9. D] Methodology followed till end of period of reporting

4) *Aconitum chasmanthum* roots (*Vatsnabha*)

Aconitum chasmanthum Stapf ex Holmes is a plant belonging to the Ranunculaceae family. It is synonymously called Indian Napellus. In Hindi it is known by the name *Mohri* and in Kashmiri *Ban-bal-nag*. Roots of Aconitum are used as a drug and have similar uses as that of *A. ferox*. The plant is found in Kashmir, Lahaul and in Chamba forest division of Himachal Pradesh.[1,2]

The toxic component of *Aconitum chasmanthum* is the alkaloid aconitine.[3] The alkaloidal content of the roots ranges from 2.98 to 3.11 per cent. The other alkaloids found in *A chasmanthum* are indaconitine, chasmaconitine, chasmanthinine, chasmanine, and homochasmaconitine. The root is used in rheumatism and fever. Rhizomes are reported to be used in fever, skin diseases, enlargement of spleen and neuralgia.[3] Aconitine is one of the most deadly poisons. Symptoms set in almost immediately after ingestion, the most common features being tingling of the tongue, mouth and stomach, which spreads to all parts of the body and is accompanied by a burning sensation followed by numbness. In the Ayurvedic system this drug is used in conditions of fever, diarrhea, dyspepsia, sprue and bronchitis.[4]

Aconitum chasmanthum roots were collected from Darjeeling, West Bengal and microscopy of the roots studied to check the authenticity of the sample. Also the roots were authenticated by Dr. H M Pandit, Botanist, Guru Nanak Khalsa College, Mumbai.

Sample Preparation: The process of sodhana was carried out on the roots of Aconitum and then the sample was subjected to the following process. 5 g of the root sample was extracted with methanol in a Soxhlet assembly till exhaustion. The volume of extract was made up to 50 ml with methanol and this was used for the quantification studies. Pre as well as post-*sodhit* samples were subjected to the same process.

Ayurvedic *sodhana prakriya* for Aconitum: The roots of aconitum (100g) were boiled in the *dolayantra* for a period of 3 *prahars* in *gomutra* (1 litre) (Cow's urine, pH between 7.8 to 8.2). Then they were washed with warm water and dried. The dried roots were then subjected to *svedana* process using cow's milk for 3 *prahars*. The samples were consequently washed with warm water and dried.[5] The samples were further used for quantitative analysis. Also the medium was retained for analysis.

Modified Method I: The roots of aconitum (25g) were boiled in the *dolayantra* for a period of 3 *prahars* in alkaline medium (pH 7-8)(250ml). Then they were washed with warm water and dried. The dried roots were then subjected to *svedana* process using chloroform/ghee/oil medium for 3 *prahars*. The samples were consequently washed with warm water and dried. The percentage of chloroform/ghee/oil in the medium was 3.5%v/v. This was adjusted to match the content of fat in cow's milk.

Modified Method II: *Terminalia chebula* fruits (50g) were boiled with 500ml of water for 3 hours to form a decoction. This decoction was filtered and further used for the detoxification of aconitum. 25g of the roots were boiled in the *T chebula* decoction for 2 hours.

Quantification Studies: The High Performance Liquid Chromatograpy (HPLC) technique was used for the quantification of the aconitine in the samples of *Aconitum chasmanthum*.

HPLC analysis was performed with a Jasco (Hachioji, Tokyo, Japan) system, using 250 mm × 4.6 mm i.d., RP-18 (5-µm particle size) column, an intelligent pump (PU-1580, PU-2080), a high-pressure mixer (MX-2080-31), a manual sample injection valve (Rheodyne 7725i), with a

flow rate of 1.00 ml/min, Injection volume loop: 20 µl., monitoring at 230 nm (UV-1575), and elution program: 15 min isocratic, (ACN:MeOH): Buffer:: (40:10):40 where Buffer: KH_2PO_4 buffer solution (10mM adjusted to pH 7.5±0.1 with 1 % triethylamine). Chromatographic data were processed with Borwin software.[6]

Samples for HPLC analysis were prepared by further diluting the various extract solutions as per the need of the process. A standard curve was set between 20-100µg/ml. Standard of aconitine was procured from ChromaDex, USA. All reagents used were of Analytical Grade or HPLC grade.

10. D] Interim modification of objectives/methodology, if any : NA

11. D] Summary on progress:

The roots of *Aconitum chasmanthum* were subjected to the Ayurvedic *sodhana prakriya*. *Aconitum chasmanthum* samples were subjected to HPLC analysis to determine the content of aconitine in these samples. In addition to the aconitum samples the media used for *sodhana prakriya* was also analyzed for the content of aconitine.

