Acute toxicity study of a polyherbal Unani formulation Habbe Shifa in experimental animal model

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Received 06.08.12, revised 11.02.13

Habbe Shifa (HS) is an important pharmacopoeial Unani formulation which is widely used in Unani system of medicine. HS contains Tukhme dhatura (Datura stramonium L.) as a major constituent. The present study investigated the acute toxicity of HS in Swiss albino mice. In the present study, hydro alcoholic extract of HS was administered to six groups of mice orally in the doses ranges from 8-42 gm/kg B wt and the animals were observed continuously for gross behavior and mortality at 0 min, 30 min, 60 min, 120 min, 240 min and 24 hrs. The numbers of animals found dead within 24 hrs in each group were recorded. The LOAEL (Lowest Observed Adverse Effect Level) for the oral dose extract was found to be 8 gm/kg B wt. The estimated oral LD-50 of hydro alcoholic extract of HS was found to be 30 gm/kg B wt in mice. From the results, it is concluded that HS is safe at normal therapeutic dose as well as at higher doses.

Keywords: Toxicity, Habbe Shifa, Acute

IPC Int. Cl.: A61K 36/00, A01D 15/00, C12N, C12P

Plants and derivatives of plant played a key role in world health and have long been known to possess biological activity. At present, it is easier to determine efficacy and safety of herbal remedies because it is known which chemical compounds are present in these plants and which of these are associated with a number of side effects. Medicinal plants behave as authentic medicines because the chemical substances of which they are formed can have a biological activity in humans. Determination of efficacy and safety of herbal remedies is necessary because many people using these agents as self medication. Since, there is limited data available about the safety of the commonly used herbal remedies, therefore, efforts to elucidate health benefits and risks of herbal medicines should be intensified1. It is the need of the hour to evaluate acute and chronic toxicities of herbal drugs. Acute toxicity testing in animals is typically the initial step in the assessment and evaluation of the health effect characteristics of a test substance, and its primary purpose is to provide information on potential health hazards that may result from a short term exposure. Acute oral toxicity is defined as the adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hrs. It is typically presented as an LD-50 value, which is a statistically derived estimate of the single dose of a substance that can be expected to cause death in 50% of the treated animals. LD-50 data are expressed in terms of amount of the test substance per unit body weight of the animal (e.g., gm or mg/kg)2,3. The Toxicity of dhatura has been mentioned by Ibn Sina4 and Ismail Jurjani5 and also reported by modern Science. So, it was assumed that overdose of Habbe Shifa (HS) might cause toxicity. Further, Unani system of medicine lacks studies related to the toxicity of drugs. A number of drugs such as arsenic, opium, lead, mercury and dhatura are used in the Unani system of medicine, are essentially toxic if used without caution. The drug under present study was HS which has Tukhme dhatura (Datura stramonium L.) as major constituent.

HS is a combination of medicinal plants including Tukhme dhatura (Datura stramonium L.), Rewandchini (Rheum emodii Wall. ex Meisn.), Zanjabeel or Sonth (Zingiber officinale Roscoe) and Samaghe Arabi [Acacia nilotica (L.) Delile. syn. *Corresponding author
Acacia arabica (Lam.) Willd.\textsuperscript{6} which is used in the treatment of Humma (fever), Iya (fatigue), Tashannuj e rewi (pulmonary spasm) and Zeequn Nafas (asthma) and opium de-addiction in Unani system of medicine\textsuperscript{6,7,8}. The analgesic, anti-convulsant and anti-pyretic activity of HS has been reported and aqueous and alcoholic extract of HS was found to produce significant analgesic, anti-convulsant and anti-pyretic activity\textsuperscript{9}, but there is no data available on toxicity of HS. Therefore, Acute Toxicity of HS was carried out.

**Methodology**

**Procurement of raw drugs**

The ingredients of HS were procured from the registered crude drug dealer, Bangalore and all the crude drugs were identified by Dr Roohi Zaman.

