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BIO-ANALYTICAL STUDIES ON THE PROCESS OF DETOXIFICATION AND SAFETY EVALUATION OF *ACONITUM LACINIATUM* AND *ABRUS PRECATORIUS* FOR USE IN AYURVEDIC PREPARATIONS

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ABSTRACT

Objective: The aim of the study was to have bio-analytical approach for detoxification process of two poisonous plant materials viz. *Aconitum laciniatum* (root) and *Abrus precatorius* (seed) which are used in Ayurvedic preparations.

Materials and Methods: For both the species proximate analysis was carried out. For the same plants HPTLC fingerprinting was compared , before and after detoxification process, using triphala quath. Infra red spectral studies for *Abrus precatorius* species (red and white) were compared, with respect to detoxification process. Protein fingerprinting was carried out for various Aconitum species available in the market. In support of the results obtained from the above methods for detoxification, safety evaluation , post single dose administration, C1- post single dose administration]was done using albino mice as the study model for *Aconitum laciniatum* (root) and *Abrus precatorius* (seeds- red and white).

Outcome Measures: Comparison of the results for the crude poisonous herbal material with the material obtained after detoxification in triphala quath.

Results: HPTLC fingerprinting, Infra red spectral studies, safety evaluation study (animal toxicity) showed that process of detoxification for the above mentioned plant materials using triphala quath is effective and less time consuming.

Conclusions: The study highlights that the commonly used poisonous crude herbal materials viz. *Aconitum laciniatum* (root) and *Abrus precatorius* (seeds - red and white) can be safely used in Ayurvedic preparations, after detoxification using triphala quath.

INTRODUCTION: Again People are shifting from the modern medicines to the ancient systems of medicines like Ayurveda. Ayurveda is a very comprehensive medical system which has been practiced for generations in India. Ayurveda is time tested system of medicine but, one must be able to explain the various processes used by our ancient system in terms of modern language and methodology acceptable to the modern world.

Various methods of detoxification are given in the authoritative books of Ayurveda for *Aconitum laciniatum* and *Abrus precatorius*; but the checks or tests for completion of the detoxification process do not find any mention in the books.

Aconitum species are very commonly used in Ayurvedic preparations like Tribhuvan kirti, Sutshekhar etc. after mitigation or detoxification. The same process is called as 'shodhan' in Ayurveda. In small dose aconitum acts as heart stimulant but in large dose the same acts as heart depressant [2]. Therefore proper detoxification process is a must.

Abrus precatorius has antifertility and anticancer activities. Overdose may cause vomiting, diarrhea and cardiac arrest [1, 3].

Methods of detoxification reported in the literature for aconitum root mainly use animal derived substances like cow milk, cow urine, goat milk etc. But the advantage in the use of triphala quath [5] is that the material being derived from plant we have a choice for the quality to be mentioned relatively easily.

A study was reported, which was done for crude aconite and with 'samskaras ' before being used in the Ayurvedic formulations [7].

In another study the roots were used after mitigation, i.e., soaking them in cow urine or milk (2-3 days) tilll they become soft [2].

Aconite tubers are traditionally macerated in cow's urine and kept in the Sun , for 3 days the urine is renewed every day .This treatment decrease the toxic alkaloid content by 60% [9].

Detoxifidcation of aconite root is done by traditional methods and studied chromatographically. Medium of detoxification used is cow urine, milk, steam cooking . TLC after detoxification revealed the presence of benzoic acid which confirmed that the process involves hydrolysis of the alkaloids to their respective amino acids [13]. C2 was any study done with all the testing methods adopted with materials of the 2 test plants processed using the Ayurvedic traditional methods cited here? What were the results? / If these have not been done/ or not being reported in this paper, please correct the reporting to factually state the same. This is important]

For Abrus species methods reported in Ayurvedic literature for detoxification were using cow milk, rice kanji and lemon juice [1, 6].

Seeds are boiled in cow milk for 6 hours then they are washed with hot water and used [5, 6, 8].

