A Comparative Pharmacological Evaluation of Antianxiety Activity of Raw and Traditionally Shodhita (Processed) Rhizome of Vacha (Acorus calamus L.)

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ABSTRACT
Background: Vacha (Acorus calamus L.; Acoraceae), one of the well-known drug of Ayurvedic pharmacopeia, is highlighted for its Medhya (brain tonic), Sanjnahapana (restores lost consciousness), Deepana (appetizer), Pachana (digestive), etc., properties and hence used extensively in therapeutics. Ayurvedic pharmacopeias like Chakradatta, Bhaishajya Ratnavali, and Ayurvedic Pharmacopoeia of India (API) have recommended Shodhana (processing) of Vacha using certain media like Gomutra, Mundi Kwatha, Gandhodaka, etc. It has been reported by us that subjecting to Shodhana is not only safe to use but also enhances the therapeutic activity of Vacha.

Aim: To assess the antianxiety activity of raw and shodhita (processed) Vacha rhizomes in different experimental animal models.

Materials and methods: Swiss albino mice of either sex weighing 24 ± 4 g, of either sex, were administered with raw and shodhita Vacha (16 mg/kg body weight) along with distilled water. Diazepam (2 mg/kg body weight) was used as a standard drug.

Results and conclusion: Pretreatment with both raw and classically processed Vacha samples exhibited significant antianxiety activity; among them, the observed activity in shodhita Vacha was found to be better. The present study confirms the antianxiety activity of raw and shodhita Vacha. But when subjected to the traditional Shodhana procedure, the efficacy of Vacha rhizomes get enhanced.

Keywords: Acorus calamus, Anxiety, Diazepam, Plus maze, Shodhana, Vacha.


INTRODUCTION
Vacha (Acorus calamus L.; Acoraceae), one of the distinguished drug of Ayurvedic pharmacopeia, was reported by Charaka¹ and Sushruta² under Lekhaneeya, Triptighna, Arshoghna, etc., and Pippalyadi, Vachadi, etc., group of drugs, respectively. This drug is used widely in Ayurvedic therapeutics due to its Deepana (appetizer), Pachana (digestive), Medhya (brain tonic), Sanjnahapana (restores lost consciousness), Kanthy (good for throat), Vedanapathan (anodyne), Vamaka (emetic), etc., properties.³

Shodhana of Vacha using different media like Gomutra (cow’s urine), Gandhodaka (decoction prepared of six aromatic herbs), Mundi kwatha (decoction of Sphaeranthus indicus L.), etc., has been recommended by Ayurvedic pharmacopeias like Chakradatta⁴ and Bhaishajya Ratnavali.⁵ The Ayurvedic Pharmacopoeia of India (API) and the ISM directory have supported the internal administration of Vacha after Shodhana (processing) in therapeutics.⁶,⁷ In Karnataka and Kerala, there are certain folklore methods in practice for the Shodhana of Vacha.⁸ The classical quotes also reveals that Shodhana not only refers to purification procedures but also to different Samskaras, through which MUNDI KWATHA

Plant Materials
The fully matured A. calamus rhizomes were collected, in the month of November, from the forest regions of Yelagiri hills, Tamil Nadu, one of their natural habitat, as per the Ayurvedic criteria for collecting rhizomes⁹ and was authenticated by the pharmacognost of IPGT and RA, Gujarat Ayurved University, Jamnagar. The properly washed and partially shade-dried rhizomes were divided into two parts. One part was coded as raw Vacha (RV) and the second part was utilized for Shodhana.

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MATERIALS AND METHODS
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As described in the Ayurvedic text, for *shodhana*, *Vacha* samples were successively boiled in *Gomutra* (cow’s urine), *Mundi* *Kwatha* (decocction prepared from whole plant of *Sphaeranthus indicus* L.), *Panchapallava kwatha* (decocction prepared from a group of five young leaves, i.e., *Amra*, *Jambu*, *Kapittha*, *Bilwa*, and *Bijapuraka*), and *Gandhodaka* (decocction prepared from a group of aromatic herbs [*Twakpati* (leaves of *Cinnamomum zeylanicum Breynt.), *Pathraka* (leaves of *Cinnamomum tamala* N.), *Ushira* (roots of *Vetiveria zizanoides* L.), *Musta* (tubers of *Cyperus rotundus* L.), *Balamuila* (roots of *Sida cordifolia* L., and *Kushtha* (roots of *Saussurea lappa* CB Clarke)]. After completion of the *Shodhana* procedure, the test drug were shade-dried for 12 days and marked as *shodhita Vacha* (*SV*). Both test drug samples, i.e., *RV* and *SV*, were pulverized and sieved through 80 mesh and kept in airtight glass containers for further study.

### Animals

The 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).

