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Evaluation of acute toxicity and intestinal transit time of *Croton tiglium* L. seeds

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The seeds of *Jayapala* (*Croton tiglium* L.) (Euphorbiaceae) is classified under *Upavisha* in classical texts of Ayurveda known for its purgative action and is being used in the management of several disorders. Ayurvedic Pharmacopoeia of India recommends its *Shodhana* prior to its internal administration. The present study was planned with an aim to evaluate the acute toxicity of raw and *Shodhita* seeds of *Croton tiglium* L. and intestinal transit time of *Shodhita* seeds on experimental animals. Acute oral toxicity study for Raw *Jayapala* (RJ) and *Shodhita Jayapala* (SJ) seed sample were carried out following OECD guidelines. Rats were administered a single dose of 2000 mg/kg orally and then observed individually for the first 30 mins, then over a period of every 2 hours for 24 hours and at least once daily for 14 days. General behaviour, adverse effects and mortality were observed throughout the experimental period. Intestinal transit time in mice was carried out at the lower dose of 2.54 mg/kg and the higher dose of 7.61 mg/kg by adopting Kaolin expulsion test and latency of the onset of kaolin expulsion in faecal matter. The results showed that the raw *Jayapala* produced mortality in a single oral dose of 2000 mg/kg while *Shodhita Jayapala* did not *Jayapala* produce any *mortality* and toxicity except behavioural changes and diarrhoea at the same dose level. In kaolin expulsion test, *Shodhita Jayapala* produced marked increase in intestinal motility in mice among which higher dose of the drug was found better which was proved by fast clearance of kaolin in faecal pellet in comparison to control group. Oral acute toxicity study suggests that *Shodhana* (processing/purifying) process must be advocated for use of *Jayapala* to eliminate the untoward effects. Further, *Shodhita* (processed) *Jayapala* seeds have markedly increased the intestinal motility confirms the *Virechana* (purgation) *karma* of drug in mice.

Keywords: Acute toxicity, *Croton tiglium*, Intestinal transit, Jayapala, *Shodhana*.
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Introduction

Jayapala (*Croton tigilium* L. Euphorbiaceae) commonly marketed in the name of *Jamalgota* is mentioned under *Upavisha* (group of semi-poisonous drugs) in classical texts of *Ayurveda*[1] and its seeds as a poisonous part in Drugs and Cosmetics Act (India), 1940[2]. Crotonic oil present in the seed causes dermatitis, severe purgation and pustules[3]. The major constituent of the crotonic oil is Phorbol 12 myristate - 13-acetate[4] and crotonalic acid being present in freestate which are a powerful irritant to skin and a purgative in the intestine in freestate[5]. Classical texts of Ayurveda have recommended different methods of *Shodhana* (processing/purifying) process to eliminate the unwanted effects from various poisonous plants[6]. Ayurvedic Formulary of India recommends the seeds of *C. tiglium* for *Swedana* (boiled) with milk for three hours followed by washing with hot water and *Bhavana* (by soaking method) with *Nimbu swarasa* (juice of *Citrus medica* L. fruit)[7]. *Jayapala* seed is known for its purgative *(Virechana*) action and also indicated in various disease conditions like *Jalodara* (ascites), *Shotha* (oedema), *Kustha* (skin disorders), *Krimi* (worm infestation), *Jwara* (fever) etc[8]. The recent review of the literature shows that neither effect of *Shodhana* on *Jayapala* seed nor purgative action have been reported. Considering the potential action of *Shodhita Jayapala* and the adverse effect of raw *Jayapala*, it was thought to find out the role of *Shodhana* on the safety of test drug during acute administration in rats. Further, to provide the scientific evidence to the therapeutic use as purgative action, an experimental study was planned for *Shodhita Jayapala* on intestinal motility in albino mice.

Material & Methods

Collection and authentification of plant material

Raw seeds of *Croton tiglium* L.were collected from its natural habitat, Udupi district, Karnataka.

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A sample specimen was identified and authenticated and preserved in Pharmacognosy laboratory of Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar (Voucher No - PHM/15-16/6204).

