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Original article

Evaluation of toxicity of 'Vatsanabha' (*Aconitum ferox*, Ranunculaceae) Before and After *Shodhana*

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ABSTRACT

Ayurvedic preparations contain toxic elements like heavy metals and other chemicals exceeding their permissible limits. Ayurvedic method of detoxification of such products involves *Shodhana*. Hence, in present paper it has been decided to replace Ayurvedic *Shodhana* process by chemical purification method and to study the benefits and/or drawbacks of the traditional Ayurvedic *Shodhana* process. Crude aconite root, Ayurvedic *Shodhana* treated aconite root and chemical *Shodhana* treated aconite root samples were evaluated for toxicity and changes by animal studies and thin layer chromatography (TLC) respectively. The results of the toxicity study suggest that the modified method of *Shodhana* is less efficient as compared to the traditional Ayurvedic *Shodhana* process. TLC studies have shown that pseudoaconitine and aconitine were converted into far less toxic substances like veratroyl pseudoaconine and benzoylaconine respectively only in traditional Ayurvedic *Shodhana*.

1. Introduction

In Ayurveda, the very first stage of purification is called *Shodhana*.[1] Chemical purification is different from this purification. In chemical purification, there is only elimination of foreign matter, however, *Shodhana* eliminates harmful matter, modifies or converts undesirable properties to desirable, enhanced therapeutic actions. By *Shodhana*, only toxic constituents from plants are either removed or made less toxic before their use in the formulation. However the *Shodhana* process requires treatment of such products with cow dung, cow urine, and cow milk, requires sunlight and special containers like Dolayantra.[1] This may not always be feasible and/or acceptable to the consumer. Also, the quality and safety of the product After *Shodhana* is not assured because of lack of standardization of the traditional method. *Shodhana* is also a lengthy and time-consuming process. Hence, there is a need to develop an alternative chemical detoxification method to give the same result as that of the traditional Ayurvedic process, which can be performed easily and is acceptable to consumers. The method to be developed should also be able to assure quality and permit standardization of the processed products. The objective of present study is to develop a chemical lab-scale method and to compare it with the traditional Ayurvedic *Shodhana* process.

2. Materials and methods

2.1. Plant material

The roots of *Aconitum ferox* (Fig. 1A) were powdered in a mixer-grinder. The powder of root is packed in a paper bags and stored in an air-tight container until use.

2.2. Shodhana by Ayurvedic method

The roots of *A. ferox* were cut into small pea-sized pieces and kept in earthen pot containing cow urine for 8 days, and on each day, cow urine was changed for 7 days. On the 8th day, cow urine was not changed. The earthen pot was kept in sunlight (Fig. 1B). Then, it was washed with cold water, the upper layer was taken out, and was again washed with warm water. The pieces of drug were dried immediately by exposing them to sunlight. The dried pieces were grinded to get the powder form.

2.3. Chemical composition of lab-prepared cow urine

Water – 95%; urea – 2.5%; minerals (nitrogen, sulfur, phosphate, sodium, manganese, carbolic acid, iron, chlorine, magnesium) and hormones (corticosteroids) – 1%; salts (malic, citric, tartaric,

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Fig. 1. (A) Aconite root. (B) Ayurvedic *Shodhana* process. (C) Chemical *Shodhana* process.

succinic, hippuric acids, calcium salts) and enzymes (urokinase) – 1.5%; vitamin A, B, C, D, E, lactose, and creatinine – 0.5%.

2.4. Shodhana by modified chemical method

The roots of *A. ferox* were cut into small pea-sized pieces of and kept in a glass beaker containing 1 l laboratory-prepared solution of cow urine[2] for 7 days. The solution was changed every day. The beaker was kept in an oven and the temperature was maintained at 37.5° C (Fig. 1C). Then, it was washed with cold water. The outer shell was removed, and washed again with warm water. The pieces were dried and ground to get the powder form. The pH of the solution was maintained at 8–8.5.

2.5. Extraction of untreated A. ferox

The crude powdered plant material was defatted with petroleum ether and then subjected to soxhlet extraction to complete 20 cycles with chloroform. The chloroform extract thus obtained was filtered and concentrated on a water bath to obtain a thick paste.