**Animals**

The acute toxicity study was carried out on Swiss albino mice of either sex weighing 20-30 gm. The animals were procured from Sri Raghvendra Enterprises, Vijayanagar, Bangalore, India and housed in polypropylene cages in animal house facility at NIUM, Bangalore. They were acclimatized to the conditions for 1 week before experimental studies. The animals were maintained in a standard environmental condition at room temperature (25 ± 2°C) with 12 hrs light/dark cycle, humidity (50-55%), and had free access to food pellets (Sai Durga Feeds & Foods, Bangalore) and tap water ad libitum under strict hygiene. The Institutional Animal Ethics Committee (IAEC), NIUM, Bangalore, Karnataka, approved the experimental protocol vide Reg. No. 953/C/06/CPCSEA.

**Preparation of extract**

The powdered HS was extracted with mixture of ethanol and water (1:1) for 6 hrs at a temperature of 70°C using soxhlet apparatus. This extract was filtered and concentrated on water bath for complete removal of solvent. The yield of this extract was 27% w/w with respect to dried powder.

**Dosage of the drug**

Dosage of the drug for Swiss albino mice was calculated by extrapolating the human therapeutic dose by conversion factor 12 which was found to be 100 mg/kg B wt\textsuperscript{10}. A pilot study was carried out with two mice for each dose of powdered material of the test drug in the form of suspension, administered in various doses in increasing dose (8, 20, 24, 30, 36 & 42 gm/kg) once orally to find out the approximate dose producing 50% mortality. Till the dose of 20 gm/kg B wt, no mortality was observed and it was very difficult to form suspension of powdered drug with higher dose due to its thick consistency. Therefore, in actual study HS extract was used to determine acute toxicity study. The hydroalcoholic extract of HS in the doses of 8, 20, 24, 30, 36 & 42 gm/kg B wt were administered once in each group by oral route with the help of gastric canula.

**Acute toxicity study**

Acute toxicity test was performed according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 420\textsuperscript{3}. Swiss albino mice of either sex weighing 20-30 gm were randomly assigned to 6 groups (I, II, III, IV, V & VI) of 6 mice each. Mice were fasted overnight (12 hrs) with free access to water prior to administration of single doses (8, 20, 24, 30, 36 & 42 gm/kg) of the extract dissolved in distilled water by oral route. After the administration of the test drug all the animals were kept in polypropylene cages singly and observed continuously for Gross behavior and mortality at 0 min, 30 min, 60 min, 120 min, 240 min and 24 hrs. The numbers of animals found dead within 24 hrs in each group were recorded. The other parameters observed include piloerection, grooming, trembling, wriggling, diarrhoea, breathing difficulty, constant changing position, immobility, asthenia, anorexia, ataxia, urination and syncope\textsuperscript{11}.

**Calculation of LD-50**

The LD-50 was calculated by Graphical method of Miller and Tainter. The observed percentage mortality was converted into probit referring to the probit table. The values thus obtained were plotted against log dose. The LD-50 value and its standard error were determined from the graph, if the line was straight enough\textsuperscript{12}.

**Results**

The effects of oral administration of single doses of HS extract in Swiss albino mice are summarized in Table 1. The animals were observed continuously for Gross behavior and mortality at 0 min, 30 min, 60 min, 120 min, 240 min and 24 hrs. The LOAEL (Lowest Observed Adverse Effect Level) for the oral dose extract was found to be 8 gm/kg B wt. Symptoms of toxicity such as piloerection, writhing, exophthalmos, initially irritability then immobility
and decreased grooming were found. Haematuria and malaena were also found at higher doses. The LD-50 value was calculated by graphical method. The acute toxicity data indicated that the estimated oral LD-50 of hydroalcoholic extract of HS was found to be 30 gm/kg B wt in mice. The results are presented in table and figure (Tables 1, 2 & Fig. 1).

