



Original Research Article

An Experimental Study to Assess Toxic Potential of Jayapala (Croton Tiglium) Beeja In Relation To Gastrointestinal Tract

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ABSTRACT

Background: *Croton tiglium Linn.* is an irritant poison. Crotin is the toxalbumin, active principle which possesses the irritant activity. The mucosa of gastrointestinal tract is having tendency of becoming hypersensitive to the *Croton tiglium linn.* which may lead to the symptoms like burning pain from mouth to esophagus, nausea, vomiting, bloody stools and dehydration which in turn may lead to weak pulse, loss of body temperature and eventually death.

Methods: An experimental study was taken to find out the toxic effect of *Croton tiglium linn.* seed on gastro intestinal tract in animal model. The LD 50 of the *Croton tiglium linn.* in rats were determined by acute oral toxicity test by following OECD guidelines 425. The repeated dose toxicity has been evaluated by selecting two doses of LD 50 and administered for 15 consecutive days.

Results: On 15th day one hour after drug administration blood samples were obtained from retro-orbital puncture and biochemical investigation has been carried out and organs has been processed for histopathological examination. LD 50 has been found to be 2000mg/kg. The haematological, biochemical and histological changes were found to be normal.

Conclusion: Current study concludes that *Croton tiglium Linn.* seeds (Jayapala beeja) has got minimal toxic effect on gastrointestinal tract of albino rats.

Key words: *Croton tiglium linn*, acute oral toxicity, haematology, GIT Toxicity.

INTRODUCTION

Jayapala (*Croton tiglium linn.*) is one among the eleven upavisha mentioned in ayurvedic literature. (Sharma Sadanand et al.) Though the whole plant is considered as visha. Shastry JLN Its seed is highly toxic. (Sharma P V et al.) It is used in many compound herbo-mineral preparations like Iccha bedhi rasa, Ashwakanchuki rasa etc.

(Vij Krishnan et al.) Though the purification of *Croton tiglium linn* [CTL] is mentioned in classics, usage of improperly purified *Croton tiglium linn* as an ingredient may lead to many harmful effects. Irrational usage of *Croton tiglium linn* without assessing kosta may lead to many adverse effects. As the seed of *Croton tiglium linn.* resembles the seed of Eranda it may be

ingested accidentally. It can also be used as homicidal poison by mixing with food substances. (Bruin, Yuri et al.)

Croton tiglium Linn is an irritant poison. Croton is the toxalbumin, active principle which possess the irritant activity. The mucosa of gastrointestinal tract is having tendency of becoming hyper-sensitive to the *Croton tiglium linn*. which may lead to the symptoms like burning pain from mouth to esophagus, nausea, vomiting, bloody stools and dehydration which in turn may lead to weak pulse, loss of body temperature and eventually death. (Ottoboni, M. et al.) Hence the study is taken up to evaluate the toxic effect of *Croton tiglium linn* seed on gastrointestinal tract in animal model.

MATERIALS AND METHODS

Plant material collection of drug & preparation: *Croton tiglium linn* seeds were collected and macroscopically authenticated vide analysis report number 474/14020401. The seeds were crushed to fine powder & used for administration to the animal.

Experimental animals: Albino rats of wistar strains of either sex between 150 to 250 g body weights were obtained from animal house attached to department of Pharmacology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved by the institutional animal ethical committee under the reference no. SDMCAU/IAEC-2013-14-HS-DJ-O6. The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%.

Acute oral toxicity test: Acute oral toxicity was performed by following OECD -425 guidelines using AOT software. Albino rats of either sex selected by random sampling were used for acute toxicity study. The animal were kept fasting for overnight and provided only with water. The test drug was administered at a dose of 175,550 up to 2000mg/kg (up and down method) and observed for 14 days. If any mortality was observed the same dose will be repeated again to confirm its toxic potential. If mortality was not observed the procedure was repeated for higher doses in the following order 175, 550 and 2000mg/kg body weight.