2.6. Exaction of Ayurvedic Shodhana-treated A. ferox

The Ayurvedic *Shodhana*-treated drug material was defatted with petroleum ether and then subjected to soxhlet extraction to complete 20 cycles with chloroform. The chloroform extract thus obtained was filtered and concentrated on a water bath to obtain a thick paste.

2.7. Extraction of chemically treated A. ferox

The powdered modified chemically treated *Shodhana* drug material was defatted with petroleum ether and then subjected to soxhlet extraction to complete 20 cycles with chloroform. The chloroform extract thus obtained was filtered and concentrated on a water bath to obtain a thick paste.

2.8. Toxicity study

For this study, Organisation for Economic Co-operation and Development (OECD) guideline 420 and some reference articles were followed.[3] With prior approval from Institutional Animal Ethical Committee (Registration No.751/03/abc/CPCSEA), toxicity studies were conducted on female albino rats (150–200 gm). Sighting study was performed to determine appropriate dose for main study. Sighting study gave an appropriate selection of starting dose for the main study. Four groups were prepared for three extracts and one control. A total of five animals were taken for each group. The test substance was administered using a stomach tube. From the sighting study, the dose of 5 mg/kg was selected for Before *Shodhana* Extract and Lab Scale-Treated Extract. This dose of 5 mg/kg of Before *Shodhana* Extract and Lab Scale-Treated Extract

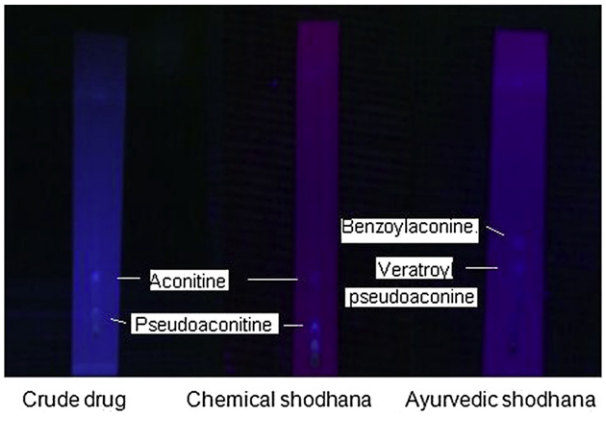

Fig. 2. Thin layer chromatography studies showing changes in chemistry of aconite root after and before different treatments. Stationary phase – Silica gel G; mobile phase – Benzene:Ethylacetate:Ethanol (15:4:1); detection – UV 366; running time: 8 cm.

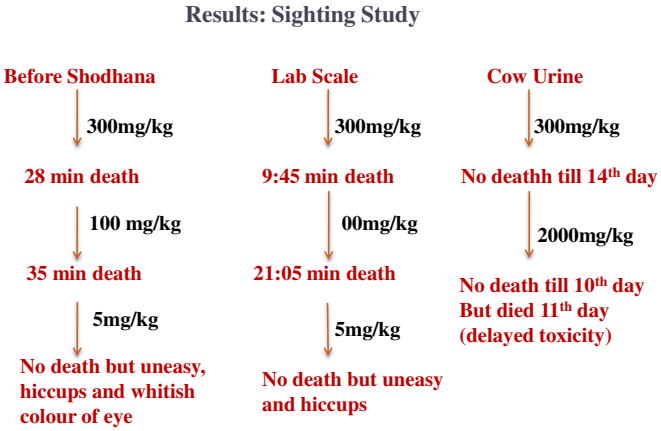

Fig. 3. Results of acute toxicity study (slighting study).

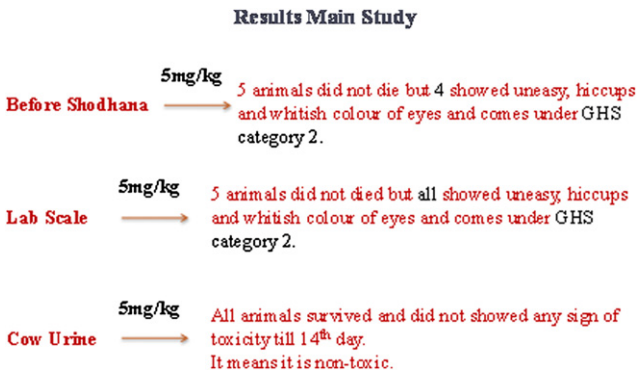

Fig. 4. Results of acute toxicity study (main study).

showed evident toxicity while 300 mg/kg for Cow Urine-Treated Extract showed no toxicity till the 14th day. After the 14th day, the blood was withdrawn from the retro-orbital plexus of rats and pathological laboratory tests were performed that might be affected by the drug.

