DISCUSSION

There are many poisonous plants reported in ancient scriptures of Ayurveda which are being still practiced widely in a number of diseases after proper Shodhana (purification/detoxification). It is reported thataconite (Vatsanabha) purified by cow’s urine is converted to cardiac stimulant, whereas raw aconite is cardiac depressant. Likewise the plant Kupeelu (Strychnos nux-vomica Linn.) known as nux-vomica, is described under the ‘Upavisa Vargas’ (poisonous group), it’s seeds have been used successfully in many diseases after proper Samaskar, known as Shodhana. Nux-vomica was introduced in Europe in the sixteenth century, but was not much used in medicine, being chiefly employed to poison dogs, cats, crows, etc. It is cited in the treatises of Ayurveda that the ‘Visha’ (poison) becomes ‘Amrita’ (nectar) after logical administration and the ancient physicians of Ayurveda successfully used this drug to combat number of diseases after proper purification in some specific media. Various Shodhana (purification) procedures have been adopted for purification of nux-vomica seeds and these methods are either mentioned in the classics of Ayurveda or practiced traditionally. The role of these purificatory procedures on the major chemical constituents of Kupeelu is yet to be evaluated.

By taking these facts as background, the present study entitled “A Comparative Pharmacognostical and Phyto- pharmacological Evaluation of Raw and Shodhita Kupeelu (Strychnos nux-vomica linn.) Seeds” has been taken and carried out in the following phases:

1. Conceptual Study
2. Pharmacognostical Study
3. Pharmaceutical Study
4. Analytical Study, and
5. Pharmacological Study.

CONCEPTUAL STUDY

- Concept of Visha & Upavisha:

Visha means sadness, grief, sorrow, disappointments, etc. Hence, it is a substance which creates “Vishada” by producing harmful effects on mind and body. It prevades the whole body immediately after ingestion or it’s entry into the body and causes vitiation of the healthy dhatus. The use of Visha dravya in Ayurvedic System
of Medicine is available since Samhita period. Brihat Trayee texts describe the types of poisons and their medicinal use in details. The toxic effects of the poisonous plants are also described. The therapeutic or the pharmacological doses of these plants are clearly elaborated. As per Charaka, poisonous dravya when used in its proper dosage and in proper manner and in proper stage of the disease can prove as a medicine. According to origin, Visha dravyas are classified into two types i.e. Sthavara Visha (plant poison), Jangama Visha12 (animal poison). Among the above two types of poisons, plant poison is most frequently used in medicine. When poisonous plants are used in the medicines they act very fast in the body in low dose according to the intensity of action. Depending upon the severity of toxic effects, Visha drugs have been classified as Visha and Upavisha. Upavishas are the substances which exert toxic effects on the body but do not cause instant morbidity. Usually they have delayed onset of action which starts within 15-45 minutes after consumption58. Kupeelu is included under Upavisha category which is having specific action on spinal cord. If Kupeelu is used in crude and unpurified form, it exerts deadly poisonous and even fatal effects. It develops restlessness, suffocation, tremors and convulsion gradually and ultimately leading to death due to respiratory arrest within 2-4hrs13. However, after the purification, the Kupeelu turned to be a very effective medicine to treat a number of diseases. Even the purified Kupeelu is claimed to be a potent drug in countering old age problems and specially recommended during senility as Rasayana (antioxidant)14.

While going through the history of modern toxicology it is revealed that by 1500 BC, hemlock, opium, arrow poisons, and certain metals were used to poison enemies or for executions. Notable poisoning victims include Socrates, Cleopatra, and Claudius. Socrates was given a potent infusion of hemlock (Conium maculatum) seeds15. These seeds contain an alkaloid coniine which is a neurotoxic agent like strychnine and causes death by blocking the neuromuscular junction. By the time certain fundamental concepts of toxicology began to take shape especially by the studies of two renowned scientists Paracelsus (~1500AD) and Orfila (~1800 AD). Paracelsus (1493 – 1541) was one of the first to distinguish between the therapeutic and toxic properties of substances in modern era. His famous words were:

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy."
Paracelsus determined that specific chemicals were actually responsible for the toxicity of a plant or animal poison. He also documented that the body's response to those chemicals depended on the dose received\textsuperscript{16}.

The similar concept was also explained by Charaka 2000 years before. Charaka opined that a deadly poison can become a very good medicine if it is administered properly i.e. used in proper dosage, in proper manner and in the proper stage of the diseases\textsuperscript{17}.

