERIALS AND METHODS

Eight different types of *Shodhana* processes done are as follows.

1. *Shodhana* in cows ghee: Seeds were fried in cows ghee on low flame till it gets *kapish* (slightly brownish) color. Then its outer covering was removed and seeds were immediately pulverized. According to *Ras Tarangini* it is the quick method of *shuddhi* (*twaritam shuddhi aayaati*).
2. *Shodhana* in cows milk: Seeds were boiled in cows milk for 3 hours by *dolayantra* method. Then its outer covering was removed and the seeds were dried in sunlight. When dried properly it was then pulverized. According to *Ras Tarangini* it is the method of attaining *uttam shuddhi*.
3. *Shodhana* in cows urine: Seeds were soaked in cows urine for 7 days. Fresh cows urine was used each day. After 7 days they were cleaned with warm water. The outer covering was removed; seeds were dried in sunlight followed by pulverization.
4. *Shodhana* in *kanji*: Seeds were soaked in *kanji* for 3 days. The outer covering was removed and seeds were properly dried in sunlight followed by pulverization. By this process *sarvartha shuddhi* is attained according to *Ras Tarangini*.

5. *Shodhana* in cow dung: Seeds were soaked in cow dung for 7 days. Fresh cow dung was taken each day. After 7 days seeds were cleaned with warm water. Then the outer covering was removed and the seeds were dried in sunlight followed by pulverization.^[21]
6. *Shodhana* in cow dung + cow ghee: Seeds were soaked in cow dung for 7 days. Fresh cow dung was taken each day. After 7 days seeds were cleaned with warm water. Then the outer covering was removed and then seeds were dried in sunlight properly followed by frying in cow ghee on low flame till it gets *kapish* (slightly brownish) color and then seeds were immediately pulverized.^[22]
7. *Shodhana* in cows urine + cows milk: Seeds were soaked in cows urine for 7 days, then seeds were cleaned with warm water, then boiled in cows milk for 3 hours by *dolayantra* method. Then the outer covering was removed and then seeds were dried in sunlight properly then pulverized. (*siddhayaoga sangraha*)
8. *Shodhana* in cows urine + cows milk + cows ghee: Seeds were soaked in cows urine for 7 days. Fresh cows urine was taken each day. Then seeds were cleaned with warm water followed by boiling in cows milk for 3 hours with *dolayantra* method. Then the outer covering of seeds was removed and then seeds were dried in sunlight properly; then fried in cows ghee on low flame till it gets *kapish* (slightly brownish) color and then seeds were immediately pulverized.^[23]

High performance layer chromatography analysis of strychnine and brucine.

High Performance Layer Chromatography analysis was done to find out Strychnine and Brucine quantity in the *nux vomica* seeds after various *shodhana* procedures. High Performance Layer Chromatography analysis (HPLC) analysis was done by using Zorbax C8, (250X4.6mm), 5 μ m column. Mixture of buffer (Dissolve 0.58gm of ammonium dihydrogen phosphate in 1000ml water and adjust the pH to 7.2 \pm 0.1 with dilute ammonium solution) and acetonitrile in the ratio of 50:50: v/v was used as mobile phase. 25.1 mg of Brucine and 25.6 mg of Strychnine dissolved in 25 ml of methanol, further diluted to get concentration 40 μ g/ml and used as standards.

Observations for Strychnine and Brucine.

Table: 1 Results of HPLC Analysis.

Sample	<i>Shodhana</i> method	Strychnine results out of 1.23%	% Decrease in Strychnine	Brucine results out of 1.55%	% Decrease in Brucine
A	cows milk	0.26%	79	0.39%	75
B	cows urine + cows milk + cows ghee	0.28%	77	0.31%	80
C	cows urine + cows milk	0.30%	76	0.34%	78
D	kanji	0.38%	69	0.49%	68
E	cows dung + cows ghee	0.42%	66	0.51%	67
F	cows ghee	0.46%	63	0.59%	62

G	cows dung	0.47%	62	0.57%	63
H	cows urine	0.52%	58	0.68%	56

When *shodhana* done in cows milk, the Strychnine percentage reduced to 0.26 % out of 1.23%. *Shodhana* done in cows urine + cows milk + cows ghee, the Strychnine percentage was 0.28%, when done in cows urine + cows milk it reduced to 0.30%, when done in *kanji* it reduced to 0.38%, when done in cows dung + cows ghee, it reduced to 0.42%, when done in only cows ghee it reduced to 0.46%, when done in cows dung it reduced to 0.47 %, when done in cows urine it reduced to 0.52%.

When *shodhana* done in cows urine + cows milk + cows ghee the Brucine percentage reduced to 0.31 %, when done in cows urine + cows milk, it reduced to 0.34%, when done in cows milk it reduced to 0.39%., when done in *kanji* it reduced to 0.49%, when done in cows dung + cows ghee, it reduced to 0.51%, when done in only cows ghee it reduced to 0.59%, when done in cows dung it reduced to 0.57 %, when done in cows urine it reduced to 0.68%.

