A comparative acute toxicity evaluation of raw and classically processed rhizomes of Vacha (Acorus calamus Linn.)

Savitha D Bhat1, B K Ashok2*, Rabinarayan Acharya1 and B Ravishankar3

1Department of Dravyaguna, 2Pharmacology Laboratory, Institute for Post Graduate Teaching & Research in Ayurveda (IPGT & RA), Gujarat Ayurved University, Jamnagar-361 008, Gujarat, India
3SDM Research Centre for Ayurveda and Allied Sciences, Kuthpady, Udupi-574 118, Karnataka, India

Received 9 November 2011; Accepted 28 August 2012

The rhizomes of Vacha (Acorus calamus Linn.) have been used in Ayurvedic medicine for the treatment of various ailments. Ayurvedic pharmacopoeia of India has advocated Shodhana (Purification) procedure to be done prior to its use. Hence in the present study comparative oral acute toxicity of raw and classically processed Vacha rhizome was undertaken to provide safety profile on acute administration. The rhizomes were collected from wild source and Shodhana procedure was done as mentioned in the Ayurvedic texts. Acute toxicity test was evaluated as per OECD 425 guidelines with 2000mg/kg as limit test. Test drugs were administered orally to overnight fasted female rats and detailed behavioural profiles were recorded throughout the study. Change in body weight, haematological and biochemical parameters were carried out on 14th day. At 2000 mg/kg dose both raw and classically processed Vacha did not produce any observable toxic effects and all animals survived 14 days of observation.

Keywords: Acorus calamus, Acute toxicity, Albino rats, Rhizome, Shodhana, Vacha.

IPC code; Int. cl. (2011.01) — A61K 36/00

Introduction

Acorus calamus Linn. (Family: Acoraceae) known as Vacha in Ayurveda has been used in the Indian medicine for thousands of years for its beneficial role as brain tonic (Medhya), appetizer (Deepaka), emetic (Vamaka), etc.1 It is commonly used to treat diarrhoea, epilepsy, oedema, headache, eye disorders, colic, piles, insomnia, loss of memory, rheumatism, toothache and respiratory ailments with proven antioxidant, nootropic, sedative and hypnotic, anti-fungal, anti-ulcer, anthelmintic, anti-inflammatory and analgesic activities.2-11 Most of these functions are attributed to the aromatic oil present in the rhizome12. The major active principles present in the A. calamus oil are α- and β-asarone, calamene, calamenenol, calameone, α-pinene, camphene, eugenol, etc.13-15, among which β-asarone is one of the most important one and has been the subject of considerable studies12. Further, most of the pharmacological actions of A. calamus are attributed to β-asarone16, but was reported to be carcinogenic in rodents and potentially genotoxic17.

*Correspondent author:
E-mail: drashokbeekay@yahoo.co.in;
Phone: 09429333816 (Mob.)

Ayurvedic classics have emphasized various methods of Shodhana (purificatory procedures) to overcome the undesired effects from various poisonous and non-poisons drugs18. Even though Vacha does not come under poisonous drug category, several Ayurvedic texts and Ayurvedic pharmacopoeia of India have recommended Shodhana for Vacha rhizome using Gomutra, Mundī Kwatha (Decoction prepared from whole plant of Sphaeranthus indicus Linn.), Panchapallava katham (Decoction prepared from a group of five leaves, viz. Amra (Mangifera indica Linn.), Jambu (Syzygium cumini Skeels.), Kapitha (Feronia limonia Linn.), Bilwa (Aegle marmelos Corr.) and Beejapuraka (Citrus medica Linn.) and Gandhodaka (Decoction prepared from a group of aromatic herbs, viz. Twak (Cinnamomum zeylanicum Breyn.), Patraka (Cinnamomum tamala Nees.), Ushira (Vetiveria zizanoides Linn.), Musta (Cyperus rotundus Linn.), Bala mula (Sida cordifolia Linn.), Kushta (Saussurea lappa C. B. Clarke)20. The reason behind this Shodhana procedure though clearly not mentioned by any of the texts, may be is to reduce the strong carminative and emetic actions of rhizome.
However, it may not be ruled out the fact that ancient Indian physicians were well aware of its toxic effects like carcinogenicity and mutagenecity and to overcome these effects they might have recommended the Shodhana procedure. Also, till date no acute toxicity profile is available for raw Vacha and purified Vacha. Hence, in this study Vacha rhizomes were subjected to classical purificatory procedure and a comparative acute toxicity evaluation of raw and classically processed rhizomes were undertaken to provide acute toxicity profile.