Various properties of *Visha* have been described in the table no. 2.1. Due to its subtleness (*Sukshma*), the poison vitiates *Rakta* (blood) because the latter has the property of moving through the subtle channels in the body. *Anirdehsya rasa* or *Avyakta rasa* (indistinct taste) is the characteristic feature of both the poison and water. The latter dominates the composition of *Kapha*. Therefore, the *Avyakta rasa* attribute to poison causes augmentation or aggravation of *Kapha*. *Marmas* or vital organs are *soumya* in nature. Therefore, the poison which has opposite properties like *tikshna* (sharpness) adversely afflicts these vital organs. The term ‘Vikasi’ is derived from the root ‘*Kas*’= ‘*hims*’ (meaning violence) with the preposition ‘*Vi*’. By implication, this term means excessive violence or the suppression of the ‘*Ojas*’ resulting in looseness of the joints. The term ‘*laghu*’ (lightness) implies instability for which therapeutic measures do not produce the desired results. The term ‘*Visada*’ means freedom from sliminess as a result of which the *dosas* do not remain adhered to the tissue elements, and therefore, constantly circulate all over the body. These attributes of the poison together exhibit specific actions like aggravation of *Vata*\textsuperscript{18}.

As stated earlier the Seers of Ayurveda have classified poisons into two types depending on their origin. Apart from the *Sthavara* and *Jangama*, there is a third type of poison called *Gara visha* which is prepared by the combination of various types of substances. ‘*Gara*’ is a technical term specifically used in the texts for this type of artificial poison which produces it effects after a long time to cause diseases and death\textsuperscript{19}.

*Susruta* has also described three categories of poisons including this artificial (*Kritrima*) prepared variety\textsuperscript{20}. When the poison is produced by the combination of two non-poisonous substances, then it is called as ‘*Gara*’. However, when the poison is produced by the combination of two poisonous materials, then it is called *Kritrima*. Another type of poison, known as ‘*Dusi visha*’ has also been described by the ancient
Acharyas. According to the Charaka, ‘Dusi visha’ vitiates blood (Rakta) and produces symptoms like Aru (eczema in the head), Kitima (psoriasis) and Kotha (urticaria). Depending upon the location of the dosas and the constitution of the patients, poison produces several other complications. This type of poison afflicts all the three dosas and may cause death of the patient\(^{21}\).

Susruta in this context also opined that a constant use of some particular time (i.e. cloudy/windy day, rainy season), particular place (i.e. marshy country) and particular diet (e.g. wine, seasamum, Kulattha etc.) sexual intercourse, excessive exercise as well as regular day sleep tends slowly to poison the fundamental Dhatus and this slow poison (Dusi visha) ultimately manifests some specific symptoms in the body\(^{22}\).

Table 2.3 shows various Upavishas as mentioned in Rasatarangini with their fatal dose & fatal period. It is evident from the table that only one raw crushed seed of Kupeelu is capable of producing death within 4 hrs. Therefore, Shodhana as well as administration in proper dose should be strictly followed prior to its use in medicine.

- **Role of Media in Shodhana**

The concept of Shodhana is reported for the first time in the Charaka Samhita in the context of Danti Dravanti Kalpadhyaya. To reduce the ‘Vikasi’ property of Danti root, Charaka mentioned a specific Samaskara (processing) by Agni\(^{23}\). Acharya Vagbhata also mentioned the Shodhana of plant drugs in detail in the context of Bhallataka Rasayana and Amrita Bhallataka\(^{24}\).

In the present study the Shodhana of Kupeelu seed was performed by following the principles of Nimajjana, Swedana & Bharjana in various media.

The media employed in the Shodhana process has very significant role for eradicating the toxic chemical components partially or by transforming them to non-toxic substances. Sometimes media act like solvent to dissolve the material thereby separating them from the insoluble impurities, like Godugdha, used for Guggulu Shodhana or Churnodaka used for the Shodhana of Manashila. Media may also provide some new organic or inorganic principles to the drug which may be responsible for enhancing their therapeutic efficacy. Previous study reveals that Gomutra Shodhita Vatsanabha is turned to be cardiac stimulant whereas the crude one is claimed to be as cardiac depressant\(^{1}\).

In this study, an attempt has been made to validate the different Shodhana procedures of Kupeelu with various media like,
Gomutra (cow urine),
Godugdha (cow milk),
Goghrita (cow ghee),
Kanji (sour gruel),
Eranda taila (castor oil),
Ardraka swarasa (fresh ginger juice) and
Water.

Though Gomutra and Kupeelu possess similar properties like Katu, Tikta, Rasa; Katu Vipaka and Ushna Veerya, yet it is used frequently for the Shodhana of Kupeelu seeds. It may be due to its Lekhana property which ultimately leads to expel out the poisonous Strychnine and Brucine from the seeds. As the cow urine is an alkali media it may also hydrolyze the toxic alkaloids Strychnine and Brucine to their less toxic derivatives.