Repeated dose toxicity: Healthy wistar albino rats were grouped into three different categories six each. Group I treated with 0.5% gum acacia and considered as normal control. Group II & III rats were administered with 200mg/kg & 400mg/kg body weight of *Croton tiglium linn*. seed powder, which is administered as a suspension in 0.5% gum acacia for 15 consecutive days. During the experimental period the following parameters has been assessed food intake, water intake and behavioral changes. On the 15th day one hour after drug administration all rats were anaesthetized and blood was collected by retro-orbital puncture. The following biochemical parameters such as LFT, KFT, and C - reactive protein and total hematology were carried out. Rats were sacrificed under deep ether anesthesia and the following organs such as stomach, small intestine and a part of large intestine has been processed for histopathological examination.

Statistical analysis: The data generated were mentioned as Mean \pm SEM. Difference among the groups were assessed by

employing one way ANOVA with Dunnet's multiple 't' test using Graph pad prism.

RESULTS AND DISCUSSION

Total ten hematological parameters related to RBC, WBC and platelets were studied. *Croton tiglium linn.* seed at 200mg/kg dose level did not produce significant effect on any of the parameters studied. Though moderate decrease in many parameters and moderate increase in two parameters were observed, it did not reach statistically significant level. At higher dose level, the only significant change observed was significant decrease in MCV (Mean corpuscular volume). This index represents the average volume of red cells in a specimen. It is elevated or decreased in accordance with average red cell size; ie, low MCV indicates microcytic (small average RBC size), normal MCV indicates normocytic (normal average RBC size), and high MCV indicates macrocytic (large average RBC size). It is to be noted that at lower dose moderate but non-significant increase was observed. The reasons for the above may be insufficient iron absorption or its utilization. The observed changes were not significant extent and are comparable with normal control and hence can be considered to be no pathological significance. Thus it can be suggested that *Croton tiglium linn* is not likely to produce any significant effect on hematological parameters in the clinical settings. (Yadav RP et al., Rostom, Alaa et al.)

The serum biochemical estimation of urea and creatinine showed significant reduction in comparison to normal control group. It indicates it may be due to the reduction in the formation. Decrease in the urea may be due to the decreased nitrogen and the decrease in the creatinine may be due to the decreased muscular activity or decreased formation in the liver. Thus the alteration in the parameters indicates there is

increased catabolism and negative nitrogen in the body. The result is linked to the elevated total protein in the serum. This might be due to the excessive destruction of mucus and smooth muscle cell of GIT by the *Croton tiglium linn.* seeds. (Arns, W et al)

The result has shown there is significant elevation in the CRP. This is an acute reactive protein elevated in response to inflammatory cytokines during inflammatory process. CRP levels have been used as a parameter of inflammation. In the present study there is a significant elevation in the CRP. It indicates the test drug *Croton tiglium linn.* has toxic role on smooth muscle by causing inflammation process. (Singh, Gurkirpal et al.)

In acute damage membrane destruction causes release of archedonic acid, which can mediate the inflow of leucocytes into inflammatory sites, leading to release of lysozomal enzymes and toxic oxygen radicals. Thus there will be elevation of ALP level in the serum. In the present study *Croton tiglium linn.* Seed has shown considerable increase in ALP level in comparison to normal control group.

The significant increase in both direct and total bilirubin indicates the excessive destruction of RBC and excessive cellular lesions produced by *Croton tiglium linn* seeds.

The result related to body weight change, food intake, dry fecal content and food conversion ratio were co-related with each other. There is an increased food intake in all in comparison to normal control rats and resulted in increased fecal content. This shows the drug has action which increased the appetite. Hence there is an increase in food intake and food conversion ratio. (Singh, G., et al.)

Even though there is an increased food intake and food conversion ratio we observed gradual decrease in the body weight. This shows the rate of anabolism

and catabolism might not be the same. Hence the body has increased catabolic action than anabolism. (Shirasaka, Tetsuhiko, et al.)

The underlying mechanism has to be explored further and validate the therapeutic usefulness of the drug in higher experimental set up.

Croton tiglium Linn., is an irritant poison. Croton is the toxalbumin, active principle which possess the irritant activity. The mucosa of gastrointestinal tract is having tendency of becoming hyper-sensitive to the *Croton tiglium* linn. which may lead to the symptoms like burning pain from mouth to esophagus, nausea, vomiting,

bloody stools and dehydration which in turn may lead to weak pulse, loss of body temperature and eventually death. Hence the study was taken up to evaluate the toxic effect of *Croton tiglium* linn. seed on gastrointestinal tract in animal model.