Table 1: Quantification of Aconitine in the various samples treated by different methods before and after the treatment along with the aconitine concentration in the medium used.

Method used	Pre-sodhit sample (Aconitine (mg) in 5g sample)	Medium (Aconitine (mg))	Post-sodhit sample (Aconitine (mg) in 5g sample)
Conventional	4.577	3.144	0.543
Chloroform	4.577	3.6395	1.236
Ghee	4.577	3.920	1.488
Oil	4.577	3.791	0.8016
T. chebula treated	4.577	Complexed with tannins	0.743

A standard curve was set up in the range of 20-100µg/ml of aconitine with a correlation coefficient of 0.981. The Rt for standard aconitine was found to be 5.04 min.

Fig 1: HPLC chromatogram for 40 µg/ml of aconitine standard.

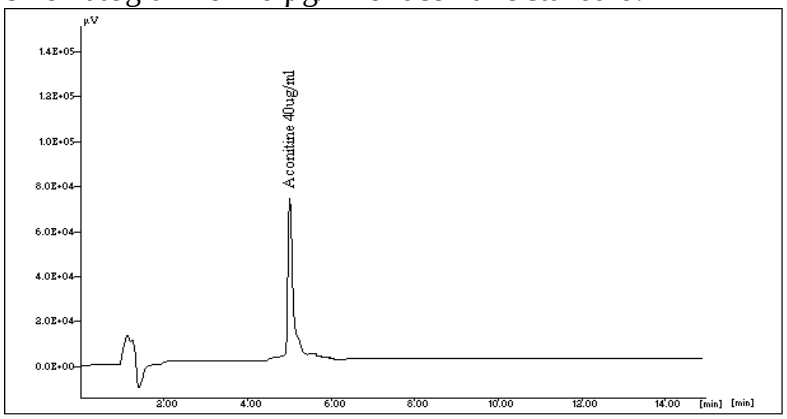
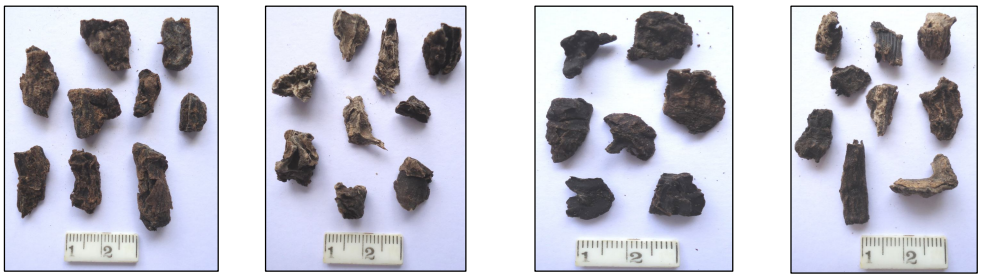


Fig 2A: *Aconitum chasmanthum* roots (a) and *Aconitum chasmanthum* root pieces prior to treatment

(a) (b)

Fig. 2B: Post-*sodhit Aconitum chasmanthum* roots treated by conventional method(a), chloroform method(b), oil method (c) and ghee method (d)



(a) (b) (c) (d)

Discussion

The roots of *Aconitum chasmanthum* have been used in Ayurveda since many years. They have been used in conditions of fever, diarrhea, dyspepsia, sprue and bronchitis. But the roots contain a toxic principle, aconitine which is alkaloidal in nature and in high doses is not recommended for internal use. Hence the roots have been subjected to the Ayurvedic *sodhana prakriya* so as to either chemically modify the component to some less toxic derivative or to reduce the concentration of that component to tolerable limits.

The screening of the pre-*sodhit* samples of *Aconitum chasmanthum* has indicated the presence of aconitine in the samples to an extent of about 0.09% *w/w* on dry weight basis. The post-*sodhit* samples have shown to contain significantly reduced amounts of aconitine. The percentage of aconitine in the conventionally treated sample was found to be 11.86% of the original concentration of that found in the sample before treatment. The concentration of aconitine in the chloroform treated sample was 27%, in ghee treated sample was 32.51%, in oil treated sample was 17.51% and in *T chebula* treated sample was 16.23% of the original content of aconitine in the pre treated sample. The results obtained from the HPLC quantification studies have indicated that both the processes namely conventional and the modified using the different media for the *sodhana prakriya* have led to a significant decrease in the content of aconitine in the sample.
The *sodhana prakriya* prescribed for Aconitum involves boiling of the sample in *gomutra* for 9 hours and then with milk for 9 hours subsequently. Since aconitine is an alkaloid, at alkaline pH it remains in its free base form. Aconitine gets solubilized in the fatty fraction of the milk. The analysis of the medium used for *sodhana prakriya* shows presence of aconitine. Also the post-sodhit samples of *Aconitum* show small quantities of the aconitine. Hence it can be concluded that the decrease in the toxicity of the Aconitum roots post- *sodhana* treatment is due to the decrease in its concentration. The mechanism involved in the above process is the solubilization of the aconitine in the milk. With this possible mechanism involved an attempt was made to put forth an alternate medium for milk and hence the sample was treated with chloroform/ghee/oil. Also the method using *T chebula* decoction leads to the complexation of the aconitine with the tannins from the decoction. Hence the aconitine percentage in the *T chebula* treated sample also shows decrease in the aconitine content. Results obtained from the alternate media are comparable to the conventional method used and hence they could be used as an alternate media for the *sodhana prakriya* in place of milk.