Godugdha possess properties like Madhura, Snigdha, Guru, Sheeta etc., which are just opposite to that of Kupeelu. This may counteract the adverse effect of the drug. The various activities of Godugdha like Preenana, Jeevana, Vajikarana, Vayasthapana etc., may convert the poisonous Kupeelu seed to therapeutically more potent one. A previous study also reported that boiling in milk converted the strychnine into less toxic isostrychnine (Cai et al., 1990).

Ghee is viewed as a superior cooking fat because it doesn’t burn during cooking, unlike butter and fats which are liquid at room temperature. When the seeds are fried with Goghrita, it may takes on the properties of the seeds due to its “Samaskarasya Anubartanat” action. Ghee also provides a soothing and cooling effect, helping to offset the irritant effect of the seeds. By frying with Ghee, seeds became swollen, soft and easily made into powder form.

Kanji is having the property to pacify Vata & Kapha and it is also used as Shoolaghna. Hence, Shodhana in Kanji may enhance the same properties of the Kupeelu seeds.

Eranda Taila is specifically used in different painful conditions like Amavata, Pristha & Guhya shula, Kati shula etc., as it is claimed as Vatahara. When the seeds are fried in Eranda taila, it penetrates readily into the seeds due to its Sukshma property and may potentiate the Vatahara & Shothara activities of the seeds. Previous
study shows that Ricinoleic acid is the main component of castor oil and it exerts anti-inflammatory effects\textsuperscript{30}.

\textit{Ardraka swarasa} is Madhura in Vipaka and it pacifies Vata & Kapha. For this reason to obtain better therapeutic efficacy, the traditional \textit{Unani} practioners use the Kupeelu seeds in acute rheumatic conditions after purifying it in \textit{Ardraka Swarasa}\textsuperscript{31}.

Water (\textit{Jala}) is used as an intermediate media for Kupeelu \textit{Shodhana} in \textit{Unani} System of Medicine\textsuperscript{32}. The main aim of \textit{Shodhana} of Kupeelu seeds is to remove the toxic alkaloids Strychnine & Brucine. It is reported that these two alkaloids are slightly soluble in cold water as well as boiling water\textsuperscript{33,34}. This may be the reason that the \textit{Unani} practitioners have selected the most common and easily available media for \textit{Shodhana} of Kupeelu seeds. Moreover the method of applying heat in \textit{Swedana} process may break these toxic alkaloids, converts and removes them from the seeds to the water and helps in converting the toxic drug into a nontoxic.

- **The Drug Kupeelu**

The drug Kupeelu was not described in the ‘\textit{Brihat Trayee}’ texts of Ayurveda. A drug described as \textit{Vishamustika} is found in the Surasadi gana of \textit{Sushruta Samhita}\textsuperscript{35} but it is not botanically identified as \textit{S.nux-vomica}\textsuperscript{36}. Dalhana also mentioned it as \textit{Raja Nimba}\textsuperscript{37}. Its uses in Ayurveda were recorded from the period of “\textit{Brinda Madhava}” (9\textsuperscript{th} A.D.). The drug \textit{Visamusti} was mentioned in the English translation of Brinda’s \textit{Siddha Yoga} edited by P. V. Tiwari, while describing ‘\textit{Vatavyadhi chikitsa}’. Later it was mentioned by different authors with number of synonyms. (Table 2.6) Synonyms like \textit{Visatinduka}, Kupeelu, \textit{Visamusti} etc., indicate toxic nature of this tree. While searching through various \textit{Ayurvedic} literatures different pharmacological actions of seeds such as S	extit{hothahara}, P	extit{utihara}, V	extit{edanasthapana}, U	extit{ttejaka}, N	extit{adibalya}, D	extit{eepana}, P	extit{achana}, G	extit{rahi}, S	extit{hoopalrashamana}, H	extit{ridayotjekah}, K	extit{aphaghna}, K	extit{asahara}, V	extit{ajikarana}, B	extit{alva}, K	extit{ushthaghna}, K	extit{andughna}, S	extit{wedapanayana} etc., are available\textsuperscript{38,39}. The other parts of Kupeelu like root, bark, leaves, and fruits are also used as medicine in various diseased conditions. Purified Kupeelu seeds are also claimed to be potent drug in countering old age problems and specially recommended during senility as \textit{Rasayana}\textsuperscript{40} (antioxidant). The plant is also found to have analgesic & anti-inflammatory\textsuperscript{41}, anti-oxidant\textsuperscript{42}, anti-tumor\textsuperscript{43}, anti-snake venom\textsuperscript{44}, anti-diarrhoeal\textsuperscript{45} and hepatoprotective\textsuperscript{46} activities when studied in animal models. Classical text books of
Ayurveda suggest certain compound formulations containing purified seeds of Kupeelu as one of the ingredient for the management in various diseases. (Table 2.7)

In a previous study, 16 alkaloids have been seperated and identified from the crude nux vomica and 80% of them are strychnine and brucine, as well as their derivatives such as isostrychnine and brucine N-oxide. Strychnine (C$_{21}$H$_{22}$O$_{2}$N$_{2}$; m.p. 286 to 288$^0$C) and brucine (C$_{23}$H$_{26}$O$_{4}$N$_{2}$; m.p. 178$^0$C) have been reported as the most important and strongly toxic alkaloids present in this, besides other minor alkaloidal constituents. It is also reported that nux vomica in large doses, producing tetanic convulsions and eventually death and in lesser doses it may manifest mental derangement. So it is mandatory to purify or properly processed nux vomica seeds prior to its administration in therapeutics. There are also few reports of previous research works advocating a variety of methods of purification of nuxvomica seeds as per Chinese, Unani and Ayurveda system of medicine.