Sample preparation

The raw *Jayapala* seeds were washed, air-dried and pulverized into fine powder and stored in the airtight glass bottle being labelled as raw *Jayapala* (RJ)[9]. *Shodhana* of the seeds were carried out by *Swedana* (boiling) method with *Godugdha* (cow milk) for three hours and after *Swedana* of *Jayapala,* seeds were washed, air-dried and pulverized into fine powder. The fine powder was further subjected to *Bhavana* with *Nimbu swarasa* for three times[10]. These *Shodhita* seed powder were shade dried and stored in an airtight glass bottle for experimental use being labelled as SJ.

Experimental animals

Charle's foster albino rats (200±20 g) and Swiss albino mice (25±5 g) of either sex were used in experimental studies. Animals were obtained from the Animal house attached to the Pharmacology laboratory of I.P.G.T. & R.A. Animals were housed in each cage made up of polypropylene with stainless steel top grill. The selected animals were kept under acclimatization for one week before the commencement of the study. Animals were exposed to 12 hours dark and light cycle with ideal laboratory condition in terms of ambient temperature (23±2ºC) and humidity (50-60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and drinking water was given *ad libitum*. Experiments were carried out in conformity with the Institutional Animal Ethics Committee (IAEC) after obtaining its permission (IAEC/19/2015/42) in accordance with the guideline formulated by CPCSEA, India.

Dose fixation

The normal dose of *C. tiglium* in human is ¼ rati (31.25 mg) according to Rastarangani[11] and in *Ichhabhedi rasa*[12], one of the compound formulations frequently used to induce purgation during *Virechana Karma*, the proportion of *C. tiglium* is 1/3rd part of total ingredients i.e. 93.75 mg taken as the higher dose of the drug. Considering this, the dose for animal experimentation was calculated by extrapolating the human dose to animal dose based on the body surface area ratio following the table of Paget and Barnes. (1964)[13].

The lower dose for mice for the experimental study was as follows:

= Therapeutic human dose × body surface area ratio (convertibility factor) for 20 g mice
= 31.25 mg × 0.0026 = 0.081 mg/20 g body weight of mice=4.06 mg/kg body weight of mice

The higher dose for mice for the experimental study was as follows:

= Therapeutic human dose × body surface area ratio (convertibility factor) for 20 g mice
= 93.75 mg × 0.0026 = 0.24 mg/20 g body weight of mice =12.18 mg/kg body weight of mice Suspension was prepared using gum acacia in distilled water. The drug was administered by oral feeding cannula.

Acute oral toxicity study

Acute toxicity studies of drugs were carried out in female Charles Foster rats by using Organization for Economic Co-operation and Development (OECD) 425 guideline with 2000 mg/kg as limit dose[14]. The rats were divided into two groups each consists of five animals and the group received Raw *Jayapala* being coded as RJ and *Shodhita Jayapala* as SJ. In test drug groups (RJ and SJ), animals in each group were dosed in sequence, usually at 48 hours intervals. Food but not water was withheld for an overnight before the experiment and further, two hours after administration of test drug. The animals were observed keenly for about 30 minutes for any signs of toxicity or mortality, and further observations were made every 2 hours for 24 hours after administration of the drugs. The body weight was noted weekly.

Intestinal transit time (kaolin expulsion test)

The selected Swiss albino mice were divided into three groups of six each comprising three male and three females. Group (I) kept vehicle control group received gum acacia in distilled water (10 mL/kg, PO), whereas, Group (II) and (III) kept as drug-treated groups received *Shodhita C. tiglium* seed powder at lower dose (2.54 mg/kg, PO) and higher dose (7.61mg/kg, PO) respectively. The effect of the test drug on intestinal transit time was carried out established on the previous study[15]. In short, one hour after drug administration, 0.1 mL of 40% kaolin suspension solution was administered with the help of an oral catheter. The animals were placed in a transparent arena and were carefully observed for the beginning of the kaolin expulsion, which begins in the form of white coloured faecal pellets.

Table 1 — Effect of test drugs on intestinal transit time in mice

Treatments and Doses (mg/kg)	Kaolin pellet expulsion time (min)	Percentage change
Vehicle Control	402.50 ± 21.50	--
Shodhita C. tiglium seed powder (2.54 mg/kg, po)	198.60 ±05.89*	50.65↓
Shodhita C. tiglium seed powder (7.61 mg/kg, po)	94.60 ±09.60**	76.49↓

Data: Mean± SEM, ↓ Decrease. *p< 0.05, **p< 0.01 when compared with the control group (ANOVA followed by Dunnett's multiple't' test)

Statistical analysis

The obtained data have been presented as mean ± standard error mean, the difference between the groups, determined by ANOVA followed by Dunnett's multiple't' test for unpaired data to assess the statistical significance between the groups. The value $P< 0.05$ is considered as statistically significant.

