INTRODUCTION:

Pharmacology can be defined as the study of the effects of drugs on the function of living systems. As a science, it was born in the mid-19th century, one of a host of new biomedical sciences based on principles of experimentation rather than philosophy that came into being in that remarkable period. Long before that—indeed from the dawn of civilization—herbal remedies were widely used, pharmacopoeias were written, and the apothecaries’ trade flourished, but nothing resembling scientific principles was applied to therapeutics. Until the late 19th century, knowledge of the normal and abnormal functioning of the body was too rudimentary to provide even a rough basis for understanding drug effects.

The motivation for understanding what drugs can and cannot do came from clinical practice, but the science could be built only on the basis of secure foundations in physiology, pathology and chemistry. It was not until 1858 that Virchow proposed the cell theory. The first use of a structural formula to describe a chemical compound was in 1868. Bacteria as a cause of disease were discovered by Pasteur in 1878. Previously, pharmacology hardly had the legs to stand on, and we may wonder at the bold vision of Rudolf Buchheim, who created the first pharmacology institute (in his own house) in Estonia in 1847.

In its beginnings, before the advent of synthetic organic chemistry, pharmacology concerned itself exclusively with understanding the effects of natural substances, mainly plant extracts and a few (mainly toxic) chemicals such as mercury and arsenic. An early development in chemistry was the purification of active compounds from plants. Friedrich Sertürner, a young German apothecary, purified morphine from opium in 1805. Other substances quickly followed, and, even though their structures were unknown, these compounds showed that chemicals, not magic or vital forces, were responsible for the effects that plant extracts produced on living organisms. Early pharmacologists focused most of their attention on such plant-derived drugs as quinine, digitalis, atropine, ephedrine, strychnine and others.

Beginning in the 20th century, the fresh wind of synthetic chemistry began to revolutionize the pharmaceutical industry and with it the science of pharmacology. New synthetic drugs, such as barbiturates and local anesthetics, began to appear, and
the era of antimicrobial chemotherapy began with the discovery by Paul Ehrlich in 1909 of arsenical compounds for treating syphilis. Further breakthroughs came when the sulfonamides, the first antibacterial drugs, were discovered by Gerhard Domagk in 1935 and with the development of penicillin by Chain and Florey during the Second World War, based on the earlier work of Fleming.

These few well-known examples show how the growth of synthetic chemistry, and the resurgence of natural product chemistry, caused a dramatic revitalization of therapeutics in the first half of the 20th century. Each new drug class that emerged gave pharmacologists a new challenge, and it was then that pharmacology really established its identity and its status among the biomedical sciences.

The 20th century was marked by development of experimental pharmacology which particularly aims to find out a therapeutic agent suitable for human use, to study the toxicity and to study the mechanism & site of action of the drug. Experimental pharmacology deals with the discovery of new drugs or to study the actions of existing drugs which involves two stages,

🗦 Pre-clinical experimental pharmacology- It includes the identification and optimization of novel chemical lead structures and testing on animals and animal tissues or organs for their biological actions.

🗦 Clinical pharmacology- It includes the administration of drugs on human volunteers and patients for assessing the pharmacokinetics, safety and efficacy in human beings.

Now a day there is a global trend among the pharmacologists to evaluate plant or plant products in experimental animals in terms of providing experimental or pharmacological basis to the drugs used in traditional system of medicine.

Searching through various research journals, text books of Ayurveda and different search engines reveals that very few pharmacological works have been reported on the analgesic & anti-inflammatory activity of strychnine & brucine. But till date no pharmacological works on Shodhita Kupeelu seed have been carried out in this regard. Even the LD50 study of Shodhita seeds have not done yet. Hence, in this study an attempt has been made for the first time to evaluate the LD50 values, analgesic & anti-inflammatory activity of Kanji purified Kupeelu seeds and Gomutra-Godugdha-Goghrita (A.F.I approved method) purified seeds in comparison to the raw seeds.
PLAN OF THE STUDY:

The present pharmacological study has been carried out in the following three phases:
- Acute toxicity study
- Anti-inflammatory activity study
- Analgesic activity study

