# A Pilot Study to Evaluate The AntidotalActivity of Nimbuswarasa(Citrus medica) on Jayapala Seed (Croton tiglium)Induced Toxicity on Wistar rats

**Dr. Shobha Bhat. K**
Associate Professor, Department of Agadtantra, Faculty of Ayurveda, IMS, BHU, Varanasi, India. 221005.

***Abstract:*** ***The present paper discusses the acute toxicity profile of Jayapala beeja choorna(seed powder) and the antidotal activity of nimbuswarasa(lime juice) on it. OECD guidelines 425 with AOT software were followed to assess acute toxicity test to find the dose of Jayapala beeja choorna. In phase-II, antidotal effect assessment of Nimbuswarasa on Jayapala induced toxicity was done.***

***The LD 50 value was calculated to be more than 2000 mg/kg from the phase-I study. In Phase-II test, body weight of rats was significantly decreased in the test group when compared to the control group which was reversed in the anti-dote administered groups. There was significant decrease in food intake in the test groupwhich was reversed by the administration of anti-dote. Administration of Jayapala caused non-significant increase in the wet faecal weight and the antidote at TED dose produced opposite effect. As regards todry faecal weight,in the antidote TED dose given group perfect antagonism was not observed but at higher dose significant reversal and significant increase in dry weight was observed in comparison to Jayapala alone group. Administration of Jayapala produced significant increase in faecal waterconfirming the watery stool formation enhancing effect of Jayapala. This effect was significantly reversed in the antidotal groups. Administration of Jayapala lead to significant decrease in food conversion ratio and significant reversal was observed in the antidotal groups. Hence, from the whole observations and from the above data, it is very clear that NimbuSwaras is an effective antidote against Jayapala induced toxicity.***

***Key words: Antidote, Jayapala beeja, Nimbuswarasa, Toxicity.***

---

**INTRODUCTION:**

Among the different classifications of visha mentioned in the samhitas, sthavara visha comprises vishas of vegetable and mineral origin. The later Acharyas of Ayurveda further classified the sthavara vishas of vegetable origin into two sub classes; Visha and Upavisha[1]. Jayapala is an organic irritant vegetable (herbal) poison[2], explained under the category of Upavisha[3], The seeds of jayapala are highly toxic due to the presence of Tiglnic acid, Crotonic acid & Crolonolic acid. Anyhow there are many Ayurvedic formulations like Icchabedi rasa, Jwarari rasa, Jalodari rasa etc. that contains jayapala as one of main ingredients. Ayurveda claims that the use of such poison in therapeutics is safe as they are processed with shodhana procedure. Improper shodhana procedure or improper use of such drugs can also lead to the manifestation of poisonous signs & symptoms[4].

Safety is the most important consideration before administration of such products. The knowledge and administration of proper antidote can be lifesaving in circumstances of its toxicity & it may help to decrease the morbidity & health care costs. The widespread availability of an affordable antidote for such drugs would revolutionize the management of toxicity. In the later Nighantus like Bhavaprakasha Nighantu, Nigantu Adarsha etc and the recent texts like Indian material medica, we get the references for the commonly available antidotes of these upavishas. Nighantu Adarsha explains the various antidotes for Jayapala as Dughda, Dadhi, Nimbuswarasa etc[5]

Nimbuswarasa is a commonly available drug and if the antidote activity gets proven, could be a cost effective remedy in jayapala induced toxicity. Hence before planning an experimental protocol, a pilot study was planned to access the antidotal effect ofnimbu swarasa on jayapala induced toxicity just based on certain GIT related parameterswhich is discussed here.

**Aims and Objectives:-**

1. To determine the acute toxicity profile of Jayapala seed.
2. To assess the antidote effect of nimbuswarasa on jayapala seed induced toxic effect, especially on GIT related symptoms.