Swiss albino mice of either sex weighing 24 ± 4 g were obtained from Institute’s Animal House. The animals were acclimatized for 7 days before commencement of the experiment in standard laboratory conditions: 12 ± 01 hour day-and-night rhythm, maintained at 25 ± 3°C and 50–70% humidity as per the CPCSEA guidelines. Animals were provided with balanced food (Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited) and water *ad libitum*. Before the experiment, the animals were fasted overnight.

### Dose Selection and Schedule

In the present study, the dose of *Vacha* samples (*RV* and *SV*) was fixed at 16 mg/kg/mouse. This dose was calculated by extrapolating the human dose (according to API, 120 mg/day, for *Vacha*) to animals, based on the body surface area ratio, by referring to the standard table of Paget and Barn, for experimental animals. The test drug was suspended in distilled water with suitable concentration depending on body weight of animals and administered orally with the help of a gastric catheter sleeved to a syringe. Diazepam was selected as a standard antianxiety drug (RS) and administered in the help of a gastric catheter sleeved to a syringe. Diazepam was depending on body weight of animals and administered orally with drug was suspended in distilled water with suitable concentration fasted overnight.

Swiss albino mice of either sex were divided into groups of six each. The first group was administered with distilled water and served as control. Second and third groups received RV and SV in calculated doses. The fourth group received diazepam (2 mg/kg) and served as the reference standard group.

### Open-field Behavior Study

The animals were individually exposed to an open-field apparatus 1 hour after drug administration. In this experiment, a specially designed square box of 96 × 96 cm with a side wall of 15 cm height was used as an apparatus. The floor is divided into 36 equal squares. The experiment was carried out by keeping the instrument in a dimly lit and quite area. Each animal was placed at the same corner and allowed to explore the arena for 5 minutes. The parameters recorded were time of exploration, number of squares crossed, number of rearing, freezing time (duration of immobility), and number of fecal pellets expelled.  

#### Antianxiety Activity Using Elevated Plus Maze

Mice were given a single oral dose of the vehicle, test drug, and standard drug 1 hour before they were placed on the elevated plus maze (EPM). The dose administration schedule was adjusted so that each mouse took its turn on the EPM apparatus 1 hour after administration of the dose. To begin a test session, mice were placed on the open arm facing the center of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5-minute test period. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus maze, could invoke maze anxiety.

#### Statistical Analysis

The values were expressed as mean ± SEM (standard error mean). The significance of differences among the groups was assessed using the one-way analysis of variance (ANOVA) and the test followed by the Dunnett’s test. *p* values less than 0.05 were considered as significant.

### Results

Both *RV* and *SV* significantly reduced the latency of the onset of exploration initiation as well as number of rearing (Table 1). The observed effect is almost equal to that of the diazepam-treated group. There is a nonsignificant increase in the number of squares crossed and decrease in the number of fecal pellets expelled by both the drugs (*RV* and *SV*). Total freezing time was significantly decreased by pretreatment with *RV*, while *SV* decreased this parameter only to a nonsignificant extent.

Administration of *RV* and diazepam significantly increased all the tested parameters, i.e., the latency of first entry, time spent in the open arm, and number of entries to the open arm in comparison to the control group (Table 2). Pretreatment with *SV* nonsignificantly reduced the latency of the first entry; however, it significantly increased the time spent in the open arm and number of entries to the open arm in comparison to the control group.

### Discussion

The normal aversion of the animal to an open, brightly lit area is well assessed by the open-field model that examines the anxiety-related behavior. Animals removed from their acclimatized cage and placed in a novel environment express anxiety and fear, by showing
alteration in all or some parameters, such as decrease in ambulation and exploration, and immobilization or freezing due to the augmented autonomic activity.\(^\text{18}\) In this test, locomotor and rearing activities are accepted as indicators for exploratory movements and defecation is regarded as an indicator of emotionality. The animals treated with both RV and SV showed increased motor activity in the open field. The numbers of squares crossed by the drug-treated animals are greater than their control counterpart. The test animal also had significantly lower latency of exploration.

The open-field activity is one of the behavioral assays of drug-induced dopaminergic actions.\(^\text{19}\) Dopamine is mainly implicated in the locomotor and exploratory activity and it has been repeatedly found that decreased activity in central dopaminergic systems of adult animals produced hypoactivity.\(^\text{20}\) The locomotor activity is reduced by dopamine receptor-blocking drugs or electrolytic lesions of ascending dopamine systems. Drugs that enhance dopaminergic transmission produced increased locomotor activity.\(^\text{21}\) Although there was no significant difference between the two samples with respect to the activity profile, SV showed better effect by decreasing the freezing time, which can be taken as one of the good parameter in terms of the antianxiety activity.