**Discussion and conclusion**

Administration of herbal drugs in crude form was the predominant before the advent of modern drugs. Still, it is the predominant in many underserved populations of various countries. Herbal drugs continue to infuse new ideas and treatments into modern medicine for the benefit of the patients. In most instances, problems arise due to inappropriate usage of herbs and supplements. Herbs and supplements can be toxic when used for inappropriate indications, or prepared inappropriately, or used in large excessive dosages, or for a prolonged duration of time. Unani system of medicine lacks studies related to the toxicity of drugs. A large number of drugs such as arsenic, opium, lead, mercury and *dhatura* are used in the Unani system of medicine which found essentially toxic, when used without caution. The major ingredient of HS is *Datura stramonium* L. It is a plant with both poisonous and medicinal properties. The neurotoxicity is attributed to the presence of tropane alkaloids which contain a methylated nitrogen atom (N-CH₃) and include the anti-cholinergic drugs atropine, and scopolamine. A wide range of medicinal values of this plant have contributed in scientific field, in Unani medicine as well as in ethnomedicine. The LOAEL (Lowest Observed Adverse Effect Level) for the oral dose extract was 8 gm/kg B wt and the estimated oral LD-50 of hydroalcoholic extract of HS was 30 gm/kg B wt in mice. According to OECD (Organization for Economic Co-operation and Development) guideline for acute oral toxicity, an LD-50 dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is found to be safe. According to ancient physicians such as Ibn Sina and Majoosi, sometimes the need of compound formulation is to reduce toxicity of any drug. Therefore, it is possible that the other ingredients of HS are helpful in reducing the toxicity of *dhatura*. Unani literature claims Samaghe arabi [*Acacia nilotica* (L.) Delile. syn. *Acacia arabica*

### Table 1— Acute oral toxicity study of HS in mice

<table>
<thead>
<tr>
<th>Treatment dose (gm/kg)</th>
<th>D/T Mice (Dead/treated)</th>
<th>Symptoms of toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>0/6</td>
<td>LOAEL (Lowest Observed Adverse Effect Level) i.e. piloerection; writhing; exophthalmos; Immobility.</td>
</tr>
<tr>
<td>20</td>
<td>1/6</td>
<td>piloerection; writhing; exophthalmos; Initially irritability then immobility; decreased grooming; haematuria.</td>
</tr>
<tr>
<td>24</td>
<td>2/6</td>
<td>piloerection; writhing; exophthalmos; Initially irritability then immobility; decreased grooming; haematuria; malaena.</td>
</tr>
<tr>
<td>30</td>
<td>3/6</td>
<td>piloerection; writhing; exophthalmos; Initially irritability then immobility; decreased grooming; haematuria; malaena.</td>
</tr>
<tr>
<td>36</td>
<td>4/6</td>
<td>piloerection; writhing; exophthalmos; Initially irritability then immobility; decreased grooming; haematuria; malaena.</td>
</tr>
<tr>
<td>42</td>
<td>6/6</td>
<td>piloerection; writhing; exophthalmos; Initially irritability then immobility; decreased grooming; haematuria; malaena.</td>
</tr>
</tbody>
</table>

### Table 2—Results of Acute oral toxicity study of HS in mice by Graphical method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (gm/kg)</th>
<th>Log dose</th>
<th>Dead/total</th>
<th>Dead %</th>
<th>Corrected %</th>
<th>Probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>0.90</td>
<td>0/6</td>
<td>0</td>
<td>4.16</td>
<td>3.25</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>1.30</td>
<td>1/6</td>
<td>16.66</td>
<td>16.66</td>
<td>4.05</td>
</tr>
<tr>
<td>III</td>
<td>24</td>
<td>1.38</td>
<td>2/6</td>
<td>33.33</td>
<td>33.33</td>
<td>4.56</td>
</tr>
<tr>
<td>IV</td>
<td>30</td>
<td>1.47</td>
<td>3/6</td>
<td>50</td>
<td>50</td>
<td>5.00</td>
</tr>
<tr>
<td>V</td>
<td>36</td>
<td>1.55</td>
<td>4/6</td>
<td>66.66</td>
<td>66.66</td>
<td>5.44</td>
</tr>
<tr>
<td>VI</td>
<td>42</td>
<td>1.62</td>
<td>6/6</td>
<td>100</td>
<td>95.83</td>
<td>6.75</td>
</tr>
</tbody>
</table>

Fig. 1—Graphical method of determination of LD-50
(Lam.) Willd] for its use as corrective for other single drugs or compound formulations\textsuperscript{16,17,18}. So, it might be possible that \textit{Samaghe arabi} reduced the toxicity of \textit{dhatura}, as it was observed that HS was found to be safe at normal therapeutic dose as well as at higher doses.

Acknowledgement

The authors would like to record their gratitude to Prof M A Jafri, Ex-Director, National Institute of Unani Medicine (NIUM) Bangalore, for providing an academic and research environment to work with excellence and meticulousness. The authors would like to thanks to library staff of NIUM for providing necessary books to accomplish this paper. The authors would like to thanks to Animal House Staff for their valuable assistance during the experimental work in the animal house.

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