MATERIALS AND METHODS:

1. Pharmaceutical Study.
2. Analytical Study.
3. Experimental Study.

MATERIALS:-

Pharmaceutical Study:

Drugs for preparation – Gunja,
Analyses of Gunja beeja,

Analytical Study:-

Different chemicals for analyses.

Experimental Study:-

Animals - 12 female Wistar rats for animal study
12 male Wistar rats for experimental Study.

Instruments for feeding the animals – Gastric tube, syringe.

Equipments for sample collection - Scalpel, Capillary Tubes,
Glass Slide, Glass Tube, EDTA bulb.

Equipments for dissection - Diethyl ether, gloves, scissor, scalpel,

Formalin for organ preservation.
METHODOLOGY.

A. PHARMACEUTICAL STUDY

1. Collection of raw drugs

2. Authentication of drugs

3. Preparation of Gunja beeja churna


1. Collection of raw drugs:
   Gunja beeja collected from natural habitat.

2. Authentication of drugs:
   Both Gunja beeja were authenticated at Department of Botany, B.V.V. Sangha’s Science College, Bagalkot. (Authentification Certificate is being attached)

3. Preparation of Gunja beeja churna:
   Gunja beeja churna: It was prepared in Bhaishajya Pharmacy, of BVVS Ayurved Medical College & Hospital, Bagalkot.

SOP for preparation:

- Dried, clean seeds of Gunja were selected.

- Initially gunja beeja are pounded in kalvayantra and later it was made into fine powder by using grinder. Prepared Powder was passed through 120 mesh size.

- It was packed in air tight container.
MATERIALS AND METHODS

Observations:

- Fine pale yellow color powder was formed.

Precautions:

- Undried, Unripened and infected seeds were discarded.
- Compulsorily gloves, mask, apron and goggle were used during preparation.
- Should clean vessels properly after preparation.

B. ANALYTICAL STUDY:

Analysis of *Gunja beeja* was carried out at Pharmacology Department, BVVsangha’s HSK College of Pharmacy, Bagalkot. *Gunja beeja* was subjected for following analyses as per standard Kandelawal text.

1. **Organoleptic characters**

   1. Part
   2. Color
   3. Odor
   4. Taste
2. Physico-chemical analyses

1. Foreign matter determination

2. Loss on drying

3. Total ash

4. Acid insoluble ash

5. Water soluble extractive

6. Alcohol soluble extractive

3. Preliminary phyto-chemical screening

1. Organic elements

2. Inorganic elements
C. EXPERIMENTAL STUDY

OECD guidelines are used for testing of chemicals are periodically received in the light of scientific progress or changing assessment practices. Guidelines 423 adopted in March 1996, as the second alternative to the conventional toxicity test, described in test guidelines.

It is a stepwise procedure with the use of three animals each of both sexes per step. This method depends on the mortality or moribund status of the animal, on average 2-4 steps may be necessary for evaluating the acute toxicity of the test substance.

This method is based on biometric evaluation with fixed doses, adequately separated to enable a substance to be ranked for classification purpose and hazardous assessment.

LD50 (Median Lethal Dose) is a single dose of substance that can be expected to cause death in 50 per cent of animal when administered by the oral route. Its value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

For this animal study, following Animal experiment protocol has been followed,

**Table 1. Protocol of Animal Experiment**

<table>
<thead>
<tr>
<th></th>
<th>Centre</th>
<th>B. V. V. Sangha’s HSK College of Pharmacy, Bagalkot, Karnataka, Recognized by CPCSEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Animal Species</td>
<td>Albino Mice</td>
</tr>
<tr>
<td>3</td>
<td>Strain</td>
<td>Swiss Albino</td>
</tr>
<tr>
<td>4</td>
<td>Source of Animal</td>
<td>B. V. V. Sangha’s HSK College of Pharmacy, Animal House, Bagalkot, Karnataka.</td>
</tr>
<tr>
<td>5</td>
<td>Weight of Mice</td>
<td>20 gms</td>
</tr>
<tr>
<td>6</td>
<td>No of Animals</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>Sex of Animal</td>
<td>50% Male, 50% Female in each group.</td>
</tr>
<tr>
<td>8</td>
<td>Diet of Animal</td>
<td>Rodent pallets</td>
</tr>
<tr>
<td>9</td>
<td>Water</td>
<td>Community tap water ad libitum</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS

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<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>Temp</td>
<td>18° – 30° C</td>
</tr>
<tr>
<td>11</td>
<td>Humidity</td>
<td>40 – 60%</td>
</tr>
<tr>
<td>12</td>
<td>Light Cycle</td>
<td>12 hrs Light 12 hrs Dark</td>
</tr>
<tr>
<td>13</td>
<td>Vehicle Used</td>
<td>Water</td>
</tr>
<tr>
<td>14</td>
<td>Period of Acclimatization</td>
<td>15 days</td>
</tr>
<tr>
<td>15</td>
<td>Period of Fasting</td>
<td>Overnight</td>
</tr>
<tr>
<td>16</td>
<td>Test Drug</td>
<td>Powder of dried Gunja Seeds (Abrous precursorius)</td>
</tr>
<tr>
<td>17</td>
<td>Dosing</td>
<td>200,300,400,500 mg/kg body weight</td>
</tr>
<tr>
<td>18</td>
<td>Route of administration</td>
<td>Oral</td>
</tr>
</tbody>
</table>

**Principle of the test:**

It is a stepwise test by using minimum number of animals (per step 3 animals). A defined dose of drug is administered orally in a single dose in experimental animal. This method depends on the presence or absence of compound related mortality of animals, dosed at one step will determine the next step.

**Description of method:**

**Selection of animal: Swiss** Albino mice are preferably used although other than rodent species also used. Based on sensitivity, adult healthy female mice species of 8-12 weeks are used for testing.

**Housing and feeding:** Animals kept in cage at ambient temperature of about 22° C with 12 hours day and 12 hours night cycle with a conventional laboratory diet and water *ad libitum.*
**Preparation of animal:** Animals are randomly selected and marked individually for identification and kept in a cage at least 5 days for acclimatization.

**Preparation of dose:** In general, test drug should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. In rodents, volume of drug should not exceed 1ml/100gm body weight, and for aqueous solution 2ml/100gm body weight can be considered.

**Procedure:**

**Administration of dose:**

Test drug is administered in a single oral dose by using stomach tube or suitable intubation canula. In unusual circumstances dosing can be given in a small fraction over a period, not exceeding 24 hours. But here single dose is given per day. Before dosing, animal should be fasted over night by withdrawing food but water is fed. And after 4 hours of dosing both water and food have to be provided.

**Number of animals and dose level:**

6 animals of both sexes (Three male and Three Female) are used for each step. Here dose level starts from one of four fixed levels 200, 300, 400 and 500 mg/kg body weight. If mortality is not found at the level of 500 mg/kg body weight, then limit test should be conducted.

**Observations:**

Observations have to be done individually during first 30 minutes, periodically during the first 24 hours, with a special attention given during the first 4 hours and daily for a period of 06 days. Toxicity signs and symptoms, its onset, recovery period and death all these observations should be observed and recorded properly.

Observations in changes in skin and fur, eyes, mucous membrane and also respiratory, circulatory, central nervous system and somatomotor system and behavior patterns. Also behavioral changes like tremors, convulsions, lethargy, salivation, diarrhea, sleep and coma all other observations are recorded during the study.
Body weight:

Animals should be weighed prior to the dosing, weekly and thereafter at the end of the study before the sacrificing the animal. Weight variation should be calculated and recorded.

Pathology:

All test animals which died during study, after sacrificing, or removed from the study for welfare reasons are subjected for gross necropsy.

Data and Reporting:

Data:

All data should be noted and all data should be summarized in tabular form, showing for each test group like the number of animals used, number of animals displaying signs of toxicity, the number of animals found died during the test or killed for humane reasons, and the time of toxic effects and reversibility and time of death and necropsy findings.

Test reporting:

It includes about the Test substance (Physical nature, purity, physico-chemical properties), vehicle, test animals (species, number, age, sex, housing, condition, diet etc.) Test condition (dosing, its volume). Results of animal observations after dosing, observations like body weight, and necropsy findings etc and also it includes discussion and interpretation of results and conclusion.