2.9. Statistical analysis

Data were analyzed using Dunnet test following one-way analysis of variance (ANOVA), using

GraphPad Prism 6, USA. $P > 0.05$ was considered as non-significant.

2.10. Chromatographic studies

Thin layer chromatography (TLC) studies have been performed to observe changes in aconite alkaloids.[4,5] The stationary phase employed was Silica gel G and mobile phase was Benzene:Ethylacetate:Ethanol (15:4:1). Detection was done in a UV cabinet using a 366-nm lamp. The running distance was 8 cm.

3. Results and discussion

Crude drug without any treatment, traditional Ayurvedic method *Shodhana*-treated drug material, and lab-scale chemically prepared cow urine-treated drug material were extracted with chloroform separately. These extracts were further evaluated by TLC to identify changes that might have occurred during *Shodhana* treatment.[6,7] TLC studies have shown that pseudoaconitine and aconitine were converted into far less toxic substances veratroyl pseudoaconine and benzoylaconine respectively only in traditional Ayurvedic *Shodhana* (Fig. 2). As aconite is a poisonous drug in Ayurveda, it was decided to observe how *Shodhana* or chemically treated drug shows changes in its toxic effects by animal toxicity studies. Pathological parameters of animal blood samples were also evaluated.[8,9] In slighting study, 300 mg/kg of Before *Shodhana* Extract (suspension of chloroform extract of Before *Shodhana* crude *A. ferox*) was given and monitored death occurred within 28 min. Another animal was dosed at 100 mg/kg of Before *Shodhana* Extract, death occurred within 35 min. Another animal again dosed at 5 mg/kg, no death was observed but the animal was found to be uneasy, had hiccups, and there was whitish color in its eyes (Fig. 3).

In a sighting study, 300 mg/kg of Lab Scale-Treated Extract (suspension of chloroform extract of After *Shodhana* modified chemically treated *A. ferox*) was given and monitored death occurred within 9:45 min. Another animal was dosed at 100 mg/kg of Lab Scale-Treated Extract and death occurred within 21:05 min. Another animal was dosed at 5 mg/kg, no death was observed but the animal was found to be uneasy and had hiccups.

In a sighting study, 300 mg/kg of Cow Urine-Treated Extract (suspension of chloroform extract of After *Shodhana* ayurvedically treated *A. ferox*) was given and monitored. No death occurred till the 14th day. The animal was found to be normal. Another animal was dosed at 2000 mg/kg of Cow Urine-Treated Extract, no death occurred till the 10th day but the animal died on the 11th day, which indicates delayed toxicity.

In the main study, the animals were observed for first 24 h at an interval of 30 min. with special attention during 1st 4 h, and daily thereafter, for a total of 14 days. Additional observations were also noted like behavior and color of eye (Fig. 4).

In the main study, 5 mg/kg of dose was defined for Before *Shodhana* Extract as per sighting study and given to one group of animals containing five animals each. The animals did not die but showed uneasiness, hiccups, and whitish color of eye. This was observed in all four animals which mean that the drug comes under Globally Harmonized System of Classification and Labeling of Chemicals (GHS) category 2.

In the main study, 5 mg/kg of dose was defined for Lab Scale-Treated Extract as per sighting study and given to second group of animals containing 5 animals each. The animals did not die but showed uneasiness and hiccups. This was observed in all four animals which means the drug comes under GHS category 2.

In the main study, 300 mg/kg of dose was defined for Cow Urine-Treated Extract as per sighting study and given to the third group of animal containing 5 animals each. All animals survived

Table 1
Results of biochemical parameters for toxicity study in female albino rats.