Results

In the present oral acute toxicity study, mortality was observed in raw *Jayapala* treated group after 140 minutes of drug administration at a dose level of 2000 mg/kg while no mortality was found in *Shodhita Jayapala* treated group during the course of study (14 days) at an oral dose level of 2000 mg/kg. In RJ treated group, the behavioural signs of toxicity such as CNS depression (hypoactivity, passivity, relaxation, and ataxia), ANS (ptosis), hypothermia, abdominal cramps were also observed during first one hour. The gross necropsy findings showed multiple ulceration and perforation and internal bleeding of the GI tract. Whereas opposite effect such as hyperactivity, Straub tail, rearing, ambulation, diarrhoea was found in SJ treated group. All the animals were normal except behavioural changes during the period of study in this group.

In Kaolin expulsion test, both the dose levels of test drug significantly shortened the intestinal transit time as observed by fast expulsion of kaolin in faecal pellet when compared to the control group. However, the higher dose produced a marked decrease in kaolin expulsion time in mice (Table 1).

Discussion

Herbal products prepared from medicinal plants in developing countries have become popular in healthcare and described as safe as they are obtained from natural sources. Though, bioactive compounds from medicinal plants are determined to be safe without understanding the possible health effects and thus commonly used as self-medication[16]. However, there is a lack of data on the toxicological profile and adverse effects of these compounds. Therefore, acute toxicity study is required not only to identify the range of doses in animal studies but also to explain the probable clinical adverse effects evoked by the test compounds under investigation. It is also an important effective parameter for calculating the therapeutic index of drugs and chemicals[17].

Raw seed kernel of *C. tiglium* L. contains 55-57% croton oil. Oil of *C. tiglium* seeds is reported to contain phorbol esters and crotonic acid along with the fatty acids. The dose of 1 mL oil produces mortality in human[18]. Both the constituents are reported to have irritant activity on the gastrointestinal tract and subsequently responsible for severe purgative action. These constituents are oil soluble and hence, may be removed by cow milk during the process of *Śhodhana* in the present study in *Shodhita Jayapala*[19]. It may be speculated that the reduction in the toxicity of *C. tiglium* seeds is due to the reduction of the level of these two constituents along with the other constituents[20-22]. In the present study, SJ treated group showed no adverse events at the dose of 2000 mg/kg which indicate that the LD_{50} was greater than 2000 mg/kg through the oral route. It also indicates that the test drug may not likely to produce any drastic degenerative changes at the therapeutic dose administered during the disease conditions.

The latency of onset of kaolin expulsion in faecal matter was selected as a parameter for the intestinal motility. Kaolin is a native aluminium silicate and has traditionally been used internally to control diarrhoea. It is reported that kaolin is insoluble and is not absorbed into the bloodstream. Instead, it acts locally in the intestines, where it absorbs toxins and relieves mild diarrhoea[23]. The kaolin used as a marker in this study in a much lower dose (40 % kaolin, 0.1 mL administered for 20 g mouse) than that of therapeutic human dose (26.2 g for every s hours) to avoid any significant action on the intestine.

It is a well-known fact that, it is not an easy task to prove the *Virechana* (purgative) action of a drug in the experimental animal model because of its broader meaning. Ayurveda defines *Virechana karma* as the action which makes the faeces watery and expels it out forcibly, either formed or not formed into a mass[24].

Kaolin was given one hour after drug administration to avoid possible interaction with test drug. The animal was carefully observed for apparent toxic symptoms if any due to kaolin as well as symptoms of *Virechana* such as uneasiness, which may likely to be produced by test drug.

According to Charaka, *Rechaka dravyas* possess *Ushna* (hot in nature), *Tikshna* (sharpness), *Sukshma* (Fineness), *Vyavayi* (spreads rapidly throughout the body) and *Vikashi* properties[25]. *Jayapala* has been reported for its *Rechaka* property (purgative action) in Ayurvedic compendia[26]. According to Sushruta, *Rechaka* drugs are *Guru* (Heaviness) in nature due to the predominance of *Prithvi* (earth) and *Jala* (water) constitution[27]. Owing to this, they have the potential to move intestinal contents towards *Adhomarga*.