In another study kanji is prepared from rice (decoction of rice in water).Seeds of *Abrus precatorius* are boiled in it for 3 hours [5, 6, 8, 10].

In another methods seeds of *Abrus precatorius* are bundled in cotton cloth and soaked in lemon juice in suspended position (dolayantra) and cooked for 3 hours [6].

The chief poisonous constituent of the seed is abrine and is inactivated by heat [2].

The study was reported with aq. extract of unprocessed and processed (milk as well as kanji shodhit) white, red and brown varieties of seeds administered i.p. in Albino mice [11]..

Acute toxicity studies of the processed and the unprocessed seeds revealed the LD_{50} to be 0.5 to 1.0 mg. and LD_{100} between 1.0 and 2.0 mg/kg. b.w. in white and red variety of the unprocessed seeds, respectively. The value increased in the processed material with both milk and kanji shodhan The LD_{50} was 1.0 to 2.0 mg. in milk shodhit and 15.05 to 20.0 mg in kanji shodhit seeds, while LD_{100} was 2.5 to 5.0 mg/kg.b.w. in milk shodhit and 20.0 to 30.0mg/kg.b.w. in kanji shodhit seeds of red and white form respectively [11].

Another reference reports that alcoholic extract of seed kernels was non toxic upto dose of 60mg/kg p.o. in rats. subcutaneously LD_{100} of water extract of seeds in mice was found to be 2mg/kg and orally 25mg/kg, caused death in 40% animals.

MATERIALS AND METHODS :

Plant Material: Roots of *Aconitum laciniatum* and seeds of *Abrus precatorius* were procured from market. Few more samples available in the market by the name aconite were also obtained for comparison purpose. For preparation of triphala quath, a mixture of fruit samples of *Terminalia chebula* Retz (Haritaki), *Terminalia belirica* Roxb. (Bibhitaka) and *Emblica officinalis* (Amalaki) in 1:1:1 w/w proportion was used in powder form.

To 1kg of this coarse powder mixture 16 lit. potable water was added and soaked for 4-5 hours. The mixture was heated till volume reduced to 4 lit. (Initially on high flame and after boiling on low flame) It was then filtered and used for detoxification process. [C3 Please state the quantum of water used, any concentration done by boiling and to what extent, what was the yield and any straining /filtration done etc]

All the above mentioned plant materials were authenticated from Agharkar Research Institute, Pune. The specimen have been deposited and the authentication certificate numbers are:

Sample Name	Certificate No.
Aconitum laciniatum Stapf	9-70
Aconitum palmatum D. Don.(Ativish)	10-85
Aconitum heterophyllum Wall ex Royle (Ativish)	10-86
Aconitum laciniatum stapf (Aconitum white,/safed bachnag)	10-87
Processed and chared sample (Aconitum black/black bachnag)	10-84
Abrus precatorius (red)	10-143
Abrus precatorius (white)	10-144
Terminalia chebula Retz	10-46
Terminalia bellirica Roxb	10-45
Emblica officinalis Gaertn.	10-44

[C4- Please state whether any specimen has been deposited with them?, and give specimen numbers]

Methods: Various bio-analytical methods were used to study the quality of plant material and the process of detoxification of roots of *Aconitum laciniatum* and seeds of *Abrus precatorius* .

1. **Proximate analysis**: It is one of the diagnostic features of the plant drug. Determination of ash values were carried out for the plant material in order to remove carbon matter and calculate the ash contents.. Extractive values of two solvents namely water and ethanol revealed percentage of active components present in the plant material.

2. **2D-Paper Chromatography**: The analysis of phenolic acids in aqueous extract of *Aconitum laciniatum* root was carried out by using two-dimensional ascending paper chromatography. The mobile phases used were Toluene: acetic acid: water (6:7:3 v/v) in the first direction and sodium formate: formic acid: water (10:1:200 v/v) in the second direction. The spray used to locate the compounds on the chromatograms were diazotized p-nitraniline or diazotized sulphanilic acid and 10% sodium carbonate overspray [16].

