

Studies on *sodhana prakriya* of *Gunja* (*Abrus precatorius* Linn.) seeds

Nabar^{1*} Manasi, Pimpalgaonkar² PB & Laddha¹ KS

¹Medicinal Natural Products Research Laboratory, Department of Pharmaceutical Science and Technology,
Institute of Chemical Technology, Matunga, Mumbai-400 019, Maharashtra

²Government Ayurvedic College, Tuljapur Road, Madhuban, Osmanabad-413501, Maharashtra

Email: ph09mp.nabar@ictmumbai.edu.in, drpramod53@gmail.com, ks.laddha@ictmumbai.edu.in

Received 01.06.10; revised 15.11.10

Ayurveda 'the Science of life' is a system of traditional medicine, native to Indian subcontinent and practiced in other parts of the world as alternate system of medicine. Ayurveda is currently followed by millions of people in India, Nepal and Sri Lanka along with many of their counterparts in the western countries. While most of the drugs referred in Ayurveda are found to be safe, there are few which contain toxic constituents in them necessitating detoxification process of '*sodhana*' prior to their use as a drug. The process of *sodhana* leads to detoxification of the drug without interfering in its therapeutic properties (*gunas*). The current paper deals with the *sodhana prakriya* for *Gunja* (*Abrus precatorius* Linn.) seeds. The study aims at evaluating the underlying principle and thereafter, to find out if any alternate process can be substituted for the conventional *sodhana prakriya*.

Keywords: *Sodhana, Abrus precatorius, Gunja*

IPC Int.Cl.: A61K 36/00, A61P 3/04, A61P 3/06

Ayurveda 'the science of life' is a system of traditional medicine native to Indian subcontinent and is currently used by millions of people in India, Nepal and Sri Lanka for their day-to-day healthcare needs along with their western counterparts¹. Ayurvedic system of medicine is well founded on the basic principles of nature and its elements after a careful and thorough study of human physiology². Most of the drugs used in the system of Ayurveda are obtained from plant, animal or mineral sources. Common examples of drugs used in Ayurveda are castor oil (*Ricinus communis*), acorus (*Acorus calamus*), aloes (*Aloe barbadensis*), guggul (*Commiphora weightii*), turmeric (*Curcuma longa*), honey, *moutik bhasma* (pearl oxide), *swarna bhasma* (gold oxide), etc. Some of the drugs used in Ayurveda are found to contain certain toxic principles. Examples of such drugs are *Bhallatak* (*Semecarpus anacardium*), *Datura* (*Datura metel*), *Gunja* (*Abrus precatorius*), *Vatsanabh* (*Aconitum ferox*), *Vekhand* (*Acorus calamus*), etc. These plant products are subjected to a detoxification process prior to their use as medicine. This process of detoxification of the drug without harming its medicinal properties (*gunas*) is referred to as the

process of '*sodhana*' or '*sodhana prakriya*' in Ayurveda. The Ayurvedic *sodhana prakriya* is formulated in such a way so as to reduce the toxic effects of the harmful constituent and also in some cases to potentiate the effect of the constituents of the drug. Some of the *sodhana* processes commonly employed in Ayurveda are *Bhavana* and *Swedana* which make use of different liquids, few of them even obtained from animal sources like *godugdha*, *gomutra* and some are the products of the fermentation process like *kanjika*.

Jequirity seeds or *Gunja* is one such drug that undergoes the process of *sodhana* prior to its use as a medicine in Ayurveda. *Gunja* have been used as a medicine in Ayurveda and contain a toxalbumin and a hemagglutinin. The process of *swedana* has been used for the detoxification of *gunja*.

The current study is related to the *Abrus precatorius* seeds. *Abrus precatorius* Linn. is also synonymously called Crab's eye, Indian Liquorice or Jequirity and is commonly known as *rati* in Hindi and *chanoti*, *gunchi* or *gunja* in Marathi. The seeds are ovoid, scarlet with a black spot round the hilum, or black with a white spot, or uniformly black or white, glossy. Current study deals with the seeds having scarlet colour with a black spot round the hilum. The

*Corresponding author

seeds of this plant are poisonous. The bruised seeds have been used for poisoning cattle, for homicidal purpose and as an abortifacient. In the indigenous system of medicine, seed extracts are used externally for the treatment of ulcers and skin affections. The seeds have also been used internally in the affections of the nervous system and their paste is applied locally in sciatica, stiffness of shoulder joints and paralysis. They are said to be useful in diarrhea, dysentery and possess anthelmintic activity. Ethanolic extract of seeds has shown anti-bacterial and anti-fungal activities. The seeds show purgative, emetic, tonic and aphrodisiac properties. Powdered seeds are said to affect the uterine functions and prevent conception in women. Poultice of the seeds is used as a suppository to bring about abortion^{3,4}.