The experimental results suggest that the *sodhana prakriya* for *Aconitum chasmanthum* roots

leads to the decrease in the concentration of the toxic component, aconitine due to solubilizaton in the treatment medium. Further the results also indicate that the proposed methods with a change in the treatment media are at par with the conventionally used Ayurvedic *sodhana prakriya* with respect to the detoxification process.

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9. E] Methodology followed till end of period of reporting:

5) *Semecarpus anacardium* fruits (*Bhallatak*)

Semecarpus anacardium Linn. is a tree belonging to the Anacardiaceae family. It is synonymously called Marking Nut Tree or Oriental Cashew. In Hindi it is know by the names *bhela* or *bhilawa* and in Marathi *bibha* or *bhilava*. The fruits of Semecarpus are used as drug in the Ayurvedic system of medicine. The tree is found all over India. [1]

The pericarp of the fruit yields a black, oily, bitter, highly vesicant oil. This vesicant liquid is known in trade as Bhilawan Nut Shell Liquid (BNSL). It contains a polyphenolic component called bhilawanol ($C_{21}H_{32}O_{2)}$, semicarpol, and other phenolic components. The toxicity of the fruit is due to these polyphenolic components found in the oil of the pericarp.

The fruits of Semecarpus are acrid, hot and anthelminthic; it is considered beneficial in ascites, tumors and warts, acute rheumatism, asthma, neuralgia, epilepsy and psoriasis. The juice of the pericarp and also of the tree trunk is a powerful counter-irritant and vesicant. It causes painful blisters that tend to spread to the adjacent areas of the skin. In Ayurvedic system of medicine it is used in skin diseases, syphilis, scrofula, worms, rheumatism and leprosy.[2] Semecarpus pericarp oil shows such toxicity hence it is used in Ayurveda only after subjecting it to the *sodhana prakriya*.

Semecarpus anacardium fruits were collected from Chhindwara, Madhya Pradesh. The authenticity of the fruits was checked by macro and microscopic studies. Further the fruit sample was authenticated by Dr. H M Pandit, Botanist, Guru Nanak Khalsa College, Mumbai.

Sample Preparation: The process of *sodhana* was carried out on the fruits of Semecarpus and then the sample was subjected to the following extraction process. Pericarps from 5 g of the fruit samples were extracted with hexane in a Soxhlet assembly till exhaustion (24 hours). The extract obtained were made up to 100ml with hexane. The extract so obtained was further used for the quantification studies. Pre as well as post-*sodhit* samples were subjected to the same process.

Ayurvedic *sodhana prakriya* for *Bhallatak*: Fruits of *S anacardium* were dropped in a beaker of water. Fruits that sunk to the bottom were used for the *sodhana prakriya*. 100g of the fruits of *S anacardium* whose thalamus was detached were buried in 500g of brick powder for 7 days. They were intermittently rubbed with the brick powder. After 7 days the fruits were wiped and then boiled in milk (500ml) for 1 *prahar*. The fruits were then washed with water and dried.[3]

Modified Method: With an aim to find an alternative to the traditional *sodhana prakriya*, studies were also carried out using alternate method. Since the toxic components were estimated to be present in the oil part of the pericarp, the modified method was devised so as to reduce the oil component. The method involves heating of the fruits and expression of the oil from the fruit by the heating process.

Fig. 1 Ayurvedic *sodhana prakriya* of *Semecarpus anacardium* fruits

Quantification Studies: UV spectrophotometric methods were used for the estimation of phenols and proteins in the different samples of Semecarpus and the medium used.