MATERIALS AND METHODS:

The Animals:

Wistar strain albino rats of either sex; weighing 210 ± 60 g and Swiss albino mice of either sex; weighing 24 ± 4g were used for the study. The animals were obtained from the animal house attached to I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar. Six animals were housed in each cage made up of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. The animals were exposed to 12 hour light and 12 hour dark cycle with the relative humidity of 50 to 70% and the ambient temperature during the period of experimentation was 22 ± 03°C. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. For their drinking purpose tap water *ad libitum* was used. The experiments were carried out in conformity with the Institutional Animal Ethics Committee (IAEC) after obtaining its permission (IAEC 06/09-11/PhD/04).

Test drugs:

The pharmacological study was carried out on two samples of *Shodhita Kupeelu* seeds i.e. KGMDG & KKJ which were prepared by A.F.I adopted Shodhana method for *Kupeelu*¹ and Kanji method² respectively. The details pertaining to this have been given in elaborate manner in the pharmaceutical study part of the thesis. The *Shodhita* sample was compared with the raw *Kupeelu* to find out the impact of the *Shodhana*. The test drugs are;
- Raw *Kupeelu* seeds (KR)
- A.F.I approved *Shodhita Kupeelu* seeds (KGMDG)
- *Kanji Shodhita Kupeelu* seeds (KKJ)
**Dose selection:**

Human dose of *Shodhita Kupeelu* seeds is 250mg/day. The purified / processed (*Shodhita*) *Kupeelu* seeds were compared with the raw *Kupeelu*. So the same dose was taken for the raw *Kupeelu* seeds also.

**Dose fixation:**

The dose selection was done on the basis of body surface area ratio using the table of Paget and Barnes (1964) and it was done as follows.

Human dose × body surface area ratio convertibility factor

Human dose of *Kupeelu* seed powder: 250 mg/day.

Dose for rats: Human dose×0.018

= 250mg×0.018

=4.5 mg/200g body weight of rat

=22.5 mg/kg/day of rat

Dose for mice: Human dose×0.0026

=250mg×0.0026

=0.65 mg/20g body weight of mice

= 32.5 mg/kg/day

**Route of drug administration:**

The test drug was administered according to the body weight of the animals by oral route. Stock solution of suitable concentration was prepared with de-ionized water freshly just prior to administration and administered with the help of suitable sized gastric catheter no. 6 sleeved onto a syringe.

1. **ACUTE TOXICITY STUDY:**

Acute oral toxicity study was carried out as per OECD guideline 425.

**Instruments used:**

Weighing scale, Monopan balance, Syringes, Needles, Catheters, Micro capillaries

**Animals:**

Female Wistar strain albino rats weighing 160 ± 30 g were used for evaluation of acute toxicity test.

**Procedure:**

Acute toxicity study was conducted using limit dose test of Up and Down procedure. Three samples of *Kupeelu* were administered once orally to overnight
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Shodhana of Kupeelu

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fasted rats at various dose levels viz., the animals of each group were dosed in graded doses i.e. 55, 175, 550 and 2000 mg/kg. Gross behaviour and mortality, if any was observed throughout the study period for 14 days.

**Examination of physical and behavioral changes:**

The animals were observed continuously for 6 hours after dosing. The careful cage side observation was done without disturbing the animal attention and at the end of every hour the animals were individually exposed to open arena for recording the behavioral changes like increased or decreased motor activity, convulsions, straub’s reactions, muscle spasm, catatonia, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation, diarrhea, writhing, mode of respiration, changes in skin color, exitus, C.N.S. depression-hypoactivity, passivity, relaxation, ataxia, narcosis, etc.

**Mortality:**

All the animals were observed at half hour, 1h, 2h, 3h, 4h, 5h, 6h, 24h and 48hrs after dosing and thereafter daily once for mortality during the entire period of the study (14 days).

2. **ANTI-INFLAMMATORY ACTIVITY:**

I. **Carrageenan induced paw edema in rats:**

Standard procedure of Winter et al., (1962) was followed for the anti-inflammatory activity of the test drugs. In this model Carrageenan was used to induce the paw oedema in rats.