The principle toxic constituents of *Gunja* are abrin, a toxalbumin and hemagglutinin. Abrin is a highly toxic protein present in the seeds to an extent of about 0.15 %. Abrin consists of two polypeptide chains A and B bound by disulphide bond. The toxic action of abrin is associated with the A-chain and that the B-chain functions as a carrier moiety necessary for binding of the toxin to the cell surface⁵. *Abrus* agglutinin is a bivalent tetramer of 134,900 Daltons⁶. The toxic effects seen after ingestion of *Gunja* are hemorrhagic, gastroenteritis with erosions, hemolysis, acute renal damage, hepatotoxicity with elevated liver enzymes and seizures⁷.

The current work aims at studying the underlying principle, with an attempt to understand the possible mechanism involved in the detoxification of the *Gunja* (*Abrus precatorius*) seeds by the *sodhana prakriya* described in Ayurveda and thereafter to find out if an alternate process can be devised for the conventional *sodhana prakriya*. With the conventional processes using different media obtained from biological sources, an attempt is also made to suggest more simpler, acceptable and non-biological media.

Methodology

Abrus seeds were collected from the local market of Mumbai. Saline (Nirlife Healthcare) was purchased from the local medical store. The process of *sodhana* was carried out on 3 samples, namely the entire seed, separated seed coat and the embryo to ascertain the localization of the hemagglutinin in the seeds. *Gunja* seeds, seed coats and embryos were then subjected to the subsequent procedures. One gm of the seed sample was macerated in water for 1 day at room

temperature. The sample was filtered and rendered isotonic and filtered through a 0.2 micron filter. The filtrate so obtained was used for the study. Pre as well as *post-sodhit* samples were subjected to the same procedure.

The entire seeds, seed coats and embryos of *Gunja* were steamed using *kanjika* in a *dolayantra* for 1 *prahar* (3 hrs). Subsequently the seed coats and embryos from the intact seeds were separated, washed with warm water and dried^{8,9}. The dried samples were then treated in a similar manner as that of the *pre-sodhit* samples and then subjected to hemagglutination assay.

With an aim to find an alternative to the traditional *sodhana prakriya*, studies were also carried out using water as the medium for the *sodhana prakriya* in place of *kanjika*. In both, the media studies were carried out in two conditions, with the seeds immersed in the medium (boiling) and with the seeds exposed to the steam produced by the medium (steaming).

Haemagglutination assay

Rat blood was used for the haemagglutination assay procedure.

Procedure for obtaining Washed Red Blood Cells (RBC's): 2 ml of rat blood was collected in Appendorff tubes containing 0.2 ml of 5% EDTA solution to prevent clotting of blood. It was centrifuged at 3000rpm for 15 min to remove soluble blood constituents. Plasma was separated and replaced with equal volume of normal saline. The resuspended components were further washed (centrifuged and resuspended alternately) four times using normal saline to obtain washed RBC's. The washed RBC's were finally suspended in normal saline to obtain a concentration of 4 % v/v. A 24 well plate was used to carry out the assay^{10,11,12}.

Assay: 0.2ml RBC suspension was added to each well along with 0.2ml of the sample. A negative control (blank) was maintained using 0.2ml of saline in place of the sample. The plate was incubated at 37± 2°C for 2 hrs. Plates were read manually at the end of the incubation period. The assay was carried out in triplicates.

Results

The presence of agglutination in the blood samples is an indication of the presence of haemagglutination factor in the sample. The results stated below are with reference to rat blood samples.

Table 1—Showing hemagglutination activity of different samples of *Gunja* (*Abrus precatorius*) seeds

	Post- <i>sodhit</i> samples				Blank	Pre- <i>sodhit</i> samples
	<i>Kanjika</i>		Water			
	1 steamed	2 boiled	3 steamed	4 boiled		
A	—	—	—	—	—	—
B	—	—	—	—	—	+
C	—	—	—	—	—	—
D	—	—	—	—	—	—

Columns 1 & 2 represent post-*sodhit* samples treated using *kanjika* as the medium. Columns 3 & 4 represent post-*sodhit* samples treated with water as the medium. Column 5 represents the blank. Column 6 represents the pre-*sodhit* samples. Samples in column 1 and 3 were steamed with the medium whereas those in columns 2 & 4 were boiled in the medium (Table 1).

Row A: seed coats separated pre-treatment,

Row B: embryo separated pre-treatment,

Row C: seed coat separated post-*sodhana prakriya*,

Row D: embryo separated post-*sodhana prakriya*.

Well 6 C represents the pre-*sodhit* entire seed sample.

Pre-*sodhit* seed coat and entire seed samples did not cause agglutination. The pre-*sodhit* embryo sample caused high degree of agglutination. All the post-*sodhit* samples did not show agglutination (Fig.1).

The results obtained with both the media, namely *kanjika* and water as well as in both the conditions; boiling and steaming were the same. All the post-*sodhit* samples did not show signs of agglutination.

