EXPERIMENTAL STUDY OF DHATTURA (DHATTURA ALBA NEES) W.S.R. ITS ACUTE TOXICITY

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ABSTRACT
The goal of Ayurved is to provide healthy and long life to human beings through effective and safest way of treatment. Our Acharyas have done detailed study on visha(poisons)- upavishas(sub-poisons) regarding their toxic effects and and also using them as medicine after proper processing called Shodhana(purification). After exhaustive screening of classical ayurvedic literature where in references are given on toxic effects of Dhattura(Dattura alba Ness). Despite of its toxic properties Dhattura is used therapeutically in many preparations after doing Shodhana. The aim of present study was to see acute toxicity effects if any, produced by Ashodhita(unpurified) Dhattura seed extract as well as Shodhita(purified) Dhattura seed extract. The test drugs were tested for acute toxicity on albino rats their lethal dose was calculated .The results revealed that the acute toxicity of Dhattura seed is reduced after Shodhana by gomutra(cows urine). The results were supported by data obtained from LD50(lethal dose) studies. Objectives: Experimental study of Dhattura (Dattura alba Ness) to see its acute toxicity. Materials and Methods: This section of study represents the Shodhana, Extraction and Experimental study. Acute toxicity studies were carried out so as to get detail data on acute toxicity of Dhattura seeds before and after Shodhana by Gomutra.

Keywords: Dhattura( Dattura alba Ness), Shodhana(purification), Gomutra(cows urine) Shodhita(purified), Ashodhita(unpurified).

INTRODUCTION:
In recent years there has been resurgence of interest in Ayurvedic drugs on account of their tremendous efficacy of curing several human ailments & almost nil side effects. So it is need of the time to get scientific data on herbal drugs regarding their Efficacy, Safety, and Toxicity.

Now days along with the popularity of Ayurvedic medicines the toxicity produced by them is also heard frequently. So considering the available literatures on Dhattura1,2 the present study has been undertaken to see the acute toxicity if any produced by Dattura alba Nees. on albino rats, before and after Shodhana.(Purification)
In ancient Ayurvedic literature lots of references are available regarding the testing of the drug and food on animals for safety of mankind. In (Charak.chikitsasthan 23/108-113,Sushrut kalpasth 1/28-334,Astanghrudaya sutrasthana7/5-185)where as the visha & upavisha are also used for therapeutic purpose after their...
shodhana. So to avail scientific data & to see toxicity if any produced by upavisha Dhattura before & after shodhana the present study was under taken.

MATERIALS AND METHODS
- Materials for solubility of Dattura alba Nees.
  Aim: To know the solubility of drug, subjecting to extraction.
  Materials: Funnels, beaker, filter paper, test tube, fine powder of Dattura alba Nees.
  Solvents: 1) Ethyl alcohol   2) Ethyl acetate
  3) Petroleum Ether 4) Chloroform
  5) Methane 6) Distilled water 7) Solvent ether 8) Acetone 9) Benzene 10) Toluene
  11) Xylene 12) Carbon tetrachloride.
  Methodology: The pinch of fine powder of Dattura alba Nees. seeds was added to the different solvent taken in a test tube and mixed well and allowed to stand for certain period. Then the mixture was filtered through filter paper kept in different funnels. The filter paper which contains fewer residues, it was considered the drug was more soluble in that solvent.

I. Extraction:
Materials:
Drug: Coarse powders of Ashodhita (unpurified) & Shodhita (purified) seeds of Dattura alba Nees.
Equipments required: Soxhlet apparatus of 1000ml, round bottom flask, water condenser with distillation apparatus. Beaker’s of 500ml, measuring cylinder, weighing machine, filter paper, magnetic stirrer, porcelain glass chips. (Boiling chips).
Chemical: Distilled water.
Methods: The air dried seeds of Dattura alba Nees. was subjected to exhaustive extraction by soxhlet apparatus around 18 hrs with Distilled water. Extraction was done in two batches, of this one batch of coarse powder with Distilled water. The extraction process was carried out for about 18 hrs to each batch. After the extraction the solvents were distilled off to obtain semisolid extract which was concentrated on magnetic stirrer. The weights of each batch extract were recorded.