Many of the strychnine poisoning resembles the clinical features of tetanus and hence strychnine poisoning has to be diagnosed from tetanus. The Table 2.8 indicates some of the distinguishing points between strychnine poisoning and tetanus.

Strychnine toxicity (LD$_{50}$ value) is also varying from one animal to another and it is reported in Table 2.9.

**PHARMACOGNOSTICAL STUDY**

Pharmacognostical study of a drug is the key for getting therapeutically potent medicines prepared of genuine raw drug. Genuine raw drug can only be obtained if it is collected from its natural habitat which grows in ideal soil. The soil which is devoid of big stones, excessive water, without Valmika (snake nest) and nearer to water tanks should be selected for this purpose. The soil which is unctuous, smooth, tight, blackish-white or reddish in color and with grass is considered as the best soil for collection of raw materials.

Ayurveda also gives special importance on the characteristics of collected drugs. The drug, collected for the medicinal usage should have some distinct characteristics like, it should not be affected by smoke, rain, air or water and it should be collected in the respective seasons. The drug should be free from Krimi (pests), Visha (poison), Shastra (weapon), Atapa (excessive sunlight), Pavana (strong wind), Dahana (fire),
Toya (excessive moisture), Sambadha (diseases) and should not be grown on Marga (road side)\textsuperscript{59}.

The raw materials used in Ayurveda shall be collected according to part used, season, potency etc., in a specific manner. Acharya Charaka recommended the collection of fruits according to their season\textsuperscript{61}. It is also advised not to collect unripen or over-ripen fruit. Fruits which are well ripened shall only be collected from the plants\textsuperscript{62}.

In the present study, fully matured Kupeelu (Strychnos nuxvomica Linn.) fruits were collected personally from the field of Bankura district, West Bengal in India during the month of December and the seeds were then taken out from the fruit pulp. The fresh seeds thus obtained, were used for pharmacognostical study both before and after Shodhana.

On comparing macroscopically the organoleptic characters of the Shodhita powdered samples with raw seeds, certain changes in color, odor and taste were observed. It indicates the impact of various media during the Shodhana processes. This might be due to the fact that during Shodhana processes, the constituents responsible for color, odor & taste of the media might have been transported into the Kupeelu seeds to a certain extent, which ultimately developed some new organoleptic characters in the Shodhita samples. The organoleptic characters are presented here in the below mentioned table, (Table 7.1)

### Table 7.1: Comparative organoleptic characters of raw and Shodhita Kupeelu

<table>
<thead>
<tr>
<th>Powdered Samples</th>
<th>Color</th>
<th>Odor</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR</td>
<td>Pale brown to yellowish gray</td>
<td>Odorless</td>
<td>Intensely bitter</td>
</tr>
<tr>
<td>KGM</td>
<td>Whitish gray</td>
<td>Characteristic of urine</td>
<td>Salty bitter</td>
</tr>
<tr>
<td>KGD</td>
<td>White</td>
<td>Characteristic of milk</td>
<td>Sweetish bitter</td>
</tr>
<tr>
<td>KGG</td>
<td>Golden brown</td>
<td>Characteristic of ghee</td>
<td>Sweetish bitter</td>
</tr>
<tr>
<td>KGMD</td>
<td>Grayish white</td>
<td>Not Characteristic of ghee</td>
<td>Bitter</td>
</tr>
<tr>
<td>KGMDG</td>
<td>Grayish brown</td>
<td>Characteristic of ghee</td>
<td>Sweetish-bitter</td>
</tr>
<tr>
<td>KET</td>
<td>Reddish brown</td>
<td>Pungent</td>
<td>Moderately bitter</td>
</tr>
<tr>
<td>KKJ</td>
<td>White</td>
<td>Characteristic of Kanji</td>
<td>Moderately bitter</td>
</tr>
</tbody>
</table>
Histochemical studies reveal the presence of Strychnine, Brucine and Hemicellulose in the raw seeds (Table 3.1). Strychnine was found to be present in the middle portion of the endosperm whereas, Brucine in the outer part of endosperm. Hemicellulose was found in the cell wall of seeds.