Extract (mg/kg)	Death/recovery	Lipid profile				
		Total cholesterol (mg%)	HDL (mg%)	LDL (mg%)	VLDL (mg%)	Triglyceride (mg%)
Control	No death till 14th day	98.378 ± 0.2668	49.045 ± 0.4601	36.530 ± 0.3724	12.930 ± 0.3679	64.003 ± 0.1841
Before *Shodhana* 5 mg/kg	Death within 28 min. at 300 mg/kg. 35 min at 100 mg/kg and no death but uneasy, hiccups and whitish color of eye at 5 mg/kg	150.00 ± 2.614***	61.648 ± 0.708***	70.268 ± 1.876***	31.573 ± 0.213***	156.69 ± 0.217***
Lab-scale 5 mg/kg	Death within 9:45 min at 300 mg/kg 21:05 min at 100 mg/kg and no death but uneasy and hiccups at 5 mg/kg	147.75 ± 3.326***	62.793 ± 0.136***	56.703 ± 3.217***	28.988 ± 0.313***	153.98 ± 0.401***
Cow urine 300 mg/kg	No death till 14th day at 300 mg/kg	101.98 ± 0.0394ns	81.230 ± 0.115ns	7.108 ± 0.147***	18.603 ± 0.201***	64.218 ± 0.201ns

Mean ± SD; $n = 5$ in each group; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns$P > 0.05$ when compared with control; HDL, LDL, VLDL.

Table 2
Results of biochemical parameters for toxicity study in female albino rats.

Extract (mg/kg)	Death/recovery	Urea (mg%)	BUN (mg%)	Creatinine (mg%)	Albumin (mg%)
Control	No death till 14th days	55.190 ± 0.08371	25.768 ± 0.04049	0.5250 ± 0.02500	2.800 ± 0.04082
Before *Shodhana* 5 mg/kg	Death within 28 min. at 300 mg/kg. 35 min at 100 mg/kg and no death but uneasy, hiccups and whitish colour of eye at 5 mg/kg	90.225 ± 0.058***	42.130 ± 0.028***	0.8750 ± 0.025***	4.750 ± 0.028***
Lab scale 5 mg/kg	Death within 9:45 min at 300 mg/kg 21:05 min at 100 mg/kg and no death but uneasy and hiccups at 5 mg/kg	88.958 ± 0.096***	41.538 ± 0.044***	0.8500 ± 0.028***	4.475 ± 0.025***
Cow urine 300 mg/kg	No death till 14th day at 300 mg/kg	55.598 ± 0.084*	25.843 ± 0.154ns	0.4000 ± 0.000*	3.000 ± 0.000*

Mean ± SD; $n = 5$ in each group; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns$P > 0.05$ when compared with control; BUN.

and did not show any sign of toxicity till the 14th day. It means it is non-toxic. Both Before *Shodhana* Extract and Lab Scale-Treated Extract significantly increased ($P < 0.001$) serum concentrations of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), triglyceride, urea, blood urea nitrogen (BUN), creatinine, and albumin. Cow Urine-Treated Extract also significantly increased the HDL and VLDL but significantly decreased the LDL serum concentration of at-administration doses compared to control. However, it did not alter the total cholesterol, triglyceride, and BUN (Tables 1 and 2).

4. Conclusion

Though treatment with cow urine, cow milk, or cow dung is the traditional method of *Shodhana*, it may not be feasible or acceptable to all. The results of the present study suggest that the modified method of *Shodhana* is less efficient as compared to traditional Ayurvedic *Shodhana* process. TLC studies have shown that pseudoaconitine and aconitine were converted into far less toxic substances veratroyl pseudoaconine and benzoylaconine respectively only in traditional Ayurvedic *Shodhana*. TLC studies also revealed that chemical purification failed to convert toxic alkaloids to less toxic alkaloids. Chemical *Shodhana* process shows toxic effects on organs like liver, heart, and kidney. Thus, it is confirmed that the Ayurvedic *Shodhana* process is one of the powerful methods of detoxification and purification. Sincere attempts should be made to develop standardized and quality assured Ayurvedic *Shodhana* processes. Further studies are focused to evaluate other alternative *Shodhana* processes that use cow milk instead of cow urine.

Conflicts of interest

All authors have none to declare.

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