EXPERIMENTAL STUDY: Aim- To see the acute toxicity of Shodhita(purified) Dhattura seeds water extract & Ashodhita(unpurified) Dhattura seeds water extract in the selected Albino Rats.

Collection of Animals:
- The Animals of either sex were collected from the animal House of B.L.D.E Medical College, Bijapur.
- They were exposed to natural day & night cycles along with ideal laboratory conditions.
- The Animals were separated according group & kept in separate cages.

Sample size: For Acute toxicity study of Ashodhita(unpurified) Dhattura total 24 Albino Rats were used where as for Shodhita(purified) Dhattura total 40 rats were used. In one group 4 Albino Rats were taken & feded with same dose according to body weight.

Materials: Material required for present study are Dolayantra, Gomutra(cows urine), Albino Rats of either sex, Gas burner, 5cc syringes, Rubber catheter, 18 no Metallic needle covered with rubber, Seizer, Scalpel forceps, plastic bottles, Small glass bottles, Cloth, Cotton swabs. Selection of Animals: Healthy Adult Albino Rats of either sex weighing from 180-250gms were used as experimental model in this study.
Preparation of Animals

For Acute toxicity study animals were with free access to food & water 4 Albino Rats were taken in a group & housed separately. They were fasted for 18 Hrs before giving the dose.

Grouping: The Ashodhita(unpurified) Dhattura seed extract was administered to 6 groups of Albino Rats, each group containing 4 Albino Rats. Shodhita(purified) Dhattura seed extract was administered to 10 groups, each group containing 4 Albino Rats.

METHODOLOGY:

A) Shodhan of Dhattura seeds

As per classified reference found in Rastarangini, the shodhan of Dhattura seeds (Dattura alba Nees.) was carried out in Dr B.N.M.E Trust Ayurvedic Medical College Pharmacy by Dolayantra method. The Dravadravya (liquid media) used was Gomutra (cows urine). 500gms of Dhattura seeds were tied in pottali. which was submerged in 4 liters of Gomutra (cows urine) so that the pottali remains fully immersed in Gomutra (cows urine) but will not touch base of Dolayantra. The Shodhana process was carried out on Mandagni (mild heat). After 1Yama (3Hours) the pottali was removed & seeds were taken out & allowed to dry after washing with warm water. They were then triturated in Khalwayantra (Morter&Pistle.)

B) Acute Toxicity studies: The aim of present Acute toxicity study was to calculate the approximate lethal Dose (LD50) of Ashodhita(unpurified) Dhattura seed extract & Shodhita(purified) Dhattura seed extract in Albino Rats.

Methodology- For the calculation of approximate LD50 of Ashodhita(unpurified) as well as Shodhita(purified) dhattura seed extract KARBER’S method was followed

Procedure – The animals were fasted for 18 hrs before starting the study.

The Ashodhita(unpurified) & Shodhita (purified) Dhattura seed extract was administered orally in single dose to 4 Albino Rats according to their body weight. After administering the extract the animals were placed separately in cage, their behavior was observed & also mortality was recorded in 24 hrs if any.

For Ashodhita(unpurified) Dhattura seed extract total 6 dose levels were used.
1) 500mg/kg 2) 1000mg/kg 3) 1500mg/kg 4) 2000mg/kg 5) 2500mg/kg 6) 3000mg/kg

For Shodhita(purified) Dhattura seed extract total 10 dose levels were used

- 500mg/kg 3) 1500mg/kg 5) 2500mg/kg 7) 3500mg/kg 9) 4500mg/kg
- 1000mg/kg 4) 2000mg/kg 6) 3000mg/kg 8) 4000mg/kg 10) 5000mg/kg

To calculate the approximate LD50 of both the extract KARBER’S method was used.

OBSERVATIOn ON EXPERIMENTNAL STUDY:

1) Observation on Shodhan of Dhattura Seeds

- Dolayantra method was followed for Shodhana (purification).For 500 gms of seeds 4 litres of Gomutra(cows urine) was used. Gomutra(cows urine) was added frequently to maintain the level above Pottali. Entire process was carried out on Mandagni.