ROLE OF MEDIAS FOR KUPILU SHODHANA

Panchagavya, erand taila, Adrak swaras are different Medias, necessary for *shodhana* which brings *gunantardhan* and *gunutkarsha*. *Shodhana* is the processing method by which the drugs get potentiated and are enhanced therapeutically because of the removal of impurities. It means that these medias brings about change in chemical constituent of drug. Studies have confirmed presence of alkaloids in cows milk used for *kupilu shodhana* signifying the importance of role of media in the same.^[24] Some studies also showed the decreased amount of Strychnine and Brucine with significant increase in iso Strychnine, iso Brucine and their N-Oxides using thermal treatment (Cia et al.,1990). Heat treatment or constant boiling for specific time in specific media modifies the chemical constituents.^[25]

From *ayurvedic* point of view, cows milk consists ten *gunas- madhura, seetha, mrudu, snigdha, bahala, slakshna, pichila, guru, manda* and *prasanna*.^[26] *Rasapanchaka* of cows milk is *madhura rasa; guru, snigdha, manda guna; madhura vipaka* and *sheeta veerya* and that of *kupilu* is *katu, tikta, kashaya rasa; laghu, rooksha, teekshna guna; katu vipaka* and *ushna veerya*. So, cows milk and *kupilu* have properties which are mostly opposite to each other. This may be the cause of reduction in the compounds after purification of *kupilu* with cows milk.

Cows urine is *katu lavan rasa, ruksha, tikshna, ushna, laghu, pittala*. Eventhough cows urine is having these properties it is *vishahara*. In Ayurveda, cow dung has been used for treating different diseases and its properties are approximately similar to cows urine.

From Ayurvedic perspective, Cows urine, cows dung, *Kanji* are *Vatakapha shamak*, which means it will predominantly act on *vatakaphaj bhava* of seeds, thereby doing its *shuddhi*. While cows milk, cows ghee are *vatapitta shamak*, which means it will act on *vatapitta bhava* of seeds, thereby doing its *shuddhi*. *Visha* predominantly have *pittaj bhava* compared to *kapha*, so may be *vata pitta shamak* medias have good results in reducing the toxicity. In process where seeds are first processed with cow dung and then with cows ghee is nothing but first to do *vatakapha shaman* and then to do *vatapitta shamana*, thereby trying to balance *tridosha* in seeds.

EFFECTS OF PROCESSES.

All these above mentioned processes involved, *swedan* (boiling), *nimmajan* (soaking), *bharjan* (frying) which resulted as below.

1. Change in the *Panchabhoutika* constitution of a substance.
2. Change in the chemical properties i.e. enhancing pharmacological actions of a substance.
3. Change in the state and nature of a substance.
4. Causes removal of impurities.
5. *Tridosha* is bounded within limit.
6. Enhanced medicinal properties and effects.
7. Mode of action becomes safe, smooth.

DISCUSSION

The Strychnine and Brucine are very potent poisons, so to reduce its poisonous effects and to enhance its therapeutic effect, *shodhana* has been mentioned in literature. In this paper eight different types of *shodhana* procedure has been carried out and its HPLC analysis has been done. In quantitative HPLC study, alkaloidal content were decreased in all the *Shodhita* samples when compared to the raw drug.

In this study the best results were found in sample A (cows milk) and B (cows urine+cows milk+cows ghee). In sample A (cows milk) 79% and 78% reduction in Strychnine and Brucine was obtained respectively. By this method *uttam shuddhi* is attained as per literature. Even in some studies cows milk *shodhita* sample proved to be the best in comparison with cows urine and cows urine + cows ghee (Mitra et al. 2012, Agrawal VK et al. 1977, Pradeep HR et al.2000). The seeds processed in cows milk showed the lowest Strychnine content in the cotyledons, exhibited marked inhibition of PTZ induced convulsions and maximal potentiation of hypnosis, and were the safest (LD50), have also been reported by Katiyar et al. 2010. Mehta N et al. 2007, evaluated presence of alkaloid in the cows milk used for *kupilu shodhana* signifying the importance of role of media in the same. Agrawal VK 1977 evaluated that the cows milk reduces the toxicity as compared to cows ghee.

