Kamal Patra as an Antidote in Dhatura Poisoning in Albino Mice - In Vivo Study

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Introduction: Dhatura (Dhatura metel) is cerebrotoxic, Deleriant poison which is also classified as Upavisha in Ayurveda. Various poisons have been described in Ayurveda along with their antidotes. Mode of action of these antidotes is not mentioned in texts. In Ayurvedic literature According to Basavrajeeayam under the heading of Vish-Prativishani, Chincha Rasa and Kamal Patra churna have been described to be possessing antidote action which may act by some way to counter toxicity of Dhatura. It is necessary to verify the efficacy of these antidotes on scientific parameters so that it can be useful in emergencies.

Objectives: To establish the action and mechanism of Kamal Patra Churna against toxic effects of Dhatura on albino mice. Methods: Swiss albino mice were selected as an animal model and antidote potential of kamal patra against datura powder is evaluated by measuring body temperature, time of convulsions and survival rate.

Results: Kamal patra administration before datutal poisoning reduced the toxic effects of datura such as hypothermia, convulsion and increased the survival rates.

Conclusion: From this study, we can conclude that Kamal Patra can resist the toxic effects of Dhatura up to some extent.

Key Words: Dhatura, Kamal Patra, Agad, Toxicity, Antidote

INTRODUCTION

In the ancient era, Ayurveda was considered as one of the advanced faculty worldwide. Agadtantra is the branch of Ayurveda which deals with the toxicity of various snakes, spiders, insects, rats etc. animals and its treatments. The word ‘Gada’ means poison and the antidote used is called as ‘Agada’. According to modern science, Toxicology is the branch which deals with the study of poison regarding their sources properties, mode of action, symptoms which they produce, lethal dose, fatal period, treatment of their detection estimation & autopsy findings. Dhatura (Dhatura metel) is cerebrotoxic, Deleriant poison which is also classified as Upavisha in Ayurveda. It is a genus of poisonous herbs shrubs, up to the height of 3–4 ft. This plant has been noted for intoxicating, narcotic properties, they produce temporary insensibility (stupefying effects) in ordinary doses. A bitter taste, dryness of mouth and throat, burning pain in the stomach dysphasia, headache and difficulty in talking are the first symptoms that are complained of. These are followed by giddiness, staggering gait, in the co-ordination of the muscles, the peculiar flushed appearance of the face, dry hot skin, photophobia, dilated pupils, delirium & drowsiness. Sometimes, exfoliations of the skin are seen over most of the body. The pulse becomes irregular and intermittent. In fatal cases drowsiness passes into stupor convulsions, coma, death occurs usually from respiratory failure.

Various poisons have been described in Ayurveda along with their antidotes. These antidotes are readily available in nature. Mode of action of these antidotes is not mentioned in texts. In Ayurvedic literature according to Basavrajeeayam...
under the heading of Vish-Pratishvahani, Chincha Rasa and Kamal Patra churna has been described to be possessing antidote action which may act by some way to counter toxicity of Dhatura.5-11 Kamal (Nelumbo nucifera) is a beautiful aquatic plant with a wide range of medicinal usage. It is Madhura, Tikta Rasatmak and Shita Virya hence reduces Pitta Dosha.12-14 Main chemical constituents of Kamal are Nuciferine,10-Nonacosanol, Nelumboside, Neferine, Nuciferin, Nelumbine Questirring,

It is necessary to verify the efficacy of these antidotes on scientific parameters5 so that it can be useful in emergencies. Hence present study entitled “Kamal Patra as an Antidote in Dhatura poisoning in mice was carried out to establish the action and mechanism of Kamal Patra Churna against toxic effects of Dhatura on albino mice.

**MATERIAL AND METHODS**

**Collection of material**

Seeds of Dhatura metel were collected in December to January so at that time seeds can be collected from dried fruit.

The collection of leaves of Kamal (Nelumbo nucifera) for the study was done. Collected leaves were dried in shade. The samples were used in powder form.

**Procedure**

1. *Dhatura metel*: 1 gm coarse powder of Dhatura seeds choodna were extracted with 70% alcohol in the warm water bath with occasional shaking for ½ hr. in closed tubes, successively with the batches of 3.2 and 1 ml of alcohol. 10μl of the pooled extract was spotted on TLC plate silica gel F$_{254}$ Merk and was developed in Toluene: ethyl Acetate: Diethylamine (7:2:1).

