



Comparative Toxicological Evaluations (Acute Toxicity) of Raw (unprocessed) and Processed (detoxified / shodhit) Extracts from Kupeelu (*Strychnos nux-vomica* linn.) in Wistar Rats

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ABSTRACT

Shodhana is the process of removal of unwanted/poisonous components of the drug for reducing the toxic ingredients and potentiating the action of the drug. Kupeelu (*Strychnos nux-vomica* Linn.) commonly known as nux-vomica, is a poisonous plant used extensively in various ayurvedic formulations, with great therapeutic significance. In the present study an attempt has been made to purify the seeds by performing three different methods (one classical and two traditional) using Cow's (urine, Milk and Ghee), *Kanji* and *Aloe vera* extract as *Shodhana* media. Post Shodhana the acute toxicity of the unprocessed and all processed seeds were assessed to evaluate and ascertain whether processing has rendered the seeds less toxic. Results thus obtained confirmed that Shodhana by three different methods had varying degrees of reduction in the toxic alkaloids as compared to the unprocessed seeds. There was a marked decrease in the toxicity of the processed seeds as compared to the unprocessed seed. The acute toxicity values of the unprocessed seed extract was between 15-50 mg/kg body weight, whereas seeds treated in Cow's urine, milk & Ghee as media showed toxicity between 300-600 mg/kg body weight and seeds processed in *Kanji* and *Aloe vera* as media, showed toxicity between 50-300 mg/kg body weight. This work can be extended to assess the subacute toxicity.

Keywords: Acute toxicity, kupeelu, shodhana, *Strychnus nuxvomica*.

INTRODUCTION

Many poisonous plants have been reported in Ayurveda, which are in practice for treating different types of ailments after properly detoxifying i.e. *Shodhana*.¹ Kupeelu (*Strychnos nuxvomica* Linn.) is one such plant used as an anti-inflammatory and anti-oxidant.² Kupeelu (*Strychnos nuxvomica* Linn.) has also been reported to have been prescribed as aphrodisiac in Unani system of medicine.³

The whole seed extract of *Strychnus nuxvomica* in low doses has been useful in neutralizing *Daboia russellii* and *Naja kaouthia* venom induced lethality, haemorrhagic, cardiotoxic and neurotoxic activity and potentiating polyvalent snake venom antiserum action in experimental animals.⁴ Shodhit (Detoxified) Kupeelu seeds were found to exhibit hepatoprotective activity against CCl₄ induced hepatic injury in Albino rats.⁵

The process of Shodhana (Detoxification) consists of removal of unwanted or excessive amount of contents from the drug compound and makes it less toxic and more potent to regulate its action.

The concepts of Shodhana treatment was highly accepted by the pioneers of Rasashastra for the purification of herbomineral drugs. Shodhana treatment has been divided into two major processes namely external and internal shodhana.

The processes underlying shodhana can be carried out either by deadulteration, dehydration and many more ways.⁶

In the present study an attempt has been made to purify

the seeds by performing three different methods (one classical and two traditional) using Cow's (urine, Milk and Ghee), *Kanji* and *Aloe vera* extract as *Shodhana* media. Post Shodhana the acute toxicity of the unprocessed and all processed seeds were assessed to evaluate and ascertain whether processing has rendered the seeds less toxic.

MATERIALS AND METHODS

Collection of Seeds

Mature *Kupeelu* (*Strychnos nuxvomica* Linn.) fruits were purchased from Parabia Remedies, Lalgate, Surat, Gujarat, India. The seeds were purchased from the said pharmacy since it is considered to be an authentic source as it is run by a qualified ayurvedic doctor and also it will contain seeds from different sources which in all likelihood shall be used by various ayurvedic practitioners around the area.

Seeds purchased from the source were cleaned properly and then ground in the grinders separately for raw (unprocessed) as well as processed (Shodhit/Detoxified) samples.

Seed Selection

The seeds purchased from the source were selected by following a simple quality check⁵. Initially seeds found broken or black in colour were manually separated. This was followed by putting the seeds in plastic bucket containing water, those seeds that floated on the surface of water were rejected and those that settled at the bottom were selected for further processing after air drying.



Preparation of Media for Shodhana

Samples were processed in three different media namely: A) Cow's urine, milk and ghee, B) Kanji and C) *Aloe vera* Extract.

- A. Cow's urine was collected freshly everyday from Surat Panjrapole Trust, Ghod Dod Road, Surat with the help of animal attendants tendering to cow's at the goushala.
- B. *Kanji* was freshly prepared by following steps: Preparation of Kulattha kwatha, Preparation of Odana, Shodhana of Hingu, Preparation of Masha Chakrika followed by mixing of all ingredients and then putting them in an earthen pot for fermentation and dipping the seeds for detoxifying process for a period of 21 days at pH of 3.0.
- C. Fresh *Aloe vera* exudates were prepared from plants collected from local garden and scrapped for removing the cover. The central transparent succulent portion was cut into pieces and made into juice by using a mixer to prepare exudates.

Methods of purification of *Kupeelu (Strychnos nux-vomica* Linn.) in different media

Each purification method was carried out as a single batch, by using three different media.

Shodhana (Detoxification) by Cow's Urine, Milk and Ghee as media

Nearly 2 kilogram of Seeds was processed by dipping in 5L of Cow's urine. The urine was changed every day to add fresh urine for a period of 7 days glass containers of 10 L capacity.

After the 7th day seeds were removed from the urine and sun dried for 48. Then the seeds were boiled in cow's milk for 3 hours followed by frying in cow's ghee on iron pan at low flame till they turned dark brown and brittle.^{5,9}

Shodhana (Detoxification) by Kanji as media

Kanji is an ayurvedic preparation which consists of a mixture of Kulattha kwatha, Odana, Shodhit Hingu, Masha Chakrika.