3. **HPTLC fingerprinting at 254 nm**:

   a) HPTLC method was used for comparing Aconitine standard with *Aconitum laciniatum* root, crude and detoxified samples. For detoxification *Aconitum laciniatum* roots were boiled for 4 hr in triphala quath.

   b) Triphala quath boiled for 4 hr, without *Aconitum laciniatum* root for 0 to 4 hr and with *Aconitum laciniatum* root for 4 hr was studied.

   c) HPTLC fingerprinting for crude *Aconitum laciniatum* root, triphala quath in which *Aconitum laciniatum* roots were boiled for 4 hr and triphala quath boiled without *Aconitum laciniatum* roots for 4 hr was compared.

Four more market species of aconitum root namely safed bachnag i.e. *Aconitum laciniatum*, black bachnag i.e. processed sample, ativish i.e. *Aconitum palmatum* root and *Aconitum heterophyllum* root were detoxified by the same method using triphala quath. For each species crude sample was compared with the detoxified sample using HPTLC method. The chromatograms of these samples were compared with chromatogram of the standard Aconitine.

Sample preparation for aconitum species (crude and detoxified) and Aconitine standard:

The Sun dried crude and detoxified samples of aconitum were powdered to 80 mesh size. To 0.5 g powder 5 cm^3 methanol and one drop of ammonia solution was added. The mixture was sonicated for 5 minutes at room temp. and filtered through ordinary filter paper. The filtrate was used for developing HPTLC fingerprint.

The standard Aconitine, about 95% pure was procured from SIGMA, Code No. A 8001. Three mg of the standard Aconitine was diluted to 3 cm3 with HPLC grade methanol to produce 1000 ppm stock solution. From 1000 ppm stock solution 100 micro lit. solution was diluted to 1 cm^3 with HPLC grade methanol to have 100 ppm working standard, which was used for further HPTLC analysis. [C5- For all these

solutions/extracts obtained, Please state the quantum of water used, any concentration done by boiling and to what extent, what was the yield and any straining /filtration done, drying process, temperatures, and quantity of dry powders obtained etc etc]

4. **HPTLC fingerprinting and IR spectra**: For *Abrus precatorius* seed, red and white coloured samples were studied. Detoxification was done by boiling seeds in triphala quath for 3 hr in 'dolayantra' after separating the seed coat and also by keeping the 80 mesh powder of samples in hot air oven at 100^{o}C for 1 hr [4] [same C5 above].

HPTLC fingerprinting and Infra Red spectroscopic studies were done for crude and detoxified samples of red and white *Abrus precatorius* seeds.

Preparation of *Abrus precatorius* seeds crude and detoxified samples for HPTLC: The Sun dried crude and detoxified samples (red and shite seeds) were popwdered to 80 mesh size. To 0.1 g powder of each sample 0.5 cm3 methanol was added. The mixture was allowed to stand for 30 minutes at room temperature and then filtered through ordinary filter paper. The filtrate was used for developing HPTLC fingerprint.

Preparation of *Abrus precatorius* seeds crude and detoxified samples for IR spectra: IR spectra were recorded in KBr pallets.

5. **Protein finger-printing**: Protein extraction was done using SDS-PAGE gel(sodium dodecyl sulphate-polyacrylamide gel electrophoresis). MALDI-TOF (Matrix Assisted Lasar Desorption Ionisation-Time of Filght) technique was used to differentiate between plant roots using medium molecular weight marker. Protein band patterns were compared for four aconitum root samples namely Aconitum black, *Aconitum laciniatum*, safed bachnag i.e. *Aconitum laciniatum* and *Aconitum heterophyllum* [Same C5 above].