For this purpose the drug was administered over a period of time Wistar albino rats and its effect on hematological, biochemical parameters along with histopathology of GI tract was carried out. The results obtained have been prepared in the form of consolidated statement for easy comparison and discussion on the activity observed under table 1- 12.

Table 1: Effect of CTL on haematological parameters

Group	Hb g/dl	WBC (10 ³ /μl)	RBC (10 ⁶ /μl)	MCV (fl)	MCH (pg)	PCV (%)	MCHC (g/dl)	RDW-CV (%)	RDWSD(fl)	Platelet (10 ³ /μl)
Normal control	14.75 ± 0.26	8200 ± 333.67	7.43 ± 0.23	53.53 ± 1.02	19.43 ± 0.38	39.63 ± 0.82	36.35 ± 0.13	14.45 ± 0.65	27.81 ± 0.46	8.02 ± 0.39
CTL 200mg/kg	14.65 ± 0.72	4916 ± 846.33	7.20 ± 0.34	56.33 ± 1.15	20.3 ± 0.35	40.66 ± 1.83	35.95 ± 0.28	13.78 ± 0.68	28.38 ± 1.03	6.04 ± 1.20
CTL 400mg/kg	15.20 ± 0.66	9200 ± 2362.4	7.72 ± 0.27	42.04 ± 1.93**	19.5 ± 0.18	41.98 ± 1.95	35.74 ± 0.18	12.94 ± 0.32	26.34 ± 0.62	6.80 ± 1.05

Data expressed in Mean ± SEM, **P<0.01, in comparison to normal control group.

Table 2: Effect of CTL on biochemical parameters

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)	Total Protein (g/dl)	c-reactive protein mg/l	Bilirubin (mg/dl)	Bilirubin Total (mg/dl)	Albumin g/dl
Normal control	144 ± 4.68	81.5 ± 6.99	437.66 ± 62.61	55.65 ± 6.19	0.71 ± 0.04	6.13 ± 0.13	0.46 ± 0.18	0.07 ± 0.0	0.13 ± 0.01	3.46 ± 0.08
CTL 200mg/kg	130.33 ± 13.10	63.5 ± 8.42	522.5 ± 158.88	20.83 ± 4.98**	0.61 ± 0.03	6.75 ± 0.12**	2.21 ± 0.13**	0.09 ± 0.0**	0.17 ± 0.0*	3.55 ± 0.13
CTL 400mg/kg	177.6 ± 22.40	90.8 ± 8.69	465.6 ± 57.23	27.8 ± 6.14**	0.6 ± 0.0*	6.66 ± 0.06*	1.48 ± 0.13**	0.1 ± 0.0**	0.2 ± 0.0**	3.40 ± 0.11

Data expressed in Mean ± SEM, *P<0.05, **P<0.01, in comparison to normal control group.

Table 3: Effect of CTL on electrolytes parameters

Group	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
Normal control	137.15 ± 1.13	4.48 ± 0.10	4.48 ± 0.10
CTL 200mg/kg	136.33 ± 1.40	4.33 ± 0.15	4.33 ± 0.15
CTL 400mg/kg	138.2 ± 0.58	4.7 ± 0.10	4.7 ± 0.10

Data expressed in Mean ± SEM

Table 4: showing the Effect of CTL on weight of stomach

GROUP	STOMACH(g) weight MEAN ± SEM	% CHANGE
Control	1.20 ± 0.12	
CTL TED	1.12 ± 0.04	6.66↓
CTL 2xTED	1.12 ± 0.07	6.66↓

P value-0.743 Data: MEAN ± SEM

Table 5: showing the Effect of CTL on weight of large intestine

GROUP	L.INTESTINE weight (g) MEAN ± SEM	% CHANGE
Control	0.50 ± 0.06	
CTL TED	0.58 ± 0.08	16↑
CTL 2xTED	0.64 ± 0.04	18↑

