

## Toxicological study of *Jaypalbeej Shodhana* (*Croton tiglium* L. Seed)

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**Abstract:** Ayurveda, ancient science of life reveals uses of many herbal, mineral and metals in medicine. As many of them, are toxic for human body, to be purified by process of shodhana. Shodhana doesn't mean to remove its active toxic principle but to make it compatible with body. Shodhana reduces its harmful effects on body and enhances pharmacological properties. This research study is about to find out effect of shodhana on LD 50 of Jaypal beej ( *Croton tiglium* seeds ). For this purpose Jaypal beej was procured from market, it was authenticated & standardized. A portion of these seeds was used to determine LD 50 of raw Jaypal beej. Another portion was processed by standard shodhana method . A portion of shuddha Jaypal beej was analyzed for changes in physical & chemical properties as compared to raw Jaypal beej. Another portion of shuddha Jaypal beej was used to determine LD 50 of shuddha Jaypal beej .A low dose toxicity study was done with both raw & shuddha Jaypal Beej given to separate animal groups to find out effects on tissues of the animals . The result of study is shodhana process reduces toxicity of "Jaypal beej."

### 1. INTRODUCTION:

Acharya Charaka explained in sutrasthana 1<sup>st</sup> chapter 126 verse, as Strong poisons can be best medicine when used properly in correct therapeutic formulation. In the same way the best medicine can affect adversely if not used properly for proper person in proper doses etc. Ayurveda has holistic approach towards all type of drugs. So that poisonous herbs as well as poisonous metal or minerals are used in medicines by reducing their harmful properties, enhancing pharmaceutical properties. Ayurved includes Jaypal in upvisha Varga (less toxic drugs). JaypalBeej is used in preparation of many Ayurvedic drugs like "Ichchhabhedi Rasa", "Chandramrut Rasa", "Mahamrutyanjaya rasa" etc. Jaypal is fatal when taken in over dose, causing severe burning sensation in digestive system, severe abdominal pain , vomiting ,diarrhea along with blood. It may also cause some psychological symptoms like drowsiness, vertigo etc. . Testing these less toxic substances chemically in animal experiments, will establish their safety of use as a medicine for mankind. Acute and low dose toxicity along with histopathology done . Two samples as before shodhana and after shodhana of jaypal beej are studied well.

Acute manifestations are

- The oil causes blistering externally.
- Burning pain in mouth and throat 10 ,16 , 43 , 44
- Dryness of mouth 10
- Nausea with vomiting 10 ,16 , 43, 57
- Severe abdominal pain 43 , 44 , 57
- Severe diarrhea along with blood 43 , 44, 57
- Imperceptible pulse.(C.no 1)
- Rise in Temperature ( C. No 1).
- Husky voice ( C. No 1).
- Burning pain at anus

#### 1.1. Late manifestations:

- Hemorrhagic gastritis. 44
- Haemolysis. 44
- Renal failure. 44

#### 1.2. Toxicity Study:

Acute toxicity study of both samples (Before shodhana & After shodhana ) of *Croton tiglium* seeds (Jaypal Beej ) was carried out in National Toxicological Center (N T C ) , Pune.

Following protocol was used for that.

## 2. MATERIALS:

1. Animal Species used - Albino mice.
2. No .of Animals - 4 for each sample testing.
3. Average weight of mice - 30 gm .
4. Sex of Animals - 50% males & 50% females.
5. No of groups - a ) Before shodhana &  
b) After shodhana.
6. Period of fasting - Overnight
7. Dosing - Oral route.
8. Sample (2) - In fine powder form.  
1) Before shodhana.  
2) After shodhana.
9. Water and feed ( rice bran) - adlibitum
10. Other Apparatus :
  - a. Test tubes.
  - b. 2ml syringes with 14 no. needle curved at 130 angles .
  - c. Corn oil

## 3. METHOD:

Samples were converted in to suspension by thorough mixing with corn oil. Samples were freshly prepared .Dose given to animals according to their body weight. After dosing, all animals were observed for 24 hrs for toxic signs & symptoms or mortality .

## 4. PROCEDURE:

Oral dose of 5000 mg / kg body weight, administered to each groups of both samples (Before shodhana & After shodhana ) after dosing animals were observed for 24 hrs. It was observed that group of Before shodhana sample (Group A ) had severe diarrhoea seen after 4 hrs after dosing . All the animals were found dead on Day 1 after dosing. 100 % mortality was seen. Group of After shodhana sample (Group B) had severe diarrhea after 4-6 hrs after dosing. 50 % of the animals were found dead on Day 1 after dosing. The dose of 5000 mg / kg body weight caused 100% mortality in Before Shodhana group & 50 % mortality in after shodhana group. As to compare toxicity of Jaypal Beej Before shodhana & After shodhana, the animals to be observed for at least 7 days after dosing & development of any pathological changes therefore low dose toxicity study was carried out.

For the purpose protocol was followed as given below,

- 2 samples (a) Before Shodhana  
(b) After Shodhana
- No. of Animals 4 in each group
- No. of Groups 2, each group was kept in separate cage
- Grouping : Group I A (Before Shodhana) Sample.  
Group II B (After Shodhana) Sample.
- Sex of Animals 50 % males & 50 % females.
- Period of Fasting Overnight.
- Dosing  
Group I Received oil suspension of Sample “A”  
i.e. before shodhana  
Group II Received oil suspension of Sample “B”  
i.e. after shodhana
- Dose distribution & Dilution : Period of consecutive dosing 1 day.
- Period of observation of animals : 6 days.
- Dose of both sample (i.e. Before shodhana & After Shodhana) were same i.e. 2000 mg / kg body weight.
- Water and feed (Rice Bran) - Adlibitum

Weighted samples were diluted in corn oil in the proportion 200mg/ml mixed thoroughly & suspension were prepared. Both samples were prepared.

Group I & Group II received one dose (OD) of 2000 mg/ kg body weight. It was found that both groups (i.e. I & II) showed severe diarrhea on day 1 after dosing.

**5. DETAILED OBSERVATION ARE AS FOLLOWS:**

Group I & Group II were listed below with observation from 1<sup>st</sup> day after dosing.

**Table No. 1: Group I – Before Shodhana Dose 2000 mg / kg on 1<sup>st</sup> day**

On 1 <sup>st</sup> Day Animals Sign	1 <sup>st</sup> Male	2 <sup>nd</sup> Male	1 <sup>st</sup> Female	2 <sup>nd</sup> Female
1. Diarrhea	+ + +	+ + +	+ + +	+ + +
2. Weight Loss	+ +	+ + +	+ + +	+ +
3. Redness at anus	+ +	+ +	+ +	+ +
4. Death	-	+	+	-

**Table No. 2 : Group I – Before Shodhana Dose 2000 mg / kg on 3<sup>rd</sup> day**

On 3 <sup>rd</sup> Day Animals Sign	1 <sup>st</sup> Male	2 <sup>nd</sup> Male	1 <sup>st</sup> Female	2 <sup>nd</sup> Female
1. Diarrhea	+ + +			+ + +
2. Weight Loss	+			+
3. Redness at anus	+			+
4. Death	-			-

**Table No. 3 : Group I – Before Shodhana Dose 2000 mg / kg on 6<sup>th</sup> day**

On 6 <sup>th</sup> Day Animals Sign	1 <sup>st</sup> Male	2 <sup>nd</sup> Male	1 <sup>st</sup> Female	2 <sup>nd</sup> Female
1. Diarrhea	-			-
2. Weight Loss	-			-
3. Redness at anus	-			-
4. Death	-			-

**Table No. 4: Group II – After Shodhana Dose 2000 mg / kg on 1<sup>st</sup> day**

On 1 <sup>st</sup> Day Animals Sign	1 <sup>st</sup> Male	2 <sup>nd</sup> Male	1 <sup>st</sup> Female	2 <sup>nd</sup> Female
1. Diarrhea	+ + +	+ + +	+ +	+ + +
2. Weight Loss	+ +	+	+	+
3. Redness at anus	+ +	+	+	+
4. Death	-	-	-	-

**Table No. 5 : Group II – After Shodhana Dose 2000 mg / kg on 3<sup>rd</sup> day**

On 3 <sup>rd</sup> Day Animals Sign	1 <sup>st</sup> Male	2 <sup>nd</sup> Male	1 <sup>st</sup> Female	2 <sup>nd</sup> Female
1. Diarrhea	+ + +	+	+	+
2. Weight Loss	+ +	-	-	-
3. Redness at anus	+ +	+	+	+
4. Death	-	-	-	-

**Table No. 6 : Group II – After Shodhana Dose 2000 mg / kg on 6<sup>th</sup> day**

On 6 <sup>th</sup> Day Animals Sign	1 <sup>st</sup> Male	2 <sup>nd</sup> Male	1 <sup>st</sup> Female	2 <sup>nd</sup> Female
1. Diarrhea		-	-	-
2. Weight Loss		+	+	+
3. Redness at anus		-	-	-
4. Death		-	-	-

**Table No. 7**

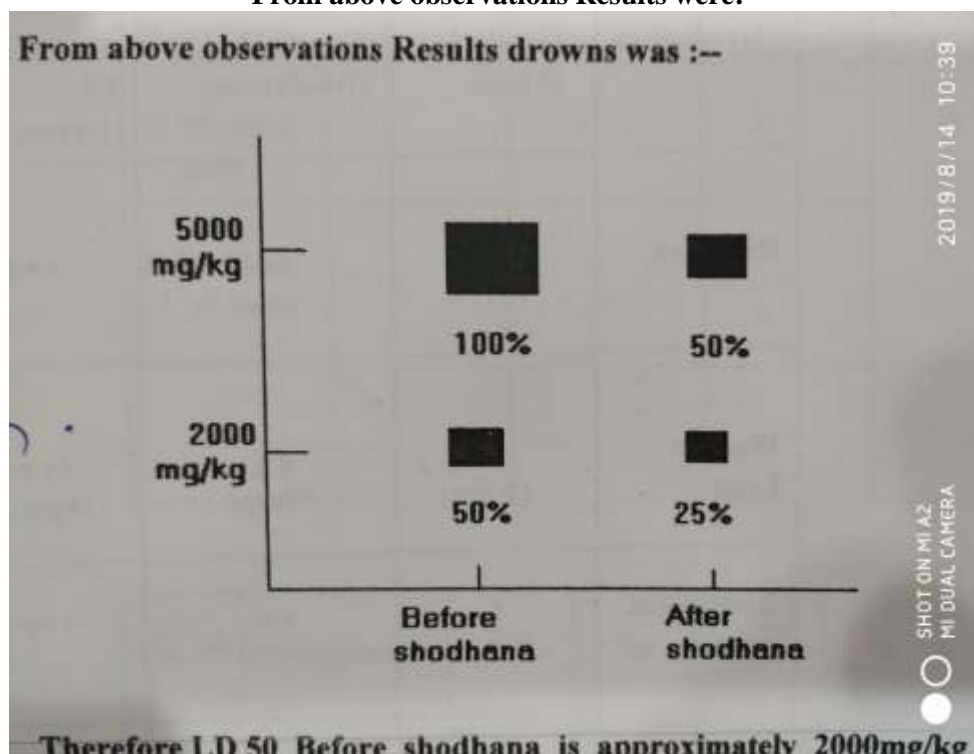
**Gradation of Toxicity signs is given below :**

Gradation	Grade I (Mild)	Grade II (Moderate)	Grade III (Severe )
1. Diarrhea	+	+ +	+ + +
2. Weight Loss	+ (2gm)	+ (4mg)	+ (6mg)
3. Redness at anus	+	+ +	+ + +
4. Weight Gain	+ (1 gm)	+ + (2gm)	+ + + (4 gm)

## 6. OBSERVATION AND RESULT:

It was observed that signs of intoxication were similar in both groups (i.e. before shodhana and after shodhana) which are diarrhoea, Weight loss, redness at anus and death. Only signs of weight gain is observed in after shodhana group. All these signs are less severe in after shodhana group. In Group I There were 1 male and 1 female who died on first day. That means % of mortality in before shodhana group is 50%. In Group II It was seen that 1 male died on 4th day. All these died animals were dissected and viscera preserved for Histopathological studies. Other surviving mice were weighted and then sacrificed by cervical dislocation. Their viscera were also preserved for histopathological studies.

From above observations Results were:



Therefore LD 50 Before shodhana is approximately 2000mg/kg LD 50; After shodhana is approximately 5000mg/kg

A) LD 50 value of Jaypal Beej after shodhana in albino mice by oral route is approximately 5000 mg/kg body weight.

B) Low dose toxicity study showed ,

Group I -50 % mortality.

Group II -25 % mortality.

(After consecutive dose at 2000 mg /kg body weight for 1 Day)

C) From autopsy finding systemic organ toxicity was also concluded

### D) Histopathological Observations:

Histopathological observation were compared and noted down as follows:

- Stomach:** Group I (Before shodhana) showed no abnormality, Lumen Contains vegetative & sloughed material. Group II Group II (after shodhana), animal no 1 & 2 showed no abnormality, but Animal no 3 & 4 showed thin mucosa of stomach.
- Small Intestine:** Group I (Before shodhana), In Animal no 1&2 of before shodhana process No abnormality detected. Group II (after shodhana) , In Animal no 1 & 3 of after shodhana process no abnormality detected, but Animal no 2 & 4 of after shodhana process showed sloughing of the mucosa & mild mononuclear cells infiltration.
- Large Intestine:** Group I (before shodhana) , In Animal no1 & 2 of before shodhana process, Mucosal gland showed vacuolated goblet cells with necrotic appearance. Group II (after shodhana) , In Animal no 1, 2, 3 & 4 of after shodhana process showed no abnormality.
- Liver :** - Group I ( before shodhana) , Animal no 1 of before shodhana process showed mild congestion & Animal no 2 showed mild focal mononuclear cells infiltrate. Group II (alter shodhana) , Animal no 1 of after shodhana process showed Regenerative activity , focal mononuclear cells infiltration, fibrotic nodule with central eosinophilic material & inflammatory cells. In Animal no 2 showed no abnormality. Animal no 3 & 4 showed mild focal mononuclear cells.

5. **Kidney** Group I (before shodhana), Animal no 1 showed mild cloudy changes & congestion, Animal no 2 showed mild congestion. Group II (after shodhana), Animal no 1, 2, 3, & 4 showed mild cloudy changes.

**Inference**: Histopathological studies show that the animals treated with *Croton tiglium* L. Seeds, Before shodhana sample showed changes greater than those treated with “*Croton tiglium* . L seeds, After shodhana sample.

## 7. DISCUSSION:

Lethal doses of *Croton tiglium* seed was found approximately 5000mg/kg body weight in Albino mice by oral route in after shodhana group. Low dose toxicity study for (2000mg/kg) body weight in Albino mice by oral group in before shodhana group showed 50% mortality and after shodhana group showed 25% mortality. LD 50 for 2000mg/kg body weight showed severe toxic effects and mortality in both samples. It means Jaypal Beej is proved fatal, if taken in large dose which signify its inclusion in “Upvisha Varga” mentioned in Ayurvedic texts. There were severe diarrhoea & marked decrease in body weight in the animals treated with before shodhana sample of *Croton tiglium*. Seeds as compared after shodhana sample. In after shodhana sample of Jaypal beej revealed increase in body weight in albino mice. It indicates that shodhana process decreases the purgative property of Jaypal Beej. It was also seen that females & males are equally resistant or sensitive to this drug in both cases (before & after shodhana). Histopathological studies of relevant organs like stomach, small intestine, large intestine, liver and kidney were compared in before shodhana group and after shodhana group.

- Liver showed mild congestion, mild focal mononuclear cells infiltration before shodhana a group while after shodhana group showed regenerative activity, mild focal mononuclear cells.
- Kidney showed mild cloudy changes & congestion. In before shodhana group, while after shodhana group showed mild cloudy changes.
- In stomach lumen contains vegetative & sloughed material in before shodhana group, while after shodhana group showed no abnormality.
- Small intestine showed No abnormality, while after shodhana group showed sloughing of the mucosa & mild mononuclear cells infiltration.
- Large intestine mucosal glands showed vacuolated goblet cells with necrotic appearance in before shodhana group, while after shodhana group showed no abnormality.

This indicates decrease in level of toxic properties of the drug. More studies can be done by using other shodhana process described in the Ayurvedic texts. So from all above tests & experiments, conclusion drawn as shodhana process is effective in reduction of toxic properties of *Croton tiglium* seeds is accepted.

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