In Microscopic study of the powdered samples most of the characters of raw Kupeelu seeds (Table 3.2) were observed in Shodhita Kupeelu samples. Only the aleurone grains were found to be absent in the powder microscopy of all the Shodhita samples. Other characters were similar to the powder drug of the raw samples. Aleurone grains are membrane-bounded storage granules found in cells of the aleurone layer in plants; contains either a protein matrix or protein-carbohydrate bodies. As they are protein in nature they may be denaturized or hydrolyzed into some other forms during the Shodhana process. Oil globules were found plenty in number in the samples like KGD, KGMD, KGG, KGMDG, and KET etc., where oil based media were used for Shodhana purpose. The powder microscopic characters of raw and Shodhita Kupeelu are summarized in the below mentioned table. (Table 7.2)

Table 7.2: Comparative powder microscopic characters of raw and Shodhita Kupeelu

<table>
<thead>
<tr>
<th>Powdered Samples</th>
<th>Diagnostic Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lignified trichomes</td>
</tr>
<tr>
<td>KR</td>
<td>+</td>
</tr>
<tr>
<td>KGM</td>
<td>_</td>
</tr>
<tr>
<td>KGD</td>
<td>_</td>
</tr>
<tr>
<td>KGG</td>
<td>_</td>
</tr>
<tr>
<td>KGMD</td>
<td>_</td>
</tr>
<tr>
<td>KGMDG</td>
<td>_</td>
</tr>
<tr>
<td>KET</td>
<td>_</td>
</tr>
<tr>
<td>KKJ</td>
<td>_</td>
</tr>
</tbody>
</table>
PHARMACEUTICAL STUDY

Shodhana of Kupeelu seeds were carried out by ten different methods for the purification of the drug. Each Shodhana procedure was repeated for three times to establish the validation of the Pharmaceutical processing.

The active constituents present in the drugs are also responsible for their toxic effects. Therefore, it is not desirable to expel them out completely from the drugs. The main aim of Shodhana process is to reduce the toxic constituents to some extent or by potentiating their chemical transformation to non-toxic or relatively less toxic substances. There may be some new principles added to the drugs which are responsible for enhancing their biological efficacy. Hence, maximum beneficial effect can be obtained by administering the Shodhita drugs within their therapeutic dosage limit.

In this study, Shodhana of Kupeelu was performed by the process of Nimajjana (dipping), Swedana (boiling), Bharjana (frying) etc., in various media.

In Nimajjana method, the Kupeelu seeds were kept immerse in some particular media like Gomutra, Kanji, Ardraka swarasa, water etc., for a specific period of time. In Swedana method the seeds were boiled under the bath of media in a Dola yantra for a definite length of time.

Principles of Nimajjana & Swedana methods are similar to the common stages of extraction process where the solvent enters through the pores into the cells resulting in the swelling of the tissues and solution of the soluble constituents takes place within the cells then there is escape of dissolved material through the solvent boundary layer by the process of diffusion – finally separation of the solution from the drug occurs. The rate of extraction depends mainly on the temperature gradient and concentration gradient across the cell membrane. The rate of extraction and solubility is increased by elevation of temperature. Rising temperature increases the concentration gradient across the cell membrane thereby increasing mass transfer of active principles from solid material to the solvent.²⁵
The principles underlying the Swedana process is similar to the process of hot continuous extraction where the continuous flow of liquid media occurs by the temperature gradient due to heating.\(^{26}\)

In Bharajana method, the seeds were fried by cow ghee in mild temperature for a specific period of time. In Bharjana process, some physical & chemical changes like reduction in hardness, increase brittleness, formation of new chemical compounds take place which ultimately make the drug body friendly.\(^{26}\)

During Shodhana of Kupeelu in various media, change in color of the media was noticed and it might be due to the removal of color containing materials like tannin from the seeds.\(^{66}\) Taste of every media became bitter after Shodhana and it might be due to the extraction of bitter principles like strychnine, brucine, etc. from the seeds. Changes in organoleptic characters of Kupeelu seeds were also noticed and the final weight obtained after each Shodhana procedure was noted accordingly. (Table 4.1-4.20) The percentage of final weight obtained and the percentage of weight lost during the each batch of Shodhana processes are duly calculated and presented in the Table 7.3.