All these ingredients were mixed in an earthen pot, fermented to attain pH of 3.0 and 2.5 kilogram seeds were dipped in the same for detoxifying for a period of 21 days after which seeds were removed from the pot and sun dried to be processed further.⁷

Shodhana (Detoxification) by Aloe vera Extract as media

Seeds were steamed for separation of seed coat and cotyledons. The processing of seeds was carried out by immersing them in exudates of fresh leaves of *Aloe vera* (ghritakumari) for a period 15 days.

This method was adopted on the basis of reported literature with slight modifications.⁸

The seeds were stirred occasionally and on the last day the seeds were washed.

The cotyledons were finally powdered for further processing.

Extraction of Samples for Toxicity Experiment

Four different samples were considered for the toxicity experiment for comparative evaluation. All the samples were dried after processing and ground in grinders for getting fine powder. These samples were:

- i. Sample I – Raw (Unprocessed/Non Shodhit) Sample
- ii. Sample II – Processed (Shodhna by Cow's Urine, Milk & Ghee as media).
- iii. Sample III – Processed (Shodhna by Kanji as media).
- iv. Sample IV – Processed (Shodhna by *Aloe vera* as media).

As mentioned dried seeds of all four samples were powdered and exhaustively extracted by adding 65 g of each sample in 750 mL of 95% ethanol, using soxhlet extractor. The resulting extracts were then evaporated in rotary evaporator. Concentrated extracts (residue) were transferred to vacuum desiccators and dried until constant weight was attained. These solvent free extracts were then used as compound for the toxicity experiments. These extracts were suspended in a vehicle (Tween 80) for a known concentration (w/v) to be used for oral dosing in Wistar rats.

Animal Experiment Design and End Points Observed

The guidelines for the animal toxicity experiment followed was OECD Guidelines for the Testing of Chemicals No. 423, Acute Oral Toxicity – Toxic Class Method.¹⁰

The experiment on animals were conducted at the animal facility of Flair Labs, Palsana, Surat which is a CPCSEA approved animal facility and the experiment was approved by the Institutional Animal Ethics Committee (IAEC) of the facility.

All the animals were given feed and water ad libitum throughout the experiment.

Following the three 'R' principle to reduce animal use, slight modifications were done to the guideline dosing without compromising with the scientific quality of the experiment.

Animal experimental design comprised of the following sets of experiment:

Sample I (Unprocessed/non shodhit): 2000, 300, 50, 15 mg/kg

Sample II (processed/shodhana by cow urine, milk and ghee as media): 600, 300 mg/kg

Sample III (processed/shodhana by kanji as media): 300, 50 mg/kg



Sample IV (processed/shodhana by *Aloe vera* as media): 300, 50 mg/kg

RESULTS AND DISCUSSION

Lethality was considered as the end points for arriving at a conclusion in the toxicity experiments. To establish the highest limit as considered by various regulatory bodies beyond which testing of toxicity is not required a limit dose of 2000 mg/kg body weight of Sample I (Unprocessed/Non-Shodhit) extract was administered to 5 males and 5 females. All the animals died within 15 minutes of exposure. Hence a lower dose with 300 mg/kg body weight was considered with only 3 females per dose following the OECD Toxic Class Method, wherein all the animals died within 45 minutes. A next lower dose of 50 mg/kg body weight with 3 females also resulted in lethality within 1 h. There was no mortality in 15 mg/kg body weight dose group treated with Sample I (Unprocessed/Non-Shodhit). This was reconfirmed with another set of females (confirmatory test) treated with 15 mg/kg body weight group treated with Sample I (Unprocessed/Non-Shodhit). Hence it was established that the LD₅₀ range for Sample I (Unprocessed/Non-Shodhit) was between 5-50 mg/kg body weight.

Based on data from Sample I (Unprocessed/Non-Shodhit), three groups of animals were exposed to a dose of 300 mg/kg body of Sample II (Shodhana by Cow's Urine, Milk & Ghee as media), Sample III (Shodhana by Kanji as media) and Sample IV (Shodhana by *Aloe vera* as media), respectively.

While there was no lethality in the group of animals treated with Sample II, lethality was established in Sample III and Sample IV at 300 mg/kg body weight dose. For Sample II the lethality was established at 600 mg/kg body weight and for both Sample III and Sample IV no lethality was observed at a lower dose of 50 mg/kg body weight.

To summaries the LD₅₀ range for Sample I (Unprocessed/Non-Shodhit) was between 5-50 mg/kg body weight, Sample II (Shodhana by Cow's Urine, Milk & Ghee as media) between 300-600 mg/kg body weight, Sample III (Shodhana by Kanji as media) and Sample IV (Shodhana by *Aloe vera* as media) between 50-300 mg/kg body weight. This establishes the fact that shodhana is an important procedure to detoxify the drug samples and also among the three processes followed, Shodhana by Cow's Urine, Milk & Ghee as media gives the least toxic drug.

CONCLUSION

From this study it may be concluded that for purification of *Kupeelu* (*Strychnos nuxvomica*), the procedure of

processing the seeds in Cow' Urine followed by boiling in Cow's milk and frying in Cow's Ghee as media for Shodhana is the best followed by processing in Kanji and *Aloe vera*.

The methods for extraction in Kanji and *Aloe vera* proved to be better as compared to data from raw (unprocessed/non-shodhit) sample but were not as good as the first processing method. These findings confirm that the process of *Shodhana* (purification) traditionally used for *Kupeelu* definitely reduces the toxic components of drug and can be used for enhancing the safety of the drug for routine human use, especially the processing in Cow's urine, milk and ghee.

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