The EPM represents one of the most widely used animal models for screening anxiolytics. This is a model that uses the natural fear of rodents to avoid open and elevated places.\(^\text{22}\) As expected, diazepam produced significant increase in time spent in the open arm and the number of entries into the open arms. Diazepam also increased the total number of entries. The increase in occupancy in the open arm and open-arm entries strongly indicate the anxiolytic activity of both RV and SV. The SV was found to be having better anxiolytic effect in comparison to that of RV. This effect might be attributed to the presence of cis-isosasarone as reported earlier\(^\text{23}\) and also by acquiring of some active principles from shodhana dravya, such as gomutra (cow’s urine) and mundi kwatha (decoration of S. indicus L.) during the Shodhana process.

Several partial agonists of 5HT1A receptors have been explored for potential utility both in anxiety disorders and in milder cases of mixed anxiety depression. The 5-HT 2c serotonin receptor is prominent in the limbic forebrain and the cerebral cortex. This receptor subtype has been postulated to be a reasonable therapeutic target for depression or anxiety.\(^\text{24}\) The possibility of test drugs acting on 5-HT receptors cannot be ruled out. The confirmation of this possibility requires further studies.

**CONCLUSION**

The present study confirms the antianxiety activity of raw and shodhita Vacha rhizomes. Further, this study also confirms the enhancement of efficacy of Vacha rhizomes, when subjected to traditional Shodhana procedures.

| Table 2: Effect on mice performance in elevated plus maze |
|-----------------|-----------------|-----------------|-----------------|
| **Groups**      | **Dose (mg/kg)**| **Latency of first entry to closed arm (seconds)** | **Time spent in open arm ** | **Number of entries from closed to open** |
| Control         | QS              | 4.50 ± 0.76     | 20.83 ± 2.97     | 4.16 ± 0.79     |
| RV              | 16              | 8.00 ± 1.52*    | 46.33 ± 6.09*    | 7.83 ± 1.25*    |
| SV              | 16              | 3.66 ± 0.61     | 76.33 ± 7.31**   | 8.33 ± 1.08*    |
| Diazepam        | 2               | 22.66 ± 2.90**  | 99.67 ± 9.00**   | 6.83 ± 0.70*    |

Data: mean ± SEM. *p < 0.05, **p < 0.01

**REFERENCES**

हिंदी सारांश

वच्या (एकरॉस कालामस एल.) के अपरिष्कृत और पारंपरिक रूप से शोधित (प्रोसेसेड) राइजोम की एंटिएंजाइटी गतिविधि का तुलनात्मक फार्माकॉलोजिकल मूल्यांकन

पृष्ठभूमि: वच्या (एकरॉस कालामस एल.,एकरॉसिस) आयुर्वैदिक फर्माकोपिया की एक प्रसिद्ध औषधि है, जिसे अपनी भूमिका (ब्रेन टोनिक), संज्ञानरूप (खोई हुई चौथा वापस साना), दीपम (एपिटाइजर), रागन (ड्राइज्स्टिव) आदि विषेषताओं के लिए जाना जाता है इसलिए इसका विकिरण में व्यापक पैमाने पर प्रयोग किया जाता है। आयुर्वैदिक फर्माकोपिया यथा चक्रवर्तित, भौतिक रत्नावली और भारतीय आयुर्वैदिक फर्माकोपिया (एपीआई) ने कुछ माध्यम यथा गाउंघूर, सूंडी क्वाय, गंधोटक आदि का प्रयोग कर वच्या के शोधन (प्रोसेसिंग) की सिफारिश की। यह रिपोर्ट किया गया कि शोधन ने केवल सुधारित है वर्तमान यह वच्या की उपचारात्मक गतिविधि में भी वृद्धि करता है।

उद्देश्य: विशिष्ट प्रयोगात्मक पषु माउडलों में अपरिष्कृत और शोधित (प्रोसेसेड) वच्या राइजोम की एंटिएंजाइटी गतिविधि का मूल्यांकन करना।

सामग्री और विधियाँ: किसी भी सेक्स की टिस्सू एल्बिनो माइस जिसका वजन 24±4 ग्राम है, को आसुत पानी के साथ अपरिष्कृत और शोधित वच्या (16 मिलीग्राम/ किलोग्राम शरीर भार) दिया गया। ड्राइजेम (2 मिलीग्राम/ किलोग्राम शरीर भार) का प्रयोग मानक औषधि के रूप में किया गया।

परिणाम और निष्कर्ष: उच्च द्रव पूर्व, दोनों अपरिष्कृत और पारंपरिक शोधित वच्या नमूनों में महत्वपूर्ण एंटिएंजाइटी गतिविधि पाई गई, जिसमें, शोधित वच्या में गतिविधि को बेहतर पाया गया। वर्तमान अध्ययन अपरिष्कृत और शोधित वच्या की एंटिएंजाइटी गतिविधि की पुष्टि करता है। परंतु जहां तक पारंपरिक शोधन प्रक्रिया का संबंध है, उसमें वच्या राइजोम की प्रभावकारिता में वृद्धि हुई है।

मुख्य शब्द: एकरॉस कालामस, एंजाइटी, ड्राइजेम, पॉल्स मेज, शोधन, वच्या