**Table 7.3: Final quantity of Kupeelu seeds obtained after Shodhana procedures**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Initial Quantity (g)</th>
<th>Final Quantity Obtained (g)</th>
<th>Loss (g)</th>
<th>Loss %</th>
<th>Average loss of 3 batches (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGM-1</td>
<td>100</td>
<td>64.50</td>
<td>35.50</td>
<td>35.50</td>
<td>34.77</td>
</tr>
<tr>
<td>KGM-2</td>
<td>100</td>
<td>66.00</td>
<td>34.00</td>
<td>34.00</td>
<td></td>
</tr>
<tr>
<td>KGM-3</td>
<td>100</td>
<td>65.20</td>
<td>34.80</td>
<td>34.80</td>
<td></td>
</tr>
<tr>
<td>KGD-1</td>
<td>100</td>
<td>70.30</td>
<td>29.70</td>
<td>29.70</td>
<td>30.73</td>
</tr>
<tr>
<td>KGD-2</td>
<td>100</td>
<td>68.50</td>
<td>31.50</td>
<td>31.50</td>
<td></td>
</tr>
<tr>
<td>KGD-3</td>
<td>100</td>
<td>69.00</td>
<td>31.00</td>
<td>31.00</td>
<td></td>
</tr>
<tr>
<td>KGG-1</td>
<td>100</td>
<td>52.30</td>
<td>47.70</td>
<td>47.70</td>
<td>49.67</td>
</tr>
<tr>
<td>KGG-2</td>
<td>100</td>
<td>48.20</td>
<td>51.80</td>
<td>51.80</td>
<td></td>
</tr>
<tr>
<td>KGG-3</td>
<td>100</td>
<td>50.50</td>
<td>49.50</td>
<td>49.50</td>
<td></td>
</tr>
<tr>
<td>KGMD-1</td>
<td>100</td>
<td>70.30</td>
<td>29.70</td>
<td>29.70</td>
<td>30.40</td>
</tr>
<tr>
<td>KGMD-2</td>
<td>100</td>
<td>69.50</td>
<td>30.50</td>
<td>30.50</td>
<td></td>
</tr>
</tbody>
</table>
After *Shodhana* with some specific media, all samples were peeled off with the help of a knife, embryo were removed and then dried properly. The dried seeds were made into powder form and the final weight of the each powdered sample was recorded. *Graph 7.1* shows that maximum loss in final yield (49.67%) was found after *Shodhana* with *Goghrita* whereas minimum loss in final yield (13.53%) was obtained after purification with boiling water. It might be due to the extraction of more soluble mass from the seeds by *Goghrita* than boiling water.
Regarding utilization of media, total volume of Ardraka swarasa was utilized during the Shodhana process. It might be due to the several factors like rate of evaporation and condensation of Ardraka swarasa regulated by the environmental temperature, humidity, surface area etc., and also might be due to the increased absorption of media by raw seeds. The percentages of average utilization of media are presented here in the Graph 7.2.

Graph 7.2: Average utilization of media during Shodhana of Kupeelu seeds
ANALYTICAL STUDY

The physicochemical analysis of different samples such as raw and *Shodhita Kupeelu* seeds were carried out followed by quantitative estimation of their toxic alkaloids viz. strychnine & brucine by HPTLC. Differences in all the physico-chemical parameters were observed among the raw and *Shodhita Kupeelu* samples. Qualitative tests reveal the absence of glycoside in *Shodhita Kupeelu* however, no other difference regarding functional group was observed among raw and *Shodhita Kupeelu* seeds.

In comparison to the raw seeds, moisture content was increased in all the *Shodhita* samples except in KAS. It indicates more removal of intracellular water portion from the raw seeds by the process of diffusion during the *Shodhana* process with *Ardraka Swarasasa*. The percentage of loss on drying of all the samples were recorded and presented here in the Graph 7.3.

**Graph 7.3: Loss on drying of raw & Shodhita Kupeelu seeds**

The Graph 7.4 indicates that maximum ash content was found in the *Gomutra Shodhita* sample and it might be due the addition of more organic and inorganic contents like nitrogen, sulphur, phosphorus, hippuric acid, NaCl etc., from cow’s urine to the raw seeds.⁶³
Water soluble extractives of all the purified samples were found to be decreased in comparison to the raw drug (KR). This might be due to the solubility of the contents like strychnine, brucine, loganin etc., in water. These water soluble contents dissolved and pulled out from the raw Kupeelu seeds during the Shodhana process by diffusion. The water & alcohol soluble extractive values of Gomutra purified sample were found to be lowest in comparison to all other samples. The result indicates maximum removal of water & alcohol soluble compounds from the raw seeds by Gomutra. The results are presented here in the Graph 7.5.

Graph 7.5: Water & Alcohol soluble extractive values of raw & Shodhita Kupeelu
pH values of all the samples were found to be acidic. It was in the range between 4.51- 6.25 (KET-4.51; KGM-6.25). (Graph 7.6) Relatively higher pH was obtained in the Gomutra purified sample which might be due to the alkalinity of cow’s urine that neutralized the acidic pH of the raw drug to some extent.