6. **Safety evaluation**: Safety evaluation was carried out using Albino Swiss Mice (female). In each group 3 animals were taken. Permission of Animal Ethics Committee was obtained, after submitting protocol for conducting safety evaluation of *Aconitum laciniatum* root and *Abrus precatorius* seeds to Ramnarain RuiaCollege (Institutinal Animal Ethics Review Committee), Mumbai. The approval was obtained vide approval letter dt. 29-1-2011. For *Abrus precatorius* seeds (red and white) Protocol No. SB100924-01 (59) and for *Aconitum laciniatum* root Protocol No. SB100924-02 (60). This was an acute single dose administration study. The study was conducted according to the OECD guidelines [12]. [C6 please state whether any Animal Ethics committee permissions were obtained, what was its number and date, did the committee ask to use 3 animals per group. Also state that this was a ACUTE, SINGLE dose administration study- PLEASE give reference to the protocol, by the by this type of study which was in official in USP is no longer is used, any comments].

Aqueous slurry of the crude and the detoxified samples of Aconitum *laciniatum* roots and *Abrus precatorius* seeds (red and white) was given to animals orally, via a gavage. Detoxification was done by boiling crude samples in triphala quath for 4 hr and 3 hr respectively [Same as C5 above].

For *Aconitum laciniatum* root the dose was 1.2g/kg body weight for crude plant material and 2.4g/kg body weight for detoxified plant material.

For *Abrus precatorius* seed, red and white the dose was 32 mg/kg body weight for crude plant material and 64 mg/kg body weight for detoxified plant material.

The dose determination was done for both the species by considering percentage of toxic phytochemical present in the plant material and $LD_{50}/_{40}$ value reported in the literature.

For aconitum species total alkaloid content is near about 1.5% [2]. LD_{50} in mice is 1.8 mg/kg orally [4]. From these two values the dose was determined for crude material, considering body weight of animals A. Double dose was used for detoxified material.

In case of *Abrus precatorius* seed, red and white, abrin present in seed is 0.15% [2] and LD_{40} is 25 mg/kg orally for water extract in mice [2]. From these values thedose for crude material was determined and double dose was used for detoxified material [3]. [C7- what was the rationale of the dose decisions, please give the basis and details].

RESULTS:

1. **Proximate Analysis**:

Results of Phytochemial Analysis of *Aconitum laciniatum*:

1. The average loss on drying: 6.07 %
2. The average total ash: 2.80 %
3. The average acid insoluble ash: 0.31 %
4. The average water soluble ash: 0.74 %
5. The average water soluble extractive: 32.29 %
6. The average ethanol soluble extractive: 11.46 %
7. The average alkaloids: 0.07 %

Results of Phytochemial Analysis of *Abrus precatorius* seed:

1. The average loss on drying: 9.30 %
2. The average total ash: 2.91 %
3. The average acid insoluble ash: 0.47 %
4. The average water soluble ash: 1.53 %
5. The average water soluble extractive: 17.29 %
6. The average ethanol soluble extractive: 4.07 %

2. **2D** Paper Chromatographic analysis for *Aconitum laciniatum* root: Phenolic acids identified in *Aconitium laciniatum*:

1. Vanillic acid
2. Cis ferulic acid
3. Trans ferulic acid
4. Cis p-coumaric acid
5. Trans p-coumaric acid

3. **HPTLC fingerprinting at 254 nm**:

a) **Comparison of Aconitine standard, *Aconitum laciniatum* root crude and detoxified sample, which was treated in triphala quath for 4 hr**: The standard Aconitine shows a single spot at Rf 0.61. Crude *Aconitum laciniatum* root also showed a spot at same Rf. In case of the detoxified *Aconitum laciniatum* root, even though the Rf is same as 0.61, AUC (area under the curve) for the spot decreases from 1 hr to 4 hr heating in triphala quath (T1 to T4). When the plate was sprayed with Dragendroff's reagent, it also indicated the presence of the alkaloid at Rf 0.61 in the standard Aconitine and in the crude. In detoxified samples AUC of alkaloid decreasesd. After 2 hr of heating it was nearly absent, indicating detoxification of *Aconitum laciniatum* root by boiling in triphala quath (**Fig. 1**, **Table 1**).