**Phlogistic agent:**

Carrageenan: 0.1ml (freshly prepared 1% in sterile saline) injected subcutaneously beneath the plantar aponeurosis in the left hind paw.

**Route of drug administration:**

The test drug was administered according to the body weight of the animals by oral route with the help of suitable sized gastric catheter no 6 sleeved onto a syringe.

**Procedure:**

Wistar strain albino rats of either sex were weighed and randomly divided into four groups of six animals in each group. First group received distilled water and served as control group. The second, third, fourth group received Raw Kupeelu...
seeds (KR), A.F.I adopted *Shodhita Kupeelu* seeds (KGMDG) and Kanji *Shodhita Kupeelu* seeds (KKJ) respectively in dose of 22.5mg/kg. The vehicle and test drugs were administered to the respective groups for five consecutive days. On fifth day, one hour after drug administration, initially left hind paw volumes up to the tibio-tarsal articulation were recorded prior to Carrageenan injection by using plethysmograph\(^7\) and then oedema was produced by injecting 0.1 ml freshly prepared 1% carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administered distilled water in the dose of 2 ml per 100 g body weight to ensure uniform hydration and hence to minimize variations in oedema formation. Paw volume was recorded at the interval of 3h and 6h after carrageenan injection. Results were expressed as percentage change in paw volume in comparison to the initial paw volumes and in comparison with control group.

II. **Formaldehyde induced paw oedema in rats\(^7\):**

The test conditions and groupings were similar to carrageenan induced paw oedema as mentioned above. The drugs were administered once daily for five consecutive days. On 5th day, initial left hind paw volumes were recorded with the help of Plethysmometer. One hour after the drug administration, 0.1 ml of 3% formaldehyde solution was injected to sub-plantar aponeurosis of the left hind limb. Paw volumes were measured at 24 h and 48 h after formaldehyde injection as described earlier. Results were expressed as percentage change in paw volume at various time intervals in comparison to the initial values.

3. **ANALGESIC ACTIVITY:**

I. **Formalin induced hind paw licking response\(^8\):**

The effect of test drugs on formaldehyde induced paw oedema was studied in Wistar strain albino rats of either sex. The selected animals were grouped into four groups of 6 rats each. First group received distilled water and served as control group. The second, third, fourth group received Raw *Kupeelu* seeds (KR), A.F.I adopted *Shodhita Kupeelu* seeds (KGMDG) and Kanji *Shodhita Kupeelu* seeds (KKJ) respectively in dose of 22.5mg/kg. The vehicle and test drugs were administered to the respective groups for five consecutive days. Pain response was induced by injecting 0.1 ml of 3% formalin in distilled water in sub plantar region
of right hind paw. The number of paw licking was noted as an index of nociception at different time intervals, 0 to 10 minutes (Early phase) and 20 to 30 minutes (Late phase).

II. Radiant heat test (Tail flick response):

Radiant heat as a source of noxious stimulus is the most frequently used stimulus for assessing analgesic activity. The instrument used for this purpose is called ‘Tail Flick Analgesiometer’. It comprises of nichrome wire which gets heated up on passing electrical current, controlled through a galvanometer. The area around the nichrome wire is cooled by passing water through an inlet and released through an outlet. When the tail of a restrained animal is placed over the heated nichrome wire, it experiences pain due to heat and flicks the tail. The time taken to flick the tail from the point of placing it over the wire is considered as reaction time. If the reaction time gets prolonged, then the test drug is supposed to have analgesic activity.

Swiss albino mice of either sex were placed on the tail flick unit so that constant heat intensity was applied to the lower third of the animal’s tail. When the animal flicked its tail in response to the noxious stimulus both the heat source and timer were stopped. A cut off time of 10 seconds was set to avoid tail damage. The basal reaction time of each mouse to radiant heat was recorded and those having TFL (tail flick latency) less than 10 seconds were selected. Selected mice were randomly divided in to four groups of six each. First group received similar volume of vehicle as test drug and served as normal control. Mice in second group were treated with raw Kupeelu (KR) in dose of 32.5 mg/kg. The Group third and fourth group were treated with processed Kupeelu viz. KGMDG and KKJ respectively in same dose 32.5 mg/kg as group second. The TFL was recorded at the intervals of 30, 60, 120, 180 and 240 minutes after drug administration.