- After completion of Shodhan process it was observed that the colour of Gomutra(cows urine) was changed from light yellow to Dark Brown.
2) Observation on Selection Of Animals:
- The healthy as well as nutritional status of Albino Rats was observed.
- Housing with breading status of experimental Albino Rats was maintained which kept them in good healthy & physical comfort.

3) Sample Size: The bifurcation of Albino Rats in their group was observed & recorded.

4) Preparation of Solution
- For Acute toxicity studies the different dose levels of Ashodhita (unpurified) & Shodhita (purified) Dhattura seed extracts were dissolved in 3ml of distilled water.

5) Observation On Selected Dose:
- The dose was divided according to the body weight of the Albino Rats.
- The related dose was given to the corresponding Albino Rats & there changes were observed.

6) Observation On Acute Toxicity Studies
- Acute toxicity studies of test drugs Ashodhita (unpurified) Dhattura & Shodhita (purified) Dhattura seeds extracts was carried out at different dose level as mentioned earlier. The main parameter to observe here was mortality, so as to calculate the LD50. Among the Ashodhita (unpurified) Dhattura group 1 out of 4 Rats was died after 10 hours drug administration at 2000 mg/kg dose level. At dose level of 2500 mg/kg 3 out of 4 Rats were died first one after 5 hours & next two after 7 hours of drug administration. At dose level of 3000 mg/kg all the 4 Rats died, first one after 3 hours next one after 3/2 hours & next two after 6 hours of drug administration. Among Shodhita (purified) Dhattura group 1 out of 4 Rats died at 4500 mg/kg dose level after 8 hours of drug administration. At 5000 mg/kg dose level 1 out of 4 Rats died after 6 hours of drug administration. The data obtained was analyzed by KARBERS method to calculate Approximate LD50 of Ashodhita (unpurified) Dhattura seeds extract & Shodhita (purified) Dhattura seed extract.

Table no. 1. Showing Acute toxicity study of Ashodhita (unpurified) Dhattura seed extract

<table>
<thead>
<tr>
<th>Dose</th>
<th>Total Number of Rats</th>
<th>Death</th>
<th>Dose Difference</th>
<th>Interval Mean Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg/kg</td>
<td>4</td>
<td>-</td>
<td>-</td>
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<tr>
<td>1000 mg/kg</td>
<td>4</td>
<td>-</td>
<td>500 mg</td>
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</tr>
<tr>
<td>1500 mg/kg</td>
<td>4</td>
<td>-</td>
<td>500 mg</td>
<td>0.5</td>
</tr>
<tr>
<td>2000 mg/kg</td>
<td>4</td>
<td>1</td>
<td>500 mg</td>
<td>3.5</td>
</tr>
<tr>
<td>2500 mg/kg</td>
<td>4</td>
<td>3</td>
<td>500 mg</td>
<td>2</td>
</tr>
<tr>
<td>3000 mg/kg</td>
<td>4</td>
<td>4</td>
<td>500 mg</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Product = Dose difference X Interval Mean Mortality
= 500 X 0.05 = 250
= 500 X 2 = 1000
= 500 X 3.5 = 1750
3000
LD50 = Maximum Dose – (product / Total number of Rats in group)
= 3000 – (3000/4)
= 3000 – 750
= 2250 mg/kg
**LD50 = 2.250 mg/kg (Approx.)**

**Table no.2. Showing Acute Toxicity study of Shodhita(purified) Dhatura seed extract**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Total Number of Rats</th>
<th>Death Difference</th>
<th>Interval Mean Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg/kg</td>
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<td>4000 mg/kg</td>
<td>4</td>
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<td>500 mg</td>
</tr>
<tr>
<td>4500 mg/kg</td>
<td>4</td>
<td>1</td>
<td>500 mg</td>
</tr>
</tbody>
</table>
| 5000 mg/kg| 4                   | 1                | 500 mg                  | 0.5

Product = Dose difference X Interval Mean mortality

\[
\begin{align*}
&= 500 \times 0.5 = 250 \\
&= 500 \times 1 = 500 \\
&\text{750}
\end{align*}
\]

LD50 = Maximum dose – (Product / Total Number of Rat in group)

\[
\begin{align*}
&= 5000 - (750/4) \\
&= 5000 - 187.4 \\
&= 4812.5 \text{ mg/kg}
\end{align*}
\]

