RESEARCH ARTICLE

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), *Sanjnasthapana* (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 *Shodana* (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*.

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Introduction

Vacha (*Acorus calamus* L.; Acoraceae), one of the distinguished drug of Ayurvedic pharmacopeia, was reported by Charaka[1] and Sushruta[2] 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), *Sanjnasthapana* (restores lost consciousness), *Kanthya* (good for throat), *Vedanasthapana* (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 *Chakradatta*[4] 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 there is *Gunaantardhana* in the primary *dravya*, rendering it safe as well as obtaining desired qualities in it.[8] This was supported by our previous research reports, where it is found that subjecting to *Shodhana* is not only safe to use but also enhance the therapeutic activity.[9,10] Bhavaprakash Nighantu reports the brain tonic (*Medhya*) effect of its rhizome.[11] In the recent past, its tranquilizing[12] and neuroprotective[13] activities have also been reported. In the present study, a comparative antianxiety activity evaluation of raw and traditionally processed rhizomes was undertaken to know the impact of the classical purificatory procedure on its efficacy, in terms of the antianxiety activity.

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Conflict of interest: None

Materials and Methods

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 rhizomes[14] and was authenticated by the pharmacognosist 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*.

As described in the Ayurvedic text, for *shodhana*, *Vacha* samples were successively boiled in *Gomutra* (cow's urine), *Mundi Kwatha* (decoction prepared from whole plant of *Sphaeranthus indicus* L.), *Panchapallava kwatha* (decoction prepared from a group of five young leaves, i.e., *Amra*, *Jambu*, *Kapittha*, *Bilwa*, *and Bijapuraka*), and *Gandhodaka* (decoction prepared from a group of aromatic herbs [*Twakpatri* (leaves of *Cinnamomum zeylanicum* Breyn.), *Patraka* (leaves of *Cinnamomum tamala* N.), *Ushira* (roots of *Vetiveria zizanoides* L.), *Musta* (tubers of *Cyperus rotundus* L.), *Balamula* (roots of *Sida cordifolia* L., and *Kushta* (roots of *Saussurea lappa* CB Clarke)].[4] 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*[6]) to animals, based on the body surface area ratio, by referring to the standard table of Paget and Barnes,[15] 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 dose of 2 mg/kg.

Swiss albino mice of either sex were divided into four 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.[16]

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.[17] 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

Table 1: Effect of raw *Vacha* and *shodhita Vacha* on the activity profile of mice in the open-field test

Groups	*Dose (mg/kg)*	*Latency of exploration initiation*	*Squares crossed*	*Rearing*	*Freezing time*	*Pellets expelled*
Control	QS	18.67 ± 2.63	79.50 ± 7.97	25.16 ± 2.98	81.00 ± 7.82	2.16 ± 0.87
RV	16	6.16 ± 1.01*	84.16 ± 15.60	17.33 ± 1.33*	38.16 ± 3.79**	1.50 ± 0.42
SV	16	5.83 ± 0.37*	97.66 ± 8.37	17.00 ± 1.21*	56.50 ± 8.83	0.83 ± 0.30
Diazepam	02	6.50 ± 0.99*	84.00 ± 14.08	19.83 ± 2.18	49.50 ± 6.82*	0.66 ± 0.33

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

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. *$p < 0.05$, **$p < 0.01$

alteration in all or some parameters, such as decrease in ambulation and exploration, and immobilization or freezing due to the augmented autonomic activity.[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.[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.[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.[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.[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-isoasarone as reported earlier[23] and also by acquiring of some active principles from *shodhana dravya*, such as *gomutra* (cow's urine) and *Mundi Kwatha* (decoction 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.[24] The possibility of test drugs acting on 5-HT receptors cannot be ruled out. The conformation 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.