STATISTICAL ANALYSIS:

The obtained data have been presented as Mean ± SEM, difference between the groups was statistically determined by student’s t test for unpaired data for the treated group with the level of significance set at $P<0.05$. The level of significance was noted and interpreted accordingly.
OBSERVATIONS AND RESULTS

1. ACUTE TOXICITY STUDY:

**Behavioural changes:** LD50 of the *Shodhita Kupeelu* samples (KGMDG & KKJ) were determined and compared with the raw *Kupeelu* (KR). During entire period of acute toxicity study, gross behavior of animals was observed. Both raw and *Shodhita Kupeelu* seed samples exhibited mild to severe features of CNS stimulation and strychnine poisoning. These features were observed up to 4h after drug administration. The features observed have been provided in following table.

**Table 6.1: Behavioural changes observed during acute toxicity test**

<table>
<thead>
<tr>
<th>Group code</th>
<th>Dose</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR</td>
<td>55mg/kg</td>
<td>Normal</td>
</tr>
<tr>
<td>KR</td>
<td>100mg/kg</td>
<td>Occasional jerks followed by convulsion</td>
</tr>
<tr>
<td>KR</td>
<td>175mg/kg</td>
<td>Exaggerated jerks followed by convulsion and death</td>
</tr>
<tr>
<td>KGMDG</td>
<td>175mg/kg</td>
<td>Normal</td>
</tr>
<tr>
<td>KGMDG</td>
<td>550mg/kg</td>
<td>Normal</td>
</tr>
<tr>
<td>KGMDG</td>
<td>1gm/kg</td>
<td>Occasional jerks</td>
</tr>
<tr>
<td>KGMDG</td>
<td>2gm/kg</td>
<td>Convulsion followed by death</td>
</tr>
<tr>
<td>KKJ</td>
<td>100mg/kg</td>
<td>Normal</td>
</tr>
<tr>
<td>KKJ</td>
<td>175mg/kg</td>
<td>Exaggerated jerks followed by convulsion</td>
</tr>
<tr>
<td>KKJ</td>
<td>550mg/kg</td>
<td>Convulsion followed by death</td>
</tr>
</tbody>
</table>

**Table 6.2: Result of LD50 study in rats**

<table>
<thead>
<tr>
<th>Group code</th>
<th>Animal No.</th>
<th>Treatment</th>
<th>LD50 (mg/kg b.w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR</td>
<td>6</td>
<td>Raw <em>Kupeelu</em> seeds</td>
<td>175</td>
</tr>
<tr>
<td>KGMDG</td>
<td>6</td>
<td>A.F.I adopted <em>Shodhita Kupeelu</em> seeds</td>
<td>1098</td>
</tr>
<tr>
<td>KKJ</td>
<td>6</td>
<td><em>Kanji Shodhita Kupeelu</em> seeds</td>
<td>265</td>
</tr>
</tbody>
</table>

Approximate LD50 values of the test drugs have been given in the Table- 6.2. LD50 of Raw *Kupeelu* was found to be 175mg/kg and it was 265mg/kg (based on an assumed sigma of 0.5) for *Kanji Shodhita* sample. In contrast to these, LD50 of A.F.I *Shodita* (A.F.I) sample was found to be 1098mg/kg.
2. **ANTI-INFLAMMATORY ACTIVITY:**

I. **Effect on Carrageenan induced paw oedema in rats:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>% Increase in paw volume at different time interval after Carrageenan injection.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 hour</td>
</tr>
<tr>
<td>Control</td>
<td>Q.S.</td>
<td>55.24 ± 3.14</td>
</tr>
<tr>
<td>KR</td>
<td>22.5</td>
<td>63.20 ± 8.59</td>
</tr>
<tr>
<td>KGMDG</td>
<td>22.5</td>
<td>59.17 ± 8.12</td>
</tr>
<tr>
<td>KKJ</td>
<td>22.5</td>
<td>60.91 ± 4.05</td>
</tr>
</tbody>
</table>