**MATERIALS AND METHODS:**

**Phase – I: Acute oral toxicity study:**

Wistar strain albino rats of either sex weighing between 150-250gm were used for the experimental study.The animals were obtained from animal house attached to the pharmacology laboratory, S.D.M Centre for Research in Ayurveda and Allied Sciences. IAEC clearance was obtained for the study and the study was conducted as per its guidelines. IAEC NO:SDMCAU/IAEC/2013-14-11.A total of 5 healthy rats of either sex, were kept under acclimatization for 7 days before dosing. They were marked with saturated picric acid solution in water for proper identification. The group number, animal number and sex of the animal were identified with the help of cage cards, as presented in the following table:

**Table 1: Details of experimental rats of acute toxicity test of Jayapala beejachoorna:**

| Sl. no | Identification of animals | Desired dose (according to AOT) | Body weight(gms ) | Calculated dose |
|---|---|---|---|---|
| 1 | Head | 175mg/kg | 230 | 0.8 |
| 2 | Neck | 550mg/kg | 248 | 2.7 |
| 3 | Back | 2000mg/kg | 240 | 2.64 |
| 4 | Base of the tail | 2000mg/kg | 230 | 3.06 |
| 5 | No mark | 2000mg/kg | 216 | 2.88 |

Rats were housed in each cage of poly propylene with stainless steel tops used as bedding material and were changed every morning.They were exposed to 12 hours light and 12 hours dark cycle with the relative humidity of 50 to70 % and the ambient temperature was 22 ± 03c.Amruth brand rat pallet feed supplied by PranavAgro Ltd was provided throughout the study period except on previous night of dosing that is (overnight) fasting before dosing. The drinking water was given in polypropylene bottles with stainless steel sipper tube.

**Preparation of test formulation for administration:**
The test drug was jayapala seed given along with gamma César used as vehicle. The test drug was made into fine suspensions in vehicle with suitable concentration. All the animals were dosed with constant dose volume (1ml/100 body weight)175mg/kg, 550mg/kg, 2000mg/kg test substance.The drug schedule was single dose per animaland the test formulation was administered through oral route at different dose levels to respective animal through oral feeding needle on to disposable syringe. The dose fixation was done according to the AOT Software.

**OBSERVATION:**

**Examination of physical and behavioural changes:**
The animal was observed continuously for 4 hours after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of the every hour the animal was individually exposed to open arena for recording the behavioural changes like increased or decreased motor activity, convulsion, straub's reaction, muscle spasm, catatonia, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing, mode of respiration, changes in skin colour, exitus, CNS depression- hypo activity, passivity, relaxation, ataxia, narcosis, etc.

**RESULT:**

**Physical and behavioural examination:**
There were no physical and behavioural changes- except observation of mild decreased motor activity, diarrhoea, straub's reaction at 2000mg/kg in all the treated animals on day one at 1, 2, 3, 4 hours intervals after dosing and there after once daily for 14 consecutive days. Thus the data obtained from the study on single dose administration of Jayapala oral administration up to 14 days of observation period did not result in any physical and behavioural changes.

**Mortality:**
All the animals were observed at 1, 2, 3,4,24h after dosing and there after daily once for mortality during the entire period of the study(i.e. 14 days).

**CONCLUSION:**
Based on the observations of the acute toxicity study, the LD 50 value of the test drug was found to be more than 2000 mg/kg.

**Phase 2 – Study of antidote effect:**

Test drug: Nimbuswarasa

Dose selection:Dose for rats:

1) **Jayapala: From AOT study**
   LD50 = 2000mg/kg, then 1/5$^{th}$ of LD50 is 400mg/kg
2) **Nimbuswarasa:For TED**
   Human dose X 0.018 for rat weighing 200g, i.e. 24 x 0.018 x 5= 2.16 ml/kg,
   i.e. 0.00216 ml/g
3) **Nimbuswarasa: For TED X 2**
   48x 0.018 x 5= 4.32 ml/kgi.e. 0.00432 ml/g

**Route of drug administration**:
The drugs were administered by oral route with the help of feeding tube for a period of 28 days.

**Statistical analysis**:
All the values were expressed as MEAN± SEM (standard error of mean). The data were analysed by one way ANOVA followed by Dunnet'smultiple't' test. A level of $P<0.05$ was considered as statistically significant. Level of significance was noted and interpreted accordingly using the Graph pad Instatsoftware.