Materials and Methods

Plant materials

Fully matured rhizomes of A. calamus were collected from its natural habitat in a particular marshy pond area in the forest regions of Yelagiri hills, Tamil Nadu in the month of November. The plant material was identified and authenticated by pharmacognosist of our institute. The rhizomes were then rubbed by a gunny cloth to remove the roots and old leaf scars. Further, they were washed thoroughly in water to remove the soil adhered to it and then dried in partial shade. The rhizomes were cut uniformly (Each in to about 2 cm pieces) and divided into parts. The first part was coded as sample RV (Raw Vacha) and the second part was utilized for Shodhana as described in Ayurvedic text Chakradatta. The process involved successive boiling (at 105°C) of Vacha rhizomes in Gomutra, Mundi kwatha and Panchapallava kwatha for three hours. It was later fomented with Gandhodaka for one hour. After Shodhana procedure the rhizomes were shade dried for 12 days and marked as sample SV. Then both RV and SV were powdered (Mesh 80) and the obtained powder was utilized for preliminary phytochemical analysis and acute toxicity evaluation.

Animals

Nineteen healthy, 10-12 week old, female, nulliparous and non-pregnant Charles Foster strain albino rats (Rattus novergicus) weighing 130 to 180 g were obtained from animal house (Registration No. 548/2002/CPCSEA) attached to Pharmacology laboratory. Six animals were housed in each cage made up of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. The animals were acclimatized for seven days before commencement of the experiment in standard laboratory conditions 12 ± 01 h day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Ltd. For their drinking purpose tap water ad libitum was used. Protocol used in this study for the use of animals was approved by the institutional animal ethics committee (Approval number; IAEC 06/09-11/PhD/08) and the care of animals was taken as per the CPCSEA guidelines.

Acute oral toxicity study

Acute oral toxicity study for both RV and SV samples were carried out as per OECD guideline 425 with 2000 mg/kg as limit test. Out of nineteen animals, five animals were allotted to control group to get normal values like body weight, behavioural profile, haematology, etc. In test drug groups (RV and SV), single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence 175, 550 and 2000 mg/kg (because no estimate of the substance’s lethality was available, dosing was initiated at 175 mg/kg) as recommended in OECD Guidelines 425. Food, but not water was withheld for overnight before the experiment and further 2 hours after administration of test drug. As there was no mortality was observed even at 2000 mg/kg, additional 4 more animals were dosed with 2000 mg/kg and observed for 14 days with different parameters. Thus, in RV and SV group seven animals each were used.

Examination of physical and behavioural changes

The animals were observed continuously for 6 h after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of every hour the animals were individually exposed to open arena for recording the behavioural changes like increased or decreased motor activity, convulsions, straub’s reactions, 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.

Mortality

All the animals were observed at ½, 1, 2, 3, 4, 5, 6 and 24 h after dosing and there after daily once for mortality during the entire period of the study (14 days).
Body weight
The body weight of each animal was recorded just prior to dosing, on day one, 7th and 14th day.

Haematological and serum biochemical parameters
On 14th day blood was collected by puncturing supra-orbital plexus by capillary tubes under ether anaesthesia for estimation of haematological and biochemical parameters. To estimate haematological parameters 0.08 ml blood was mixed with 0.02 ml of EDTA (33.33 mg/ml) and fed to the auto analyzer (ERBA CHEM-5, Trans Asia). The parameters measured were: total WBC, neutrophil percentage, lymphocyte percentage, eosinophil percentage, monocyte percentage, haemoglobin content, packed cell volume, total RBC, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). For estimation of biochemical parameters, serum was separated from collected blood and requisite quantity of serum was fed to the auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai) which was automatically drawn in to the instrument for estimating different parameters. Biochemical parameters like blood sugar serum total cholesterol serum triglyceride, serum urea, serum creatinine, serum alkaline phosphatase, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase, total protein, serum albumin and serum globulin, serum total bilirubin, direct bilirubin and uric acid were estimated.