Graph 7.6: pH values of raw & Shodhita Kupeelu

Alkaloids, oil, carbohydrate, protein and tannin are present in raw sample as well as purified samples. Steroid, Flavonoid are absent in raw and the purified samples. Glycoside was found to be absent in all the purified samples in comparison to the raw drug. It might be due to the hydrolysis of loganin into an aglycone loganetin, or converted into secologanin. Secologanin in turn is condensed with tryptamine to produce strictosidine, the central precursor of many monoterpenoid indole alkaloids.53

Picture a.

Schematic presentation of the chain reaction in Picture a. indicates that Tryptophan (1) is decarboxylated by tryptophan decarboxylase to yield tryptamine (2), which reacts with secolloganin (3) to form strictosidine (4). After numerous rearrangements, strictosidine (4) is converted into a variety of monoterpene indole alkaloids, such as 19,20-dihydroakuammicine (5), ajmalicine (6), tabersonine (7) and catharanthine (8).

HPTLC profiles of raw and Shodhita Kupeelu indicate that some peaks disappeared and some new peaks appeared after Shodhana processes. In raw sample, total 4 peaks were found whereas 3-8 peaks were observed in the purified samples under 254 nm. This disappearance and newly appearance of peaks suggest the extraction of some components like strychnine, brucine, loganin etc., and formation of some new compound during Shodhana process. The Rf values of Strychnine and Brucine standard were found as 0.54 & 0.34 respectively which were also present in all the samples. (Table 5.3 and Graph 7.7)

Graph 7.7: Peaks observed under 254 nm in raw & Shodhita Kupeelu seeds

Decrease in Strychnine and Brucine content was found in all the Shodhita samples when compared to the raw drug. Strychnine and Brucine contents were found to be lowest in the sample purified by Gomutra-Godugdha (KGMD) when compared to the other samples. (Table 5.4 and Graph 7.8) It might be due to the reason that during Shodhana processes, some amount of Strychnine and Brucine were removed by diffusion process into cow’s urine. Further boiling in cow’s milk also initiated more
diffusion of the alkaloids from the seeds as well as some amount of Strychnine and Brucine might have been converted into their N-oxidative derivatives with lesser toxicity\textsuperscript{67}. Removal of some constituents from the raw seeds were also confirmed by observing the changes in organoleptic characters like color, odor \\& taste occurred in the media as well as in the samples during \textit{Shodhana} processes.

\textbf{Graph 7.8: \% of Strychnine \\& Brucine in raw \\& Shodhita Kupeelu seeds}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{graph7.8.png}
\caption{\% of Strychnine \\& Brucine}
\end{figure}

\textbf{PHARMACOLOGICAL STUDY}

Pharmacological study was carried out in two parts viz., acute toxicity study \\& efficacy related study.

\textbf{Part I: Toxicity study:}

\textit{Kupeelu} in crude and unpurified form exerts poisonous and even fatal effects. It develops restlessness, suffocation, tremors and convulsion gradually and ultimately leading to death due to respiratory arrest within 2-4hrs. The main toxic alkaloids present in the seeds are Strychnine and Brucine which cause tetanic convulsion. Normally inhibitory neurons in the spinal cord called Renshaw cells release glycine at inhibitory synapses with motor neurons. This inhibitory input to motor neurons prevents excessive muscular contractions. Strychnine binds to and blocks glycine receptors which cause massive tetanic contractions. All skeletal muscles, including the diaphragm contracts fully and remains contracted. Because the diaphragm cannot relax, the person cannot breath. The normal delicate balance between excitation and inhibition in the CNS is disturbed and motor neurons are firing without restraint\textsuperscript{68}.
As expected raw *Kupeelu* sample (KR) treated animals showed severe convulsions followed by death at the dose of 175mg/kg. Even at the dose of 100mg/kg also, animals showed convulsions. This may be because of strychnine contents of seed which blocked glycine receptors and lead to synchronized firing of neurons. Contrast to this observation, between the two *Shodhita* samples, *Kanji Shodhita Kupeelu* (KKJ) showed LD50 around 265mg dose level whereas, the sample purified through A. F. I approved method (KGMDG) showed it at a dose level more than 1g/kg. (Table 6.2 and Graph 7.9) An increase in LD50 value indicates that subjecting to *Shodhana* procedure is having the property of removing the toxic alkaloids strychnine & brucine from the seeds. A. F. I method is found to be more effective than Kanji method in reducing the toxicity of the drug. In quantitative HPTLC study it was revealed that strychnine & brucine content were more reduced in KGMDG sample than KKJ sample which also support the above results. (Table 5.4 and Graph 7.8)