**Grouping**:
The rats were grouped into 4, with 6 animals in each group.
Group I - Vehicle Control
Group II - Jayapala seed ($1/5^{th}$ of $LD_{50}$ As per requirement)
Group III -Jayapala seed + NimbuSwarasa in TED found using Paget &Barnes
GroupIV - Jayapala seed + NimbuSwarasa in double the TED

**Drug preparation**: Jayapala beeja churna is taken in the weight of 750mg mixed with 25mg of gum acacia- in 10ml of tap water as a stock solution for each group and required amount as calculated by the dose calculation was administered to the rats. For Jayapala with Nimbuswarasa(TED) group and Jayapala with Nimbuswarasa TEDX2 group, to both grouparound20ml of Nimbuswarasa was prepared and the required dose was administered to the rats by the oral route with the help of syringe.

**Drug administration**:
Control and test drugs were administered for 28 days including experiment day in the morning session between 9-10 AM orally.

**EXPERIMENTAL PROTOCOL:**
Animals were kept on acclimatization for 7days. The test formulation was administered orally once a day for 28 consecutive days. Thefollowing parameters were assessed weekly:
Food and water intake
Body weight gain.
Wet faecal matter weight
Dry faecal matter weight
Faecal water content (Faecal wet wt.– Faecal dry wt.)
Food conversion ratio (Food intake / faecal dry weight)

**RESULTS AND DISCUSSION:**

**Discussion on the rationale behind the dose of the antidote- Nimbuswarasa:**
References regarding the use of nimbuswarasa as an antidote in jayapala poisoning is available in the later text books. But its dose is not mentioned anywhere. Usually, in case of acute poisoning, antidotes are administered in repeated doses until the symptoms are under control. In the present study, a sub acute study was planned where in the acute poisoning symptoms of jayapala toxicity was not so evident. Moreover the form of the antidote used was phala rasa which is equalent to the form of swarasa. This swarasa is niragni siddha. Hence, the dose of niragni siddha swarasakalpana as mentioned in the samhitas was selected as the dose of the antidote.( ½pala =24ml).[6] This dose was converted to the rat dose and the study planned with TED AND 2xTED dose.

**Table2:Body weight changes in different groups:**

| **Groups** | **Body weight changes in different groups:** | | | |
|---|---|---|---|---|
| | $7^{th}$ Day | $14^{th}$ Day | $21^{st}$ Day | $28^{th}$ Day |
| Jayapala seed | NSD | NSD | NSD | **SD** |
| Jayapala seed with nimbuswarasa(TED) | NSI | NSI | NSI | NSI |
| Jayapala seed with nimbuswarasa (TED x 2) | NSI | NSI | **SI** | **SI** |

As could be observed that administration of Jayapala lead to gradual decrease in body weight gain. Body weight has been increased in a non-significant manner in the test group (2) when compared to the test group (1) on 7th, 14th, 21st and 28th day which is statistically non-significant. Body weight has been increased significant manner on 7th, 14th day in the test group (3) when compared to the test group (1) which is statistically non-significant. On 21st and 28thday it has been observed that the body weight is increased when compared to the test group(1) which is statistically significant.

When the experimental rats are fed with their normal diet such as rat pellet, weekly a proportionate amount of increase in their body weight is expected. The rate at which the rats attain that weight gain is considered as increasing gradient of the body weight. The data presented above clearly shows that administration of Jayapala causes decrease in body weight gain. Body weight gain decrease might be due to decreased food intake or decreased absorption of the nutrients. This effect of Jayapala was non-significantly reversed in Jayapala seed with Nimbuswarasa(TED) and significantly in Jayapala seed with Nimbuswarasa(TED) x 2. This shows dose dependent reversal of the adverse effect of Jayapala by the anti-dote administered. This can be considered as the major evidence for the efficacy of the anti-dote.

**Table 3: Effect on food intake:**

| **Groups** | **Effect on food intake** | | | |
|---|---|---|---|---|
| | **7th Day** | **14th Day** | **21st Day** | **28th Day** |
| Jayapala seed | **SD** | **SD** | NSD | NSD |
| Jayapala seed with nimbuswarasa(TED) | **SI** | NSI | NSI | NSI |
| Jayapala seed with nimbuswarasa(TED x 2) | NSD | NSI | **SI** | NSI |