**LD50 = Above 4.8125 gm/ kg (Approx.)**

**DISCUSSION ON EXPERIMENTAL STUDY**

**Shodhan Of Dhatura Seeds**

The *shodhan* of Dhatura seeds was carried out by Dolayantra method, using Gomutra(cows urine) as dravadravya for 1 yama. By doing *shodhana* of Dhatura seeds the unwanted visible substances like sand, dust, stores will be separated & then after subjecting to *shodhana* the unwanted invisible constituents will get separated & thus it will do detoxication of toxic qualities of the drug; making the drug fit for administration. The *Shodhan* process is also supposed to increase the therapeutic qualities of a dravya. The *vishesh shodhan* of Dhatura was done by using Gomutra(cows urine).

The drug Dhatura is having Madkari (narcotic) effect because of presence of alkaloids like hyoscine, hyscyamine, and atropine. So when it is used in unpurified form, it exerts poisonous & in high dose it may be even fatal. When Dhatura seeds were subjected for Shodhan it was observed that after completion of Shodhan process the colour of Gomutra(cows urine) which was light yellow had changed into Dark Brown colour which indicates that some of the constituents of Dhatura with some extent were transferred to Gomutra(cows urine). After shodhan treatment the drug becomes deprived of its all demerits & untoward properties which existed before, when used with Yukti i.e.due consideration, will become ‘Pranadayi’ &
Rasayana. Hence only purified Dhattura is told to be used in therapeutic formation.

**Acute Toxicity Studies**

These tests were performed on Healthy albino rats weighing from 180-250 gms of either sex. The aim of the study was to calculate the Lethal Dose (LD50) of Ashodhita (unpurified) Dhattura seed extract & Shodhita (purified) Dhattura seed extract. The test drugs were administered orally at various dose levels ranging from 500mg/kg to 3000mg/kg with dose interval of 500 mg/kg, for Ashodhita(unpurified) Dhattura seed extract. The dose was given to total 6 groups of albino rats with 4 animals in each group. The animals were fasted for 18 hours before giving the doses. Distilled water was used as vehicles in the dose of 3ml for each dose level. The three dose levels i.e., 2000mg/kg, 2500mg/kg & 3000mg/kg caused mortality of 1,3 & 4 Albinorats respectively. By the data obtained the LD50 was calculated by using ‘Karbers’ method & it was found to be 2.250gm/kg body weight of Albino rat. For Shodhita(purified) Dhattura seed extract total 10 groups of Animals were administered by the test drug. The dose levels were ranging from 500mg/kg to 5000 mg/kg. According to the O.E.C.D guidelines the maximum limit test is upto 5000mg/kg of the animals & the maximum number of animals to be used in a group is 5. At 4500 mg dose level & 5000mg/kg dose level 1 rat died in each dose. The study was stopped as 5000mg/kg is the maximum dose level suggested by O.E.C.D. The vehicle used was Distilled water in dose of 3ml for each dose level. All the doses were given slowly by oral rout using no-3 rubber catheter. By the data obtained the probable LD50 was calculate by Karbers method & it was found to be above 4.815 gm/kg body weight of albino rats.

The Ashodhita(unpurified) Dhattura extract after administration produced signs of toxicity above 1500mg/kg which were observed & they were biting of nails, unsteady gait, and increased respiratory rate. In case of Shodhita(purified) Dhattura seed extract biting on nails & increased respiratory rate were observed of dose level above 4500mg/kg. Thus the LD50 of dhattura seed extract was increased from 2.250 gm/kg to above 4.815 gm/kg.

**CONCLUSIONS**

- Dhattura(Dhattura alba Nees.) is having toxic properties.
- In acute toxicity studies the LD50 of Ashodhita(unpurified) Dhattura extract was 2.250 gm/kg and LD50 of Shodhita(purified) Dhattura seed extract was above 4.8125 gm/kg. Thus it was observed that LD50 was increased from 2.250 gm/kg to above 4.8125 gm/kg after shodhana of Dhattura seeds. By compairing LD50 studies it can be concluded that acute Toxicity of Dhattura has reduced after Shodhan.

**ACKNOWLEDGEMENT**

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