The functional gastrointestinal disorders are high in the general population[28,29]. Recent evidence suggests that blockage of CCK receptors and stimulation of motilin receptors are also promising avenues to increase gastrointestinal motility. *C. tiglium* used as a cathartic in Ayurvedic system of medicine. A previous study suggests that drug exhibited a dose-dependent cathartic effect and also showed an increase in gut movement with an increased contractile movement which is partially blocked by atropine. Thus, croton dried nuts may elicit a purgative effect by increasing the gut motility, partially via muscarinic receptor activation[30,31]. It can be proposed that the test drug may be antagonizing the effect of the sympathetic system. It is also possible that it may increase the intestinal motility by cholinergic stimulation or stimulation of 5-HT4 receptors[32]. Intestinal motility activity may be activities similar to that of opioid antagonists which act at μ and σ receptors in the gastrointestinal tract to alter both motility and secretion[33]. Another possibility is prostaglandin stimulating activity of *C. tiglium.* Prostaglandins are known to stimulate intestinal fluid secretion and intestinal motility.

Conclusion

In acute oral toxicity, raw *Jayapala (C. tiglium)* seed at the dose of 2000 mg/kg showed toxic effects and mortality while *Shodhita* (processed/purified) seeds produced behavioural changes and increase the faecal pellet, its consistency and diarrhoea like activity but did not produce any toxicity and mortality in rats. From the present study, it is concluded that *Jayapala* must be administered after *Shodhana*. *Shodhita* (processed/purified) seeds of *Jayapala* markedly increase the intestinal motility confirms the *Virechana karma* in mice.

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Refernces

1 Sharma S, *Rasatarangini*, 24/163-64,11th edn, edited by Shastri K (Motilal Banarasidas, Varanasi), 2012, 676.
2 Malik V, Drugs and Cosmetics act 1940 with Drugs and Cosmetics rules, 18th edn (Eastern Book Company, Lucknow), 2006, 282.
3 Clinton H T, Thomas J H, *Clinical toxicology*, 4th edn, (Lea & Febiger), 1964, 189.
4 Parikh C K, *Parikh's textbook of Medical Jurisprudence Forensic Medicine and Toxicology*, 6th edn, (CBS publishers & distribution), 1999, 930.
5 Sujatha K, Revanasiddappa S S and Swetha S, Analytical study on shodhana of Jayapala, *Int J res Ayurveda Pharm*, 2013, **4**(6), 805-808.
6 Ilanchezhian R, Roshy J C and Acharya R N, Importance of media in Shodhana (Purification/ Processing) of poisonous herbal drugs, *Anc sci life*, 2010, **30**(2), 54-57.
7 Anonymous, *The Ayurvedic Formulary of India*, Department of Indian System of Medicine & Homeopathy, (Ministry of health and family welfare, Govt of India, New Delhi, 2003, 2nd edn, Part 1, 364.
8 Sharma S, *Rasatarangini*,24/ 318-19,11thedn, edited by Shastri K (Motilal Banarasidas, Varanasi), 2012, 706
9 Anonymous, *The Ayurvedic Formulary of India*, 2nd edn, Part I-A, Churna, Department of AYUSH(Ministry of Health and Family Welfare, Government of India, New Delhi), 2008, 304.
10 Acharya Y T, *Rasmritam*, Parishishtam 7, 1st edn, edited by Devnathsinh G (Chaukamba Surbharati Prakashana, Varanasi), 2008, 175.
11 Sharma S, *Rasatarangini*, 24/320, 11thedn, edited by Shastri K, (Motilal Banarasidas, Varanasi), 2012, 706.
12 Anonymous, *The Ayurvedic Formulary of India*, 2nd edn Part I-A, Formulations 20:6, Department of AYUSH (Ministry of Health and Family Welfare, Government of India, New Delhi), 2008, 666-7.
13 Paget G E and Barnes J M, Evaluation of drug activities, edited by Lawrence D R and Bacharach A L, *Pharmacometrics*, **1**, (Academic Press New York), 1964, 161.
14 OECD Guidelines, OECD guidelines for testing of chemicals, Test No. 425, Acute toxic class method; 2008
15 Ashok B K, Bhat S D and Ravishankar B, Screening of intestinal transit time of *Euphorbia fusiformis*Buch.-Ham. ex D. Don In Swiss albino mice, *Indian J Nat Prod Resour*, 2012, **3**(4),547-550.
16 Vaghasiya Y K, Shukla V J and Chanda S V, Acute oral toxicity study of *Pluchea arguta* Boiss extract in mice, *J Pharmacol Toxicol*, 2011, **6,** 113-123.
17 Rang H P, Dale M and Ritter J M, *Pharmacology*, (Churchill Livingstone, New York), 4th Edn, 2001, **13**, 312