The results in sample B (cows urine+cows milk+cows ghee) obtained was 77% and 80% reduction in Strychnine and Brucine respectively. The study done by same process revealed 71.49% and 54.02% reduction in Strychnine and Brucine respectively (Mitra *et al.*, 2012). This is supposed to be the best and official method of *shodhana* which reduces maximum alkaloid from seeds (Ayurvedic pharmacopoeia of India). The Strychnine (68%) and Brucine(65%) content were decreased to about 1/3rd in this *shodhana* process when compared with raw drug. In the same study the LD50 value of cows urine+cows milk+cows ghee (2600 mg/kg) purified sample was found to be increased in comparison to the raw drug 260 mg/kg. Hence, showing the effectiveness in reducing the toxicity of the drug. In the Pharmacological study, It was observed that each step of *shodhana* process enhanced the analgesic potency of the nux-vomica seeds in this method.^[27]

The results in samples C (cows urine+cows milk) showed 76% and 78% reduction in Strychnine and Brucine respectively as against in Mitra *et al.*, 2012 study they found 91 % and 88% reduction respectively. The alkaline nature of cows urine (pH 8-10) initiated the extraction process which means amount of Strychnine and Brucine might have been removed by diffusion process into cows urine. And further boiling in cows milk the amount of Strychnine and Brucine might have been converted into isoStrychnine and iso Brucine and their N-oxidative derivatives which are having lesser toxicity. (Cai *et al.*, 1990)

In sample D(*Kanji*) the Strychnine and Brucine contents showed reduction 69% and 68% respectively while in Mitra S *et al.* 2011 study it was found to be reduced by 39.25% and 17.60% respectively. As *kanji* (pH 3.4) being acidic in nature may facilitate extraction of alkaloids like Strychnine and Brucine, along with other chemical components from nux vomica, thus stating that *kanji* is best extraction media compared to *adrak swarsa* by Mitra S *et al.* 2011. The seeds purified with *Kanji* revealed better anti-inflammatory activity. Hence, it can be suggested that for the management of acute inflammatory conditions *Kanji shodhita* samples can be preferred. In sample G (cows dung) the results obtained was 62% and 63% reduction in Strychnine and Brucine respectively, whereas in sample E (cows dung +cows ghee) it was 66% and 67% reduction respectively. The cows dung may be playing a role of extracting media whereby decreasing the alkaloids like Strychnine and Brucine, along with other chemical components from nux vomica and further processing with cows ghee might be producing their N-oxidative derivatives which are having lesser toxicity. This study deals for the first time with *shodhana* of *kupilu* using cows dung. It includes the results of not only cows dung (pH 6.62) *shodhita* sample but also cows dung +cows ghee *shodhita* sample. The results for sample F (cows ghee) was 63% and 62% reduction in Strychnine and Brucine respectively. Very few studies have been done with only cows ghee.

The results for sample H (cows urine) was 58% and 56% reduction in Strychnine and Brucine respectively. Mitra *et al.* 2012 evaluated 50% and 80% reduction, Agrawal VK *et al.* 1977 evaluated 69% and 52 % reduction while Pradeep HR *et al.* 2000 also showed significant reduction in Strychnine and Brucine respectively on *shodhana* done with cows urine.

INFERENCE

It can be inferred that, on an average all *shodhana* process brings about atleast more than 50% reduction in Strychnine and Brucine. Amongst which cows milk, cows urine+ cows milk+ cows ghee, and cows urine+cows milk reduces around 75% of Strychnine and Brucine. By means of this analysis, the vishahara property of pachagavya, is well understood.

As per mentioned in Ras Tarangini, the *shodhana* of *kupilu* in cows milk gives *uttam shuddhi* where in 79% and 75% of Strychnine and Brucine reduction has been found. *Kupilu* seeds are having vatapittolban properties. This *Uttam shuddhi* is obtained as, cows milk is vatapitta shamana thereby reducing predominantly the vatapittaj bhava from seeds.

In the *shastrokta vidhi* with cows urine+cows milk+cows ghee mentioned in Ayurvedic pharmacopoeia of India is also helpful in achieving *uttam shuddhi* in which 77 % and 80 % of Strychnine and Brucine reduction has been found. In this process vata kapha shaman and vata pitta shamana in the seeds is been achieved, thereby binding tridoshas in limits.

Kanji mentioned for *shodhana* for *sarvartha shuddhi* by *Ras Tarangini* is also well understood in which 69% and 68% of Strychnine and Brucine reduction has been found. In this process the vatakapha shaman in seeds is achieved predominantly.

Twari shuddhi word used for cows ghee *shodhita kupilu* is also well understood in which 63% and 62% of Strychnine and Brucine reduction has been found. In this *shuddhi* vatapitta shaman in the seeds is achieved predominantly.

CONCLUSION

1. All the medias used for *shodhana* purpose brings about more than 50% reduction in Strychnine and Brucine.
2. Cows ghee may be considered as the better media for *Shodhana* when compared to the others on the basis of easy applicability and time saving.
3. Cows milk and cows urine+ cows milk+ cows ghee brought about *uttam shuddhi* thereby reducing more than 75% of Strychnine and Brucine.
4. The *shodhana* of *kupilu* using cows dung also brings about around 60% *shuddhi*.
5. The *shodhana* of *kupilu* using cows dung followed by *bharjan* in cows ghee also brought around 65% *shuddhi*,

which was been used by traditional *vaidyas* as single drug therapy.

6. It can be used safely as single drug therapy after its *shodhana*.

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