Administration of Jayapala leads to significant decrease in food intake till 14thday and then reached statistically non-significant level by 28th day. The reason may be instability in the digestive tract leading to disturbance and decreased food consumption. In Jayapala seed with Nimbuswarasa(TED) group significant increase in food intake was observed on 7th day and the tendency continued till 28th though albeit in a reduced manner. In Jayapala seed with Nimbuswarasa (TED) x 2 on 7th day non-significant decrease was observed, non-significant increase was observed on 14th and 28th day of administration and significant increase was observed on 21st day. The above activity profile is in conformity with the expectation that Jayapala due to GI tract irritation may interfere with the food absorption and along with this effect because of irritation food consumption may be reduced. This is reversed by the administration of antidote – Nimbuswarasa- thus again providing an un-equivocal evidence for the effectiveness of the anti-dote. This can be considered as an experimental evidence for clinical efficacy of the anti-dote.

Here in the test group(2) when prolonged use of Jayapala seed followed with Nimbuswarasa as an antidote there was increase in food conversion ratio due to Nimbuswarasahaving the property of pachan, deepana and Agnivardakaguna[7]. Might be for this reason the food intake was increased.

**Table 4: Effect on faecal weight changes:**

| **Groups** | **Effect on faecal wet weight** | | | |
|---|---|---|---|---|
| | **7th Day** | **14th Day** | **21st Day** | **28th Day** |
| Jayapala seed | NSI | NSD | **SD** | NSD |
| Jayapala seed with nimbuswarasa(TED) | NSD | NSD | **SI** | NSI |
| Jayapala seed with nimbuswarasa(TED) x 2 | NSD | NSI | NSD | **SI** |

Administration of Jayapala caused non-significant increase in wet faecal weight on 7thday, non-significant decrease was observed on 14th day and significant decrease on 21st day and again non-significant decrease by 28th day. Wet weight increase may be due to decreased absorption of water and increased undigested material. As could be observed from the results summary the antidote at TED dose produced opposite effect to the one observed in only jayapala treated group. The significant decrease observed on 21st day was antagonized in a significant manner and significant increase was observed. In TED x 2 group also similar effect was observed- the only difference being the reversal was significant with respect to 28th day output but not for 21st day output. This activity profile can be considered to be indicative of the anti-dote effect of the test drug against Jayapala induced effects on GI tract.

**Table5: Effect on faecal dry weight:**

| Groups | Effect on faecal dry weight | | | |
|---|---|---|---|---|
| | **7th Day** | **14th Day** | **21st Day** | **28th Day** |
| Jayapala seed | NSI | NSD | NSI | **SD** |
| Jayapala seed with nimbuswarasa(TED) | **SI** | NSD | **SD** | NSI |
| Jayapala seed with nimbuswarasa (TED x 2) | NSD | NSI | NSD | **SI** |

Administration of Jayapala caused non-significant increase in dry faecal weight on 7th and 21st days; non-significant decrease was observed on 14th day and significant decrease on by 28th day. Dry weight decrease may be indicative of more of watery composition in the formed stools rather than solids; water content gets evaporated due to drying leading to decreased weight. This can be suggestive of watery diarrhoea to Jayapala administration. In anti-dote TED dose given group perfect antagonism was not observed but at higher dose significant reversal and significant increase in dry weight was observed in comparison to Jayapala alone group. This can be taken as another definitive evidence for the Anti-dote effect of nimbu rasa.

The Jayapala due to its intestinal motility enhancing activity might have increased de-hydration by reducing this effect. Nimburasa might have increased the consistency of the stools leading to increased wet and dry weights.

**Table 6: Effect on faecal water:**

| Groups | Effect on faecal water | | | |
|---|---|---|---|---|
| | **7th Day** | **14th Day** | **21st Day** | **28th Day** |
| Jayapala seed | NSI | NSD | **SI** | **SI** |
| Jayapala seed with nimbuswarasa(TED) | NSI | NSD | **SD** | **SD** |
| Jayapala seed with nimbuswarasa (TED x 2) | **SD** | **SD** | **SD** | NSD |

Administration of Jayapala produced significant increase in faecal water by 21st day and it continued till 28th day. This again corroborates the suggestion made under faecal output discussion. This confirms the watery stool formation enhancing effect of Jayapala. This effect was significantly reversed with late onset with low dose anti-dote and with higher dose anti-dote the reversal was observed by 7th day itself indicating early onset. This activity profile can be considered as another good evidence for the Anti-dote activity of nimbuswarasa. The increase in the faecal water content may be due to decreased absorption of water along with increased intestinal motility.

