

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 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

Dhattura^{1,2} 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 kalpasthan 1/28-33⁴, Astanghrudaya sutrasthana 7/5-18⁵) 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 & fed 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* seeds⁶

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 *Dra-vadravya* (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 *IYama* (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 method⁷ 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 EXPERIMENTAL 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 breeding 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 car-

ried 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

Dose	Total Number of Rats	Death	Dose Difference	Interval Mortality	Mean
500 mg/kg	4	-	-	-	
1000 mg/kg	4	-	500 mg	-	
1500 mg/kg	4	-	500 mg	-	
2000 mg/kg	4	1	500 mg	0.5	
2500 mg/kg	4	3	500 mg	2	
3000 mg/kg	4	4	500 mg	3.5	

$$\begin{aligned}
 \text{Product} &= \text{Dose difference} \times \text{Interval Mean Mortality} \\
 &= 500 \times 0.05 = 250 \\
 &= 500 \times 2 = 1000 \\
 &= \frac{500 \times 3.5}{3000} = 1750 \\
 \text{LD50} &= \text{Maximum Dose} - (\text{product} / \text{Total number of Rats in group}) \\
 &= 3000 - (3000/4) \\
 &= 3000 - 750 \\
 &= 2250 \text{ mg/kg}
 \end{aligned}$$

LD50 = 2.250 mg/kg (Approx.)

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

Dose	Total Number of Rats	Death	Dose Difference	Interval Mean Mortality
500 mg/kg	4	-	-	-
1000 mg/kg	4	-	500 mg	-
1500 mg/kg	4	-	500 mg	-
2000 mg/kg	4	-	500 mg	-
2500 mg/kg	4	-	500 mg	-
3000 mg/kg	4	-	500 mg	-
3500 mg/kg	4	-	500 mg	-
4000 mg/kg	4	-	500 mg	-
4500 mg/kg	4	1	500 mg	0.5
5000 mg/kg	4	1	500 mg	1

$$\begin{aligned} \text{Product} &= \text{Dose difference} \times \text{Interval Mean mortality} \\ &= 500 \times 0.5 = 250 \\ &500 \times 1 = \underline{500} \\ &750 \end{aligned}$$

$$\begin{aligned} \text{LD50} &= \text{Maximum dose} - (\text{Product} / \text{Total Number of Rat in group}) \\ &= 5000 - (750/4) \\ &= 5000 - 187.4 \\ &= 4812.5 \text{ mg/kg} \end{aligned}$$

LD50 = Above 4.8125 gm/ kg (Approx.)

DISCUSSION ON EXPERIMENTAL STUDY

Shodhan Of Dhattura Seeds

The *shodhan* of *Dhattura* seeds was carried out by *Dolayantra* method, using *Gomutra*(cows urine) as *dravadravya* for 1 *yama*. By doing *shodhana* of *Dhattura* 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 *Dhattura* was done by using *Gomutra*(cows urine).

The drug *Dhattura* is having *Madkari* (narcotic) effect because of presence of alkaloids like hyoscine, hysecyamine, and atropine. So when it is used in unpurified form, it exerts poisonous & in high dose it may be even fatal, When *Dhattura* 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 *Dhattura* 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 Albino rats 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 used were ranging from 500mg/kg to 5000 mg/kg. According to the O.E.C.D guidelines⁸ 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 route 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*(*Dhatura 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 comparing LD₅₀ studies it can be concluded that acute Toxicity of *Dhattura* has reduced after *Shodhan*.

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