18 Pandey G, Dravya guna Vijnana, Krishnadas Academy, Varanasi, **1**(2), 2004, 860.

19 Pal P K, Nandi M K and Singh N K, Detoxification of *Croton tiglium* L. seeds by Ayurvedic process of *Shodhana*, *Anc Sci Life*, 2014, **33**(3), 157-161.

20 Wang X, Lan M, Wu H P, Shi Y Q and Lu J, *et al.,* Direct effect of croton oil on intestinal epithelial cells and colonic smooth muscle cells, *World J Gastroenterol*, 2002, **8**(1), 103-107.

21 Hu J, Gao W Y, Gao Y, Ling N S and Huang L Q *et al.,* M3 muscarinic receptor- and Ca^{2+} influx-mediated muscle contractions induced by croton oil in isolated rabbit jejunum, *J Ethnopharmacol,* 2010, **129**(3), 377-380

22 Misiak J I, Wieczorek P P and Kafarski P, Crotonic acid as a bioactive factor in carrot seeds (*Daucus carota* L.), *Phytochemistry,* 2005, **66**(12), 1485–1491

23 Berardi R R, Kroon L A and McDermott J H, *Handbook of Nonprescription Drugs*, 15th edn, Washington, DC: American Pharmacists Association, 2006, 340, 357, 358, 769.

24 Sharangadhara, *Sharangadhara Samhita*, Madhyama Khanda 6/14th edn, edited by Vidyasagar P S (Chaukhambha Orientalia, Varanasi), 2000, 178.

25 Agnivesha, *Charaka Samhita*, Vol. II KalpaSthana 12/6, edited by Bramhanand T, (Chaukhamba Surbharati Prakashan,Varanasi), 2009, 1132.

26 Bhavmishra, *Bhavaprakasha Nighaṇṭu*, Guduchyadi Varga-202, edited by Chunekar K C (Chaukhambha Bharati Academy, Varanasi), 2010, 386-387.

27 Sushruta, *Sushruta Samhita,* Part 1, Sutra Sthana 41/6, edited by Shastri A (Chaukhamba Sanskrit Sansthan, Varanasi), 2012, 199.

28 Drossman D A, Li Z, Andruzzi E, Temple R D and Talley N J *et al.,* U. S. householder survey of functional gastrointestinal disorders, Prevalence, sociodemography, and health impact, *Dig Dis Sci*, 1993, **38**(9), 1569-1580.

29 Russo M W, Wei J T, Thiny M T, Gangarosa L M and Brown A *et al.,* Digestive and liver diseases statistics, *Gastroenterology,* 2004, **126**(5), 1448-1453.

30 Jamalgota: Purging croton uses, medicines, side effects, available from http://easyayurveda.com/2017/05/09/jamalgota-purging-croton-croton-tiglium/ (Accessed on 7th December 2018).

31 Pillai N R, Gastro Intestinal effects of *Croton tiglium* in experimental animals, *Anc Sci Life*, 1999, **18**(3&4), 205-209.

32 Sternini C, Patierno S, Selmer I S and Kirchgessner A, The opioid system in the gastrointestinal tract, *Neurogastroenterol Motil,* 2004, 16(2), 3–16.

33 Musch M W, Arvans D L, Paris H and Chang E B, α2- Adrenergic Receptors Attenuate Secretagogue- Induced Endocytosis and Promote Exocytosis of Intestinal NHE2 and NHE3, *J Pharmacol Exp Ther*, 2009, **330**(3), 818-825.