Statistical analysis
The results were presented as Mean ± SEM for six rats in each group. Statistical comparisons were performed by unpaired student’s t-test for all the treated groups with the level of significance set at P<0.05.

Results
Both raw and classically processed Vacha samples at a dose of 2000 mg/kg body weight did not produce any mortality and other toxic effects during entire duration of study and all animals survived till 14 days of observation period. However, only features of CNS depression (mild sedation) were observed in both the samples administered groups at 2-6 h intervals after drug administration.

Normal progressive body weight gain was observed in normal control rats in the period of 14 days (Table 1). In both Vacha samples administered groups also an apparent increase in body weight was observed, among them the weight gain occurred in RV treated group found to be statistically significant in comparison to control group.

Among the haematological parameters, significant decrease in neutrophil percentage (P<0.001) and significant increase in lymphocyte percentage (P<0.001) was observed in both RV and SV administered groups in comparison to control group (Table 2). Out of thirteen biochemical parameters studied, none of the parameter was affected to a significant extent in comparison to control group (Table 3).

Discussion
The drugs irrespective of their origin (synthetic, animal, plant or mineral) intended to be used therapeutically, should be subjected to extensive toxicity evaluation before they are considered safe for use in the human beings. This is important because incomplete knowledge about the toxicity profile of a putative drug will entail certain amount of risk to the recipient.

Prior to the toxicity evaluation, various physicochemical parameters, qualitative tests for different functional groups, heavy metal analysis and pesticide residue were carried out by referring standard procedures. Phytochemical tests revealed the presence of carbohydrates, alkaloid, flavonoid, tannin, glycoside and terpenoid/sterols. Heavy metals and pesticide residue were not detected in the samples indicating its safety for further use in therapeutics.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (per kg body weight)</th>
<th>Initial body weight</th>
<th>7th day</th>
<th>14th day</th>
<th>Actual change</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>144.66 ± 2.16</td>
<td>156.00 ± 3.22</td>
<td>165.33 ± 2.56</td>
<td>20.66 ± 0.214</td>
<td>--</td>
</tr>
<tr>
<td>Raw Vacha</td>
<td>2g</td>
<td>152.00 ± 6.53</td>
<td>169.33 ± 5.33</td>
<td>182.00 ± 6.15</td>
<td>30.00 ± 0.89*</td>
<td>45.20†</td>
</tr>
<tr>
<td>Shodhita Vacha</td>
<td>2g</td>
<td>157.00 ± 5.92</td>
<td>168.00 ± 6.08</td>
<td>180.00 ± 6.04</td>
<td>23.00 ± 0.246</td>
<td>11.32†</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM, †-Increase, *P<0.05 (n=5 in each group)
Guidelines 425 was conducted using a single sex (Preferably females) in order to reduce variability and as a means of minimising the number of animals used. This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between the sexes but, in those cases where differences were observed, females were generally slightly more sensitive.37 Hence in present study acute toxicity was evaluated in female rats.

Body weight indicates health status of living beings. Changes in body weight are an important factor to monitor the health of an animal. Loss of body weight is frequently the first indicator of the onset of an adverse effect. The dose, at which body weight loss is by 10% or more, is considered to be a toxic dose, irrespective of whether or not it is accompanied by any other changes38,39. In present study in test drug administered groups like control group, gain in body weight was observed and the magnitude of body weight gain was comparatively high in raw Vacha treated group. Further, at 2000 mg/kg, sample did not produce any observable toxic effects during entire duration of study and all animals survived 14 days of observation. Both the samples did not affect the serum biochemical parameters to significant extent. The only matter of concern in this study which can be taken as toxic effect is increase in the lymphocyte percentage and decrease in neutrophil percentage; however exact cause for observed changes is a matter of further research.
Conclusion
From the present study it can be concluded that, oral administration of rhizomes of *Vacha* in the form of *Churna* (powder) as acute dose even at 2000 mg/kg in albino rats is relatively safe. As the rhizomes (but not the isolated essential oil) have been used in India for thousands of years without reports of cancer which suggests that using the whole herb is safe. However, further toxicological evaluation like chronic toxicity studies, etc. are required to provide complete safety profile.

Acknowledgement
Authors are thankful to Prof. M.S. Baghel, Director, IPGT & RA, Gujarat Ayurved University, Jamnagar for the constant support and providing financial support and facilities to carry out this study.

References