b) **Comparison of triphala quath boiled without *Aconitum laciniatum* root for 0 to 4 hr and with *Aconitum laciniatum* root for 4 hr**: Triphala quath was boiled for 4 hr and samples were withdrawn at 0, 1, 2, 3, 4 hr. They were spotted on HPTLC plate and developed under conditions mentioned in **Table 2** Triphala quath was boiled with *Aconitum laciniatum* root for 4 hrs and sample was withdrawn after 4 hr. It was spotted on HPTLC plate and developed under similar experimental conditions. The spot at Rf 0.61 was not observed in blank triphala quath at 0, 1, 2, 3, 4 hrs., while it was observed in a sample drawn at the end of 4 hr., when triphala quath was boiled with *Aconitum laciniatum* root [9] **(Fig. 2).** This may indicate the transfer of alkaloid Aconitine from crude material to triphala quath during the detoxification process.

c) **Comparison of crude *Aconitum laciniatum* root, triphala quath boiled with *Aconitum laciniatum* root and triphala quath boiled without *Aconitum laciniatum* root for 4 hr:** HPTLC comparison proved that spot at Rf 0.61 for alkaloid Aconitine was present in crude *Aconitum laciniatum* root and in triphala quath boiled with *Aconitum laciniatum* root for 4 hr (tB). The same spot was absent in triphala quath boiled without *Aconitum laciniatum* root for 4 hr (t4). This may suggests that the toxic alkaloid from crude sample was extracted in triphala quath during process of detoxification, as the same was absent in triphala quath.

TABLE 1: CHROMATOGRAPHIC CONDITIONS FOR HPTLC PLATE TREATED WITH DRAGNDROFF'S REAGENT (FOR FIG. 1)

Parameter	Description
Instrument	CAMAG HPTLC
Stationary Phase	Silica Gel 60 F 254 precoated plates
Band Width	8.0 mm
Spotting Volume	20, 30 micro lit. for Aconitine Std. 10 micro lit. for other samples
Mobile Phase	Toluene : Ethyl acetate : Diethylamine (7:2:1 v/v)14
Volume of Mobile Phase	10 cm3
Development Mode	CAMAG Twin Trough Chamber
Development Distance	8.0 cm
Chamber Saturation Time	30 minutes

TABLE 2: CHROMATOGRAPHIC CONDITIONS FOR HPTLC PLATE AT 254nm (FOR FIG. 2)

Parameter	Description
Instrument	CAMAG HPTLC
Stationary Phase	Silica Gel 60 F 254 precoated plates
Band Width	8.0 mm
Spotting Volume	10 micro lit.
Mobile Phase	Chloroform:Methenol (9:1 v/v)15
Volume of Mobile Phase	10 cm3
Development Mode	CAMAG Twin Trough Chamber
Development Distance	8.0 cm
Chamber Saturation Time	30 minutes
Scanning Wavelength	254 nm

TABLE 3: CHROMATOGRAPHIC CONDITIONS FOR HPTLC PLATE AT 254nm (FOR FIG. 3)

Parameter	Description
Instrument	CAMAG HPTLC
Stationary Phase	Silica Gel 60 F 254 precoated plates
Band Width	8.0 mm
Spotting Volume	10 micro lit.
Mobile Phase	Toluene : Ethyl acetate : Diethyl amine (7:2:1 v/v)14
Volume of Mobile Phase	10 cm3
Development Mode	CAMAG Twin Trough Chamber
Development Distance	8.0 cm
Chamber Saturation Time	30 minutes
Scanning Wavelength	254 nm

d) **Comparison of four more market species of Aconitum root:** Four species of aconitum namely safed bachnag (*Aconitum laciniatum* root), black bachnag (processed sample), and two species of ativish (*Aconitum palmatum* and *Aconitum heterophyllum)* were compared by HPTLC method.