Detection of Rf Values of the spot was done by using 5% methanolic sulphuric acid reagent. Rf value of visualized spots was given in table no.7

2) *Nelumbo nucifera*: 1 gm coarse powder of Kamal Patra Choorna were extracted with 70% alcohol in the warm water bath with occasional shaking for ½ hr. in closed tubes, successively with the batches of 3.2 and 1 ml of alcohol. 10μl of the pooled extract was spotted on TLC plate silica gel F$_{254}$ Merk and was developed in Toluene: methanol: pyridine (8:1:1). Rf value of visualized spots was given in table no.8 and 9.

**Animal Experiment**

**Material for In Vivo study**

Swiss Albino mice of age 90-100 days and weight ranges 20-25 gm were procured. Poisoning was induced by Dhatura seed and test drug was kamal patra powder.

**Determination of acute toxicity of Dhatura**

Acute toxicity test (LD$_{50}$ determination) of the extract of Dhatura seeds is performed as per guideline of OECD in Swiss Albino mice.

**Determination of protective action of Kamal Patra against toxicity of Dhatura seed**

Reduction in mortality and change in behaviour of mice due to a lethal dose of Dhatura seed after administration of Kamal leaves are determined.

**Dose Calculation**

**Dose Calculation for Albino Mice**

The conversion factor from man to mice is 0.0026 so according to this, all the doses were calculated. Dose for mice was obtained from the following formula

Dose of mice = 0.0026 X Dose of Man

**Dose Calculation for Dhatura Seeds**

Human fatal Dose for Dhatura Seeds is considered as 100 – 120 crushed seeds i.e. calculated as 1400 mg. According to the conversion factor, fatal dose in albino mice for Dhatura is 3.64 mg

Hence per kg wt. Fatal dose for albino mice = 03.64 x 50

= 182 mg/kg

**Dose Calculation for Kamal Patra Powder**

Human therapeutic dose of Kamal Patra is considered as 3 gm – 6 gm. According to the conversion factor, fatal Dose in albino mice for Kamal Patra is 7.8 to 15.6 mg

The dose I: Per kg wt. dose for albino mice = 7.80 x 50

= 390 mg/kg

Dose II: Per kg wt. dose for albino mice = 15.6 x 50

= 780 mg/kg

**Procedure**

Drug samples were converted into suspension by careful mixing with distilled water. Both drugs were administered orally. After administering the dose all animals were observed for 24 hours for toxic sign and symptoms or mortality till 7 days. First preliminary drug toxicity study for Dhatura was done. In each group weight of each animal was taken and noted, simultaneously dose of Dhatura and Kamal Patra were calculated accordingly. Samples of Toxic drug and Antidote were given by oral route. After dosing: the animals were observed for 24 hrs and up to 7 days. Comparative observations were tabulated (Table 2).
Group I: Acute toxicity of *Dhatura Seed churna* (N=6)

Group II: *Kamal Patra churna* (Dose 1) + *Dhatura Seed churna* (N=6)

Group III: *Kamal Patra churna* (Dose 2) + *Dhatura Seed churna* (N=6)

**Parameters:**
- i) Change in Temperature
- ii) Appearance of Convulsions
- iii) Dilatation of Pupils
- iv) Survival period (death)

**RESULTS AND DISCUSSION**

**Analytical Study**

Laboratory experiments were carried out to obtain values of following parameters for *Dhatura seeds, Kamala Patra* the observation and results follow (Table 1-5)

| Parameters                           | Sample          | Results                          |
|--------------------------------------|-----------------|----------------------------------|
| i) Change in Temperature             |                 |                                  |
| ii) Appearance of Convulsions        |                 |                                  |
| iii) Dilatation of Pupils            |                 |                                  |
| iv) Survival period (death)          |                 |                                  |