References

1. Agnivesha. Samhita C. In: Shastry K, Chaturvedi G, ed. Varanasi: Chaukambha Bharati Academy; 2001. pp. 72, 80, 81, 83, 94 and 791.
2. Sushruta, Samhita S. In: Shastri KA, ed. Varanasi: Chaukhambha Sanskrit Sansthan; 2002. pp. 13, 143, 145 and 147.
3. Kaiyadeva. Kaiyadeva Nighantu, Oushadhi varga/1215–1217. In: Sharma PV, ed. Varanasi: Chaukhambha Orientalia; 2001. pp. 224–225.
4. Chakrapanidatta. Chakradatta. In: Dwivedi R, ed. Varanasi: Chaukamba Sanskrit Samsthan; 2005. p. 155.
5. Govind Das. Bhaishajya Ratnavali. In: Mishra B, ed. vol. 19. Varanasi: Chaukhambha Surabharati Prakashan; 2008. p. 570.
6. Anonymous. The Ayurvedic pharmacopoeia of India, Part 1. 1st ed. vol. 2. New Delhi: Ministry of Health and Family Welfare, Govt. of India; 1999. pp. 168–170.
7. Anonymous. Herb directory, Annual Report: Department of ISM and H. vol. 1(X); 1998. pp. 18–25.
8. Savitha D Bhat, Ashok BK, Rabinarayan Acharya, Exploring the concept of *Vacha (Acorus calamus* Linn.) *Shodhana* in Ayurveda, IJRAP. vol. 3(3); May–June, 2012.
9. Bhat SD, Ashok BK, Acharya R, et al. A comparative acute toxicity evaluation of raw and classically processed rhizomes of *Vacha (Acorus calamus* Linn.). Indian J Nat Prod Res 2012;3(4):506–511.
10. Bhat SD, Ashok BK, Acharya R, et al. Anticonvulsant activity of raw and classically processed *Vacha (Acorus calamus* Linn.) rhizomes. AYU 2012;33(1):119–122. DOI: 10.4103/0974-8520.100328.
11. Bhavamishra. Nighantu B. In: Pandey GS, ed. Varanasi, India: Chaukhambha Bharati Academy; 2006. p. 44.
12. Agarwal SL, Dandiya PC, Sing KP, et al. A note on the preliminary studies of certain pharmacological actions of *Acorus calamus*. J Am Pharm Assoc 1956;45(9):655–656. DOI: 10.1002/jps.3030450921.
13. Shukla PK, Khanna VK, Ali MM. Protective effect of *Acorus calamus* against acrylamide induced neurotoxicity. Phytother Res 2002;16(3):256–260. DOI: 10.1002/ptr.854.
14. Pandit Narahari. Rajanighantu. In: Tripathi I, Varga D, ed. Varanasi: Chowkhamba Krishnadas Academy; 2003. p. 26.
15. Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, ed. Pharmacometrics, vol. 1. New York: Academic Press; 1964. p. 161.
16. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents: I-anti-anxiety agents. Indian J Exp Biol 1997;35(6):565–575.
17. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 1987;92(2):180–185. DOI: 10.1007/BF00177912.
18. Novas ML, Wolfman C, Medina JH, et al. Proconvulsant and anxiogenic effects of n-butyl-h-carboline-3-carboxylate, on endogenous benzodiazepine binding inhibitor from brain. Pharmacol Biochem Behav 1988;30(2):331–336. DOI: 10.1016/0091-3057(88)90463-7.
19. Frussa-Filho R, Palermo-Neto J. Effect of single and long term droperidol administration on open field and stereotyped behaviour of rats. Physiol Behav 1991;50(4):825–830. DOI: 10.1016/0031-9384(91)90024-I.
20. Maj J, Rogoz Z, Skuza G, et al. Repeated trimipramine induces dopamine D2/D3 and alpha 1-adrenergic upregulation. J Neural Transm 1998;105(2–3):329–342. DOI: 10.1007/s007020050062.
21. Carlsson A, Lindquist M, Magnusson T, et al. On the presence of 3 hydroxytyramine in brain. Science 1958;127(3296):471. DOI: 10.1126/science.127.3296.471.
22. Weiss SM, Wadsworth G, Fletcher A, et al. Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. Neurosci Biobehav Rev 1998;23(2):265–271. DOI: 10.1016/S0149-7634(98)00027-X.
23. Cupp MJ. Toxicology and clinical pharmacology of herbal products. Totowa, New Jersey, USA: Humana Press; 2000. pp. 171–175.
24. Gupta V, Bansal P, Kumar S, et al. Therapeutic efficacy of phytochemicals as anti-anxiety-a review. J Pharm Res 2010;3(1):174–179.

हिंदी सारांश

वचा (*एकोरस कालामस* एल.) के अपरिष्कृत और पारंपरिक रूप से *शोधित* (प्रोसेस्ड) राइजोम की एंटिएंक्जाइटी गतिविधि का तुलनात्मक फ़ार्माकॉलोजिकल मूल्यांकन

पृष्ठभूमि: *वचा* (*एकोरस कालामस* एल.,एकोरेसि) आयुर्वेदिक फर्माकोपिया की एक प्रसिद्ध औषधि है, जिसे अपनी *मेध्य* (ब्रेन टॉनिक), *संज्ञास्थापन* (खोई हुई चेतना वापस लाना), *दीपन* (एपिटाइजर), *पाचन* (डाइजेस्टिव) आदि विशेषताओं के लिए जाना जाता है इसलिये इसका चिकित्सा में व्यापक पैमाने पर प्रयोग किया जाता है। आयुर्वेदिक फर्माकोपिया यथा *चक्रदत्त, भैषज्य रत्नावली* और भारतीय आयुर्वेदिक फर्माकोपिया (एपीआई) ने कुछ माध्यम यथा *गौमूत्र, मुंडी क्वाथ, गंधोटक* आदि का प्रयोग कर *वचा* के *शोधन* (प्रोसेसिंग) की सिफारिश की। यह रिपोर्ट किया गया कि *शोधन* न केवल सुरक्षित है बल्कि यह *वचा* की उपचारात्मक गतिविधि में भी वृद्धि करता है।

उद्देश्य: विभिन्न प्रयोगात्मक पशु मॉडलों में अपरिष्कृत और *शोधित* (प्रोसेसेड) *वचा* राइजोम की एंटिएंक्जाइटी गतिविधि का मूल्यांकन करना।

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

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

मुख्य शब्द: *एकोरस कालामस,* एंक्जाइटी, डाइजेपेम, प्लस मेज़, *शोधन, वचा*