It was found that safed bachnag (*Aconitum laciniatum* root) was poisonous, containing toxic alkaloid, which was detected by spot at Rf 0.61. After treatment with triphala quath for 4 hr the spot for toxic alkaloid Aconitine was absent.

In case of black bachnag, the sample was processed. This may prove that the sample was previously treated for the process of detoxification, hence the spot of toxic alkaloid at Rf 0.61 was absent.

For two other species *Aconitum palmatum,* and *Aconitum heterophyllum* spot at Rf 0.61 was absent in crude root sample.

The above observations indicate that, in the case of aconitum species, detection of poisonous nature can be easily done by HPTLC method, by comparing with the standard alkaloid Aconitine. Detoxification of poisonous species can also be done easily by boiling in the triphala quath. [C8- For all the HPTLC and Paper chromatography testing done, please geive details of the mobile phase, solutions of reference substances and the test materials made, dilutions obtained, quantity of solutions applied for each spot, mobile phase used, saturation time, length of solvent front allowed to flow, etc.]

4. **HPTLC fingerprinting and IR spectra for *Abrus precatorius* seeds, red and white coloured**

a) HPTLC fingerprinting for *Abrus precatorius* seeds, red and white coloured.

Detoxification was done by two methods.

i) *Abrus precatorius* seeds (red and white) were boiled with triphala quath in 'dolayantra' for 3 hr.

ii) Powders (120 mesh) of red and white coloured *Abrus precatorius* seeds were kept in hot air oven for 1 hr at 100^{o}C [4].

It was found that for both the samples of seeds i.e. red and white, when compared with crude, there was not much difference in HPTLC pattern, when the samples were kept in oven for 1 hr at 100^{o}C [4]. The toxic protein abrin might be stable in crude form, while unstable in pure form when kept at 100^{o}C for 1 hr.

In case of detoxification using triphala quath, the results of detoxification were acceptable, as almost all peaks from the crude seed samples disappeared in triphala quath treated samples indicating detoxification.

TABLE 4: CHROMATOGRAPHIC CONDITIONS FOR HPTLC PLATE AT 254NM (FOR FIG. 4)

Parameter	Description
Instrument	CAMAG HPTLC
Stationary Phase	Silica Gel 60 F 254 precoated plates
Band Width	8.0 mm
Spotting Volume	10 micro lit.
Mobile Phase	Ethyl acetate : Methanol (1:1 v/v) (Developed in this work)
Volume of Mobile Phase	10 cm3
Development Mode	CAMAG Twin Trough Chamber
Development Distance	8.0 cm
Chamber Saturation Time	30 minutes
Scanning Wavelength	254 nm

b) **I R spectra of *Abrus precatorius* seeds**: Spectra were recorded in KBr pallets. The results of IR spectroscopy also supported the above conclusions. Spectra obtained in case of crude seeds and detoxified in oven were almost similar. While IR spectra in case of seeds treated with triphala quath showed variation at functional group region of the spectra, at wavelength 2349 cm^{-1} for both red and white seeds of *Abrus precatorius.* [C9 – for IR please give the method used was it KBr disc method or knujol method]

5. **Protein fingerprinting**: DNA fingerprinting is used for the identification of the correct plant species. However it does not help to identify the desired part of the same plant, for which protein fingerprinting is a complementary technique, since protein expression is different in different parts of the same plant. In the present study protein fingerprinting technique was used to identify roots of Aconitum black, *Aconitum laciniatum* (aconitum white), *Aconitum laciniatum, Aconitum heterophyllum.*

