Work plan and methodology

Time schedule preparation and adherence to the schedule strictly is very useful in keeping our work – train on the track and in time

1. Review of literature
2. Collection and preparation of starting material for compound unani formulation.
3. Pharmacological evaluation of compound unani formulation.

METHODOLOGY

Review of literature
This will be a continuous process from starting till the finalization of the work. The sources utilized would be Books, Standards like IP, USP, Extra Pharmacopeia WHO guidelines etc., Journals including National and International, Patent, historically established and Internet.

Collection and preparation of starting material for compound unani formulation.
It is planned to collect the natural drug material from a reliable source and checked cautiously as per the need. Starting material will be separated and then preserved and improved shelf life of the constituents.

Pharmacological evaluation of compound unani formulation.
This will be done with an intention to confirm the study of Novel Unani Formulation prevent gallstone formation in mice (C57BL/6) susceptible to Cholediasis by lithogenic diet. Also this activity can give better options for its commercial use in near future research.
The compound unani formulation would be studied for its lithotriptic activity in c57bl/6 female mice. The mice will be induced with lithogenic diet for specified periods of time for formation of gallstones. Control group will fed on standard pellet diet and the lithogenic group will fed on lithogenic diet and the lithogenic diet plus drug group will fed on lithogenic diet and UCF will be given simultaneously, and the drug group will fed on drug alone with standard diet. The biochemical parameters (serum concentrations of Cholesterol, Triglycerides, HDL, Bilirubin, AST / SGOT, ALT /SGPT, Alkaline Phosphatase, Total Protien and Albumin) will be assessed at 8th week and 12th week of induction with lithogenic diet. Histopathological findings will also record.

36 female C57BL/6 mice weighing 12-15 grams will be divided into 4 groups.

**Group 1**-control group n=6  
**Group 2**- lithogenic diet group n=12 (LD GROUP)  
**Group 3**-lithogenic diet + ucf group (n=6). (LD+UCF group)  
**Group 4**-UCF group (n=6, to be taken from LD group at the end of 8 weeks).

Study is divided into two phases.  
Phase-I and phase-II.

Induction of gall stones:  
Gall stones will induce using lithogenic diet with the composition for a period of 8 weeks.  
Group 1-control group (n=12):  
Mice in this group will feed on standard pellet diet with free access to the water for 8 weeks.  
Group 2-lithogenic diet group (n=12). (LD group):  
Mice in this group will feed on lithogenic diet for a period of 8 weeks with free access to water.
Group 3-lithogenic diet +UCF group (n=6). (LD plus UCF group):
Mice in this group will feed on lithogenic diet along with UCF with dose 12gms/kg/day for a period of 8 weeks.

Group 4-UCF group (n=6, to be taken from LD group at the end of 8 weeks):
Mice in this group will be taken from LD group at the end of 8 weeks and will be given UCF at the dose of 12gm/mg/day along with standard pellet diet for next 4 weeks.

Procedure:

Phase-I
At the end of 8 weeks mice from group I, group II, group III will be sacrificed under ether anaesthesia and the samples will collected. blood samples subject at 10,000rpm and the serum will separate and stored at -20 centigrade for further analysis.

Phase-II:
After 12 weeks of treatment mice from UCF will be sacrificed under ether anaesthesia and the samples will collect. blood samples subject at 10,000rpm and the serum will separate and stored at -20 centigrade for future analysis.

Composition of lithogenic diet:
A lithogenic diet is one that can increase formation of gallstones, although it’s typically used in laboratory animals. It has got inhibitory effect on the rate limiting enzyme of cholesterol gallstone biosynthesis.
The below ingredients constitute the lithogenic diet approx to 1000gms

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200gm</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50 gm</td>
</tr>
<tr>
<td>Sucrose</td>
<td>260 gm</td>
</tr>
<tr>
<td>Starch</td>
<td>260 gm</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>150 gm</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>40 gm</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>15 gm</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>10 gm</td>
</tr>
<tr>
<td>Vitamins</td>
<td>10 gm</td>
</tr>
<tr>
<td>d-l methionine</td>
<td>3 gm</td>
</tr>
</tbody>
</table>

The comparisons will be done as follows—at 8 weeks- Group I with Group II. Group I with Group III, Group II with Group III. At the end of 12 weeks the comparison will be done between, Group I with Group III, and Group II with Group IV.

**Drug administration and dosage:**

The quantification of daily weight consumption of the lithogenic diet (LD) will be done and thereby calculation of the concentrations of drug necessary to compound with daily feeds.

The calculation of dose was done using dose conversion factor for mice -7 as well as per unit mg/kg. i.e 12gm/kg of body weight.
The ingredients used for the preparation of the UCF are as follows:

1. Allium sativum (garlic/lahsun). 5gms.
2. Dolichos biflorus (horsegram/kulthi). 5gms.
3. Echinops echinatus (camel thistle/oont katara). 5gms.

**Procedure for collecting samples:**

Before sacrificing the mice external physical inspection will be done.

After 12 weeks of induction with gall stones animals will be sacrificed.

Mice would fast over night and anaesthetized with ether in the anaesthetizing chamber.

Upper midline abdominal incision will be given and the gall bladder and other organs would be inspected.

The size of the different gall bladders will be measured in all the three groups.

Gall bladder would be then excised and will store at -80 degree before analysis.

Liver will excise and, rinsed with 0.15M Nacl to remove blood and will store at -80degree before analysis.

Blood would be collected from retro –orbital sinus as well as from left ventricle, centrifuged at 10,000 revolutions per minute for 6 minutes and serum would be collected and stored at -20 degree for further analysis.

Slides would be prepared for histopathological examination of the organs.