**Table 1: Loss on drying and Ash value**

| Samples          | Wt. of samples at room temp. | Wt. of samples after drying at 105°C | Percentage loss on drying at 105°C |
|------------------|------------------------------|------------------------------------|-----------------------------------|
| 1. Dhatura seeds | 2.80 gm                      | 2.60 gm                            | 5.86%                             |
| Kamal Patra      | 1.45 gm                      | 1.37 gm                            | 6.2%                              |
| 2. Ash Value     | Total ash                    | Water-soluble ash                  | Acid insoluble ash                |
| 1. Dhatura seeds | 2.73%                       | 0.32%                              | 0.23%                             |
| 2. Kamal patra   | 0.9%                        | 0.13%                              | 0.05%                             |

**Table 2: Alcohol and Aqueous extractive values at Dhatur seeds, Kamal Patra**

| Samples          | Alcohol extractive values   |
|------------------|-----------------------------|
| 1. Dhatura seeds | 6.39%                       |
| 2. Kamal Patra   | 10.72%                      |

**Table 3: Showing preliminary phytochemical screening of Dhatura seeds**

| Sr. No. | Plants constituents | Test/reagent                  | Alcohol extract | Water extract |
|---------|---------------------|------------------------------|-----------------|---------------|
| 1.      | Steroids            | Salkowski reaction           | + ve            | + ve          |
|         |                     | Liebermann-Burchard          | - ve            | - ve          |
| 2.      | Alkaloids           | Dragendorff’s reagent        | + ve            | + ve          |
|         |                     | Mayer’s reagent              | + ve            | + ve          |
| 3.      | Tannin              | Ferric chloride test 5%      | - ve            | - ve          |
|         |                     | lead acetate test            | - ve            | - ve          |
| 4.      | Flavonoids          | Shinoda test                 | - ve            | - ve          |
| 5.      | Carbohydrates       | Molish’s test                | + ve            | + ve          |
|         |                     | Barfoets test                | - ve            | - ve          |
| 6.      | Amino acids         | Ninhydrin test               | - ve            | - ve          |
| 7.      | Proteins            | Biuret test                  | + ve            | + ve          |

**Effect of poisoning and kamal patar in animals**

**Changes in body Temperature**

The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes (Table 6A and B). The mean change in body temperature in group 1 was 38.14±0.43. While in Group 2 it reduced
to 37.44±0.37 and further in Group 3 it reduced down to 37.08±0.33. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1 with Group 2 and Group 2 with Group 3 suggested statistically Non-Significant changes. While Comparison of Group 1 with Group 3 suggested Mean difference of 1.0630 and Q value 5.43 which is statistically significant.

Table 6A: Changes in body temperature (°C) after Acute toxicity of Dhatura in Albino mice (n=6)

| Group | Mean±SD | SEM | P-value | Inference |
|-------|---------|-----|---------|-----------|
| Gr. -1 | 38.14±0.43 | 0.25 | 0.0378 | |
| Gr. -2 | 37.44±0.37 | 0.31 | Significant | |
| Gr. -3 | 37.08±0.33 | 0.19 | | |

Table 6B: Showing Tukey Kramer Multiple comparison tests within the groups

| Comparison | Mean difference | Q value | P-value | Inference |
|------------|----------------|---------|---------|-----------|
| Gr.-1 vs Gr.-2 | 0.6967 | 3.144 | > 0.05 | Not significant |
| Gr.-1 vs Gr.-3 | 1.0630 | 4.798 | < 0.05 | Significant |
| Gr.-2 vs Gr.-3 | 0.3667 | 1.655 | > 0.05 | Not significant |

Table 7A: Time of appearance of Convulsions (Min) after Acute toxicity of Dhatura in Albino mice (n=6)

| Group | Mean±SD | SEM | P-value | Inference |
|-------|---------|-----|---------|-----------|
| Gr. -1 | 153.32±7.33 | 4.23 | | |
| Gr. - 2 | 159.32±4.91 | 2.85 | 0.1617 | Not Significant |
| Gr. - 3 | 166.43±8.74 | 5.04 | | |

Table 7B: Showing Tukey Kramer multiple comparison test within the groups

| Comparison | Mean difference | Q value | P-value | Inference |
|------------|----------------|---------|---------|-----------|
| Gr.-1 vs Gr.-2 | -6.000 | 1.448 | > 0.05 | Not Significant |
| Gr.-1 vs Gr.-3 | -13.107 | 3.163 | > 0.05 | Not Significant |
| Gr.-2 vs Gr.-3 | -7.107 | 1.715 | > 0.05 | Not Significant |