**Graph 7.9: Result of LD50 study in rats**

*Part II: Efficacy study:*

It has been well established fact that Ayurvedic classics have emphasized various methods of *Shodhana* (purificatory procedures) to overcome the undesired effects from various poisonous drugs. The same is very true about *Kupeelu* seeds for which various Shodhana methods are mentioned in Ayurveda. In the Pharmacological study, *Kupeelu* seeds were subjected to two classical methods of *Shodhana* (Kanji and AFI methods) and evaluated for analgesic and anti-inflammatory activities to know whether classical purificatory procedure affect the efficacy or not i.e., whether efficacy will remain same or it will enhance the efficacy or it will decrease the efficacy.
**Anti-inflammatory activity:** In carrageenan induced paw oedema test, the first phase (up to 3 hours after injection of carrageenan) results from the concomitant release of mediators: histamine, serotonin and kinins on the vascular permeability and the second phase (up to 6 hours after injection of carrageenan) is correlated with leukotrienes formation and release. From the results, as depicted in the Table-6.3, both raw and possessed *Kupeelu* seeds at the given dose level, failed to exhibit any significant response at the first phase. However, the test drugs KR, KGMDG and KKJ non-significantly inhibited the second phase of carrageenan induced inflammatory response indicates mild action of test drug on leukotrienes formation and release.

The formalin-induced inflammation in the rats foot may be conveniently divided into two parts, the first involving 5-hydroxytryptamine as mediator and the second mediator which is unrelated to 5-hydroxytryptamine. In contrast to results obtained in carrageenan induced paw oedema, both KR and KKJ significantly suppressed paw oedema at both time intervals, which were found to be statistically highly significant. The result shows that decrease in paw volume is nearly equal in both the samples after 24 h whereas the decrease is more in raw drug than the *Kanji* purified sample after 48h of Formaldehyde administration (Table-6.4). Thus, the result indicates that these two samples of *Kupeelu* (KR & KKJ) having significant anti-inflammatory activity against formalin induced inflammation. However, the test drug KGMDG non-significantly inhibited both the phases of formalin induced inflammatory response indicates mild action of the test drug on proliferative phases of inflammation.

- **Analgesic activity:** In analgesic activity study, the analgesic testing protocol was selected such that both centrally and peripherally mediated effects could be ascertained by adopting formalin induced pain and tail flick response methods. Tail flick model, which is thermal induced nociception, indicates narcotic involvement, which is sensitive to opioid receptors (Abbott and Young, 1988). The ability of the drugs to prolong the reaction latency to thermally induced pain in mice further suggests central analgesic activity. Table-6.5 shows the data related to the tail flick response in mice. In control group, the tail flick response showed increase at various time intervals in comparison to the basal values. In-group KGMDG elevation in pain threshold was observed at all the time intervals studied except for the observation related to 60 min, at which a mild decrease was observed when compared to the control group. Although the values are statistically
insignificant, yet it can be assumed that the drug KGMDG may has mild analgesic activity against tail flick test. In the group treated with KR, a decrease in pain threshold was observed at all the time intervals studied except for the observation related to 60 min. at which a mild increase was observed when compared to the control group. These values are statistically insignificant suggest that the drug KR has failed to prove any sort of analgesic effect in tail flick test. Results observed in-group KKJ shows elevation in pain threshold at 60 min., 120 min., and 180 min. of post drug administration in contrast to the decrease in pain threshold at 30 min. and 180 min. interval. These values are not statistically significant but the percentage wise increase in pain threshold suggests mild analgesic effect of the drug at particular time intervals.

Formalin injection to plantar aponeurosis of rats shows pain response in two phase’s viz., initial and late phase. The initial phase lasts for 0-10 min. of formaldehyde injection; it is supposed to be mediated through modulation of neuropeptides. The second phase, which is observed 20-30 minutes of formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin etc. Data related to the effect of test drugs on experimental pain induction through Formalin is depicted in the Table- 6.6. In the first phase (0-10 min.) all, the three-test drugs (KR, KGMDG and KKJ) exhibit a decrease of 19.59%, 36.29% and 16.65% respectively in the number of paw licking response. However, the decrease in-group KGMDG was only found to be statistically significant when compared to the control, which indicates the presence of marked analgesic activity in the drug mediated through modulation of neuropeptides. Although, the percentage wise decrease in other two groups i.e., KR and KKJ also indicates presence of mild analgesic activity in these two drugs. While in the second phase of reading season, i.e. 20-30 min. after drug administration, a significant decrease of 34.01% was observed in-group KR indicating its significant suppression effect on pain of inflammatory origin. However, non-significant decrease i.e. 29.50% was observed in-group KGMDG and an increase of 11.31% was found in-group KKJ that is also statistically insignificant. Though the result found in-group KGMDG is not statistically significant but the percentage wise decrease in number of paw licking is suggestive that the drug KGMDG has mild inhibitory activity against inflammatory pain.
References:

17. Ibidem (ref.5), Cha. Su. 1/126.


58. Ibidem (ref.11), 247p.

59. Ibidem (ref.35), Su. 36/2

60. Ibidem (ref.35), Su. 38/80

61. Ibidem (ref.5), Kalpa. 1/10.