**Table 7: Effect on food conversion ratio:**

| Groups | Effect on food conversion ratio | | | |
|---|---|---|---|---|
| | **7th Day** | **14th Day** | **21st Day** | **28th Day** |
| Jayapala seed | **SD** | NSD | NSD | NSI |
| Jayapala seed with nimbuswarasa(TED) | NSI | NSI | **SI** | NSI |
| Jayapala seed with nimbuswarasa (TED x 2) | **SI** | NSI | **SI** | NSI |

Administration of Jayapala lead to significant decrease in food conversion ratio by 7th day, this get converted to non-significant by 14th and 21st day and non-significant increase was observed by 28th day. In TED dose anti-dote given group non-significant reversal was observed on 7th and 14th day and the intensity reached significant level by 21st day and into non-significant increase by 28th day. At higher dose level significant reversal was observed on 7th day and 21st day. By 28th day there was no difference between Jayapala and anti-dote given groups. Thus confirming the pattern observed with other parameters the above activity profile can be considered as an un-equivocal evidence and support the anti-dote activity of nimbu rasa.

Decreased food conversion ratio is indicative of decreased digestion and assimilation of the food material in to body components. The decrease may be due to decreased food intake probably caused by irritant property of the Jayapala. Further food conversion ratio indicates the weight of food required to produce a unit gain in the live weight of an animal. From ayurvedic point of view the observed decrease may be due to agnimandyadue to attenuation of thejataragnifunction or intensity- leading to incomplete digestion of the food. Because of incomplete digestion food assimilation in to dhatusmay be incomplete. Nimbu rasa has significant anti-dote effect against this effect of Jayapala. Nimbuswarasa having the property of pachan, dipana and Agnivardakaguna might enhance metabolism leading to better food conversion. One interesting finding is that the food conversion ratio decrease was found to be attenuated and reached almost normal level by 28th day in Jayapala group. This might be due to the oaksathmya (Adjustment to particular diet) effect for prolonged use of Jayapala seed .

**Probable mode of action of nimbuswarasa on jayapala induced toxicity:**

Croton tigliumisused as a cathartic in Ayurvedic system of indigenous medicine. Seeds, leaves, bark and root are said to be used in traditional medicine for various ailments. Phytochemical studies reported earlier show that the seeds contain a fatty fixed oil, the croton oil which is composed of crotonoleic acid, the active principle,tiglic acid or methyl crotonic acid, crotonal, a non-purgative fraction, several volatile acids and fatty acids. Crotonoleic acid is a mixture of croton resin with inactive fatty acids. The activity ofcroton oil as a vesicant externally and purgative internally is attributed to thepresence of crotonoleic acid which is said to occur in free state. It is freely soluble inalcohol and in combination as a glyceridethis free acid is a powerful irritant to skinand intestine.

Crotonoleic acid which is a mixture of croton resin with inactive fatty acids may act as a powerful irritant to the intestinal mucosa and thereby causing enteropoolingeffect. Previous reports showed that the essential oil of Croton tiglium had purgative, analgesic, antimicrobial, and inflammatory properties.[8]Histology changes induced by croton tiglium seed oil viewed using electron microscope depicted some swollen epithelial cells, many pyknotic nuclei, congested intramucosal and submucosal small vessels and many infiltrated lymphocytes in mucosa of jejunum and colon after 2 weeks of administration[9].

Considering the action of nimbuswarasa on jayapala, researches hypothesize that limonoids would involve in inflammatory pathway via modulating p38 MAP kinase activity at various extent in smooth muscle cells. Results demonstrated that the different functional groups containing limonoids had differential effects on the p38 MAP kinase activity.p38 MAP kinase are responsive to stress stimuli like osmotic shock as created by jayapala.[10]Above research concludes that Nimbuswarasa(containing limonoids) decreases p38 MAP kinase activity which is therefore sort for possible therapeutic effect on the inflammatory changes in the gut created by administration of jayapala.