Discussion

Gunja contains an agglutinating factor that is responsible for the agglutination of the erythrocytes from the blood. The screening of the pre-*sodhit* samples of seed coat, embryo and the entire seed led to the conclusion that only the embryo contained the hemagglutinating factor. The hemagglutination assay carried out using the various post-*sodhit* samples indicated that the treatment, steaming or boiling with *kanjika* or with water led to the destruction of the hemagglutinating factor, the factor being protein in nature. The *sodhana* procedure prescribed for the detoxification of *Gunja* involves treatment at elevated temperature. Hence, the reduction in the toxic properties of the samples can thus be correlated with the denaturation of this protein factor leading to destruction of the hemagglutinating activity. Water when used as an alternate medium in place of *kanjika* has also led to destruction of the hemagglutinating

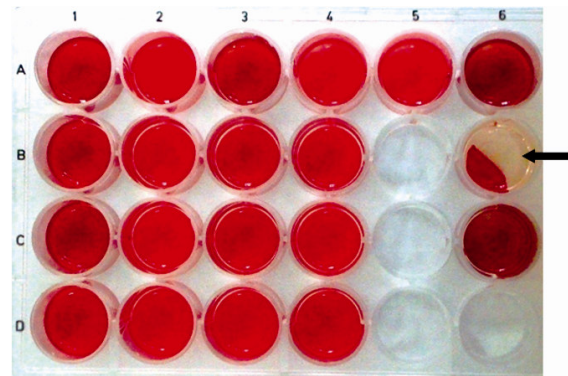


Fig.1—Agglutination studies in the various pre and post-*sodhit* samples of *Gunja* (*Abrus precatorius*) seeds and arrow indicating the well in which agglutination has occurred

factor. So it has a potential to be used as an alternate medium in the *sodhana* procedure for *Gunja*. But animal studies need to be carried out to evaluate the therapeutic effects (*gunas*) of seeds treated with water. This can help determine whether the therapeutic effects of *Gunja* are retained even on treatment with water in place of *kanjika*. A result to this effect can lead us to the conclusion that since the detoxification of *Gunja* seeds is due to the denaturation of the protein factor at elevated temperature, *kanjika* can be replaced with water for the *sodhana prakriya*.

Conclusion

Conventionally *kanjika* is used as a medium for *sodhana prakriya* of *gunja* (*Abrus precatorius* seeds), an attempt was made to find out an alternate medium for this *sodhana prakriya*. Studies carried out using both, *kanjika* as well as water as the medium for *sodhana prakriya* have shown similar results. The process of detoxification of *gunja* is based on the denaturation of the protein factor at elevated temperature. Both the media have shown to be effective in the detoxification of *gunja* and hence the results suggest that water could be used as an alternate medium in place of *kanjika* for the *sodhana prakriya* of *gunja*.

Acknowledgement

Authors are thankful to the Central Council for Research in Ayurveda and Siddha for providing research grant for the studies.

References

- 1 Patwardhan K, Gehlot S, Singh G & Rathore HCS, The Ayurveda education in India: How well are the graduates exposed to basic clinical skills?, *Evid Based Complement Alternat Med*, (E publication, England), 2009.
- 2 Jain S, Gill V, Vasudeva N & Singla N, Ayurvedic medicines in treatment of cancer, *Chinese Int Med*, 7(11) (2009) 1096-1099.
- 3 Anonymous, *The Wealth of India*, Revised edition, Vol: I, (National Institute of Science and Communication, New Delhi), 2005, 18-20.
- 4 Chopra R N, Nayar S L & Chopra I C, *Glossary of Indian Medicinal Plants*, 4th reprint, (National Institute of Science Communication), 1996, 1.
- 5 Refsnes D, Olsnes S & Pihl A, On the toxic proteins Abrin and Ricin, *J Biol Chem*, 249(11) (1974) 3557-3562.
- 6 Anonymous, *The Merck Index*, 14th edn, (Merck & Co, Inc., U S A), 2006, 4.
- 7 Subrahmanyam D, Mathew J & Raj M, An unusual manifestation of Abrus precatorius poisoning: A report of two cases, *Clin Toxicol (Phila)*, 46 (2008) 173-175.
- 8 Sekar S, Traditional alcoholic beverages from Ayurveda and their role on human health, *Indian J Tradit Knowle*, 6(1) (2007) 144-149.
- 9 Chunekar K C & Pandey G S, *Bhavaprakasha Nighantu*, 8th edn (Chaukhambha Bharati Academy Varanasi, India), 1988, 354-356.
- 10 Vogel H G, *Drug Discovery and Evaluation: Pharmacological assays*, 2nd edn, (Springer-Verlag Heidelberg, Germany), 2002, 288-289.
- 11 Islam M R & Funatsu G, Purification and Characterization of the Constituent Polypeptide chains of *Abrus precatorius* Agglutinin, *Agric Biol Chem*, 52(5) (1988) 1217-1222.
- 12 Carlini C R & Udedibie A B, Comparative effects of processing methods on hemagglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds, *J Agric Food Chem*, 45(11) (1997) 4372-4377.