Time of Dilation of Pupil

The mean change in the time of dilatation of Pupils in group 1 was 111.89±6.79. While in Group 2 it increased to 119±6.04 and further in Group 3 it increased up to 133.58±7.57. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested significant changes. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1 with Group 2 and Group 3 suggested statistically Non-Significant (Graph 1, 2).
Table 8A: Time of Dilation of Pupil (Min) after Acute toxicity of Dhatura in Albino mice (n=6)

| Group | Mean±SD | SEM | P-value | Inference |
|-------|---------|-----|---------|-----------|
| Gr.-1 | 111.89±6.79 | 3.92 |         |           |
| Gr.-2 | 119.75±6.04 | 3.48 | 0.0217  | Significant |
| Gr.-3 | 133.58±7.57 | 4.37 |         |           |

Table 8B: Showing Tukey Kramer Multiple comparison tests within the groups

| Comparison      | Mean difference | Q value | P-value | Inference       |
|-----------------|-----------------|---------|---------|-----------------|
| Gr.-1 vs Gr.-2  | -7.870          | 1.99    | > 0.05  | Not Significant |
| Gr.-1 vs Gr.-3  | -21.69          | 5.50    | < 0.05  | Significant     |
| Gr.-2 vs Gr.-3  | -13.82          | 3.50    | > 0.05  | Not Significant |

Duration of Survival period

The mean change in the time survival Period (Death) in group 1 was 370.36±24.99. While in Group 2 it increases to 402.35±17.88 and further in Group 3 it increases up to 424.51±22.72. The observations were analyzed statistically by one way ANOVA Test. The P-value suggested Non-significant changes.

Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1, Group 2 and Group 3 suggested statistically Non-Significant. Further, the groups were compared with each other with the help of Tukey Kramer Multiple Comparison Test. Comparison of Group 1 with Group 2 suggested Mean difference of 31.99 and Q value 2.51 which is statistically Non-Significant. Comparison of Group 1 with Group 3 suggested Mean difference of 54.15 and Q value 4.25 which is statistically Non-Significant. Comparison of Group 2 with Group 3 suggested Mean difference of 22.16 and Q value 1.73 which is statistically Non-Significant.

The observations were analyzed statistically by one way ANOVA Test. The P-value suggested Non-significant changes (Table 9A and B).

Table 9A: Duration of Survival period (Min) after Acute toxicity of Dhatura in Albino mice (n=6)

| Group | Mean±SD | SEM | P-value | Inference       |
|-------|---------|-----|---------|-----------------|
| Gr. – 1 | 370.36±24.99 | 14.42 |         |                 |
| Gr. – 2 | 402.35±17.88 | 10.32 | 0.0623  | Not quite significant |
| Gr. – 3 | 424.51±22.72 | 13.12 |         |                 |

Table 9B: Showing Tukey Kramer multiple comparison test within the groups

| Comparison      | Mean difference | Q value | P-value | Inference       |
|-----------------|-----------------|---------|---------|-----------------|
| Gr.-1 vs Gr.-2  | -31.99          | 2.51    | > 0.05  | Not Significant |
| Gr.-1 vs Gr.-3  | -54.15          | 4.25    | > 0.05  | Not Significant |
| Gr.-2 vs Gr.-3  | -22.16          | 1.73    | > 0.05  | Not Significant |

CONCLUSION

Dhatura is neurotoxic cerebral deliriant poison which is also classified as Upavisha in Ayurveda with ‘9 Ds’ toxic effects. The active principle is dhaturine containing hyoscyamine, hyoscymine and atropine. It blocks the acetylcholine receptors and thus produces sympathomimetic or parasympatholytic actions (Anticholinergic actions). In vivo study of Antidote properties of Kamal Patra shows, hyperthermia caused due to toxicity is significantly reduced, duration of the appearance of convulsions is increased slightly but it is statistically insignificant, duration of dilatation of the pupil is significantly increased; rise in duration if the survival period is statistically insignificant but a slight rise was seen in it.

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