P value 0.543 Data: MEAN ± SEM

Table 6: showing the Effect of CTL on weight of small intestine

GROUP	S.INTESTINE weight (g) MEAN ± SEM	% CHANGE
Control	0.67 ± 0.06	
CTL TED	0.61 ± 0.06	8.95 ↓
CTL seed 2xTED	0.86 ± 0.08	28.35 ↑

P value-0.073 Data: MEAN ± SEM

Table 7: showing the Effect of CTL on food intake

GROUP	FOOD INTAKE(g) MEAN ± SEM	% CHANGE
Control	5.92 ± 0.19	
CTL TED	6.86 ± 0.62	15.87 ↑
CTL 2xTED	7.50 ± 0.88	26.68 ↑

P value-0.240 Data: MEAN ± SEM

Table 8: showing the Effect of CTL on wet fecal content

GROUP	Wet .FECES(g) MEAN ± SEM	% CHANGE
Control	2.60 ± 0.15	
CTL TED	2.54 ± 0.40	2.30 ↓
CTL 2xTED	2.56 ± 0.22	1.53 ↓

P value-0.989 Data: MEAN ± SEM

Table 9: showing the Effect of CTL on dry fecal content

GROUP	DRY FEACAL CONTENT(g) MEAN ± SEM	% CHANGE
Control	1.13 ± 0.52	
CTL TED	1.36 ± 0.17	20.35 ↑
CTL 2xTED	1.37 ± 0.09	21.00 ↑

P value-0.973 Data: MEAN ± SEM

Table 10: showing the Effect of CTL on food conversion ratio

GROUP	FCR(g) MEAN ± SEM	% CHANGE
Control	4.61 ± 0.12	
CTL TED	4.98 ± 0.42	8.02 ↑
CTL 2xTED	5.26 ± 0.36	14.09 ↑

P value-0.404 Data: MEAN ± SEM

Table 11: showing the Effect of CTL on fecal water content

GROUP	F.WATER(g) MEAN ± SEM	% CHANGE
Control	1.26 ± 0.10	
CTL TED	1.32 ± 0.22	4.76 ↑
CTL 2xTED	1.24 ± 0.13	1.58 ↓

P value -0.937 Data: MEAN ± SEM

Table 12: showing the Effect of CTL on body weight

GROUP	BODY WEIGHT gain (g) MEAN±SEM	
Control	13.74± 2.03	
CTL TED	-10.85±3.89	178.6↓
CTL 2 ×TED	-3.89±4.04	127.9↓

Data: MEAN ± SEM

Histopathology

Stomach: microscopic examination of the stomach sections from both Jayapala TED

dose and 2 x TED dose groups showed normal cytoarchitecture without any degenerative changes.

Small intestine: In TED dose group (group I) – cytoarchitecture was found to be normal in sections from two rats. In one rat epithelial disruption, oedema and cell depletion of moderate to severe intensity was observed. In 2 xTED group oedema was observed in one rat and shortening of epithelium in another rat. Cytoarchitecture of small intestine was found to be normal in other rats.

Large intestine: in group I (TED dose) in one rat marked oedema, necrosis was observed, and mild shortening of epithelium was observed in another rat. Remaining rats exhibited normal cytoarchitecture. In group II 2 X TED group- mild epithelium erosion was observed in one rat and in another rat shortening of the epithelial layer was observed. Remaining rats exhibited normal cytoarchitecture.

CONCLUSION

Haematological study revealed that there was a significant effect of Ashodhita Croton tiglium linn. on MCV at the double quantity of therapeutic dose, however other parameters were not affected to significant level. Biochemistry study revealed that a significant effect of Croton tiglium linn. seed at both dose levels, on serum urea, c-reactive protein, total protein, direct and indirect bilirubin. There was a significant decrease in the body weight of the wistar rats of both. Administration of Croton tiglium linn. did not lead to significant changes in food intake, food conversion ratio and wet fecal weight in both the groups. Histopathology revealed that mild to moderate inflammation in the stomach, small intestine and large intestine at both dose level. This study showed that ashodhita Croton tiglium linn. has got minimal toxic effect on gastrointestinal tract.

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