For protein extraction reagents and chemicas used were liquid nitrogen, 1M Tris HCl pH 7.5, sucrose, Triton X-100. Phenyl methyl sulfonyl fluoride, EDTA, Tri chloro acetic acid. Protein extraction buffer is prepared from these reagents. Appropriate (20-25 micro gram) concentration of the protein from four different aconitum species was loaded on a 15% SDS PAGE gel (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). Protein bands of four aconitum root species were compared with medium molecular weight marker. The two species namely *Aconitum laciniatum* and safed bachnag showed the same protein bands, indicating that both the roots are same. Aconitum black and *Aconitum heterophyllum* showed presence of different molecular weight protein, hence represent roots of different plant species. [C10- please give details of solution used, gel media etc] [6]. Safety evaluation of *Aconitum laciniatum* root and *Abrus precatorius* seeds (red and white). [C6 please state whether any Animal Ethics committee permissions were obtained, what was its number and date, did the committee ask to use 3 animals per group. Also state that this was a ACUTE, SINGLE dose administration study- PLEASE give reference to the protocol, by the by this type of study which was in official in USP is no longer is used, any comments]

a) **Safety evaluation of *Aconitum laciniatum* root**: Animal model selected for the study was Albino Swiss Mice – female, with weight around 25 g.

Three groups of animals were made with three animals per group. First group was control group. Group 2 was given aqueous slurry of roots of *Aconitum laciniatum*. The dose was 1.2g/kg.body weight (single dose). Group 3 was given aqueous slurry of detoxified roots (in triphala quath) of *Aconitum laciniatum* 2.4gkg.body weight (single dose). The study duration was 14 days acclimatization and 14 days post dosing observations.

Mortality observations: For the Group 2, with crude plant material mortality was 66.66%. While for Group 3 with detoxified plant material, no mortality was observed even though the dose was double.

b) **Safety evaluation of *Abrus precatorius* seeds-red**: Experimental conditions were the same as above (a), only the dose was different.

First group was control. Group 2 was given aqueous slurry of seeds of *Abrus precatorius* - Red . The dose was 32 mg/kg.body weight (single dose). Group 3 was given aqueous slurry of detoxified seeds (using triphala quath) of *Abrus precatorius* - Red. The dose was 64 mg/kg body weight.

Mortality observations: For the Group 2, with crude plant material mortality was 33.33%. While for Group 3 with detoxified plant material, no mortality was observed even though the dose was double.

c) **Safety evaluation of *Abrus precatorius* seeds-white**: Experimental conditions were same as above (a), only the dose was different.

First group was control. Group 2 was given aqueous slurry of seeds of *Abrus precatorius* - White. The dose was 32 mg/kg. body weight (single dose). Group 3 was given aqueous slurry of detoxified seeds (using triphala quath) of *Abrus precatorius* -White. The dose was 64 mg/kg body weight.

Mortality observations: For the Group 2, with crude plant material mortality was 100%. While for Group 3 with detoxified plant material, no mortality was observed even though the dose was double.

In all the above three cases of the mortality, it was due to respiratory disorders and breathlessness. For other animals there was gradual increase in body weight. Daily water and food intake was also normal.

DISCUSSION: Various poisonous materials are used by our ancient systems of medicines like Ayurveda, Unani and Siddha. The literature available may tell us which methods we should follow for detoxification but, the scientific reasoning behind the process was unexplained. Hence, this was the attempt to scientifically study the process of detoxification for *Aconitum laciniatum* root *and Abrus precatorius* seeds (red and white) using triphala quath as detoxifying solvent.

Proximate analysis indicates the quality of crude material. Results of various methods used for analysis like HPTLC fingerprinting, IR spectra, safety evaluation were supporting the results for the method of detoxification used. Protein fingerprinting, 2-D paper chromatography was useful in authentication of the roots of herbal materials. In case when poisonous materials are used after detoxification in drug preparations, the materials must be critically evaluated in terms of modern scientific parameters. These plant derived materials should be carefully standardized and their efficacy and safety for a specific therapeutic application should be checked.

In the present research work safety evaluation was done with single dose administration, and that more detailed acute and chronic studies will be required before finalizing this 'Triphala quath' based purification/detoxification to be adopted..11- Please make it clear that the safety evaluation was done with single dose administration, and that more detailed acute and chronic studies will be required before finalizing this TRPHALA quath based purification to be adopted. Authors to consider making this part reflect in the title of the paper also.

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