While the data presented in the changes in body weight are observed, it is evident that administration of Jayapala has caused decrease in body weight gain. Body weight gain decrease might be due to decreased food intake or decreased absorption of the nutrients resulted by the inflammatory pathology which was also exhibited in the food conversion ratio which showed statistically significant decrease.

In the Jayapala administered group, significant increase in faecal water content was noticed which is due to its purgative effect.On the other hand, in both the antidote administered groups the above changes were reversed.

Apart from this,the decreased body weight gain was reversed in both the antidote administered groups. Decreased food conversion ratio is indicative of decreased digestion and assimilation of the food material into body components. Nimbuswarasa administered groups had significant antidote effect against this effect of Jayapala.

As regards to the faecal water content, this effect was significantly reversed with late onset with low dose anti-dote and with higher dose anti-dote the reversal was observed by 7th day itself.

CONCLUSION:

Based on the above data, it can be concluded that probably nimbuswarasaacts as a physiological antidote against jayapala, acting on the tissues of the body and producing symptoms exactly opposite to those caused by Jayapala in sub acute toxicity.

**LIMITATIONS OF THE STUDY:**

This was just a pilot study where in only the parameters related to the GIT of rats were studied. The hypothesis of this pilot study that nimbuswarasa acts as an antidote for jayapala seed induced toxicity stays strongly positive and based on the positive results of this pilot study, a detailed protocol may be planned for the assessment of the antidotal activity of nimbuswarasa against Jayapala seed induced toxicity involving the assessment of different haematological, biochemical parameters along with the ponderal changes(after sacrifice) in different organs in experimental animals.

**ACKNOWLEDGEMENT:**

The author acknowledges the effort of Mr. RAVI. M M.Sc. (Med. Pharmacology) and Dr.Mouneshwari for conducting this pilot study.

**REFERENCES:**

**[1]** PanditKashinathShastry, Rasa Tarangini, Taranga 24/ 6, Edition: 16, 2004, MotilalBanarasidas Publications, P-648.

**[2]** PanditKashinathShastry, Rasa Tarangini, Taranga 24/163 Edition: 16, 2004, MotilalBanarasidas Publications, P: 375.

**[3]** Sri Bhavamishra. Bhavaprakasha, Pu.Kha.3Guduchyadivarga, with 'Vidyotini' Hindi commentary, edited by Sri BrahmasankaraMisra and Sri RupalaljiVaisya, Varanasi; Choukamba Sanskrit bhavana p-401-402.
**[4]** Agnivesha. "CharakaSamhita", Chikitsasthana 23/4, Revised by Charaka and Dridhabala with the Ayurveda Dipika commentary of Chakrapanidatta, edited by AcharyaYadhavjiTrikamaji. Varanasi: Chaukhambha Sanskrit Sansthan, Ed. Reprint, 2006, P:470.
**[5]** BapalalG.Vaidya; NighantuAdarsha; ChaukhambaBharati Academy; Varanasi; Vol.2; 2009; p-44.
**[6]** PanditParashurama; SharangadharaSamhita; Prathamkanda 7/169, ChoukambaOrientalia; Varanasi; 7th edition 2010; p 123.
**[7]** Agnivesha. "CharakaSamhita", Chikitsasthana 23/4-6, Revised by Charaka and Dridhabala with the Ayurveda Dipika commentary of Chakrapanidatta, edited by AcharyaYadhavjiTrikamaji. Varanasi: Chaukhambha Sanskrit Sansthan, Ed. Reprint, 2006, P:570-571.
**[8]** Kalyan K. Banerjee and A. Sen.1983.Glycolipid-dependent agglutination of liposomes by Croton tigliumlectin.Biochem. J,5(6):418-422.
**[9]** Thiago M. Moraesa, HélioKushimaa, Fábio C. Moleiroa, Raquel C. Santosa et al. 2009.Effects of limonene and essential oil from Citrus aurantium on gastric mucosa: Role of prostaglandins and gastric mucus secretion. Chemico-Biological Interactions,180, (3): 499–505.
**[10]** Jinhee Kim, Guddadarangavvanahally K. Jayaprakasha, MariappanMuthuchamy, Bhimanagouda S. Patil . 2011. Structure–function relationships of citrus limonoids on p38 MAP kinase activity in human aortic smooth muscle cells. European Journal of Pharmacology, 670(1): 44–49.