**Data:** Mean ± SEM, ↑ - Increase, ↓ - Decrease

Data related to the effect of test drug on carrageenan induced paw oedema in albino rats have been given in table-6.3. Both raw and *Shodhita Kupeelu* seeds failed to inhibit the carrageenan induced paw oedema at both time intervals in comparison to control group.

II. **Effect on Formaldehyde induced paw oedema in rats:**

<table>
<thead>
<tr>
<th>Group code</th>
<th>Dose (mg/kg)</th>
<th>% increase in paw volume at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hour</td>
</tr>
<tr>
<td>Control</td>
<td>Q.S.</td>
<td>32.56 ± 2.38</td>
</tr>
<tr>
<td>KR</td>
<td>22.5</td>
<td>20.12 ± 2.30**</td>
</tr>
<tr>
<td>KGMDG</td>
<td>22.5</td>
<td>27.43 ± 5.28</td>
</tr>
<tr>
<td>KKJ</td>
<td>22.5</td>
<td>19.43 ± 2.62**</td>
</tr>
</tbody>
</table>

**Data:** Mean ± SEM, **P<0.01, ***P<0.001(compared with control group)

Data related to the effect of test drug on formaldehyde induced paw oedema in albino rats have been given in table- 6.4. A significant inhibition of paw oedema was observed in KR and KKJ sample treated groups in comparison to control group at both 24 h and 48h, while non-significant inhibition was observed in KGMDG group.
3. ANALGESIC ACTIVITY:

I. Effect of test drugs on tail flick response (TFL) in mice:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>TFL after drug administration (sec.)</th>
<th>0-10 min</th>
<th>20-30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial % change</td>
<td>30 min % change</td>
<td>60 min % change</td>
</tr>
<tr>
<td>Control</td>
<td>Q.S.</td>
<td>1.91 ± 0.15</td>
<td>--</td>
<td>1.99 ± 0.17</td>
</tr>
<tr>
<td>KR</td>
<td>32.5</td>
<td>2.03 ± 0.18</td>
<td>06.28 ↑</td>
<td>1.73 ± 0.43</td>
</tr>
<tr>
<td>KGMDG</td>
<td>32.5</td>
<td>2.13 ± 0.13</td>
<td>11.52 ↑</td>
<td>2.63 ± 0.55</td>
</tr>
<tr>
<td>KKJ</td>
<td>32.5</td>
<td>2.50 ± 0.24</td>
<td>30.89 ↑</td>
<td>1.97 ± 0.25</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM, ↑ - Increase, ↓ - Decrease

II. Effect of test drugs on Formalin induced paw licking response in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Number of paw lickings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-10 min</td>
</tr>
<tr>
<td>Control</td>
<td>Q.S.</td>
<td>17.00 ± 1.53</td>
</tr>
<tr>
<td>KR</td>
<td>22.5</td>
<td>13.67 ± 1.23</td>
</tr>
<tr>
<td>KGMDG</td>
<td>22.5</td>
<td>10.83 ± 1.40*</td>
</tr>
<tr>
<td>KKJ</td>
<td>22.5</td>
<td>14.17 ± 2.50</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM, ↑ - Increase, ↓ - Decrease, *P<0.05, **P<0.01 (compared with control group)
Data related to the effect of test drug on formalin induced paw licking response in rats have been given in table- 6.6. All the three samples of Kupeelu apparently inhibited the first phase of formalin induced pain response, among them only the inhibition observed in KGMDG treated group is found to be statistically significant. In second phase, raw and KGMDG administered groups show apparent inhibition of formalin induced pain response, among them only the inhibition observed in KR treated group is found to be statistically significant. Further KKJ administered group failed to inhibit the second phase of pain response.

REFERENCES: