

EFFECT OF SHODHAN PROCESS ON TOXICITY OF ERAND BEEJ (RICINUS COMMUNIS LINN. SEEDS)

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ABSTRACT

Agadtantra is one of the important branches of *Ashtang Ayurveda* which is related with study of poisons (*Visha*). *Ayurveda* have stated various types of poisons as *Visha*, *Upavisha*. These poisons can be used as medicines directly or can be added in various formulations only after proper *Shodhan* (Purification) procedures. *Eranda Beej* (*Ricinus communis* Linn. Seeds) are considered as one of the most potent poisons known till today to mankind but not included in any type of poison in *Ayurveda* but *Shodhan Procedure* of *Eranda Beej* is mentioned in the texts and it stated that *Eranda beej* is to be used only after *Shodhan* process. To test efficiency of *Shodhan* process by chemical and animal experiments and to establish the safe use of

Eranda Beej after *Shodhan* as a medicine to mankind; current paper aims towards the topic - Effect of *Shodhan* Process on Toxicity of *Eranda Beej* (*Ricinus communis* Linn. Seeds).

KEYWORDS: Shodhan, Ricin, Pottali, Erand Beeja.

INTRODUCTION

Agadtantra is one of the important branches of *Ashtang Ayurveda* and has its own importance in *Ayurveda* treatment system.

As defined by *Acharya Sushruta*, *Agadatantra* is the name of that branch which describes the features of bites by poisonous animals like snakes, insects, spiders, rat etc. diseases due to different kinds of poisons and their treatment.

In *Agadtantra* 'gada' i.e. *Visha* (poison) is the substance which when enters the body spreads fastly and vitiates *Doshas* causing acute damage to 7 *Dhatus* like *Rasa*, *Rakta*, etc. and degrades health of human beings and resulting in destruction of life.

Though these poisons are harmful and dangerous to life, *Ayurveda* has mentioned the uses of poisonous drugs therapeutically as medicine after *Shodhan* procedure. It is stated that poisons can be used as a medicine when used properly in correct therapeutic dose, formulation and a good medicine can also effect adversely if not used properly for proper person in proper dose etc.

Those impurities that are present in impure (before *Shodhan*) drugs are not found in pure (after *Shodhan*) drugs; so the *Vishadravyas* should be used in medicinal preparation only after *Shodhan* procedure.

These procedures are based on scientific knowledge and basic principles in *Ayurveda*. Thus *vishadravyas* are used effectively in formulations after their *Shodhan*. As stated by *charakacharya*, Means a poison can be used as medicine if used properly and a medicine can prove hazardous if not used properly.

Shodhan procedure of *Eranda Beej* (*Ricinus communis*, Linn. Seeds) is mentioned only in *Yogaranakara* as boiling in coconut water for 3 hours.

Eranda is not included in *Visha* or *Upvisha* according to *Ayurveda* but according to modern science included in one of the most poisonous plants known till today to mankind but Surprisingly used widely in *Ayurveda* treatment in various diseases especially in *Vatavyadhi*.

Various parts of *Eranda* plant such as leaves, fruits and seeds are used for treatment directly or in preparation of various formulations.

Accepting all above facts, there are tremendous innovations in 21st century. Thus the old concepts are needed to be tested against current developments and available parameters.

To test efficiency of the *Shodhan* process by chemical and animal experiments and to establish the safe use of toxic drugs after *Shodhan* as a medicine to mankind.

With this view selected this topic - Effect of *Shodhan* Process on Toxicity of *Eranda Beej* (*Ricinus communis* Linn. Seeds).

AIM

To study acute oral toxicity of *Erand Beeja* (*Ricinus communis* Linn. seeds) before & after *Shodhan*, in albino mice.

OBJECTIVES

- 1) To assess LD 50 of *Erand Beeja* (*Ricinus communis* Linn. seeds) before & after *Shodhan*, in albino mice.
- 2) To assess quantitative change in Ricin after *Shodhan* process.

MATERIALS AND METHODOLOGY**A) *Shodhan* process of *Erand Beej***

- 1) *Erand Beeja*(*Ricinus communis* Linn. seeds).
- 2) Coconut water.
- 3) Cotton cloth.
- 4) *Dolayantra*.
- 5) Grinder.
- 6) Containers.
- 7) Measuring flasks.
- 8) Gas stove.

Procedure

1. 300gm of authenticated sample of *Erand Beej* (*Ricinus communis* Linn. Seeds) and 4000ml of authenticated sample of fresh coconut water were taken.
2. These seeds were cleaned with dry cotton cloth and divided into two groups of 150gm each.
3. From first group seeds were directly processed in grinder and fine paste was prepared and named as **Sample A (Before *Shodhan*)**.
4. Outer layer/Shell of the seeds from second group was removed.
5. Coconut water was taken in *Dolayantra*.
6. The seeds from second group were kept on a cotton cloth and four corners of the cotton cloth were taken together and tied the knot to form a small bag (*Pottali*). This small bag (*Pottali*) containing seeds was dipped into coconut water and precaution was taken that *Pottali* gets fully immersed into coconut water.
7. This *Dolayantra* containing coconut water and *Pottali* was kept on gas fire, with the help of low flame for 3 hours and coconut water was added repeatedly in *Dolayantra* as required.

8. This *Shodhan* process was done on 07/01/2013 started at 12.10pm.
9. After *Shodhan* process seeds were removed from *Pottali* and kept in shadow for drying for 24 hours.
10. These dried seeds were processed in grinder and fine paste was prepared and named as Sample 'B' (After *Shodhan*).



Fig. 1: Erand Plant.



Fig. 2: Seeds of Erand.



Fig. 3: Coconut fruit.



Fig. 4: Impurified seeds without shell.



Fig. 5: Pottali.



Fig. 6: Shodhan Process (Dolayantra).



Fig. 7: Shodhan Process.



Fig. 8: Color change in coconut water.



Fig. 9: Pottali removed from dolayantra.



Fig. 10: Purified seeds.

B) Animal Experimentation

Acute oral toxicity study was conducted according to OECD guidelines 423 at National Toxicology Centre Pune. Housing, feeding conditions and preparations were as follows:-

- a) Age of Mice- 8 Weeks old.
- b) Sex-Non pregnant nulliparous female.
- c) Environmental conditions.

Room temperature- in the experimental animal room was 22°C ($\pm 3^{\circ}\text{C}$)

Relative humidity- $55 \pm 5\%$

Lighting was artificial; and illumination cycle was set to 12 hours light and 12 hours dark

C) Diet- Pelleted feed was provided ad libitum during acclimatization and during the study

d) Water-RO treated water was provided ad libitum in bottles with stainless steel sipper tubes with an unlimited supply of water

e) Three albino mice were group caged by dose and clear observations were made of each mouse. (E.g. 300mg/kg and 2000mg/kg)

f) Preparation of doses-

Vehicle used-Corn oil

Sample – in fine paste form.

- 1) 202-A-Before *Shodhan*
- 2) 202-B-After *Shodhan*

(202 was the project no. given by NTC, Pune)

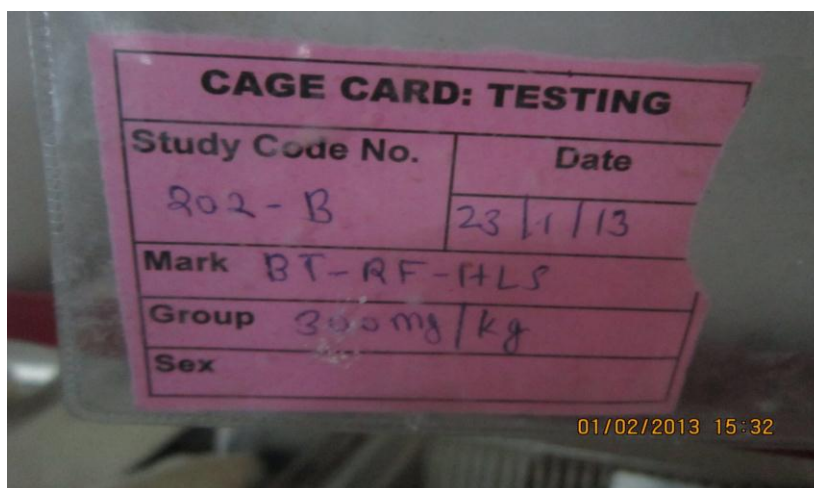
For 300mg/kg dose drug was prepared as 30mg/ml and for 2000mg/kg dose it was 200mg/ml

Dose given- in both the conditions dose was given as per body weight like 1ml for 100gm animal means for 20gm mouse it was 0.20 ml.



CAGE CARD: TESTING	
Study Code No. 202-A	Date 23/1/13
Mark H-B-T	
Group 300 mg/kg	
Sex Female	

01/02/2013 15:31



C) Physicochemical Tests

Following physicochemical tests were done for both the samples 202-A & 202-B as per methods mentioned in API

- I. Total ash value.
- II. Water soluble extractive.
- III. Total ash value.
- IV. Water soluble extractive.
- V. Acid insoluble ash.
- VI. Alcohol soluble extractive.
- VII. Ph of coconut water.
- VIII. Thin layer chromatography.
- IX. Thin layer chromatography of coconut water.
- X. HPLC of Alcoholic extract.
- XI. HPLC of filtered alcoholic extract.

OBSERVATIONS

1) Table showing difference in morphological characters of *Erand Beejaa*(*Ricinus communis*, Linn.seeds)& coconut water (*Cocos nucifera*, Linn.) before & after Shodhan.

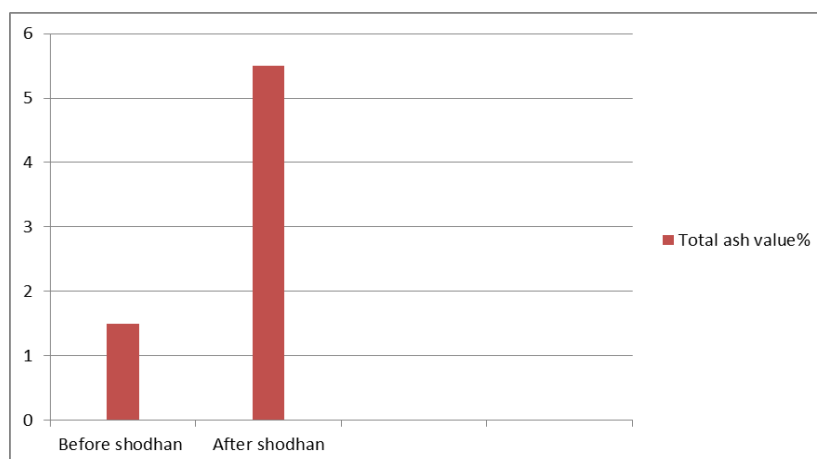
Samples	Process	Colour	Smell
1. Seeds of <i>Ricinus communis</i> , Linn.	Before Shodhan	white (without shell)	Smell of Ricinus seeds
	After Shodhan	Yellowish brown	Smell of coconut water
2. Coconut water	Before Shodhan	No colour	No smell
	After Shodhan	Dark brown	Smell of Ricinus seeds
3. Paste of <i>Ricinus communis</i> , Linn.seeds	Before Shodhan (with shell)	Brown	Pungent
	After Shodhan	Yellowish brown	Sweet smell

2) Following observations were noted during *Shodhan* process.

- 1) Pottali was floating (not fully immersing) in coconut water initially.
- 2) Boiling started at 12.28pm.
- 3) As boiling started white coloured froath formed which was disappeared at 12.35pm.
- 4) Colour of coconut water started changing from clear to faint yellowish at 12.43pm.
- 5) Typical odour of coconut started at 12.53pm.
- 6) Colour of coconut water started to change slightly brownish at 1.17pm.
- 7) Colour changed to dark brown at 2.00pm
- 8) After drying in shadow for 24 hrs, colour of seeds remained yellowish brown.

3) Table showing difference between total ash values.

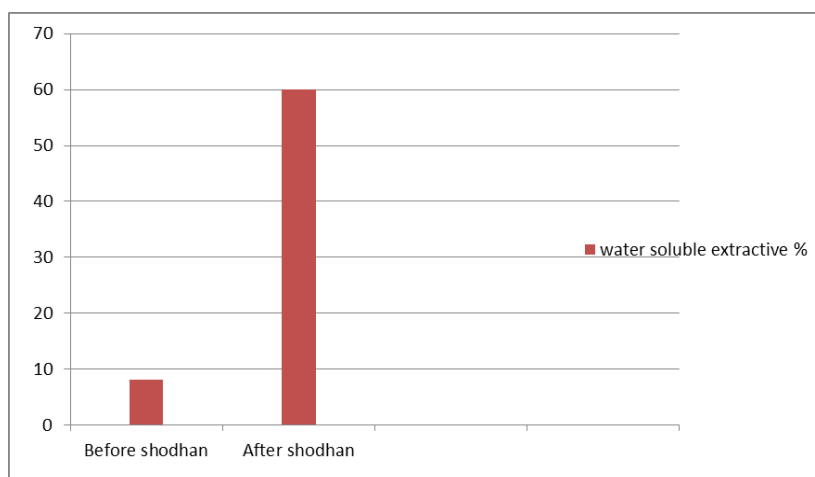
	Before <i>Shodhan</i> (with shell)	After <i>Shodhan</i>
Total ash value %	1.5	5.5

Column Diagram showing difference between ash values.

Total ash value of *Erand Beeja* changed from 1.5 to 5.5.

4) Table showing difference between water soluble extractives.

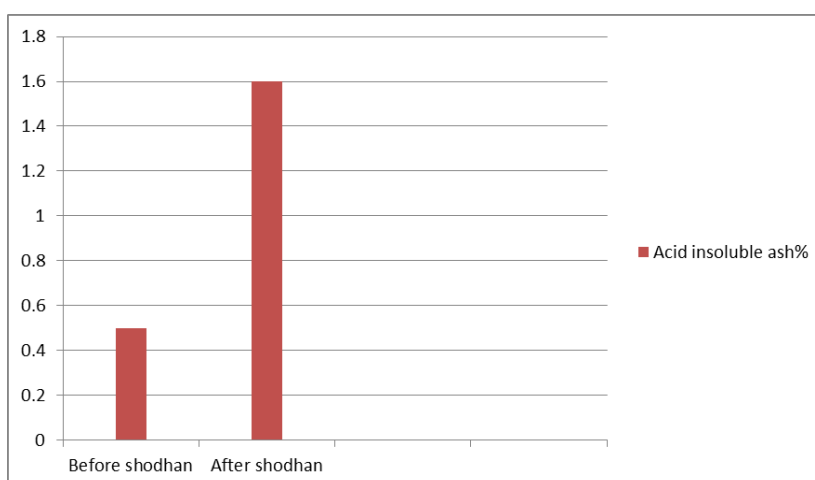
	Before <i>Shodhan</i> (with shell)	After <i>Shodhan</i>
Water soluble extractives%	8	60

Column Diagram showing difference between water soluble extractives %.

Water soluble extractive% of *Erand Beeja* increased from 8 to 60.

5) Table showing difference between Acid insoluble ash %:

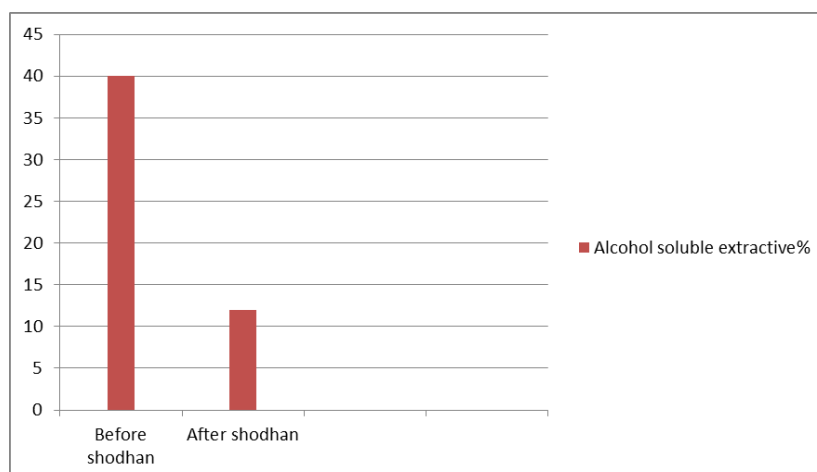
	Before Shodhan (with shell)	After Shodhan
Acid insoluble ash %	0.5	1.6

Column Diagram showing difference between Acid insoluble ash %.

Acid insoluble ash % of *Erand Beeja* changed from 0.5 to 1.6.

6) Table showing difference between alcohol soluble extractive %.

	Before Shodhan (with shell)	After Shodhan
Alcohol soluble extractive %	40	12

Column Diagram showing difference between alcohol soluble extractive.

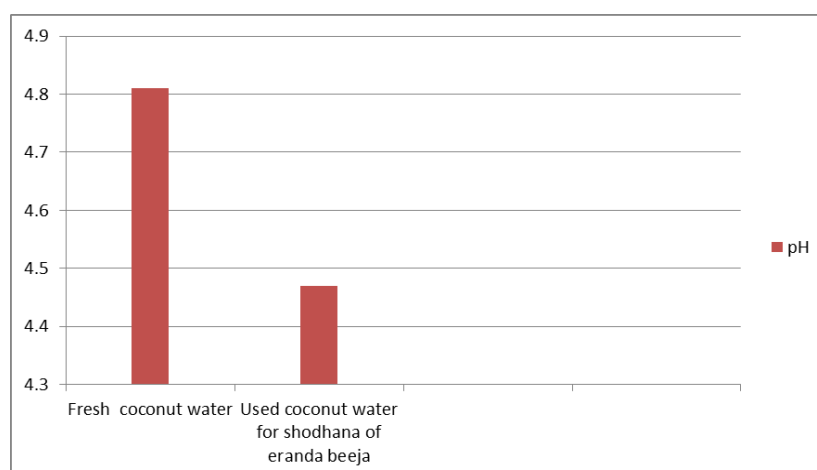
Alcohol soluble extractive% of *Erand Beeja* changed from 40 to 12.

7) Observation on alcoholic extraction of *Erand Beeja* (*Ricinus communis*, Linn. Seeds)

Extract from apparatus A (after *Shodhan* sample) was pinkish brown in colour & from apparatus B (before *Shodhan* sample) was dark brown in colour.

8) Table showing difference in pH of coconut water before & after *Shodhan*:

	Fresh coconut water (<i>Cocosnucifera</i> Linn.)	Used Coconut water (<i>Cocosnucifera</i> Linn.) for <i>Shodhan</i> of <i>Erand Beeja</i>
pH	4.81	4.47

Column Diagram showing difference between pH of coconut water

pH of coconut water reduced from 4.81 to 4.47 i.e. acidity of coconut water increased due to *Shodhan* process.

9) Table showing difference between Rf values during TLC of *Erand Beeja* (*Ricinus communis*, Linn. Seeds) & coconut water (*Cocos nucifera*, Linn.) before and after Shodhan.

B (Before Shodhan).

Sr. No.	Sample Spot	R.F. Value
1.	0.39	0.083
2.	4	0.85
3.	4.4	0.93

A (After Shodhan).

Sr. No.	Sample Spot	R.F. Value
1.	0.4	0.085
2.	3.9	0.83
3.	4.2	0.89
4.	4.6	0.9

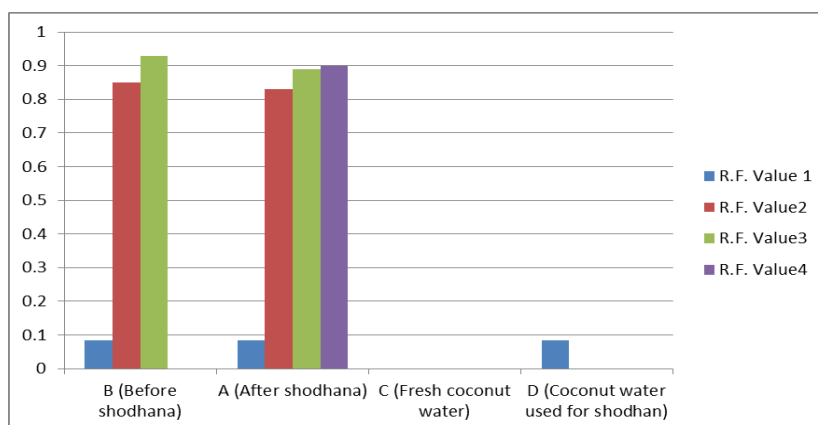
C (fresh coconut water).

Sr. No.	Sample spot	R.F. value
1.	No spot	-

D (coconut water used for the Shodhan of Erand Beeja).

Sr. No.	Sample spot	R.F. value
1.	0.4	0.085

Column Diagram showing difference between Rf values during TLC of *Erand Beeja* (*Ricinus communis*, Linn. Seeds) & coconut water (*Cocos nucifera*, Linn.) before and after Shodhan.



In sample B- 3 spots were observed.

In sample A- 4 spots were observed.

In sample C- no spot was observed.

In sample D- 1 spot was observed.

10) Table showing difference in peak area before & after *Shodhan* in HPLC

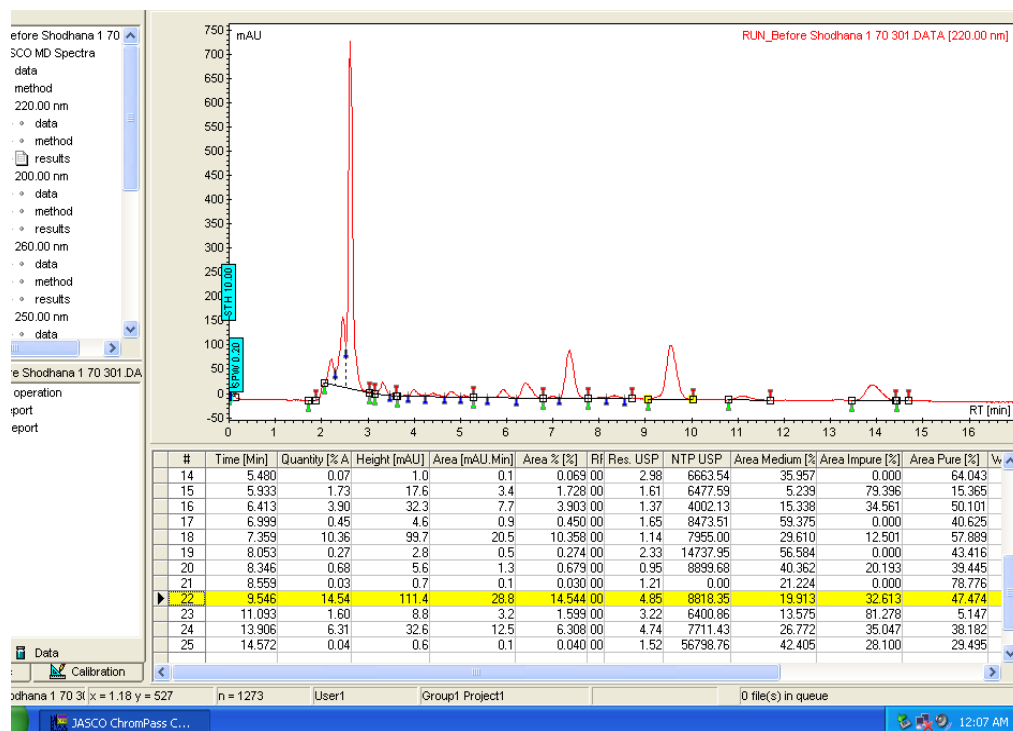
Peak area (mAU).

Before <i>Shodhan</i>		After <i>Shodhan</i>	
1 st inj. Of filtered alcoholic extract	3.2	1 st inj. Of filtered alcoholic extract	1.4
2 nd inj. Of filtered alcoholic extract	9.3	2 nd inj. Of filtered alcoholic extract	1.8
1 st inj. Of alcoholic extract	138.6	1 st inj. Of alcoholic extract	—
2 nd inj. Of alcoholic extract	5.2	2 nd inj. Of alcoholic extract	—

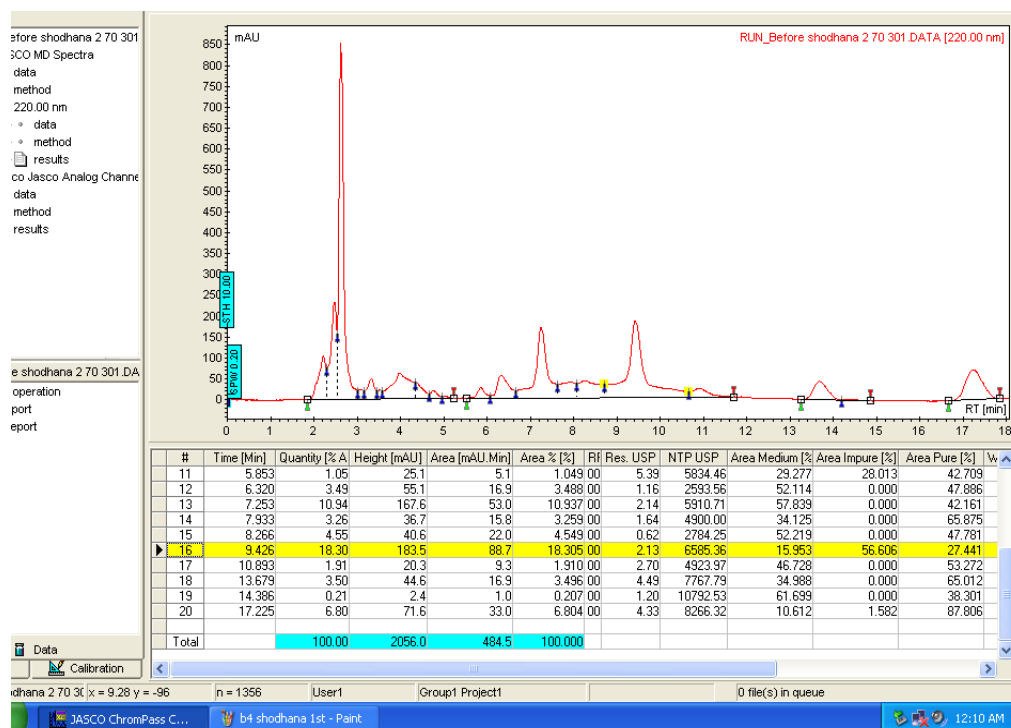
Table showing retention time before & after *Shodhan* in HPLC

Retention time

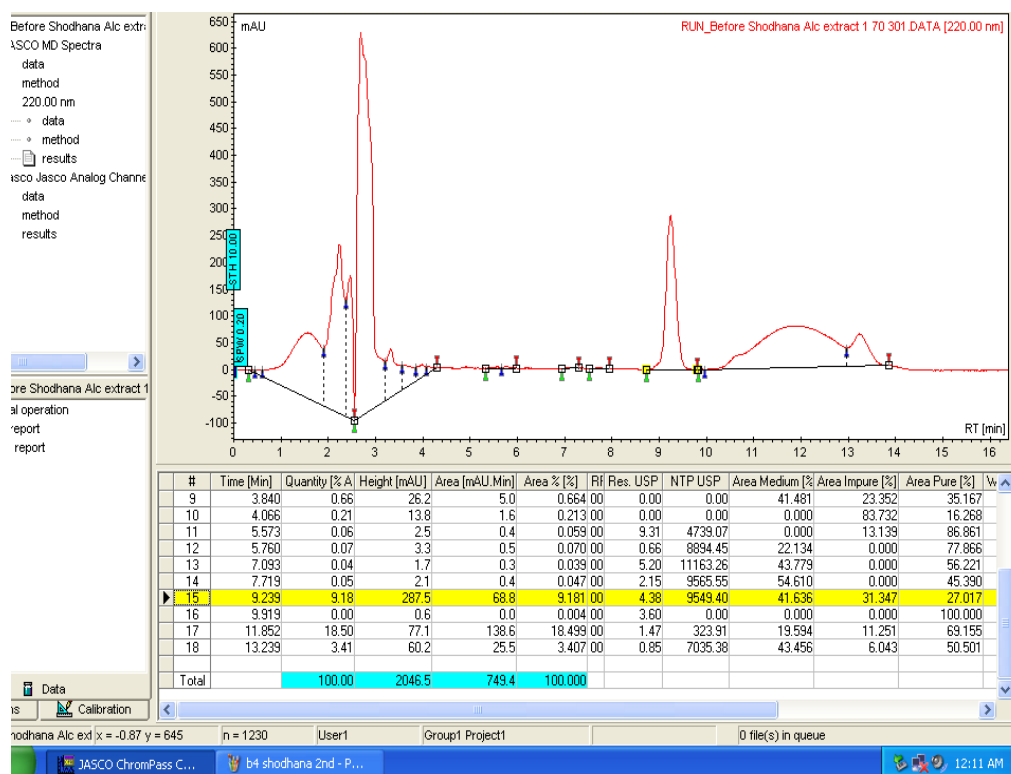
Before <i>Shodhan</i>		After <i>Shodhan</i>	
1 st inj. Of filtered alcoholic extract	11.093	1 st inj. Of filtered alcoholic extract	10.86
2 nd inj. Of filtered alcoholic extract	10.89	2 nd inj. Of filtered alcoholic extract	10.72
1 st inj. Of alcoholic extract	11.85	1 st inj. Of alcoholic extract	10.87
2 nd inj. Of alcoholic extract	10.51	2 nd inj. Of alcoholic extract	10.88

HPLC graphs of extracts of *Erand Beejaa* are as followsHPLC graph of 1st injection of filtered alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn. seeds) before *Shodhan*.

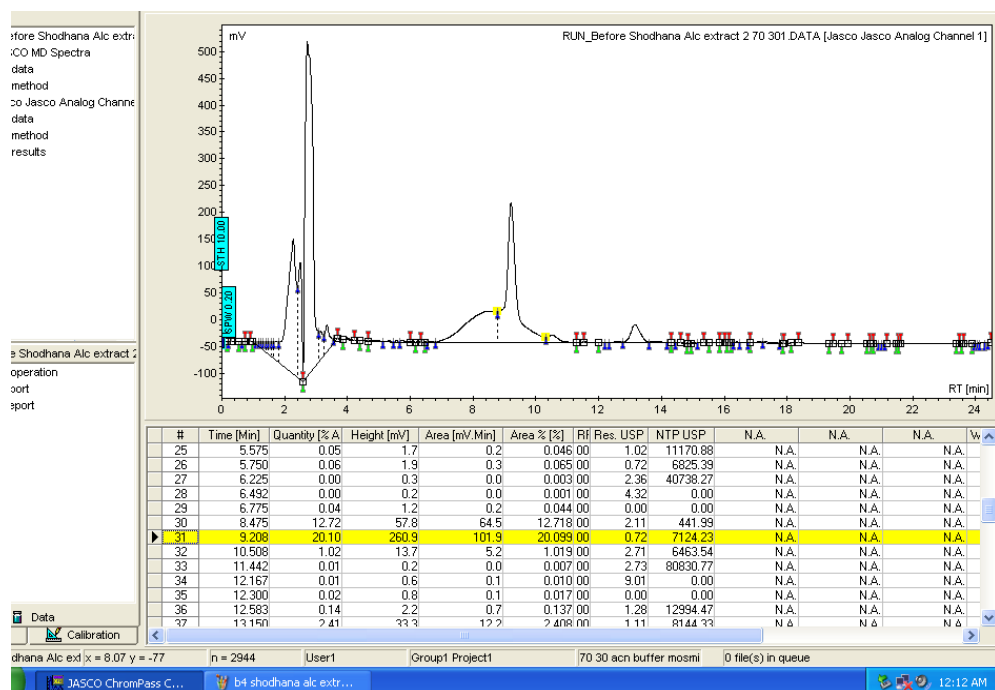
HPLC graph of 2nd injection of filtered alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn. seeds) before Shodhan.



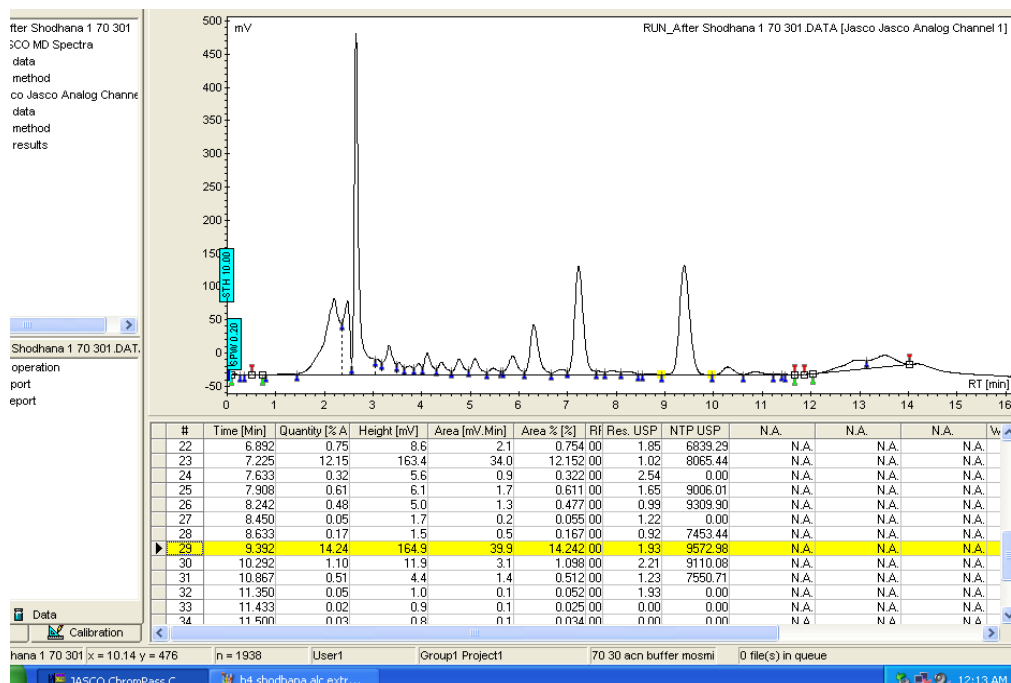
HPLC graph of 1st injection of alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn. seeds) before Shodhan.



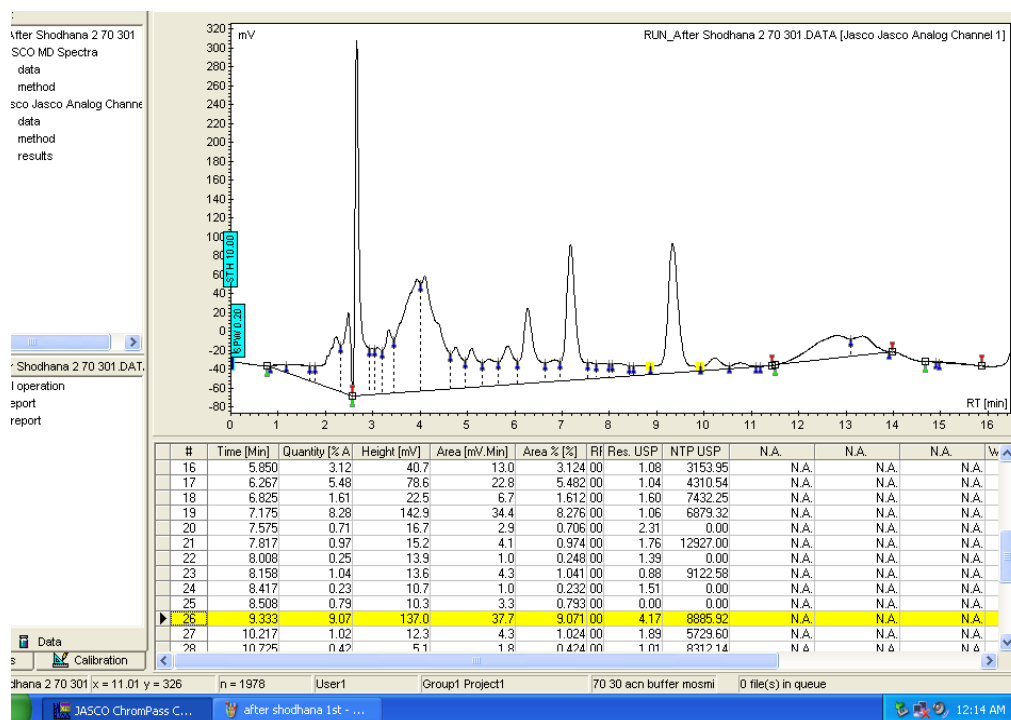
HPLC graph of 2nd injection of alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn. seeds) before Shodhan.



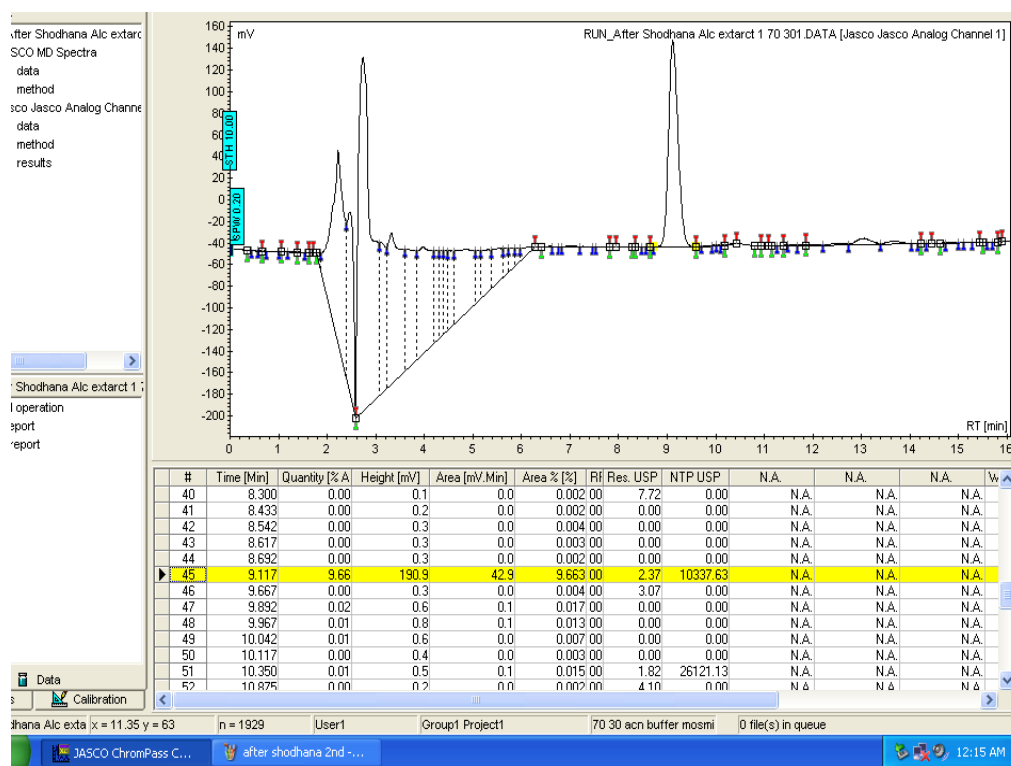
HPLC graph of 1st injection of filtered alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn seeds) after Shodhan



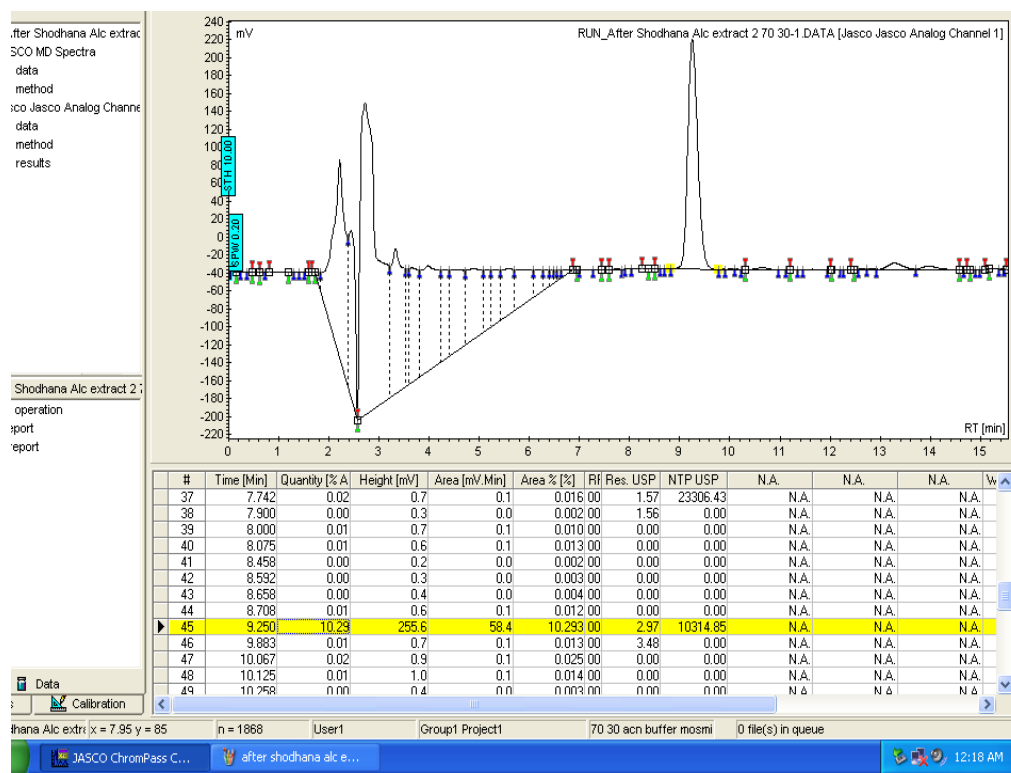
HPLC graph of 2nd injection of filtered alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn. seeds) after Shodhan.



HPLC graph of 1st injection of alcoholic extract of *Erand Beeja* (*Ricinus communis*, Linn. seeds) after Shodhan.



HPLC graph of 2nd injection of alcoholic extract of *Erand Beeja (Ricinus communis, Linn. seeds)* after Shodhan.



11) Observation on acute oral toxicity study: Starting dose was decided as 300mg/kg for both the samples and mice were observed for continuous 14 days daily at each dose level on the basis of following parameters.

- i. Weight.
- ii. Salivation.
- iii. Diarrhoea.
- iv. Urination.
- v. Respiratory Changes.
- vi. Convulsions.
- vii. Coma.
- viii. Death.

A) At the dose of 300mg/kg for both the samples 202-A & 202-B no signs & symptoms of toxicity were observed in both the groups.

B) At the dose of 2000mg/kg of sample 202-A one mouse from the group had loose motions 8 episodes during first 8 hrs. after dosing. The mouse was in comatose stage after 8hrs & 20 minutes of dosing, & had gasping breath after 9 hrs & 30 minutes of dosing. The mice died

after 10 hrs of dosing while for the sample 202-B all the three mice were remained healthy for all fourteen days & no signs & symptoms of toxicity or diarrhoea were observed.

C) One mouse from group of 202-A died at the dose of 2000mg/kg after 10 hrs of dosing, hence dose of 2000mg/kg repeated in next 3 mice as per OECD guidelines.

D) At the dose of 2000mg/kg of 202-A sample in next 3 mice, 2 mice had 6 & 10 episodes of diarrhea before death while other remaining mouse showed 3 episodes of diarrhea and weight loss in 14 days. Hence acute oral toxicity study was stopped at this dose level for said sample.

E) At the dose of 2000mg/kg of 202-B sample; all the three mice were remained healthy for fourteen days & no signs & symptoms of toxicity were observed.

F) All the three mice from control group were remained healthy for fourteen days & no signs & symptoms of toxicity were observed.

G) Autopsy findings of dead animals were suggestive of internal hemorrhages in liver, spleen, kidneys, brain and lungs.



Fig.11: Autopsy finding in animal.



Fig.12: Visceral organs removed after autopsy, look for haemorrhagic changes.

DISCUSSION

Especially the plant *Eranda* was selected for this study. This plant is easily available everywhere. It is mentioned in *Ayurveda* texts since thousands of years & is one of the major oil crop in the India since last many years but came in the focus of the world in last decade since it (Ricin) was used as biological or chemical warfare agent in USA in 2004.

Shodhan of *Eranda* seeds has not been mentioned anywhere in any text, except reference from *Yogaratanakara*. According to *Ayurveda* every part of the plant is useful but according to modern science the entire plant of *Eranda* is poisonous. The toxicity of any substance related to *Ayurveda* medicine can be reduced by *Shodhan* process. Hence *Shodhan* of *Eranda* seeds was carried out as per description is explained in *Yogaratanakara*.

The process of converting harmful *gada* in to useful medicine is *Shodhansanskara*. *Shodhan* process (purification) is one of the procedures, which in spite of its use in *Ayurveda* system has not been yet scientifically explored & very little work has been done on *Shodhan* using modern techniques. So realizing importance of *Shodhan* process, I have tried to prove it in this study & acute oral toxicity study was conducted in albino mice, before & after *Shodhan* of *Eranda* seeds.

For *Shodhan* of *Eranda* seeds, *Narikeludaka* (coconut water) was used. Coconut water & *Eranda* seeds remain in contact with each other for considerable time. Therefore changes in morphological, physical, chemical, biological properties of *Eranda* seeds may be taking place due *Shodhan* process. Hence effect on these properties were studied & compared before & after *Shodhan*.

Mice were selected for acute oral toxicity study because it is having high homogeneity with human beings, it is a mammal & having fast reproduction rate, their small size, low cost, ease of handling, uniformity in breeding, sensitive to low dose & they are commonly used in acute toxicity studies.

In physicochemical study total ash value, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value, TLC & HPLC were performed. For these tests sample before *Shodhan* was fine paste prepared from *Ricinus communis*, Linn. Seeds with shell processed in grinder and sample after *Shodhan* was fine paste prepared from *Ricinus communis*, Linn. Seeds without shell boiled in coconut water for three hours and then processed in grinder.

Logically speaking both the samples was totally different from each other hence difference between the values was observed. It clearly suggests that use of *Erand Beejain* medicines is expected as after *Shodhan* only but never as crude seeds.

Acute oral toxicity study was performed according to OECD 423 guidelines. Starting dose was selected as 300mg/kg. No signs & symptoms of toxicity were observed at dose of 300mg/kg in both the groups of mice in 14 days. Hence dose of 2000mg/kg was given in three mice in group 'a' & 'b'. One out of first three mice from group 'a' died after 10 hrs at the dose of 2000mg/kg of 202-A (before *Shodhan* sample). While all the three mice from group b were survived & was healthy at the dose of 2000mg/kg of 202-B (after *Shodhan* sample). Hence dose of 2000mg/kg of both samples (202-A & 202-B) was repeated in next three mice. Of that two mice from group 'a' died after 4hrs 30 min & 22hrs 30 min each & one mouse showed diarrhoea while all the three mice from group 'b' remained healthy & survived. Signs & symptoms were observed for 14 days. All the mice of control group remained healthy. Hence LD50 of sample 202-A is 2000mg/kg & sample 202-B is safe at the dose of 2000mg/kg. Limit dose was considered as 2000mg/kg. Hence acute oral toxicity study was terminated at this stage only.

It means that *Shodhan* process reduced toxicity of *Erand Beeja* at a significant extent.

CONCLUSION

From the above observations and discussion we can conclude that

1. *Erand Beej (Ricinus communis, Linn. Seeds)* are toxic & can produce harmful effects if taken in large quantity. So it is mentioned as highly toxic drug according to modern science. This conclusion can be drawn by acute oral toxicity study.
2. The LD50 value of *Erand Beej (Ricinus communis, Linn. Seeds)* before *Shodhan* is 2000mg/kg while it is safe at the dose of 2000mg/kg after *Shodhan*.
3. To perform the *Shodhan* process regarding any material as mentioned in the literature is very much necessary, because this *Shodhan* process brings definite change in physical, chemical, biological properties of material. Regarding *Erand Beej (Ricinus communis, Linn. Seeds)* the LD50 of after *Shodhan* sample is increased, that means toxicity is reduced, so *Shodhan* is very helpful.

4. After performing HPLC for Ricin of both the samples before & after *Shodhan* it can be concluded that *Shodhan* process definitely reduces Ricin content of the seeds quantitatively so the toxicity is reduced significantly.

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