Phenolic component analysis: A solution of catechol of concentration 2mg/ml in acetone was made. From this stock solution of concentration 0.1 mg/ml was prepared. Aliquots of concentration range 10-50µg/ml were prepared by transferring 1, 2, 3, 4, 5 ml of stock solution in five different 10ml volumetric flasks. To it, added 0.5ml of $TiCl_4$ reagent (20% $TiCl_4$ in HCl) and then volume was made up to 10ml with acetone. Similarly, blank was prepared with acetone and reagent. The solutions were thoroughly mixed on vortex for 15 sec. A coloured complex between phenolic compounds and $TiCl_4$ formed immediately, the absorbance of which was measured at 430nm against an equivalent blank.

Preparation of $TiCl_4$ reagent (20% $TiCl_4$ in HCl): 10ml of $TiCl_4$ was transferred to a 50ml volumetric flask containing little quantity of HCl. The transfer of $TiCl_4$ was kept slow to avoid its solidification. Finally volume was made up to 50ml.

Protein assay: Bio-rad Protein Assay Dye reagent concentrate was diluted 5 times to form the working dye solution. Casein standard solutions between 20-100µg/ml were prepared. For the assay 100µl of each of the std. solutions and the samples were taken in different test tubes. To this added 5 ml of the working dye solution and mixed and kept for 15 min after which the absorbance of the complex formed was measured at 595nm. A blank was also prepared by omitting the sample.
All reagents used were of Analytical Grade.

10. E] Interim modification of objectives/methodology, if any : NA

11. E] Summary on progress:

The fruits of Semecarpus were subjected to the Ayurvedic *sodhana prakriya*. *Semecarpus anacardium* samples were subjected to UV spectrophotometric analysis to determine the percentage of the phenols in them and the medium used was also analyzed by this method to determine the percentage of protein in the samples.

Table 1: Quantitative analysis of phenols in the *S anacardium* samples. Phenol content of 5g of sample.

Method used	Pre-sodhit sample (mg)	Post-sodhit sample (mg)	Difference
Conventional	67.3815	43.2765	24.105

Standard curve for catechol was set in the range of 10-50 with a correlation coefficient of 0.9999. Absorbance was measured at 430nm.

Table 2. Quantity of free protein in 25 ml of the medium prior to treatment and that after the treatment.

Content of free protein in the medium		Content of protein used for complex formation (mg)
Prior to treatment (mg)	Post treatment (mg)	
535	6.5	528.5

Fig. 2 Pre-*sodhit Semecarpus anacardium* fruits (a), post-*sodhit* conventionally treated *Semecarpus anacardium* fruits (b) and post-*sodhit* heat treated *Semecarpus anacardium* fruit pericarps (c).

(a) (b) (c)

Discussion

The fruits of *Semecarpus anacardium* have been used in Ayurveda since many years. They have been used in skin diseases, syphilis, scrofula, worms, rheumatism and leprosy. But the oil from the pericarp of the fruits contain toxic phenols, cause dermal toxicity. Hence the fruits have been subjected to the Ayurvedic *sodhana prakriya* so as to decrease their toxicity.

The screening of the pre-*sodhit* samples of *Semecarpus anacardium* have indicated the presence of higher quantities of polyphenols in the sample. The post-*sodhit* samples have shown to contain significantly reduced amounts of polyphenols. The percentage of polyphenols in the milk treated sample was found to be 35.77% of that of the original polyphenol content.

0.535g of free protein was found in 25ml of the milk medium used for the treatment of 5 g of Semecarpus sample. The protein content of the medium left after the treatment was 6.5 mg. Hence the difference in the protein before and after the treatment ie.528.5 mg, was proposed to

be used for the complexing of the phenol from the sample during treatment.

The toxic component of *S anacardium* are the polyphenols present in the pericarp of the fruit. The conventional method of *sodhana* involved boiling of the fruits in milk, which leads to the complexation of the polyphenols with the milk proteins leading to decrease in the concentration of these polyphenols from the fruit pericarp. Thus the toxicity of *S anacardium* is reduced.[4] The polyphenols present in the pericarp are the major components of the oil, hence the modified method developed for *S anacardium* involves the reduction in the quantity of oil present in the pericarp of the fruit and consequent decrease in the toxicity.

The experimental results suggest that the *sodhana prakriya* for *Semecarpus anacardium* fruits leads to the decrease in the concentration of the toxic component, polyphenols due to the complexation with the milk proteins. Further since the toxicity of *Semecarpus anacardium* is due to the polyphenols present in the pericarp the decrease in their percentage could lead to decrease in the toxicity of the *sodhit* sample and the alternate method also targets the decrease in the oil content of the pericarp consequently leading to the decrease in the polyphenol content. Hence it can be used as an alternate method to the Ayurvedic *sodhana prakriya*.

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